The effect of aspirin and lipopolysaccharide binding protein on hypercoagulability induced by lipopolysaccharide

by

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It always seems impossible until it's done. -Nelson Rolihlahla Mandela

Declaration and originality

I, Sthembile Valencia Sibanda, hereby declare that I have read and understood the University of Pretoria's policy on plagiarism. I declare that this dissertation is my own original work. Where other people's work has been used (either from a printed source, internet or any other source), this has been properly acknowledged and referenced in accordance with the requirements of the Department of Physiology and the Faculty of Health Sciences. I have not used work previously produced by any other student or any other person to present as my own. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Sthembile Valencia Sibanda (Principal investigator)

Abstract

Inflammation is a well-known underlying cause in many diseases. Chronic inflammation has a role in a host of common and often deadly diseases, including Alzheimer's disease, rheumatoid arthritis, cancer, type 2 diabetes, heart disease, and even conditions like depression. A highly potent inflammagen might be one of the drivers of systemic inflammation, when present in circulation and it is the bacterial component called Lipopolysaccharide. It is known that lipopolysaccharide is located on the outer membrane of gram-negative bacteria. Recently, research has shown that this bacterial (and other) membrane components, when in circulation, might have a direct effect on blood hypercoagulability, which is a prominent hallmark of chronic, systemic inflammation. In this study it is shown that Lipopolysaccharide caused marked changes in the nature of the fibrin network formed. Furthermore, irregular red blood cells' membrane, platelets activation and formation of microparticles were seen when examining with scanning electron microscope and confocal microscope. Addition of Lipopolysaccharide caused significant hypercoagulation and changes to viscoelasticity of whole blood, as noted using Thromboelastograph. Airyscan confocal microscopy was used to study abnormal fibrin(ogen) protein folding in the presence of Lipopolysaccharide, using a well-known amyloid protein marker of anomalous blood protein clotting, called Thioflavin T. Previously, it was shown that this anomalous clotting of fibrin(ogen) fibres is amyloid in nature. Mopping agents are needed to reverse the hypercoagulation induced by Lipopolysaccharide. Biochemical agents (mopping agents) that will prevent anomalous blood clotting are therefore needed to reverse the hypercoagulation induced by Lipopolysaccharide. Such agents could, in future be used to prevent anomalous clotting in inflammatory diseases, where increased Lipopolysaccharide levels are known to be present.

The first Lipopolysaccharide mopping agent of choice is Lipopolysaccharide binding protein. The Lipopolysaccharide binding protein is a serum molecule that arbitrates cellular activation in reaction to endotoxin by ensuring the delivery of Lipopolysaccharide to either soluble or membrane bound forms of CD14 also known as mCD14. The second Lipopolysaccharide mopping agent in this study, is aspirin. Aspirin is an anticoagulant and commonly used anti-inflammatory agent. In literature aspirin has been found to increase fibrin clot porosity and susceptibility to lysis. Lipopolysaccharide binding protein, and the combination with aspirin, and the effects on anomalous blood clotting, is novel, and has not previously been studied.

The research questions that this thesis thus aims to address is, at what minimal concentration can the mopping agent Lipopolysaccharide binding protein be used to effectively "mop up" Lipopolysaccharide, and can Lipopolysaccharide binding protein be used in combination with Aspirin, an anticoagulant agent, to reduce the signs of hypercoagulation produced by Lipopolysaccharide *ex vivo*? Which techniques can be used to visually see the difference in structure and elastic function of fibrin(ogen) as well as cells in the coagulation system, namely red blood cells and platelets?

The results shown here reveal that Lipopolysaccharide binding protein can reduce the effect of Lipopolysaccharides on fibrin(ogen) and cells of the coagulation system at an exposure concentration of $2ng.L^{-1}$. The coefficient of variation from Airyscan confocal between control and treatments indicated a decrease in the intensity of samples treated with Lipopolysaccharide binding protein mixed with Lipopolysaccharide and a decrease in intensity from whole blood treated with aspirin compared to control ($p=0.0106$). The coefficient of variation calculated from Scanning electron microscopy, showed a distinct alteration in the clots from samples treated with Lipopolysaccharide binding protein and aspirin alone and mixed together gave (more variation of light coming from fibres, neatly branched forming three layers) as significant difference, when aspirin was mixed with Lipopolysaccharide showed less variation light from fibres fussed into one layer with the contact surface and the same with aspirin mixed with Lipopolysaccharide (less light variation) compared to the control (untreated PPP) ($p<0.0001$).

From the Thromboelastography® results there was a statistically significant differences in clot strength between whole blood and whole blood mixed with Lipopolysaccharide binding protein and Lipopolysaccharide, ($p=0.0412$). This shows a decrease in platelet and/or fibrin(ogen) interaction resulting in a less dense clot that is less rigid in fibrinogen or platelets. The time from clot initiation to maximum clot formation decreased when whole blood was mixed with aspirin together with Lipopolysaccharide binding protein and Lipopolysaccharide as compared with naïve whole blood, ($p=0.0071$). This usually occurs in hypercoagulation seen by decreased time from clot initiation to maximum clot formation. The amount of thrombus total resistance decreased significantly when whole blood was mixed with aspirin and Lipopolysaccharide verses whole blood alone, ($p=0.0078$). This shows that the addition of aspirin to Lipopolysaccharide can lead up to hypocoagulable state were the clot strength decreases.

When measuring fibre thickness it was found that Lipopolysaccharide binding protein alone and in combination with aspirin effectively reduced the large fibres produced by Lipopolysaccharide, almost back to normal size which is +-110nm. The difference was statistically significant ($p<0.05$).

Lipopolysaccharide binding protein and aspirin showed impeccable results reducing the signs of Lipopolysaccharide-induced hypercoagulation, both in plasma and whole blood. Many non-communicable diseases have been shown to have a bacterial component. The amyloid protein created with Lipopolysaccharide, can be reversed in the blood model with the study's novel combination of Lipopolysaccharide binding protein/aspirin could be implemented in applications for treatment of conditions where there is presence of Lipopolysaccharide particularly sepsis.

Key words: Aspirin, chronic inflammation, confocal microscopy, Fibrin, Lipopolysaccharide, Lipopolysaccharide binding protein, Hypercoagulation, Thromboelastography®

Table of Contents

List of Tables

List of Figures

List of abbreviations

Abbreviations	Names
AD	Alzheimer's disease
ASA	Acetyl salicylic acid (aspirin)
BSE	Back Scattered Electrons
CD14	cluster of differentiation 14
$CaCl_2$	Calcium (II) Chloride
Co-Cr	Coated cobalt chromium
COX	Cyclooxygenase
CV	Coefficient of variation
CLSM	Confocal laser scanning microscopy
CD	Crohn's disease
DAMPs	Damage associated molecular pattern molecules
DIC	Disseminated Intravascular Coagulation
DM	Diabetes Mellitus
DMDs	Dense matted deposits
EAA	Endotoxin activity assay
E. coli	*Escherichia coli*
EDX	Energy dispersive x-ray
Hb	Hemoglobin
OH	Hydroxyl radical chemical formula
ICU	Intensive care unit
IL1,3&6	Interleukins

KDO	keto- deoxyoctulosonate
(LAL) Assay	Limulus amebocyte lysate
LPS	Lipopolysaccharide
LBP	Lipopolysaccharide-binding protein
MD2	human MD-2 molecule
MetS	Metabolic Syndrrome
MPs	Microparticles
MRTG	Maximum rate of thrombin generation
NSAID	Non-steroidal Anti-inflammatory Drugs
NF-kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
OMVs	Organization pour la Mise en Valeur du fleuve Senegal
PD	Parkinson's disease
aPTT	Partial thromboplastin time
PAMP	Pathogen associated molecular pattern
PDLLA	Poly (DL-lactic acid)
PrPSc form	PRion Protein SCrapie
PDLLA	Poly (DL-lactic acid)
PPP	Platelets Poor Plasma
PT	Prothrombin time
RBCs	Red Blood Cells
RA	Rheumatoid arthritis
Rho-kinase	Rho-associated protein kinase
RS-LPS	Rhodobacter sphaeroides Lipopolysaccharide

SEM	Scanning Electron Microscope
TF α	Tissue factor α
TLR4	Toll-like receptor 4
TMRTG	Time to maximum rate of thrombus generated
TEG	Thromboelastography®
ThT	Thioflavin
TNF	Tumor necrosis factor
TPA	Tissue plasminogen activator
TTG	Total thrombus generation
UC	Ulcerative Colitis
WB	Whole Blood

Chapter 1: Introduction

Inflammation is a well-known fundamental cause in many diseases. Chronic inflammation has a role in a host of common and often deadly diseases, including Alzheimer's disease (AD), Rheumatoid Arthritis (RA), cancer, type 2 diabetes mellitus (T2DM), heart disease, and even conditions like depression. A highly potent inflammagen might be one of the drivers of systemic inflammation, when present in circulation and it is the bacterial component called lipopolysaccharide (LPS). It has been shown through research that LPS is located on the outermost layer of gram-negative bacteria and plays a vital role in inciting low-grade inflammation (Glaros et al., 2013). Low grade or also known as metabolic endotoxemia refers to sub-clinically elevated levels of circulating blood endotoxin, this has also been found in chronic inflammatory diseases. (Terawaki et al., 2010, Moreno- Navarrete et al., 2010, Cani et al., 2008, Wiedermann et al., 1999).

Recently, research has shown that this bacterial (and other) membrane components, when in circulation, might have a direct effect on blood hypercoagulability, which is a prominent hallmark of chronic, systemic inflammation. The presence of LPS in circulation from inflammatory diseases, may have a variety of origins, including permeable gut, periodontitis and gingivitis and ultimately an uncharacteristically functioning immune system. The quantitative valuation of LPS concentrations in blood can be difficult. The Presence of LPS may have crucial clinically relevant effects on the blood microenvironment and may be essential in the cure/moderation of inflammatory conditions (Kell and Pretorius, 2015d, Potgieter et al., 2015a, Kell et al., 2015b, de Punder and Pruimboom, 2015, Latta et al.2015). Lipopolysaccharide was also found to have played an essential role in chronic inflammation like in AD and Parkinson's disease (PD) patients (Potgieter et al., 2015, Pretorius et al., 2017b, Pretorius et al., 2018b).This will be further discussed in the literature review.

Lipopolysaccharide is the key component of the outer membrane of Gram-negative bacteria. LPS is in unencapsulated strains, exposed on the cell surface. It is believed that LPS induces a spectrum of biological effects, which may be detrimental or advantageous for the host (Chan et al., 2018, Freudenberg and Galanos, 1990, Jiao et al., 1989). Within Gram- negative bacteria, the outer membrane of LPS shields the bacterium counter to the action of bile salts and lipophilic antibiotics.

The dysfunction of hepatobiliary transporter systems caused by endotoxin-induced autoantibodies is known to be involved in the development of postoperative jaundice associated with bacterial infection after major hepatectomy (Chan et al., 2018). Lipopolysaccharides are heat stable endotoxins and have long been recognized as a key factor in septic shock in humans and more generally, in causing a strong immune response in ordinary mammals' cells (van Heesch et al., 2013, Moraes et al., 2018).

Lipid A is the biologically active part of the LPS molecule, which has been identified as critical to the endotoxin activity of LPS. This was demonstrated by (Jiao et al., 1989) who found identical bioactive outcomes, including endotoxic activity, among synthetic and natural-sourced *E. coli* lipid A preparations. The active receptor for LPS has been recognized as the CD14/TLR4/MD2 receptor complex, which promotes the secretion of proinflammatory cytokines including tumor necrosis factor-α and interleukin-1 (Dai et al., 2018, Barnea et al., 2016, Aldrich and Sevick-Muraca, 2013). While the lipid A component is primarily responsible for immune response activation, the polysaccharide component of *Salmonella enterica* LPS is also necessary for NF-Kb.

Previous research demonstrated that LPS triggers hypercoagulation of whole blood (WB) (Li et al., 2018, Luo et al., 2018, van Heesch et al., 2013, Zang et al., 2018) and in plasma using thromboelastography® (TEG) (Pretorius et al., 2016d) this was also visible when viewing the ultrastructure of WB and fibrin fibres using scanning electron microscopy (SEM) (Pretorius et al., 2016d, Pretorius et al., 2017a). It was shown that LPS when added to PPP it caused dense matted mass deposits (DMDs) and unusual fibrin fibres in shapes and sizes when viewed with SEM the outcome were published (Pretorius et al., 2016a). The same pattern of irregular fibres was seen commonly in chronically inflammatory patients with AD and PD (Potgieter et al., 2015). The formation of amyloid in fibrin(ogen) proteins and the major clotting protein in plasma, was also seen with confocal microscopy (Pretorius et al., 2016d, Pretorius et al., 2017a).

The first LPS mopping agents of choice is Lipopolysaccharide binding protein (LBP). The LBP is a serum molecule that arbitrates cellular activation in response to endotoxin by ensuring the delivery of LPS to either soluble or membrane bound forms of CD14 also known as mCD14 (Thompson et al., 2003, Tobias et al., 1993). Natural LBP is a 58KD glycoprotein produced in the liver. It binds at lipid A of LPS with high affinity (10-9M) and lowered the cellular LPS effects at CD14+ cells (IL1ß, IL6, TNF-α) (Kim et al., 2016, Thompson et al., 2003)

It plays as opsonin for GRAM negative cells, LPS, neutrophils and granulocytes (Pretorius et al., 2016a, Pretorius et al., 2018b, Thompson et al., 2003). Aside from this activating role, previous work has shown that LBP and LPS can bind to cells by forming large groups which are secured by mCD14. This binding phenomenon does not correlate with cellular activation. Researchers have shown that neutralization of LBP achieved by blocking either the binding of LPS to LBP or the binding of LPS/LBP complexes to CD14 protects the host from LPS-induced toxicity, confirming that LBP is a critical component of innate immunity (Le Roy et al., 1999).

The second LPS mopping agent of choice is acetylsalicylic acid (aspirin). It is an anti-inflammatory drug, and it was introduced for treating human maladies about 100 years ago (Ohno et al., 2002, Cingoz and Gurel, 2016). It has remained the most commonly used drug for relieving pain, inflammatory symptoms, and fever (Cingoz and Gurel, 2016). Aspirin also has established efficacy for inhibiting myocardial infarction and ischemic stroke, as well as for treating acute myocardial infarction (Lopes et al., 2016). A recent animal study found a vasodilating and blood pressure lowering effect of aspirin independent of cyclooxygenase (COX), (these are granules important for protein translation) but mediated by inhibition of the Rho-associated protein kinase (RhoA/Rho kinase) signaling pathway (Leithäuser et al., 2012)**.** RhoA/Rho-kinase pathway plays an essential role in various pathological conditions. The RhoA partakes in the control of smooth muscle tone and activates many downstream kinases. The best characterized are the serine/threonine kinase isoforms (Rho-kinase or ROCK), ROCKα/ROCK2 and ROCKβ/ROCK1. ROCK is necessary for varied purposes such as local blood flow, arterial/pulmonary blood pressure, airway resistance and intestinal peristalsis (Mason et al., 2004). ROCK activation permits actin/myosin interactions and smooth muscle cells reduction by maintaining the activity of myosin light-chain kinase, independently of the free cytosolic calcium level. The sensitization of smooth muscle myofilaments to calcium has been implicated in many pathological states, such as hypertension, diabetes, heart attack, stroke, pulmonary hypertension, erectile dysfunction, and cancer. (Nunes et al., 2010). Aspirin also acetylates lysine residues in fibrinogen resulting in increased fibrin clot permeability and enhanced clot lysis as well as directly promoting fibrinolysis with high-dose aspirin (Undas et al., 2007, Undas et al., 2006).

The question that arose was, is there a mopping agent for LPS in humans?

The research questions that this thesis thus aims to address, at what minimal concentration can the mopping agent LBP be used to effectively "mop up" LPS , and can LBP be used in combination with aspirin an anticoagulant agent to reduce the signs of hypercoagulation produced by LPS *ex vivo*? What techniques can be used to see the difference in structure and elastic function of fibrin(ogen) as well as cells in the coagulation system, namely red blood cells (RBCs) and platelets? In order to address these questions, 0.2 ng. L^{-1} of bacterial LPS from *Escherichia coli* (*E. coli*) was added to either platelet poor plasma (PPP) or WB. Aspirin was added to a final concentration of 0.5mM and LBP was at a final exposure concentration of $2ng.L^{-1}$.

The null hypothesis (H_0) of the study was that the effect of LPS on blood clotting and hypercoagulation, including RBC and platelet structure, cannot be reversed, or prevented by LBP and aspirin, using healthy blood and plasma.

The alternative hypothesis (H_1) of the study was that at low physiological levels of LBP ($2ng.L^{-1}$) and aspirin (0,5mM) final exposure concentrations will reduce the adverse effects induced by LPS both in plasma and WB.

The novelty of the study

Amyloid fibrils are formed by soluble plasma proteins, which assemble to form insoluble fibres that are resistant to degradation. Their formation can accompany disease and each disease is characterized by a specific protein or peptide that aggregates (Kell and Pretorius, 2015a, Kell and Pretorius, 2015b, Kell and Pretorius, 2017). Our research group was the first to use the Thioflavin T (ThT) marker to mark amyloid protein strain and viewed with Airyscan. In recent years our research group was the first to report that fibrinogen molecules change their biochemical structure to become amyloid in the presence of a bacteria component such as LPS (Pretorius et al., 2016a, Kell and Pretorius, 2017).

Research has shown that aspirin plays a role in coagulation as an antiplatelet agent, as well as an anti-inflammatory agent and may part take in reducing microparticle formation, whereas LBP has been reported to bind to LPS inhibiting the binding of LPS to plasma fibrin(ogen) proteins (Kell and Pretorius, 2015b, Pretorius et al., 2016a, Pretorius et al., 2017a).

Currently there are only just two papers with research that is in close relation to this study (Osnes et al., 2000) and (Pernerstorfer et al., 1999). The following is a brief summary on their outcomes and how their study is different to this current study:

1). *The effect of aspirin on LPS incubated of WB was investigated by Osnes and team, year 2000*. The authors used a concentration of 5mM aspirin in attempt to compact LPS-induced symptoms. Aspirin induced a concentration dependent increase 2.5-5-fold at 5 mM aspirin in LPS-induced appearance of TNF-alpha and fibrinopeptide in plasma. They reported that aspirin raised the levels of LPS-induced TF-mRNA and TNFalpha-mRNA in monocytes isolated from WB. These researchers showed that the presence of LPS in aspirin had pro-inflammatory effects (Osnes et al., 2000).

This current study support's their outcome as we had similar results as aspirin couldn't reduce hypercoagulation of whole blood and plasma that was induced by LPS. The two studies are different since in this current study aspirin was tested alone and in combination with LBP to compact hypercoagulation. This study focuses on the morphology of RBCs, platelets, the structural defects on fibrin molecules and the folding of fibrinogen into final fibrin mesh seen in clots. The clot structure gives us an indication of the state (hypercoagulable/hypercoagulable) and nature of the sample tested. Confocal shows the nature of the fibrin fibres through identifying amyloid proteins. For this research physiological concentration levels of aspirin was used instead of a high dose therapy level. Literature shows that using an increase in aspirin concentration may cause damage to the endothelial layer (Brighton et al., 2012, Zhang et al., 2014) hence we used physiological levels of 0.5mM.

2). *Endotoxin-Induced Activation of the Coagulation Cascade in Humans by Pernerstorfer and team, year 1999.*

The second was written by a group of researchers who studied the effects of aspirin on systemic coagulation that was activated by LPS. The researchers used human life models and there were given LPS intravenously (4 ng/kg IV) after intake of either placebo or aspirin (1000 mg). They also gave their volunteers a dosage of 100 mg of acetaminophen 1000 mg was given to a third group to control for possible effects of antipyresis. Their results showed that aspirin or acetaminophen could not inhibited LPS-induced coagulation (Pernerstorfer et al., 1999).

They used blood cell counts, flow cytometry, albumin levels and commercialised tissue factors (TF) in vitro to study the type of TF affected during coagulation before and after LPS was administered and with aspirin.

In this current PhD study aspirin and LBP were used in combination and separately to compact the effect of LPS (hypercoagulation). This was an *ex vivo* study, and TF were not used to asses coagulation, TEG was used to study the viscoelastic properties of each participants reaction to LPS, LBP and aspirin. The TEG results showed the total thrombus generation TGG (dcs) decrease when aspirin was mixed with LPS compared to control. In this way it is shown that LPS and aspirin resulted in a faster clotting time. We are in agreement with this study, however the study's design and objective are different.

Our group (where I was a co-author) was the first to show that by using LBP to LPS incubated blood or inflammatory conditions we can selectively affect fibrin fibres and hence regulate blood clotting (Pretorius et al., 2018a, Pretorius et al., 2017a, Pretorius et al., 2018b). In recent years many non-communicable diseases have been shown to have a bacterial component. The amyloid protein created with LPS, can be turned around in the blood model with the study' novel combination of LPB/aspirin it could have far reaching applications for treatment of conditions where LPS presence is implicated in particularly sepsis.

Chapter 2: Literature review

This literature review will focus on discussing LPS, LBP, and aspirin in more detail, and why LPS might be an important target in clinical studies of inflammatory conditions.

Lipopolysaccharide as potent inflammagen

Humans and animals all have endotoxins, the most popular typer is LPS (Diacovich and Gorvel, 2010, Parija, 2009). Dormant microbes and colonizing bacteria shed off LPS during an infection (Marshall et al., 2002). Through research LPS as an endotoxin is well established as a strong inflammatory agent (De Castro et al., 2010, Płóciennikowska et al., 2015). The production of inflammatory cytokine causes damage ranging from low grade inflammation to sepsis is a result of replacement and production of LPS (Kitchens and Thompson, 2003, Marshall et al., 2002).

Lipopolysccahride is made of fats and polysaccharides it is a lipoglycan (Polanowska-Grabowska et al. 1993, Kell and Pretorius, 2015c). For the bacterial existence the structure of gram-negative bacteria is important (Tzeng et al., 2002), the O-polysaccharide is a sugar component and contains repeating glycan polymers (Holst, 2007). This region is serves as a target for detection by host antibodies and it is found on the outer domain of the LPS molecule and (De Castro et al., 2010, Rittig et al., 2003). The arrangement of the 0-chain and occurrence determines if LPS is rough or smooth (Rittig et al., 2003). It is the entire length of the O-chain that makes LPS smooth, however when it is absent LPS is classified as being rough (Rittig et al., 2003, Kell and Pretorius, 2015c). Lipopolysaccharide that is classified as rough are known to have penetrable cell membranes to hydrophobic antibiotics (Rittig et al., 2003).

Continuing with the structure of LPS, the latter region is known as lipid A. It is phosphorylated with many fatty acids and these rich hydrophobic regions are useful in conferring the LPS into its bacterial membrane (Ding et al., 2013, Müller- Loennies et al., 2003, Holst, 2007, De Castro et al., 2010). The core domain of this region contains sugar molecules called keptose and 3-deoxy-D-mannoctulosonic acid also known as KDO (keto-deoxyoctulosonate) (Kell and Pretorius, 2015c, Holst, 2007). During infections or illnesses, the bacterial cells are cut off by the immune system into tinny particles. It is these membrane fragments that contain the destructive and harmful lipid A. They are exposed to the circulation and trigger inflammatory symptoms such as fever, diarrhea to more serious conditions like fetal endotoxins shock (Ding et al., 2013, Diacovich and Gorvel, 2010, Van Oosten et al., 2001).

The non-automatic release of LPS from bacterial walls is due to a physiological activity of membrane vesicle trafficking in the process of bacterial outer membrane vesicles (OMVs), these also may contain other viruses and proteins (Kell and Pretorius, 2015c, McBroom et al., 2006).

As mentioned in the introduction chapter, LPS increases the negative charge of the cell membrane as it constitutes the major part of the outer membranes from the outer gram-negative bacteria (Abrahamsson et al., 2014, Cuaz-Pérolin et al., 2008, McBroom et al., 2006). The LPS helps to steady the overall arrangement, any alterations that occur in the outer membrane is primarily for physical integrity and securing of the membrane from chemical impairments/harms (Moran et al., 1996). In the case of activation of inflammatory response cell, the LPS in it host cells binds to CD14/TLR4/MD2/ receptor complex to immune cells (mononcytes, B-cells, dendritic cells and macrophages). The active immune cells support the secretion of pro-inflammatory cytokines, eicosanoids and nitric oxide (O'Neill, 2014, Park et al., 2009, Poltorak et al., 2000, Muroi and Tanamoto, 2002).

The LPS when it is inside the host, it stimulate the secretion of cytokines for inflammation which can lead to increased chronic inflammatory reactions such as auto immune diseases and lower-grade inflammation present in AD patients (Bester et al., 2015b, Park et al., 2009) . The superoxide is produced as part of cellular reaction, it is known as a major reactive oxygen species (ROS). Lipopolysaccharide stimulates the ROS species in numerous TLR4 expressing cell types (Beutler et al., 2001, Termeer et al., 2002, Płóciennikowska et al., 2015).

The *Neisseria meningitides* are clinical signals of infections with pathogenic Gram-negative bacteria. These pathogens trigger meningococcal disease involving meningococcemia, Waterhouse-Friderichsen set of symptoms, Haemophilus influenzae and meningitis ((Tzeng et al., 2002, Moran et al., 1996, Van Oosten et al., 2001). In the core part of sugars (oligosaccharides) in *Neisseria* spp. of LPS copy the carbohydrate moieties of glycosphingolipids present in human cells. These imitations may work to mask the bacterial surface from the host (Moran et al., 1996).

LPS and bacterial components as drivers of inflammation in inflammatory conditions

A major health risk nowadays is Chronic inflammatory disease. Most of the disorders are known to be supplemented by long-term inflammation, and most of them are characterized by an increase in serum ferritin (SF) and hypercoagulability (Bester et al., 2015b, Kell and Pretorius, 2015c, Schumann and Zweigner, 1999).

Typically, these inflammatory symptoms are triggered by pro-inflammatory cytokines, although the cause of this inflammation is still unknown. Researchers found that bacteroides species are not abundant, but they are dominate in Finnish and Estonian infants. Therefore, LPS exposures came from bactericides rather than from *E. coli*. They revealed that bactericides LPS is structurally different from *E. coli* LPS and prevents innate immune signaling and endotoxin tolerance (Ghanim et al.,2009). Furthermore, unlike LPS from *E. coli, B. dorei* LPS does not decrease the occurrences of autoimmune diabetes in non-obese diabetic mice (Ghanim et al., 2009, Zhou et al., 2015).

A typical principal description for systemic inflammation in non-infectious diseases, is a dormant microbiome which is accompanied by a continuous chronic dysbiosis of the gut that can shed the inflammatory LPS. This in the literature reviews from papers by our group (Kell et al., 2015b, Kell and Pretorius, 2015a, Potgieter et al., 2015a). Due to the discovered information that all inflammatory conditions show a pathology in hematology and clotting cells, our research group investigated the impact of LPS on the cardiovascular system by studying the fine morphology of RBCs, clot structure as well as platelets using SEM, fibrin networks, platelets, as well as the viscoelastic parameters were also determined using TEG were low physiological concentrations of LPS was added to healthy participants' blood *ex vivo*, this research output was published (Pretorius 2016). This current study is a continuation of this research output. The current study now focuses on the treatment of hypercoagulation induced by LPS.

Figure 2.1 is representative of the action pathway taken by LPS and the role it plays during inflammatory diseases. This is a schematic diagram of the different sections discussed in this chapter.

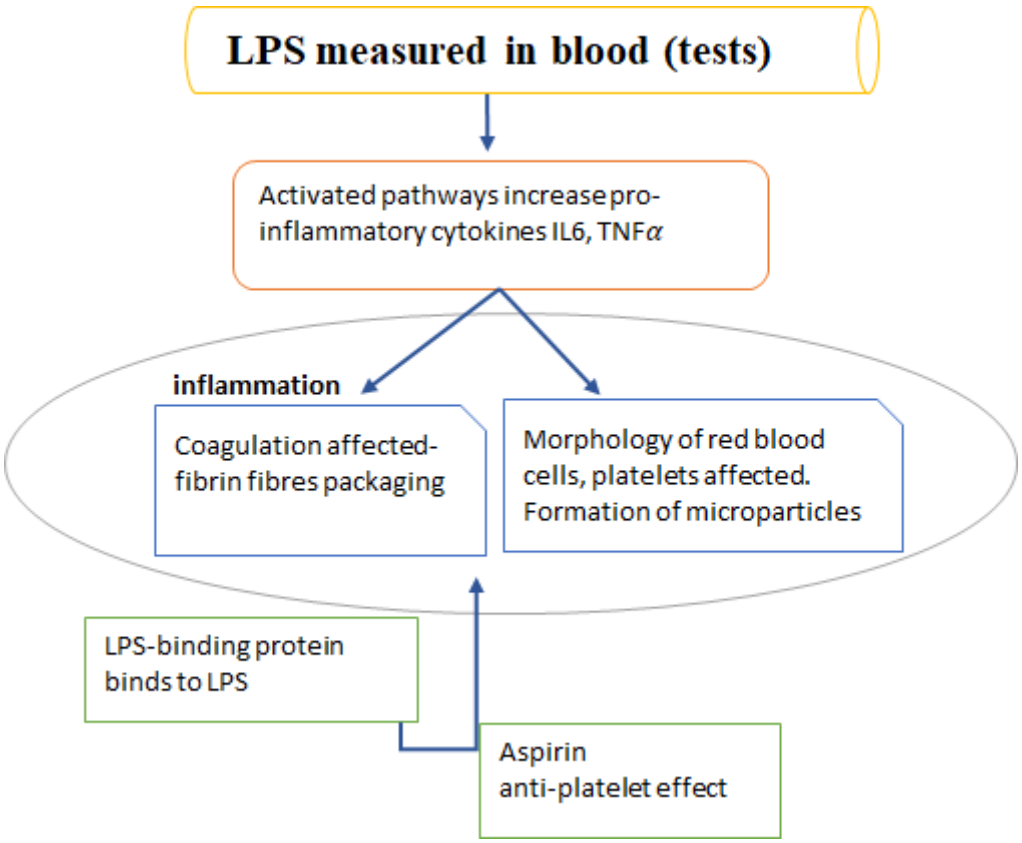


Figure 2. 1.A pathway followed by LPS in activating pro-inflammatory cytokines, to induce inflammation and the effects of LPS on red blood cells and fibrin fibres. This figure will be used repeatedly to visually illustrate the structure of the thesis**.**

The occurrences of LPS during hypercoagulable states in research shows that LPS can play a vital role in the development of the diseases in humans and animals see (Hagar et al., 2013, Liu et al., 2005, Sandahl et al., 2014, Shi et al., 2014). Hence the detection methods for levels of active and circulating LPS are crucial for treatment and maintaining of LPS levels. In the following paragraph the finding methods for LPS will be discussed, the figure below has a red box highlighting the endotoxins test assay box.

Testing for Endotoxaemia in blood

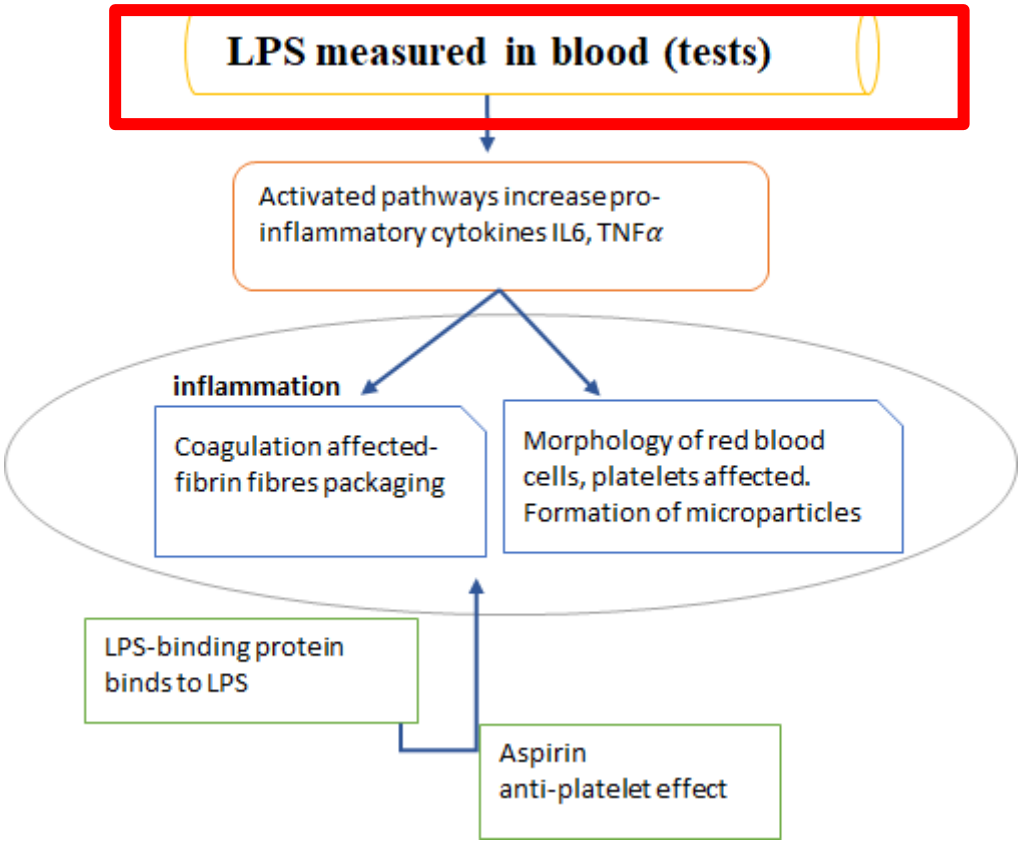


Figure 2. 1.A pathway followed by LPS in activating pro-inflammatory cytokines, to induce inflammation and the effects of LPS on red blood cells and fibrin fibres. The red box highlights the next section in this literature review which will be on the detection methods for LPS.

Due to limitations of existing endotoxin assays Gram-negative infection and the clinical set of symptoms of sepsis have been challenging to determine (Morrison and Ulevitch, 1978). Therefore, researchers have used critically ill patients from medical or surgical intensive care unit (ICU) to quantify the total LPS in their blood. The manner in which they conduct the test is that on the day of ICU admittance, they determined endotoxin concentrations using a novel chemiluminescent assay see (Marshall et al., 2002) this was the chromogenic alteration of the limulus amoebocyte lysate (LAL) assay. They studied 74 patients who were admitted consecutively. Endotoxin levels increased in patients with sepsis than in patients that clinically presented with a condition other than sepsis concentrations were 470 ± 57 pg/ml and 157 ± 140 pg/ml respectively. Endotoxaemia was considerably associated with Gram-negative infection and no patient with a Gram-negative infection had an endotoxin level below 50 pg/ml. White blood cell counts of patients with EAA-detected endotoxaemia were significantly elevated to ($15.7 \pm 9.1 \times 10^9$ cells/l for endotoxaemic patients versus $10.8 \pm 6.2 \times 10^9$ cells/l for patients without endotoxemia).

These researchers suggested that EAA could be a diagnostic tool for the studying invasive Gram-negative infection and early sepsis. Researchers found more deviancies in the assay to mark it as incomplete, they suggested better assays with better accurate results however, the concentrations are normally assayed using the EAA method (Jiang et al., 2009, Harte et al., 2010a, Su and Ding, 2015). When using the assay some researchers found that white blood cell counts of patients with endotoxin activity assay EAA detected was significantly higher (Marshall et al., 2002, Hurley et al., 2015) even elevated levels were found in patients with diabetes (Pussinen et al., 2011). However, the LPS blood concentration were not clear blood due to it hydrophobic nature, little or no LPS is actually free (Kell and Pretorius, 2015d). although acceptable in simple conditions, this test is not reliable in blood, due to results that are inconsistent, even between controls and patients (Andrä et al., 2004, Ketchum and Novitsky, 2000, Mattsby- Baltzer et al., 1991, Novitsky, 1998). Nevertheless, it is still important to know in which diseases LPS is most prevalent and what concentrations are found in each inflammatory disease. **See Table 2.1** for a detailed list of all conditions that have been linked with LPS.

Table 2. 1. Diseases associated with LPS and quantified LPS concentrations taken from (Kell and Pretorius, 2015c)

Disease	**LPS in disease**	**LPS in controls for studies**	**Tissue type**	**Reference**
Acute coronary syndrome	0.61 $\mu g.mL^{-1}$	1.87 $\mu g.mL^{-1}$	Serum chlamydial lipopolysaccharide (cLPS)	(Tiirola et al., 2007)
Inflammatory bowel disease	12.6 $pg.mL^{-1}$ (5·9−16·2)	12·2 $pg.mL^{-1}$ (3·8−26·3)	Serum	(Funderburg et al., 2013)
Non-alcoholic fatty liver disease	7.8 -14.8 EU/mL	3.2 - 5.2 EU/mL	Serum	(Harte et al., 2010b)

Sepsis	300 .mL^{-}	.mL^{-1}	Plasma	(Opal et al., 1999)
Type 1 diabetes				
Microalbuminuria group	31–60 EU/mL	-	Plasma LAL assay	(Nymark et al., 2009)
Normoalbuminuric group	38–74 EU/mL	-	Plasma LAL assay	
Type 2 diabetes				
Non-obese Postmenopausal Women	-	0.37±0.02 EU/mL	Plasma LAL assay	(Zaman and Zaman, 2015)
Diabetic non-obese Postmenopausal women	0.39±0.03 - EU/mL		Plasma LAL assay	
Insulin-treated diabetes	6.6–10.7 EU/ml	3.1–5.1 EU/mL	Serum	(Al-Attas et al., 2009)

The occurrence of LPS has essential and clinically relevant effects on the blood microenvironment, and most importantly the care of inflammatory conditions (Kell and Pretorius, 2015d, Potgieter et al., 2015a, Kell et al., 2015b, de Punder and Pruimboom, 2015, Latta et al.2015) due to the inaccuracy of the EEA test to quantify LPS concentrations in WB can be difficult. The foundations of blood endotoxin may be derived from permeable mucosal barriers and concentrated (Glaros et al., 2013), this is known to trigger a non- resolving and chronic inflammatory state (Maitra et al., 2011, Laugerette et al., 2011, Maitra et al., 2012). This may explain the development of chronic inflammatory diseases linked with low dose endotoxemia (Glaros et al., 2013). **See Table 2 .2** for list and quantified measurement of LPS found in tissues.

Table 2. 2. Quantified concentrations of LPS found in different tissues taken from (Kell and Pretorius, 2015c)

Tissue type	(LBP) concentration	(LBP): control	REFERENCE
Bacterial gastrointestinal infections	28.5 ± 16.5 μg.mL^{-1}	-	(Elsing et al., 2011)
Crohn's disease (CD) and ulcerative colitis (UC)	57·11 μg.mL^{-1} (49·4−65·8)	50·01 μg.mL^{-1} (37·1−63·9)	(Funderburg et al., 2013)
Diabetes type 2	19.78 ± 6.40 μg.mL^{1}	20.53 ± 6.99 μg.mL^{-1}	(Zhou et al., 2015)
Endocarditis			
Infectious endocarditis	Median 33.41 mg ɪ $^{-1}$	Median 5.61 mg ɪ $^{-1}$	(Vollmer et al., 2009)
Noninfectious heart valve diseases	Median 6.67 mg.L^{-1}		
Inflammatory bowel disease	52·7 μg.mL^{-1} (45·4−64·6)	39·1 μg.mL^{-1} (32·1−43·7)	(Funderburg et al., 2013)

The potential causes of blood endotoxin and localization of the LPS in blood and tissues has been reviewed in the above section. The next paragraph focuses on LPS and its role in pro-inflammatory cytokine production as highlighted by the red box in the figure below.

Lipopolysaccharide activating pro-inflammatory cytokines

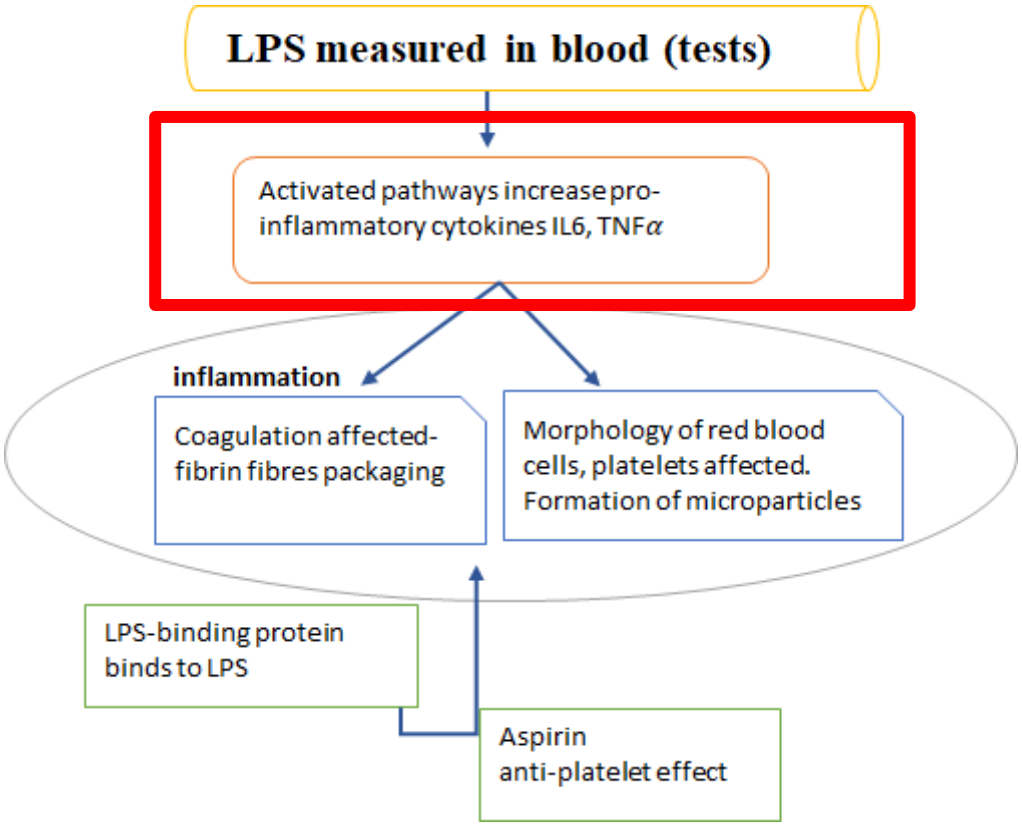


Figure 2. 1.A pathway followed by LPS in activating pro-inflammatory cytokines. The topic of the section to be reviewed is highlight of with the red box. This section will focus on the direct pathway LPS takes to activate pro-inflammatory cytokines.

Lipopolysacchairde is known to trigger inflammatory cells, to release a large number of proinflammatory cytokines such as IL-8, IL-6, IL-1β, IL-1, IL-12, and IFNγ (Ramachandran, 2014a, Tracey and Lowry, 1990, Beutler, 1993). Moreover researchers have also incorporated TNFα in the list as it also plays a part during endotoxic shock and initiates tissue damage (Ramachandran, 2014a, Beutler et al., 1985). Lipopolysaccharides in combination with TNFα, are known to induces cell death on the endothelium layer in several tissues including intestine, and thymus (Haimovitz-Friedman et.al., 1997a).

Through research LPS is now identified as a leading pathogen-associated molecular pattern (PAMP) that triggers the body's innate immune response to pathogens (Li et al., 2016, Zhang et al., 2016). Researchers observed that when LPS was injected in mice, it would stimulate a cytokine reaction and transient stimulation of the proinflammatory cytokines TNF- and IFN- (Haimovitz-Friedman et al., 1997b, Ramachandran, 2014b) these are the acute-phase response and Verstrepen with team found an enhanced increase in IL- 6, which peaked at 3 to 4 hours after LPS injection (Verstrepen et al., 2008).

The host's cells distribute damage-associated molecular pattern molecules (DAMPs) as markers that signal the innate immune system to unexpected cell death to invading microbial (Li et al., 2016).

Lipopolysaccharide binds to TLR4 to stimulates an innate immune response, these cells are single membranes commonly found in macrophages and can be dendritic cells which locates molecules derived from bacteria (Poltorak et al., 1998, Beutler et al., 2001, Park et al., 2009, O'Neill, 2014). Normally, LPS bounded to LBP in blood is carried by CD14 co- receptor to TLR4 receptors. It was found that innate immunity in AD patients was also associated with the upregulation of CD14 co-receptor (Reed-Geaghan et al., 2009, Heppner et al., 2015) also this has been discussed in the introduction, it is repeated here to emphasize the importance of these cells-receptors binding in the generation of inflammation. The magnitude of cytokine activation shows the binding strength to CD14/TLR4 (Tsutsumi-Ishii et al., 2008). Hence the stronger the attachment of LPS to CD14/TLR4 the greater the activation of cytokines. These inflammatory cytokines are triggered by a set of pathways illustrated in **Figure 2.2** with the transcription factor NF-kB and it role adapted from (Tak et al., 2001, Noort et al., 2015).

A series of kinases are disabled when extracellular signals coming from binding of LPS(Tiirola et al., 2007) , more particular the phosphorylation of IKB to release the NF-KB which translocate in blood to LPB. Here LPS is transported to the TLR4 receptor via CD14 co-receptor (Reed-Geaghan et al., 2009, Heppner et al., 2015, Liu et al., 2005). Research has shown that the inhibitor IkB protein can inactivate the NF-KB in the cytoplasm (Tiirola et al., 2007) particularly phosphorylates the IkB and in so doing releasing the NF-kB that can translocate to the nucleus to convert on a large variety of other genes, as well as TNF-a and IL-6 (Kellum et al., 2007) see **Figure 2.2** diagram of inflammatory cytokine that are activated due to presence of LPS. Interestingly there is also a not related LPS activation pathway which is not dependent of TLR4 (Hagar et al., 2013, Kayagaki et al., 2002, Shi et al., 2014) it occurs at elevated external concentrations of LPS. However, this study will not be looking into this pathway because of the low physiological concentrations' levels of LPS used for the study showing the effects of LPS at physiological concentrations.

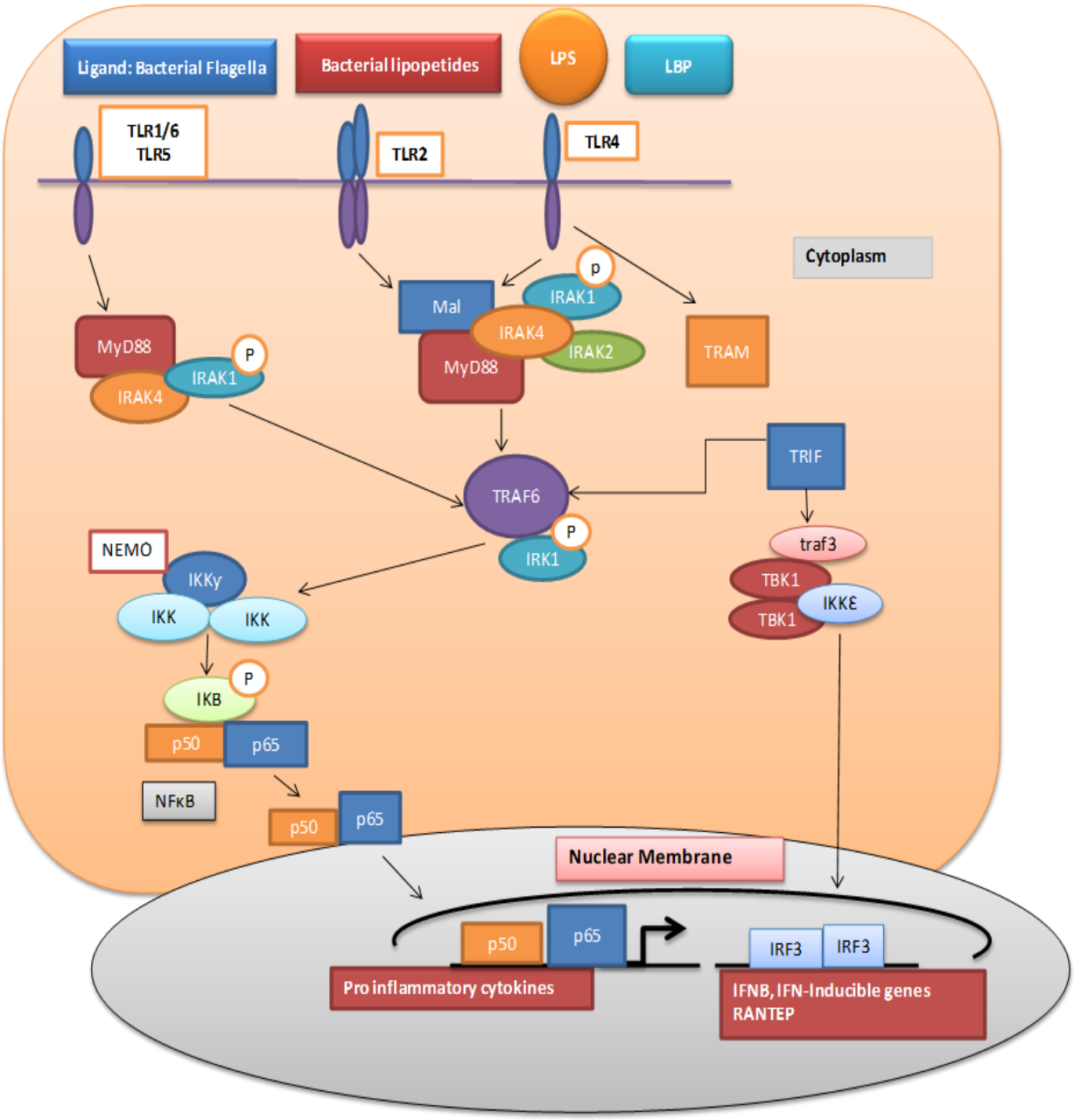


Figure 2. 2. Inflammatory cytokines activated by LPS adapted from (Kell and Pretorius, 2015c)

In the section that follows the presence of LPS in blood will be reviewed as well as how this endotoxin might bind to RBCs, keeping in mind that RBCs do NOT have the regular LPS binding receptors present on the cells (Viriyakosol and Kirkland, 1995).

Effect of LPS on RBCs may Propagate Inflammation via changes in RBC structure and function

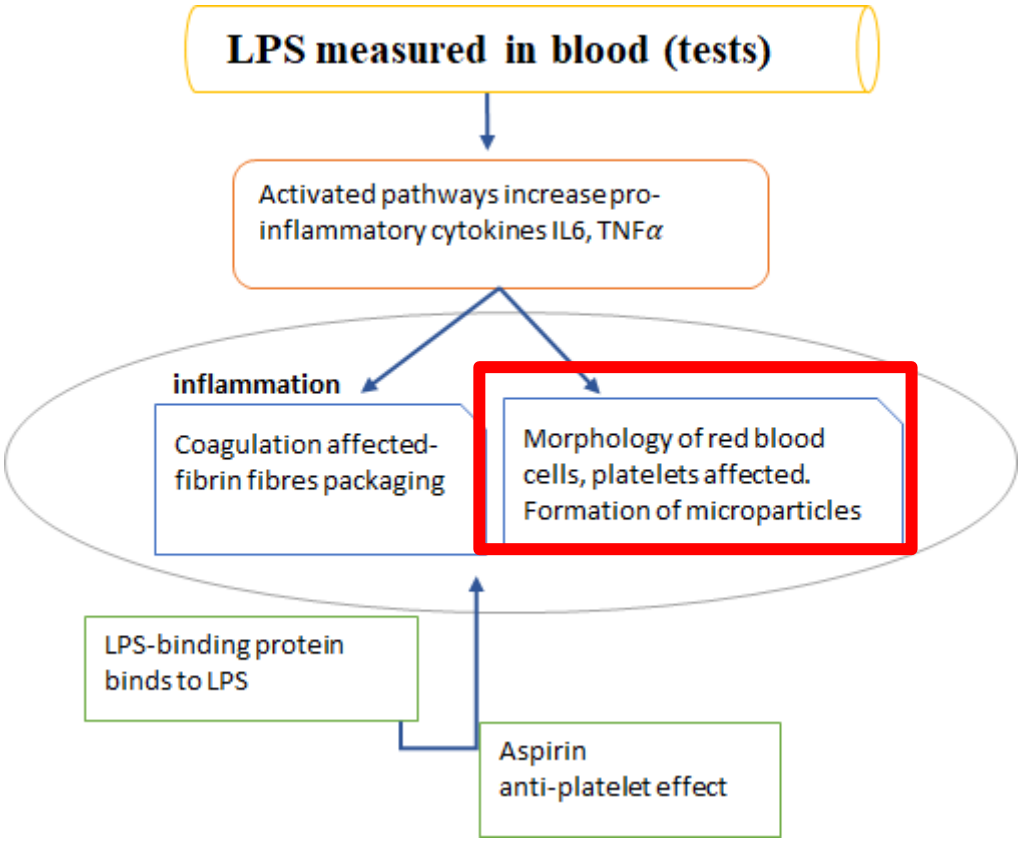


Figure 2. 1. A pathway followed by LPS in activating pro-inflammatory cytokines, to induce inflammation and the effects of LPS on red blood cells and fibrin fibres. The red box highlights the next section in this literature review which is the effect LPS has on the structure of red blood cells and platelets.

As it has been shown from previous paragraphs the LPS is controlled by numerous serum proteins, including LBP, bactericidal/permeability-increasing protein and soluble CD14 (sCD14) (Juffermans et al., 1998, Goldblum et al., 1994). Through research, experts found that the binding of LBP to LPS increases the binding of LPS to CD14 in blood, which then released on monocytes and neutrophils and but not on RBCs (Viriyakosol and Kirkland, 1995, Czerkies et al., 2013). Researchers has proven that it is the carbohydrate portion of LPS which attaches or binds to the CD14 protein (Pugin et al., 1994, Heumann et al., 2001). This is due to the hydrophilic regions on the CD14 that are on the outer surface on the N-terminal region which is next to the regions of carbohydrate in LPS (Kim et al., 2005, Liu et al., 2005). Researchers (Zanoni and Granucci, 2013) found that the malleable. CD14 structure could explains how it could bind different LPS species with the same affinity.

Although RBCs lack the CD14 binding sites, it is believed that LPS does bind to the cell, but it is thought that binding of LPS on to the RBC membrane surface is due to the hydrophobic regions in both LPS and the RBC (Brauckmann et al., 2016b, Myung et al., 2016).

In literature it has been proven that LPS can mix with lipoproteins because it can change misfolded proteins (prions- proteins misfolded but can change back to their natural shape) to it more toxic form PrPSc (these are particles consisting of irregular isoform) of normal abundant cellular proteins (De Castro et al., 2010). A study conducted by a group of researchers used gas chromatography to study the potential or possible binding of LPS to RBCs by assessing the amide linked hydroxyl acid. In their first set of results the exposure rate was 82% for LPS and 79% for lipid A when they were in a buffer solution. In washed RBCs' membrane the exposure rates of LPS and lipid A were 0.03% and 0.5% respectively and in RBCs' membrane of WB the exposure rate was 2.69% (more in this group) their results confirmed what the other expect proved that at a small minimal level LPS can bind to WB or RBCs (Schlichting et al., 1996, CONLON, April 1995, Myung et al., 2016, Brauckmann et al., 2016b). When LPS binds to RBCs abnormalities arise, evidence from a decrease in the amount of hydroxymyristic acid which was measured in RBCs (Pöschl et al., 2003).

Brauckmann and team found that there was an increase in free hemoglobin (Hb) and LDH activity when blood cells were exposed to LPS (Brauckmann et al., 2016a).The membrane integrity of RBCs was lowered, this was shown by a reduction in osmsotic resistance, formation of frgaments of RBCs (stocytes) and a decrease in RBCs' cell wall rigidity. The non-toxic RS-LPS would block the LPS-evoked increased Hb concentration of the supernatant (clear liquid overlying RBC deposits). The removal of plasma material in washed RBCs assays failed to decrease hemolysis. Here Brauckmann show direct physicochemical of LPS with RBCs' membrane *ex vivo.*

These researchers hypothesized, that not all effects of LPS are mediated by TLR and may also explains LPS toxicity in cells missing TLR, like RBCs (Brauckmann et al., 2016). Complete competitive inhibition of LPS activity is probable at a 100-fold excess of the antagonist. The LPS-RS does not induce TLR4 signalling but is exposed by the LAL assay, the standard endotoxin detection assay. On the contrary to LPS-RS standard, ultrapure LPS-RS does not activate the TLR2 pathway (Coats CR 2005).

In another study conducted by (Myung et al., 2016) the researchers evaluated the changes in RBC accumulation and deformability over 24 hours. Their attempt suggests specific shear stress values for detecting RBC deformability in a mouse endotoxemia template using LPS. Six-week-old male BALB/c mice received LPS (20mg/kg) intraperitoneally. Combination indices (AIs) and T1/2 were measured to assess RBCs aggregation, and elongation indices (EIs) were used to assess RBC deformability at shear stress values of 0.3, 0.5, 1, 3, 7, 10, 15 and 20

Pascals (Pa) 0,30 min, 1, 2, 4, 6, 9, 12, 18 and 24 hours after the LPS injection. No significant differences were detected in the AIs during the study period, however, T1/2 shortened significantly 2, 6, 12, 18, and 24 hours after the LPS injection. The EIs grew significantly 24 hours after LPS injection at 0.5 and 1 Pa shear stress, whereas it decreased significantly at 10 Pa of shear stress 24 hours after the LPS injection.

Altered RBC deformability was noticeable 24 hours after the LPS injection and T1/2 this was a sensitive marker for detecting changes in RBC aggregation. This study detected changes in RBC deformability in LPS-induced septic mice (Myung et al., 2016). In the following section the role of LPS in coagulation will be discussed. This is to compare some key factors altered in coagulation that are present in systemic inflammation. This is highlighted with a red box on the figure below.

LPS effects on coagulation and development of thrombosis

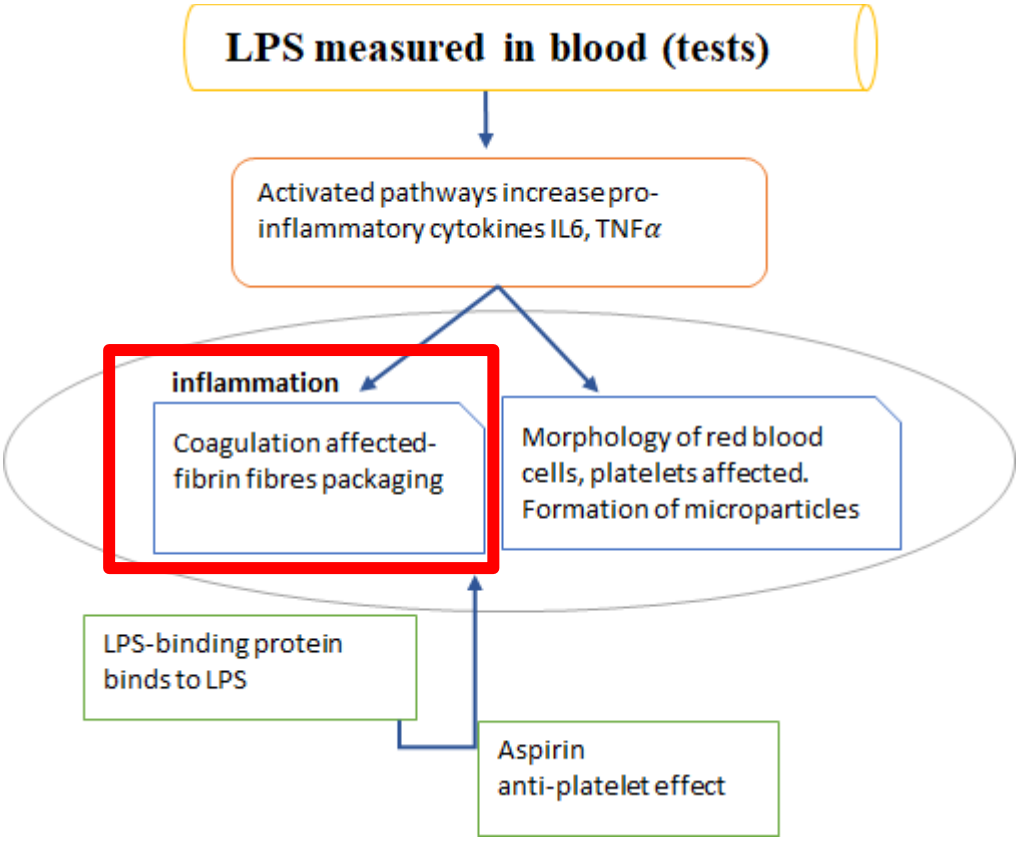


Figure 2. 1. A pathway followed by LPS in activating pro-inflammatory cytokines, to induce inflammation and the effects of LPS on red blood cells and fibrin fibres. The next section is highlighted with a red box, which will be on the effect LPS has on fibrin fibres hence affecting coagulation.

Many chronic inflammatory diseases commonly show both hypercoagubility and hypofibrinolysis (lack of or impaired breaking of clots) in patients when tested (Kell and Pretorius, 2015h). However, both the kinetics and the end product structure of the clotting procedure can affect the biochemical nature of the clot, free iron and the fibrin concentration (Kell and Pretorius, 2014b, Pretorius et al., 2014b, Pretorius, 2013). It is clear from the literature that LPS is a procoagulant via direct process (a precursor of blood factors needed for clotting) (Wu et al., 2014, Simmons and Pittet, 2015). Lipopolysaccharide is implicated in a unfavorable change coagulation profile hence it is a precursor for LPS induced coagulation (Chaby, 2004). Furthermore, Branger and team in year 2003 they intravenously injected LPS in mice and examined the changes. The found that LPS was related with encouragement of the coagulation system, which was increase in the plasma concentrations of the prothrombin fragment (Branger et al., 2003). Also Pretorius and coworkers have shown in several articles that inflammation affects the coagulation profile, the RBC shape and fibrin formation (Buys et al., 2013, Kell and Pretorius, 2014c, Kell and Pretorius, 2015i, Pretorius et al., 2014a, Pretorius and Kell, 2014b, Pretorius and Lipinski, 2013b, Pretorius et al., 2014d, Pretorius et al., 2014e, Pretorius et al., 2014f, Pretorius et al., 2013b).

A thrombotic state is a feature of systemic inflammation and is classified by hypercoagulation. It is usually the underlying of all thrombotic conditions (heart diseases, strokes and venous thromboembolism) (Raskob et al., 2014). Hypercoagulable state coincides with a small fibrin concentration and an increase in D-dimer levels (Nelson and Junge, 2015, Halder et al., 2015). These changes are seen as markers for development of heart diseases (Tonkin et al., 2015, Halaby et al., 2015, Hou et al., 2012). **Figure 2.3 is** a flow diagram shows the effects of LPS on coagulation in the presence of blood.

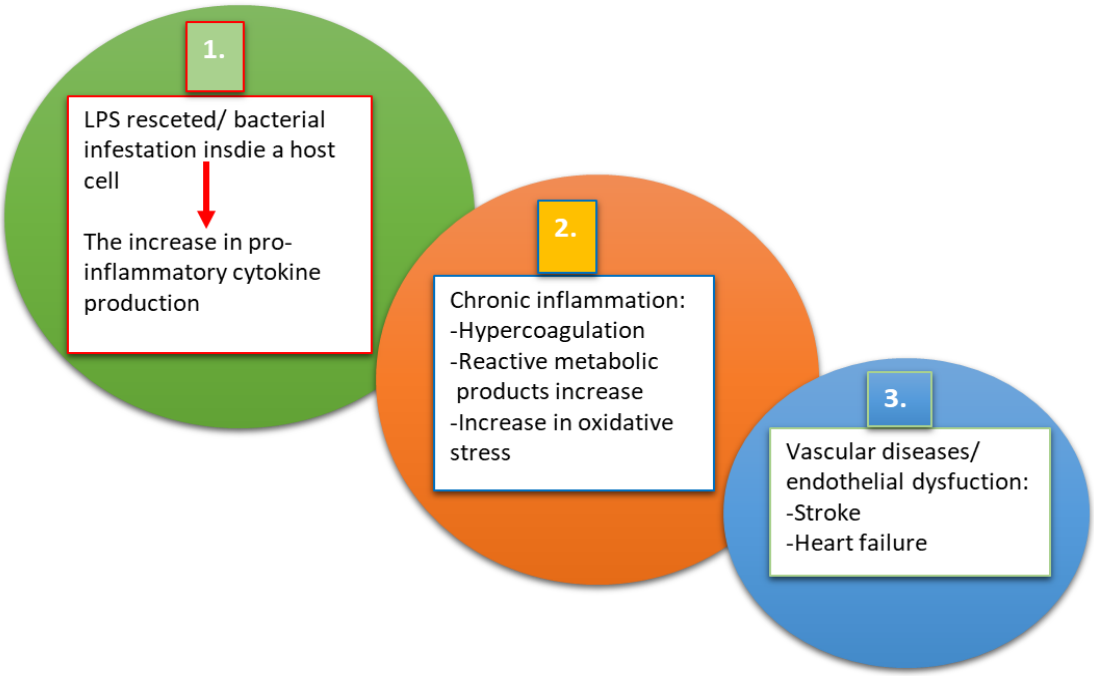


Figure 2. 3. Flow diagram where there is presence of LPS in blood. 1. Up-regulation of cytokines production. 2. Chronic inflammatory disorders. 3. Vascular diseases results.

Since LPS has been proven to have cytokine-dependent effects, now a question arises that can LPS be the cause of hypercoagulation by working on the coagulation pathway directly? One direct path to cause hypercoagulation in blood is via TF upregulation. It is known that TF is associated with the cytokine receptor class II family and is active early in the (extrinsic) coagulation cascade, a step in the clotting cascade where it is essential to recruit thrombin construction from prothrombin (Rao and Pendurthi, 2005, Monroe and Key, 2007). The upregulation of TF plays a central role in driving a thrombosis-inflammation route plus hypercoagulability (Chu, 2006b, Chu, 2006a). This is known as coagulation-dependent on inflammation (Strukova, 2006).

Researchers (Koch and team) established that when they added 100ng.mL^{-1} of LPS to newborn babies' umbilical cord it induced TF-mediated activation of hemostasis (Koch et al., 2009). Agreeingly there were researchers (Landsem and team) who added LPS from *E.coli* (100 ng.mL^{-1}) to stimulate the clotting cascade in blood through the complement system as well as the CD14-dependent increase of TF. This led to prothrombin activation and eventually hypercoagulation (Landsem et al., 2013, Landsem et al., 2015). Research conducted by Landsem and colleagues also revealed that when WB was mixed with LPS and *E. coli* there was an increased surface TF expression on monocytes (Landsem et al., 2013) more TF expression and hypercoagulation of blood.

However, this was seen just after 2 hours of incubations, thus it wasn't an acute route (Landsem et al., 2015). Together the complement system and the ability of phagocytic cells to clear out pathogens from an organism, and TF play a key role starting role in coagulation and are activated in sepsis (Landsem et al., 2013, Lin et al., 2010). The complement system is also stimulated competently by whole Gram- negative bacteria, while absolute LPS can trigger the complement system only at elevated concentrations (Brekke et al., 2007, Landsem et al., 2013). However, in addition to alterations in TF expression by LPS, the process could include the direct binding of the lipophilic LPS (Calabrese et al., 2015, Maeshima and Fernandez, 2013) to pass around plasma proteins, like fibrinogen, this instant binding is also known to initiate abnormalities in clotting (Lin et al., 2010). This would be independent of the slower TF activation, and thus an acute and relatively speedy process. Here it is evident that LPS can affect coagulation via indirect and direct pathways.

During healthy blood clotting the intrinsic and extrinsic pathways are both present (Norris, 2003, Smith, 2009, Pretorius and Kell, 2014), they all converge in the final steps were the prothrombin is transformed to thrombin followed by the conversion of specific molecules of soluble fibrinogen to insoluble by thrombin-catalysed, the two fibrinopeptides are conveter to an insoluble, multimolecular complex of in the form of fibrin. This is the chief component of the blood clot (Pretorius and Kell, 2014, Norris, 2003). Thrombin also activates factor XIII to produce factor XIIIa, which in turn catalyses the intermolecular crosslinks between fibrin fibrils to steady the clot so formed (Narayanan, 1999, Adam et al., 2009b, Smith, 2009). In the extrinsic pathway, where deficiencies affect the prothrombin time (PT), as well as the intrinsic pathway that allows recreation and calculations that mimic the results of the activated partial thromboplastin test (aPTT) (Narayanan, 1999, Norris, 2003, Pretorius and Kell, 2014). **Figure 2.4.** shows how thrombin acts a crucial role in starting the polymerisation of fibrinogen. The processes with the standard breakdown of the fibrin via lysis is catalysed by plasmin formed from the tissue plasminogen activator-catalysed activation of plasminogen this figure was taken from (Kell and Pretorius, 2015b).

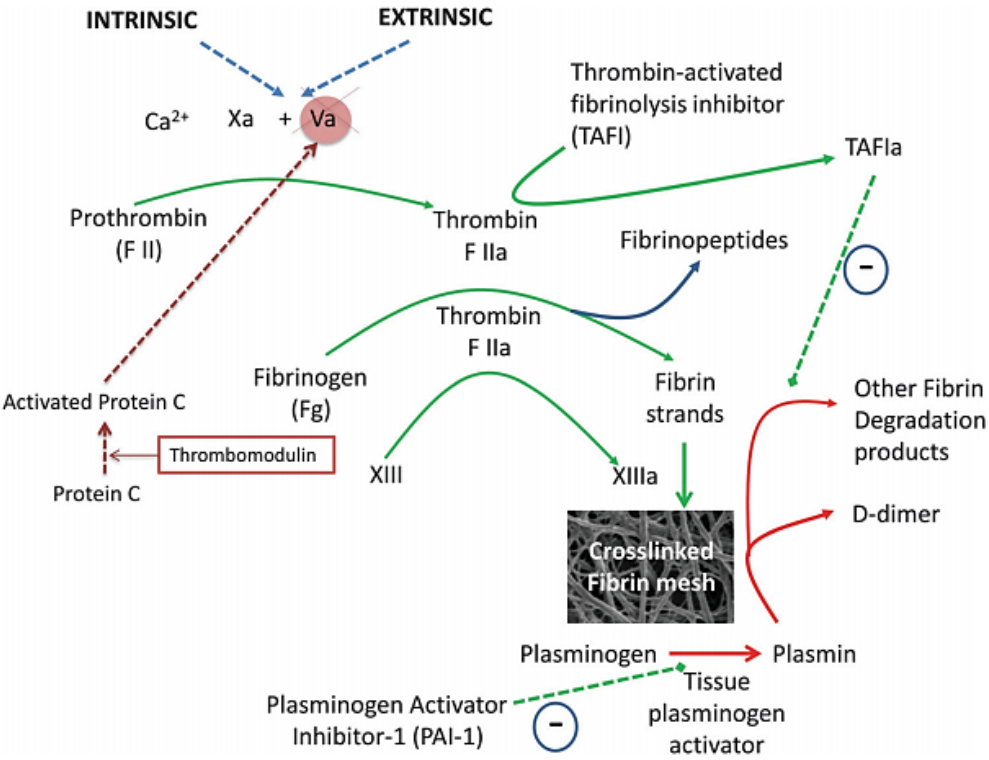


Figure 2. 4. The latter stages of the various coagulation pathways, showing places where thrombin plays a crucial role in initiating the polymerisation of Fibrinogen (Kell and Pretorius, 2015b).

For a closer look at how fibrinogen folds into the final product that is the cross-linked fibrin mesh see **Figure 2.5.** This typical fibrin meshes will be seen again in the SEM chapter as the major component of this study is about how fibrin mesh is affected by different reagents.

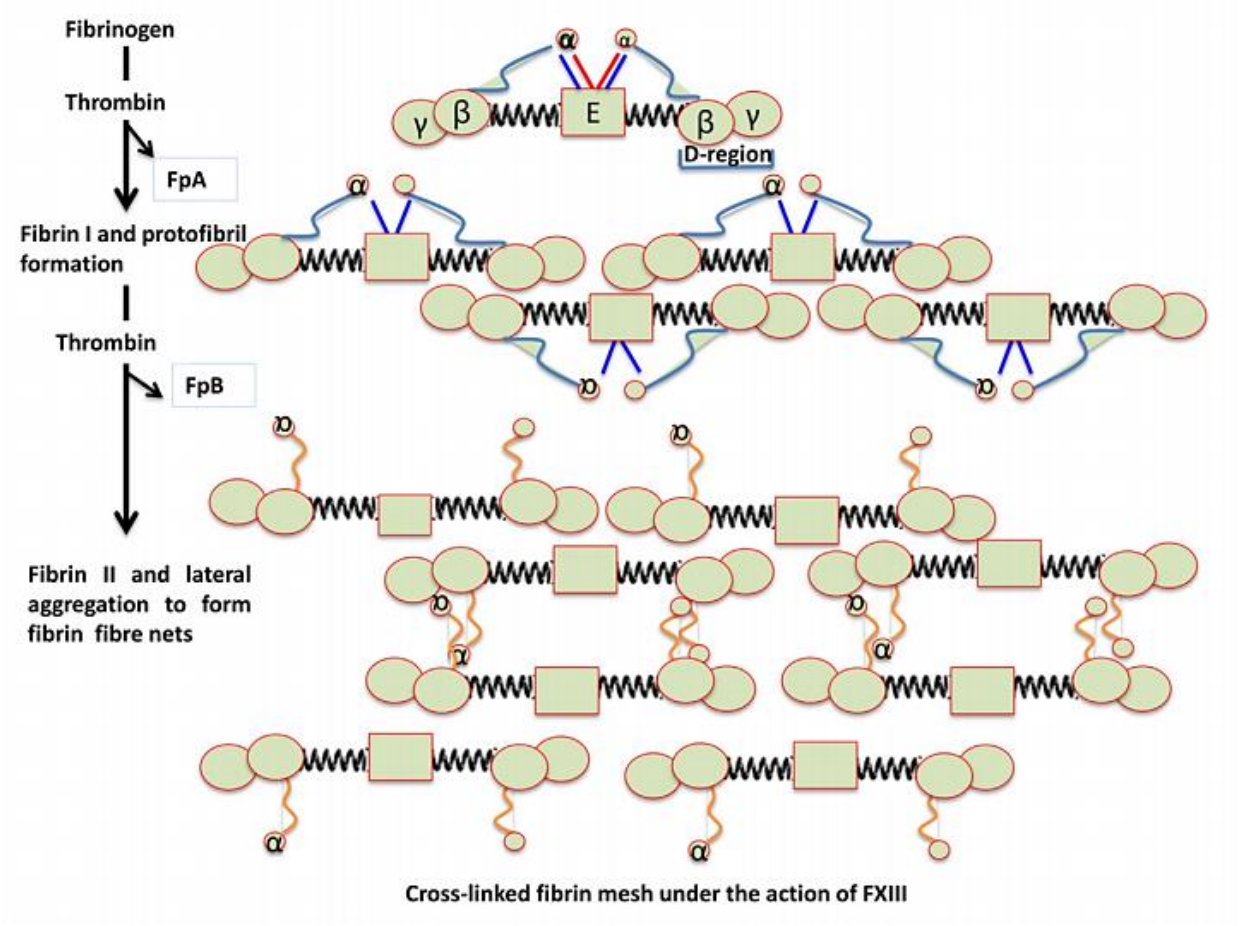


Figure 2. 5. Diagrammatic representation of fibrinogen folding into the final product, the cross-linked fibrin mesh. This meshes will be seen through the thesis. This diagram was taken from (Kell and Pretorius, 2017)

In order to understand how hypercoagulation occurs, the focus will be on the typical protein molecule structure of fibrin fibres. It was previously shown that a healthy protein structure contains lots of alpha colis and little Beta sheets. It is clear that the precise variations that happen during inflammation in the a-helix and b-sheet interaction involve major changes in secondary structure (Kell and Pretorius, 2015a, Kell and Pretorius, 2015b, Kell and Pretorius, 2017). *In vivo*, as a section of normal wound healing. The clot is detached through the fibrinolytic system and this process is facilitated by the serine protease plasmin, which cuts the fibrin molecule at specific positions (Bucay et al., 2015, Draxler and Medcalf, 2015, Smith, 2009); to form a variety of degradation products described to as D-dimer (Adam et al., 2009a, Walker and Nesheim, 1999). Using an inverted optical microscope and fluorescently-labeled fibers suspended between micropatterned ridges, researchers have directly evaluated the lysis of individual fibrin fibers (Bucay et al., 2015). During fibrin polymerisation, typically obscure plasminogen and plasmin binding sites are exposed.

The C regions that include lysine-dependent tPA- and plasminogen-binding sites (Bucay et al., 2015, Draxler and Medcalf, 2015, Medved and Nieuwenhuizen, 2003). During fibrinolysis, plasmin originally cuts the C regions, and then splits the three polypeptide chains connecting the E-domains and the D-domains (Bucay et al., 2015, Nieuwenhuizen, 2001, Adam et al., 2009b). The accurate procedure of fibrinolysis is controlled by various structural arrangements and physical properties of the clot itself. These properties include clot density, rigidity and fibrin fibre diameter (Collet et al., 2003, Weisel, 2007, Weisel, 2005). Bucay and co-workers in 2015 discovered that if fibres are exposed to plasmin, thin fibres are simply cut, also that thicker fibres sprouted in length during fibrinolysis. Thus the susceptibility of lysis of the fibers is directly related to the intrinsic strain on the fibre resulting from the polymerisation process (Bucay et al., 2015). The resistance of hydrolysis of abnormal fibrin clots can be precisely related to this 'hypohydrolysis" (proteinase K resistance) distinctive of PrPSc. Researcher Campbell and colleagues (Campbell et al., 2010), discovered that diameter can affect fibrinolysis rates: "Fibre diameter and network density play significant roles in clot dissolution (Weisel and Litvinov, 2008). Researchers found that thick fibres help quicker plasmin generation rates compared to thin fibres. However thin fibres lyse faster than thick fibres due to coarse networks of thick fibres lyse faster than tight networks of thin fibres (Collet et al., 2003, Campbell et al., 2010). It does not yet seem to be known, but seems probable, that the form of fibrin in Disseminated intravascular coagulation (DIC) which are a serious syndrome were the proteins that control blood clotting become overactive is indeed a b-amyloid.

When inflammagens like LPS are present in circulation, it has the propensity to bind to the fibrin(ogen) protein molecules. This initiates the alpha coils touncoil into beta sheets see **Figure 2.6.** for a visual representative of transition in fibrin formation under deformation, this image was taken from (Kell and Pretorius, 2017).

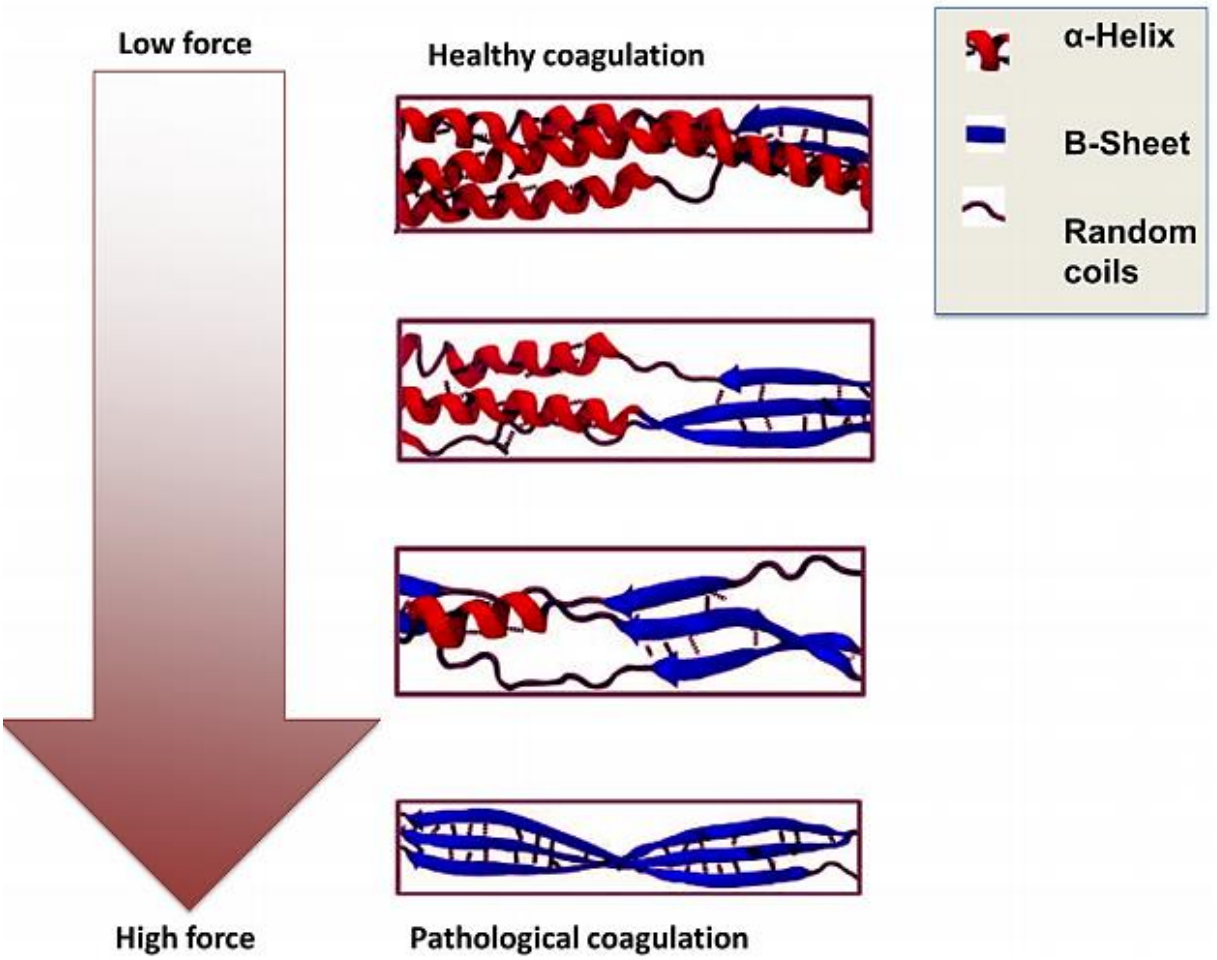


Figure 2. 6. Transition from a-helices to b-sheets phase in fibrin formation under deformation of low to high force that is healthy coagulation to pathological coagulation (Kell and Pretorius, 2017).

A previous novel finding by Page and co-workers (Page et al., 2019b) was that hypercoagulated areas or anomalous clotted fibrinogen show auto-fluorescence in purified and FITC (fluorescent fibrin(ogen) as well as pooled plasma clots. Moreover, these areas have similarities to the areas where ThT amyloid markers binds. This is in line with previous findings which shows fluorescence in the obvious range which develops during the accumulation of a range of polypeptides, including the disease-related human peptides amyloid-β (Chan et al., 2013, Tikhonova et al., 2018).

The necessary action mechanisms of LPS have been reviewed in a more biochemical manner as well as the physiological effects of LPS when present in host cells. The following sections will be based on possible treatments to the LPS predicament, solutions such as Lipopolysacchaide binding protein (LBP) and/or aspirin that may hinder the binding of LPS to blood components.

Lipopolysaccharide binding protein might prevent the uncoiling of the alpha coils into beta sheets, when added to the blood model, and also prevent LPS to directly bind to RBC and platelet membranes, that might result in abnormal cellular structure and function.

Lipopolysaccharide binding protein (LBP) defense against LPS

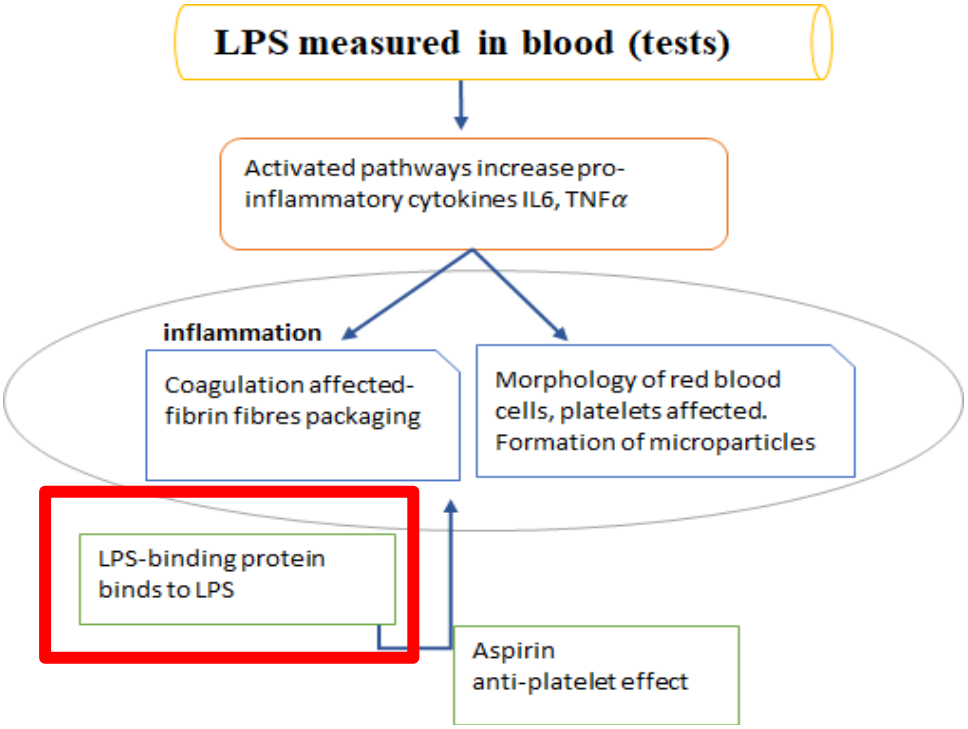


Figure 2. 1.A pathway followed by LPS in activating pro-inflammatory cytokines. The topic of the next section is highlighted with a red box which is the action and effect of LBP binding to LPS.

Lipopolysaccharide binding protein is a protein that is originate in humans and is programmed by the LBP gene and it is a soluble acute-phase protein that binds to bacterial LPS to elicit immune responses by presenting the LPS to important cell surface design recognition receptors called CD14 and TLR4 (Le Roy et al., 1999, Klein et al., 2015). It is known that LBP is a hepatically synthesized, acute-phase protein that shuttles endotoxin molecules to effector cells bearing CD14 on their cell surfaces (Tapping and Tobias, 1999). During the acute phase, LBP can be expected to bind gram-negative bacteria and bacterial fragments and promote the interaction of coated bacteria with phagocytes (Wright et al., 1989). It has been shown that LBP-knockout mice have markedly diminished responses to endotoxin (Wurfel MM 1997) yet are rendered susceptible to systemic Salmonella infection (Jack RS 1997). Activation of the TLR4 signaling complex requires the coordinated function of LBP, CD14, MD-2, and TLR4 (Mitchell et al., 2018, Poikonen et al., 2009, Peri et al., 2010, Moody and Cotton, 2017).

Researchers have shown that therapeutic administration of high doses of LBP may protect normal mice from endotoxin challenge (Lamping, N 1998, Dedrick RL 1996). On various types of cells, TLRs are also important membrane receptors and are members of the IL-1 receptor family that participate in activation of cells by bacteria and bacterial products (Beutler et al., 2001, Means et al., 1999).

Moreover, there are several reports which indicate that TLR proteins mediate cellular activation by bacterial LPS via a signaling pathway is largely shared by the type I IL-1 receptor (Means et al., 1999). However, research shows that previously TLR2 associates with the high affinity LBP membrane CD14 to serve as an LPS receptor complex, that LPS treatment enhances the oligomerization of TLR2 Related with receptor and the IL-1R-associated kinase (IRAK) is recruited to the TLR2 complex (Yang et al., 1999).

Studies show that heterotrimeric G proteins have been implicated in TLR4 signaling in macrophages and endothelial cells, however, the guanine nucleotide binding protein G(i) subunit alpha-1 and alpha-3 (Gαi1/3) are required for LPS responses remains unclear. Researchers have found that Gαi1/3 deficiency caused LPS tolerance in mice (Li et al., 2015). The host response to endotoxin is dependent on the physicochemical characteristics of the endotoxin molecule (Hurley JC.1995), the relative concentrations of endotoxin-binding proteins (Opal et al., 1994), and the degree of cellular responsiveness to endotoxin (Sweet MJ 1996). The principal plasma protein responsible for transporting endotoxin to immune effector cells is LBP (Fenton MJ 1998). Lipopolysaccharide binding protein is a serum molecule that mediates cellular activation in response to endotoxin by ensuring the delivery of LPS to either soluble or membrane bound forms of CD14 also known as mCD14 (Coats et al., 2005, Thompson et al., 2003). Aside from this activating role, previous work has shown that LBP and LPS can bind to cells by forming large aggregates which are anchored by mCD14. This binding phenomenon does not correlate with cellular activation. The results show that neutralization of LBP accomplished by blocking either the binding of LPS to LBP or the binding of LPS/LBP complexes to CD14 protects the host from LPS-induced toxicity, confirming that LBP is a critical component of innate immunity (Le Roy et al., 1999). To further characterize these events, researchers have generated a biologically active radiolabeled LBP ligand with high specific activity. The novel receptor for L BP and LPS complexes has been detected on many cell types and may be a receptor required for the cellular clearance of LPS (Tapping and Tobias, 1999).

In a study conducted by (Steven M. Opal 1999) they showed that during inflammation LBP levels were significantly elevated in 97% patients with severe sepsis. The LBP is able to bind and transfer LPS to a variety of acceptors and, is a candidate as an alternative to CD14 if the function of CD14 is simply to concentrate LPS at the cell surface (Erenler and Yardan, 2015, Means et al., 1999). However, the activation of LBP expressing cells has been shown to be less efficient than activation of the mCD14 expressing cells and minimally more efficient than activation of empty vector transfected cells (Tapping and Tobias, 1999, Tsutsumi-Ishii et al., 2008, Means et al., 1999). This slight effect is likely to be due to the presence of a small amount of soluble CD14 (sCD14) and the ability of LBP to promote formation of LPS-sCD14 complexes which are agonistic for these cells. Thus, it is clear that LBP cannot act as an efficient surrogate for CD14, and the function of CD14 is more complicated than the simple concentration of LPS at the cell surface (Means et al., 1999).

To ensure successful reversal of LPS an anti-inflammatory agent might be needed, the review of the use of aspirin, a well-known anti-inflammatory drug alone or in combination with LBP work together to decrease the harmful effect of LPS. **See Figure 2.7.** For the possible actions of LPS on hypercoagulation.

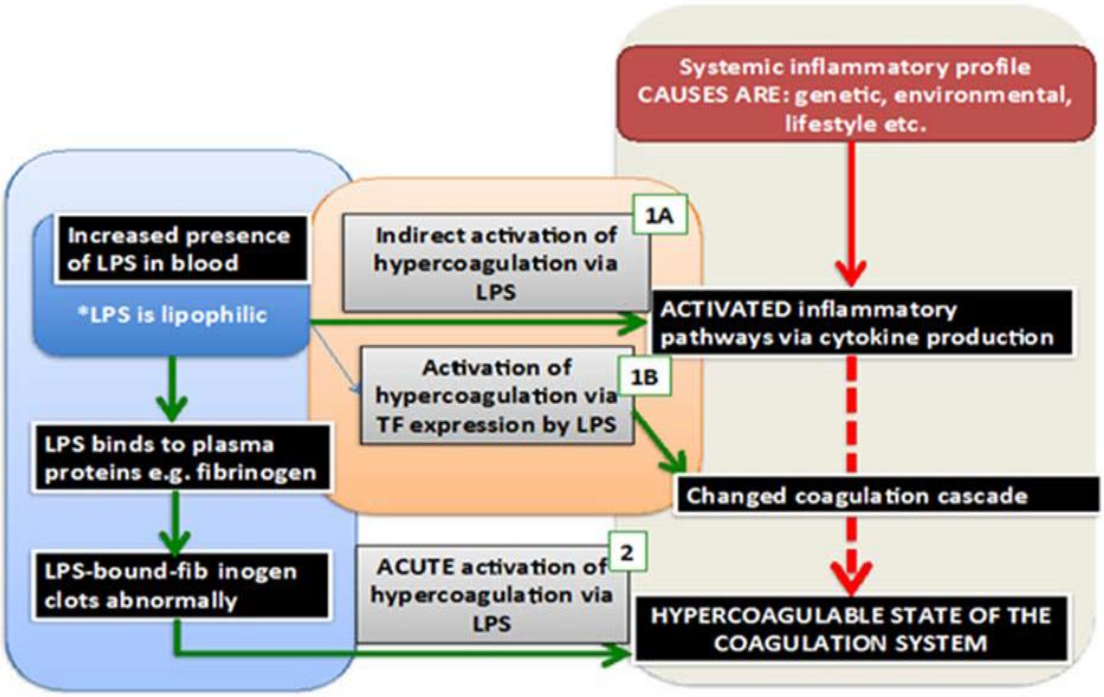


Figure 2. 7. The effect of LPS on coagulation were (1A) labels the indirect activation of LPS (1B) shows the direct activation of coagulation thorugh LPS affecting TF direct (2) Acute activation of hypercoagulation occurs when the LPS binds to fibrinogen taken from (Pretorius et al., 2016d).

The next section focuses on anti-coagulant and anti-platelets agent commonly used all over the world to reduce signs of hypercoagulation. Aspirin is available commercially and can be purchased over the counter without a prescription in some areas of the world. The next section is a review on how it has been used in the medical science world in attempt to combat hypercoagulation as a major hallmark of inflammation. The next section focuses on the role of aspirin as an anticoagulant, this is highlighted with a red box in the figure below.

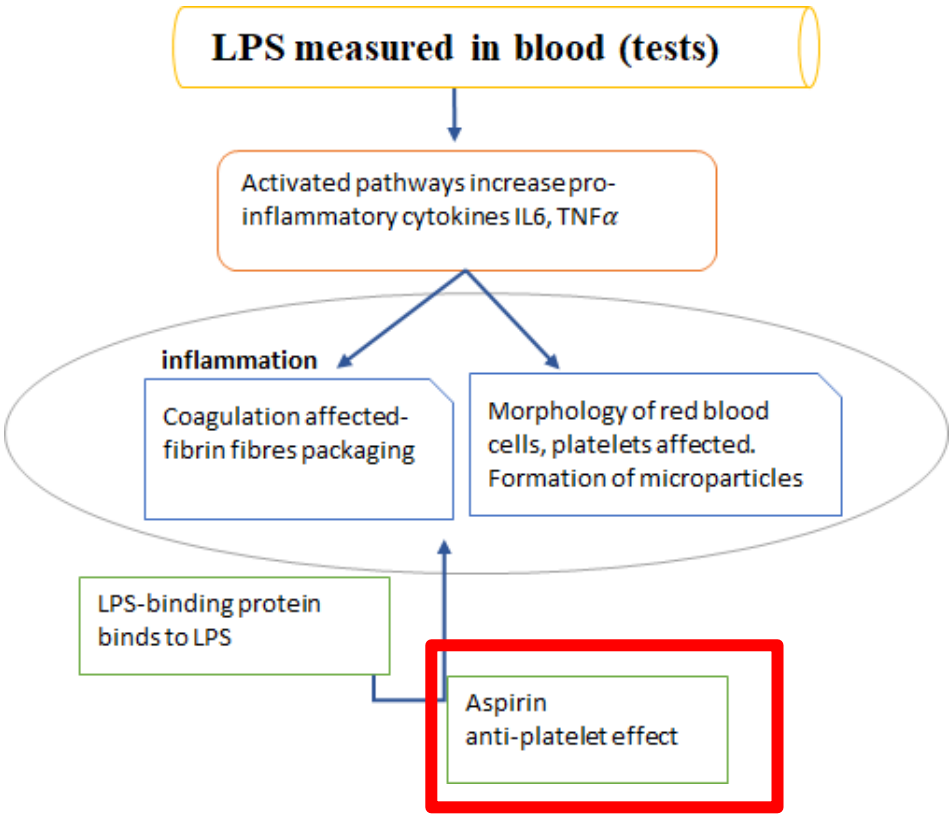


Figure 2. 1.A pathway followed by LPS in activating pro-inflammatory cytokines. The next section is highlighted in red border -the role of aspirin as an anti-inflammatory agent.

Aspirin is one of the most widely manufactured drugs in the world. Its chemical name is acetylsalicylic acid and it is prepared by the reaction of acetic anhydride with salicylic acid, in the presence of an acid catalyst to speed up the reaction (Borthwick et al., 2006, Koohshekan et al., 2016).

Salicylic acid and aspirin have the same analgesic and antipyretic properties, but salicylic acid is more acidic than aspirin and irritating to the stomach, mouth and mucous membranes (Petrenko et al., 2016, Paterson et al., 2008). Salicylic acid was identified in willow bark extracts as an active anti-inflammatory compound over a century ago (Ohno et al., 2002).

Due to its bitter taste, chemical derivatives of salicylic acid (2-hydroxybenzoic acid) were synthesized and tested. Acetylsalicylic acid (aspirin) eliminated the bitter taste but retained the anti-inflammatory action, and it was introduced for treating human maladies about 100 years ago (Ohno et al., 2002, Cingoz and Gurel, 2016). It has remained the most commonly used drug for relieving pain, inflammatory symptoms, and fever (Cingoz and Gurel, 2016). Aspirin also has established efficacy for preventing myocardial infarction and ischemic stroke, as well as for treating acute myocardial infarction (Lopes et al., 2016).

A variety of peculiar agents, such as leeches and bark, were used to prevent thrombosis, such as Hirudin which was used during the 19th century (Baskurt and Meiselman, 2012). Previously clinicians have used heparin, strepokinase, urokinase, tissue plasminogen activator (TPA), dicumarol, warfarin, aspirin, ticlopidine, Clopidogrel, SSHA and SP54 provoked huge advances in anticoagulation (Baskurt and Meiselman, 2012, Cuaz-Pérolin et al., 2008). Although the risk after a thrombotic episode is now highly reduced, blood clots still present damaging or even lethal consequences in human organisms (Tsoucalas et al., 2016). Antiplatelet and antithrombotic therapies are systematically considered to prevent restenosis following coronary stent implantation (Bagoly et al., 2016). Currently, patients receiving medicated stents (a splint placed temporarily inside a duct, canal or blood vessel to aid healing obstruction) are prescribed to orally take anticoagulants and antiplatelet drugs such as aspirin and prasugrel (PRAS) (Bagoly et al., 2016, Spencer et al., 2007, van Rooy et al., 2015). Drug coated surfaces were found to resist the adsorption of fibrinogen when compared to bare or coated cobalt-chromium (Co-Cr) Molybdenum is one of numerous metals are used in making orthopedic implants. or poly (DL-lactic acid (PDLLA) these are polymers of low molecular weight and can be combined with drugs like antibiotics or growth factors to establish a locally acting drug-delivery system (Hans 2003). Interestingly, ASP- and PROP-containing substrates not only showed reduced adhesion of platelets and delayed coagulation time, but also drastically reduced the expression level of IL-8 and IL-6 (Lih et al., 2016).

Although the established antiplatelet agents, such as aspirin and clopidogrel, have been shown to be beneficial in treating thromboembolic diseases, they have considerable limitations (Yen et al., 2016). Recently aspirin has been used and compared to dual antiplatelet therapy with clopidogrel and aspirin is frequently used for the prevention of recurrent ischemic events.

The aspirin monotherapy influenced ADP-induced platelet aggregation and secretion but did not have an effect on vasodilator-stimulated phosphoprotein (VASP) phosphorylation and on the ADP(PGE1) platelet aggregation test (Bagoly et al., 2016). Anticoagulants, low-dose aspirin, NSAIDs are associated with increased risk of upper and lower gastrointestinal bleeding. Non-steroidal anti-inflammatory drugs is a class of analgesic medication that reduces pain, fever and inflammation. Since most episodes of back pain involve inflammation, NSAIDs such as ibuprofen and naproxen are often an effective treatment option. Use of anticoagulants appears to be the strongest risk factor for gastrointestinal bleeding (Lanas et al., 2015).

However, as the investigation propels further it is found that researchers used therapeutic aspirin concentrations (0.5 mM), at this level aspirin had no detectable effect on endothelial cell viability or proliferation (Salvado et al., 2013, Zhang et al., 2017). Leithauser and team reported that aspirin caused a striking reduction in tubule formation in a three-dimensional collagen angiogenesis assay. This was also seen with equimolar concentrations of salicylate, while selective Cox inhibitors did not inhibit angiogenesis in this assay either alone or in combination. Furthermore, high doses of aspirin or salicylate (5mM), well above therapeutic plasma concentrations, lead to endothelial cell apoptosis (Leithäuser et al., 2012). Research suggested that aspirin, at therapeutic concentrations, directly inhibits angiogenesis via a Cox- independent mechanism, which may significantly contribute to its neoplastic protective effects (Borthwick et al., 2006). Amongst all different kinds of analgesic drugs, aspirin (acetylsalicylic acid) is most commonly used to relieve minor pains, reduce fever and to prevent inflammation. It might be suggested that in the presence of high concentrations of H_2O_2, aspirin can protect (Bovine Liver catalase) BLC activity through a mechanism in which the inactive form of the enzyme (compound II) is reduced to the active form (Koohshekan et al., 2016). The primary established effect of aspirin on hemostasis is to impair platelet aggregation via inhibition of platelet thromboxane A_2 synthesis, thus reducing thrombus formation on the surface of the damaged arterial wall (Undas et al., 2007, Mason et al., 2004). Growing evidence also indicates that aspirin exerts additional antithrombotic effects, which appear to some extent unrelated to platelet thromboxane A_2 production. Aspirin can also reduce thrombin generation with the subsequent attenuation of thrombin-mediated coagulant reactions such as factor XIII activation (Undas et al., 2007, Undas et al., 2006). Important and central to our study, aspirin also acetylates lysine residues in fibrinogen resulting in increased fibrin clot permeability and enhanced clot lysis as well as directly promoting fibrinolysis with high-dose aspirin (Undas et al., 2006, Undas et al., 2007).

The variable effectiveness of aspirin in terms of clinical outcomes and laboratory findings, which has been termed aspirin resistance, may be related to these additional antithrombotic effects that are altered when associated with common genetic polymorphisms such as the Leu33Pro β_3-integrin or Val34Leu factor XIII mutations. However, the clinical relevance of these observations is still unclear (Undas et al., 2007). Here one of the aims is also to determine, in the presence of LPS and/or LPB, if aspirin can prevent amyloid fibrin(ogen) from forming in healthy blood model.

The effect of Aspirin on red blood cells

A recent animal study found a vasodilating and blood pressure lowering effect of aspirin independent of COX, but mediated by inhibition of the RhoA/Rho kinase signaling pathway (Leithäuser et al., 2012). Aspirin at a dosage of 500 mg/d has an impact on vaso- regulation in the microcirculation (Elblbesy et al., 2012).The results showed that in the aspirin group after 7 days a significant increase of capillary RBC velocity was found at rest and during hyperemia (Elblbesy et al., 2012).

Aspirin therapy significantly inhibited platelets since cyclooxygenase 1 derived thromboxane generation levels were reduced by 99% (Borthwick et al., 2006). The level of vascular cell activation can be measured by the number and phenotype of microparticles found in the circulation (Leithäuser et al., 2012).

Interestingly in a study conducted by Chiva-Blanch and his team they found that aspirin therapy inhibits vascular wall cell activation and microparticle shedding. Microparticles derived from RBCs, activated monocytes, and smooth muscle cells were significantly reduced after 10 days of aspirin administration (Chiva-Blanch et al., 2016). This was highly useful as it is known that LPS causes microparticles (Øvstebø et al., 2014, Stief, 2009, Chapman and Wideman Jr, 2006, Ståhl et al., 2009). **Figure 2.8.** shows how endotoxic effects of LPS and the possible protective role played by LBP and aspirin in preventing the binding of LPS to blood components (from the reviewed literature).

For this study the prime investigating techniques were TEG and confocal microscopy. The LBP and aspirin were used to combat the effect of LPS individually and in combination. Thromboelastography® was used to study the viscoelastic properties of WB when treated with different reagents such as LPS, LBP and aspirin. The viscoelastic properties of treated blood will be compared with untreated WB from the same individual.

The second most important techniques that was used was super resolution confocal microscopy as this was a novel way of testing amyloid formation and treatment. Using Airyscan technique for super resolution of fibrin fibre network.

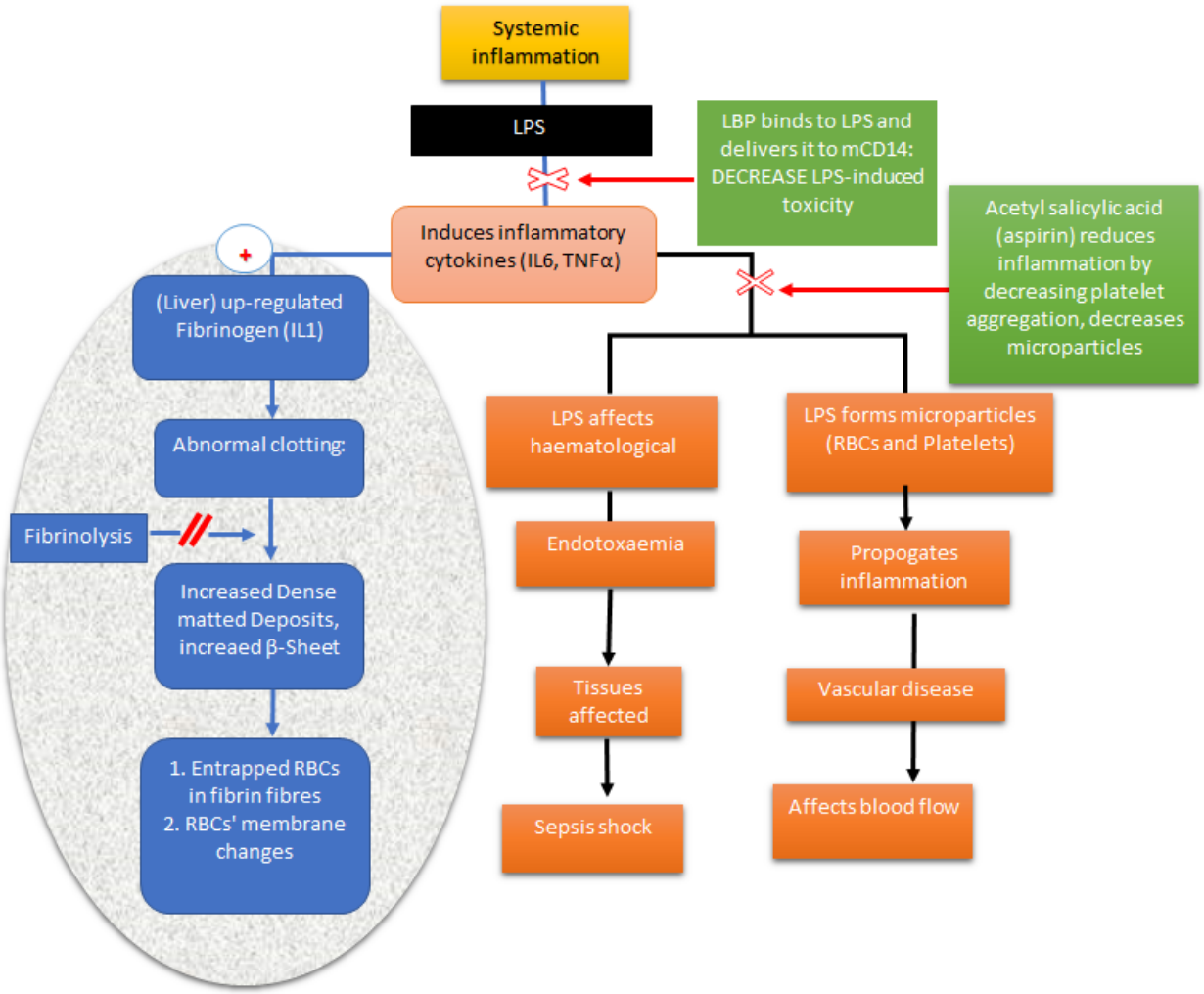


Figure 2. 8. Schematic diagram showing the endotoxic effects of LPS and the possible protective role played by LBP and aspirin in preventing the binding of LPS to blood components. Diagram was constructed from literature reviewed in this chapter

Major detection techniques

Thromboelastography® and coagulation review

In the recent years, TEG® has become a popular monitoring device for haemostasis and transfusion management in major surgery, trauma, and haemophilia. Thromboelastography® is performed in WB/PPP and assesses the viscoelastic property of clot formation under low shear conditions (Pretorius et al., 2017c, Ramchand et al., 2016, Swanepoel et al., 2015). Thromboelastography® can be performed with a variety of activator and inhibitors at different concentrations representing the most important factors for different intervals and clot formation variables reported in multiple studies and algorithms. Furthermore, fibrinogen levels and platelet counts have a major influence on thromboelastographic variables (Bolliger et al., 2012). In addition, differences in patient populations, devices, and preanalytical conditions contribute to some conflicting findings in different studies.

Researchers used the TEG® to study the viscoelastic properties of the blood, before and after addition any kind of reagents that will be investigated. Standard TEG procedures were followed and calcium chloride ($CaCl_2$) was added to activate the clotting process done previously by (Nielsen, 2007, Nielsen, 2008, Nielsen and Pretorius, 2014b, Nielsen and Pretorius, 2014a). For this study TEG was conducted with WB treated with LBP which is known to be commonly found in blood (Gonzalez-Quintela et al., 2013). The LPS was added to the LBP and WB as LBP and CD14 are believed to enhance binding of LPS (Kitchens and Thompson, 2003). The mixture that was created was believed to cause hypercoagulation. While other conventional tests evaluated the coagulation pathway until the formation of the first fibrin strands, the TEG continuously evaluates clot formation, its strength, platelet function until eventual clot lysis or retraction (Bolliger et al., 2012). See chapter 4 for TEG experiments and results. Previous experiments showed that LPS might add to an upregulated coagulation system, (remove) through two pathways: (i) via a direct and acute binding to plasma proteins (e.g. fibrinogen) or (ii) by an indirect or chronic (longer-term) this was published (Pretorius et al., 2016a) . The **Figure 2.9.** below is a diagram adapted from (Bolliger et al., 2012) illustrating tracings and parameters that explains the viscoelastic properties when using the machine.

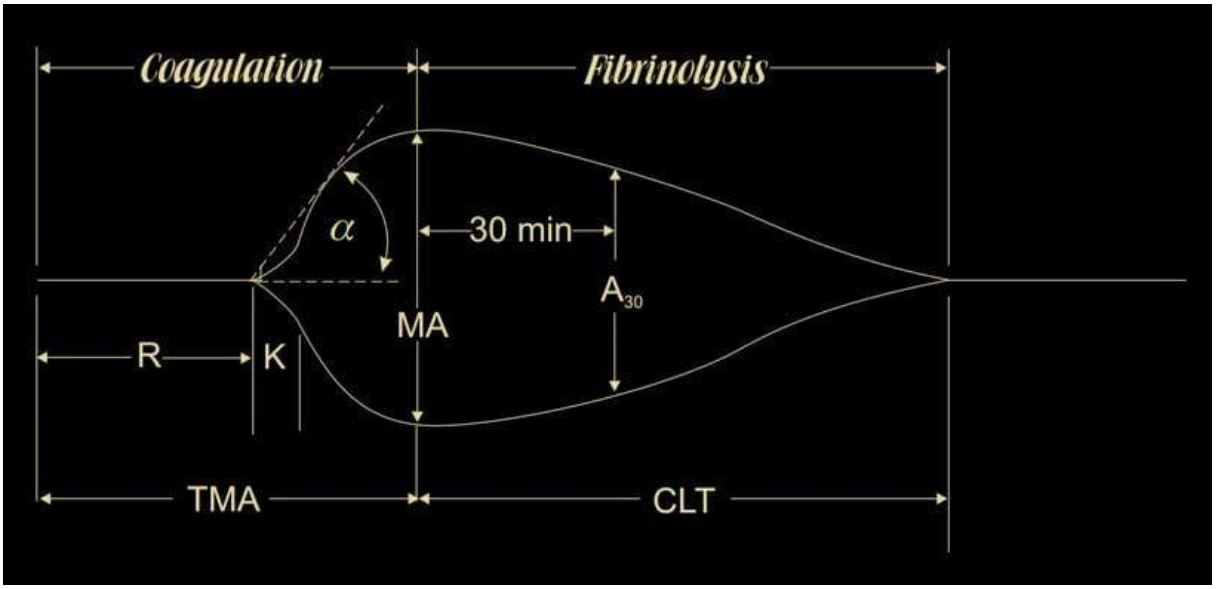


Figure 2.9. Image showing the interpretations of Thromboelastographic parameter. Showing the global analysis of the viscoelastic properties of whole blood. Normal Thromboelastography® Tracing, Depicting Rate of Formation and Degradation of Clot as well as Maximum Amplitude (MA), R Time, α Angle, and K Value adapted from (Bolliger et al., 2012)

Pretorius and team showed that by using small concentrations of LPS that amounted in molar terms to less than 10^{28} relative to fibrinogen, and demonstrated it by both viscoelastic and ultrastructural methods (Pretorius et al., 2016a). Experiments were conducted and confirmed that LPS can change the viscoelastic properties of PPP within 30 seconds of its addition. Furthermore, WB with added LPS, but without thrombin activation, showed spontaneously formed, amyloid-like matted deposits (these are sometimes known as Nets, but for the purpose of this study matted mass deposits would be used). Purified fibrinogen experiments with different serotypes of *E. coli* LPS such as o111:b4 LPS and o26:b6 LPS, with and without added thrombin showed the same results as seen with the patient's fibrinogen eliminated patients' bias to the LPS (this erases any arguments that some people could be bias to LPS or react differently to LPS) (Pretorius et al., 2016d).

Confocal microscopy measuring defects and reversal of defects review

As mentioned in the introduction amyloid fibrils formation can accompany disease and each disease can have a specific protein or peptide type (Kell and Pretorius, 2015a, Kell and Pretorius, 2015b, Kell and Pretorius, 2017).

The marker of choice for this study is ThT (mark amalyiod protein made by LPS) (Pretorius et al., 2016a) which was added at a final concentration of 5 µM to 100 µL PPP in this study. Autofluorescence is the natural emission of light by biological structures when they have absorbed light, and is used to distinguish the light originating from artificially added fluorescent markers (fluorophores) (Monici, 2005). Free ThT fluoresces faintly with excitation and emission maxima of 350 and 440 nm, respectively, whereas upon interaction with amyloid fibrils an increase in ThT fluorescence is observed, with excitation and emission maxima at about 440/450 and 480/490 nm (Groenning, 2010, Kuznetsova et al., 2016). It was shown from ThT measurements that LPS could massively affect the formation of β-sheets during fibrin packaging (Pretorius and Lipinski, 2013a). Only a limited number of autocatalytic mechanisms can disclose this, that which is essentially a very rapid form of amyloidogenesis and autocatalytic structural rearrangement to a β-rich conformation (LeVine Iii, 1997, Kuznetsova et al., 2016). From the above literature review, it is clear that LPS is involved in most inflammatory diseases, and that it causes hypercoagulation forming low-grade inflammation, either as a systemic inflammation or as neuroinflammation. In the current study, investigation of the effect of LBP and aspirin on LPS induced hypercoagulation will be revealed.

Sample types and descriptions are as follows:

- Naïve PPP/WB (this the untreated PPP or WB)
- PPP/WB +LBP (this is PPP/WB that has been incubated with LBP at final exposure concentration of 2ng.L^{-1})
- PPP/WB + LPS (this is the PPP/WB that has been incubated with LPS at a final exposure concertation of 0, 2ng.L^{-1})
- PPP/WB + LPS+ LBP (this is PPP/WB that has been incubated with LPS mixed with LBP, both reagents were mixed to be added at a final exposure concentration of 2ng.L^{-1})
- PPP/WB + Aspirin (this is PPP/WB that has been incubated with aspirin at a final exposure concentration of 0.5mM)
- PPP/WB +Aspirin + LBP (this is PPP/WB that has been incubated with aspirin mixed at a final exposure concentration of 0.5mM followed by mixing with LBP at a final exposure concentration of 2ng.L^{-1})

- PPP/WB + Aspirin + LPS (this is PPP/WB that has been incubated with aspirin at a final exposure concertation of 0,5mM then mixed further with LPS to give a final exposure of 0, 2ng.L^{-1})
- PPP/WB + Aspirin + LPS + LBP (this is PPP/WB that has been incubated with aspirin to give a final exposure concentration of 0,5Mm then further mixed with LPS and LBP both at a final exposure concentration of 2ng.L^{-1}).

Aim of the study

The aim of the study was to show if aspirin and LBP could influence the LPS triggered adverse clot formation in whole blood and frozen platelet poor plasma as well as membrane ultrastructure of red blood cells and platelets.

The following objectives directed this study, that is to investigate:

1. The viscoelastic properties of each participants before any reagents were added to their WB using TEG. These results were also used as health margins for each participant.
2. To investigate the effect of LPS on viscoelastic properties in each participant using TEG.
3. The investigation of the combined effect of aspirin and LBP on inhibiting the effects of LPS using TEG.
4. To mark amyloid fibril proteins (anomalous clotted fibrin(ogen) deposits) formed in pathologies and compare them to normal control blood, using super resolution confocal microscopy. The ThT was the fluorescent marker of choice for this study as it marks best amyloid fibrils formed in pathological fibres. In the presence of fibrin fibre deformation.
5. To investigate the effect of aspirin on LPS, to assess if aspirin will prevent pathological fibrin fibre formation and changes to RBCs ultrastructure. Scanning electron microscope together with super resolution confocal microscopy techniques were used to give a visual representative of clot structure.
6. The effect of LBP, LPS and aspirin on WB coagulability this includes RBC structure and clot structural changes using SEM.

7. Investigate the hypercoagulable state of matted mass deposits (anomalous clotting called amyloid) and structure of fibrin fibres networks after the addition of LPS to control naïve PPP; this was to create a positive control. The network was visualized with SEM.
8. The effect of LBP mixed with LPS, this was to assess if LBP alone can “mop up” or uptake and bind LPS to prevent pathological clot structure and changes to plasma
9. The effect of LPS, aspirin and LBP on the thickness of fibres network using Image J software 2000.

Chapter 3: Demographics

Introduction

Demographics is the study of a population based on factors such as age, race and gender. The study is not gender-based and very much age specific. This chapter will explore the study design, participant recruitment methodology, as well as participant and control demographics.

Study design

This study was conducted in collaboration with Dr Morne´ Strydom a total number 48 healthy participants were recruited. For this study stored platelet poor plasma from patients who participated in previous study were used, ethical approval was granted at the University of Pretoria Ethics number 169/2016 (Research Ethics Committee: Faculty of Health Sciences).

All patient information was handled anonymously. Each participant signed a consent form giving permission to use their blood for the study. Ethical clearance was obtained from the University of Pretoria, Research Ethics Committee, Faculty of Health Sciences Protocol number 213/2015. Ethical approval letter, amendment approval letters and renewal approval letter are all attached on the Index pages.

Sample size and collection

A total of 48 individuals participated in this study, two citrate tubes were drawn from each participant. The blood was drawn by Dr M Strydom a medical practitioner. All citrate tubes had to stand for 30 min after blood was drawn before any work could be done. This was to allow for complete binding of calcium. Samples were not refrigerated they were kept at room temperature until processing – this was done within 24 hours of blood drawing. Whole blood was used for SEM studies to analyse surface membrane of platelets and RBCs. Whole blood was also used for TEG study to analyse the viscoelastic properties of participants when WB was treated with LPS, ASA and LBP. Due to laboratory times constraints not all 48 participants' blood was used for TEG. The second citrate tube was centrifuged for 30 min at 2590 rpm for PPP. The PPP was kept and stored at -80ºC until further use. Stored PPP was used for SEM and confocal microscopy to look at the fibrin fibres formation, clot structure before and after all treatments. Blood was drawn in a good healthy sterile environment.

The participants could leave the study at any time they wished to, the main aim was to collect blood and make people feel at ease. For a direct comparative study, each individual was used as their own control before and after treatment addition. Below is a list of criteria that were used when selecting participants. The criteria were used for all blood collections.

The exclusion criteria:

- Smokers (smokes tobacco or a tobacco-related product)
- Chronic medication (any prescriptions or over the counter medication that is taken at a daily basis)
- Known inflammatory chronic diseases (asthma, Tuberculosis, Human immunodeficiency virus)
- Females who were on hormonal contraceptives
- Pregnant females
- Individuals younger than 18 years
- Elderly people above 65 year of age

Inclusion criteria:

- All races and genders were accepted

Table 3.1 is obtained from the non-parametric Mann Whitney test analysis together with graph in **Figure 3.1** the test was to determine the population age mean and to determine if the difference in ages of males verses female numbers had any significance difference.

Table 3. 1. Mann-Whitney test results study population

P value	0.8777
Exact or approximate P value?	Exact
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	616.5 , 511.5
Mann-Whitney U	265.5
Difference between medians	
Median of column A	34.00, n=26
Median of column B	33.00, n=21
Difference: Actual	-1.000
Difference: Hodges-Lehmann	0.000

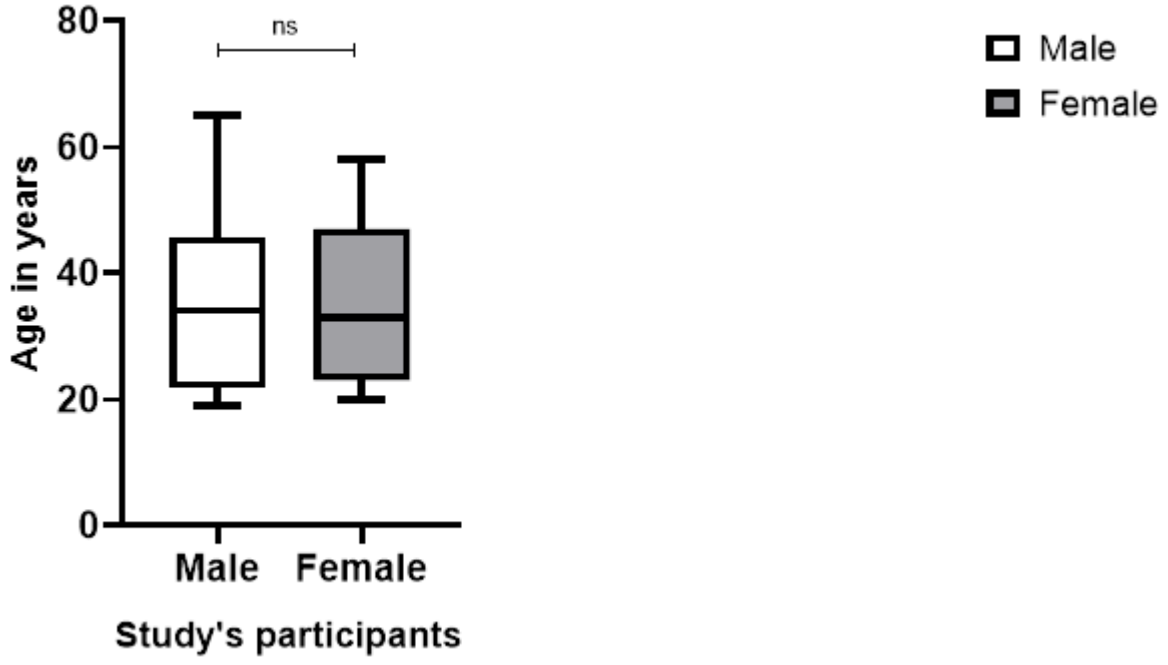


Figure 3. 1. Participant demographics showing age and gender box plots. The box plots indicate minimum age of 18 years for participants and a maximum of 65 years of age. The box plot shows a mean for both genders to be 35 years of age. The study was biased towards the +-35 years age group. There was no significance between males and female (P<0.05). Seen with non-parametric Mann Whitney test. (**ns- is non-significant**)

Discussion and conclusion

For this study TEG strictly required fresh WB and due to time constraint only three participants were taken at a time. Typically, in the ideal laboratory conditions, citrated WB is analysed after 30 min of collection, whilst PPP also be prepared after 30 minutes of collecting, followed by storing at −80 °C, until use for SEM or confocal microscopy. Hence, a lot more plasma was stored in the form of PPP that could be used for confocal microscopy. and SEM analysis and less fresh blood (WB) was available. According to the table with the Mann Whitney analysis of the study's participants, there was no statistically significant difference between the ages of males and females. This justifies that the study's population was selected strictly according to people's ages, but it was not selective on gender. From **Figure 3.1.** shows box plot of the study population indicating min max of the gender's ages and the median. The mean and standard deviation of their age groups were taken into consideration. According to statistical tools there were no significance deference between the age groups of male's verses females.

The reason for conducting statistics of age was to the certainty that it will provide the differences in age groups of males' verses females who participated in the study. Almost all diseases or health outcomes occur at different age groups. Most chronic diseases, including most cancers, occur more often among older people. Other outcomes, such as many types of injuries, occur more often among younger people (Norris, 2003, Pretorius and Kell, 2014). The age distribution determines what the most common ages were in the population and fitness to this study. This was to show that the study was age biased.

Chapter 4: Thromboelastography®

Introduction

Thromboelastography® is a well-known technique that is an important point-of-care method and it is widely used in medical research laboratories. The process of blood clotting has been studied for centuries. The current knowledge pertaining to haemostasis and the blood components, including platelets and fibrin networks which are closely involved in coagulation will be discussed in this chapter. Special emphasis is placed on tissue TF, calcium and thrombin since these components have been implicated in both the coagulation process and inflammation (Swanepoel et al., 2015). Analysis of platelets and fibrin morphology indicate that calcium, TF and thrombin at concentrations used during viscoelastic analysis with TEG bring about alterations in platelet and fibrin network ultrastructure, which is similar to that seen in inflammation (Swanepoel et al., 2015, Pretorius et al., 2017c). For this experiment citrated WB was used.

Hypercoagulability goes hand in hand with inflammation and is strongly influenced by the fibrinogen concentration (and *vice versa*), this can be mediated via interleukin 6. Poorly liganded iron is a significant feature of inflammatory diseases, and hypo-fibrinolysis may change as a result of changes in the structure and morphology of the clot, which may be mimicked *in vitro*, and may be caused *in vivo*, by the presence of unliganded iron interacting with fibrin(ogen) during clot formation (Kell and Pretorius, 2015f, Campbell et al., 2010, Pretorius and Kell, 2014a, Pretorius et al., 2011b). Many of these phenomena are probably caused by electrostatic changes in the iron-fibrinogen system, though some researchers have found that hydroxyl radical ($OH^{\cdot}$) formation can also contribute under both acute and chronic conditions. Hydroxyl radical is highly reactive and consequently short-lived; however, they form an important part of radical chemistry. (Pretorius et al., 2013a, Protopopova et al., 2015).

Clotting parameters and clot structure are informative measures of overall haematological healthiness of an individual (Kell and Pretorius, 2015f, Nielsen et al., 2004, Nielsen et al., 2007, Pretorius et al., 2017c, Swanepoel et al., 2015). One of the current point-of-care methods and research techniques that characterizes the viscosity and elasticity of either WB or PPP is the TEG (Nielsen et al., 2004, Nielsen et al., 2007, Pretorius et al., 2017c, Swanepoel et al., 2015). This chapter discusses the investigation that was conducted using TEG to study the various thrombus formation influenced by: LPS; LBP and aspirin. **Table 4.1** describes the various viscoelastic parameters when using the TEG.

Table 4. 1.TEG clot parameters for whole blood. This table was taken from (Pretorius et al., 2017c)

Parameters	Explanation
R value: reaction time measured in minutes	Time of latency from start of test to initial fibrin formation (amplitude of 2 mm); i.e. initiation time
K : kinetics measured in minutes	Time taken to achieve a certain level of clot strength (amplitude of 20 mm); i.e. Amplification
A (Alpha): Angle (slope between the traces represented by R and K) Angle is measured in degrees	The angle measures the speed at which fibrin build up and cross linking takes place, hence assesses the rate of clot formation; i.e. thrombin burst
MA: Maximal Amplitude measured in mm	Maximum strength/stiffness of clot. Reflects the ultimate strength of the fibrin clot, i.e. overall stability of the clot
Maximum rate of thrombus generation (MRTG) measured in Dyn cm−2 s−1	The maximum velocity of clot growth observed or maximum rate of thrombus generation using G, where G is the elastic modulus strength of the thrombus in dynes per cm^{-2}
Time to maximum rate of thrombus generation (TMRTG) measured in Minutes	The time interval observed before the maximum speed of the clot growth
Total thrombus generation (TTG) measured in Dyn.cm^{-2}	The clot strength: the amount of total resistance (to movement of the cup and pin) generated during clot formation. This is the total area under the velocity curve during clot growth, representing the amount of clot strength generated during clot growth

Table 4.2. was adapted from (Pretorius et al., 2017c), and modified to have ranges of each of the TEG parameters that was tested for this investigation. This table was used as a guideline for the investigation, to determine hypercoagulable verses hypocoagulable state from the results.

Table 4. 2. TEG parameters with ranges: guideline to identify pathology adapted from (Pretorius et al., 2017c)

WB	Hypercoagulable		Hypocoagulable
R min (9-27)	Clot forms faster	↑	Clot forms slower
K min (2-9)	Clot reaches a set (20 mm) strength quicker	↑	Clot reaches a set (20 mm) strength slower
A Alpha (22-58)	An increased thrombin burst resulting in more cross-linking of fibrin fibres	↓	A decreased thrombin burst resulting in less cross-linking of fibrin fibres
MA mm (44-64)	Increased platelet and/or fibrin(ogen) interaction resulting in a denser clot that is more rigid	↓	Decreased platelet and/or fibrin(ogen) interaction resulting in a less dense clot that is less rigid
MRTG[b] Dyn cm^{-2} s^{-1} (0-10)	Increased clot growth	↓	Decreased clot growth
TMRTG[b] min (5-23)	Decreased time from clot initiation to maximum clot formation	↑	Increased time from clot initiation to maximum clot formation
TTG[b] Dyn.cm^{-2} (251-1014)	Increased clot strength	↓	Decreased clot strength

Chapter Aim

The aim of this chapter was to investigate the viscoelastic properties of WB using TEG before and after WB treatments with; LPS, LBP alone and mixed with LPS, aspirin with LBP, aspirin with LPS and aspirin with LBP mixed LPS, to combat the inflammatory effect induced by LPS.

Chapter objectives

- To investigate the efficiency of blood coagulation parameters prior to adding any of the test molecules, determining the coagulation pattern of each participant using WB.
- To investigate the viscoelastic properties after exposure to LPS (used as reference of hypercoagulation).
- To investigate the viscoelastic properties in participants after exposure of the following: LBP, LPS and LBP, Aspirin, Aspirin and LBP, Aspirin and LPS, Aspirin and LPS+LBP. to establish the protective roles played by aspirin alone or in combination with LBP.

Materials and Methods

A health margin for all participants was marked by their coagulation. The viscoelastic properties of each participant were used as a health margin. The TEG is a method of testing the viscoelastic properties of blood coagulation. TEG can be used for a point of care test to measure viscoelasticity of the blood (Nielsen et al., 2007, Pretorius et al., 2017c, Swanepoel et al., 2015).

Reagents for the experiment

- **Lipid binding protein**

  1µg of LBP was reconstituted in 1000ml of ultrapure distilled water to make up a stock solution. Up to 0.2 µg/ml LBP mediates binding of FITC-LPS (0.5µg/ml) to CD14 CHO transfectants. Purchased from Sigma-Aldrich® South Africa.

- **Lipopolysaccharides**

  Lipopolysaccharide from bacterium called *Escherichia coli* 0111:B4 Lyophilized was used in this study.

The lyophilized powder was reconstituted in ultrapure distilled water to make up a final stock solution of 10ng.L^{-1}. Purchased from Sigma-Aldrich® South Africa.

- **Sodium salicylate**

  (Aspirin) Reagent Plus®,_>99.5% S3007-500G. Formula: C7H5NaO3 and formula Weight: 160.10 g.mol^{-1}.

- **Calcium Chloride activator**

  A clot activator that was used in the TEG. Catalogue nr 4905145, Qiegen, concentration of 2M purchased from Sigma-Aldrich® South Africa.

Method

A volume of 333.2µl of WB was aliquoted into an Eppendorf tube. To this tube a volume of 6.8µl of LBP (final exposure concentration of 2ng.L^{-1}) was added making a total volume of 340µl. To test the effect of LPS on coagulation and its effects on viscoelasticity a final exposure concentration of 0.2 ng.L^{-1} LPS was added to 333.2µL of WB, this served as the positive control. To study the effect of LBP on LPS, and to see how LBP in direct exposure to LPS will affect coagulation and viscoelasticity. A volume of 326µL WB was added with 13,6µL of LPS and LBP both reagents at a final exposure concentration of 2ng.L^{-1}.To study the effect of aspirin on coagulation 25µl of aspirin finale exposure concentration of 0.5mM was added to 475µl of WB. To study the effect of LBP and aspirin on coagulation 25µl of aspirin finale exposure concentration of 0.5mM was added to 475µl of WB, Only 2ng.L^{-1} final exposure concentration of LPB was added. To study the effect of aspirin on LPS, 25µl aspirin (final exposure concentration of 0.5Mm) was added to 475µL WB. Only 0, 2ng.L^{-1} final exposure concentration of LPS was added. To study the effect of combined therapy aspirin mixed with LPS and LBP, 25µl aspirin (final exposure concentration of 0.5Mm) was added to 475µL WB. Only 0, 2ng.L-1 final exposure concentration of LPS was added and LBP was added to a final exposure concentration of 2ng.L^{-1}. A volume of 340 µl of WB with treatment or naïve, was placed in the TEG cups. This was followed by the addition of 20µl activator calcium chloride ($CaCl_2$) into the cup and rotated gently six times a minute by the TEG. This was to induce shear forces on the samples. and activate coagulation. A thin torsion wire was used to measure clot formation. The speed and strength of clot formation was measured. The speed at which the sample coagulates depends on the activity of the plasma coagulation, platelet function, fibrinolysis and other factors, which can be affected by genetics, illness, environment and medications (Kim et al., 2015, Swanepoel et al., 2015).

Steps on how to operate the TEG:

The participants details were entered onto the TEG system and database. Once the sample types were defined eg. WB naïve, LBP, LPS, aspirin, aspirin and LPS, aspirin and LBP and aspirin with LPS and LBP. The cup was loaded and pin into each channel. The tracings were viewed offset or superimposed.

The patterns of changes in strength and elasticity in the clot provided information about how well the blood performed haemostasis, and how well or poorly different factors were contributing to clot formation.

Results

These are results obtained from the TEG. Each tracing/curve represents a thrombus obtained with or without treatment that has been added to the WB. **Figure 4.1 to Figure 4-7** shows the tracing example from naïve samples this is WB without treatments. Refer to index pages for visual representation of how a normal TEG curve verses curve from inflammatory condition.

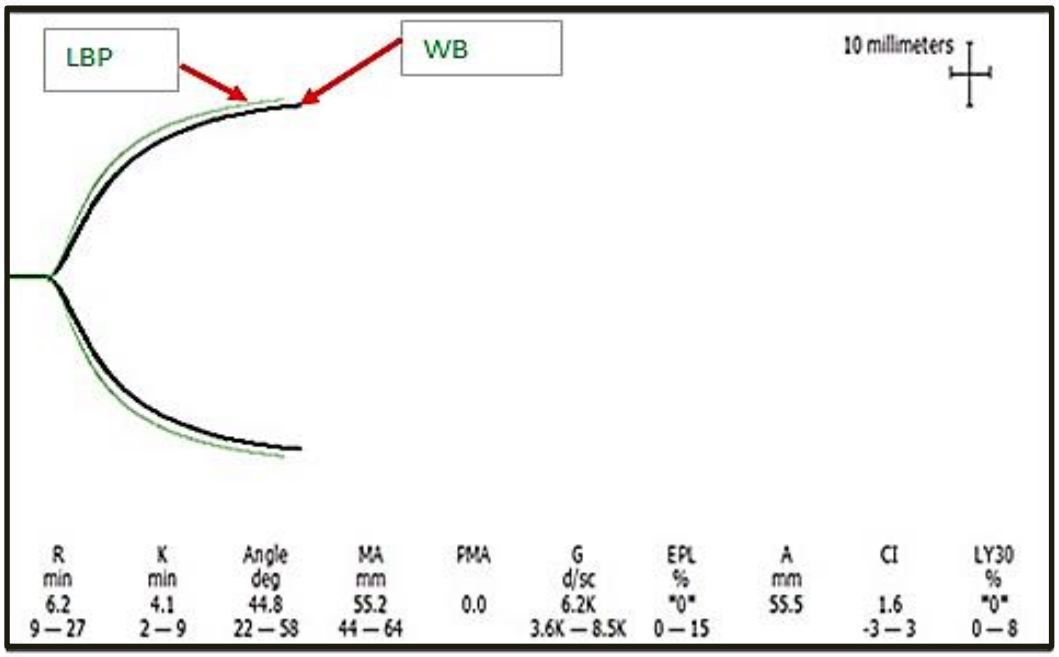


Figure 4. 1.TEG tracings from WB (black curve) and WB mixed with LBP (green curve) both curves are normal shaped, and all parameters were almost identical between LBP and WB.

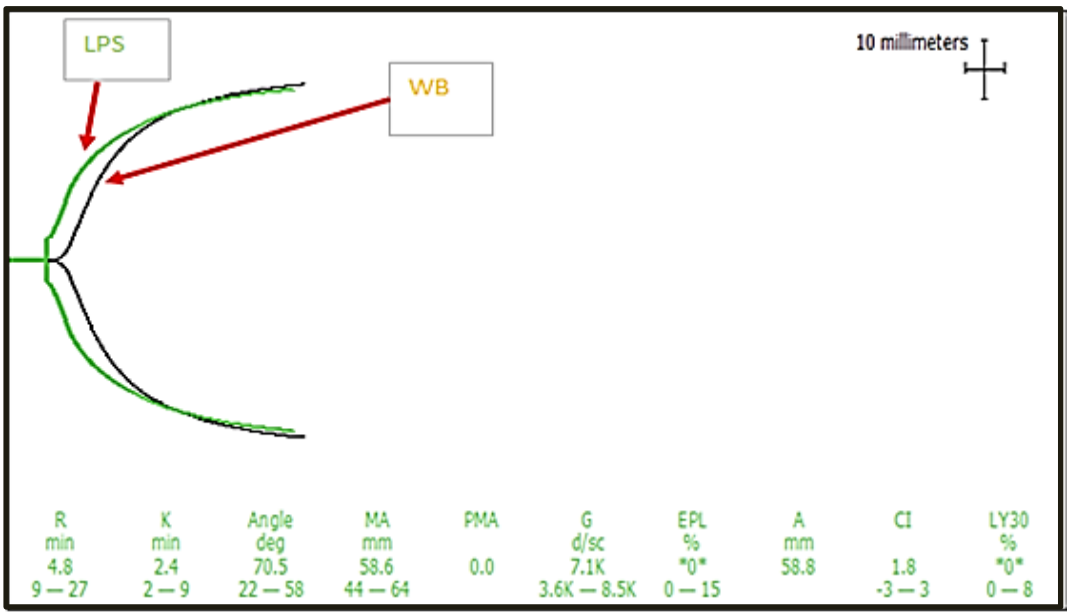


Figure 4. 2.TEG tracing from WB (black curve) and WB with LPS (green curve). Showing a hypercoagulable abnormal curve when LPS was added in WB.

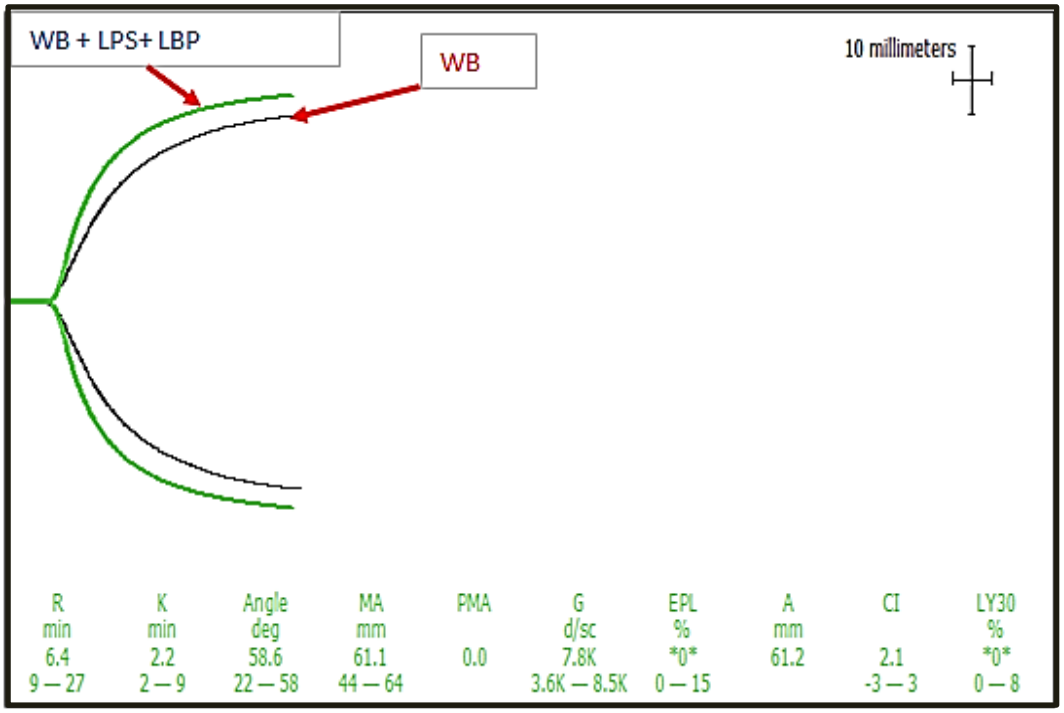


Figure 4. 3.TEG tracings of WB (black curve) and WB +LPS+LBP (green curve). The two curves are normal shape but when LBP is added with LPS in WB the thrombus forms faster with less disruptive interactions of fibrin (MA decreases).

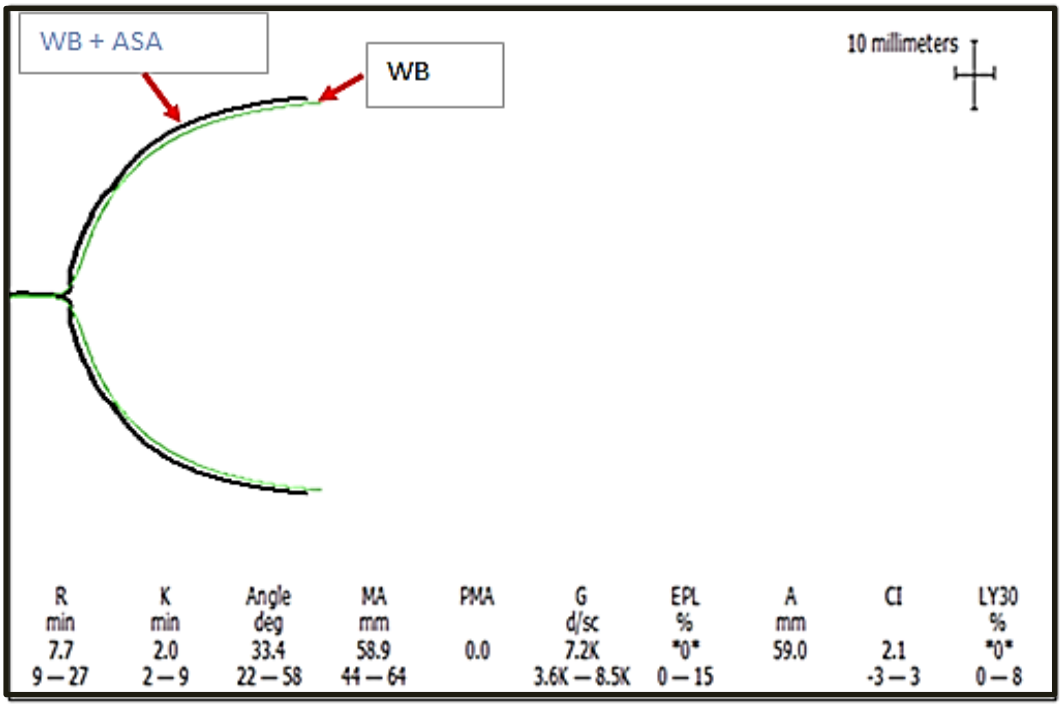


Figure 4. 4.TEG tracings of whole blood (WB) (green curve) and aspirin (ASA) mixed with WB (black curve). Almost the same size and same shape as with the WB. No significant variations in viscoelastic parameters between aspirin treated samples verses WB.

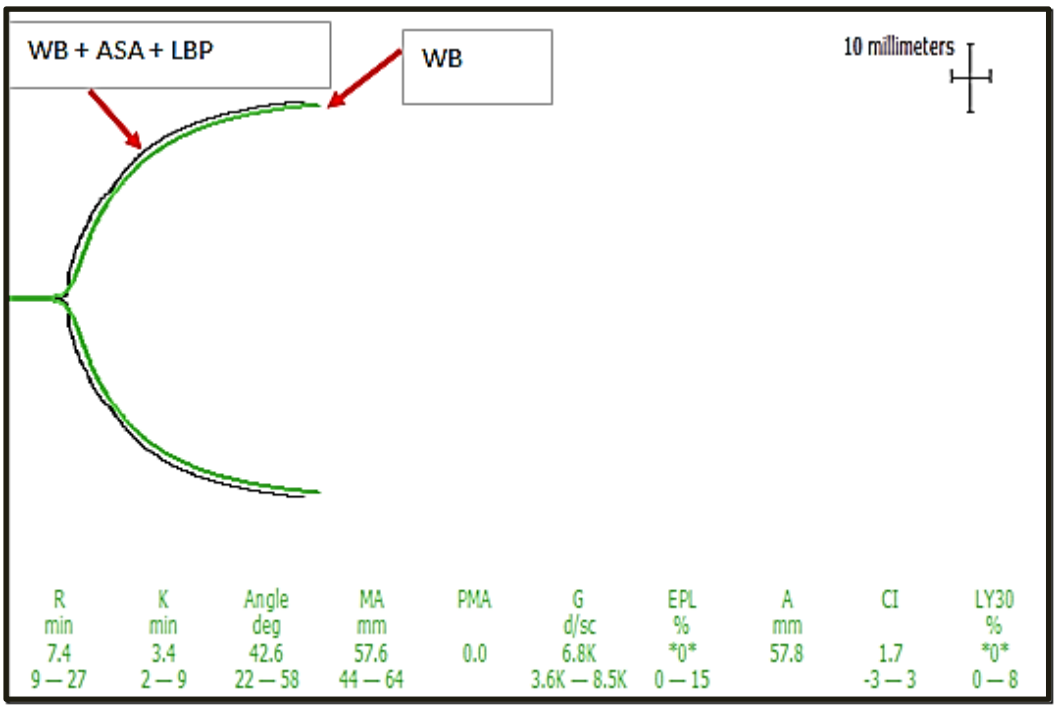


Figure 4. 5.TEG tracings of whole blood (WB) (green curve) and WB mixed with Lipopolysaccharide binding protein (LBP) and aspirin (ASA) (black curve). The two curves have a normal shape almost identical viscoelastic parameter.

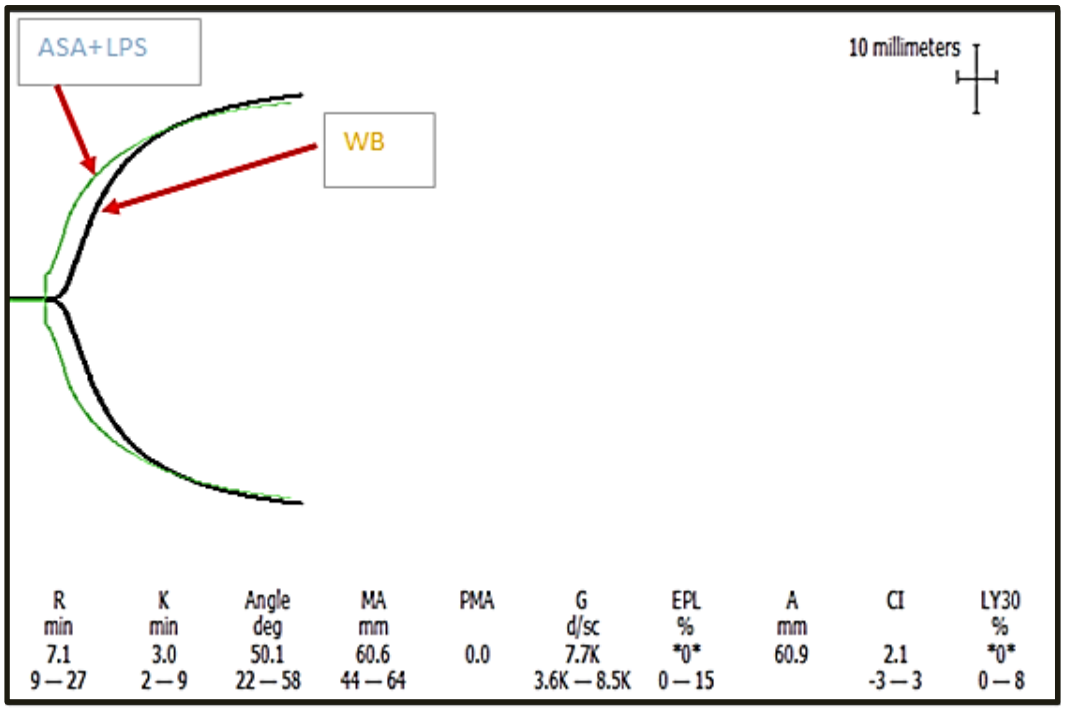


Figure 4. 6.TEG tracing of whole blood (WB) mixed with aspirin (ASA) also mixed with Lipopolysaccharide (LPS) green curve verses WB mixed (black curve). The aspirin and LPS mixed with WB give an abnormal curve symbolizing a hypercoagulable curve. Viscoelastic parameters when comparing with naïve WB shows ASA+ LPS in WB thrombus forms at a faster rate (TGG decreased).

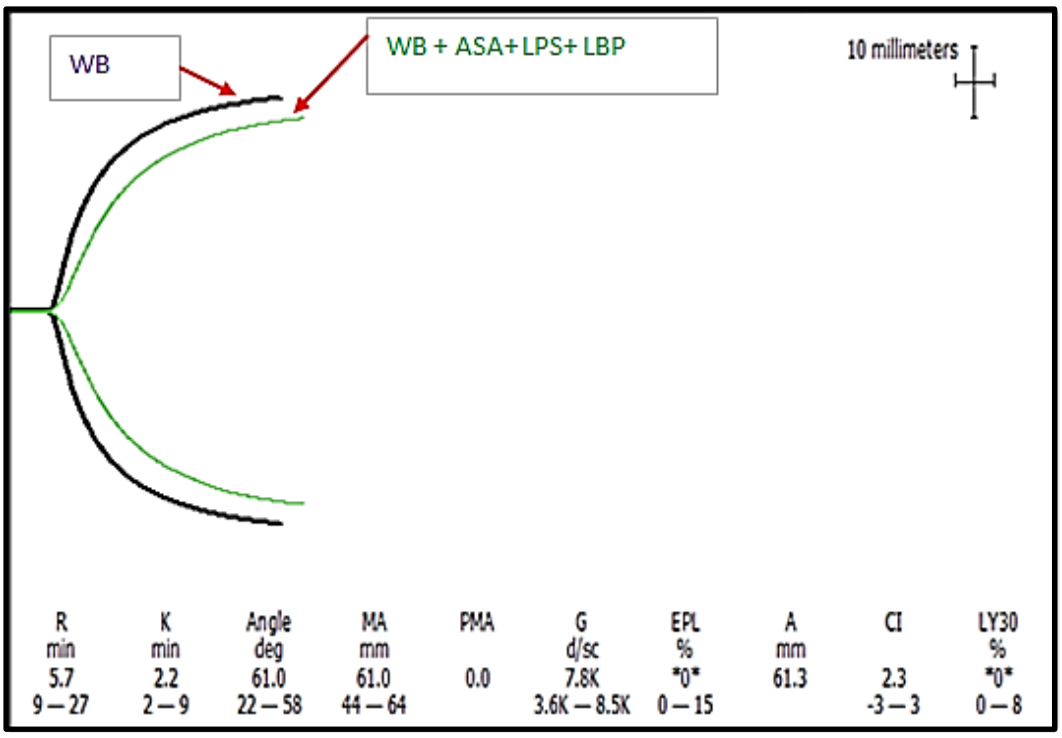


Figure 4. 7.TEG tracings of whole blood (WB) black curve and WB mixed with aspirin (ASA)+ Lipopolysaccharide (LPS) + Lipopolysaccharide binding protein (LBP) green curve. The curves that form when LPS is added with LBP and ASA is a normal curve with which forms slightly faster thrombus then when WB is tested (TMRTG decreased).

Statistical Analysis

Quantitative analysis of each parameter described in **Table 4.3** provided continuous datasets for each sample. The baseline or naïve WB sample was used as the control and to which all subsequent treatments were compared. Therefore, following the D-Agostino and Pearson normality test (to determine Gaussian distribution of data), repeated measures ANOVA was conducted to determine if treatments significantly influenced the outcome with the level of significance set at $p<0.05$. From the TEG results the mean, standard deviation and median of each of TEG parameter was taken. The median is a measure of central tendency. It represents the value for which 50% of observations are lower and 50% are higher. In other words, it is the value at the center of the sorted observations. See **Table 4.3.** for median, mean and standard deviation of WB with and without treatments. All WB samples treated or untreated were then analyses by a Thromboelastograph 5000 Haemostasis Analyzer System.

Since all datasets did not pass the normality test for Gaussian distribution, the median and interquartile ranges (IQR) were represented in **Table 4.4 -4.8** followed by **Figures 4.8 – 4.13**.

Table 4. 3. The median, mean and standard deviation of WB with and without treatments from TEG

		Naïve WB	**LBP**	**LPS**	**LBP + LPS**	**ASA**	**ASA+ LBP**	**ASA + LPS**	**ASA + LPS+LBP**
R (min)	Median	5.5	5.7	5.7	5.9	6.2	5.5	4.7	5.1
	Mean	5.99	6.29	6.14	6.35	6.36	5.49	4.79	5.25
	±SD	2.01	1.76	1.57	1.94	1.97	1.26	1.72	1.01
K (mm)	Median	2.6	2.7	2.6	2.8	2.2	2.85	2.6	2.2
	Mean	2.77	2.74	3.01	3.79	2.58	3.96	3.55	2.52
	SD	1.01	0.98	1.62	3.66	1.34	4.36	3.50	1.12
Angle (deg)	Median	56.9	51.1	50.4	50.1	53.9	51.8	53.2	57.4
	Mean	56.89	51.01	51.55	48.34	52.17	51.92	52.24	56.11
	SD	11.079	10.55	10.53	12.93	12.38	10.66	15.52	12.84
MA (mm)	Median	61.3	57.0	54.0	54.4	58.9	57.6	56.0	54.8
	Mean	59.62	55.72	54.13	52.19	58.54	53.47	53.55	53.67
	SD	6.94	5.01	7.42	9.32	7.35	11.48	12.36	8.18
MRTG (dcs)	Median	6.02	4.24	4.99	4.09	5.73	4.74	4.83	5.32
	Mean	5.99	5.09	4.60	4.37	5.54	5.63	8.61	6.0
	SD	2.40	2.50	1.49	1.84	1.72	3.43	14.07	3.78
TMRTG (min)	Median	7.67	8.42	8.25	8.42	7.67	8.33	6.33	6.67
	Mean	8.91	8.89	8.32	8.82	8.23	8.00	6.38	6.83
	SD	2.78	1.69	2.22	2.62	2.58	2.23	2.26	1.26
TGG (dcs)	Median	792.12	664.28	649.16	599.57	696.01	681.89	630.65	608.67
	Mean	769.07	645.14	644.07	577.51	716.28	624.82	603.16	609.51
	SD	194.76	125.13	191.62	168.80	191.88	225.93	373.68	173.91

Table 4. 4. Dunnett's test multiple comparison R (min) of all treatments with control

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
WB vs LBP	-0.6889	1.130	No	-2.334 to 0.9557
WB vs LPS	-0.4556	0.7471	No	-2.100 to 1.189
WB vs LBP + LPS	-0.7333	1.203	No	-2.378 to 0.9113
WB vs ASA	-0.1889	0.3098	No	-1.834 to 1.456
WB vs ASA + LBP	0.6778	1.112	No	-0.9668 to 2.322
WB vs ASA + LPS	0.5333	0.8746	No	-1.111 to 2.178
WB vs ASA + LBP + LPS	0.6333	1.039	No	-1.011 to 2.278
ANOVA table	P value			
Treatment (between columns)	P=0.0975			
Individual (between rows)	P<0.0001			

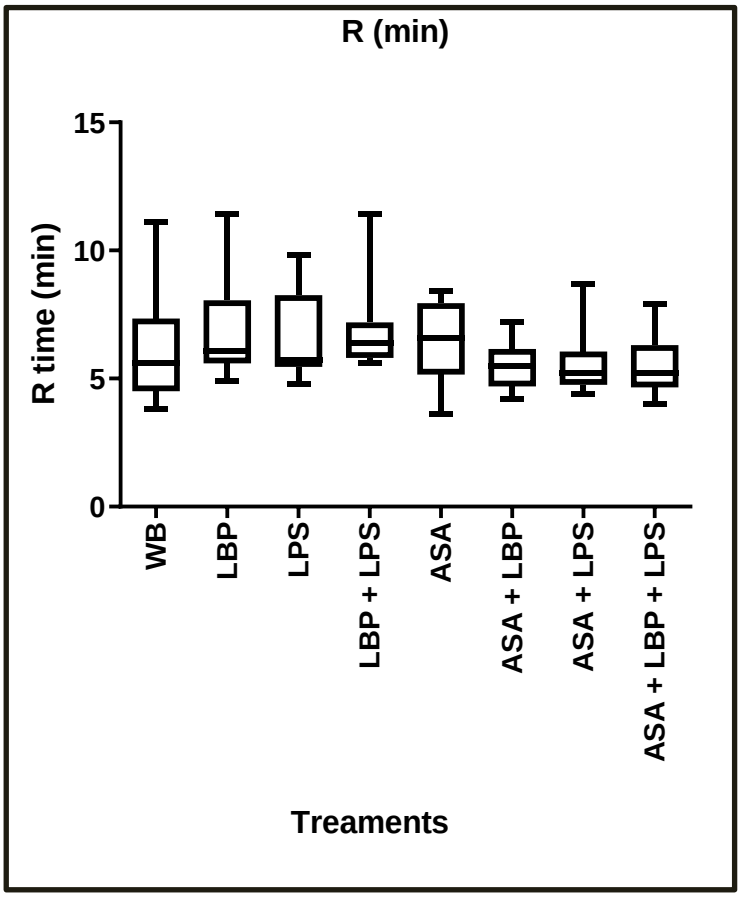


Figure 4. 8. The R time (min) measuring how fast the clot formed between treatments and control was not significantly altered following a repeated measures ANOVA and Dunnet's multiple comparison posttest (p>0.05)

Table 4. 5. Dunnett's test multiple comparison K (min) of all treatments with control

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
WB vs LBP	0.03333	0.09598	No	-0.9034 to 0.9700
WB vs LPS	0.2333	0.6719	No	-0.7034 to 1.170
WB vs LBP + LPS	0.08889	0.2559	No	-0.8478 to 1.026
WB vs ASA	0.6333	1.824	No	-0.3034 to 1.570
WB vs ASA + LBP	0.1889	0.5439	No	-0.7478 to 1.126
WB vs ASA + LPS	0.2333	0.6719	No	-0.7034 to 1.170
WB vs ASA + LBP + LPS	0.7333	2.112	No	-0.2034 to 1.670
ANOVA table	P value			
Treatment (between columns)	P=0.3041			
Individual (between rows)	P=0.0005			

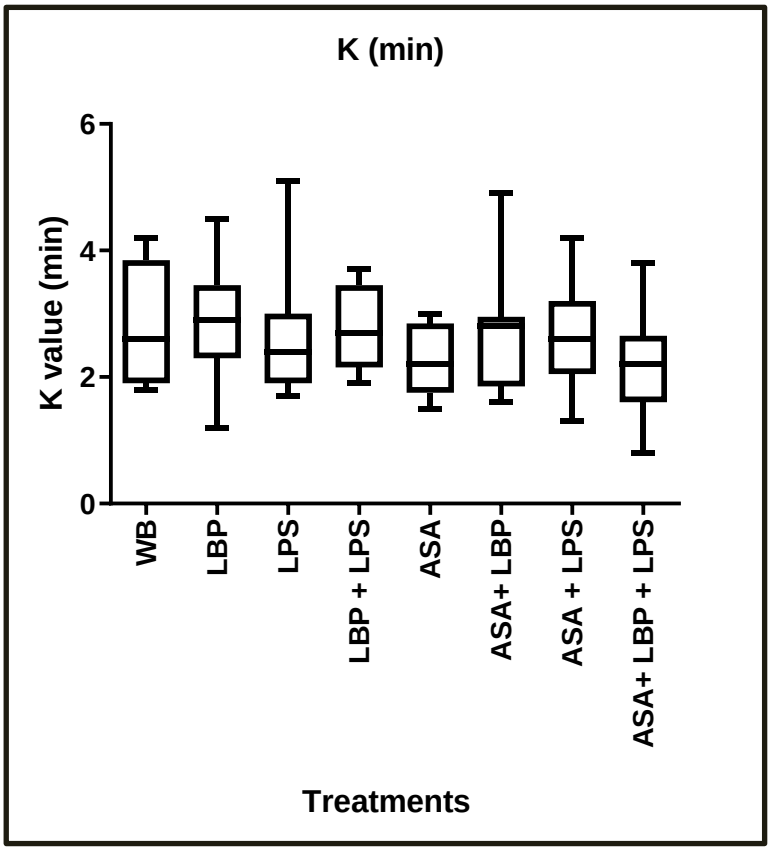


Figure 4. 9. The kinetics (min) indicative of the time take for the clot to achieve an amplitude of 20mm between treatments and control was not significantly altered following a repeated measures ANOVA and Dunnet's multiple comparisons posttest (p>0.05).

Table 4. 6. Dunnett's test multiple comparison MA (mm) of all treatments with control

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
WB vs LBP	2.611	0.9783	No	-4.587 to 9.810
WB vs LPS	3.189	1.195	No	-4.010 to 10.39
WB vs LBP + LPS	7.311	2.739	Yes	0.1127 to 14.51
WB vs ASA	-1.433	0.5370	No	-8.632 to 5.765
WB vs ASA + LBP	2.778	1.041	No	-4.421 to 9.976
WB vs ASA + LPS	5.989	2.244	No	-1.210 to 13.19
WB vs ASA + LBP + LPS	3.567	1.336	No	-3.632 to 10.77
ANOVA table	P value			
Treatment (between columns)	P=0.0412			
Individual (between rows)	P<0.0001			

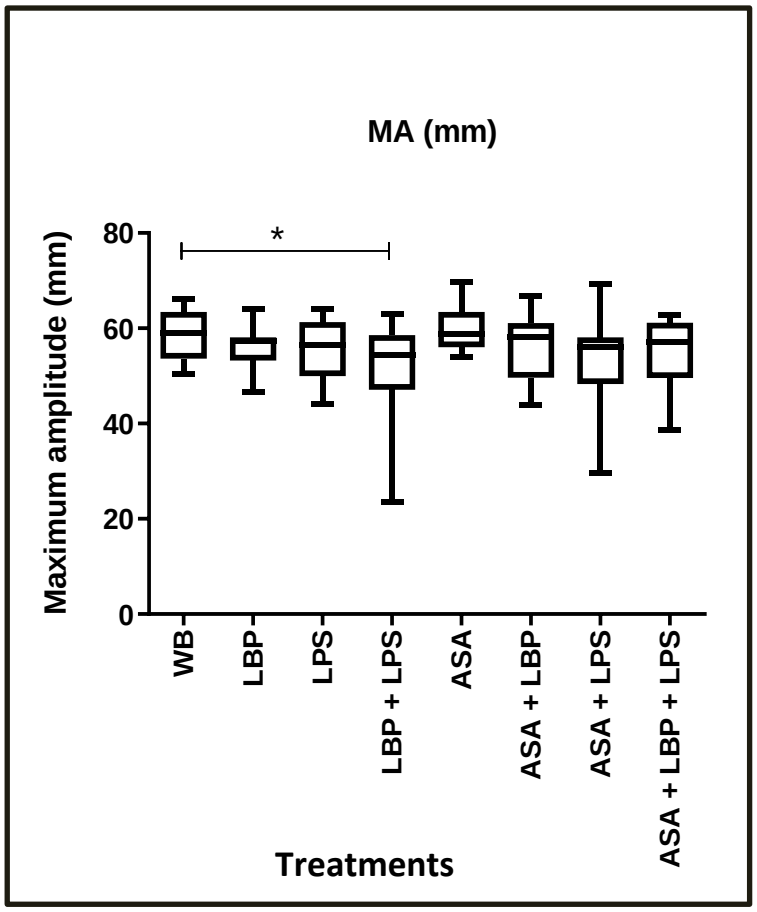


Figure 4. 10. The maximum amplitude (mm) measuring the clot strength and fibrin interaction formed between treatments and control was only significantly altered in the LBP + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest (p=0.0412).

Table 4. 7. Dunnett's test multiple comparison MRTG (dcs) of all treatments with control

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	q
WB vs. LBP	1.118	-0.9650 to 3.200	No	1.454
WB vs. LPS	0.9038	-1.179 to 2.986	No	1.176
WB vs. LBP + LPS	1.528	-0.5550 to 3.610	No	1.987
WB vs. ASA	0.1175	-1.965 to 2.200	No	0.1528
WB vs. ASA + LBP	0.5300	-1.553 to 2.613	No	0.6894
WB vs. ASA + LPS	1.649	-0.4338 to 3.731	No	2.145
WB vs. ASA + LBP + LPS	0.1313	-1.951 to 2.214	No	0.1707
ANOVA table	P value			
Treatment (between columns)	P=0.2138			
Individual (between rows)	P=0.0067			

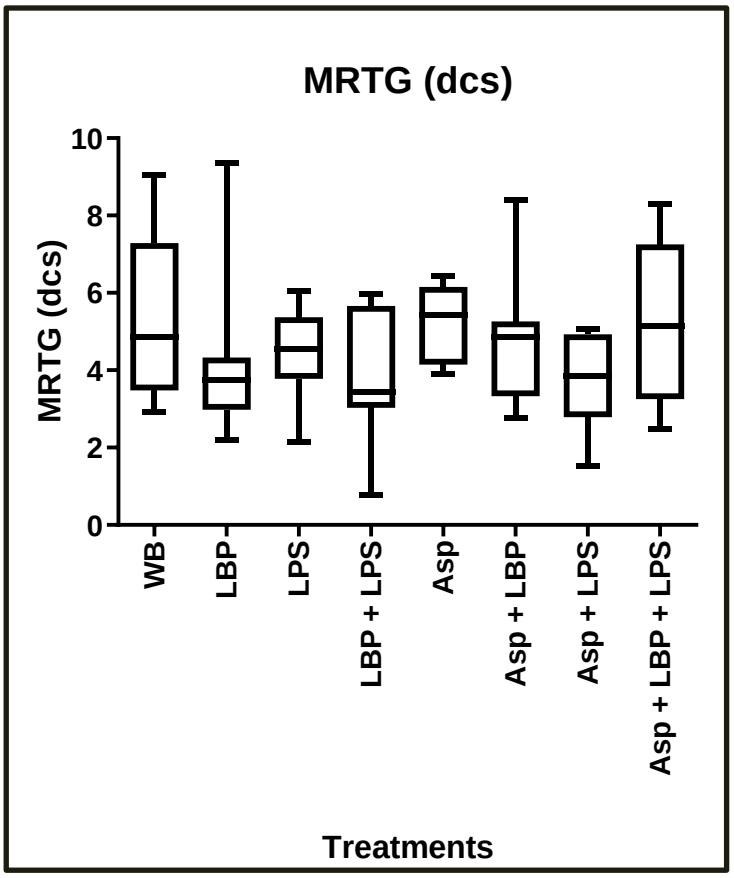


Figure 4. 11. The maximum rate of thrombus generation (dcs) between treatments and control was not significantly altered following a repeated measures ANOVA and Dunnet's multiple comparisons posttest (p>0.05).

Table 4. 8. Dunnett's test multiple comparison TMRTG (min) of all treatments with control

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
WB vs LBP	-0.3144	0.3898	No	-2.490 to 1.861
WB vs LPS	0.3256	0.4035	No	-1.850 to 2.501
WB vs LBP + LPS	0.2122	0.2631	No	-1.964 to 2.388
WB vs ASA	1.121	1.390	No	-1.055 to 3.297
WB vs ASA + LBP	1.704	2.113	No	-0.4715 to 3.880
WB vs ASA + LPS	1.981	2.456	No	-0.1948 to 4.157
WB vs ASA + LBP + LPS	2.390	2.962	Yes	0.2141 to 4.566
ANOVA table	P value			
Treatment (between columns)	P=0.0071			
Individual (between rows)	P=0.0006			

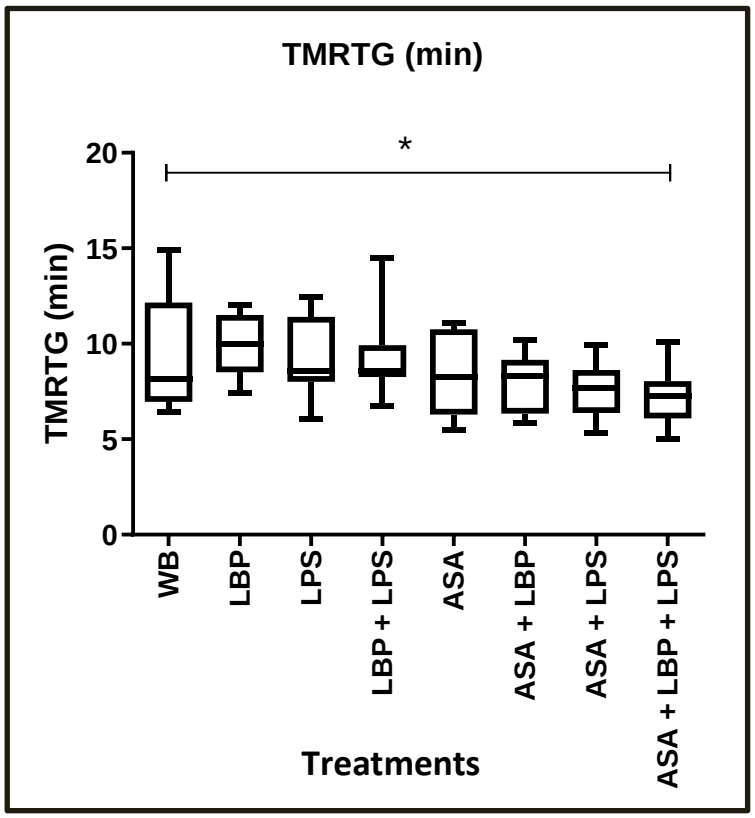


Figure 4. 12. The time taken to maximum rate of thrombus generation (min) between treatments and control was only significantly lower in the ASA + LBP + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest (p=0.0071).

Table 4. 9. Dunnett's test multiple comparison TGG (dcs) of all treatments with control

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
WB vs LBP	73.23	1.055	No	-114.0 to 260.5
WB vs LPS	76.90	1.108	No	-110.4 to 264.2
WB vs LBP + LPS	152.2	2.192	No	-35.06 to 339.4
WB vs ASA	-52.56	0.7570	No	-239.8 to 134.7
WB vs ASA + LBP	61.42	0.8846	No	-125.8 to 248.7
WB vs ASA + LPS	231.0	3.327	Yes	43.74 to 418.3
WB vs ASA + LBP + LPS	79.93	1.151	No	-107.3 to 267.2
ANOVA table	P value			
Treatment (between columns)	P=0.0078			
Individual (between rows)	P<0.0001			

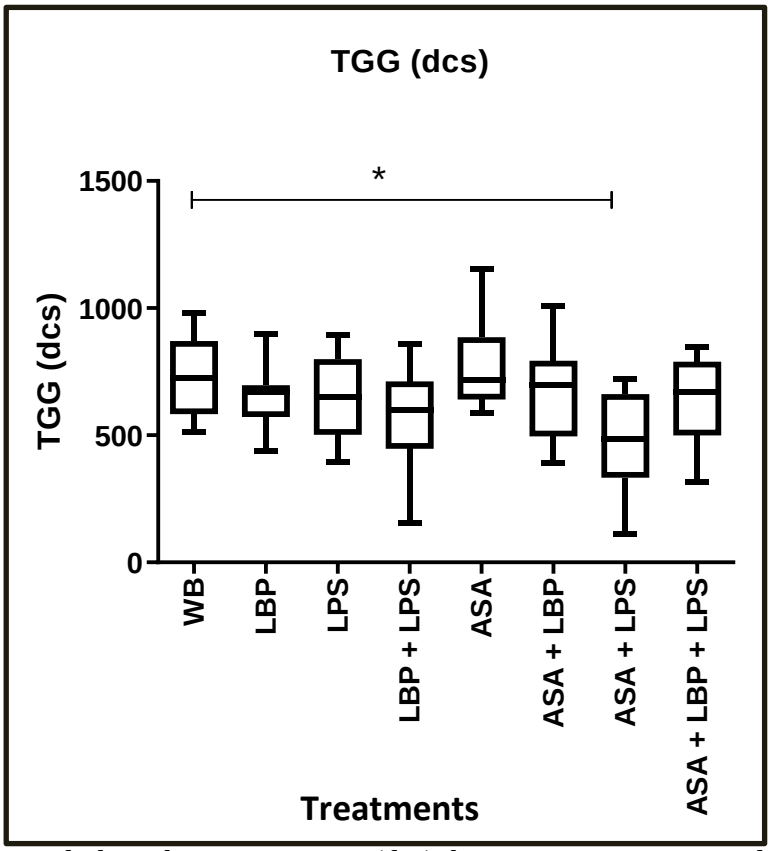


Figure 4. 13. The total thrombus generation (dcs) between treatments and control was only significantly reduced in the ASA + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest (p=0.0078).

Discussion

The aim was to detect the viscoelasticity properties of participants *ex vivo* before and after treatments. **Table 4.1** is a report of all the TEG clot parameters of WB and explanations of each parameter. This table was used to explain and understand the results. Followed by **Table 4.2.** which shows the ranges of each TEG parameter to give guidelines when identifying clotting pathologies. **Table 4.3** shows the mean, median and standard deviation from the TEG results of WB and tests which was used in the statistical program to obtain significance. The TEG curves shown in **Figure 4.1 – Figure 4.7** are directly from the TEG software and a representative of how the clot forms in the machine. Since all datasets did not pass the normality test for Gaussian distribution, the median and interquartile ranges (IQR) were represented in **Figures 4.8. – Figure 4.13**. There was no single treatment which influenced clot kinetics in a similar trend.

In **Figure 4.8** box plot shows the R time (min) measuring how fast the clot formed. The difference between treatments and control was not significantly altered following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p>0.05$). The R(min) of the TEG parameter is explained as the time of latency from start of test to initial fibrin formation (amplitude of 2 mm); i.e. reaction time measured in minutes.

The kinetics (min) indicative of the time take for the clot to achieve an amplitude of 20mm. The difference between treatments and control was not significantly altered following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p>0.05$). **Figure 4.9.** shows K (min) kinetics measured in minutes. Some of the samples show unexpected results (with insignificant) due to unknown reasons.

Angle (deg) which measures the speed at which fibrin build up and cross linking takes place, hence assesses the rate of clot formation; i.e. thrombin burst and there is also the MA (mm) which is the maximum strength/stiffness of clot, reflects the ultimate strength of the fibrin clot, this is the overall stability of the clot. The results obtained from the MA(mm) reading, were the difference in clot strength between MA from naïve WB and MA from WB mixed with LBP together with LPS was significant, suggesting that the mixture of LBP when mixed with LPS causes a decrease in the strength of the clot and the rate of the clot formed as seen in **Figure 4.10.** a box plot that shows the maximum amplitude (MA) measured in (mm) measuring the clot strength formed between treatments and control was only significantly altered in the LBP + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p=0.0412$). A statistically significant decrease in MA of WB and WB with LBP + LPS shows that LBP decreases platelet and/or fibrin(ogen) interaction resulting in a less dense clot that is less rigid, thus showing an improvement**.**

In **Figure 4.11.** the Maximum rate of thrombus generation (MRTG) measured in Dyn cm−2 s−1 comparative results is shown. The MRTG between treatments and control was not significantly altered following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p>0.05$).

In **Figure 4.12.** the time taken to maximum rate of thrombus generation (min) (TMRTG) is measure from each TEG tracings. Whereby MRTG measures the maximum velocity of clot growth observed or maximum rate of thrombus generation using G, where G is the elastic modulus strength of the thrombus in dynes per cm^2. The TMRTG between treatments and control was only significantly lower in the ASA +LBP +LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p=0.0071$). This means that when ASA was added with LBP and LPS it decreases the initiation to maximum clot formation, causing a hypercoagulable state. One of the most damaging forms of hypercoagulation is known as DIC (Asakura, 2014, Bick, 2002, Wada et al., 2012, Levi and van der Poll, 2013) It is essentially a runaway form of hypercoagulation, and it too may be induced by LPS (endotoxin) (Asakura, 2014, Duburcq et al., 2015, Wu et al., 2012, Yu et al., 2013). Disseminated intravascular coagulation has a common pathogenesis in terms of persistent widespread activation of coagulation in the presence of underlying disease, but the degree of fibrinolytic activation often differs by DIC type. DIC with suppressed fibrinolysis is a DIC type usually seen in sepsis (Asakura, 2014). The addition of LPS together with ASA and LBP could have initially causing DIC which is easily detected by the TEG.

The TTG, indicates the total thrombus generation (TTG) measured in $Dyn.cm^{-2}$ from TEG tracings of WB to all reagents. This TEG parameter measures the clot strength, the amount of total resistance (to movement of the cup and pin) generated during clot formation. This is the total area under the velocity curve during clot growth, representing the amount of clot strength generated during clot growth as seen in Figure **4.13.** The TTG between treatments and control was only significantly reduced in the ASA + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p=0.0078$). This means that when ASA was added with LPS it caused the clot strength along with growth of clot to be less.

There are seven TEG parameters that can be measured using either PPP or WB, only clotting and not clot lyses were measured. Not all these parameters need to change to indicate pathological coagulability. The degree of pathological coagulability can be related to the number of TEG parameters (one to seven) that are influenced. It is therefore important to look at each of the parameters individually, to follow a truly individualized approach.

Clotting properties can be particularly important when determining the inflammatory status of individuals, mostly after a major event like thrombo-embolic ischemic stroke or after diagnoses of e.g. type II diabetes, where disease progression or treatment regimens are of great value. (Ramchand et al., 2016), and is typically used during intensive care procedures (Abuelkasem et al., 2016, Kim et al., 2015, Ramchand et al., 2016).

Chapter conclusion

From these results it was deduced that although the multiple comparisons between most of the tests were statistically non-significant, there was a statistically significant differences in clot strength (MA) between WB mixed with LBP and LPS, and naïve WB. This shows a decrease platelet and/or fibrin(ogen) interaction resulting in a less dense clot that is less rigid in fibrinogen or platelets. The TMRTG was statistically significantly lower when WB was mixed with aspirin and LBP mixed with LPS as compared with naïve WB. This means the time from clot initiation to maximum clot formation decreased. The TTG analysis which measured the clot strength, significantly decreased when WB was added with aspirin and LPS. Aspirin can reduce the hypercoagulable thrombus formed in the presence of LPS. These results suggest that separately aspirin and LBP have an effect in decreasing hypercoagulable thrombus/clot formed, this could serve as a base defense approach in hypercoagulable conditions (exception of the TMRTG results) when the clot is large, and highly dense. These results will be used again in the final chapter as part of the conclusion discussion of all results.

Chapter 5: Confocal microscopy

Introduction

Confocal laser scanning microscopy (CLSM), is an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of using a spatial pinhole to block out-of-focus light in image formation (Amos and White, 2003, Amin et al., 2017). Capturing multiple two-dimensional images at different depths in a sample enables the reconstruction of three-dimensional structures (a process known as optical sectioning) within an object (Qiao et al., 2017). This technique is used extensively in the scientific and industrial communities and typical applications are in life sciences, semiconductor inspection and materials science (Amos and White, 2003, Bagheri et al., 2018).

Light travels through the sample under a conventional microscope as far into the specimen as it can penetrate, while a confocal microscope only focuses a smaller beam of light at one narrow depth level at a time (Bagheri et al., 2018, Cardenas-Perez et al., 2018). The CLSM achieves a controlled and highly limited depth of focus (Amos and White, 2003, Bagheri et al., 2018, Cardenas-Perez et al., 2018, Hoffman et al., 2006). A fluorescence microscope is an optical microscope that uses fluorescence and phosphorescence instead of, or in addition to, reflection and absorption to study properties of organic or inorganic substances (Yoneyama et al., 2017, Shao et al., 2012).

The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope, or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image (Mortensen et al., 2016, Ni et al., 2017, Nwaneshiudu et al., 2012).Super-resolution microscopy in light microscopy, is a term that gathers several techniques, which allow images to be taken with a higher resolution than the one imposed by the diffraction limit (Mortensen et al., 2016, Pant et al., 2017).

Due to the diffraction of light, the resolution in conventional light microscopy is limited (Zou et al., 2018). In this context, a diffraction-limited microscope with numerical aperture (N.A.) and light with wavelength (λ) reaches a lateral resolution of $d = \lambda/(2\ N.A.)$ - a similar formalism can be followed for the axial resolution (along the optical axis, z-resolution, depth resolution) (Jiang et al., 2018, Ni et al., 2017, Zou et al., 2018)

The new ZEISS LSM 880 with Airyscan technology

In this study the Airyscan technology was used to image the smallest structures, it can capture the faintest signal. For some researchers they have used Airyscan to assists them by getting accurate data from live cells or other weakly-labelled samples (Amos and White, 2003, Chetverikov et al., 2015, He et al., 2016). Each photon of emission light is precious, with Airyscan it collects all precious emission light without compromise and get a unique combination of gentle super resolution imaging and high speed (Chetverikov et al., 2015, He et al., 2016, Hoffman et al., 2006). Researchers have found that by using Airyscan technology they can use multicolour samples with any label and get image quality (Amos and White, 2003, Chetverikov et al., 2015). This new technology allows scientists and researchers to get perfect optical sections and resolve structures of 120 nm (in x, y) and 350 nm (in z) even in thick samples. Plus, Airyscan gives an instant 4 – 8× improvement of signal-to-noise (SNR) (Chetverikov et al., 2015, He et al., 2016). The sample that have been used on this type of microscope are; WB smears to look at RBCs , platelet rich plasma to study platelets (Olumuyiwa-Akeredolu et al., 2017, Pretorius et al., 2014b) and their ultrastructure, platelet poor plasma to look at the fibrin fibres clot formation and ultrastructure (Strydom et al., 2016, Pretorius et al., 2016a, Pretorius et al., 2017a). For the purpose of this study the fibrin fibres were investigated using confocal microscope to identify amyloid formation. Amyloid fibrils are formed by soluble proteins, which assemble to form insoluble fibres that are resistant to degradation (Kell and Pretorius, 2017, Pretorius et al., 2016d). Their formation can accompany disease and each disease is characterized by a specific protein or peptide that aggregates (Itzhaki et al., 2016a, Biancalana and Koide, 2010). In this study LPS was used as an inflammogen to induce inflammatory symptoms such as amyloid fibrils (as previously reported Pretorius 2016) in 'control' participants' blood *ex vivo*. A marker that can be used to mark these amyloid fibrils is ThT which was added at a final concentration of 5 u M.. Amyloid formation is seen by increase i n the fluorescent marker ThT on fibrin(ogen) (Biancalana and Koide, 2010, Biancalana et al., 2009). This marker is known to be the most suitable in pathologies that involve hypercoagulation when treating blood with LPS (Pretorius et al., 2016d).

In this chapter the methods and standard operating procedures will discussed when using the confocal ZEISS LSM 880 airy scanning technology. The PPP was mixed with a variety of reagents and treatments to investigate the clotting pattern before and after treatments.

The statistical outcomes are scientific and relevant which reveal the significance of the investigation, finally the chapter conclusion will be drawn based on the outcomes of the investigation. See **Figure 5.1.** for a schematic diagram of the chapter.

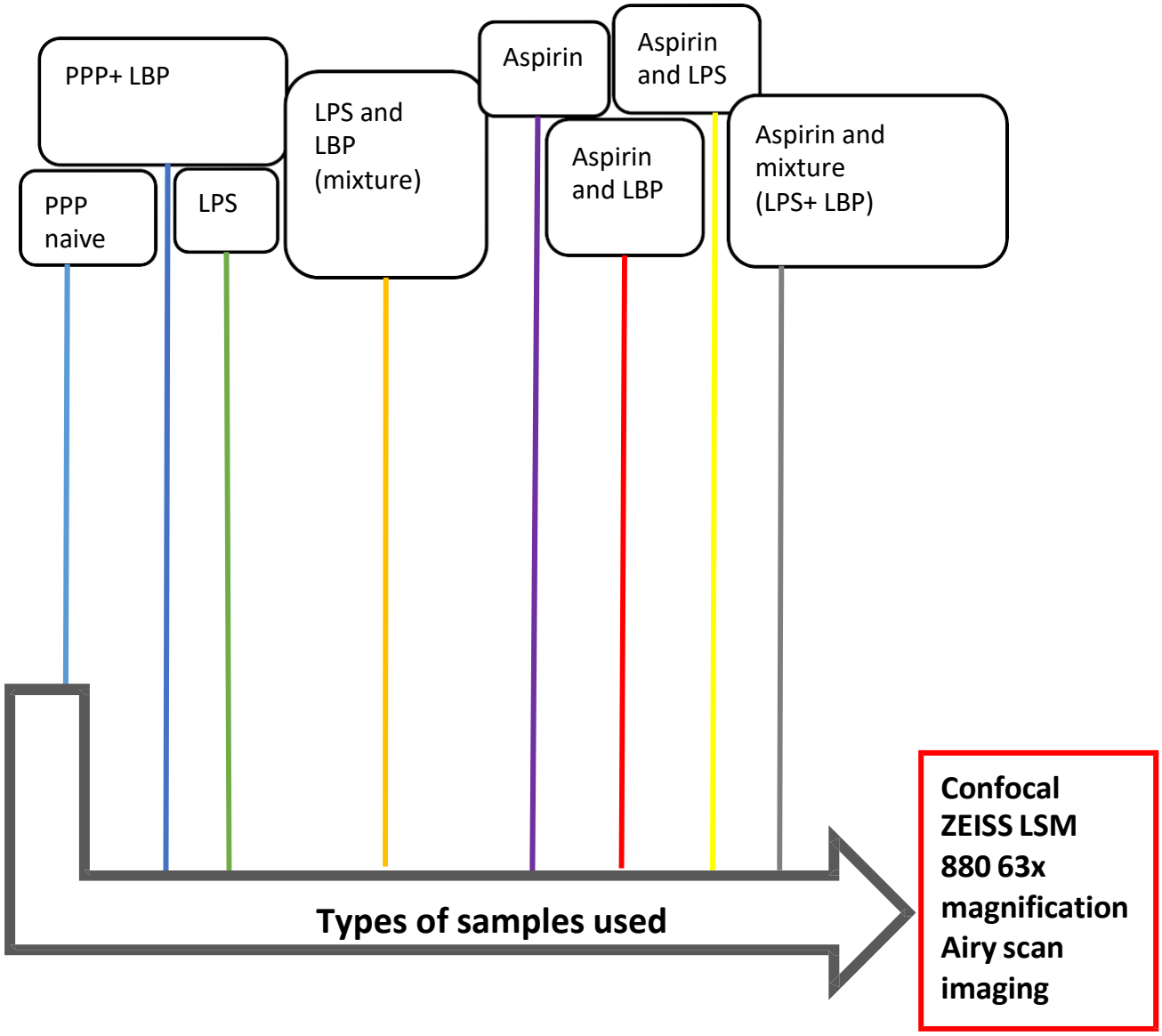


Figure 5. 1. Schematic diagram of this chapter

Chapter Aim

The aim of this chapter is to investigate the effects of LPS, LBP, aspirin individually and mixed on the integrity and morphology of the formation of fibrin fibres and to quantify anomalous clotted fibrin(ogen) or amyloid formation of the fibrin(ogen) plasma clotting protein, by marking the fluorescence intensity produced by the fluorescent marker ThT that marks amyloid-like protein structure. During anomalous clotting, fibrin(ogen) fibres unfold into a changed protein conformation. See **Figure 2.7.** for a transition in fibrin formation under deformation from healthy coagulation to pathological coagulation in the literature review for clarity.

Chapter objectives

- To determine the fibrin(ogen) formation and state or healthiness of each participant by looking at fibrin fibres formed when adding naïve PPP with 5μM of ThT.
- To determine the integrity of fibres, after all treatment using ThT marker that fluoresces when there is a presence of β-sheet exposed during amyloid formation. Using Image J v2.0 FIJI software (National Institutes of Health, USA).
- To obtain quantitively results of micrographs fluorescence intensity from naïve (untreated) plasma fibrin fibres along with those treated with LPS, LBP, LPS and LBP, aspirin, aspirin and LBP, aspirin and LPS, aspirin and LBP with LPS. Using Image J v2.0 FIJI software (National Institutes of Health, USA)

Materials and Method

Confocal microscopy was used to investigate the ultrastructure of fibrin fibres predisposed to LPS, LBP and aspirin in combinations or individually. The following materials and methods were used.

Reagents for the experiment

- **Lipopolysaccharide binding protein**

  Natural LBP is a 58KD glycoprotein produced in liver.. Purchased from Sigma-Aldrich® South Africa.

- **Lipopolysaccharides**

  Lipopolysaccharide from a bacterium called - *Escherichia coli* 0111: B4 Lyophilized Powder. Purchased from Sigma-Aldrich® South Africa.

- **Acetyl salicylate**

  Aspirin reagent Plus®, _>99.5% S3007-500G. Formula: C7H5NaO3 and formula Weight: 160.10 g.mol^{-1} and purchased from Sigma-Aldrich® South Africa.

- **Thioflavin T**

  Thioflavin T is a benzothiazole dye that increases in fluorescence upon binding to amyloid. Thioflavin T has been used in histology and for protein characterization. (Biancalana and Koide, 2010, Biancalana et al., 2009, Pretorius et al., 2016a). Purchased from Sigma-Aldrich® South Africa.

- **Human Thrombin**

  Purchased from South African Blood Service. Appearance (Color) White to Off-White Appearance (Form) Powder.

Method

A volume of 196µl of PPP was aliquoted into an Eppendorf. To this tube a volume of 4µl of LBP (final exposure concentration of 2ng.L^{-1}) was added making a total volume of 200µl. To test the effect of LPS on direct fibrin fibres formation. In an Eppendorf a volume 196µL of PPP was mixed with 4µL LPS (a final exposure concentration of 0.2 ng.L^{-1} LPS), this served as the positive control. To study the effect of LBP on LPS, and to see how LBP in direct exposure to LPS will affect fibrin fibre formation and ultrastructure. A volume of 192µL PPP was added with 8µL of mixed (LPS and LBP) both reagents at a final exposure concentration 2ng.L^{-1}. All reagents were added to make up a volume of 200µL. To study the effect of aspirin on fibre formation 10µl of aspirin final exposure concentration of 0.5mM was added to 190µl of PPP. To study the effect of LBP and aspirin on coagulation and fibre formation, 10µl of aspirin (final exposure concentration) of 0.5mM was added to 190µl of PPP, followed by adding 2ng.L^{-1} final exposure concentration of LBP was added. To study the effect of aspirin on LPS, 10µl aspirin (final exposure concentration of 0.5Mm) was added to 190µL PPP. Then only 0, 2ng.L^{-1} (final exposure concentration) of LPS was added.

To study the effect of combined therapy aspirin mixed with LPS and LBP, 10µl aspirin (final exposure concentration of 0.5Mm) was added to 190µL PPP. Followed by adding 0, 2ng.L^{-1} (final exposure concentration) of LPS was added and LBP was added to a final exposure concentration of 2ng.L^{-1}. The described procedures/ techniques were used to create different study treatments. Thereby each sample from everyone with and without treatments can be compared with each other. All Eppendorf's were incubated for 10 minutes before adding 0.2µL of ThT (Abcam, ab120751) final concentration of 5µM ThT. For all participants this was the marker used. The ThT was the marker used for the first time to mark amyloid protein formation during a previous study which was published in nature (Pretorius et al., 2016d). Clots were made from the naïve or treated samples by adding thrombin. Thrombin was solubilized in PBS containing 0.2% human serum albumin to obtain a concentration of 20 U.mL^{-1} and was used at a 1:2 ratio to create extensive fibrin networks.

Confocal microscopy sample preparations

A pipetted volume of 10 µl sample PPP was placed onto a microscope slide/ glass slide frosted one end size 25mm X 75mm. A volume of 5 µl of thrombin (final exposure concentration of 5mM) was pipetted onto the PPP drop first .The tip of a bent pipette was used to carefully swirl the droplet and spread only touching the droplet, not the glass. The sample was dried at room temperature for up to 1 minute. The Airyscan detector increases the resolution by a factor of 1.7, achieving super-resolution of 140 nm. The statistical tools that was used is One-Way ANOVA with Multiple Comparison's Test for the confocal analysis (controls vs. LBP vs. LPS vs LBP mixed with LPS vs aspirin vs aspirin with LBP vs aspirin with LPS vs aspirin with LPS mixed with LBP), comparing the mean of each column with the mean of every other column (GraphPad 8). The non-parametric test was done for the Airyscan analysis (control naïve PPP). For each picture, the histogram of intensities (8-bit scale) was obtained using the histogram function of ImageJ. From this, the coefficient of variation (CV, as standard deviation/mean) calculated.

All relevant data (Excel data files and the raw data GraphPad stats file) to replicate the study's findings are available in the Indexes. Histogram analysis of micrographs was carried out using Image J v2.0 FIJI software (National Institutes of Health, USA). An average of three images were used per participants. Per group of treated samples, 3 images were used to calculate CV and all micrographs were taken under the same gain of 680m the offset between images remained the same intensity (min max). As these factors are known to influence the fluorescence observed with confocal microscopy.

Results

Platelets poor plasma was mixed with human thrombin to create a clot that was viewed with the confocal microscope. The fluorescence marker ThT amyloid was used **Figure 5.2** and **Figure 5.3** shows the morphology of a healthy clots, these PPP samples were not treated. These micrographs were used as the refence point. All images from the confocal microscope in this thesis were enhanced by adding more brightness for better visuals. However, the gain settings were kept the same for all Image J analysis and statistical analysis. The added brightness did not change the original data from the image thus did not have an influence in the processing of the images in image J

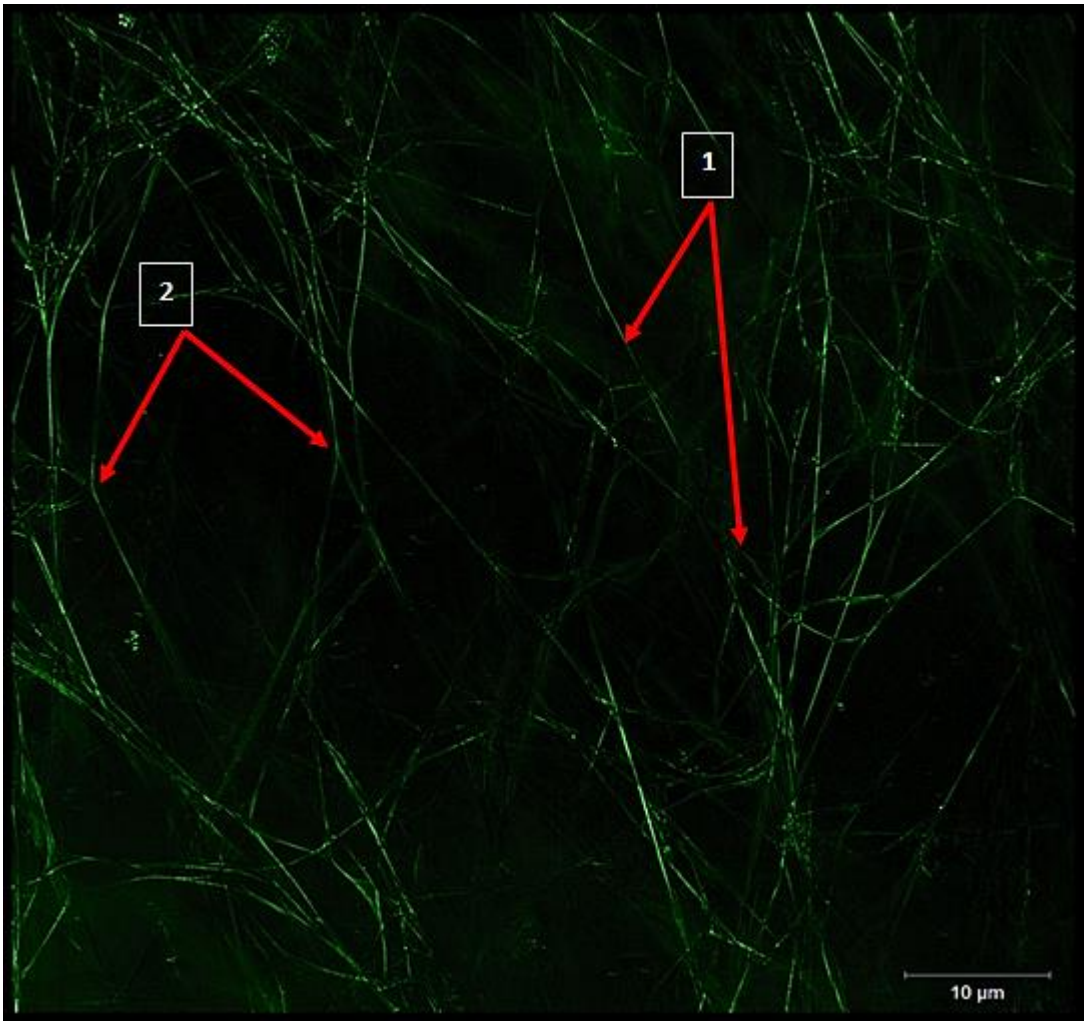


Figure 5. 2. Airyscan micrographs of PPP with added thrombin to form extensive fibrin fibres. Thioflavin T (ThT) final exposure concentratin of 5 µM to mark possible amyloid fibres formation. Micrographs were taken with a Zeiss LSM 510 META confocal microscope with a Plan-Apochromat 63x/1.4 Oil DIC objective. Micrograph of normal healthy fibres showing individual fibre network. Typical phenotype of normal healthy clot ultrastructure. Red arrows with (1) long thin strands of fibres network. (2) branch point of fibres. Scale bar 10µm.

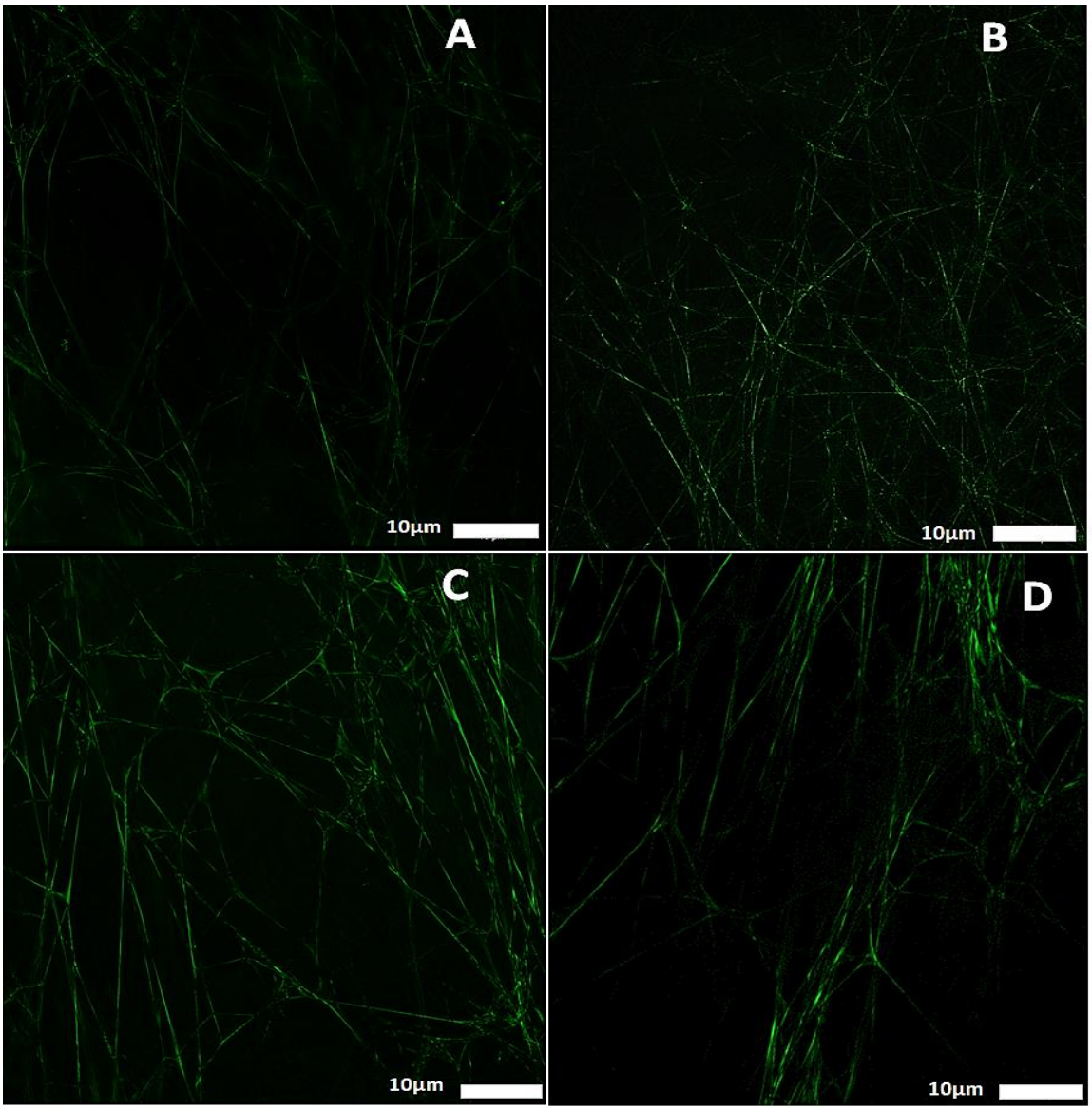


Figure 5. 3. Micrographs of healthy fibrin network (A-D). Clot was formed by adding 5µl of human thrombin to 10µl PPP visible individual fibres seen indicate a health state of the clot. Scale bar 10µm.

Lipopolysaccharide binding protein

Lipopolysaccharide binding protein was added to PPP to show that it had no negative effect on the fibres as it is found at low physiological levels in humans (Pretorius et al., 2017a, Schroder et al., 2000). In **Figure 5.4.** below, LBP incubated with PPP are shown. These resemble fibres of naïve PPP.

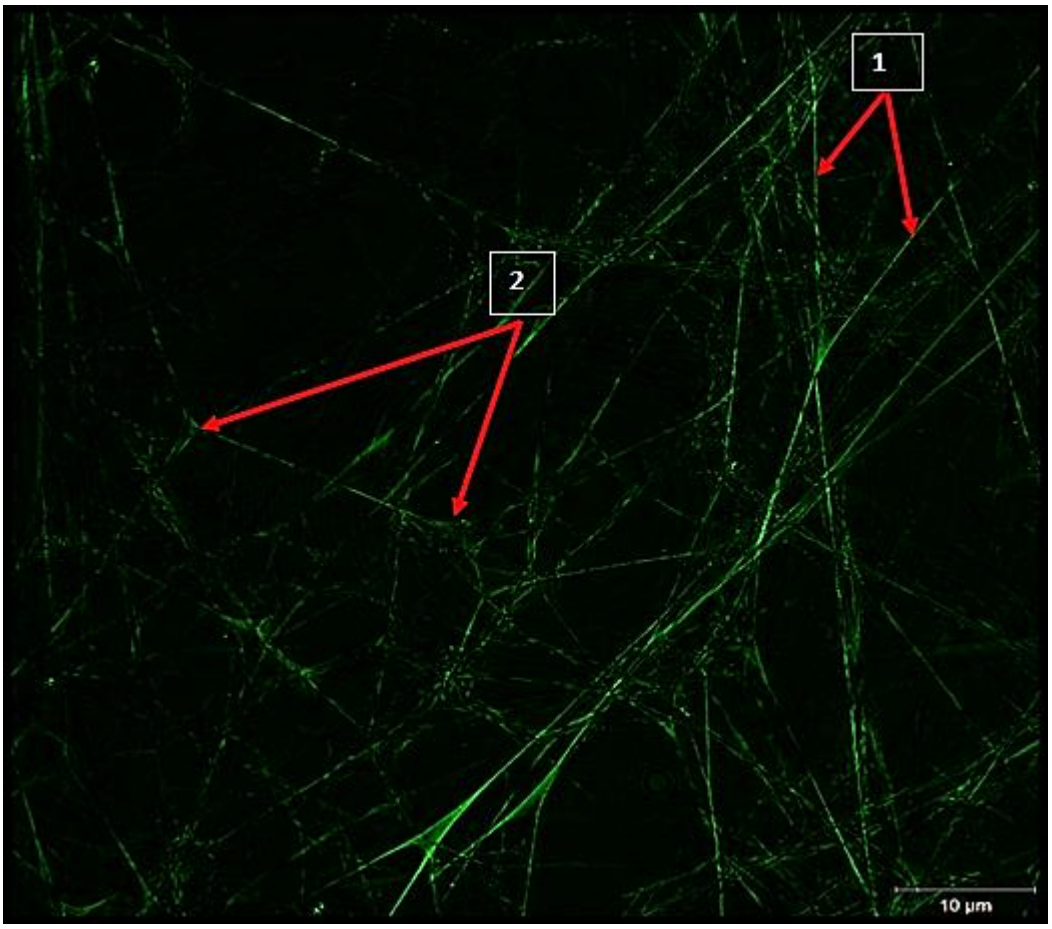


Figure 5. 4. Micrograph showing fibres network from PPP incubated with LBP (final exposure concentration of 2ng.L^{-1}). Typical phenotype of normal healthy clot ultrastructure. Red arrows with (1) showing long thin strands of fibres network. (2) the individual branch point of fibres. Scale 10µm

Figure below contains a group of fibres, clots that were made by incubated LBP together with PPP, followed by adding ThT and then thrombin. **Figure 5.5** shows how LBP affects the formation of fibres and the clot morphology.

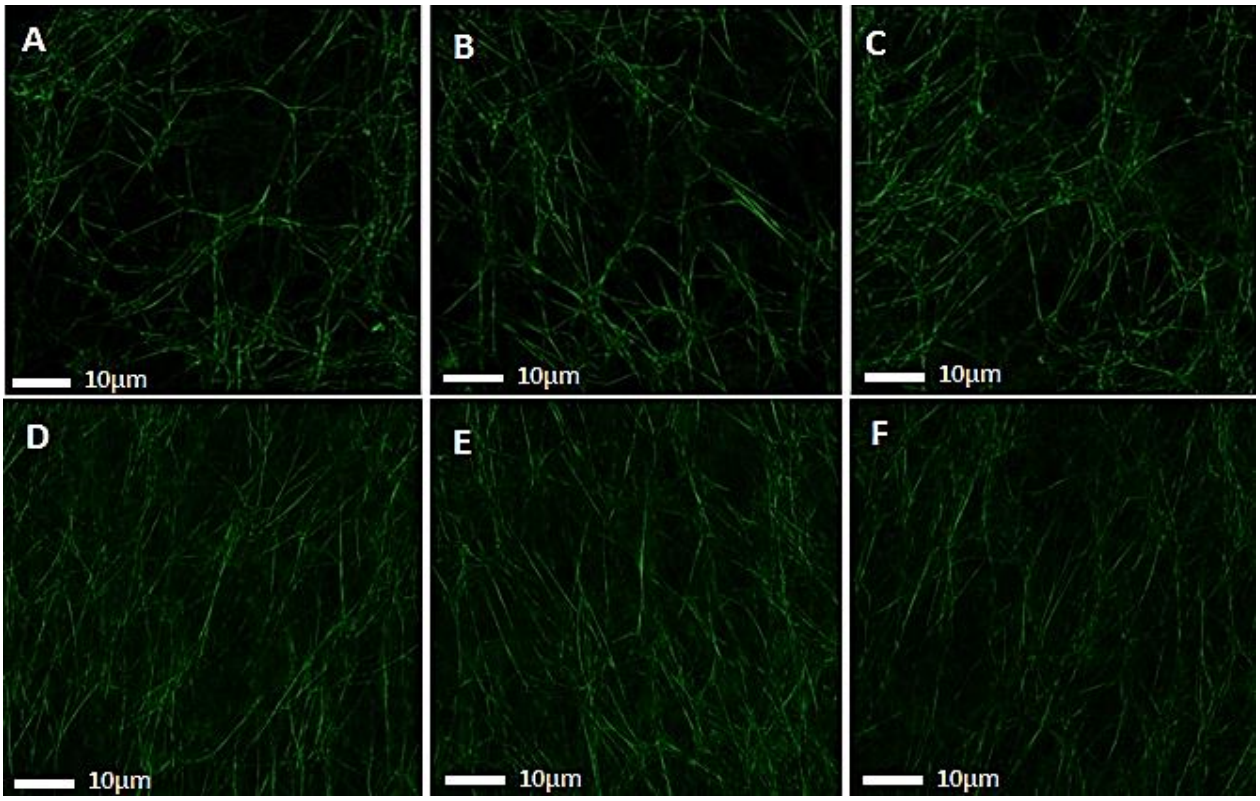


Figure 5. 5. Micrographs of PPP incubated with LBP showing healthy individual fibres. (A-F) Different fibres formed from different healthy individuals after incubating PPP with LBP followed by ThT marker for 5 minutes. Scale bar 10µm

Effect of Lipopolysaccharide on fibrin fibre formation

Lipopolysaccharide's effect on fibrin fibres formation was tested. A final exposure concentration 5mM of amyloid marker ThT was added to naïve PPP and LPS final exposure concentration of 0. 2ng.L^{-1}. When viewing with the confocal microscope an increased binding of ThT to β-sheet-rich areas on the fibrin(ogen) was evident, **Figure 5.6.** shows the β-sheet were LPS has attached to fibrin(ogen). Short fibres are visible.

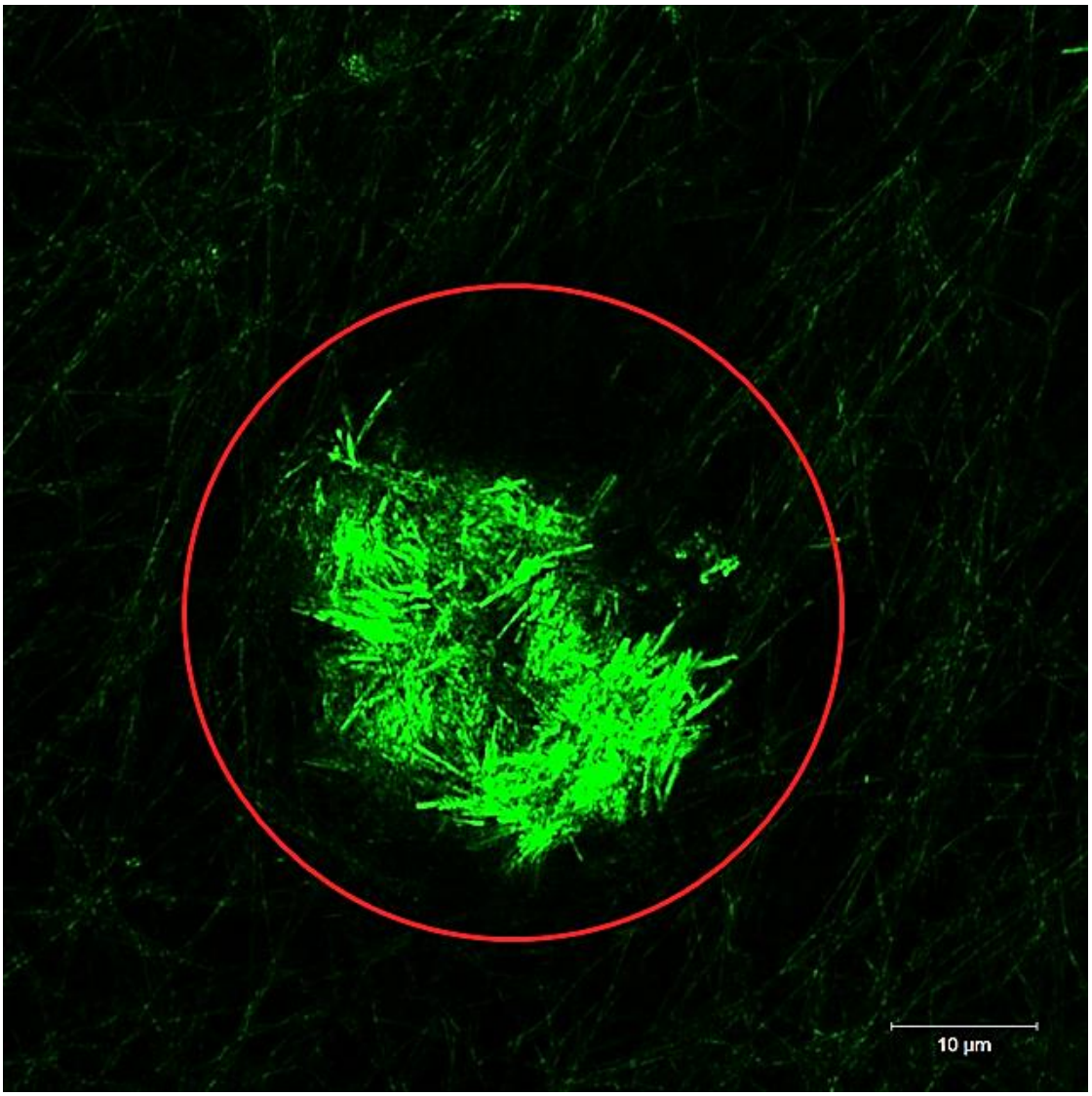


Figure 5. 6. Micrograph of fibrin fibres were PPP with added final exposure concentration of 0.2ng.L^{-1} LPS. Highly dense fibres and increased fluorescence, visible abnormal short fibres highlighted with red circle. Scale is 10µm

Below in **Figure 5.7.** micrographs of different participants' PPP after incubation with LPS shows how LPS affects fibres differently. Mostly shortened fibres cling together as seen in micrographs.

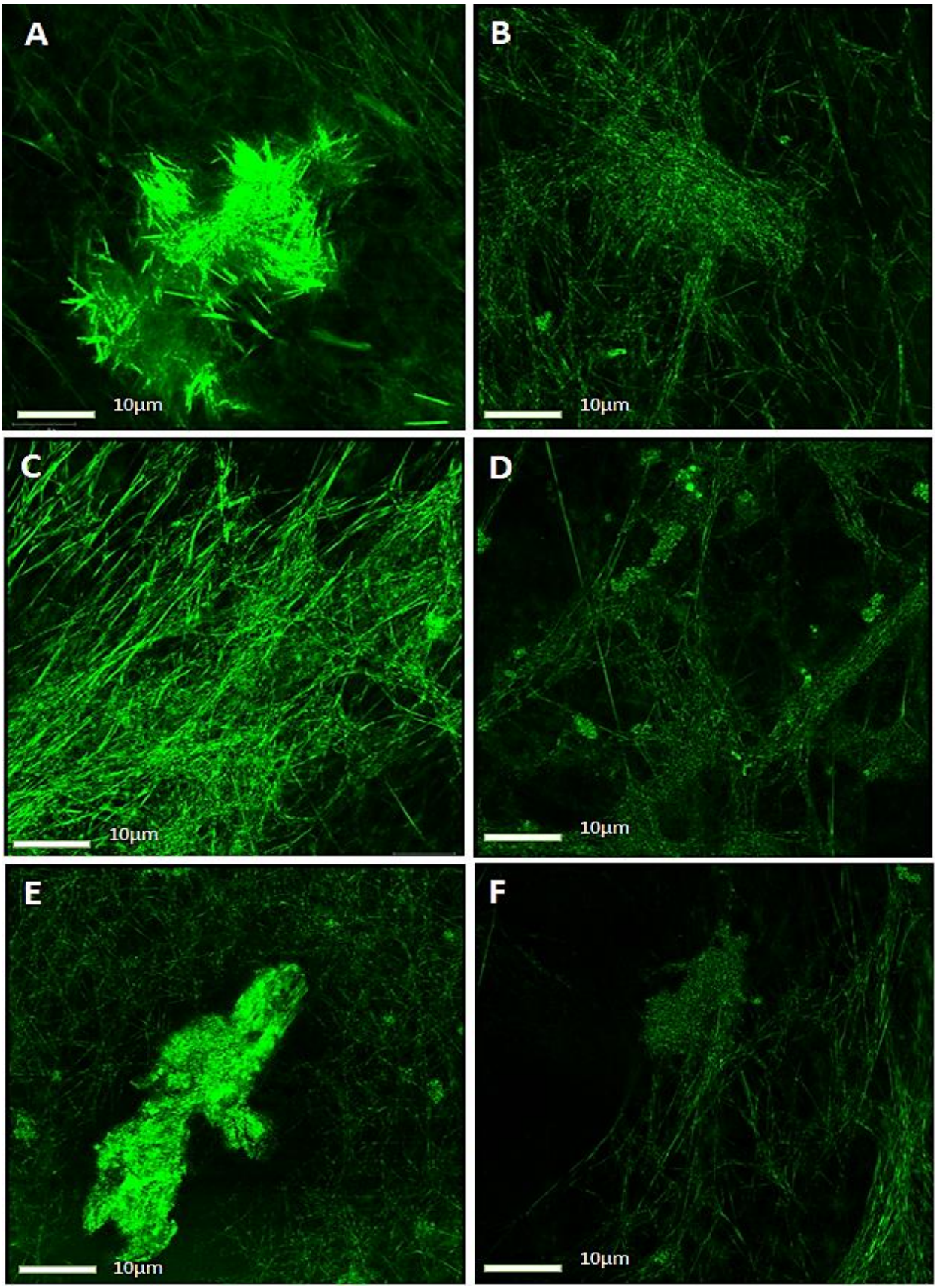


Figure 5. 7. Micrograph showing fibrin fibres after incubating PPP with LPS 0.2ng.L^{-1} final exposure concentration. (A) lumpy, short, broken fibres-amyloid structures formed. (B, C, D, E, F) different structures of dense matted mass deposit of fibres. Scale bar 10µm.

Lipopolysaccharide binding protein and LPS (mixture)

As the investigation continues a mixture was formulated that contained both LPS and LBP. The results showed identical fibre network to control (untreated plasma). In **Figure 5.8**. Shows typical phenotype of normal healthy clot ultrastructure.

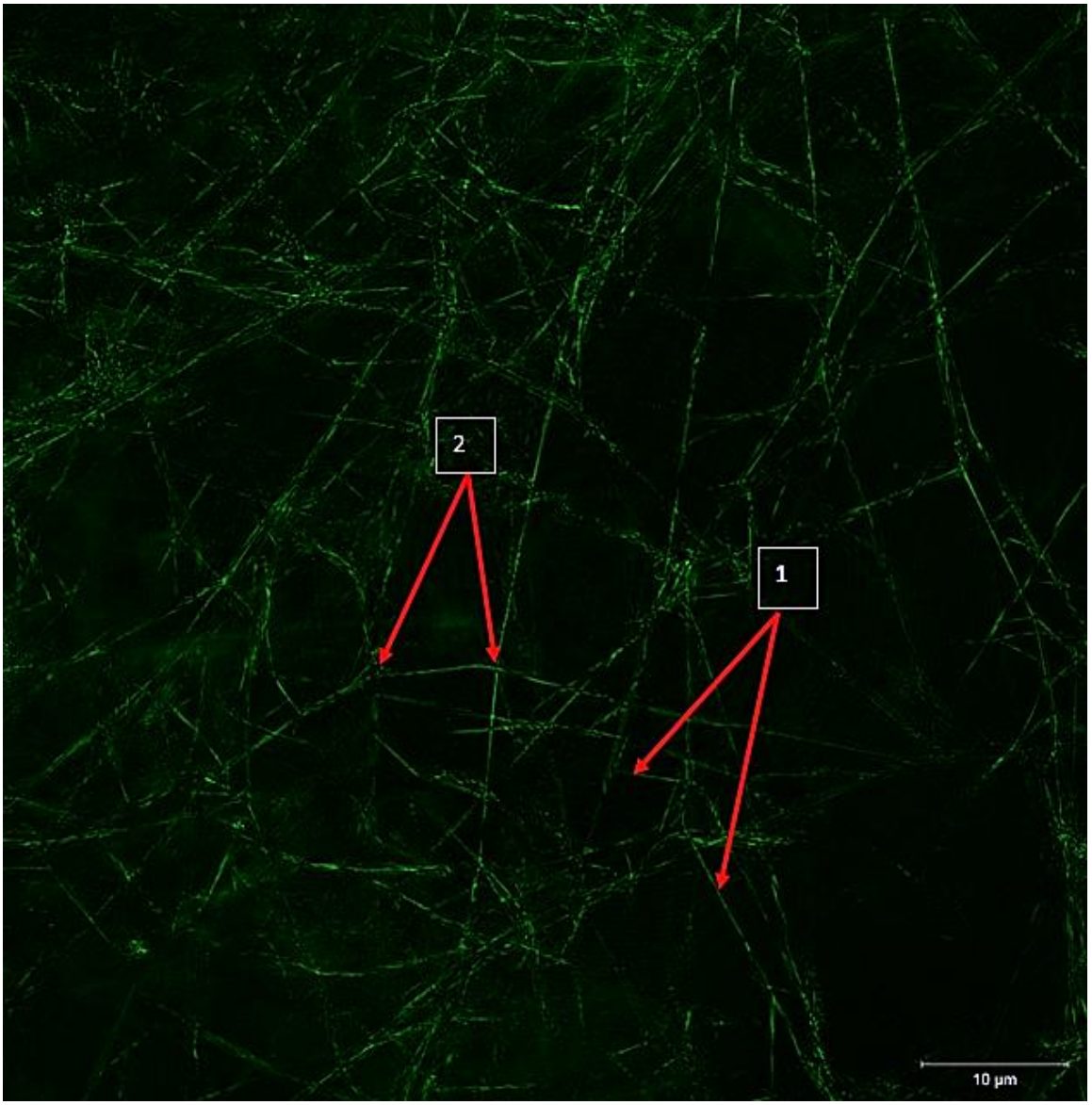


Figure 5. 8. Micrograph showing fibres network from PPP incubated with LBP and LPS. Red arrows with (1) showing long thin strands of fibres network. (2) the individual branch point of fibres. Scale 10µm

Figure 5.9 shows the direct comparison between the LBP mixed with LPS and naïve (untreated) PPP from the same participant. In both micrographs individual fibrin fibres and clear branching point are visible.

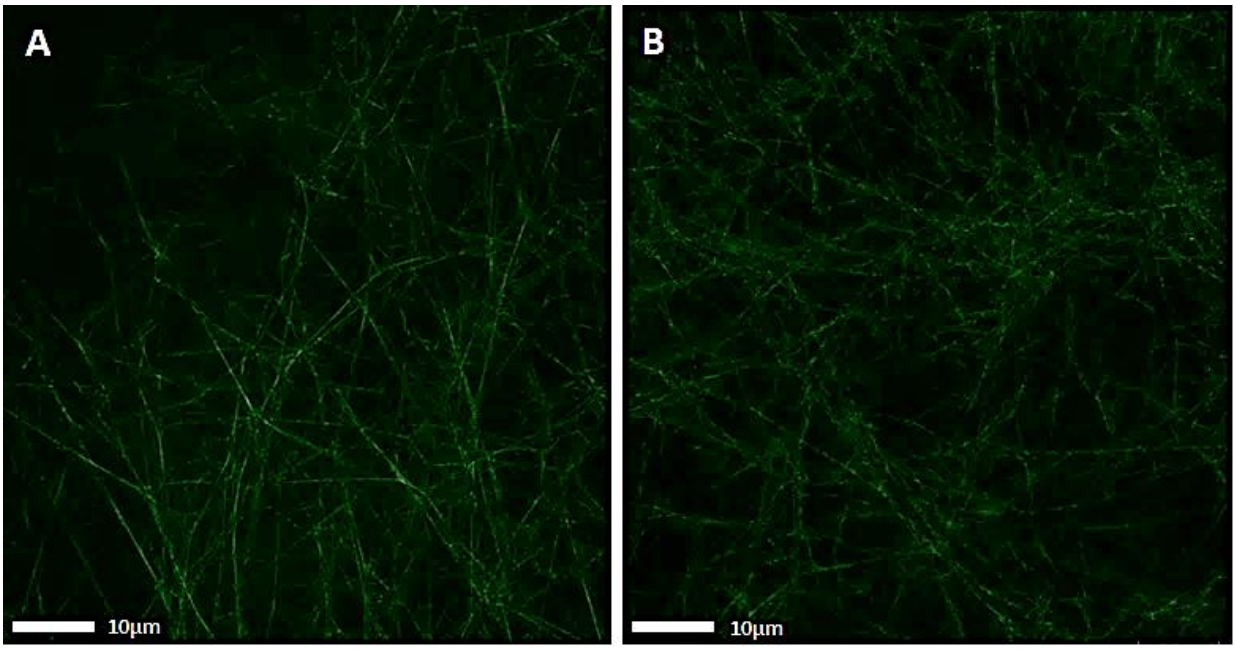


Figure 5. 9. Micrograph of fibrin fibres, clots were achieved by adding human thrombin of concentration 5uM to the **(A)** naïve PPP, shows healthy normal fibres network with very little fluorescence **(B)** PPP mixed with LPS and LBP. Fibres with very little fluorescence. This shows normal clot which is normally seen in healthy individuals. Micrograph. Scale bar 10µm

The investigation continued to study the differences between the LBP when added to PPP and when added with LPS together (mix). See **Figure 5.10.** below micrographs of fibrin fibres made from PPP treated with LBP comparing with fibres from PPP incubated with LPS mixed with LBP. Further will follow is **Figure 5.11**. micrographs of PPP clots from PPP treated with LPS mixed with LBP verses fibres from PPP treated with LPS.

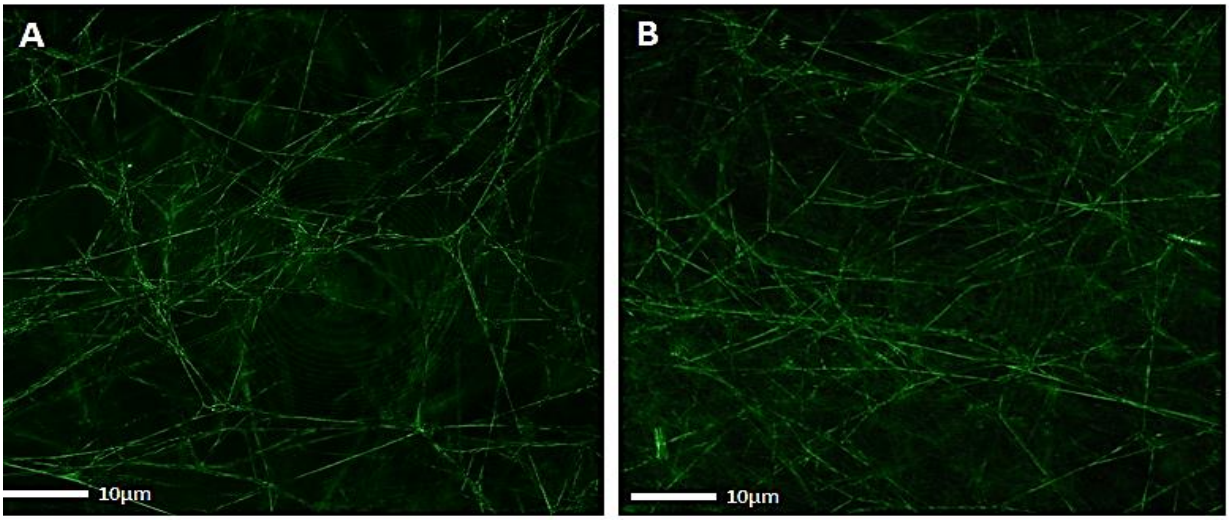


Figure 5. 10. Fibrin fibre network (A) LBP mixed with LPS. less to no harmful effect on the nature and structure of fibres. (B) PPP incubated with LBP, resembles naïve PPP ultrastructure. The two groups (A) and (B) looked almost identical phenotypically. Scale bar 10 µm.

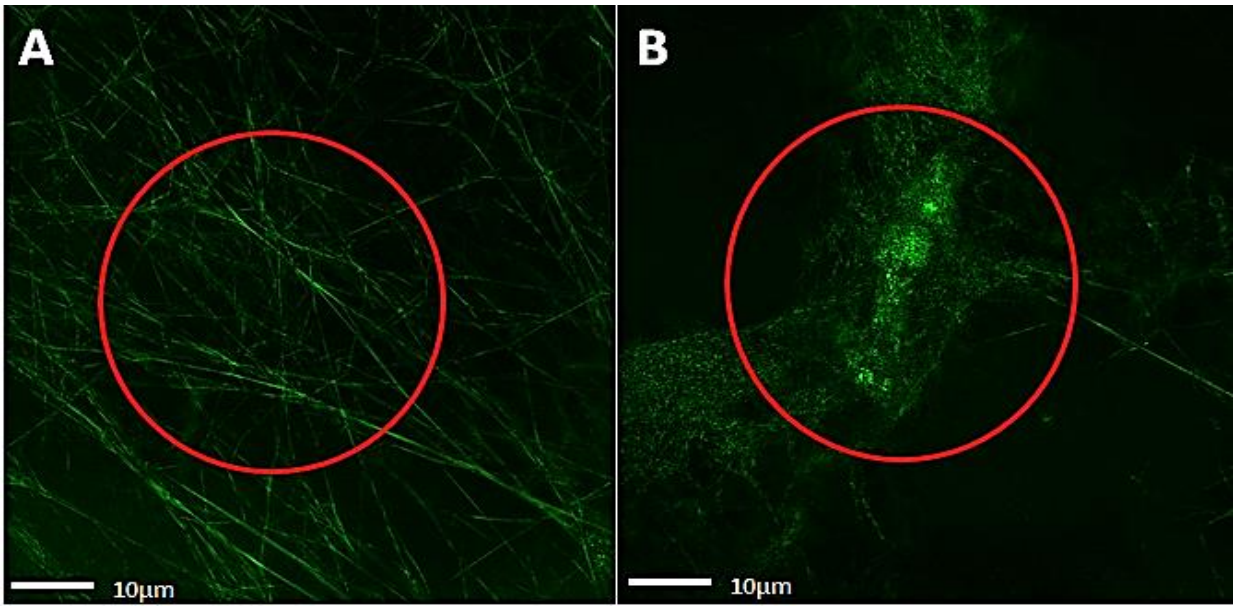


Figure 5. 11. Fibrin fibre network (A) PPP treated with LPS and LBP micrograph showing healthy fibrin network highlighted with red circle. (B) Fibrin fibres formed after incubating PPP with 0. 2ng.L $^{-1}$ exposure concentration of LPS, showing matted mass deposit indicated with a red highlighted circle and strong fluorescence with ThT marker. Scale bar 10µm

The effects of acetyl acetate (aspirin) on the formation of fibrin fibres and clot morphology

Aspirin at a final exposure concentration of 0.5mM was incubated with PPP. The results are shown in **Figure 5.12.** healthy individual fibrin network is visible, a characteristic seen in healthy individuals. Aspirin had no negative effect on the fibrin(ogen) formation.

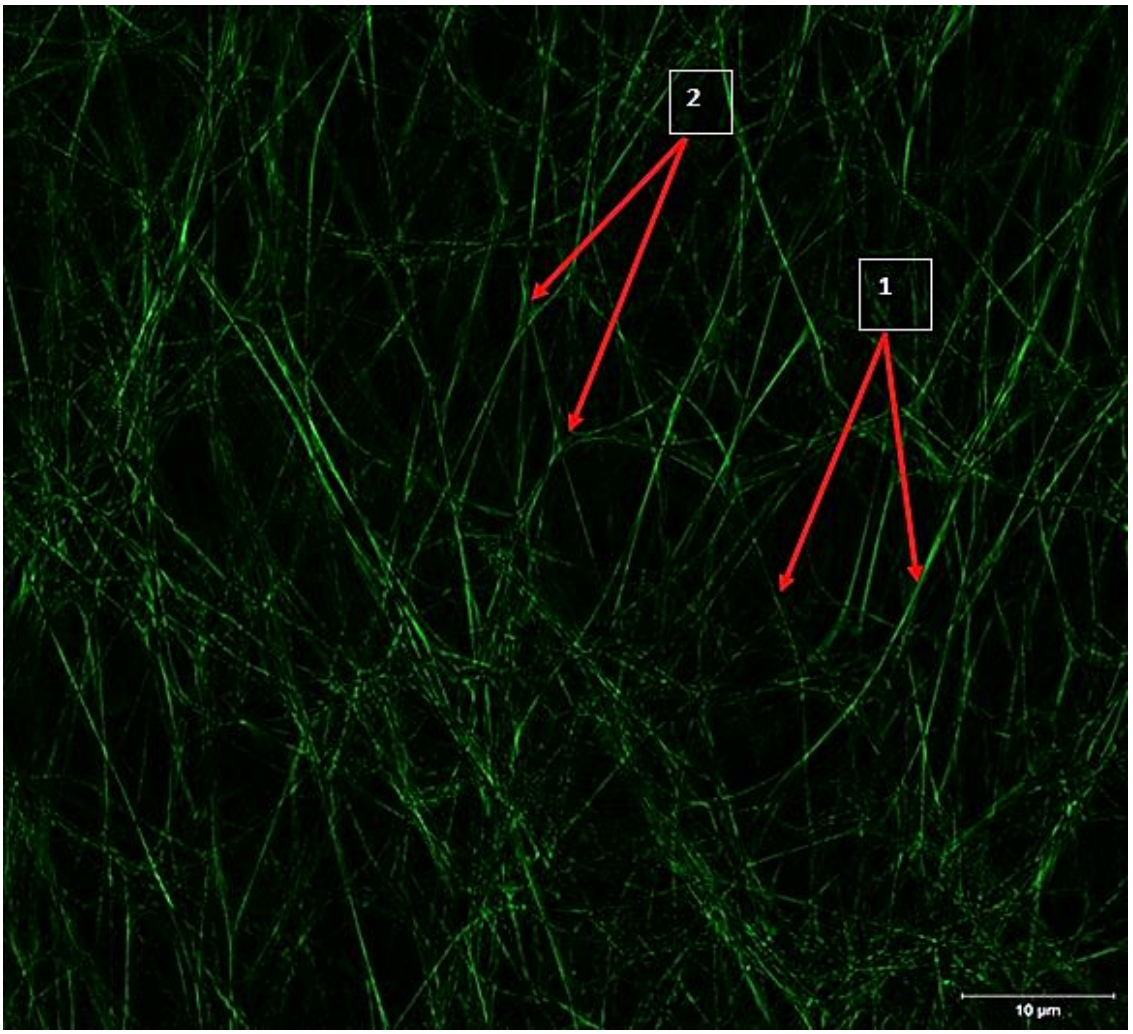


Figure 5. 12. Micrographs of fibres PPP was added with aspirin 0,5mM final exposure concentration, incubated for 10 minutes, followed by addition of 5mM ThT. Only 5 µl of thrombin was added to activate and imitate the clotting process. Scale bar 10µm

More micrographs of aspirin mixed with PPP are seen in **Figure 5.13. (A-D)** showing clear visible fibrin fibres and less fluorescence from the ThT marker.

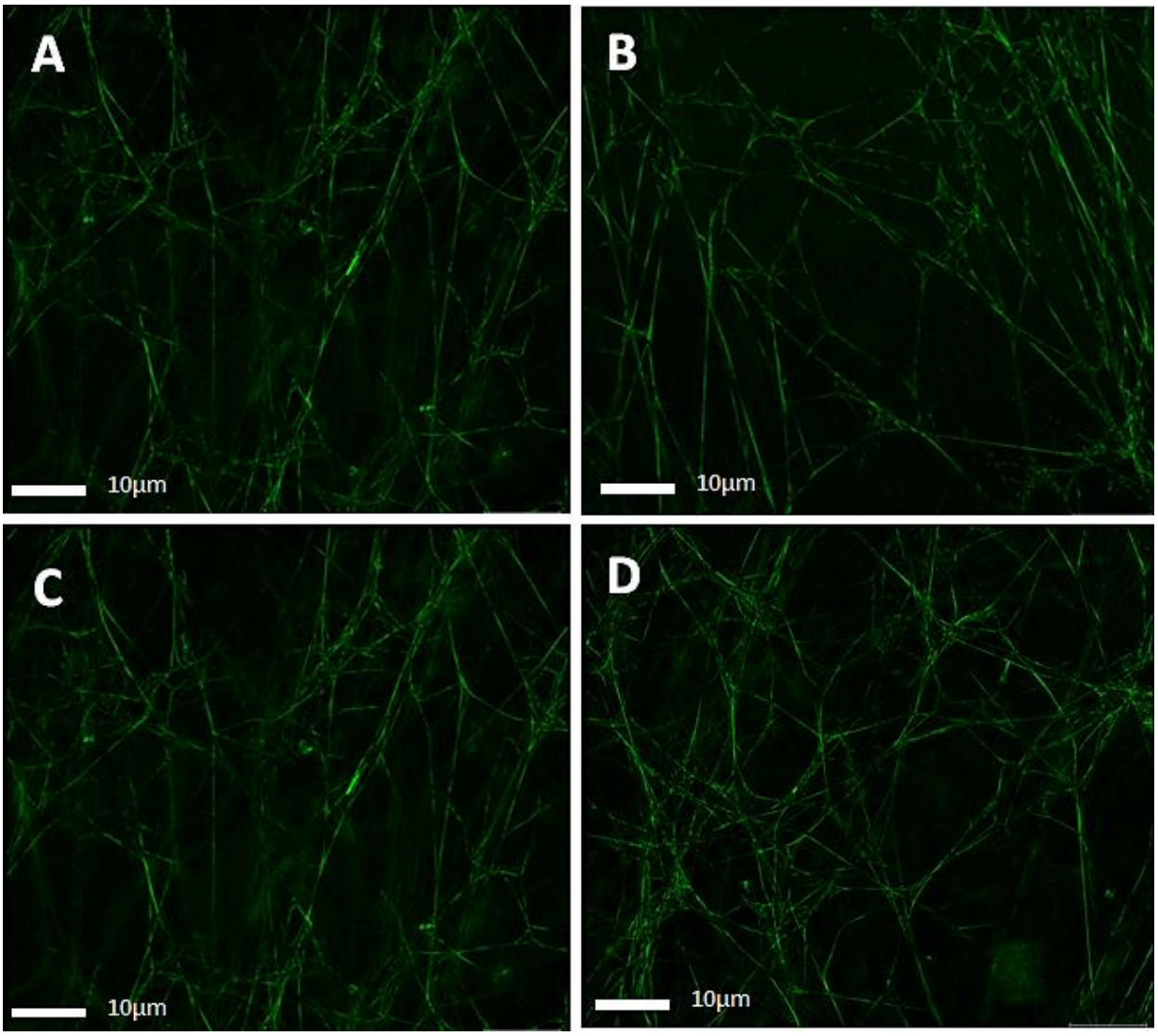


Figure 5. 13. Micrographs (A-D) aspirin incubated with PPP final exposure concentration of ASA (0,5mM). To make the clot human thrombin 5µM was added. Only ThT marker of concentration 5Mm was added to mark amyloid formation. Scale bar 10µm

The effect of aspirin and Lipopolysaccharide on fibrin fibres formation

Platelet poor plasma was treated with aspirin. To this mixture LBP was added at a final exposure concentration of $2ng.L^{-1}$. After 10 minutes of incubation samples were prepared for confocal. In a dark room ThT was added to these PPP treated Eppindorf. This was to see the role of aspirin together with LBP on fibrin(ogen) formation and morphology, the results are seen in **Figure 5.14.**

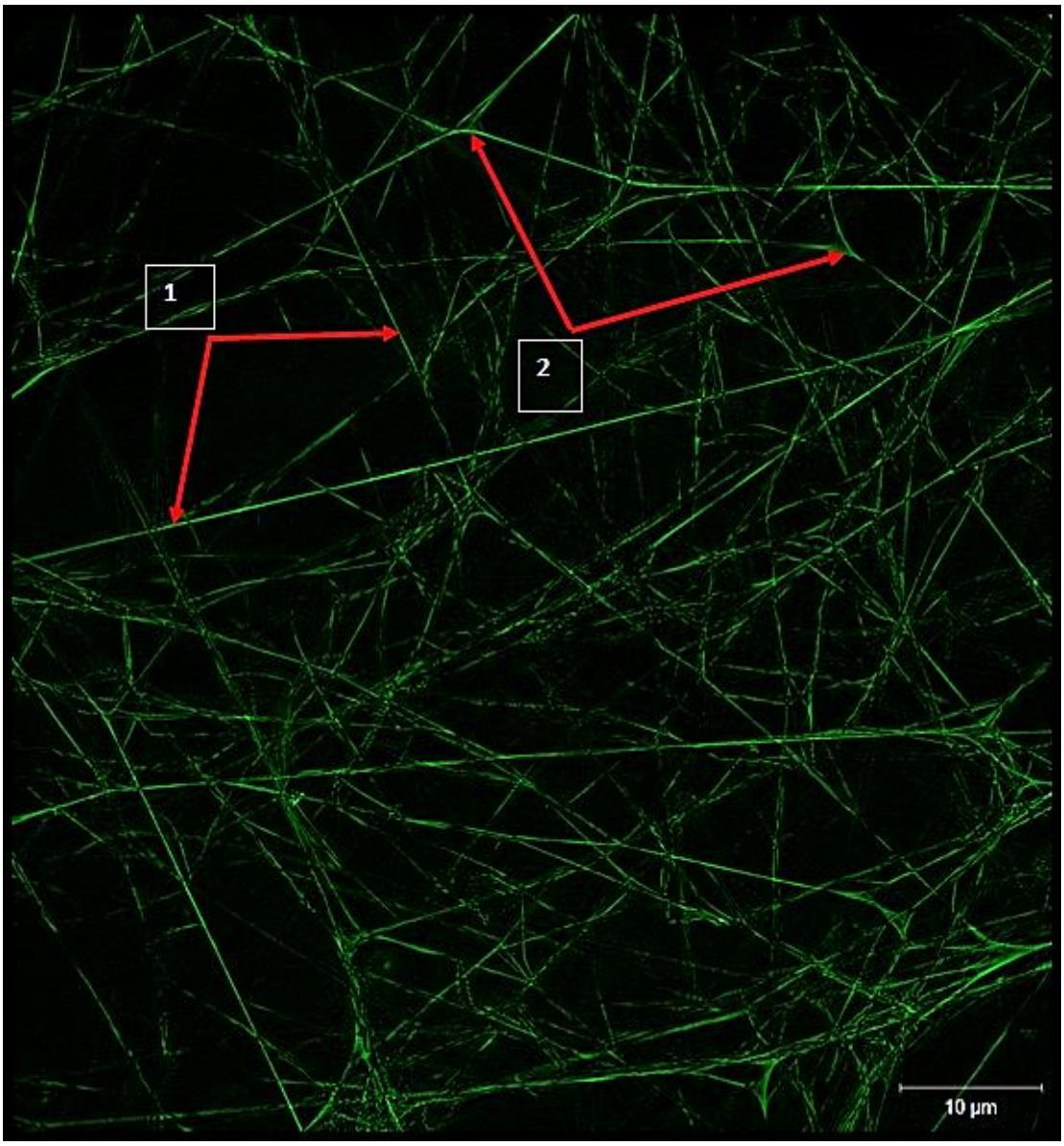


Figure 5. 14. Micrographs of PPP added with aspirin 0,5mM final exposure concentration and LBP 2ng.L^{-1}, incubated for 10 minutes, followed by addition of final exposure concentration 5mM of ThT. Only 5 µl of 5mM thrombin was added to activate and imitate the clotting process. Scale bar 10µm

More detailed micrographs of fibres from PPP incubated with aspirin mixed with LBP are shown in **Figure 5.15.**

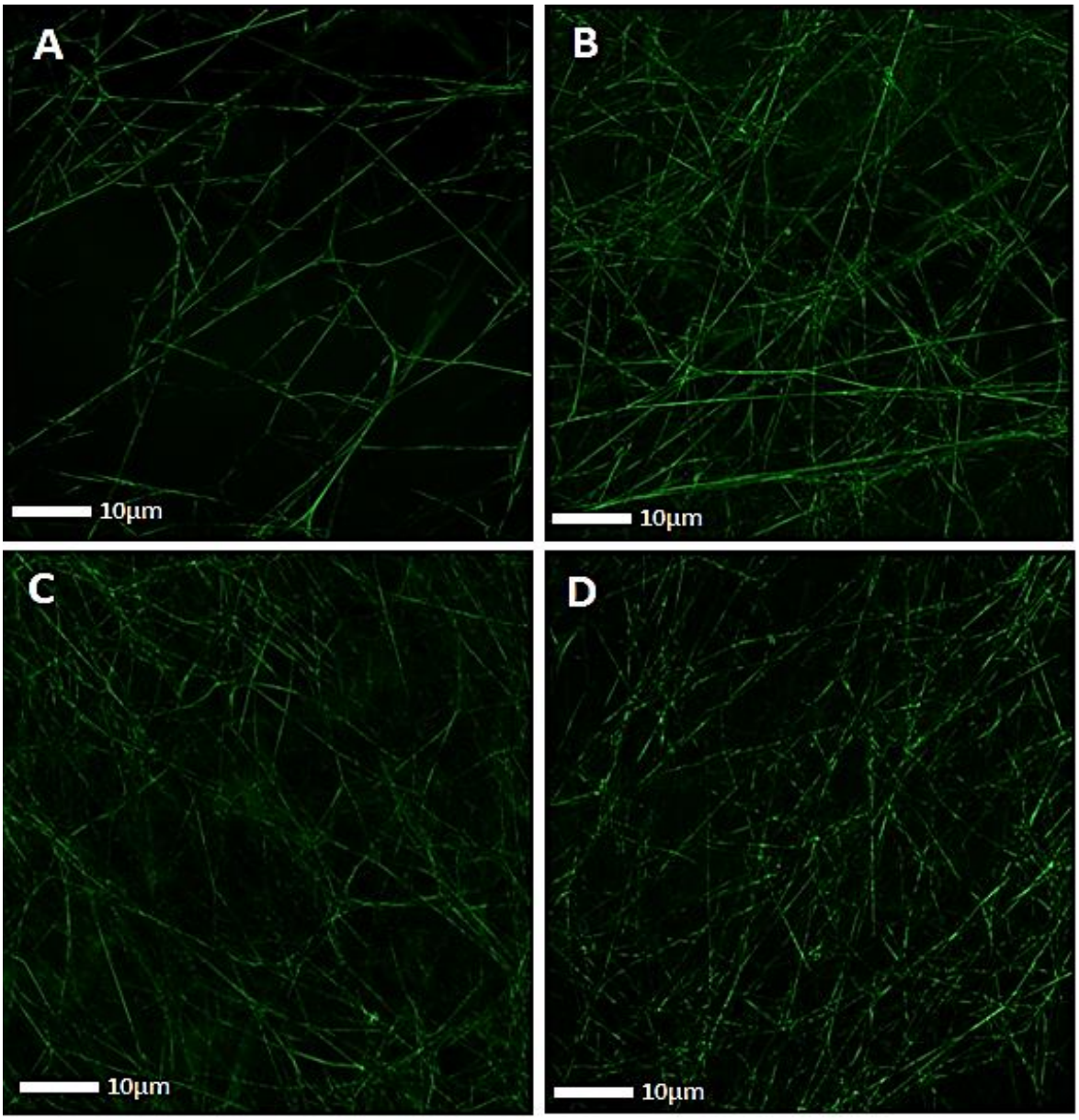


Figure 5. 15.(A-D) Micrographs of fibrin fibres, PPP with aspirin 0,5mM final exposure concertation, incubated for 10 minutes and then incubated with LBP to a final exposure concentration of 2ng.L^{-1}. Only 5 µl of 5mM thrombin was added to activate and imitate the clotting process. Scale bar 10µm

The effect of aspirin on hypercoagulability induced by Lipopolysaccharide in platelet poor plasma

Aspirin and PPP were mixed with an inflammatory agent activator LPS. Lipopolysaccharide was added to make a final exposure concentration of 0, 2ng.L^{-1}. After 10 minutes of incubation samples were prepared for confocal. In a dark room ThT was added to each PPP treated. This was to see the role of aspirin played in fibrin(ogen) formation and morphology during an hypercoagulation induced by LPS, the effects are seen **Figure 5.16.**

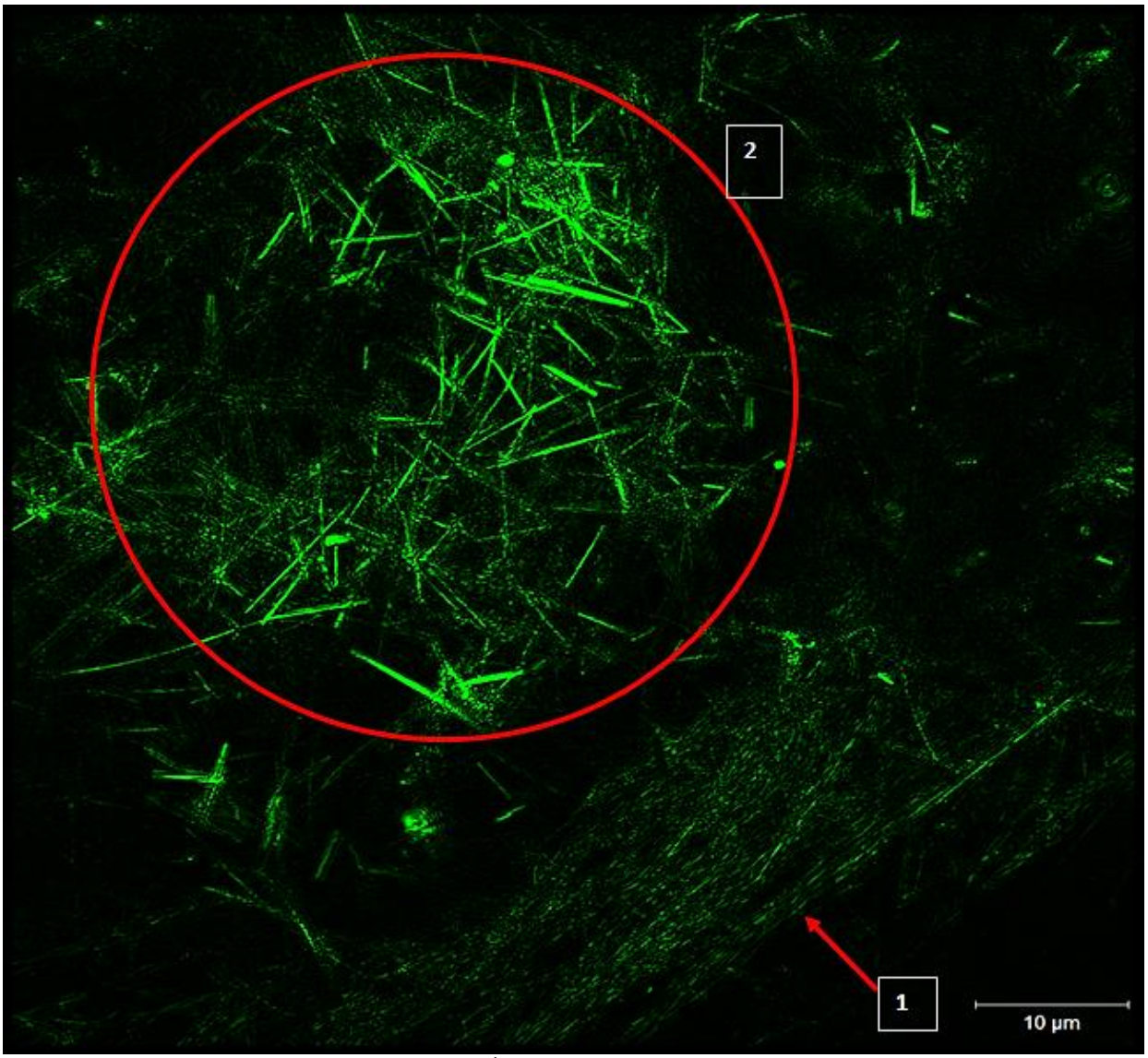


Figure 5. 16. Aspirin mixed with 0.2ng.L^{-1} LPS, 0,2µL of (5µM) ThT was added to mark amyloid structure (1) Matted mass deposits, (2) shows short anomalous fibrin(ogen) amyloid formation. Scale bar 10 µm

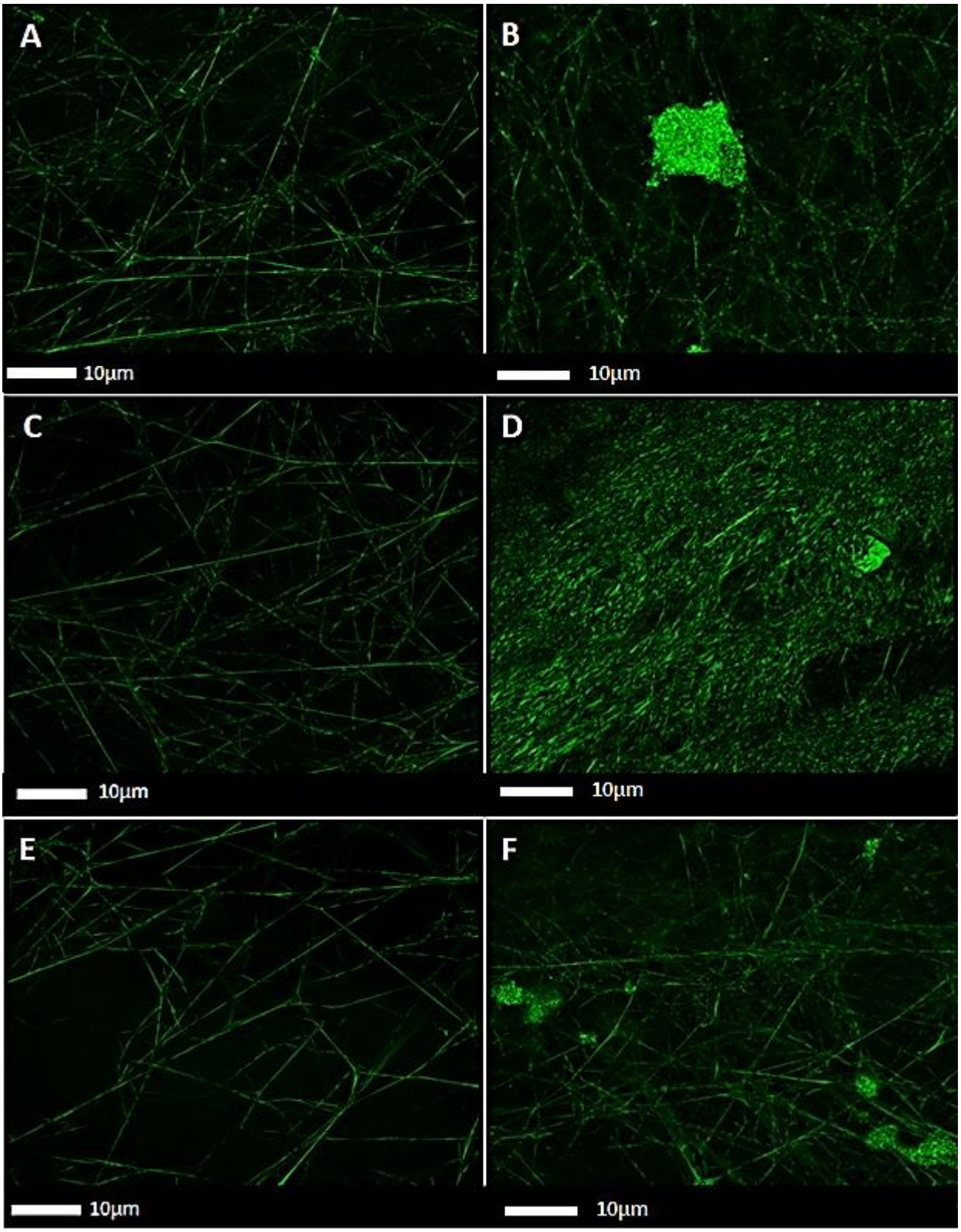


Figure 5. 17. Micrographs (A,C,E) of fibrin fibres here plasma was incubated with aspirin final exposure concentration of 0,5Mm and with LBP of final exposure concentration of 2ng.L^{-1} verses micrographs (B,D,F) aspirin incubated with LPS for 10 minutes prior to adding ThT the selective marker. All clots were prepared as described and human thrombin was added to imitate the clotting process. Scale bar 10µm

The combined effect of aspirin and Lipopolysaccharide binding protein mixed with LPS on combatting hypercoagubility induced by Lipopolysaccharide of plasma clot

To solve the research question (what reagents could be used to combat inflammatory symptoms induced by LPS) the next paragraph focuses on the possible solutions to the research question proposed. **Figure 5.18**. micrograph of naïve PPP treated with aspirin and LBP added with $0.2 ng.L^{-1}$ final exposure concentration of LPS. The micrograph shown here reveals fibrin fibres that have low fluorescence and evenly branched out, these fibres did not form pathological dense masses as seen when incubated with LPS. These fibrin fibres resembled the morphology of naïve PPP as seen previously**,** thin evenly branched and less fluorescence fibrin network.

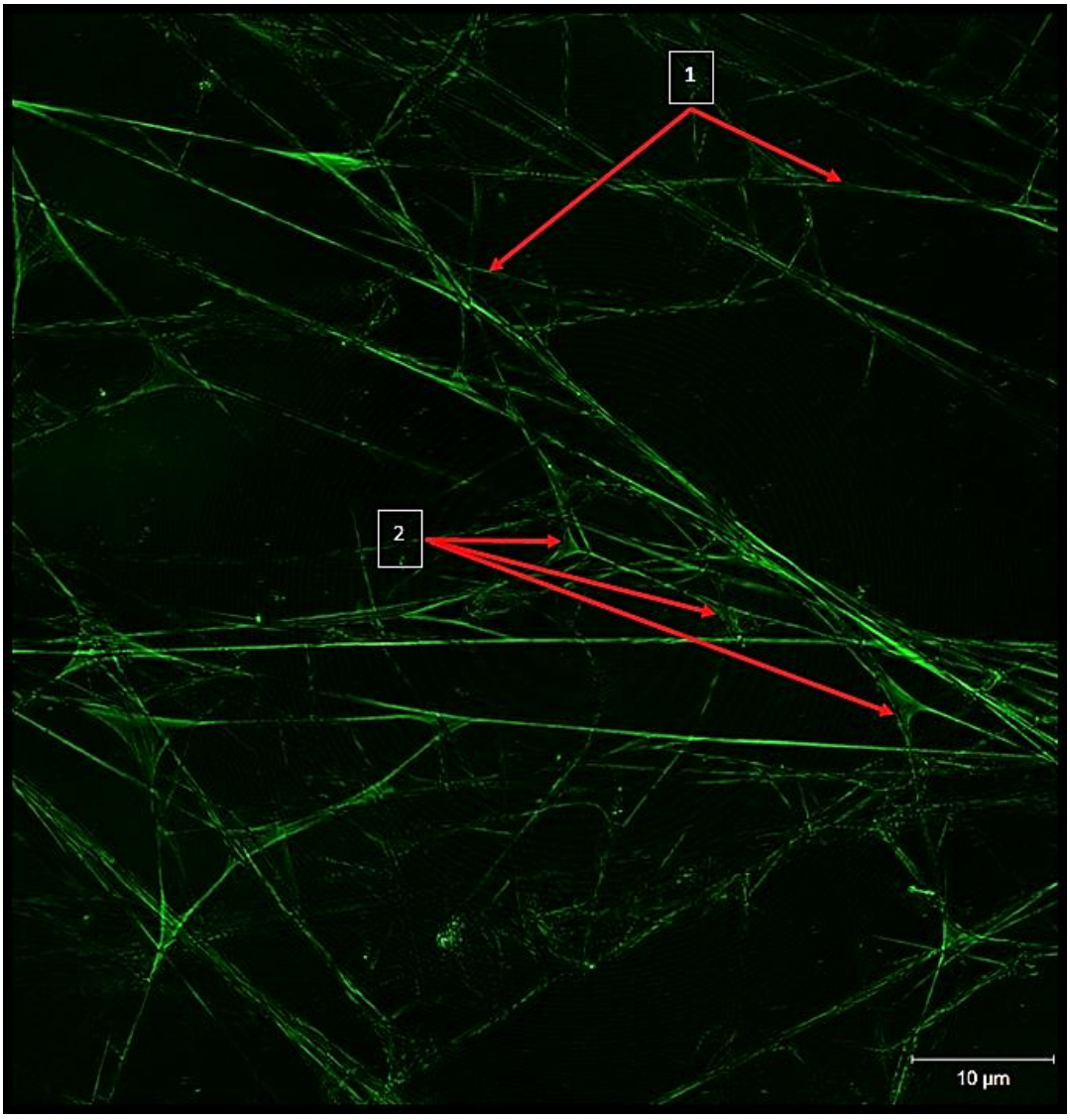


Figure 5. 18. Micrographs of PPP with added aspirin 0,5mM and a final exposure concentration of $2ng.L^{-1}$ LBP and $0.2ng.L^{-1}$ of LPS. Human thrombin 5mM of ThT was added to make the clot. Scale bar 10µm

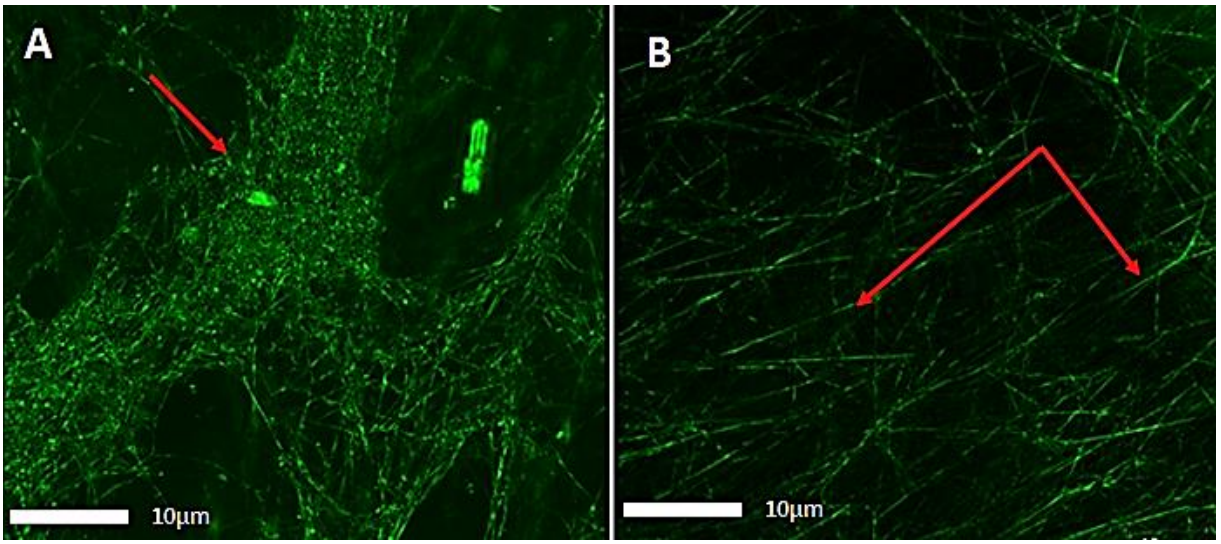


Figure 5. 19. Confocal micrograph of (A) PPP incubated with aspirin and LPS the red arrow indicates the joining of fibres in an abnormal manner. Forming matted mass deposits and increase staining of ThT marker (B) PPP incubated with final exposure concentrations of; 0.5mM aspirin and 0.2ng.L^{-1} LPS mixed with LBP. The red arrows show strands of normal, healthy fibres. Scale bar 10µm

Results from the aspirin mix (LPS and LBP) versus aspirin incubated with LPS shows a vast difference in phenotype between the two groups. From the results it is evident that aspirin incubated with LPS has big and dense clots see **Figure 5.20. (B)** The fibres were thick and were highly fluorescent with amyloid marker ThT in comparison to results obtained from PPP mixed with aspirin and mixture (LBP and LPS). In this group the fibres are less dense and had marginal fluorescence (see **Figure 5.20.(A)**).

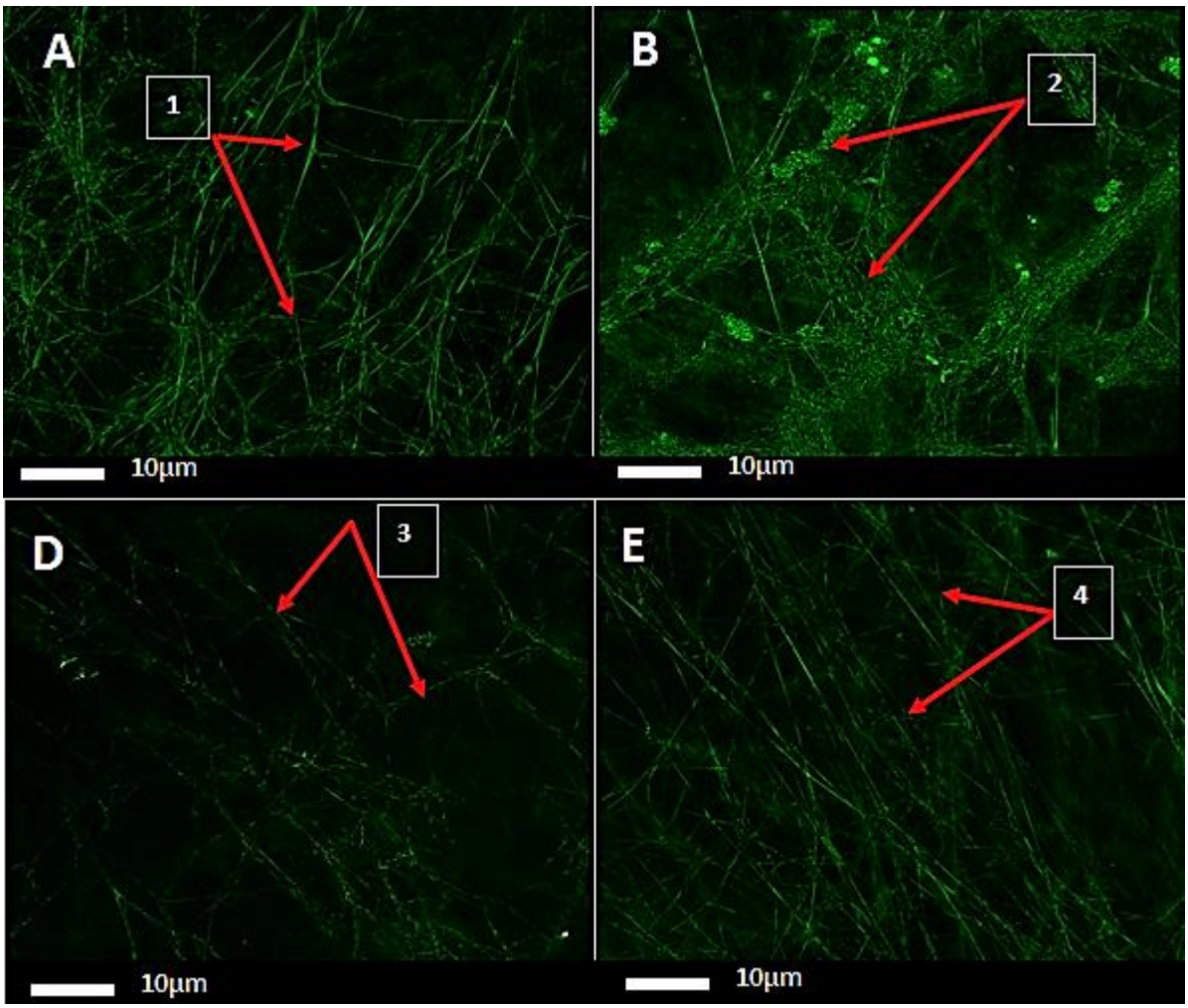


Figure 5. 20. Micrographs (A,C,D) of fibrin fibres incubated with aspirin and mixture (LPS and LPS-binding protein). Red arrow (1, 2,4) shows thin fibres evenly branched out in contrast to the red arrow (4) in micrograph (B) matted mass, unbranched fibres shows fibres from PPP incubated with aspirin with LPS for 10 minutes then followed by the addition of 0.2µm of ThT. Scale bar 10µm

To exclude most of the autofluorescence micrographs were taken of PPP without a marker. Then viewed under normal settings. Any fluorescent areas seen are thus auto-fluorescent. Free ThT fluoresces faintly with excitation and emission maxima of 350 and 440 nm, respectively, whereas upon interaction with amyloid fibrils an increase in ThT fluorescence is observed, with excitation and emission maxima at about 440/450 and 480/490 nm (this was mentioned in the Literature review in this thesis).The point is to show that in this study, there are also autofluorescent areas that are thus amyloidogenic (producing or tending to produce amyloid deposits) and that these anomalous clotted areas both show autofluorescence as well as fluorescence with ThT See **Figure 5.21.** below.

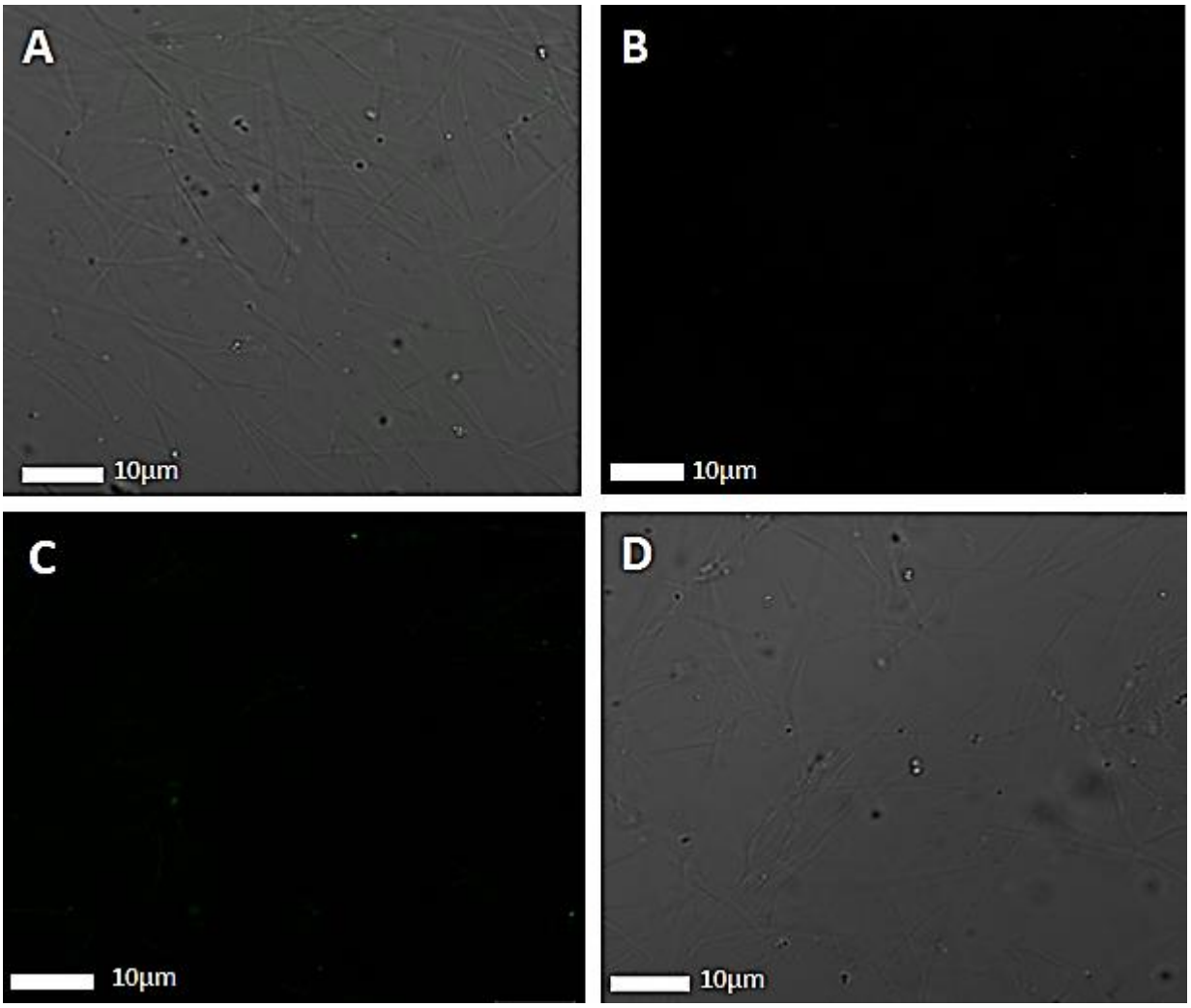


Figure 5. 21. Micrographs (B and C) fluorescence of naïve PPP with 5µl of thrombin to create a clot. These samples did not have the ThT marker therefore little to no fluorescence is seen. The micrograph (A and D) are of the same location on the clot as with the fluorescence, but they were taken under white light to locate fibres. Scale bar 10µm

Histogram

Confocal micrographs of ThT treated fibrin gels taken at 63 X were analysed for 8-bit pixel intensity using the histogram function of FIJI. These histograms measure the average distribution of green wavelength and black pixels per 8-bit area in an image which can then be used to calculate the coefficient of variation. In other words, the variation between green (representing fluorescence of β-amyloid positive region) and black (areas either devoid of fibrin or devoid of β-amyloid positive regions) in the image as an indicator of the overall presence of amyloid fibrils rich in β-sheets in the sample. Depending whether "Weighted RGB Conversions" is checked or not in. With 16-bit images, the range of gray values between the Min and Max values is divided into 256 bins. With RGB colour images, the green channels are visible. See **Figure 5.22.** for histogram of PPP, LBP, LPS and LBP with LPS.

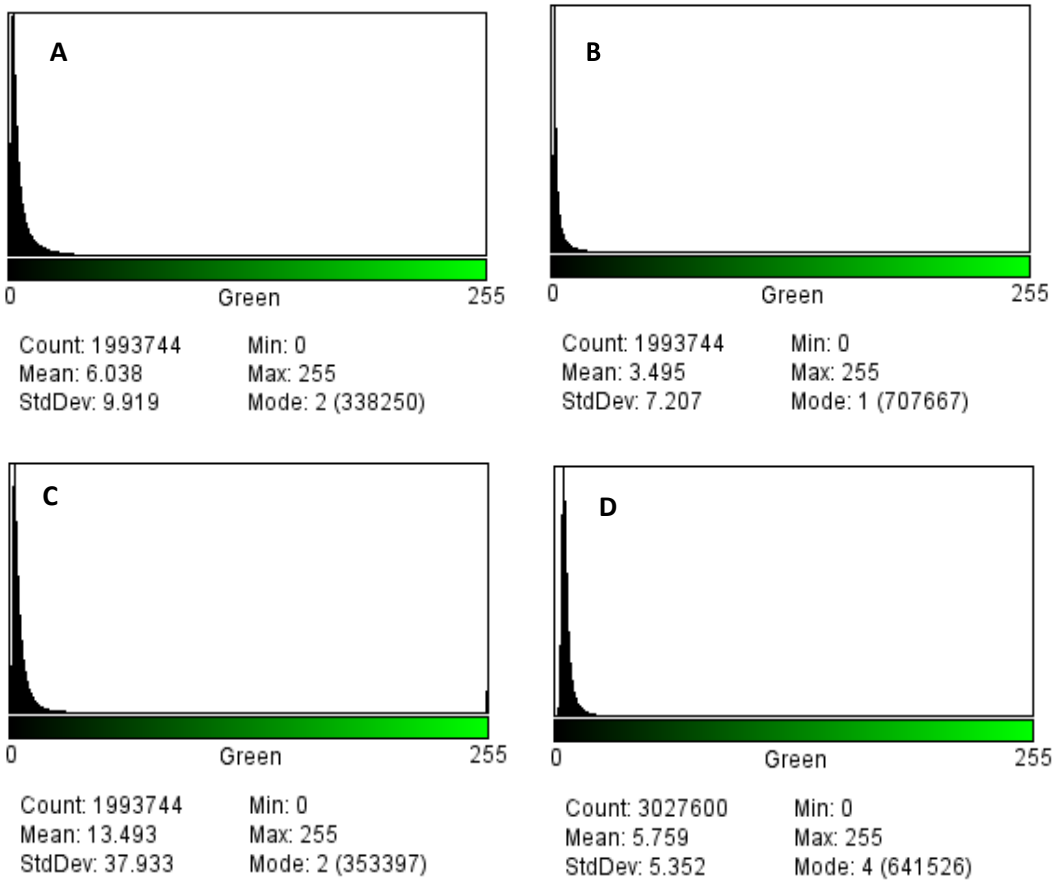


Figure 5. 22. Histogram showing the 8-height intensity, (A) naïve PPP, (B) PPP incubated with LBP, (C) PPP incubated with LPS (D) PPP incubated with LPS mixed with LBP

Next, are histogram taken from treatments with aspirin, see **Figure 5.23** below.

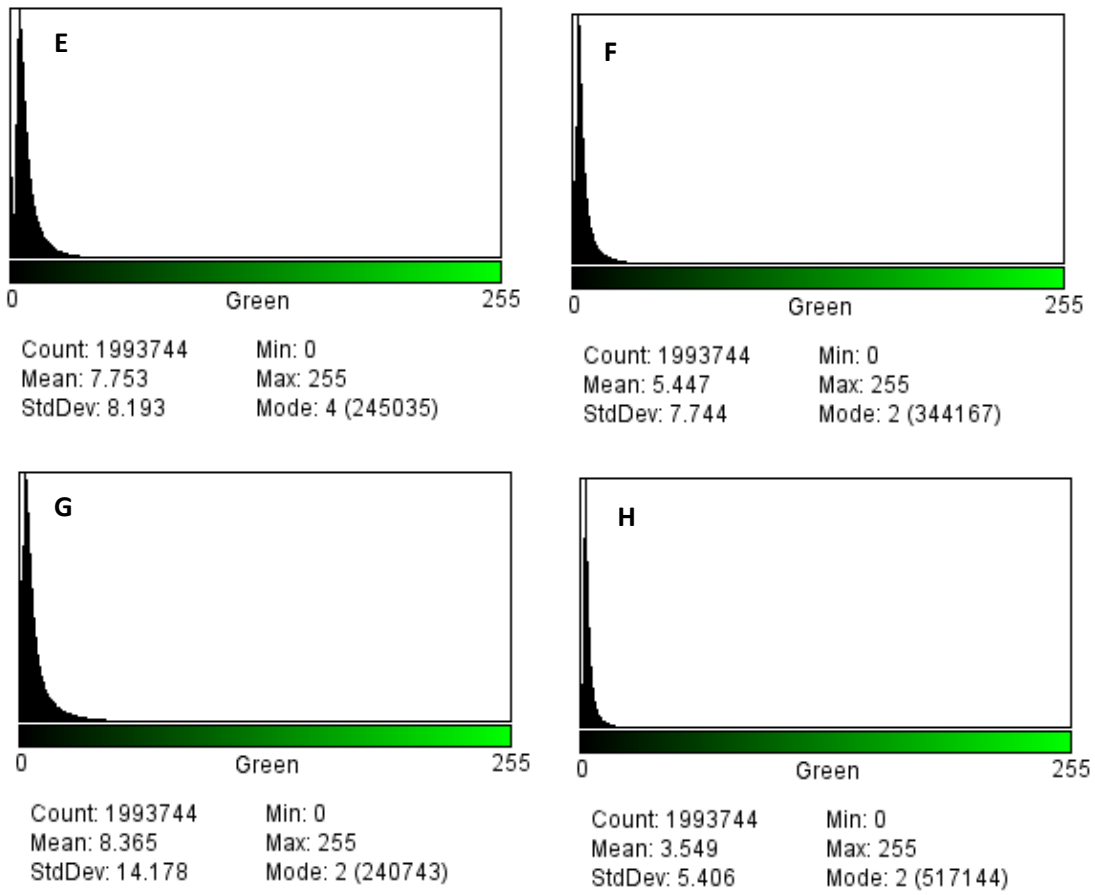


Figure 5. 23. Histogram showing the 8-height intensity, (E) PPP incubated with Aspirin, (F) PPP incubated with aspirin added with LBP, (G) PPP incubated with aspirin with LPS (H) PPP incubated with aspirin and pre-mixed LPS and LBP.

Statistical Analysis

ImageJ was used to calculate the mean and standard deviation of the intensity of the pixels in the images of the clot, using the histogram function, followed by the calculation of the coefficient of variation (CV) (i.e. SD/mean) of the intensity of the clot structure. This is a standard method that has been utilized by many researchers (Bester et al., 2016, Pretorius et al., 2014b, Pretorius and Kell, 2014, Pretorius et al., 2016a, Pretorius et al., 2017a, Pretorius et al., 2012)

Table 5. 1. Multiple comparison of 8-height intensity from confocal histogram of treated coefficient of variance verse control

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
PPP vs LBP	-0.002881	0.01907	No	-0.4015 to 0.3957
PPP vs LPS	0.3238	2.143	No	-0.07482 to 0.7224
PPP vs LBP + LPS	0.4146	2.744	Yes	0.01599 to 0.8132
PPP vs Asp	0.4446	2.943	Yes	0.04597 to 0.8432
PPP vs Asp + LBP	0.2524	1.671	No	-0.1462 to 0.6510
PPP vs Asp + LPS	0.2390	1.582	No	-0.1596 to 0.6376
PPP vs Asp + LBP + LPS	0.3822	2.530	No	-0.01636 to 0.7808
ANOVA table	P value			
Treatment (between columns)	P=0.0106			
Individual (between rows)	P=0.1719			

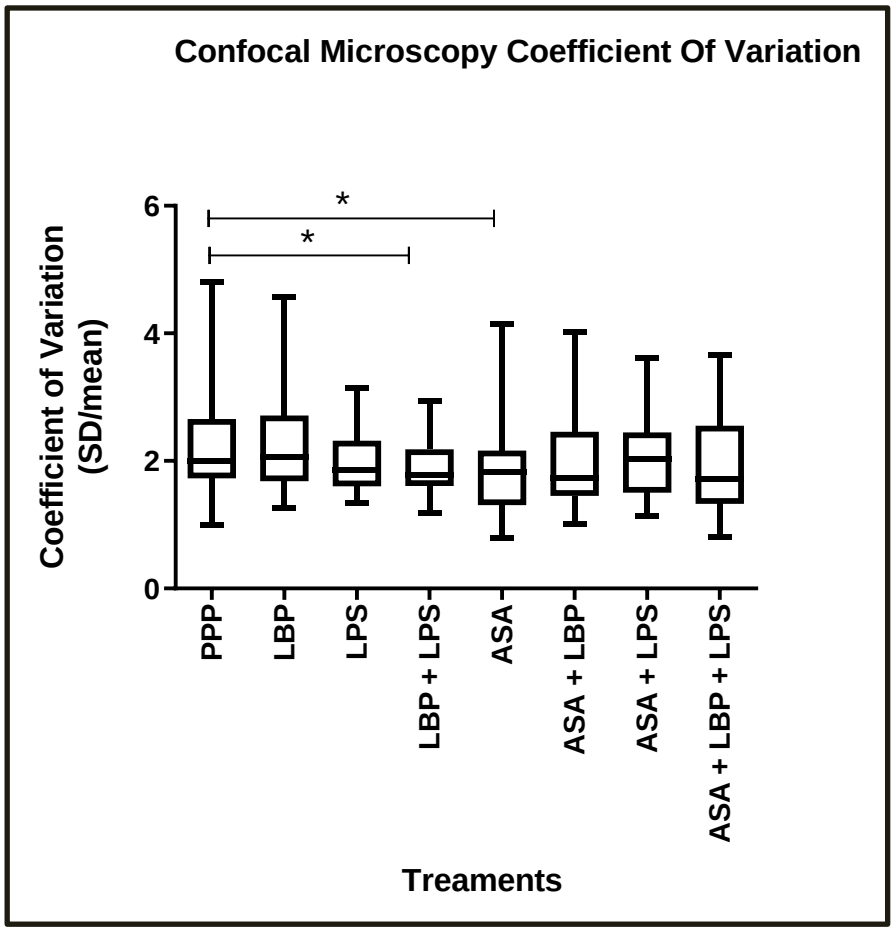


Figure 5. 24. The coefficient of variation determined between control and treatments indicated a decrease in the intensity of samples treated with LBP+LPS and ASA (p=0.0106) following a repeated measures ANOVA and Dunnet's multiple comparisons test.

Discussion

A marker that was used to mark the amyloid fibrils formed by LPS is ThT. Free ThT fluoresces faintly with excitation and emission maxima of 350 and 440 nm, respectively. See **Figure 5.2 and Figure 5.3.** these are confocal micrographs of untreated PPP from healthy participants, showing healthy individual fibre networks visible branch points with very little to no fluorescence. Previously it was noted that in healthy PPP, the presence of ThT, little fluorescence was present, with only occasional very small patches of fluorescence (Pretorius et al., 2016d, Kell and Pretorius, 2017). This might be because in all individuals (even if they are perceived to be healthy) there might be minor areas of misfolding of fibrin(ogen). This is however different when LPS had been incubated in healthy PPP, fluorescence was greatly enhanced, suggesting increased binding of ThT to β-sheet-rich areas on the fibrin(ogen) as seen in **Figure 5.6**. From these results, it is evident that LPS causes the fibrin(ogen) to polymerize into a form with a greatly increased amount of β-sheet (in the presence of thrombin), reflecting amyloid formation. This causes a strong fluorescence observable (when excited 440 nm) in the presence of ThT (Kell and Pretorius, 2017, Pretorius et al., 2016d) see **Figure 5.7.** β-sheet-rich areas or amyloid, anomalous clotted areas, are seen in fibrin(ogen) in clots created from PPP incubated with LPS**.** These findings were also supported by (Biancalana and Koide, 2010, Biancalana et al., 2009). The β-sheet or anomalous clotted areas in fibrin(ogen) were not seen when PPP was incubated with; LBP; or when LPS was added with LBP; aspirin; aspirin with LBP; aspirin added to PPP with LPS and LBP treatments. To 'mop up' LPS a mixture was formulated that contained both LPS and LBP. All results showed typical phenotype of normal healthy clot ultrastructure. See **Figure 5.8** a representative image of the LBP and LPS in plasma.

Researchers also found that the binding of LPS to LBP or the binding of LPS/LBP complexes to CD14 protects the host from LPS-induced toxicity, confirming that LBP is a critical component of innate immunity (Le Roy et al., 1999). However, as we do not have cells in the PPP and therefore no CD14, this is the first experiment that investigates the action of LPS/LBP in a cell free healthy blood model, suggesting that LBP van also protect fibrin(ogen) protein structure when in circulation.

The second reagent/treatment (anti-inflammatory) that was introduced to plasma was aspirin. Aspirin and PPP were mixed with an inflammatory agent activator LPS. Lipopolysaccharide was added to make a final exposure concentration of 0, 2ng.L^{-1}.

The effects are seen in **Figure 5.16** were shows *(*1) matted mass deposits, (2) cut short β-sheets of fibrin(ogen) amyloid formation. Aspirin was not able to mob up the harmful effect of LPS in plasma alone.

Confocal micrographs of ThT treated fibrin clots taken at 63X were analysed for 8-bit pixel intensity using the histogram function of FIJI. It is evident here from the histogram the STDev of all four are different, starting with that from **Figure 5.22. -(A)** naïve (untreated) plasma (B) LBP mixed with PPP, and (D) PPP incubated with LBP and LPS all three histogram show a lower intensity and hence lower STDev in comparison to the high intensity and greater STDev from (C) PPP with LPS. This shows a high fluorescence stain from our amyloid marker ThT, greater presence of amyloid proteins in this group of fibres.

Furthering investigation of aspirin on hypercoagulation induced by LPS, histogram of aspirin treatments, here similarities in the STDev of (E) aspirin, (F) aspirin with LBP and (H) aspirin with LPS and LBP they all range within the same range (according to their CV's) as have seen with the naïve PPP, LBP and LPS with LBP in **Figure 5.23**. All these treatments had similar light intensity and their CV's were similar when looking at the raw excel data in the Index pages. The statistical test One-way ANOVA Dunnet's test showed the difference in CV's taken from PPP naïve and PPP mixed with aspirin to have a lower intensity which was significantly different from the naïve PPP. According to the One-way ANOVA Dunnet's test the difference between the light intensity from LBP and LPS verse PPP naïve was also statistically significantly lower. This justifies the study hypothesis that LBP can be used to combat inflammation where the intensity would be greater due to ThT binding to amyloid structures in inflammatory conditions. Pretorius and team did a study were LBP was added to parkisons' and diabetes type II patients and in both studies the flourscence decreased when LBP was added to the affected plasma (Pretorius et al., 2017b, Pretorius et al., 2018b).

The use of CV metric to quantify and discriminate between clots from healthy PPP and clots from LPS, LBP, mixture (LBP + LPS), aspirin, aspirin and LBP, aspirin and LPS and finally aspirin with the mixture, gives accurate statistical data (Pretorius et al., 2016b, Pretorius et al., 2017b). ImageJ was used to calculate the mean and standard deviation of the intensity of the pixels in each of the randomly selected images, x 3 clot images per treatments per participant was used. This was followed by the calculation of the CV (i.e. SD/mean) of the intensity of the clot structure. **Fig 5.24.** shows boxplots showing minimum and maximum values per group of CV's of Airyscan confocal clot with all the treatments. For the statistical analysis of results see **Table 5.1.** CV's of all data obtained to allow for meaningful comparison between all of the magnitudes of variations. Researchers observed irregularities in the types of fibrin fibres produced in the plasma of patients with various inflammatory diseases such as amyloid in PD, T2DM, AD and many more inflammatory diseases as assessed by ThT staining, and its sensitivity to LBP. The production may be driven by intestinal LPS (Kell et al., 2015a), while gut microbiota-derived short-chain fatty acids may also have a role (Moreno- Navarrete et al.,2013). Because the addition of LBP to plasma of PD individuals improves the pathological state of the fibrin(ogen). The action of LBP is to remove tiny amounts of LPS, and related molecules, Pretorius and researchers have shown LPS to have this massive sub-stoichiometric effect which influences clotting (Pretorius et al., 2017a, Pretorius et al., 2017b, Pretorius et al., 2018). This is targeting intestinal LPS possibly to not only to reduce the production of to target pathological clotting in inflammation.

The role of aspirin was to decrease hypercoagulation induced by LPS in blood. Here the focus is on the role aspirin has on LPS. Furthering the investigation of the effect aspirin had on clot structure, aspirin was incubated with PPP then with LBP. The difference between the aspirin treated plasma along LBP with naïve PPP was statistically insignificant seen from the boxplot but when viewing the micrographs. See **Figure 5. 25.**(A-D) Micrographs of fibrin fibres, PPP with aspirin 0,5mM final exposure concertation, incubated with LBP to a final exposure concentration of 2ng.L^{-1}. Aspirin was then added with LPS in **Figure 5.16.** a major difference morphologically was seen between the two groups. The results illustrated that aspirin alone cannot reverse or reduce the effect on LPS on plasma, the micrographs were similar to those taken from PPP incubated with LPS see **Figure 5.17 (B,D,E).** which shows fibres that are condensed, dense and highly fluorescence with ThT. A signal that β-sheet have been exposed from fibrin(ogen) and proteins denatured forming amyloid proteins (Biancalana and Koide, 2010, Biancalana et al., 2009, Kell and Pretorius, 2017).

When doing the ANOVA multiple comparison Dunnet's test the difference between the PPP naive and PPP with aspirin mixed LPS was statistically insignificant. Structurally the images of aspirin + LBP verse aspirin + LPS were drastically different. This could have been due to some plasma reacting differently to LPS and aspirin causing less binding of ThT to some and hence no changes in value. When looking at the difference in CV's of aspirin+ LPS+ LBP verse PPP CV naïve the difference was insignificant, this correlate with the visual confocal images, suggesting a possible break through LPS inflammation.

Chapter conclusion

The discovery that the addition of LBP to plasma influences the structure of fibrin(ogen), furthermore lends credibility to the possible role of LPS in the etiology. The possible removal of amyloid by LBP is gaining more strength in this research. In this chapter it was also found that aspirin has no adverse effect on plasma protein at low physiological levels and according to AiryScan clot CVs.

Chapter 6 Scanning electron microscopy

Introduction

Scanning electron microscpy in the 1900s was a major invention in microscopic imaging as this instruments with it highest resolution for imaging the delicate surface aspect of objectives (HAYAT, 1974). This microscope produces images by scanning the surface of the samples with a focused beam of electrons. The electrons interact with the atoms in the sample which in turn produces various signals that can be picked up by a detector to create an image of the sample's topography and composition. The SEM is a useful technique to analyze the fine ultrastructure of RBCs, they are the most manipulable cells in our bodies, and due to their short life span, might form a vital indicator of health (Gyawali et al., 2015).

Scanning electron microscope is also used to study fibrin fibres network, the clots morphology has been used by researchers to study inflammation (Binder et al., 2017), this technique can be used as a health margin of the coagulation of individuals (Thompson et al., 2003, Tobias et al., 1993, Undas et al., 2006, Wada et al., 2012). Fibrin is produced by the polymerisation of the protein fibrinogen under the action of thrombin, that removes two fibrinopeptides, this now allows the fibrinogen to self-assemble by a 'knobs and holes' mechanism into fibres (Weisel, 2005, Wolberg, 2007, Cilia La Corte et al., 2011, Undas and Ariëns, 2011, Wolberg, 2012), but with no particularly marked changes in secondary structure (Averett et al., 2008, Weisel, 2005, Yermolenko et al., 2011, Protopopova et al., 2015). A final, transglutaminase-based crosslinking step (Dickneite et al., 2015) ensures the stability of the clot.

For this study SEM was used to study the morphology of RBCs and platelets of participants' and fibrin(ogen) fibre network. Scanning electron microscope provided quality results of fibrin fibres structure and thickness which could be quantified. The usage of SEM for ultrastructural study of all the cells is common, most researchers use it for on a routine diagnostic (Terzakis et al., 2005, Metzger et al., 2016, Nedela et al., 2016, Page et al., 2019a, Pretorius and Lipinski, 2013, Strydom et al., 2016)

This chapter reveals SEM results from blood samples of healthy individuals whose blood have been exposed to different treatments. The following paragraph states the chapter's aim

Chapter Aim

The first aim is to visualize the morphology of WB cells after treatment with different reagents listed in the introduction chapter. The second aim was to investigate the clot formation and nature of fibrin(ogen) and fibres formation after and before PPP has been exposed to different treatment on the hypercoagulable clot formed by LPS.

Chapter objectives

- To study the surface membrane of RBCs together with platelet activation before and after WB has been treated with LPS, LBP, LPS+LBP, aspirin, aspirin+ LBP, aspirin+ LPS and aspirin + LPS+ LBP. – (A qualitative study)

- To determine the fibrin(ogen) formation and state or healthiness of each participant by looking at fibrin fibres clots created when adding naïve PPP with thrombin

- To compare the naïve fibrin fibres clot ultrastructural morphology with those treated with LPS, LBP, Aspirin, Aspirin and LBP, aspirin and LPS, aspirin and LBP and LPS of the same individual (direct comparison), using SEM.

- To compare fibrin fibre thickness after each treatment to determine direct effect of each treatment on phenotype morphology and size of fibres

Materials and Methods

Reagents for the experiment

- **Lipopolysaccharide binding protein**

  It was reconstituted in 40 µL of ultra-pure distilled H_2O. The final exposure concentration of LBP was $2ng.L^{-1}$. Purchased from Sigma-Aldrich® South Africa

- **Lipopolysaccharides**

  Lipopolysaccharide from bacteria called *E.coli* 0111: B4 Lyophilized Powder. Purchased from Sigma-Aldrich® South Africa.

- **Sodium salicylate**

  (Aspirin) Reagent Plus®,_>99.5% S3007-500G. Formula: C7H5NaO3 and formula

Weight: 160.10 $g.mol^{-1}$.

- **Phosphate buffer solution (PBS)**

  was diluted with distilled/ultra-filtered water to a 10 % final dilution.

- **Fixative: is a 4% formaldehyde**

  This was obtained by diluting 38 % formaldehyde with PBS

  The solution can be aliquoted and frozen or stored at 2-8 °C for up to one month.

- **Distilled water**

- **1% Osmium Tetroxide (Kistenmacher et al., 1976)(very toxic, corrosive)**

  Osmium Tetroxide, chemical formula OsO_4, is a useful chemical compound that finds application as a staining and fixing agent for use in electron and scanning microscopy. Purchased at Sigma-Aldrich. Osmium tetroxide for microscopy can be used to visualize unsaturated lipids. A part of the lipids is immediately blackened by the influence of osmic acid solution (Kistenmacher et al., 1976, Wright et al., 1981)

- **100% ethanol**

  Ethanol, a primary alcohol, is an important raw material for the synthesis of various compounds. It can be synthesized either from molasses by fermentation or by hydration of ethylene. To obtain different percentages of ethanol, the alcohol was mixed with distilled water.

- **99% HMDS**

  Hexamethyldisilazane (HMDS) is used to trimethylsilylate alcohols, amines, thiols. This was used as final fixing stage. Purchased from Sigma-Aldrich® South Africa

- **Human Thrombin**

  Purchased from South African Blood Service. Appearance (Colour) White to Off-White Appearance (Form) Powder.

Method

A volume of 98µl of PPP/WB was aliquoted into a separate Eppendorf tube. To this tube a volume of 2µl of LBP (final exposure concentration of 2ng.L^{-1}) was added making a total volume of 100µl. To test the effect of LPS on direct fibrin fibres formation and RBCs' ultrastructure only 0.2 ng. L^{-1} LPS (a final exposure concentration) was added to 98µL of PPP/WB, this served as the positive control. To study the effect of combined therapy LBP and aspirin on PPP/WB and fibrin fibres, 25µl of aspirin was added to 475µl of PPP or WB (master tube that will be used to disperse out further required samples of WB/PPP and aspirin). To study the effect of aspirin on thrombin formation and RBCs ultrastructure after effect of LPS, 25µl aspirin (final exposure concentration of 0,5mM) was added to 475µL of PPP or WB to. From these mixed bloods 98µL of PPP or WB was aliquoted to a separate Eppendorf tube were 2µl of LBP was added. In a separate Eppendorf tube, an amount of 96µl of PPP or WB from the same PPP/WB and was mix with aspirin. To this to mixture of LBP to final concentration of 2ng.L^{-1} and LPS to final exposure concentration of 0. 2ng.L^{-1}. The described procedures/ techniques were performed on the naïve sample as well as samples treated samples. Therefor each sample from each individual with and without treatments can be compared with each other.

SEM sample preparation

A pipetted volume of 10 µl sample (PPP/WB) was placed onto a 10 mm round cover slip. When preparing with thrombin, a volume of 5 µl was pipetted onto the cover slip first and then followed by10 µl sample (PPP/WB) into the thrombin droplet. The tip of a bent pipette was used to carefully swirl the droplet and spread only touching the droplet, not the glass. The sample was dried at room temperature for up to 1 minute. With WB samples the outer ring was dry to roughly half the radius. With WB and thrombin, the distance was one quarter of the radius. For PPP and thrombin, the cover slip was lifted to eye level to observe extent of drying. If a gel-like layer was formed the sample was washed immediately. The cover slips were transferred into a new well plate and covered with PBS for 15 min. The buffer was discarded, and the sample was covered with fixative (4% formaldehyde); for 30 min**.** The fixative was discarded, all cover slips were washed 3 x 3 min buffer wash. When the samples were still wet, sample were agitated by shaking the well plate slightly.The cover slips were then transferred to the fume cupboard to discard the third buffer. Then cover slips were washed followed by placing 4 - 5 drops of osmium tetra-oxide formula O_SO_4 in short abbreviation OT, directly onto the cover slip. A total of 4-5 drops of ddH_2O was added, the well plate was covered and left in the fume cupboard for 15 minutes.

The OT/ddH_2O was discarded into the OT waste. Followed by washing 3 x 3 min with Buffer, the first wash was discarding into OT waste. The samples were then dehydrated with ethanol. Ethanol series dehydration:30 %, 50 %, 70 %, 90 % and 100 % ethanol for 3 mins each. The sample was dehydrated repeatedly with 100 % ethanol twice. At this stage, the closed well plate was sealed with parafilm and the samples stored for 3 hours. In the fume cupboard, the ethanol was discarded, and samples were covered with HMDS for 30 minutes. The HMDS was then discarded into HMDS waste bin then one drop of HMDS was placed directly onto the sample. For better coverage the well plate was tilted and if any excess HMDS fluid was seen it was drawn out. The edge of the cover slip was lifted for a few seconds, allowing the bottom to dry (the bevelled edge of a blood collecting needle worked well for this). The cover slip was left to dry in the fume cupboard overnight (12 hours) for the HMDS to evaporate. The well plate was labelled with date and name of the investigator. The well plate was sealed with parafilm until ready to be mounted and coated. The specimen was mounted on a metal stub using a sticky carbon tape which increases conductivity. The cover slips are mounted on the aluminium stubs with carbon tape. To prevent charge buildup on specimen surface, it was coated with a conductive carbon. Carbon is applied in a controlled manner in a sputter coater. It is critical that the coating is thick enough to prevent charging (typically around 10 nm) but not thick enough to obscure specimen surface details.Samples were viewed SEM an average of five micrographs were taken per cover slip that is per sample for a total of 30 controls blood.

Results of Scanning electron microscopy of Whole blood

The morphology and ultrastructure of RBCs were investigated using SEM. **Figure 6.1. shows** a RBCs with normal healthy-looking cell membrane. The surface membrane of the RBC found in healthy individual was smooth. **Figure 6.2.**A group of normal healthy RBC and inactivated platelets. The background was clear were the RBCs were scattered evenly, for normal heathy untreated WB it is common to find less RBCs on the surface of the glass cover slips this however is different in the case of inflammation and disease cases, also a rough surface membrane, later seen in **Figure 6.4**.

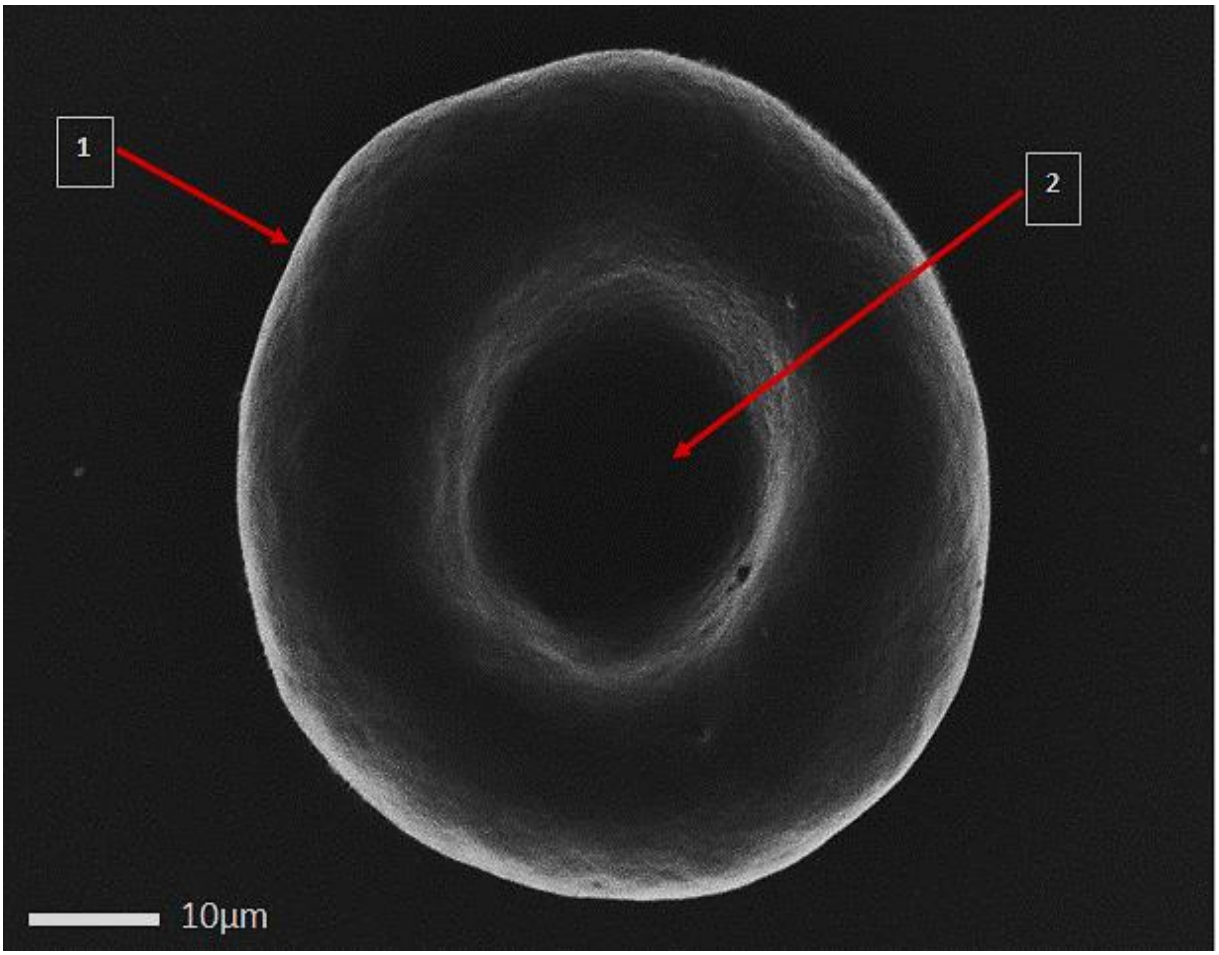


Figure 6. 1. Micrograph of healthy RBC that was not treated., red arrow (1) points to smooth membrane surface of RBC on a clear surface. Red arrow (2) hollow sphere/doughnut shape a normal RBC. Scale bar 10µm

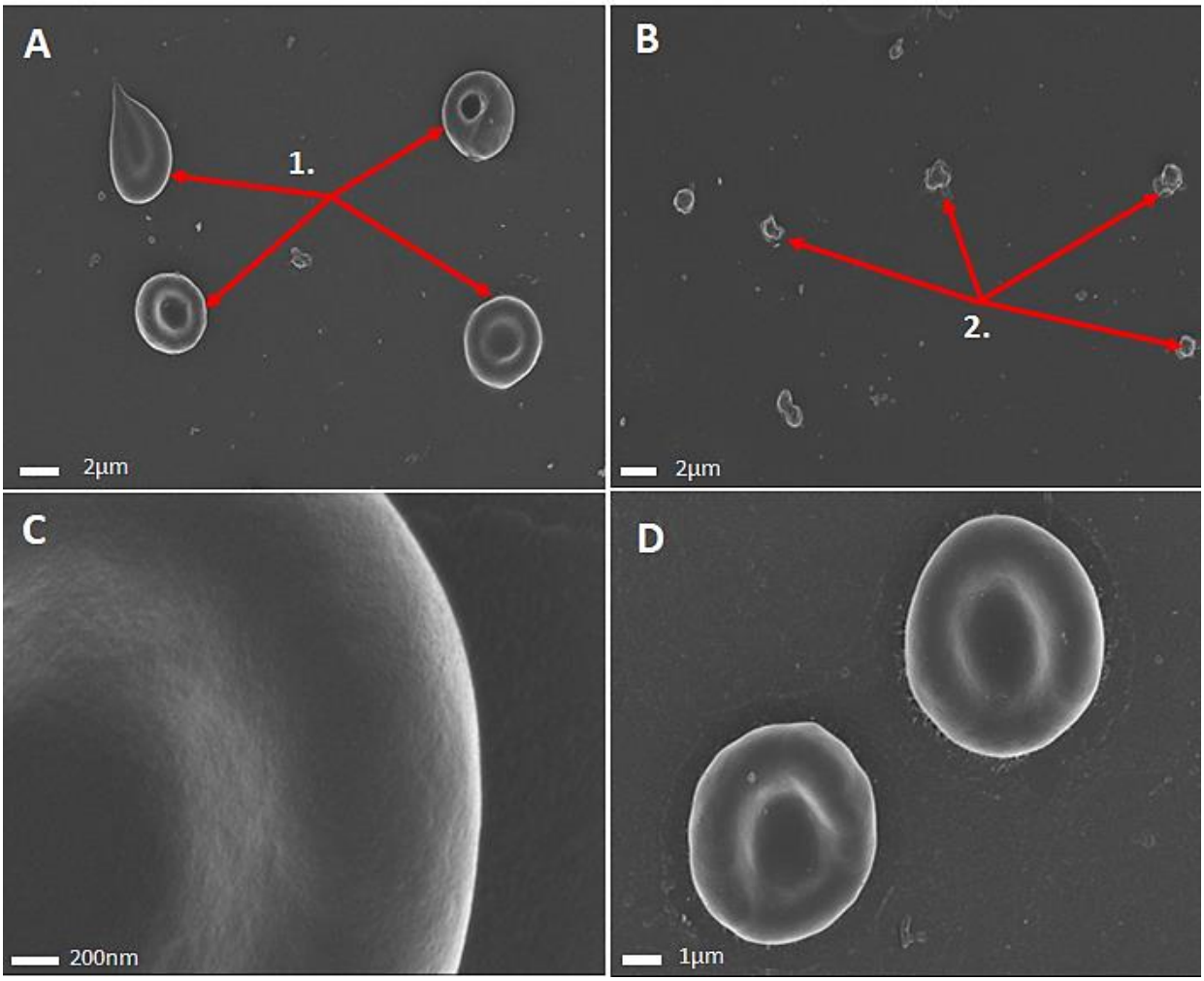


Figure 6. 2. Whole blood smears**. (**A) group of normal healthy RBC and arrows (2) show inactivated platelets. Micrograph (1) red arrows, showing healthy matured RBCs at different stages of maturation scale 2µm. **(B)** the red arrows (2) shows platelets from healthy individuals that are inactivated scale 2µm**. (C)** a mature RBC closer view looking at membrane surface area Scale 200 nm. **(D)** Micrograph shows two matured normal healthy-looking RBCs scale is 1µm.

The next section focuses on the reaction between a small physiological concentration of LBP incubated with WB.

The effects of Lipopolysaccharide binding protein on whole blood

For this investigation an exposure concentration of 2ng.L^{-1} of LBP was added with 98µL of WB from healthy participants. The results are seen in **Figures 6.3**. typical biconcave shapes of RBCs from different participants with the same treatment. These RBCs resembled those found in naive WB samples. A smooth surface membrane in all RBCs was seen.

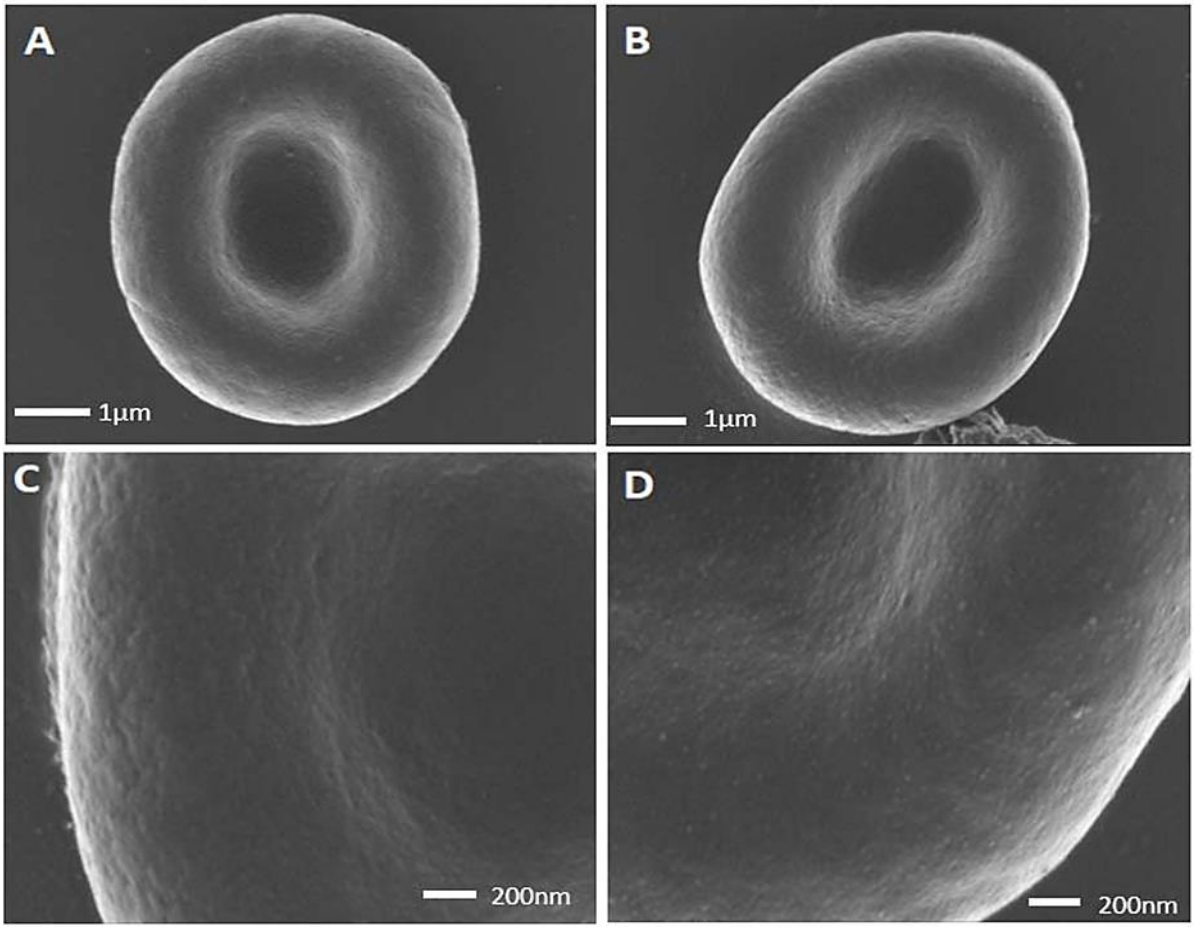


Figure 6. 3. Whole blood mixed with LBP. (A and B) RBCs with smooth surface membrane. Micrographs (C and D) are RBCs from different individuals, on higher resolution to show the smooth surface membrane of each RBC.

Lipopolysaccharide binding protein effect on platelets and RBCs interaction.

No interaction of platelets with RBCs and no evidence of activation of platelets by LBP was seen. The following sections reveal results from LPS and WB experiment. The focus was how LPS reacted with cells from healthy WB.

The effect of Lipopolysaccharide on Whole blood

When a small concentration of LPS was added to WB it affected cells platelets and RBCs. In **Figure 6.4.** shows RBC with microparticle formation on the surface of the RBC's membrane. Lipopolysaccharide was added a final exposure concentration of 0. 2ng.L^{-1}.

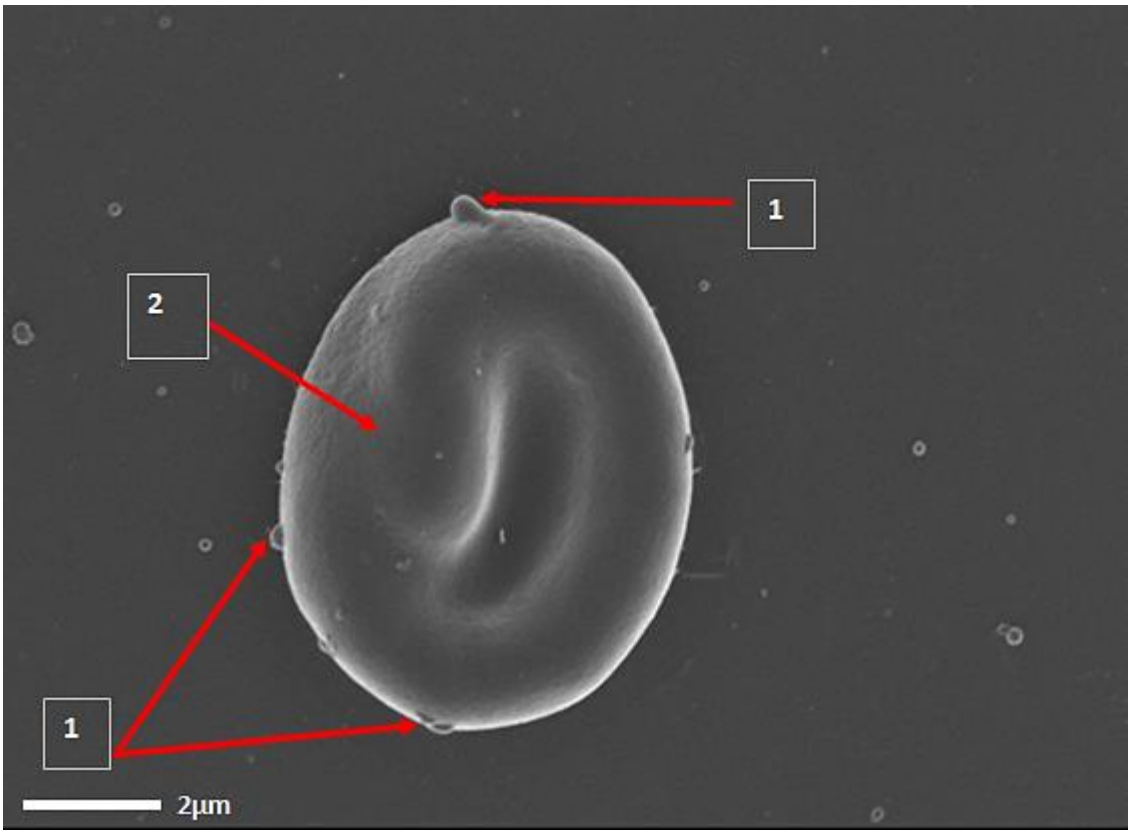


Figure 6. 4.A micrograph of RBC when WB was incubated with LPS (0. 2ng.L^{-1}) final exposure concentration. (1) Visible bulging membrane particles, (2) folding of surface membrane bulging inwards. Scale 2µm.

One of the common phenotypes seen in inflammatory diseases is the altered structure of RBCs. The morphology of RBCs after LPS has been incubated with WB using SEM shows irregularities in the shapes of RBCs and rough prickly surface membranes, see **Figure 6.5.** micrographs of RBCs from different individuals affected by LPS.

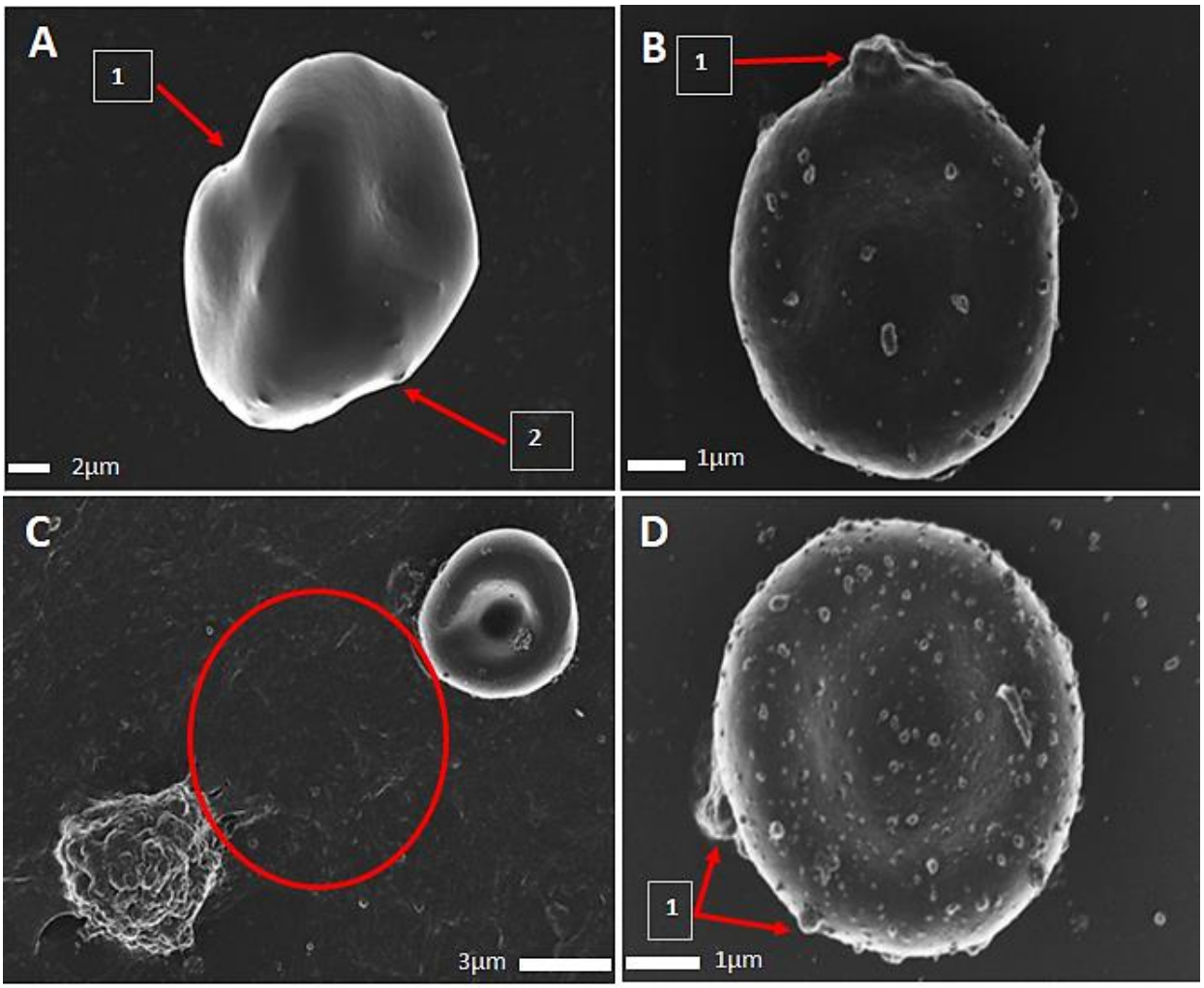


Figure 6. 5. Micrographs taken from WB incubated with LPS. Micrographs (A) (1) irregular discoid shape of RBCs with (2) bulging surface membrane on RBCs. (B) (2) Bulging surface membrane. Scale 2µm and 1µm respectively. (C) shows thick mass deposits indicated with a red circle, spontaneous formation of fibres (without the addition of thrombin). Scale 3µm. Micrograph (D) a normal shaped matured RBC it contained (1) tiny rough particles that were formed by the shedding of other platelets or RBCs OR bulging surface particles from the RBC's membrane. Scale 1µm

The next section reveals results of RBCs from WB treated with LBP and LPS. This was to identify changes in the ultrastructure of RBCs and compare them with those obtained from naïve WB.

The effect of Lipopolysaccharide binding protein mixed with Lipopolysaccharide in whole blood

For this investigation LPS was mixed with LBP prior to mixing with WB of participants. This was to investigate the hindering effect of LBP on LPS in WB. The **Figure 6.6. and Figure 6.7.** show RBCs after incubating the WB with LPS and LBP. A great difference in the morphology and membrane of RBCs was seen when WB was mixed with LPS this will be seen in later part of this chapter.

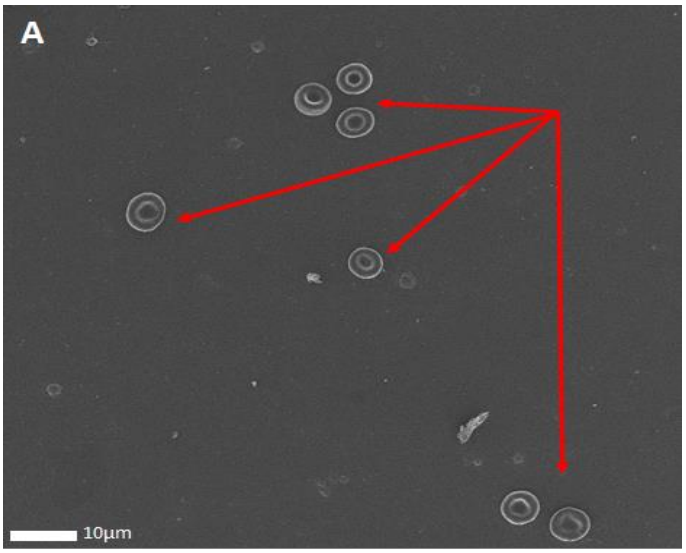


Figure 6. 6. Whole blood with mixture (LBP mixed LPS), red arrows show RBCs biconcave shape remains, and the spontaneous spindle formation hypercoagulable state (without adding thrombin) is not present. Scale bar 10µm

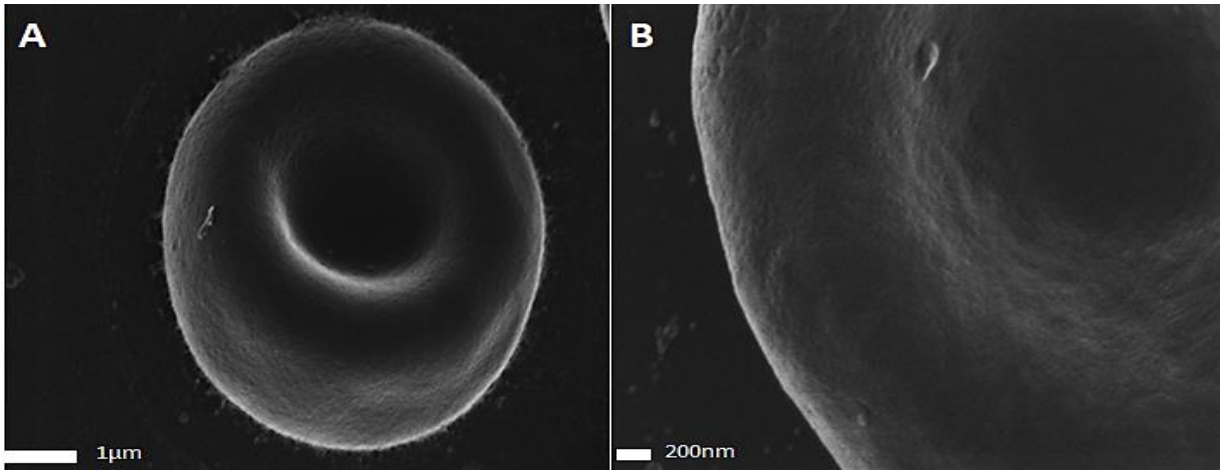


Figure 6. 7.Whole blood incubated with mixture (LPS and LBP) (A) shows a normal RBC scale 1µm (B) shows smooth surface membrane with higher magnification, scale 200nm.

The following section reveals the effect of physiological levels of aspirin on healthy WB.

The effect of aspirin on whole blood

Furthermore, the investigation continued to low physiological level of aspirin 0,5. mM (final exposure concentration) with WB. **Figure 6.8** below shows RBCs taken from samples of WB treated with aspirin. The low physiological level of aspirin had no effect on RBCs as seen from the figures below.

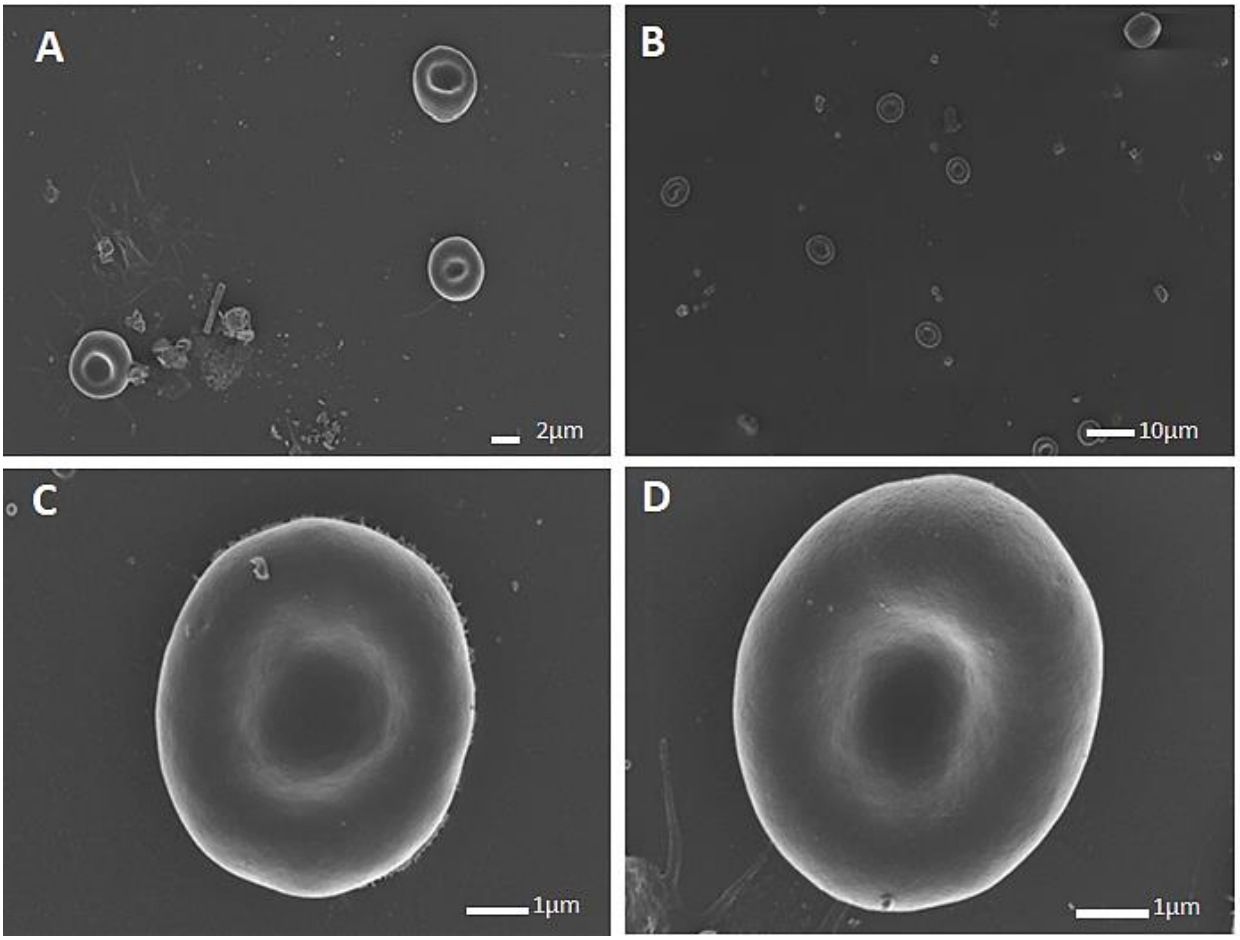


Figure 6. 8.SEM micrographs of RBCs incubated with aspirin. (A-D) show smooth surface membrane of RBCs, typical biconcave shape of RBCs as seen from naïve WB. Scale 2µm, 10µm,1µm respectively.

From the results seen above it is evident that aspirin had no adverse effect on the shape and size of RBCs. The next investigation was to test the effect of aspirin together with LBP on RBCs ultrastructure. This next section focuses on the effects of the two reagents combined. Closely looking at the morphology of RBC particularly surface membrane.

The effect of aspirin in combination with Lipopolysaccharide binding protein on whole blood

Aspirin and WB were incubated prior, followed by adding LBP to the mixture. The **Figure 6.8.** shows the surface membrane of RBCs after exposure to aspirin and LBP for 10 minutes. Using the SEM, there were no drastic changes to the surface membrane and integrity of RBCs.

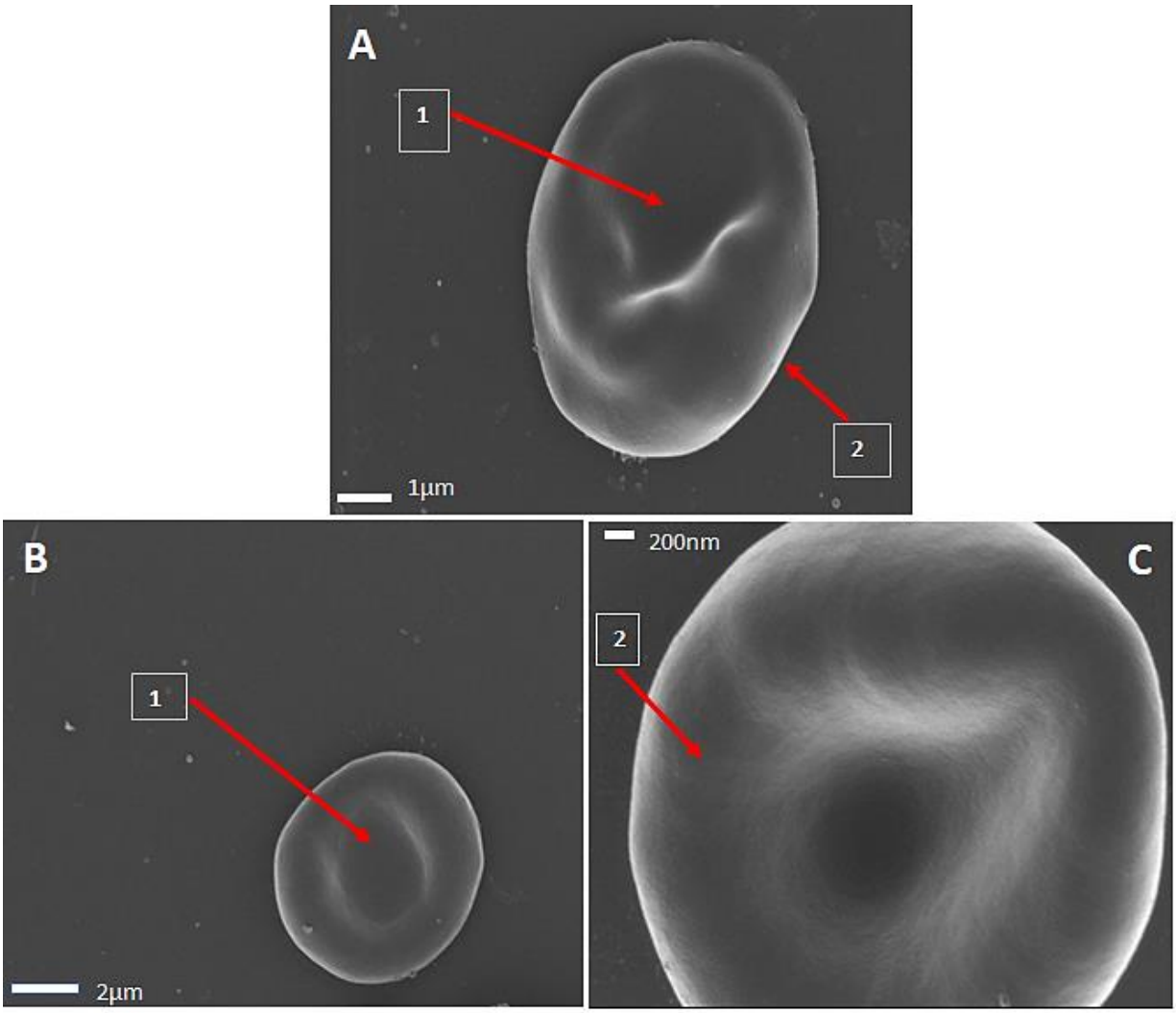


Figure 6. 9. Micrographs of RBCs with aspirin and LBP arrow (1) typical shape of the RBC. (2) Red arrows in all micrographs points to the smooth surface membrane. Micrograph (A) shows the top surface view of RBC the RBC scale is 1µm. Micrographs (B) shows RBC 2µm. Micrograph (C) top surface view of RBC on higher magnification. scale 200nm

The next section focuses on the protective role played by aspirin against LPS in WB.

The effect of aspirin on inflammation induced by Lipopolysaccharide on whole blood

One of the study's objectives was to investigate the protective role of aspirin against LPS in WB. When aspirin was added to WB followed by LPS microparticle formation was seen and irregular shapes of RBCs, see **Figure 6.10.** a group of RBCs and microparticle coming from the membranes of RBCs or other cells, there is spontaneous formation of fibres without thrombin and unhealthy binding of RBCs with platelets.

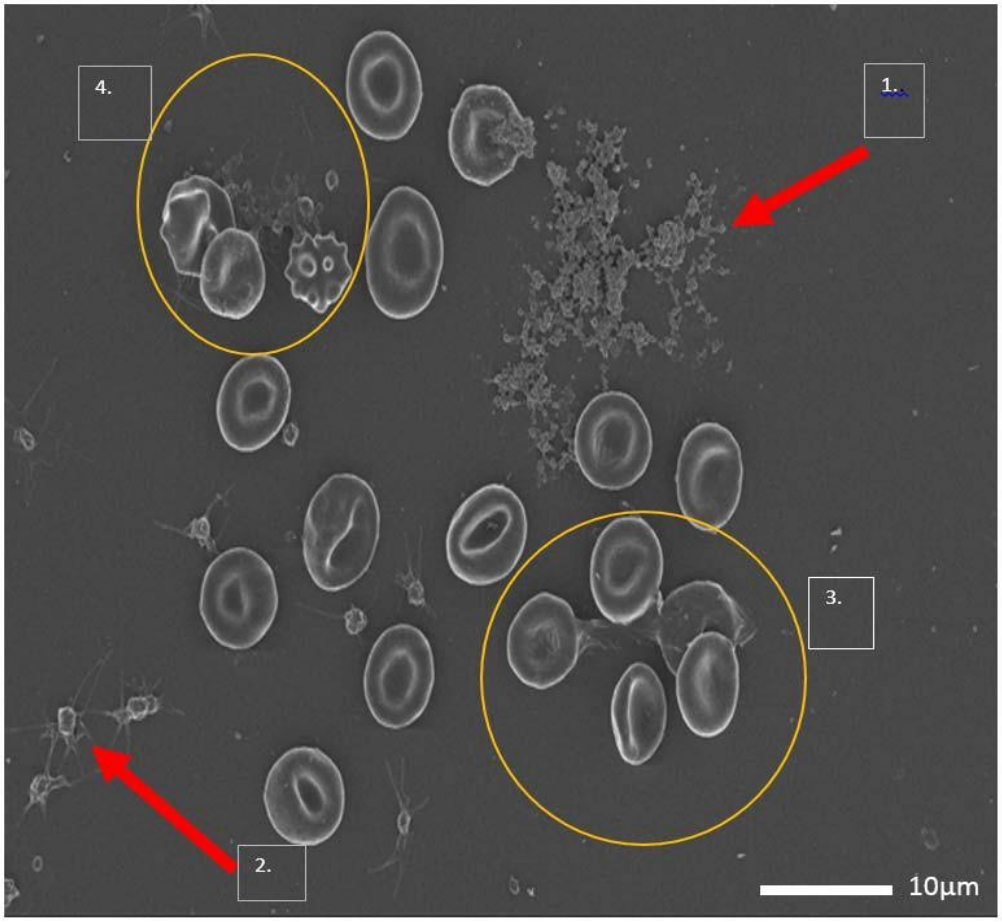


Figure 6. 10. Micrograph taken from WB treated with aspirin and LPS. (1) Arrow shows microparticles that could have shed off from neighboring RBCs and platelets. (2) pseudopodia from activated platelets. (3) yellow circle is indicating RBCs that are bonded by a thick mass and the membrane of some of the RBCs are thinned out and unsmooth. (4) Yellow circle showing a rare firm attachment of RBCs, and thick mass deposits. Scale 10µm.

The following micrographs are of RBCs interacting with platelets showing cells after incubating aspirin with WB and LPS. An increase in platelet activation, irregular shapes of RBCs commonly found in inflammatory diseases as seen in this part of the investigation **see Figure 6.11.**

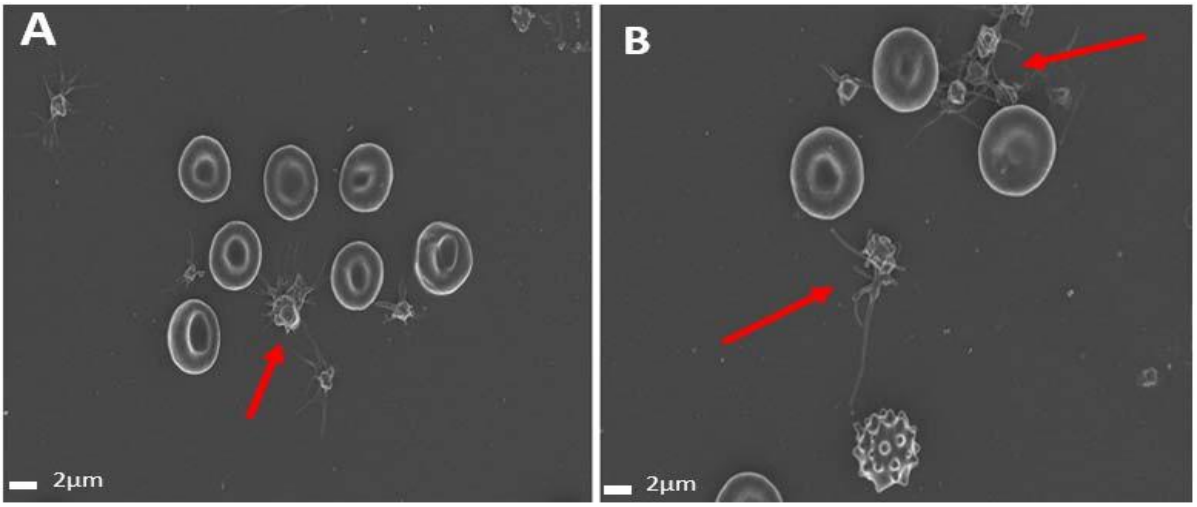


Figure 6. 11. Micrograph (A +B) are from WB that was incubated with Aspirin and LPS. Red arrows show pseudopodia from activated platelets. Scale 2µm

Scanning electron micrographs continued to show the difference in irregularities of RBCs' surface membrane presented after incubating WB with aspirin and LPS. **See Figure 6.12.** shows RBCs are trapped by the pseudopodia from activated platelets and spontaneous masses occurring on the surface. Spontaneous fibres formation on WB can be due to inflammation and since it is known that LPS triggers inflammatory cytokines, this is normally seen as inflammatory symptoms (Kell et al., 2015, Kell and Pretorius, 2015b).

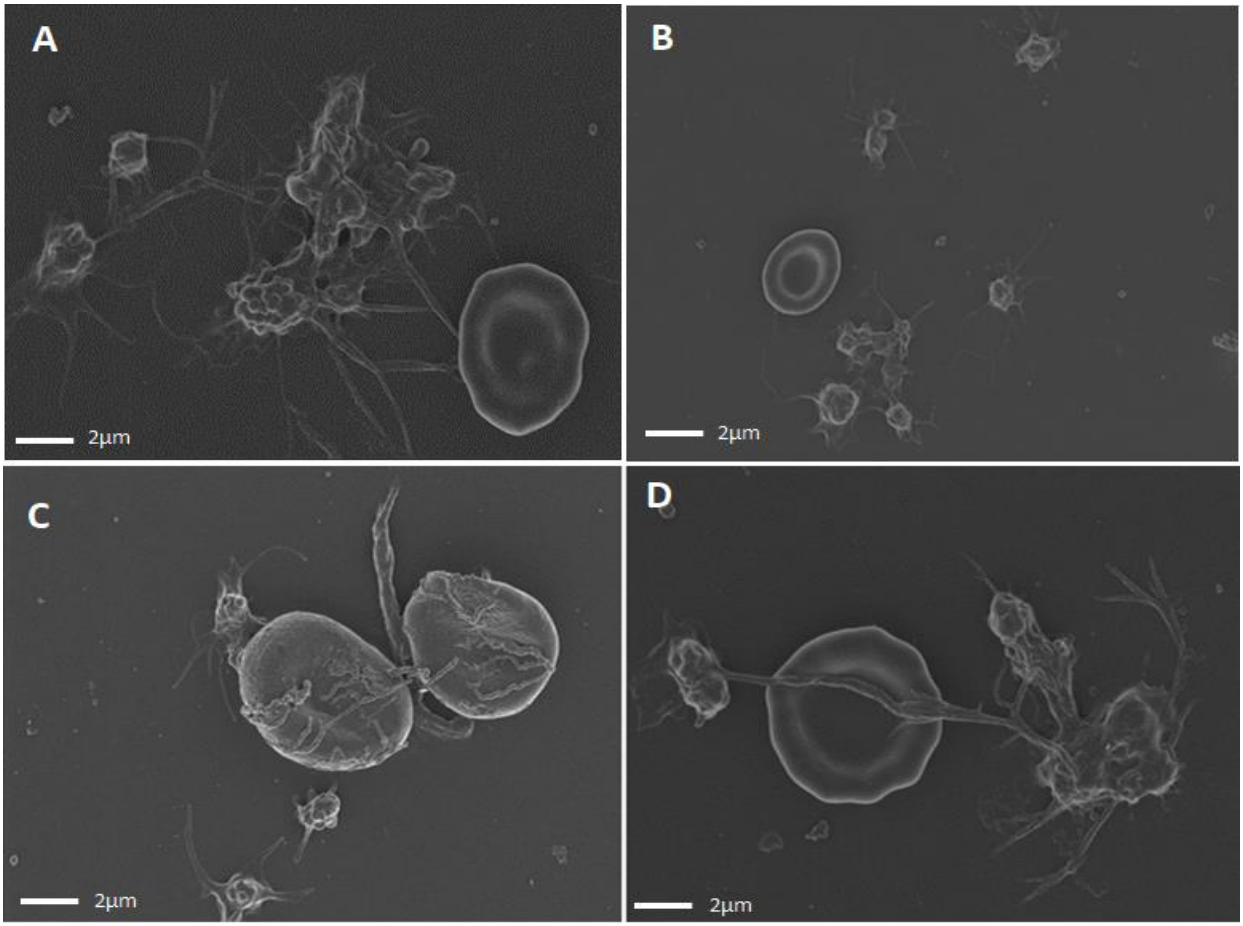


Figure 6. 12. Whole blood incubated with aspirin and LPS for 10 minutes. Here no thrombin was added to WB but fibres are visible in (A and D) masses develop on the surface as more pseudopodia from activated platelets seen. (A-D) RBCs have lost their membrane integrity, (C and D) activated platelets bounded pseudopodia. Scale 2µm

The next section focuses on how RBCs were affected when mixed with LPS and LBP and aspirin.

The effect of aspirin and Lipopolysaccharide binding protein on Lipopolysaccharide in whole blood

Aspirin and WB were incubated prior to incubation with LPS and LBP, the results show surface membrane of RBC smoothen, and clearly the decreased amount of microparticles formation on the surface. See **Figure 6.13.**(**A & B**) showing the decrease in microparticle formation and micrographs **Figure 6.13.**(**C&D**) show the zoomed surface membrane of RBCs from different individuals after exposing their WB to aspirin and LBP and LPS.

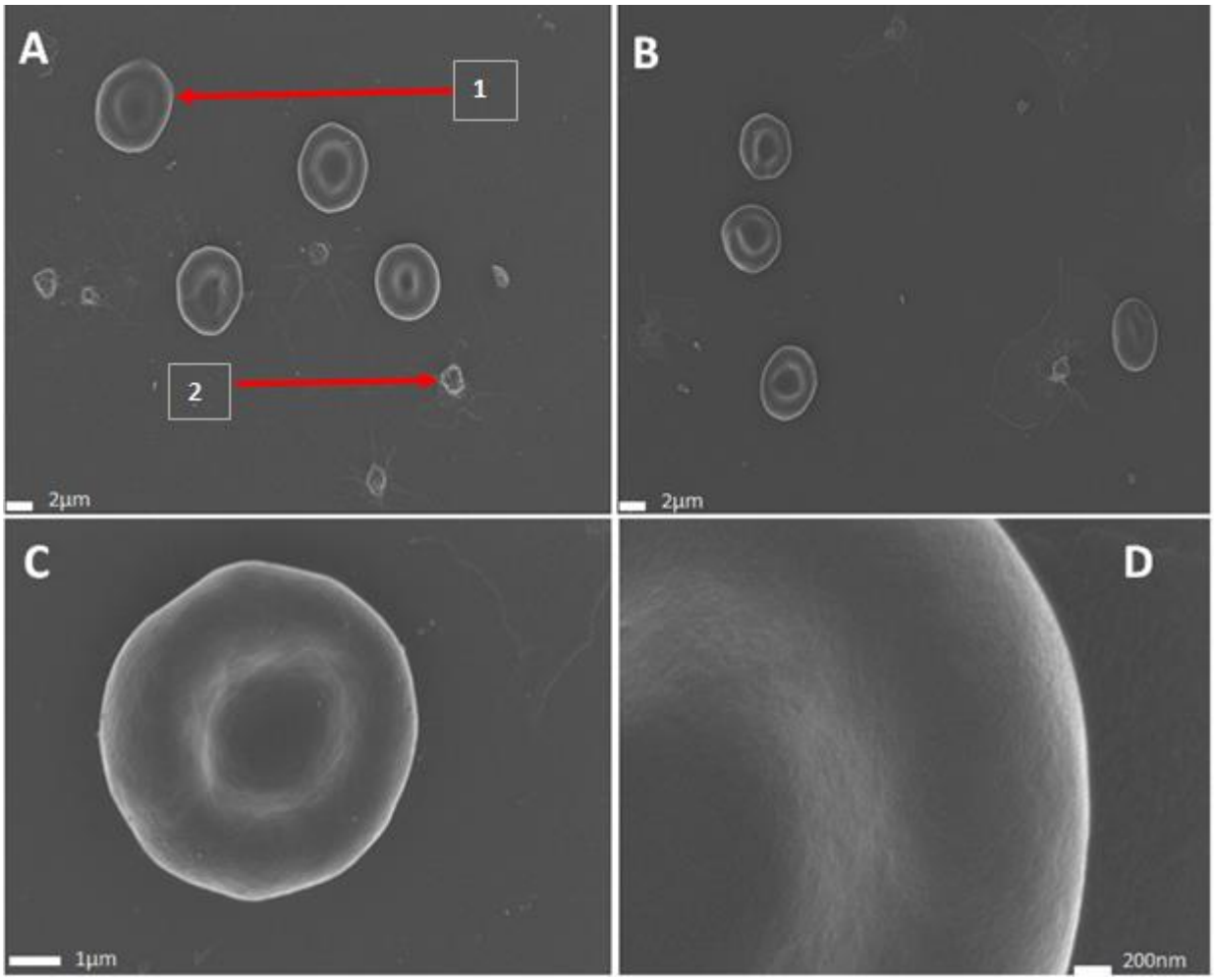


Figure 6. 13. Micrographs showing RBCs after incubating WB with aspirin and mixture (LBP and LPS) incubated for 10minutes. (A and B) looking at RBCs surface membrane and platelets. Red blood cells regain their shape. (1) Red arrow shows RBCs with its original normal shape, (2) red arrow shows activated platelets seen by the pseudopodia Scale 2µm. Micrograph (C) RBC smooth surface membrane, scale 1µm. (D) Surface membrane of RBC higher resolution, showing a smooth surface membrane Scale 200nm.

The next section focuses on how fibrin(ogen) and clot morphology was affected by the same treatments *i.e.* LPS, LBP, aspirin, aspirin and LBP, aspirin and LPS and finally aspirin and LBP with LPS.

Results of scanning electron microscopy of platelet poor plasma

First, the focus was on normal fibrin fibres that has not been treated with any substance except for human thrombin to make the clot. This acted as a health measurement of each participant and also control for each test See **Figure 6.14.** phenotypic normal clot with long branched fibres network.

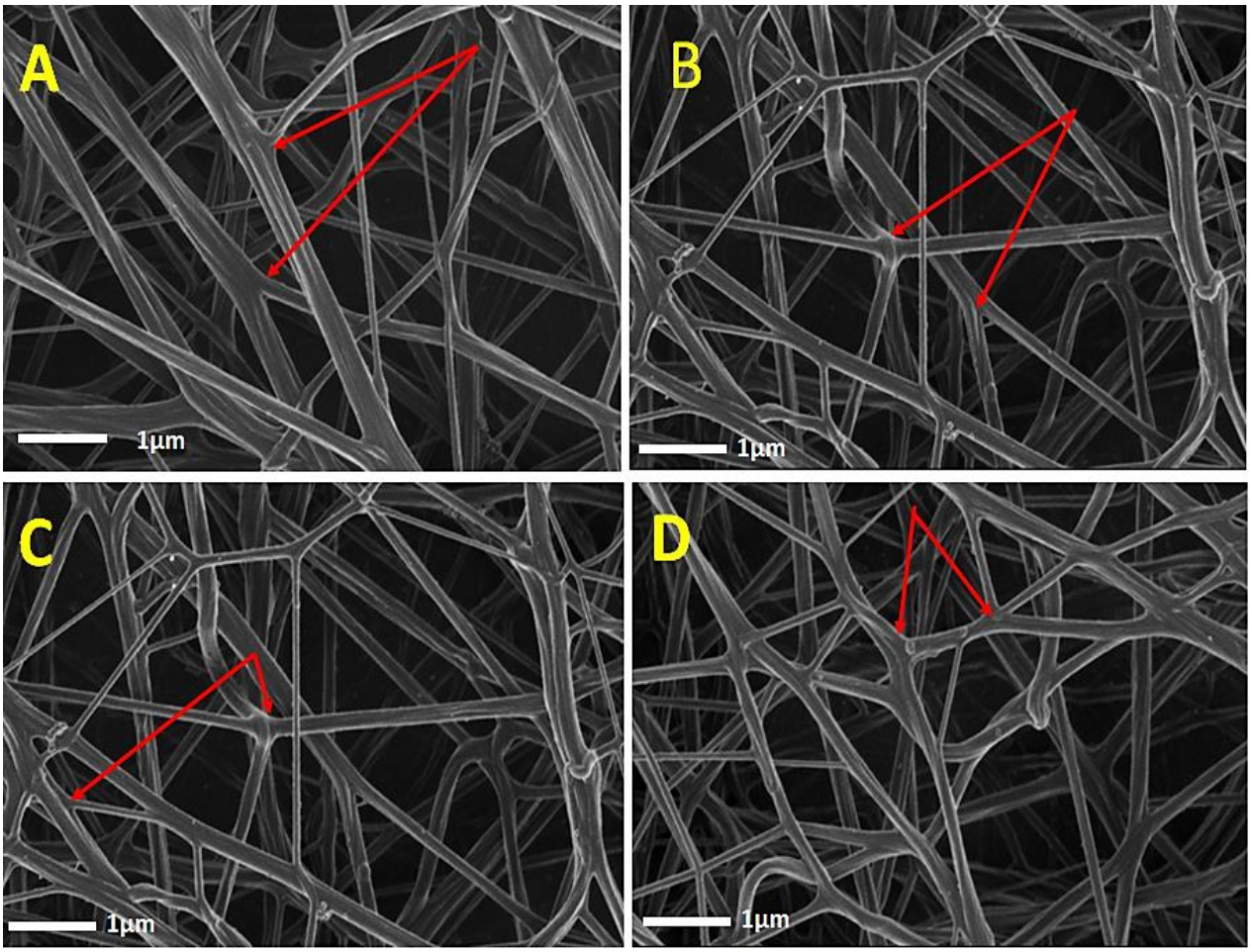


Figure 6. 14. Micrographs of fibrin fibres after incubated for 10 minutes with 5Mm thrombin (A-D) these are normal healthy-looking fibres from different healthy participants. The red arrows display point of branching of the fibrin networks. Scale 1µm for all micrographs above.

The effect of Lipopolysaccharide binding protein incubated with PPP on fibrin fibres and clot morphology

When LBP was added to PPP at a final exposure concentration of 2ng.L^{-1}, fibres that were generated were similar to those seen in PPP untreated samples see, **Figure 6.15.** evenly branched fibrin network of fibers. These micrographs were taken from different individuals.

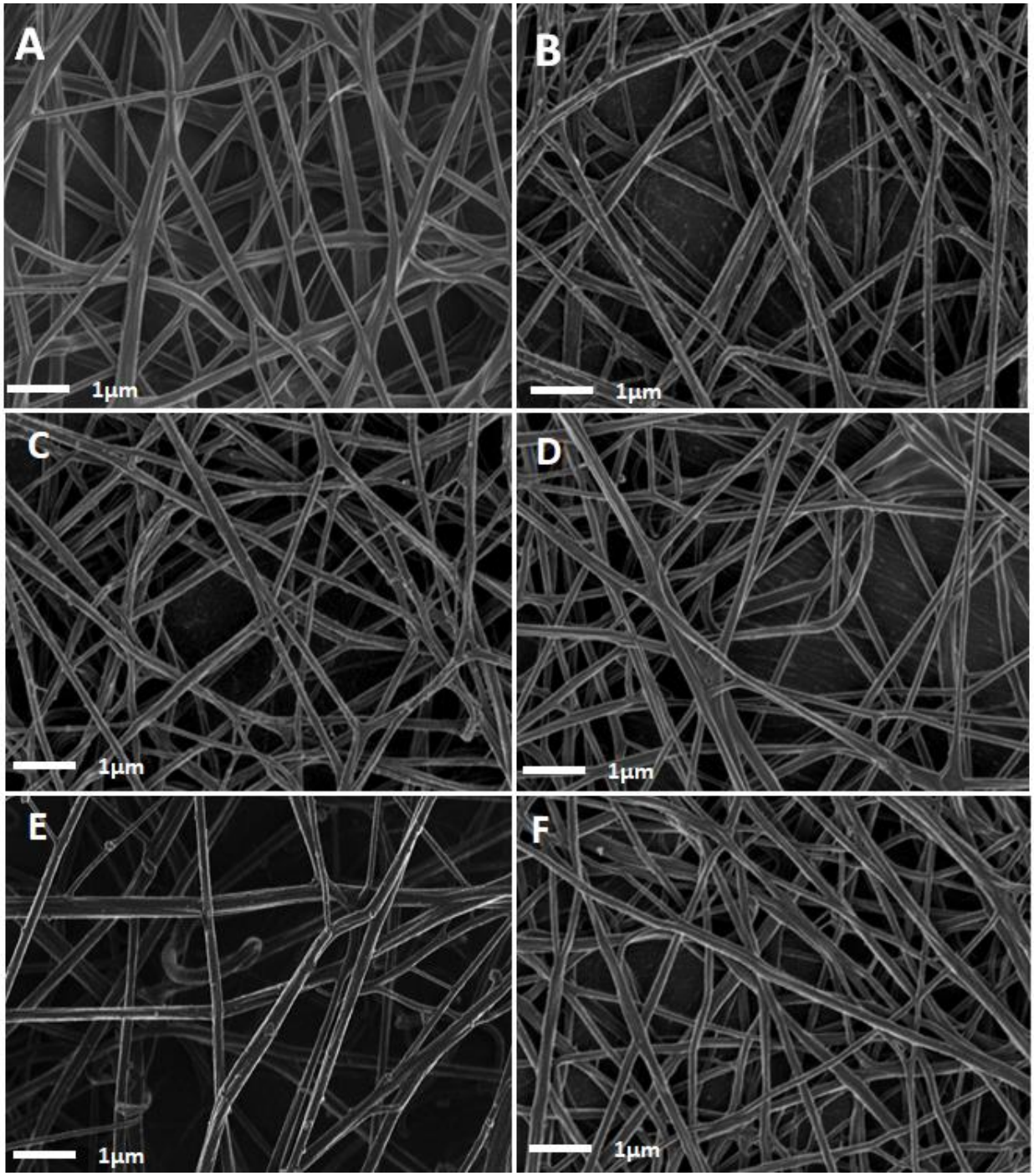


Figure 6. 15. Micrographs of fibrin fibers (A) normal healthy fibers from naïve PPP with added thrombin (B-F) micrographs of PPP with added LBP and thrombin to create a clot, this resembles fibres seen in normal untreated PPP with thrombin. Scale bar 1µm

The effect of Lipopolysaccharide on fibrin fibre formation and clot ultrastructure

When LPS was incubated with PPP the aim was to investigate the effect of LPS on normal healthy clot structure. **Figure 6.16.** shows a clot that was formed after incubating 0. 2ng.L^{-1}. LPS with PPP and added 5µl of thrombin to initiate the clotting process. In **Figure 6.17.** shows fibers of normal healthy individual verse those from the LPS clot structure.

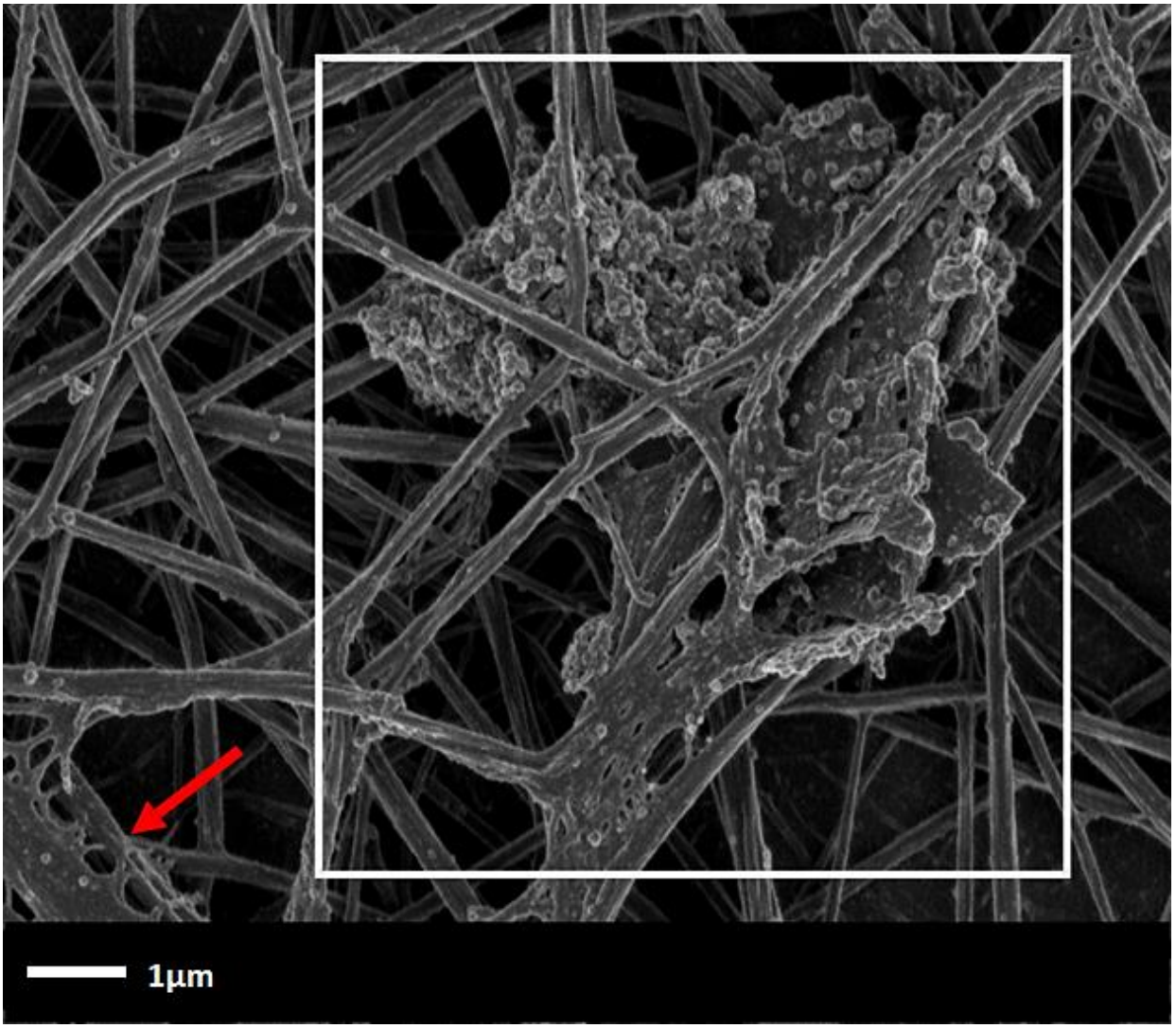


Figure 6. 16. Micrograph of LPS influenced clot, showing irregular fiber formation. The white box indicates the uncharacteristic regrouping and clustering of fibers. The fibers form a matted mass deposit shown by the red arrow, indicating a hypercoagulable state. Scale bar 1µm

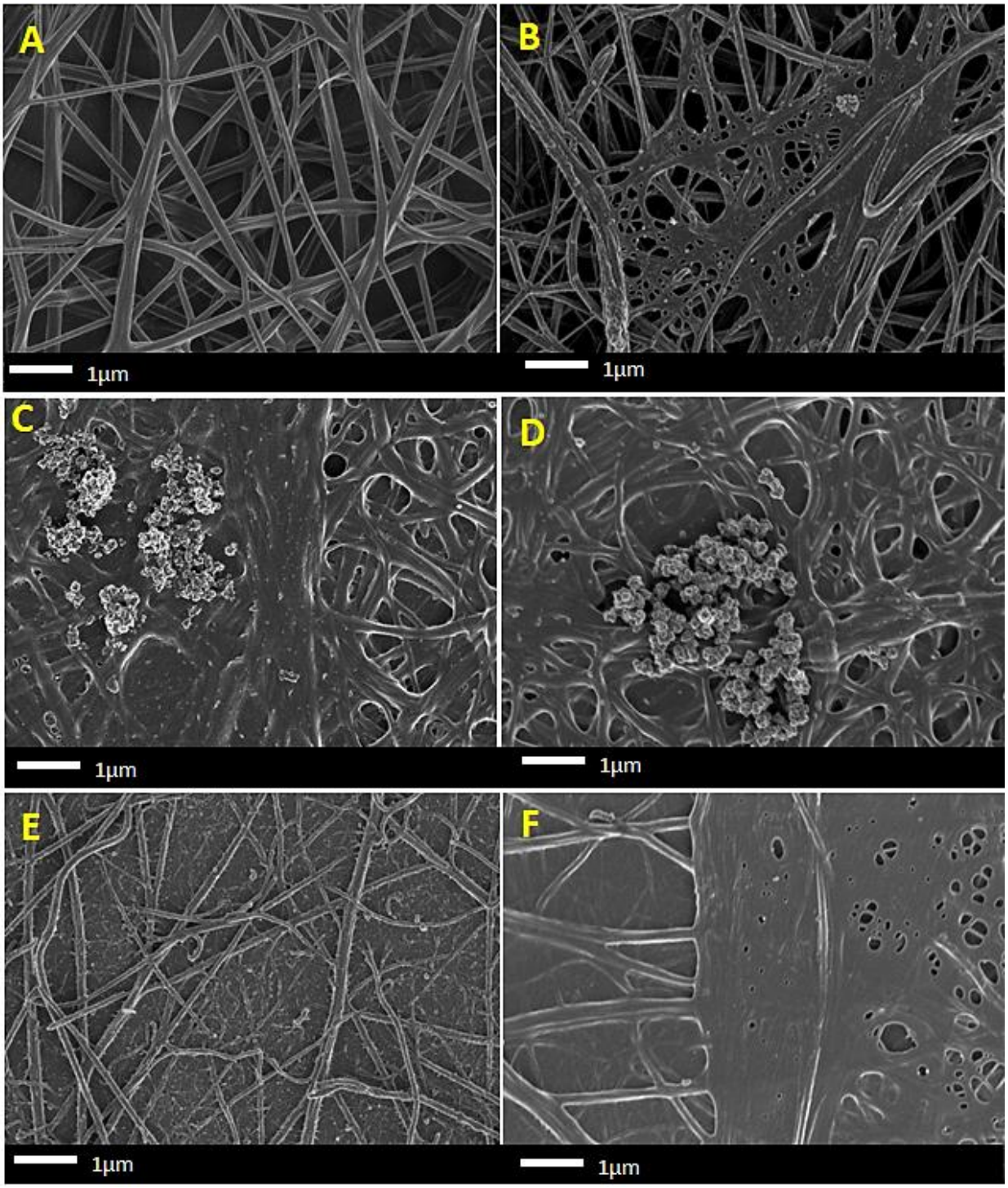


Figure 6. 17. Micrographs of clots formed (A) naïve PPP with added thrombin to create a clot, showing healthy individual fibers. (B-F) PPP treated with final exposure concentration of 0,2ng.L^{-1} LPS, visible clear formation of pathological clots. Different clots resemble different clotting pattern in different individuals. Scale bar 1µm

It is seen also that LPS can cause of fibrin fibers to decrease or increase in width see **Figure 6.18.** a variety of thin fibers resulted after incubating PPP with LPS.

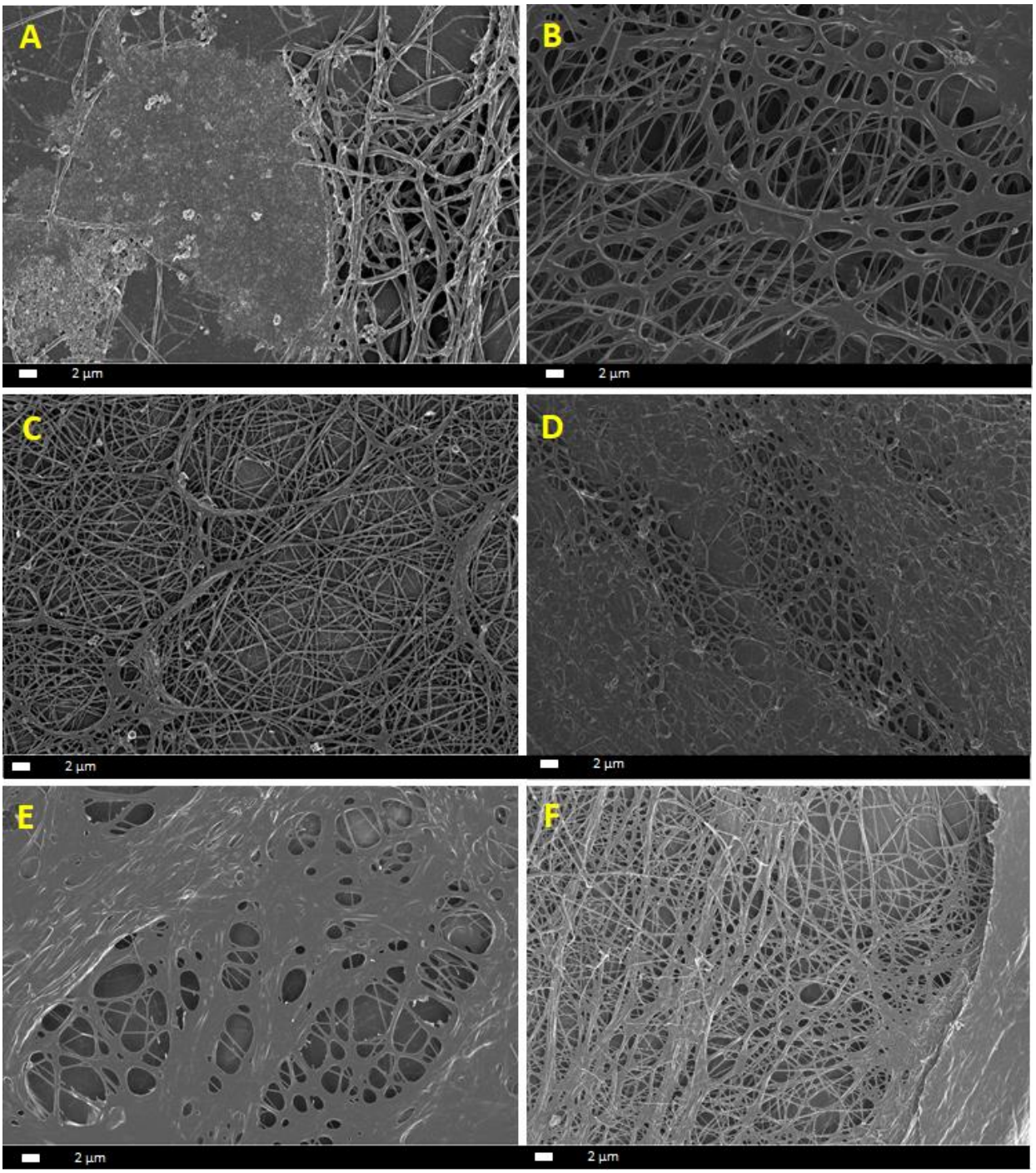


Figure 6.18. Micrographs of thin fibers after incubation with LPS. All micrographs were taken at 10.0k magnification and scale bar is 2µm.

The next section focuses on how LBP and LPS affect fibrin fibre formation and fibre clots' ultrastructure.

The combined effect of Lipopolysaccharide binding protein and Lipopolysaccharide on fibrin fibre formation and clot morphology.

With the following investigation the focus was on how LPS will affect the fibrin fibers formation and clot ultrastructure when LBP is present. The study hypothesis was that LPS will bind to LBP and hence reduces the amount of LPS binding to fibrin(ogen) hence reduces inflammatory symptom. **Figure 6.19.** shows fibrin fiber formation after incubation with the LPS and LBP along with the addition of thrombin to create fiber network.

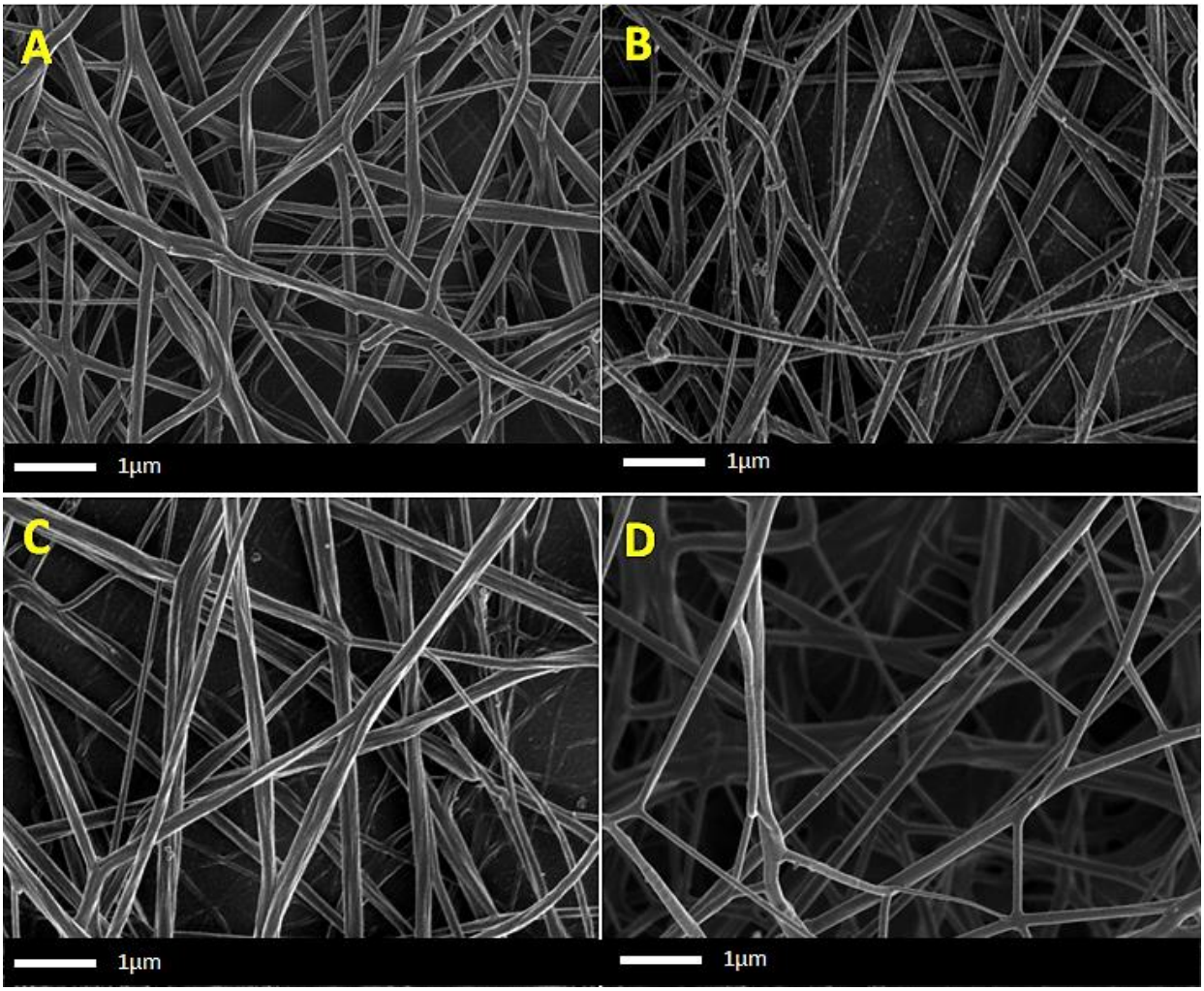


Figure 6. 19. Micrographs (A-D) LBP together with LPS mixed prior to adding to PPP of healthy individuals. These were taken from different individuals. Scale bar 1µm

The following section will then look at the effect of physiological levels of aspirin on the clotting of PPP.

Investigating the effect of aspirin on fibrin fibre formation and ultrastructure of the clot

To study the protective role that aspirin plays against LPS firstly 0.5mM final exposure concentration levels of aspirin were tested to see if it will cause any damage to the cell involved in clotting process. Below in **Figure 6.20.** micrographs of fibrin fibers taken from naïve PPP with added thrombin and fibres taken from samples that had PPP incubation with aspirin and added thrombin.

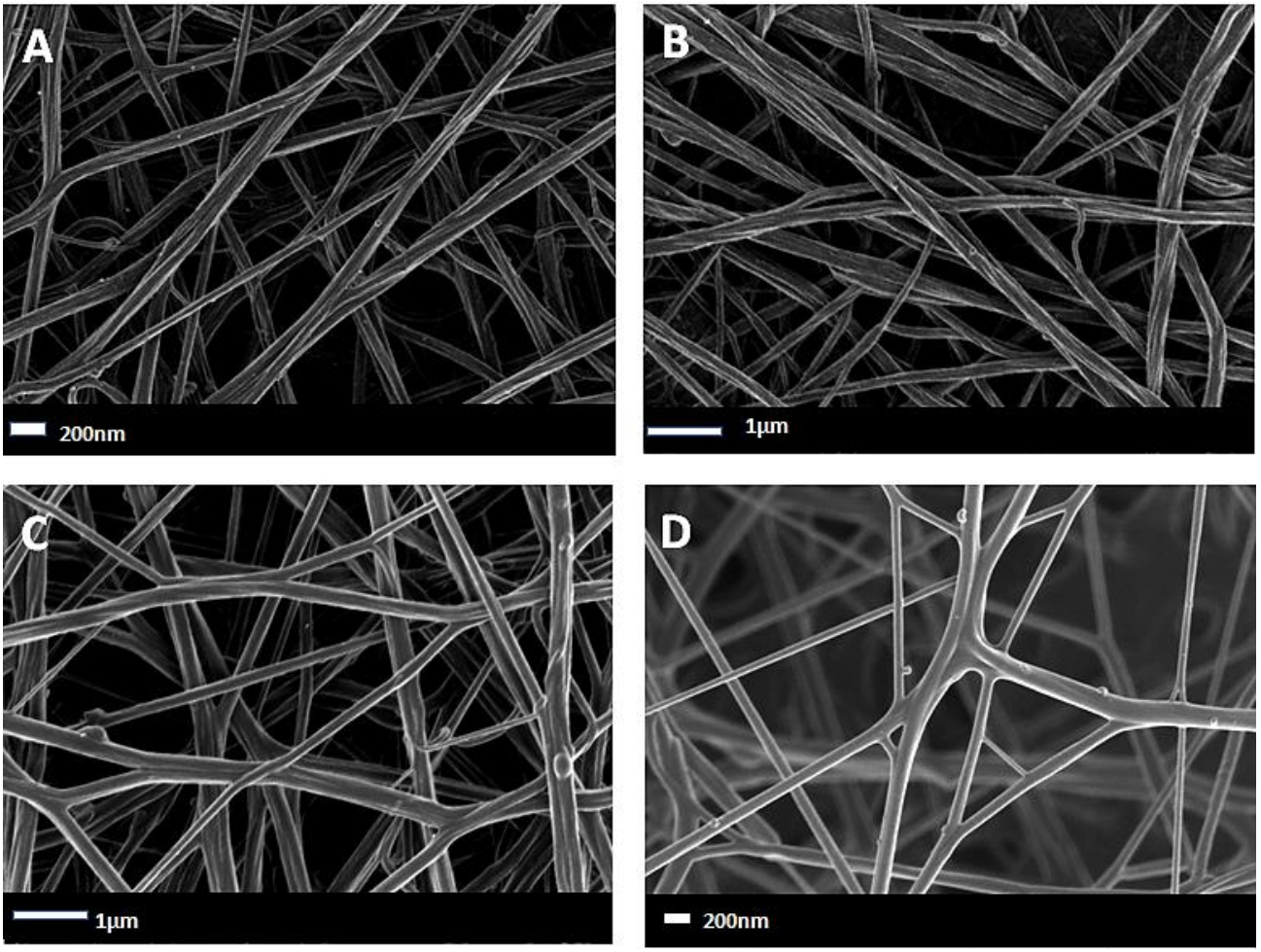


Figure 6. 20. Micrographs of PPP after incubating with 5µl of thrombin to activate clotting (A) shows normal fibrin fibres Scale bar 200nm. For direct verses micrographs (B,C,D) show healthy individuals' plasma after treating PPP with aspirin the scale bar in (C) 1µm and (D) 200nm respectively.

Below are results obtained when 0. 2ng.L^{-1} LPS (final exposure concentration) was incubated with low physiological levels of (0,5mM) aspirin and LBP.

The effect of aspirin with Lipopolysaccharide binding protein on clot and fibres formation

Furthermore, aspirin was incubated with PPP followed by mixing with LBP for 10 minutes. **Figure 6.21.** shows fibrin fibres before and after treatment with aspirin and LBP. These micrographs were taken from different individuals.

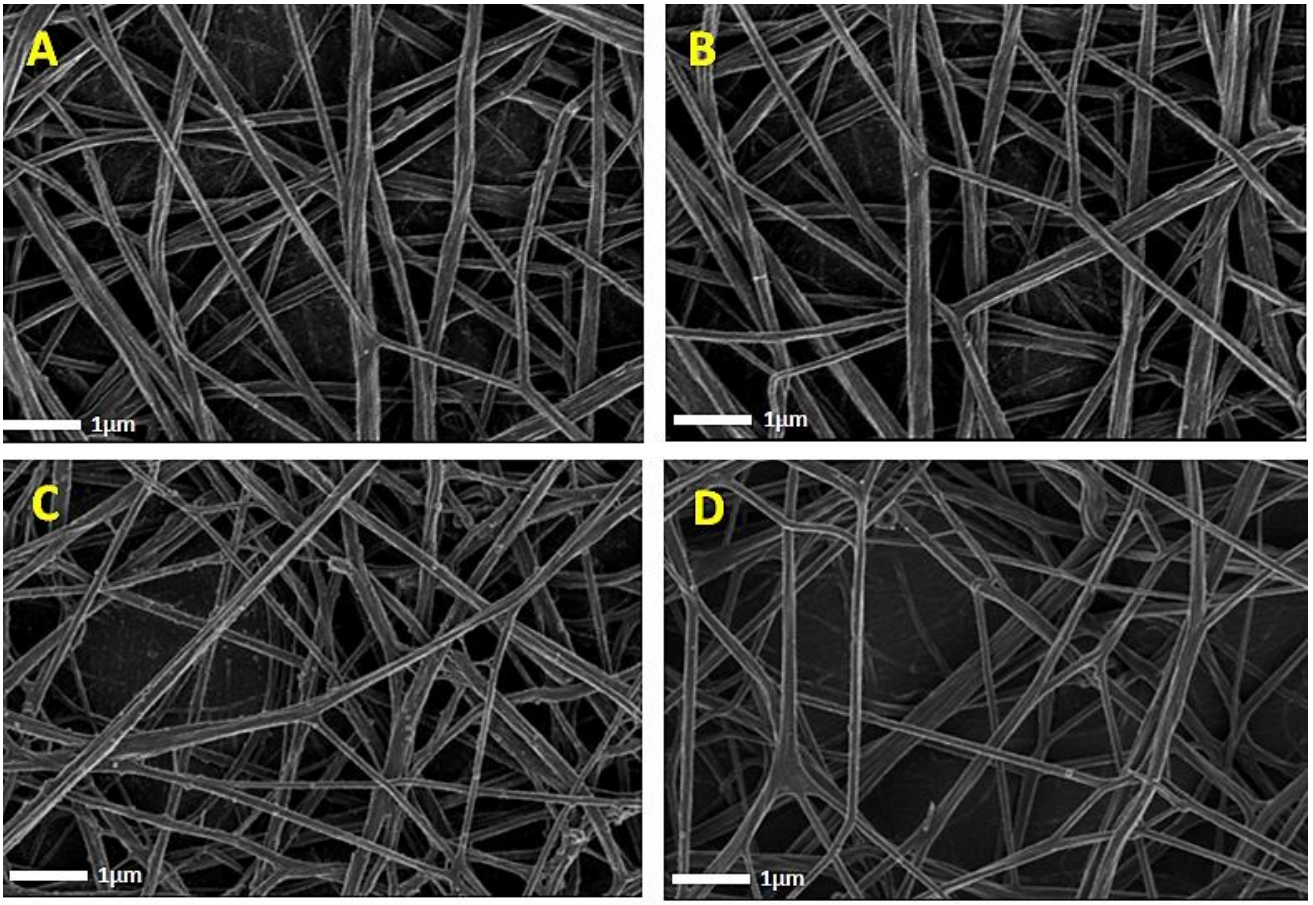


Figure 6. 21. Micrographs of aspirin incubated with LBP which was similar to PPP micrographs of untreated PPP and those from PPP treated with aspirin. (A-B) normal fibres fibrin, (C-D) PPP with aspirin and LBP, fibres are branched out venlywith clear vision of fine fibres. Scale bar 1µm

The effect of aspirin incubated with LPS is revealed in the next section.

The effect of aspirin on hypercoagubility induced by Lipopolysaccharide on plasma

It is known that aspirin plays a role in anti-platelets aggregation, and anti-inflammatory here the next section of this thesis is to investigate the effect aspirin might have on the inflammatory LPS in fibrin(ogen). The **Figure 6.22.** below show how LPS and aspirin play a role in clot formation.

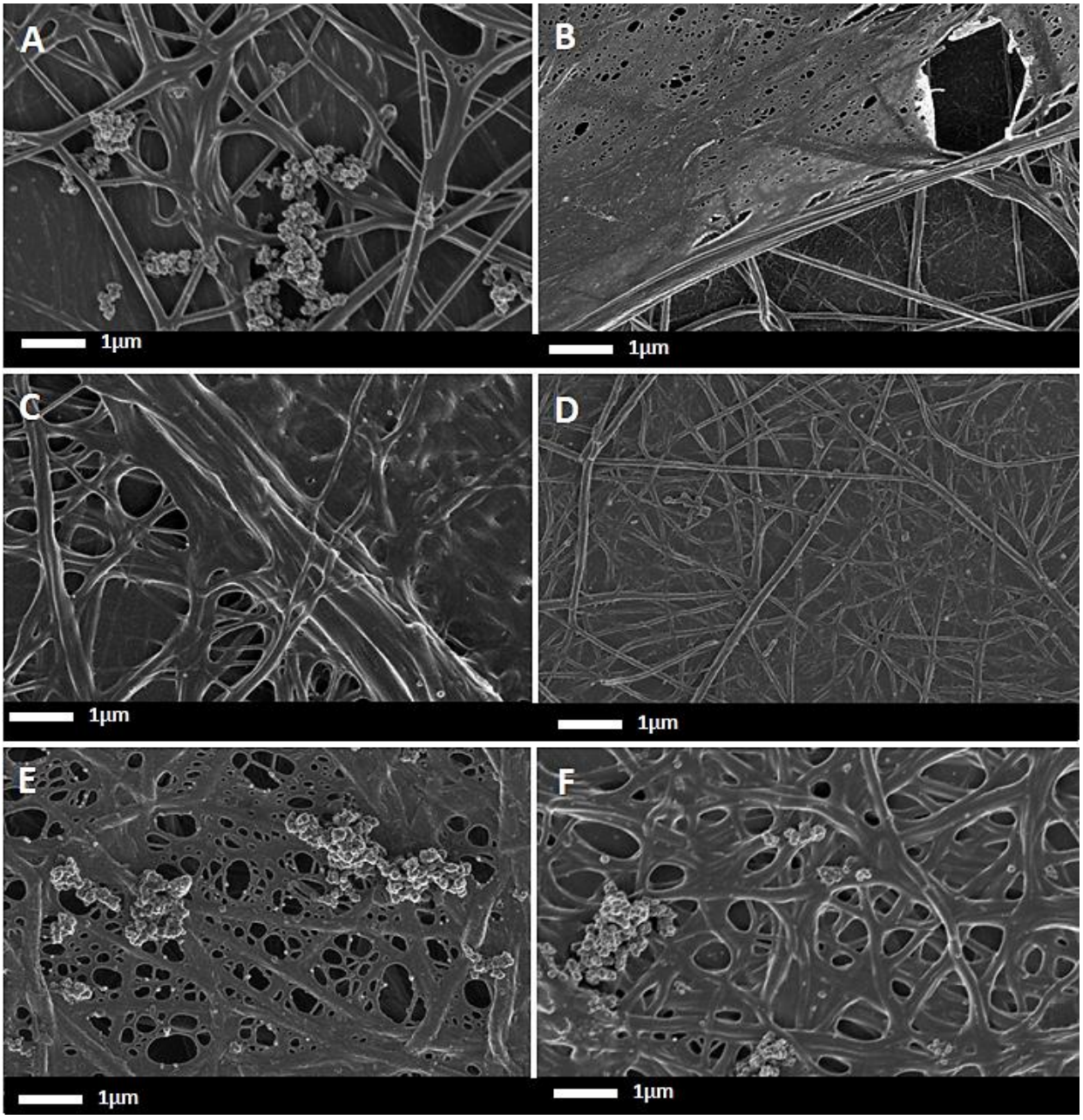


Figure 6. 22. Micrographs of (A-E) different PPP from controls that were incubated with aspirin mixed with LPS, (F) micrograph of PPP that was incubated with LPS, showing the same inflammatory patterns as when aspirin was added to LPS and PPP. Scale bar 1µm

Figure 6.23. shows the differences seen between these two groups of samples that is LPS treated PPP clot verses aspirin incubated with LPS treated PPP clots.

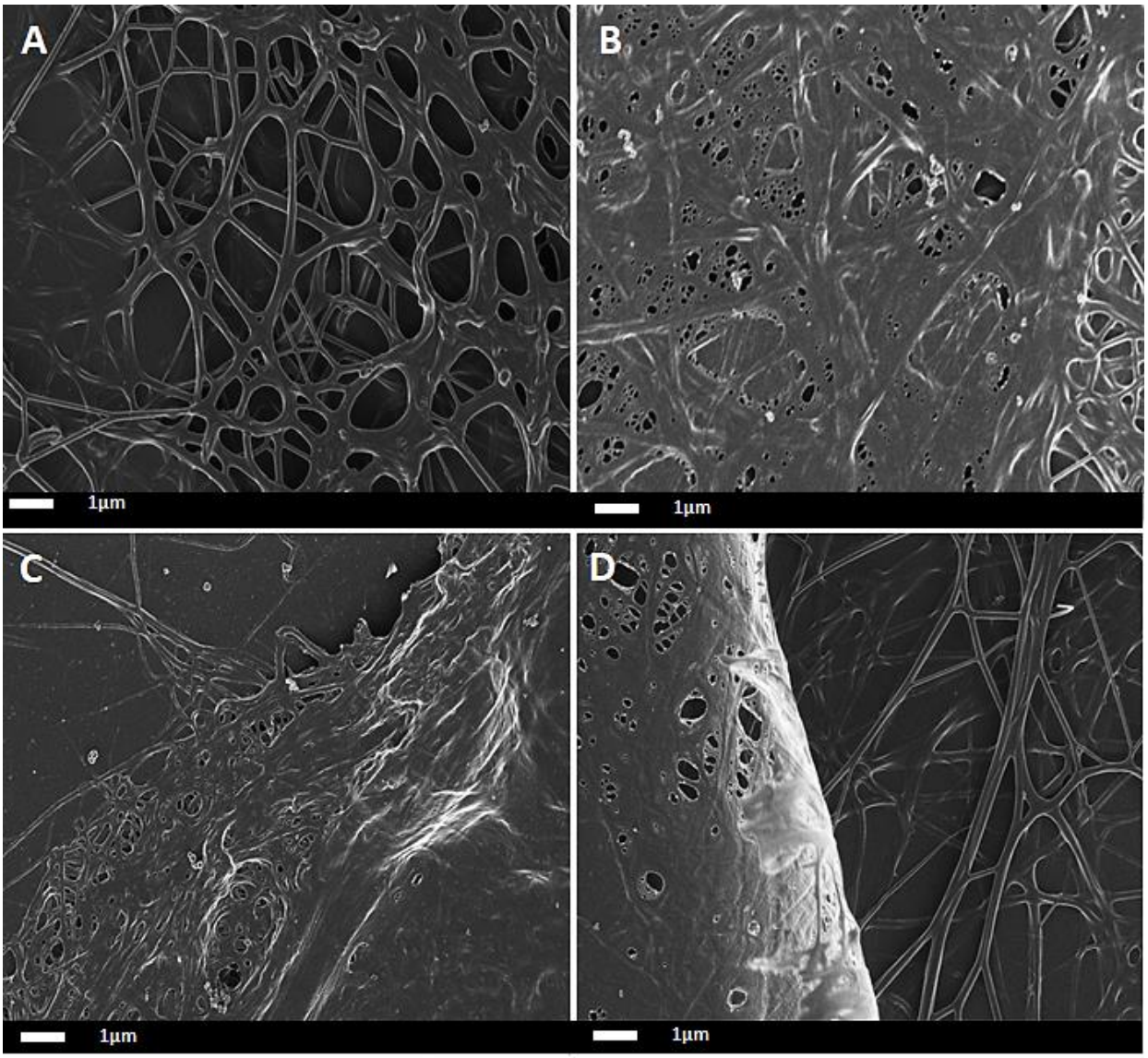


Figure 6. 23. Micrographs of fibrin fibres clots (A-B) Fibrin fibres from PPP incubated with LPS with added thrombin, unbranched fibrin fibres thick mass of fibres stuck onto the surface. (C-D) micrographs showing fibres when aspirin was incubated with LPS and PPP. The clot morphology is similar, a highly dense clot structure result. Scale 1µm

Next section focuses on the clot ultrastructure when PPP was incubated with aspirin and the mixture (LBP together with LPS).

The effect of aspirin and Lipopolysaccharide binding protein and Lipopolysaccharide on fibres formations and nature of platelets poor plasma clot

Our aim for this study was to investigate the effect of aspirin in combination with LBP on the formation of the clot, structure and viscoelastic properties of the clot formed. Thus, the (H_0) hypothesis states that LPS might be inhibited or mobbed up by the action of aspirin and LBP. In the **Figure 6.24.** shows just how the LBP together with aspirin acted together to reduce the inflammation induced by LPS.

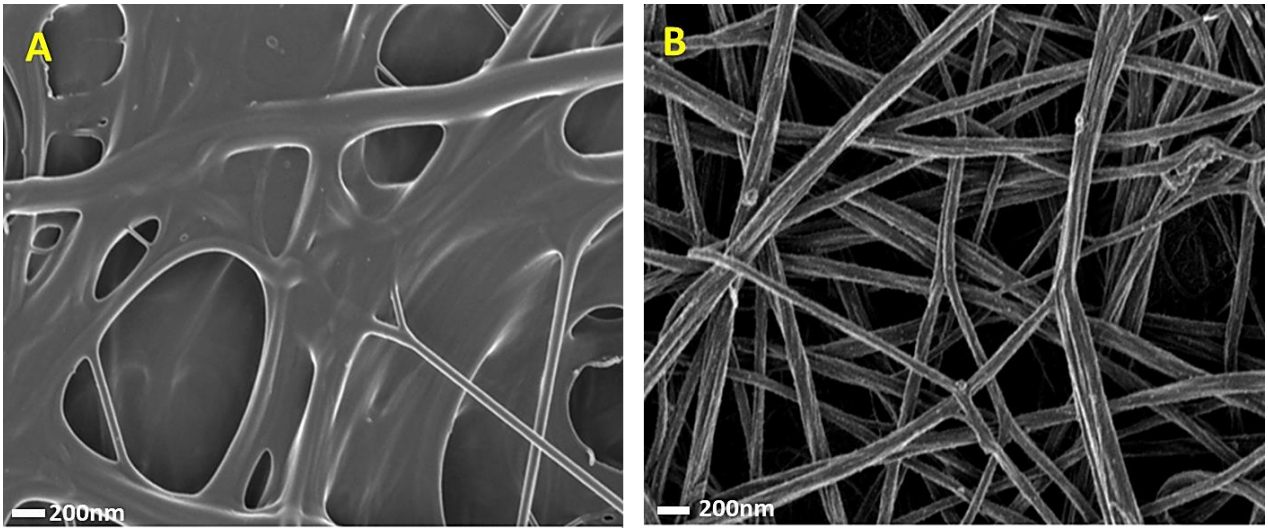


Figure 6. 24. Micrographs of PPP incubated with (A) small concentration of 02.ng. L^{-1} LPS, creating a dense hypercoagulable clot. (B) shows how the dense thick clot structure is reversed when we add LBP and aspirin to PPP. Scale 200nm

The following of micrographs were taken of PPP **Figure 6.25.** clots after incubating with LBP, LPS and aspirin.

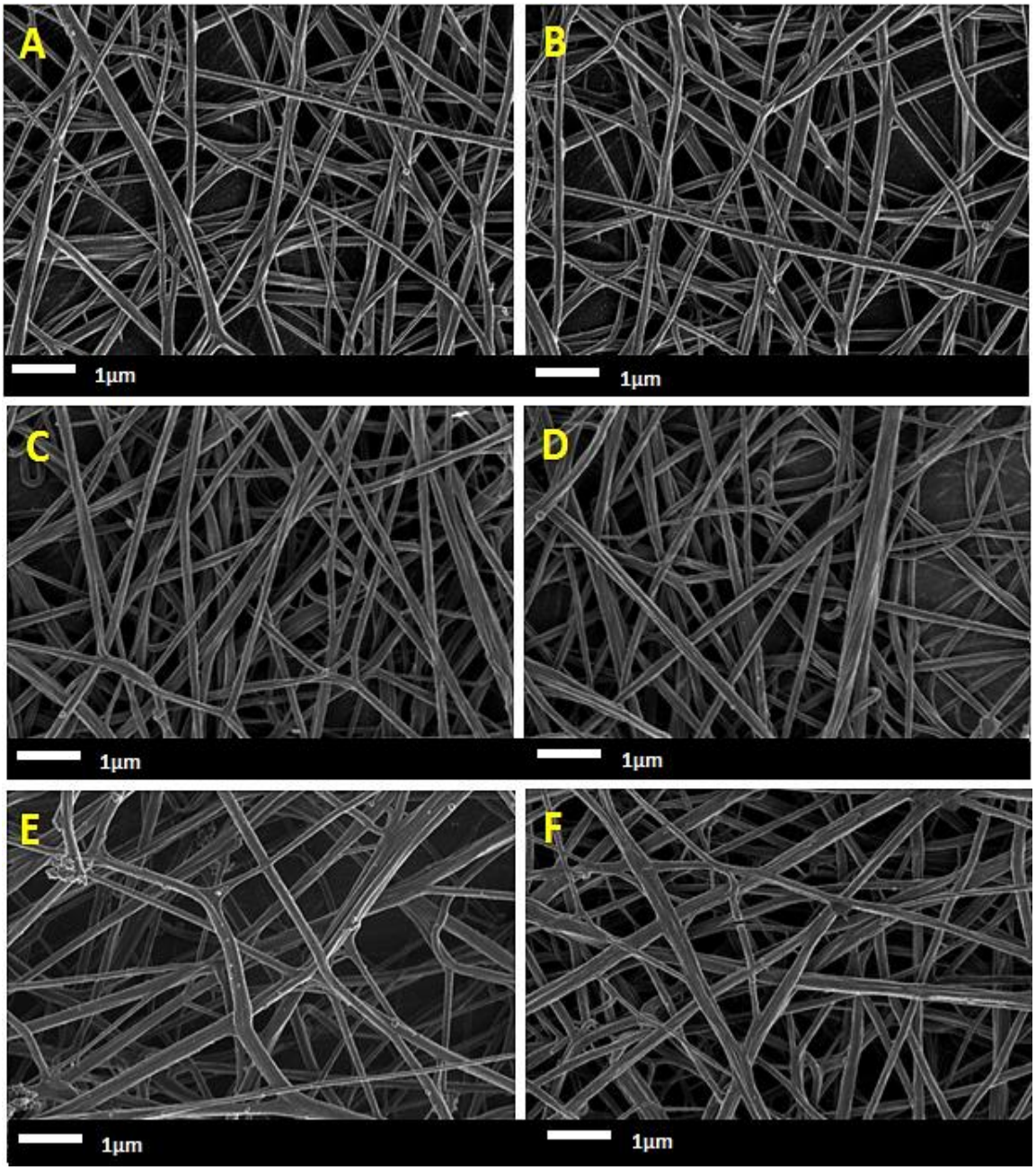


Figure 6. 25. Micrographs of PPP clots after incubating with physiological levels of aspirin and LBP together with LPS. Scale 1µm

Histogram analysis

The SEM micrographs of fibrin that were analysed were taken at X 35K and 1 kV and analysed for 8-bit pixel intensity using the histogram function of FIJI. These histograms measure the average distribution of grey and black pixels per 8-bit area in an image which can then be used to calculate the coefficient of variation. In other words, the variation between grey (representing fibrin) and black (areas devoid of fibrin or between fibrin strands) in the image as an indicator of overall density.

Coefficients of variation were analysed by means of parametric and non-parametric two tailed t tests with significance defined as $\alpha = 0.05$ (CI = 95 %) using GraphPad Prism 8 software. Following analysis, it was found that the PPP incubated with LPS generated clots that were slightly denser with fibrin fibres compared to healthy untreated naïve controls, ($P < 0.0001$) see **Figure 6.26.** for histograms taken from PPP naïve, LBP, mixtures of LBP with LPS and **Figure 6.27.** all the listed treatments mixed individually with aspirin.

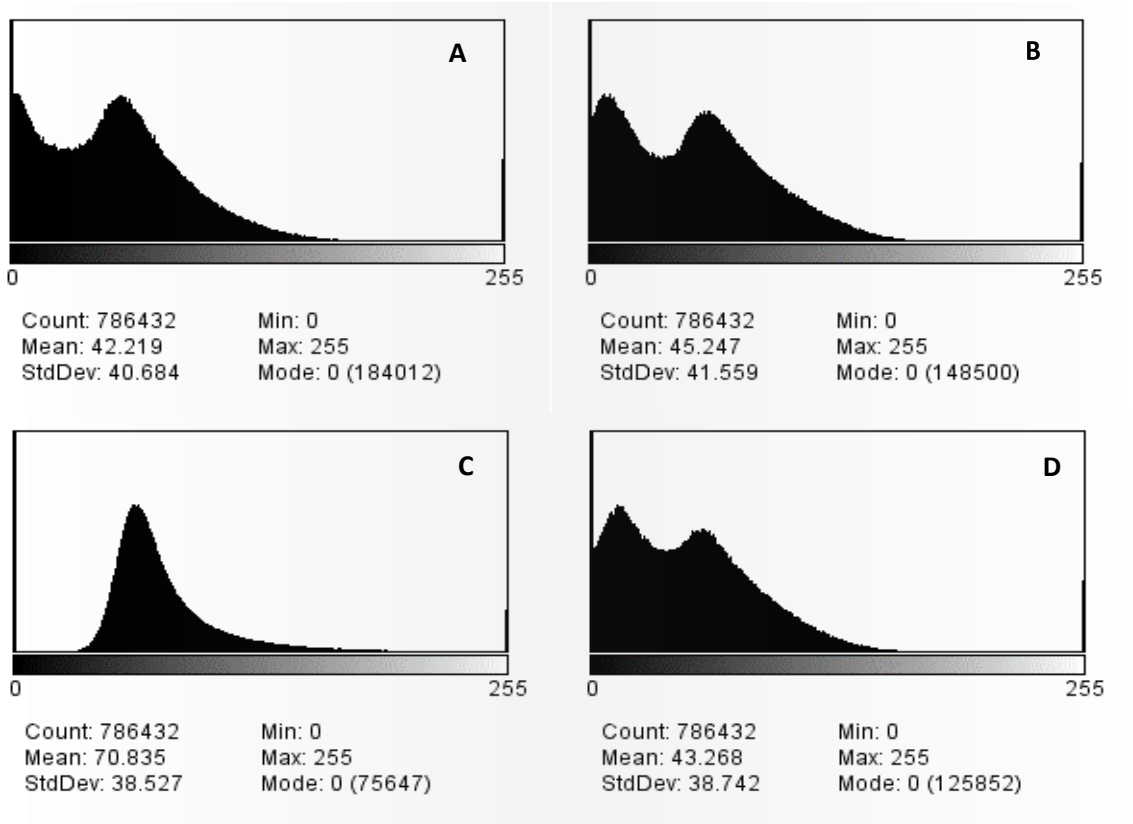


Figure 6. 26. Histogram the average distribution of grey and black pixels per 8-bit area (A) naïve PPP with no treatment, (B) PPP incubated with LBP, (C) LPS incubated with PPP (D) PPP incubated with LPS mixed with LBP

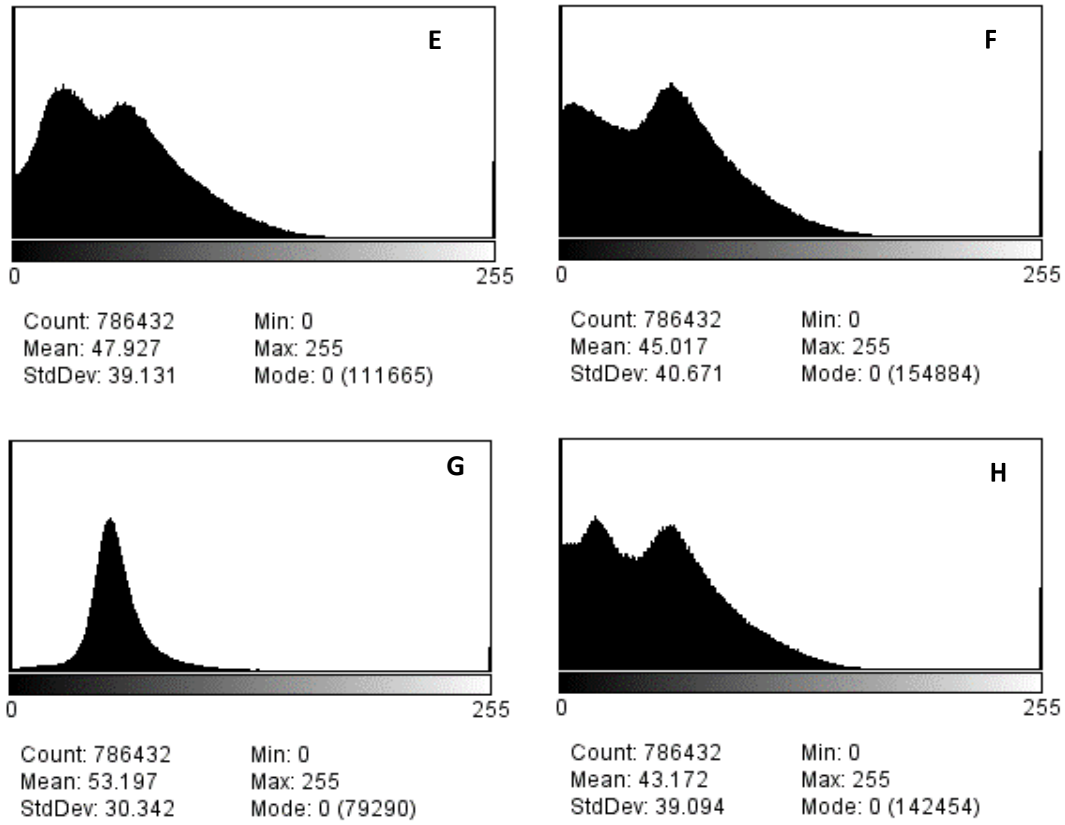


Figure 6. 27. Histogram the average distribution of grey and black pixels per 8-bit area (E) naïve PPP with low physiological levels of aspirin final exposure concentration of 0.5mM, (F) PPP and aspirin incubated with LBP, (G) aspirin incubated with LPS (H) PPP and aspirin incubated with LPS mixed with LBP

Table 6. 1. Coefficient of Variance from Scanning electron microscopy histogram analysis

	CV: controls	CV: PPP + LBP	CV: PPP + LPS	CV: LPS + LBP mix	CV: aspirin	CV: aspirin + LBP	CV: aspirin + LPS	CV: aspirin + LPS and LBP
(N) Total	147	147	147	147	147	147	147	147
Minimum	0,4620	0.4830	0.3924	0,4445	0,4583	0,3696	0.4693	0.383
Maximum	0.6304	0.6522	0.4824	0.6045	0.6704	0.6144	0,5387	0.6330
Range	0.6876	0.7696	0,5289	0.7227	0,8299	0.7689	0,6544	0.7279
Mean	0.8168	0.8542	0.5956	0,8315	0.8873	0.9235	0,9095	0,8116
Std. Deviation	0.9668	1.052	0.8257	1,093	1.056	1.203	0,5693	1.043
Std. Error of	0.7137	0,7554	0.5444	0.798	0.7797	0.7713	0.1181	0.7254

Table 6.2. Show**s** multiple comparison of CVs from each data set comparing control (naïve) to each treatment column and followed by **Figure 6. 28.** The 8 box plots of all the SEM's CVs from all treatments used in the investigation

Table 6. 2. Dunnett's multiple comparison of all the Coefficient variation from Scanning electron microscope histogram analysis

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	95% CI of diff
PPP vs LBP	-0.04175	2.920	Yes	-0.07923 to -0.004271
PPP vs LPS	0.1693	11.84	Yes	0.1318 to 0.2068
PPP vs LBP + LPS	-0.006151	0.4303	No	-0.04363 to 0.03133
PPP vs Asp	-0.06605	4.620	Yes	-0.1035 to -0.02857
PPP vs Asp + LBP	-0.05765	4.033	Yes	-0.09513 to -0.02017
PPP vs Asp + LPS	0.1444	10.10	Yes	0.1069 to 0.1819
PPP vs Asp + LBP + LPS	-0.01172	0.8201	No	-0.04920 to 0.02576
ANOVA table	P value			
Treatment (between columns)	P<0.0001			
Individual (between rows)	P<0.0001			

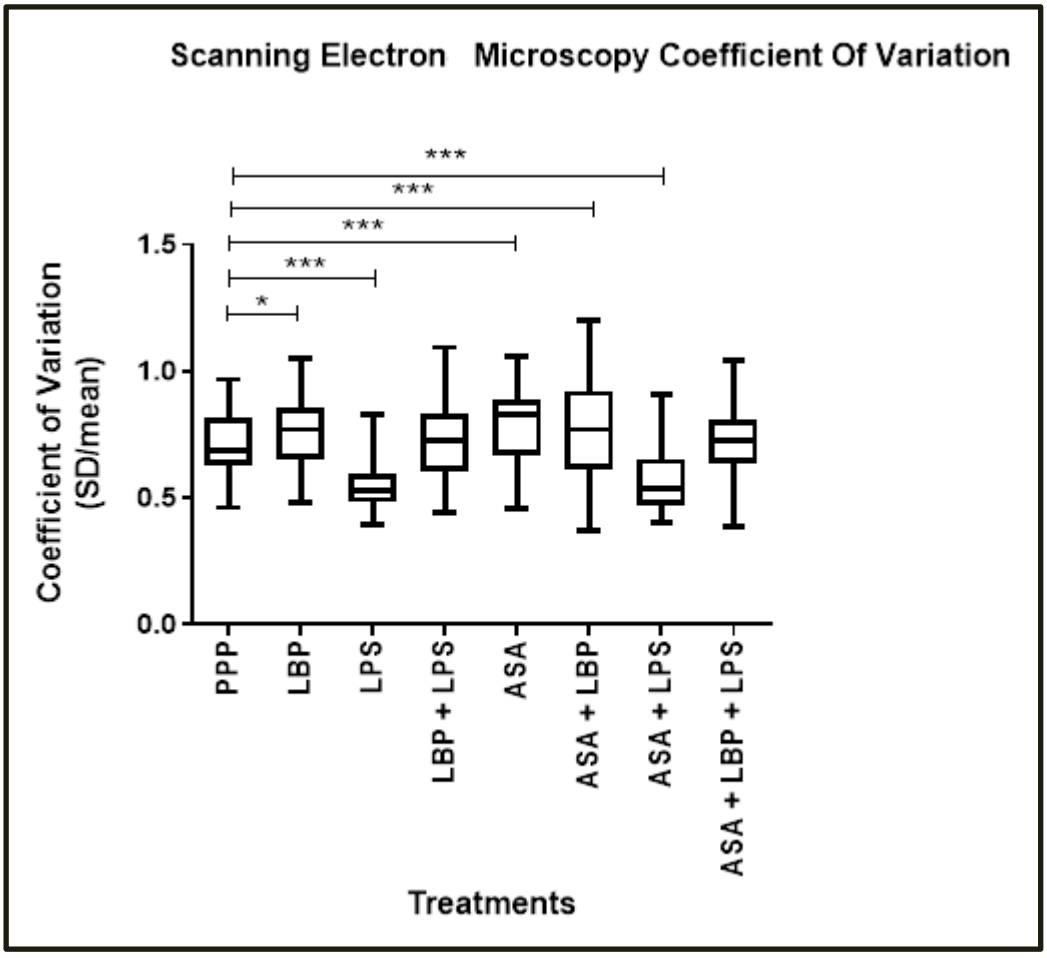


Figure 6. 28. The coefficient of variation calculated from data obtained from the histogram analysis shows a distinct alteration in the clots from samples treated with LBP, LPS, ASA, ASA +LBP and ASA +LPS compared to the control (p<0.0001) following a repeated measures ANOVA and Dunnet's multiple comparisons test.

Ultrastructure correlation of fibre network using SEM and Confocal microscopy

Confocal results were correlated with the SEM results histogram and ultrastructural changes to draw a conclusion of the differences in fibres formation after treatments. The following figures (**Figure 6.29– Figure 6.36**.) are used strategically to generate an understanding of how the results can be used to prove the hypothesis.

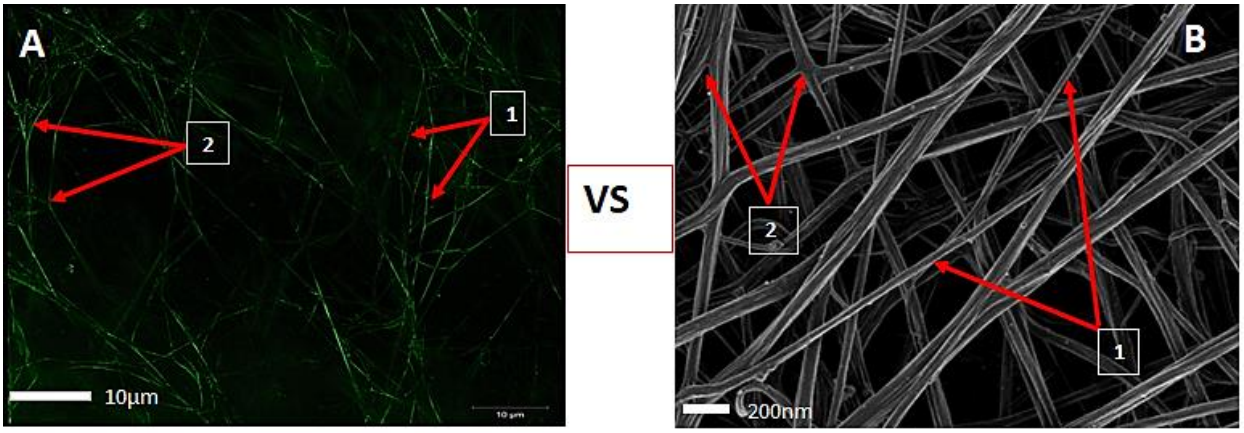


Figure 6. 29.(A) Confocal image of clot created by naïve (untreated) PPP verses (VS) image (B) of clot from SEM. Both these images show healthy normal looking network of fibres. Arrows (1) Individual fibrin fibre (2) branch point of fibres

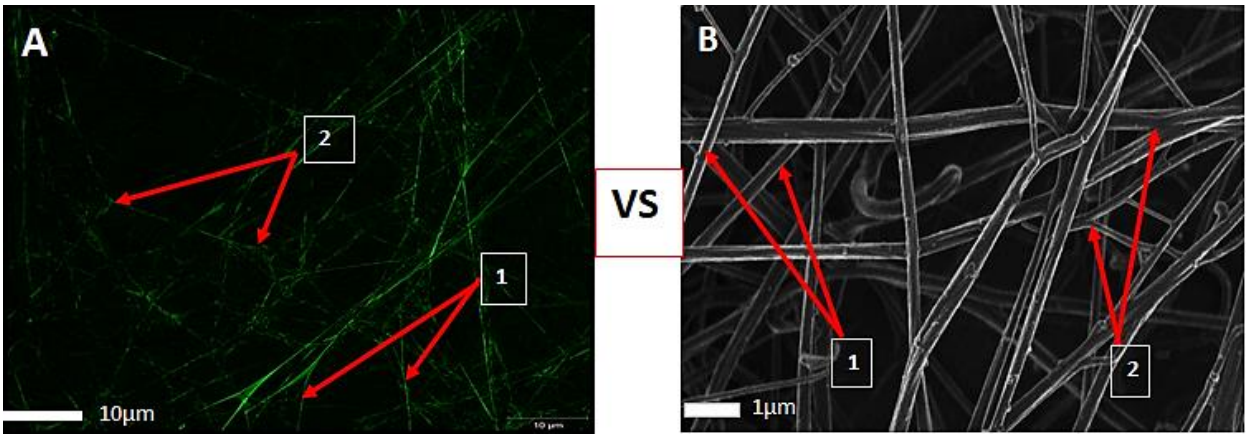


Figure 6. 30.(A) Confocal image of fibre clot formed of PPP treated with LBP verses (VS) SEM image(B) clot of fibres made from PPP mixed with LBP. Thin evenly branched fibres on both micrographs. Arrows (1) Individual fibrin fibre (2) branch point of fibres

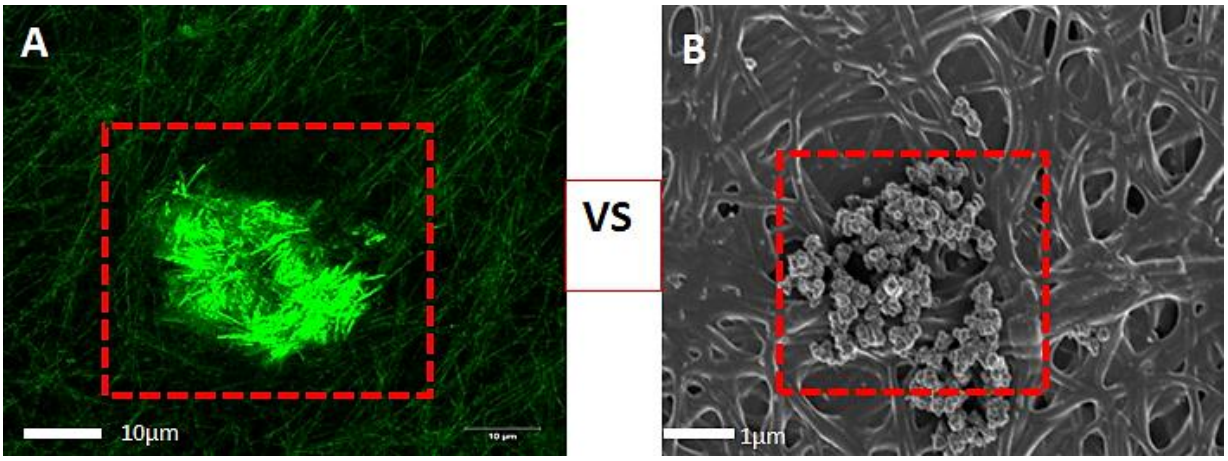


Figure 6. 31.(A) Confocal image of fibre clot formed of PPP treated with LPS. The red dotted highlighted squares indicate the amyloid β-sheets, intensely fluorescence with amyloid marker Tht verses (VS) SEM image(B) clot of fibres made from PPP mixed with LPS. Red square (A) shows highly dense fibres mashed up (B) fibres high dense area with dense matted deposits.

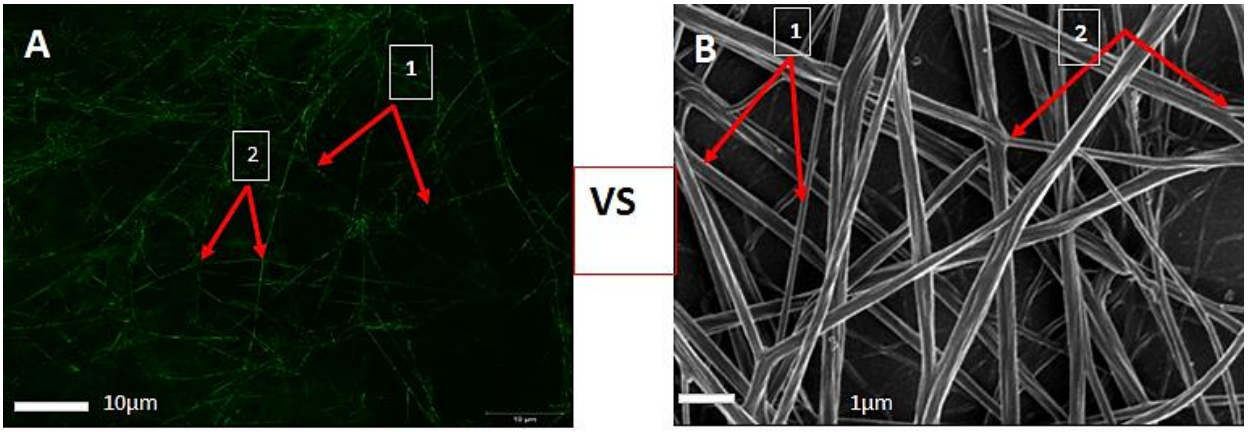


Figure 6. 32.(A) Confocal image of clot created by PPP incubated with LPS mixed with LBP verses image (B) of clot from SEM of fibres made from PPP with LPS and LBP Both these images show healthy normal looking network of fibres. Arrows (1) Individual fibrin fibre (2) branch point of fibres

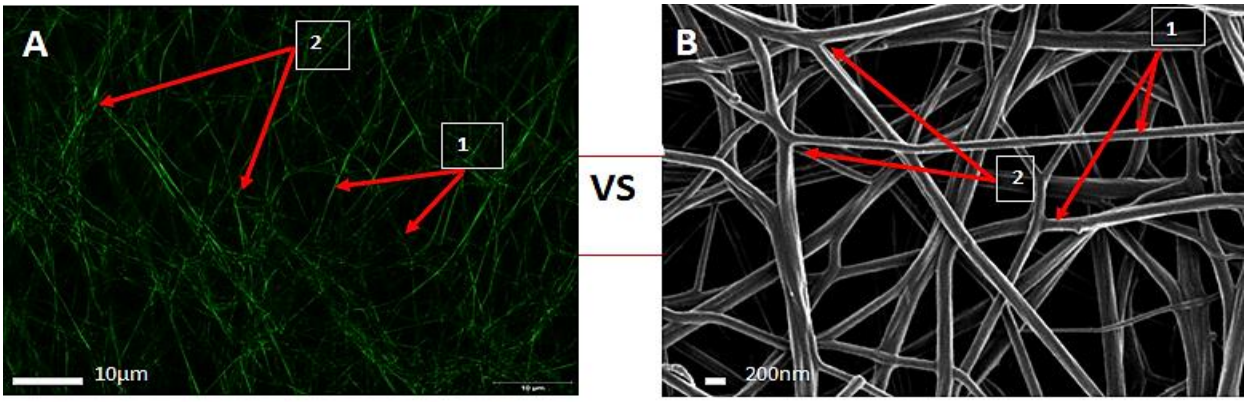


Figure 6. 33.(A) Confocal image of clot created by PPP incubated with aspirin verses image (B) of clot from SEM of fibes made from PPP incubated with Aspirin. Both these images show healthy normal looking network of fibres. Arrows (1) Individual fibrin fibre (2) branch point of fibres. The two techniques correlates.

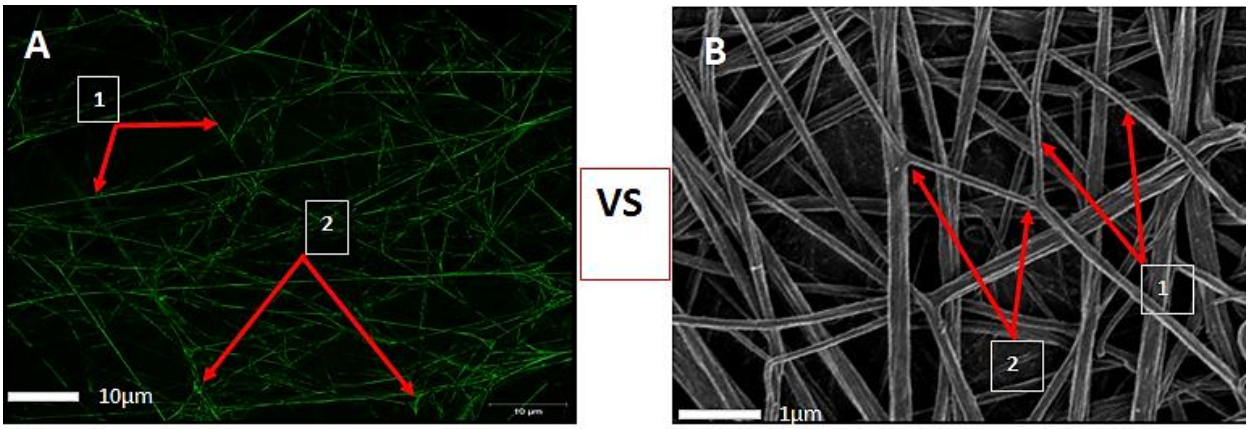


Figure 6. 34.(A) Confocal image of clot created by PPP incubated with aspirin mixed with LBP verses image (B) of clot from SEM of fibres made from PPP incubated with aspirin mixed with LBP. Both these images show healthy normal looking network of fibres. Arrows (1) Individual fibrin fibre (2) branch point of fibres. The two techniques correlates.

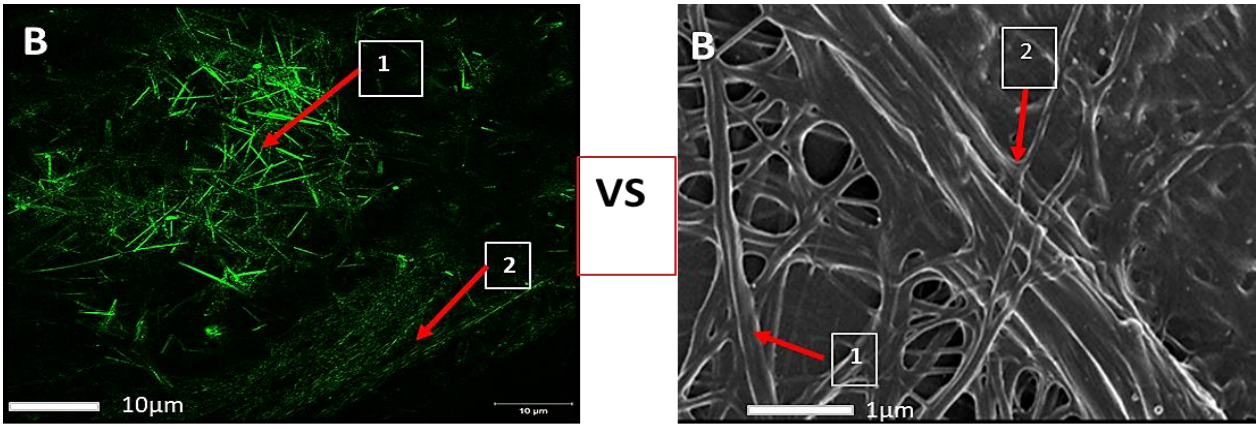


Figure 6. 35.(A) Confocal image of clot created by PPP incubated with aspirin mixed with LPS verses image (B) of clot from SEM of fibres made from PPP incubated with aspirin mixed with LPS. Both these images show abnormal formation of fibres, the clots show mashed fibres. Arrows (1) Individual fibrin fibre (2) branch point of fibres. The two techniques correlates.

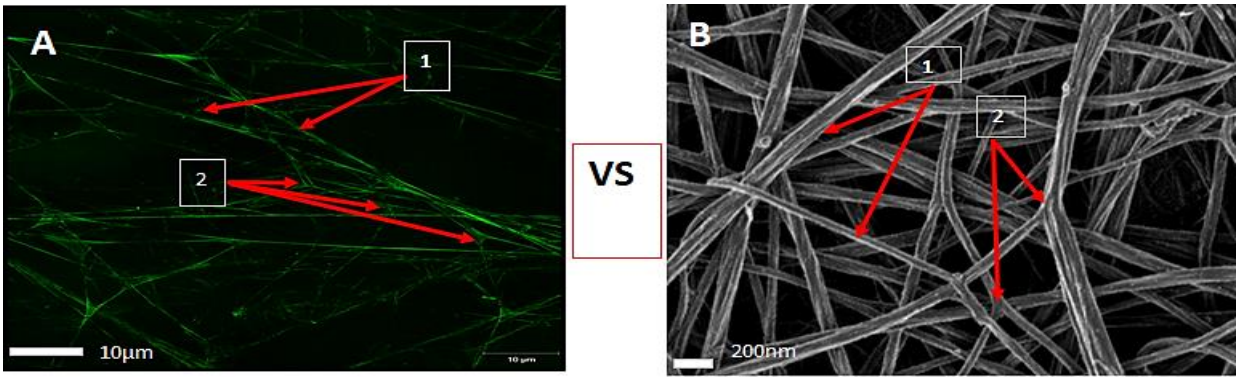


Figure 6. 36.(A) Confocal image of clot created by PPP incubated with aspirin mixed with LPS and LBP verses (VS) image (B) of clot from SEM of fibres made from PPP incubated with aspirin mixed with LPS and LBP. Arrows (1) Individual fibrin fibre (2) branch point of fibre. Both these images show healthy normal looking fibres.

When correlating only the significant differences obtained from the coefficient of variations confocal verses SEM, see **Figure 6.37.** Closely looking at the statistical differences.

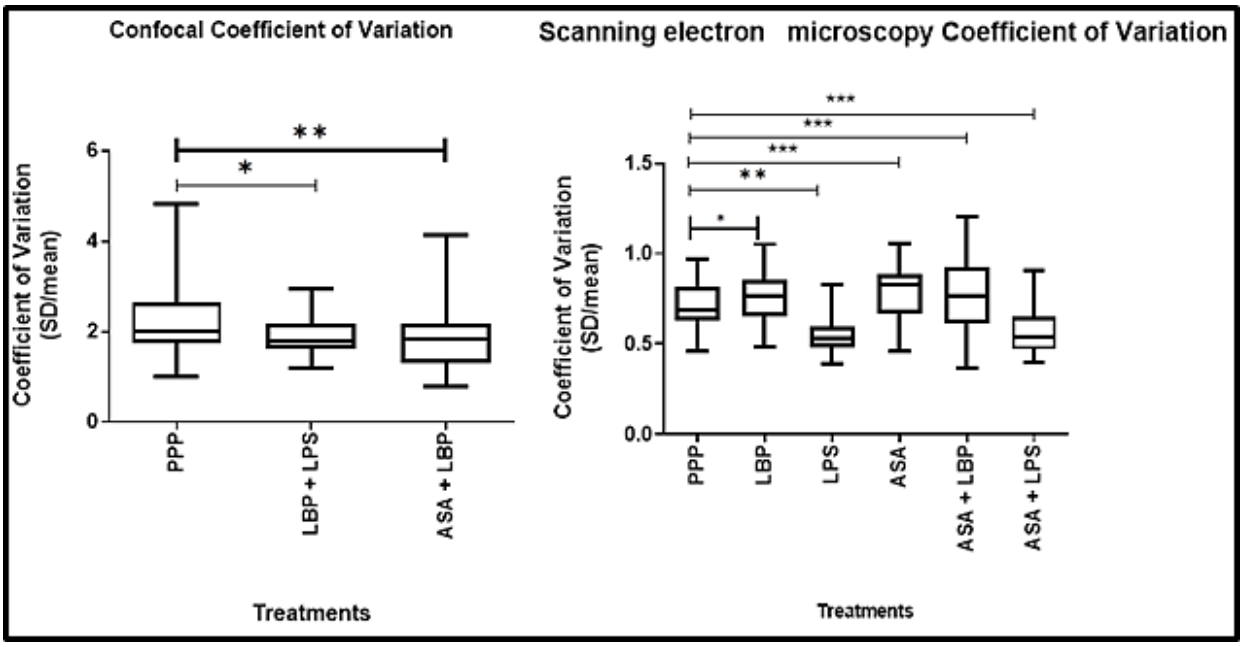


Figure 6. 37. Boxplots of the distribution of the coefficients of variation in the pixel intensities of the SEM and Airyscan clot images. Confocal technique shows less pixel intensity obtained with PPP and LBP+LPS also when plasma was mixed with aspirin and LBP. Showing properties of the treatment to have no effect in amyloid formation. SEM boxplot shows less variation of background light intensity with LPS and CVs from LPS mixed aspirin, respectively . All the other treatment gave CVs that had greater variation in light intensity according to SEM's CVs.

Fibrin Fibre thickness measurements

Micrographs of fibrin fibres of all participants were measured comparing fibrin fibres before and after treatments. This was to determine if the fibres diameter would change with the addition of the treatment combinations. The micrographs were chosen randomly from the samples. Measurements were performed on the fibrin fiber diameters using ImageJ (ImageJ is a public domain, Java-based image processing program developed at the National Institutes of Health: http://rsbweb.nih.gov/ij/); for each participant 50 fibrin fibres were measured (width in nm) taken from each sample type (naïve to treatment). **Table 6. 4.** Shows the average, median and standard deviation of all fibrin fibres diameter from Image J calculations.

Table 6. 3. Average, median and standard deviation of all fibrin fibres diameter from untreated healthy naïve to treated fibrin fibres.

Participants	Naïve	LBP	LPS	LPS+ LBP	Aspirin	Aspirin + LBP	Aspirin + LPS	Aspirin + LBP + LPS
01								
Average	110,81	113,24	1532,12	126,44	105,43	109,24	2132,62	109,90
Median	109,74	116,45	1308,99	120,11	107,99	109,44	1595,30	106,22
SD	24,56	19,93	970,12	31,14	29,10	23,47	1723,01	33,06
02								
Average	129,63	104,25	1355,33	122,06	112,93	92,32	3399,26	124,44
Median	136,20	104,42	996,75	118,30	105,70	91,37	3020,88	105,45
SD	32,57	37,89	920,06	49,57	42,39	33,68	1295,06	108,98
03								
Average	119,53	97,84	1491,41	90,84	96,44	172,26	1767,28	154,88
Median	103,63	87,44	1408,16	89,60	94,32	104,68	1223,67	116,45
SD	60,91	55,86	753,88	33,93	33,46	176,41	1313,69	210,93
04								
Average	92,99	95,18	933,03	113,69	113,33	102,28	693,02	122,20
Median	94,89	88,39	760,05	107,24	112,18	94,03	575,90	116,67
SD	40,42	26,96	488,71	63,90	36,91	47,27	545,96	49,85
05								
Average	106,85	100,08	542,05	103,42	112,87	113,45	568,02	116,50
Median	108,47	100,43	390,43	94,03	112,42	108,74	330,07	113,61
SD	37,07	31,58	408,24	38,35	37,00	30,75	584,34	33,68
06								
Average	129,96	131,52	897,22	162,40	137,11	115,22	805,34	155,78
Median	126,72	125,64	610,47	146,93	125,42	105,45	605,61	143,37
SD	45,93	40,00	748,94	66,25	51,19	51,65	588,48	49,10
07								
Average	134,74	132,35	775,33	122,54	114,36	125,95	1025,62	135,40
Median	128,83	129,45	728,27	111,70	109,74	125,21	970,15	129,24
SD	33,99	45,46	395,57	50,86	40,66	42,98	414,62	41,00
08								
Average	105,61	93,64	1298,79	136,86	107,94	120,48	1218,22	149,33
Median	105,70	95,99	1068,10	132,16	104,16	113,62	1007,39	137,78
SD	34,72	33,64	911,06	35,52	33,22	43,77	842,59	58,50
09								
Average	96,66	97,73	1544,89	92,96	112,46	110,59	1106,32	109,00
Median	92,82	96,03	1167,46	87,14	107,99	105,19	991,90	104,16
SD	34,72	28,25	1391,06	24,84	38,03	28,87	705,59	30,66
10								
Average	121,94	118,97	1321,65	129,98	107,23	123,97	387,37	102,32
Median	122,09	117,15	897,97	121,63	104,42	122,09	238,31	93,17
SD	32,73	37,21	1264,91	50,65	33,65	40,08	424,64	39,12
11								

Average	124,08	105,94	942,70	94,51	129,00	132,54	1143,79	215,08
Median	114,32	105,45	900,13	88,39	127,99	126,07	833,97	166,13
SD	48,77	32,91	421,13	31,10	52,10	47,36	728,96	169,76
12								
Average	123,03	120,34	413,54	157,76	130,29	115,74	524,19	126,33
Median	125,21	108,67	218,85	153,79	125,42	118,76	405,17	125,21
SD	36,44	45,43	389,08	52,96	41,78	38,81	365,43	38,26
13								
Average	116,09	123,86	1296,77	113,58	125,40	105,13	269,52	116,20
Median	105,19	121,88	1044,96	110,40	125,42	106,45	189,77	108,74
SD	43,27	47,53	939,63	39,13	38,60	37,20	251,86	41,26
14								
Average	107,01	117,57	805,44	95,86	153,82	134,80	912,34	139,72
Median	98,81	112,67	453,58	89,60	152,69	120,72	704,32	141,36
SD	34,76	44,64	750,52	44,83	48,36	47,92	519,84	45,85
15								
Average	97,66	101,49	521,73	126,85	124,37	115,17	1171,07	132,65
Median	95,18	103,63	457,42	119,88	115,05	118,30	1067,97	119,66
SD	34,91	37,00	272,03	40,36	48,51	40,73	570,24	45,88
16								
Average	111,66	117,89	1228,77	123,63	140,77	148,07	546,20	94,37
Median	107,48	116,45	1132,85	121,47	134,60	125,42	495,94	93,74
SD	36,40	37,96	866,50	30,93	47,94	66,23	291,61	28,83
17								
Average	118,44	119,22	389,61	110,06	105,04	121,54	765,79	98,64
Median	106,71	107,48	322,22	106,73	91,67	110,73	559,81	93,17
SD	47,56	55,91	212,87	57,06	43,87	47,22	598,62	33,23
18								
Average	135,30	117,20	491,93	106,63	133,87	119,30	465,01	149,17
Median	140,90	115,75	442,99	108,99	135,41	118,76	358,17	121,69
SD	46,43	41,63	254,52	29,62	43,79	35,62	343,49	76,91
19								
Average	132,07	129,86	424,63	108,85	129,65	143,77	777,54	165,68
Median	126,72	123,67	379,91	101,21	122,74	147,30	668,03	156,93
SD	36,93	52,67	226,15	35,42	46,80	47,97	521,16	74,48
20								
Average	115,09	140,41	947,90	114,75	101,74	122,43	784,92	143,72
Median	114,58	137,97	824,19	107,24	104,16	123,23	746,99	143,19
SD	33,84	45,00	730,43	35,74	38,06	31,22	398,95	47,23
21								
Average	106,23	110,62	719,25	105,73	113,00	121,95	715,48	124,06
Median	100,10	108,99	397,24	111,70	106,73	117,61	436,49	126,29
SD	41,03	37,97	618,52	30,43	39,33	41,54	741,17	33,93
22								
Average	103,85	105,45	745,31	108,02	117,18	117,92	1135,70	127,66
Median	104,16	105,70	672,74	107,99	114,32	111,70	1097,31	128,19

SD	32,02	32,24	460,53	37,27	34,06	34,86	480,08	37,77
23								
Average	94,57	110,67	526,70	125,78	117,85	106,33	578,42	113,40
Median	94,32	105,96	440,37	126,07	109,74	103,37	416,03	112,67
SD	31,68	33,04	376,15	39,32	39,42	34,09	504,66	32,52
24								
Average	115,48	135,30	942,29	121,33	123,17	126,97	716,46	139,84
Median	118,30	125,42	751,60	110,73	125,42	117,15	488,63	127,14
SD	37,06	66,70	667,49	49,00	35,34	40,34	590,96	46,25
25								
Average	165,85	137,71	767,42	136,25	126,96	163,19	457,32	153,30
Median	159,00	135,61	671,08	127,14	125,21	149,85	432,59	148,22
SD	66,62	36,66	477,50	41,67	48,45	99,52	195,54	52,30
26								
Average	107,68	104,46	159,57	122,51	111,97	121,35	383,29	117,51
Median	102,59	102,59	120,11	117,61	104,92	114,56	303,86	118,96
SD	31,58	33,25	149,67	36,65	37,30	41,13	235,28	36,83
27								
Average	147,82	129,64	614,72	104,18	133,54	137,73	709,69	138,41
Median	146,75	130,71	530,15	104,16	134,60	137,97	682,45	133,99
SD	52,38	57,39	376,36	31,34	39,20	48,71	291,11	45,22
28								
Average	133,85	130,34	519,59	133,62	126,77	138,69	444,52	117,27
Median	135,81	131,13	466,69	117,15	122,74	131,11	408,31	107,99
SD	41,26	39,73	325,78	57,33	39,41	46,12	223,93	42,05
30								
Average	119,83	118,89	724,31	106,53	107,75	113,11	870,86	125,96
Median	115,75	118,76	755,19	104,16	105,19	110,73	594,05	115,75
SD	40,85	40,05	233,02	39,42	28,54	32,39	862,00	44,30
31								
Average	137,57	128,32	111,96	134,34	110,61	134,70	144,46	107,06
Median	134,40	122,95	105,45	137,19	103,63	125,42	64,19	102,85
SD	43,82	42,51	42,62	43,11	41,14	49,98	362,49	32,91

Looking closely at **Figure 6.38**.(constructed using Microsoft excel office 2000) the median of each of the 50 participants' fibres, the thickness is measured from treated/ naïve micrographs.

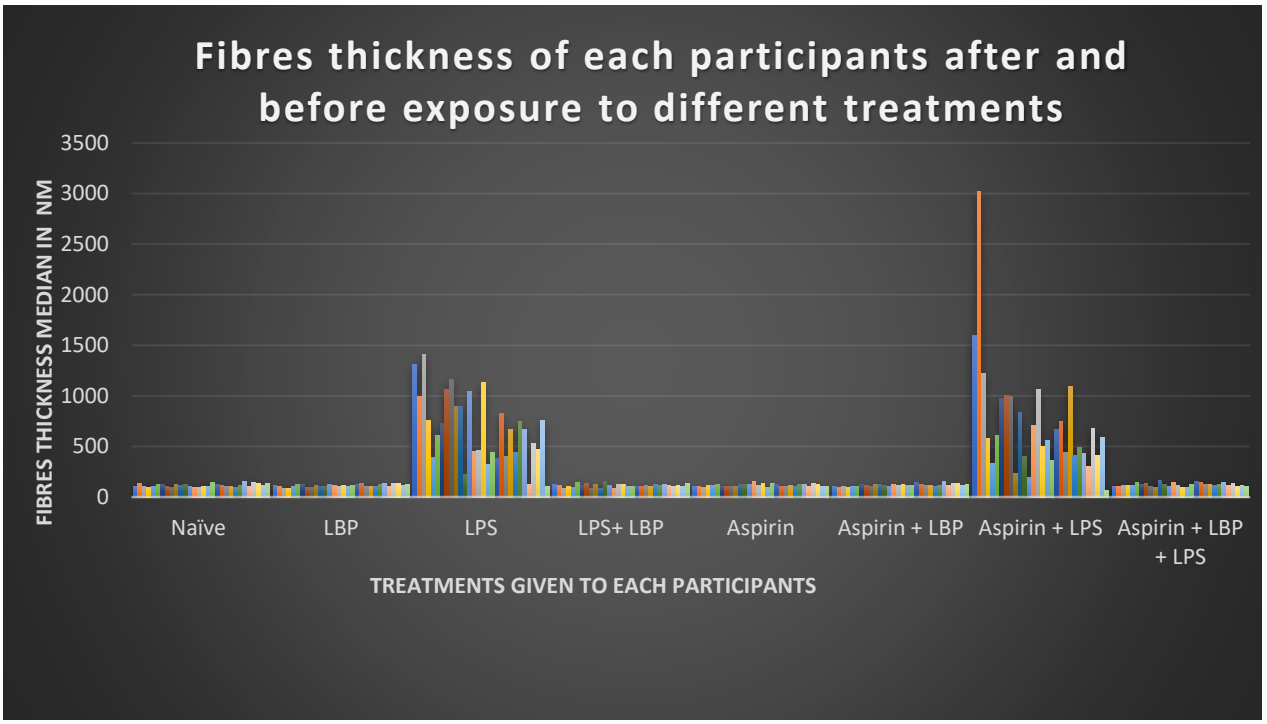


Figure 6. 38. Median thickness of all fibres from 30 participants were each PPP sample was treated with LBP, LPS, LPS and LBP , aspirin, aspirin with LBP, aspirin with LPS, and aspirin with LPS and LBP. The fibres thickness was measured using image J the values were ploted on the excel program and the median were calculated using excel 2000.

For statistical analysis One-Way Anova Multiple Comparison's Test for SEM was performed, comparing the mean of each column (control/ untreated) with the mean of every other column treated samples (GraphPad 8). See **Table 6.5** for the One-Way Anova results. Then after is **Table 6.6.** Dunnett's multiple comparison of all fibre thickness from SEM's histogram analysis following by a box plot see **Figure 6.39.**

Table 6. 4. One-Way ANOVA Results

ANOVA summary	
F	41.23
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.5544
Brown-Forsythe test	
F (DFn, DFd)	18.86 (7, 232)
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes
Bartlett's test	
Bartlett's statistic (corrected)	859.8
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes
ANOVA table	SS
Treatment (between columns)	15301408
Residual (within columns)	12300611
Total	27602019
Data summary	
Number of treatments (columns)	8
Number of values (total)	240

Table 6. 5. Dunnett's multiple comparison of all fibre thickness from Scanning electron microscope histogram analysis

Dunnett's multiple comparisons test	Mean Diff.	Significant?	q	Adjusted P Value
Naïve vs. LBP	2.418	No	0.04067	>0.9999
Naïve vs. LPS	-565.0	Yes	9.503	<0.0001
Naïve vs. LPS+ LBP	2.497	No	0.04200	>0.9999
Naïve vs. ASA	0.1125	No	0.001892	>0.9999
Naïve vs. ASA + LBP	-1.323	No	0.02225	>0.9999
Naïve vs. ASA + LPS	-601.3	Yes	10.11	<0.0001
Naïve vs. ASA + LBP + LPS	-6.087	No	0.1024	0.9999

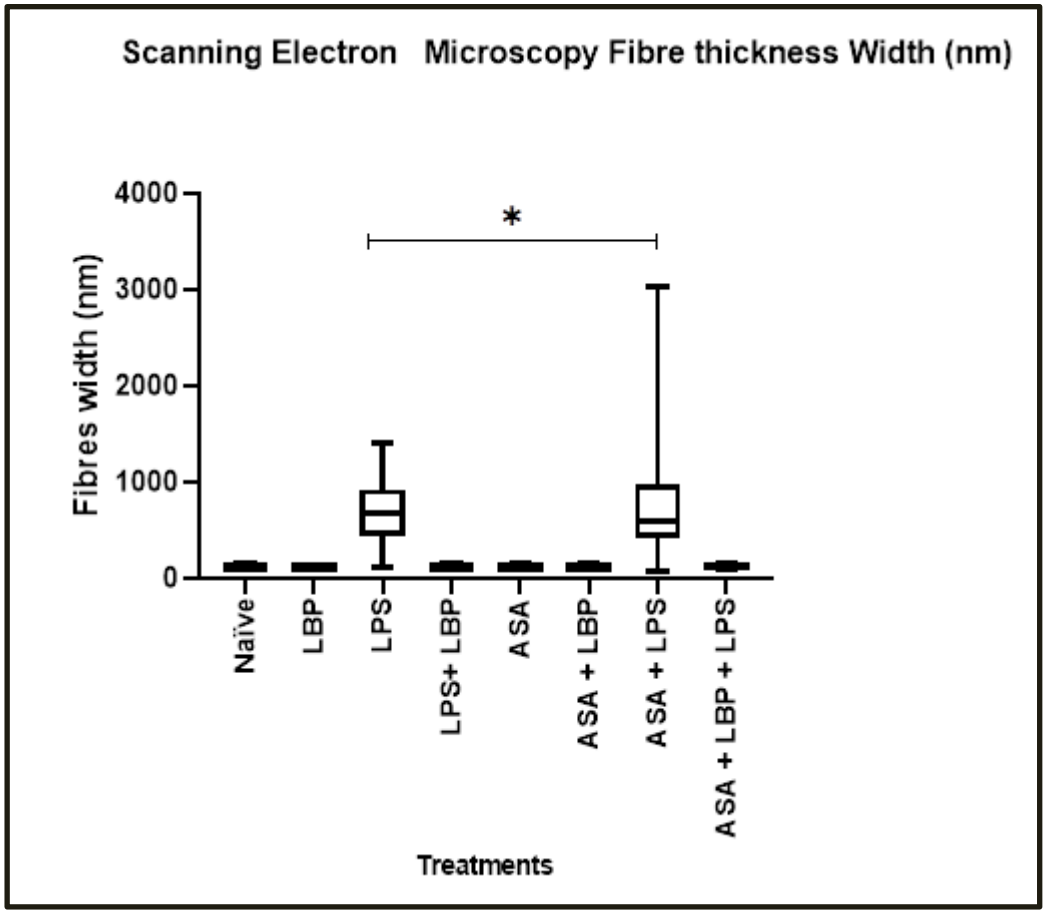


Figure 6. 39. Box plot of fibres width thickness measured (nm) shows a distinct alteration in the fibres from PPP treated with LPS and ASA along with fibres width from ASA +LPS compared to the naïve. Following a repeated measures ANOVA and Dunnet's multiple comparisons test.

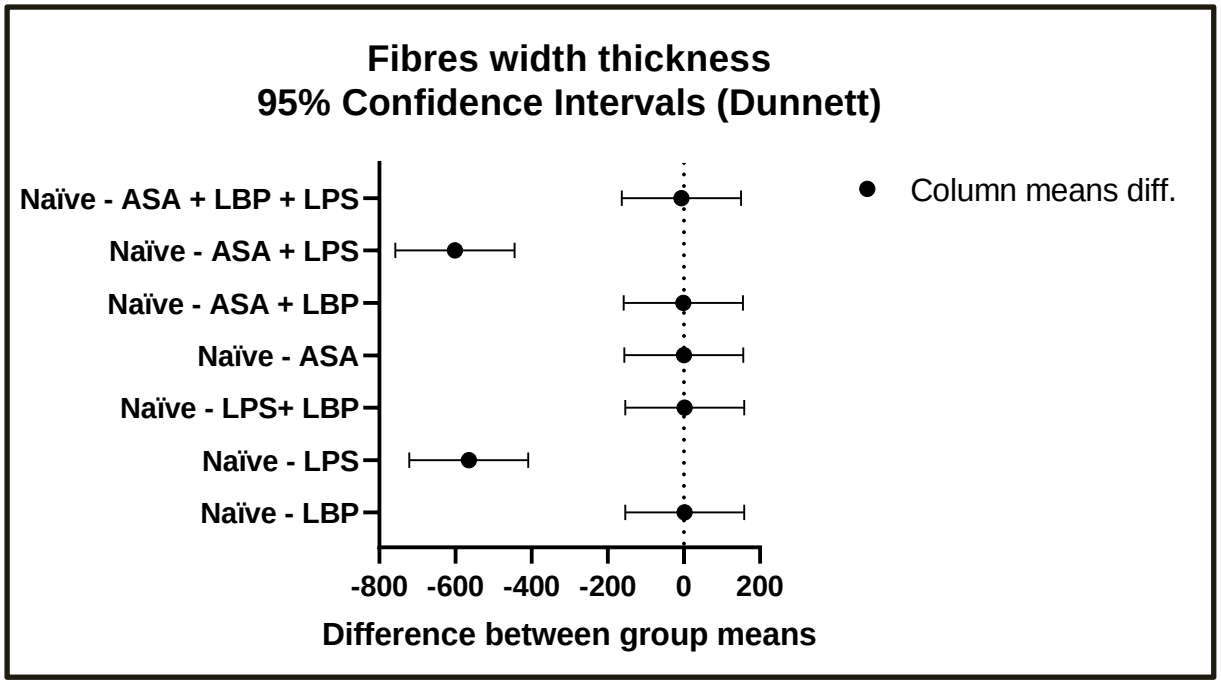


Figure 6. 40. The difference between group means of fibres width thickness measurements (nm) shows a distinct difference between fibres from samples treated with LPS and naïve fibres value of <-6000. A distinctive difference is seen also between fibres from ASA +LPS treated plasma and naïve fibres. Following One Way ANOVA test.

Discussion of Scanning electron microscopy of Red Blood Cells

Mature RBCs are highly specialized cells they are of discoid shape as seen in **Figure 6.1** The micrograph was taken from WB sample that was not treated. **Figure 6.2.** RBCs that are discoid and biconcave shape and healthy-looking platelets which have not been activated. In the blood film, RBCs seem like round elements with central cave see (in **Figure 6.1.** and **6.2**) healthy platelets and RBCs were used as positive controls and reference for all the RBCs and platelets from treatments. (Blum, 2009, Kikuchi et al., 1996). These are cells in the blood that carry oxygen from lungs to the rest of the body (Blum, 2009). Whole blood was mixed with LBP final exposure concentration of $2ng.L^{-1}$. This was to investigate if LBP had any adverse effect on the cell's morphology and function. **Figure 6.3. (A-B)** shows RBCs with biconcave structures and hollow central caves as typically seen in normal healthy individuals. Moreover, more researchers noted defects in RBCs when WB was mixed with LPS, by looking at their ultrastructure using both light microscopy and SEM (Kikuchi et al., 1996, Metzger et al., 2016, Nedela et al., 2016, Pretorius and Lipinski, 2013b). As with SEM analysis in **Figure 6.4.** shows RBC's surface membrane bulging after LPS exposure.

Similar results were also seen by Kell and team that in the presence of LPS it causes platelets and RBCs to shed off pieces of the surface membrane (Kell and Pretorius, 2015e).

The induced inflammation can lead to changes in the shape and structure of RBCs and platelets (Kikuchi et al., 1996, Kell and Pretorius, 2015e, Pretorius et al., 2016). The LBP protective role was seen when LBP was mixed with LPS prior to adding it to WB. The micrographs in **Figure 6.6.** and **Figure 6.7.** shows a few RBCs with normal healthy-looking surface membrane on clean surface (cover plate) with no microparticles. The LBP is critically involved in innate immune responses to Gram-negative infections. A study conducted by Wright showed that LBP binds to the surface of living Salmonella and to LPS coated erythrocytes, strongly improves the connection of these particles to macrophages. Lipopolysaccharide binding protein bridges LPS-coated elements to macrophages (MO) by first binding to the LPS, then binding to MO. Pretreatment of ELPS with LBP enabled binding to MO, but pretreatment of MO had no effect. Furthermore, MO did not distinguish erythrocytes coated with LBP unless LPS was also added, thus implying that the interaction of LBP with LPS results in a conformational change in LBP that allows recognition by MO (Wright et al., 1989).

This justifies what researchers state, that LBP has no adverse effect on cells in the body when mixed with WB and LPS it helps reduces the irregularities seen on the surface of RBCs' membrane as seen from figure 6.3 and more in the fibres section of this chapter, indeed LBP played a protective role (Lamping et al., 1998, Zweigner et al., 2001, Schroedl et al., 2001). For centuries aspirin has been used as an anti-inflammatory, to treat acute phase inflammations like headache and painful limbs (Salvado et al., 2013, Zhang et al., 2017). A small concentration of aspirin (0,5mM final exposure concentration) was incubated with WB. **Figure 6.8.** shows RBCs with smooth surface membrane and the shape of RBCs was the typical biconcave with hollow center, the same applied when looked at aspirin and LBP on WB, the micrographs in **Figure 6.9.** show all RBCs were normal, these reagents did not alter the morphology of RBCs.

The investigation went further to study the protective role of aspirin when LPS was incubated with WB. From the results aspirin cannot inhibit LPS from inducing inflammation, platelets remain activated even after adding aspirin to the WB see **Figure 6.10.** Micrograph taken from WB treated with aspirin and LPS.

A study done by researchers demonstrate change in RBCs morphology in metabolic syndrome (MetS) was associated with increased oxidative stress and inflammation (Gyawali et al., 2015). Whole blood viscosity, RBCs aggregation, RBCs deformability, lipid profile and blood sugar level, oxidative stress markers (RBCs reduced glutathione, superoxide dismutase, urinary isoprostanes) and inflammatory markers (high sensitivity C-reactive protein) were measured. Morphologically abnormal RBCs were significantly correlated with oxidative stress and chronic inflammation markers (Gyawali et al., 2015). Pretorius and colleagues found that the RBC membrane changes and resulting DMD interactions play a pivotal role in the persistent presence of thrombi (Pretorius et al., 2014c). During ischemic stroke, RBCs undergo oxidative and proteolytic changes resulting not only in inflammation but also in changes in cellular flow and deformation (Pretorius and Lipinski, 2013b). Moreover, the processing of RBCs' images was obtained and from these the shape parameters area, perimeter and form factor were obtained (Edison et al., 2011). This is crucial for the current as the shape and morphology or RBCs determine disease state of the participant.

The tendency of aberrant cells to stick to the endothelium indicates the surface elements not only of the RBCs but also of the endothelial cells. Sickle cell disease is a sample of a disorder where the RBC is under stress, ischemic, oxidative, or shear stress, that causes alterations in the RBC morphology (Myung et al., 2016, Schlichting et al., 1996, Poola and John, 2017, Pretorius et al., 2014a). This change leads ultimately to increased RBCs-endothelial cell adhesion see **Figure 6.11.** Reactive oxygen species generated by cytokine-activated inflammatory cells oxidize lipoproteins such as LDL and lipoprotein(a) contained by the vessel wall, facilitating uptake of these particles by activated macrophages and smooth muscle cells, with conversion into lipid-laden foam cells (Pretorius et al., 2016b, Myung et al., 2016, Brauckmann et al., 2016a). Particularly, the membranes of sickle RBCs have endured excessive cytoskeletal protein thiol oxidation, and sickle RBCs are unusually prone to vesiculation during mechanical stress *in vitro* and apparently *in vivo*. It may possibly be that the generation of reactive oxygen species in atherosclerosis activates RBCs, and macrovesicles of RBCs are formed, improving the activation of the vascular endothelium and leading to vascular inflammation and atherogenesis (Blum, 2009).

Researchers Kikuchi and team suggest that studies of platelets are more useful in the diagnosis of DIC at early stages of impaired organ function than are other indicators of inflammation such as the level of C-reactive protein (Kikuchi et al., 1996).

Investigation continued to see the combined effect of LBP and aspirin on inflammation induced by LPS in WB, **Figure 6.12.** Whole blood incubated with aspirin and LPS for 10 minutes. Here no thrombin was added to WB but spindle fibres formation was seen, typical sign of inflammation in all four micrographs. (A and C) huge masses develop on the surface as more pseudopodia from the platelets connect together. (A-C) RBCs here have lost their membrane integrity, (D) activated platelets bounded pseudopodia, most seen in chronic inflammation. When looking at WB that was treated with LBP + LPS+ aspirin the signs of inflammation had decreased, microparticles formation has decreased significantly (although there were no test conducted to quantify the amount of microparticle formation, only through visual morphology this was deduced), shape of RBCs almost regains it full complete normal structure. This is seen in **Figure 6. 13.**. Micrograph (C) RBC starting to regain it smooth surface membrane, still the integrity of the RBC's membrane is almost complete remains unchanged. Surface membrane of RBC higher resolution shows a smooth surface membrane with no bulging particles as seen when LPS was present alone.

Discussion of scanning electron microscopy of platelet poor plasma

Fibrin is produced by the polymerisation of the protein fibrinogen under the action of thrombin as discussed in the introduction of this chapter. The transglutaminase-based crosslinking step ensures a more stable clot see **Figure 6.14** healthy looking fibres with fine branches of fibres. Similarly, this was seen when PPP was incubated with LBP at low physiological levels of $2ng.L^{-1}$, the results showed no adverse effect, see **Figure 6.15.** PPP and LBP, evenly spaced fibrin fibres with clear visible branching points are present.

Many chronic inflammatory diseases are complemented by signs of inflammation such as disrupted fibres (and may be exacerbated, and possibly even largely caused) by amyloid fibril development (Chiti and Dobson, 2006, Herczenik and Gebbink, 2008, Rambaran and Serpell, 2008, Eisenberg and Jucker, 2012, Knowles et al., 2014, Tipping et al., 2015). **Figure 6.16.** shows large big matted mass of fibrin fibres bunched collectively.

This has been reported previously as broken fibres forming β-sheet fibrils also known as the development of amyloid fibril (Kell and Pretorius, 2015f, Kell and Pretorius, 2015e). An amyloid fibril (protein) is characterized as: "a protein that is deposited as insoluble fibrils, mainly in the extracellular spaces of organs and tissues as a result of subsequent changes in protein folding that ensue in a condition known as amyloidosis" (Sipe et al., 2014). The manner the LPS affects or binds to fibrin differs from individual to individual see **Figure 6.17.(B-F)** were fibres sizes changed, some individual had large fibres re-branching forming the matted mass. A visible thick mass covering fibres is seen. **Figure 6.18.** thin fibres form due to LPS introduced inflammation this was also seen by these researchers (Undas and Ariens, 2011, Pretorius et al., 2013b, Pretorius et al., 2011b).

The nature and position of the protein amyloid typically reflects the disease, such that PD, that is in addition to systemic blood hypercoagulability, also accompanied by amyloid forms of synuclein in the substantia nigra pars compacta (Olanow and Brundin, 2013, Uversky et al., 2001, Vilar et al., 2008), Alzheimer's disease, by fibrillar forms in numerous parts of the brain (Fändrich et al., 2009, Petkova et al., 2005, Serpell, 2000), and T2DM a disease with rare genome-wide associations (Fuchsberger et al., 2016) by amylin fibrils in the insulin-producing cells of the pancreas (Cooper et al., 1987, Cooper et al., 1988, Lorenzo et al., 1994, Jaikaran and Clark, 2001, Loomes, 2011, Höppener and Lips, 2006, Pillay and Govender, 2013, Zhang et al., 2014

Although fibrin too is an insoluble fibre, fibrinogen has generally not been considered as amyloidogenic, nor (healthy) fibrin as an amyloid protein although it can become so in the presence of a rare mutation in the fibrinogen a chain (Serpell et al., 2007, Picken, 2010, Stangou et al., 2010, Haidinger et al., 2013). However, as part of an extensive study of anomalous blood clotting (e.g. (Bester et al., 2015a, Kell and Pretorius, 2014a, Kell and Pretorius, 2015g, Pretorius et al., 2013b, Pretorius et al., 2014c, Pretorius and Kell, 2014c, Pretorius et al., 2015, Pretorius et al., 2016e)), it was recently found (Pretorius et al., 2016c, Pretorius et al., 2016e, Kell and Pretorius, 2016b, Kell and Pretorius, 2016a) that the anomalous clotting was in fact amyloid in nature.

The fact that it could be caused by tiny amounts of LPS was seen as consistent with a role for dormant bacteria in the aetiology of diseases (Potgieter et al., 2015b, Kell et al., 2015c, Kell and Pretorius, 2015b, Itzhaki et al., 2016b, Kell and Kenny, 2016, Kell and Pretorius, 2016c, Pretorius et al., 2016a, Pretorius et al., 2016b).

A particularly strong pointer was that the amyloid or anomalous fibrin(ogen) clot formation, was reduced when the LPS was mixed together with LBP as seen from **Figure 6.19** other researchers also found these findings (Pretorius et al., 2016e). For a schematic model of a healthy clot structure see **Figure 6.20.** where mixed PPP with aspirin. The aspirin had no harmful effect on PPP, all the fibres presented here were smooth, healthy, evenly branched a typical is clot created by adding thrombin to healthy PPP. In normal healthy clots it has been shown to consist of three layers almost like a 3-dimension view seen in **Figure 6.21** aspirin to PPP and LBP. These layers vary in thickness and structure depending upon whether the plasma was from a healthy individual or an individual with inflammation. A healthy clot consists of a bottom layer, which is caused by contact activation of the coagulum and the glass substrate (Pretorius et al., 2017a). The second layer is the actual clot, consisting of a fibrin fibre network of elongated fibres. Often, the contact activation layer is visible through the spaghetti-like fibres. Depending on the preparation procedures, a third, top layer may form on the spaghetti-like fibres. This layer naturally rinses away during the fixing and dehydration procedure.

However, it may at times be noticeable as patches of coagulum, similar to that of the contact activation layer at the bottom. In cases of induced hypercoagulation by LPS, the bottom contact activation layer is not easily distinct from the middle clot area, and bare spaghetti-like fibres are fused into the contact activation layer see **Figure 6.22.** shows when LPS was added to PPP (inducing inflammation) and aspirin. It is visible here that aspirin could not alone reverse the action of LPS. This fused layer of matted fibrin may consist of thin branching individual fibres, with very small openings between the individual fibres, seen in **Figure 6.23. (A-B)** PPP and LPS micrographs which looks similar to those from **Figure 6.23 (C-D)** PPP with LPS, and aspirin. This view gives the impression of a matted plate. This complex, hypercoagulable clot structure, DMDs was published (e.g. (Lipinski et al., 2012, Pretorius et al., 2013b, Pretorius et al., 2016e, Kell and Pretorius, 2016a)). In T2D, when viewing the smear, a top netted layer is often observed (Pretorius et al., 2017a). It primarily does not rinse away in the course of the numerous fixing and dehydration steps, and it forms instantly when mixing thrombin with plasma to generate the clot. This is due to the hypercoagulable nature of the plasma.

For this study the aim was to investigate the effect of LBP in combination with aspirin, to understand a possible protective role they may play separately and in combination to reduce the hypercoagulation induced by LPS.

Comparison was made in **Figure 6.24 (A)** PPP with LPS versus **Figure 6.24 (B)** PPP with LPS, LBP and aspirin. The collective micrographs from **Figure 6.25.** evidence of clear visible thin fibres, with no matted mass deposits present. This proves the hypothesis that LBP can reduce hypercoagulation.

It is evident from the histograms that LPS causes matted mass deposits that flatten the fibres thereby on the surface hence light phases and dark phases/layer are merged are seen. Only one thin or thick layer is seen from the SEM this is supported by Pretorius study (Pretorius et al., 2017a) were they found that in inflammation the fibres form mashes and unbranched fibres forming one layer on the surface. This on the grey and white scale shows greater light emission, more pixels in a undeviating, a one distribution curve is seen on **Figure 6.26** histogram (C) and **Figure 6.27** histogram (G) even in the presence of aspirin , LPS still thins out or thicken fibres (inflammation symptoms) this according to the ANOVA Dunnet's test it was found to be statistically significant ($P<0.0001$) when compared to the naïve CVs from untreated PPP. The naïve PPP histogram and the PPP with LBP their CVs differences were found to be statistically significant ($P<0.05$). However, their mean difference suggests a very small difference between the two groups (-0.04175) This could have been the due to focus when taking the micrographs. Lipopolysaccharide binding protein together with LPS when incubated in PPP gave a normal histogram **Figure 6.26 (D)** it still along the ranges of a normal light intensity as with the naïve samples and this is supportive with findings from the Dunnet's test comparing. Their CVs differences were found to be insignificant statistically ($P<0.05$). When naïve PPP is compared with aspirin see histogram in **Figure 6.27 (E)** there was a slight which was statistically significant but the height of intensity was almost the same as seen in naïve samples, this could have been due to certain elements activated during the preparation steps a small mean difference is seen here again (-0,06605). Histogram in **Figure 6.27 (F)** shows aspirin with LBP to have the same intensity as naïve fibres but when evaluated with the ANOVA multiple comparison Dunnet's test the CVs differences were seen to be significant ($P<0.05$) however thee mean differences of the two groups was seen to be (-0.05765). The difference here is far less than the mean difference between micrograph of PPP and LPS verse naïve PPP (mean difference was 0.169). **Figure 6.27 (E)** histogram of fibres from PPP incubated with LPS and aspirin, a heightened light intensity is seen similar to the intensity of PPP with LPS. Here the Dunnet's test agreeing showed that the differences in CVs between PPP+ LPS+ aspirin had greater differences.

They were statistically significant (P<0.05). This shows that aspirin alone cannot combat the effect of LPS. When looking at histogram from PPP with aspirin and LPS together with LBP, here the histogram is the same light intensity as seen from naïve fibres and the Dunnet's test agreeable showed a statistically insignificance between CVs of naïve fibres verses fibres from aspirin with PPP and LPS with LBP (P<0.05). The **Table 6.2** shows descriptive statistics of all CV data of PPP SEM. The number of families was 1, the number of comparisons per family was 7 (that is all the 7 treatments used for this experiment) and alpha **(α)** 0.05. This was taken from the graph shown in **Figure 6.28.** The coefficient of variation calculated from data obtained from the histogram analysis following a repeated measures ANOVA and Dunnet's multiple comparisons test.

Discussion of fibrin fibre thickness measured after each treatment /untreated plasma

The fibre thickness was measured using image J. for each participant 50 fibres were measured (width in nm) taken from each sample type (naïve to treatment). **Table 6.4.** shows the average, median and standard deviation of all fibrin fiber diameter for the healthy naïve and treated fibrin fibres. The naïve (untreated) fibres median was +-112,02 nm different with the averages of fibre thickness obtained from the LPS influenced fibre and aspirin with LPS influenced fibres. The latter two groups have higher median of +-671,91 nm from LPS and the highest median of +-584,97 nm from aspirin with LPS. Under normal conditions, the fibres forming the clot appear in a SEM like spaghetti, and the fibres have diameters of the order of 80-110 nm. In some cases, continuous fibre plates are formed, where no individual fibres could be seen or measured (Pretorius et al., 2016a, Pretorius et al., 2017a). Fibre width thickness measurements (nm) shows a distinct alteration in the fibres from samples treated with LPS and untreated PPP aswell as PPP treated with ASA +LPS compared to the naïve (untreated samples). Following a repeated measures ANOVA and Dunnet's multiple comparisons test. The differences in the Means of fibres width thickness of LPS treated PPP is compared with naïve (untreated PPP) fibres value of <-6000. The same distinctive difference of (<-6000) is seen also between fibres from ASA +LPS treated plasma and naïve fibres. Increases in fibre thickness. Intimations of β-sheet formation induced by flow can be seen in (Badiei et al., 2015, Bucay et al., 2015), while in a very striking study, Campbell et al. (Campbell et al., 2010) saw a vast increase in the flow-induced diameter of fibrin fibres, from a mean of 79 -226 nm.

It must be taken into consideration that these (measured in this study) fibres were measured as presented, no fibres were excluded due to mashed network (LPS influenced plasma).

Chapter conclusion

The study aimed at investigating the effect of LBP in combination with aspirin to combat hypercoagulation induced by LPS. The samples made from the mixture of LPS-binding protein, LPS and aspirin, were analysed a vast improvement in the structure of fibres was visible when compared with those fibres formed from LPS incubated with PPP or PPP with LPS and aspirin. With the naïve PPP, LBP, and LBP mixtures alone and with LPS there were clear visible thin fibres, with no matted mass deposits present. Statistically the differences were insignificant ($P>0.05$). When comparing fibre thickness it is clearly visible that aspirin mixed with LPS caused thicker fibres which was found to be significantly different to fibres from naïve samples.

Chapter 7: Conclusion

Aim of the chapter

The aim is to critically evaluate current LPS combating reagents/treatments and make suggestions based on the results of the cumulative viscoelastic analyses and morphological analyses of the cohort of LPS inflammatory symptoms.

The null **hypothesis** (**H0**) of the study was that LPS cannot be combated by LBP and aspirin.

The alternative hypothesisº (H_1) of the study was that at low physiological levels of LBP and aspirin they will reduce the adverse effects induced by LPS both in plasma and WB

Closing Discussion

The conclusion discussion will be divided into three phases to help with the clarity of the outcomes of this study.

Phase (1): *The effect of LBP and aspirin have on RBC structure and platelets and the role these reagents play in combatting hypercoagulation caused by LPS in WB.*

When viewing with SEM, it was evident that LPS changed the morphology of RBCs and activated platelets. More microparticles were seen in WB treated with LPS than with any other treatments (LBP or aspirin). Although RBCs do not have the CD14 binding sites, it is alleged that LPS does bind to the cell, but it is assumed that binding of LPS to RBCs membrane links with the presence of hydrophobic regions present in LPS and RBCs (Peters et al., 2009). From the SEM analysis of WB, spontaneous fibrin network formed after incubating WB with LPS or when aspirin was incubated with LPS (without the addition of thrombin). This is most commonly seen in inflammatory diseases such as AD, PD and T2DM. Huge fibrin masses develop on the surface as more fibres and pseudopodia from activated platelets connect together. One of the study's objective was to see the combined effect of LBP and aspirin on inflammation induced by LPS in WB**.** When looking at WB that was treated with LBP + LPS+ aspirin, the signs of inflammation had significantly decreased, microparticles formation had decreased (this was only visualized with SEM no further tests were conducted and no quantitative methods were used to analyze the MPs). It was believed as similar results were found when Pretorius and Kell reported in numerous of publications that what is visible on the surface of RBCs and surrounding are MPs (Kell and Kenny, 2016, Kell and Pretorius, 2015b, Kell and Pretorius, 2015a). Lastly the shape of RBCs almost regained their full complete normal ultrastructure by looking at their surface membrane.

Phase (2): *The protective role of LBP on LPS alone and in combination with aspirin on fibrin fibres formation and hypercoagulation.*

Results from SEM showed that LBP can mop up LPS and prevent LPS from binding receptors CD14 on the fibrin(ogen). This was seen with an improved clot ultrastructure. More stable, uniform branched fibers network was seen. The samples made from the mixture of LBP, LPS and aspirin, were analyzed. A vast improvement in the structure of fibres clot was visible when compared with those fibres formed from LPS incubated with PPP or PPP with LPS and aspirin. The coefficient of variation calculated from data obtained the histogram analysis showed a distinct alteration in the clots from samples treated with; (1) LBP, (2) LPS, (3)ASA, (4)ASA+LBP and (5) ASA+LPS compared to untreated plasma CV ($p<0.0001$). To distinguish more differences in these groups, the mean difference was used to compare difference in fibres obtained from the statistical different given groups. It was found that the mean difference between fibres from LPS and fibres from LPS and aspirin treated plasma were very high. Moreover, fibrin fibres from LPS and LPS mixed with aspirin showed a lower intensity light coming from a background that has no variation in fibres. Everything was matted and unevenly spaced fibres (this was also seen in (Pretorius et al., 2018b), the difference was statically significant from untreated PPP CVs. Further analyses were needed of the fibres obtained with SEM, therefore fibre thickness was measured to obtain quantitative data based on the actual thickness of fibres after direct interaction with the treatments.

The final objective from the SEM chapter was to compare fibrin fibre thickness after each treatment to determine direct effect of each treatment on morphology and size of fibres using Image J and Graphpad prism. Micrographs of fibres network of all participants were measured before and after treatments. The micrographs were chosen randomly from the samples. The value for the healthy naïve fibres was almost identical to the fibre width that were formed after incubation with LBP. The distinctive fibres were seen from samples of PPP incubated with LPS, the average fibres width here was +/-832,86nm which is significantly higher than the fibres width from naïve PPP or PPP with LBP. When comparing fibres thickness it was clearly visible that LPS and aspirin mixed with LPS caused thicker fibres which was found to be scientifically significantly different to fibres from naïve (untreated plasma) samples.

Moreover, with the confocal microscopy there was less fluorescence, less amyloid formation, as seen with the fluorescent marker ThT throughout the naïve samples to samples with aspirin and LBP, however an increase in fluorescence was seen in samples with LPS and LPS with aspirin.

When there was presence of fibre deformation, β-sheets exposed showed to increase in fluorescence as compared to normal healthy fibres which had very little to no fluorescence. According to the statistical analysis there was statistical significance deference when comparing PPP naïve verses PPP that was incubated with LPS and LBP, the LBP with LPS produced a far less fluorescence light therefore producing lower CV's. Suggesting that LBP may play a role in lowering the binding of amyloid marker ThT to fibres, marking a less disrupted fibres. Part of the objectives from the confocal chapter was to add aspirin to plasma, this was to see if the low physiological concentration of 0,5mM aspirin had an adverse effect on fibrin fibers clot structure. The low physiological levels of aspirin were seen not to cause harm to fibres. From the confocal microscopy results it was deduced that aspirin decreased the binding of ThT fibres hence did not cause harm to fibres formation. These results were found to be statistically significant ($P<0.05$). Boxplots of the distribution of the CVs in the pixel intensities of the SEM and Airyscan clot images was used to analyse and distinguish results from both techniques. The LBP and aspirin treatments have no effect in amyloid formation (possible good treatment plans for agents such as LPS known to cause amyloid).

Phase (3): *Combining the two sections discussed into one big conclusion using the aid of viscoelastic properties of clots generated by the TEG.*

Scanning electron microscopy together with super resolution confocal microscopy techniques were used to give a visual representative of clot structure and also get quantitative data. Aspirin alone could not decrease the hypercoagulation caused by LPS, it could not mop up LPS both in WB and PPP. This was seen by an increase in fluorescence ThT after the incubation of LPS and aspirin on the confocal. With the SEM the clot structure still resembled the clot formed when LPS was incubated with PPP, matted mass deposits and unbranched fibres. When the WB was incubated with LPS and aspirin the RBCs denatured, membrane still with irregularities in **Fig 6.10** indicates microparticle formation when LPS was added to WB and aspirin. According to the TEG®, the (TMRTG) time taken for maximum rate of thrombus generation (min) between treatments and control was only significantly lower in the ASA + LBP + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p=0.0071$). Interestingly the clot strength MA was decreased when LPS was added with LBP in WB. It was seen that LBP had an effect in decreasing a strong hyper dense clot as seen in hypercoagulable state. The TGG (dcs) between treatments and control was only significantly reduced in the ASA + LPS treatment following a repeated measures ANOVA and Dunnet's multiple comparisons posttest ($p=0.0078$).

This also shows that aspirin on LPS incubated WB has an effect to loosen the size of the thrombus and weaken the thrombus during hypercoagulable states. This is prominence since the purpose of the study was to see whether aspirin alone or with LBP can reduce hypercoagulable state, and it is proven that both these reagents can reduce high dense clot. Although and as mentioned in the literature that aspirin has been found to increase fibrin clot porosity and susceptibility to lysis, this study focused at what concentration can aspirin do this and what techniques can be sued to quantify and qualify the results. More research is needed for detection and correction of coagulopathy is linked to the clot breaks down (lysis) this has been used by researchers to link clot formation and lyses to disease (Curry et al., 2018, Verma and 2017). For TEG Lysis 30 and Lysis 60 (LY30 and LY60) these are percent reductions in the area under the TEG curve, when MA stays constant, the Lys continues occur 30 and 60 min after MA giving full result of the dynamics of the clots (Whiting and DiNardo, 2014). Also the addition of Rheometry for additional mechanistic parameters on clot formation, could enables a proper description of the mechanical properties of the fibrin network when exposed to different treatments(Verma and 2017, Mohammadi Aria et al., 2019).

The amyloid fibrin(ogen) protein created with LPS, can be reversed in the blood model with the study' novel combination of LPB and aspirin, and these results could have far-reaching applications for treatment of conditions where LPS presence is implicated. Conditions include sepsis and even traditional systemic inflammatory conditions like T2DM, AD and PD. This study's novel discovery might therefore be useful to treat hypercoagulability in non-communicable diseases where a bacterial component is suspected.

Below **Figure 7.1.**a mind map with results from all experimental chapters in this study. Followed by **Figure 7.2.** a conclusion diagram.

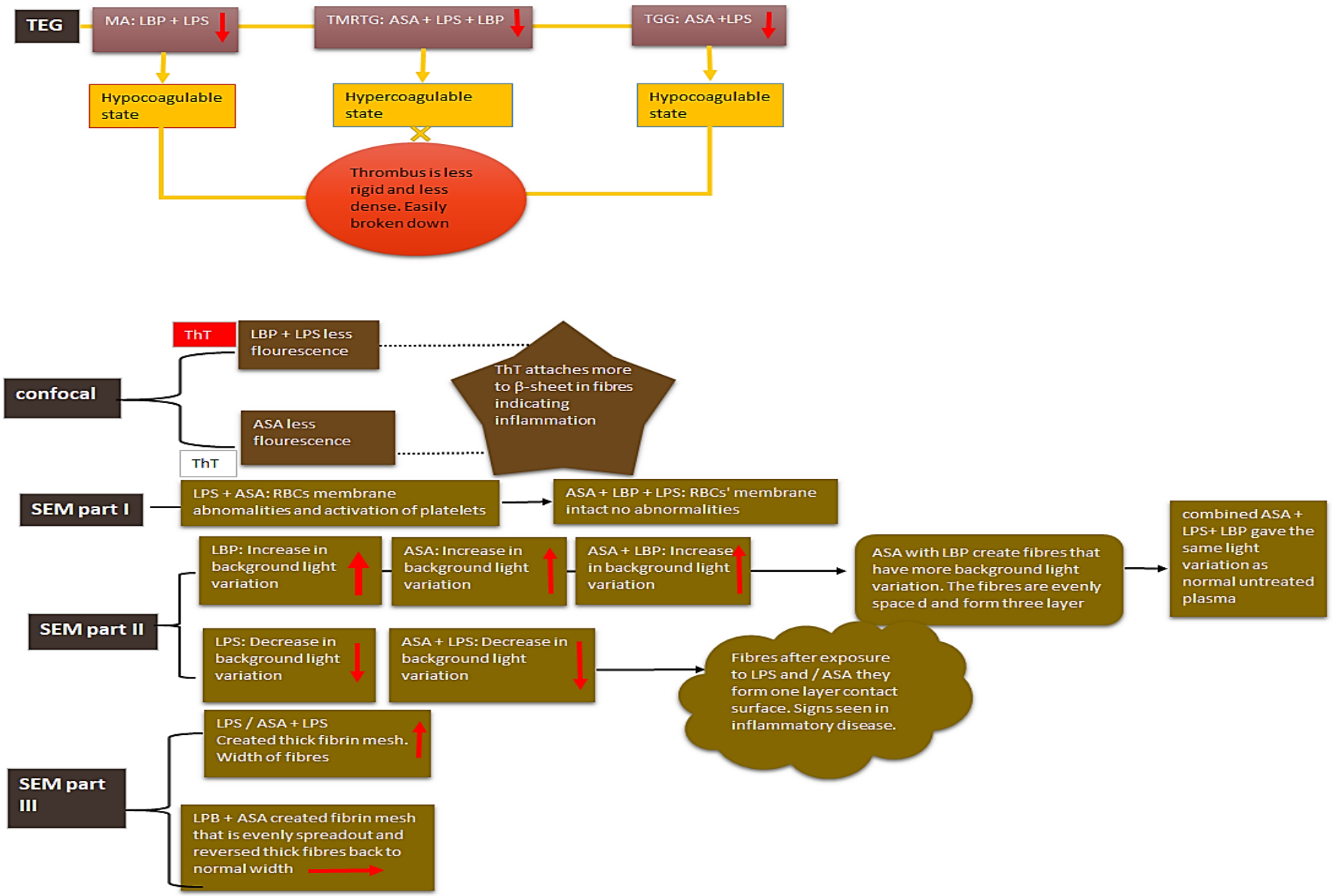


Figure 7. 1. Mind map of the study's outcomes per experimental chapte

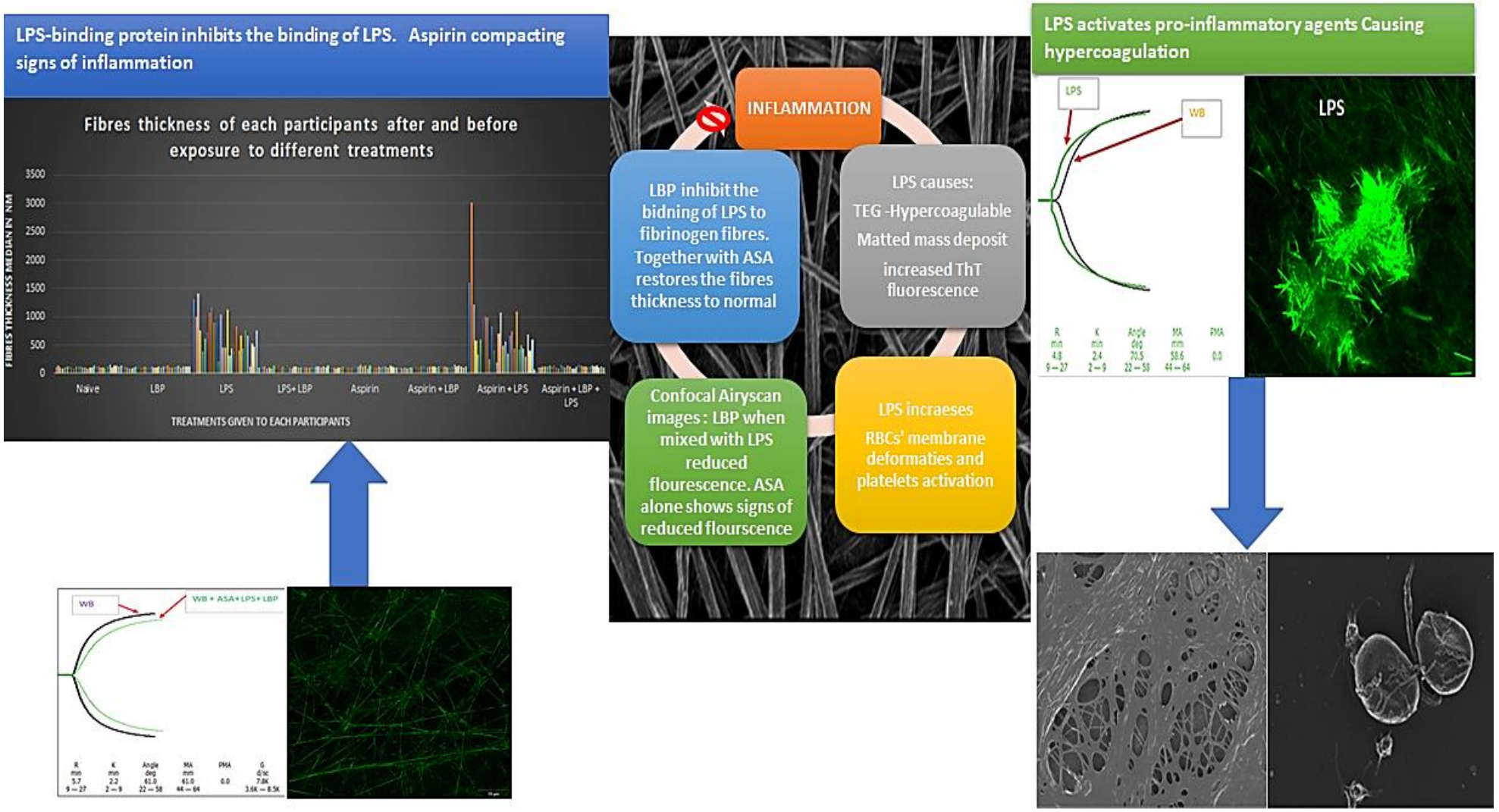


Figure 7.2. Conclusion diagram

References

ABRAHAMSSON, T. R., JAKOBSSON, H. E., ANDERSSON, A. F., BJÖRKSTÉN, B., ENGSTRAND, L. & JENMALM, M. C. 2014. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clinical and Experimental Allergy,* 44, 842-850.

ABUELKASEM, E., LU, S., TANAKA, K., PLANINSIC, R. & SAKAI, T. 2016. Comparison between thrombelastography and thromboelastometry in hyperfibrinolysis detection during adult liver transplantation. *Br J Anaesth,* 116, 507-12.

ADAM, S. S., KEY, N. S. & GREENBERG, C. S. 2009b. D-dimer antigen: current concepts and future prospects. *Blood,* 113**,** 2878-87.

AL-ATTAS, O. S., AL-DAGHRI, N. M., AL-RUBEAAN, K., DA SILVA, N. F., SABICO, S. L., KUMAR, S., MCTERNAN, P. G. & HARTE, A. L. 2009. Changes in endotoxin levels in T2DM subjects on anti-diabetic therapies. *Cardiovascular Diabetology,* 8.

ALDRICH, M. B. & SEVICK-MURACA, E. M. 2013. Cytokines are systemic effectors of lymphatic function in acute inflammation. *Cytokine,* 64, 362-9.

ALEXANDER, K. S., MADDEN, T. E. & FARRELL, D. H. 2011. Association between gamma' fibrinogen levels and inflammation. *Thromb Haemost,* 105, 605-9.

ALZAHRANI, S. H. & AJJAN, R. A. 2010. Coagulation and fibrinolysis in diabetes. *Diabet Vasc Dis Res,* 7, 260-73.

ALZAHRANI, S. H., HESS, K., PRICE, J. F., STRACHAN, M., BAXTER, P. D., CUBBON, R., PHOENIX, F., GAMLEN, T., ARIËNS, R. A. S., GRANT, P. J. & AJJAN, R. A. 2012. Gender-specific alterations in fibrin structure function in type 2 diabetes: associations with cardiometabolic and vascular markers. *J Clin Endocrinol Metab,* 97, E2282-7.

AMIN, H. H., MEGHANI, N. M., PARK, C., NGUYEN, V. H., TRAN, T. T., TRAN, P. H. &

LEE, B. J. 2017. Fattigation-platform nanoparticles using apo-transferrin stearic acid as a core for receptor-oriented cancer targeting. *Colloids Surf B Biointerfaces,* 159, 571-579.

AMOS, W. B. & WHITE, J. G. 2003. How the confocal laser scanning microscope entered biological research. *Biol Cell,* 95, 335-42.

ANDRÄ, J., GARIDEL, P., MAJERLE, A., JERALA, R., RIDGE, R., PAUS, E., NOVITSKY, T., KOCH, M. H. & BRANDENBURG, K. 2004. Biophysical characterization of the interaction of Limulus polyphemus endotoxin neutralizing protein with lipopolysaccharide. *Eur J Biochem,* 271, 2037-46.

AVERETT, L. E., GEER, C. B., FUIERER, R. R., AKHREMITCHEV, B. B., GORKUN, O. V. & SCHOENFISCH, M. H. 2008. Complexity of "A-a" knob-hole fibrin interaction revealed by atomic force spectroscopy. *Langmuir,* 24, 4979-88. ASAKURA, H. 2014. Classifying types of disseminated intravascular coagulation: clinical and animal models. *Journal of intensive care,* 2**,** 20.

BADIEI, N., SOWEDAN, A., CURTIS, D., BROWN, M., LAWRENCE, M., CAMPBELL, A., SABRA, A., EVANS, P., WEISEL, J. & CHERNYSH, I. 2015. Effects of unidirectional flow shear stresses on the formation, fractal microstructure and rigidity of incipient whole blood clots and fibrin gels. *Clinical hemorheology and microcirculation,* 60**,** 451-464.

BAGHERI, H., MANSHAEI, F. & REZVANI, O. 2018. Three-dimensional nanofiber scaffolds are superior to two-dimensional mats in micro-oriented extraction of chlorobenzenes. *Mikrochim Acta,* 185, 322.

BAGOLY, Z., HOMORÓDI, N., KOVÁCS, E. G., SARKADY, F., CSIBA, L., ÉDES, I. & MUSZBEK, L. 2016. How to test the effect of aspirin and clopidogrel in patients on dual antiplatelet therapy? *Platelets,* 27, 59-65.

BARNEA, E. R., HAYRABEDYAN, S., TODOROVA, K., ALMOGI-HAZAN, O., OR, R., GUINGAB, J., MCELHINNEY, J., FERNANDEZ, N. & BARDER, T. 2016.

PreImplantation factor (PIF*) regulates systemic immunity and targets protective regulatory and cytoskeleton proteins. *Immunobiology,* 221, 778-93.

BASKURT, O. K. & MEISELMAN, H. J. 2012. Iatrogenic hyperviscosity and thrombosis. *Seminars in Thrombosis and Hemostasis,* 38, 854-864.

BELLARY, S., ARDEN, W. W., SCHWARTZ, R. W. & ANDERSON, K. W. 1995. Effect of lipopolysaccharide, leukocytes, and monoclonal anti-lipid a antibodies on erythrocyte membrane elastance. *Shock,* 3, 132-136.

BESTER, J., VAN ROOY, M. J., MBOTWE, S., DUIM, W. & PRETORIUS, E. 2016. Transient ischemic attack during smoking: The thrombotic state of erythrocytes and platelets illustrated visually. *Ultrastruct Pathol,* 40**,** 57-9.

BESTER, J., SOMA, P., KELL, D. B. & PRETORIUS, E. 2015b. Viscoelastic and ultrastructural characteristics of whole blood and plasma in Alzheimer-type dementia, and the possible role of bacterial lipopolysaccharides (LPS). *Oncotarget,* 6, 35284-35303.

BEUTLER, B. 1993. Endotoxin, tumor necrosis factor, and related mediators: new approaches to shock. *New Horiz,* 1, 3-12.

BEUTLER, B., DU, X. & POLTORAK, A. 2001. Identification of toll-like receptor 4 (Tlr4) as the sole conduit for LPS signal transduction: Genetic and evolutionary studies. *Journal of Endotoxin Research,* 7, 277-280.

BEUTLER, B., MILSARK, I. W. & CERAMI, A. C. 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science,* 229, 869-71.

BIANCALANA, M. & KOIDE, S. 2010. Molecular mechanism of Thioflavin-T binding to amyloid fibrils. *Biochim Biophys Acta,* 1804, 1405-12.

BIANCALANA, M., MAKABE, K., KOIDE, A. & KOIDE, S. 2009. Molecular mechanism of thioflavin-T binding to the surface of beta-rich peptide self-assemblies. *J Mol Biol,*

385, 1052-63.

BICK, R. L. 2002. Disseminated intravascular coagulation: a review of etiology, pathophysiology, diagnosis, and management: guidelines for care. *Clinical and applied thrombosis/hemostasis,* 8**,** 1-31.

BINDER, V., BERGUM, B., JAISSON, S., GILLERY, P., SCAVENIUS, C., SPRIET, E., NYHAUG, A. K., ROBERTS, H. M., CHAPPLE, I. L. C., HELLVARD, A., DELALEU, N. & MYDEL, P. 2017. Impact of fibrinogen carbamylation on fibrin clot formation and stability. *Thromb Haemost,* 117**,** 899-910.

BLUM, A. 2009. The possible role of red blood cell microvesicles in atherosclerosis. *Eur J Intern Med,* 20, 101-5.

BOLLIGER, D., SEEBERGER, M. D. & TANAKA, K. A. 2012. Principles and practice of thromboelastography in clinical coagulation management and transfusion practice. *Transfus Med Rev,* 26, 1-13.

BORTHWICK, G. M., JOHNSON, A. S., PARTINGTON, M., BURN, J., WILSON, R. & ARTHUR, H. M. 2006. Therapeutic levels of aspirin and salicylate directly inhibit a model of angiogenesis through a Cox-independent mechanism. *FASEB Journal,* 20, 2009-2016.

BRANGER, J., VAN DEN BLINK, B., WEIJER, S., GUPTA, A., VAN DEVENTER, S. J. H., HACK, C. E., PEPPELENBOSCH, M. P. & VAN DER POLL, T. 2003. Inhibition of coagulation, fibrinolysis, and endothelial cell activation by a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. *Blood,* 101, 4446-4448.

BRAUCKMANN, S., EFFENBERGER-NEIDNICHT, K., DE GROOT, H., NAGEL, M., MAYER, C., PETERS, J. & HARTMANN, M. 2016. Lipopolysaccharide-induced hemolysis: Evidence for direct membrane interactions. *Scientific reports,* 6, 35508-35508.

BREKKE, O. L., CHRISTIANSEN, D., FURE, H., FUNG, M. & MOLLNES, T. E. 2007. The

role of complement C3 opsonization, C5a receptor, and CD14 in E. coli-induced up-regulation of granulocyte and monocyte CD11b/CD18 (CR3), phagocytosis, and oxidative burst in human whole blood. *J Leukoc Biol,* 81, 1404-13.

BRIGHTON, T. A., EIKELBOOM, J. W., MANN, K., MISTER, R., GALLUS, A., OCKELFORD, P., GIBBS, H., HAGUE, W., XAVIER, D., DIAZ, R., KIRBY, A. & SIMES, J. 2012. Low-dose aspirin for preventing recurrent venous thromboembolism. *N Engl J Med,* 367**,** 1979-87.

BOLLIGER, D., SEEBERGER, M. D. & TANAKA, K. A. 2012. Principles and practice of thromboelastography in clinical coagulation management and transfusion practice. *Transfus Med Rev,* 26, 1-13.

BUCAY, I., O'BRIEN, E. T., III, WULFE, S. D., SUPERFINE, R., WOLBERG, A. S., FALVO, M. R. & HUDSON, N. E. 2015. Physical Determinants of Fibrinolysis in Single Fibrin Fibers. *PLOS ONE,* 10**,** e0116350.

BUYS, A. V., VAN ROOY, M. J., SOMA, P., VAN PAPENDORP, D., LIPINSKI, B. &PRETORIUS, E. 2013. Changes in red blood cell membrane structure in type 2 diabetes: a scanning electron and atomic force microscopy study. *Cardiovasc Diabetol,* 12, 25.

CALABRESE, V., CIGHETTI, R. & PERI, F. 2015. Molecular simplification of lipid A structure: TLR4-modulating cationic and anionic amphiphiles. *Mol Immunol,* 63, 153-61.

CAMPBELL, R. A., ALEMAN, M., GRAY, L. D., FALVO, M. R. & WOLBERG, A. S. 2010. Flow profoundly influences fibrin network structure: implications for fibrin formation and clot stability in haemostasis. *Thromb Haemost,* 104, 1281-4.

CAMUS, S. M., DE MORAES, J. A., BONNIN, P.2015. Circulating cell membrane microparticles transfer heme to endothelial cells and trigger vasoocclusions in sickle cell disease. *Blood,* 125, 3805-3814.

CANI, P. D., BIBILONI, R., KNAUF, C., WAGET, A., NEYRINCK, A. M., DELZENNE, N.

M. & BURCELIN, R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, 57, 1470-81.

CARDENAS-PEREZ, S., CHANONA-PEREZ, J. J. 2018. Structural, mechanical and enzymatic study of pectin and cellulose during mango ripening. *Carbohydr Polym*, 196, 313-321.

CHABY, R. 2004. Lipopolysaccharide-binding molecules: transporters, blockers and sensors. *Cell Mol Life Sci*, 61, 1697-713.

CHAN, F. T., KAMINSKI SCHIERLE, G. S., KUMITA, J. R., BERTONCINI, C. W., DOBSON, C. M. & KAMINSKI, C. F. 2013. Protein amyloids develop an intrinsic fluorescence signature during aggregation. *Analyst*, 138**,** 2156-62.

CHAN, K. M., CHENG, C. H., WU, T. H., LEE, C. F., WU, T. J., CHOU, H. S. & LEE, W. C. 2018. De Novo Endotoxin-Induced Production of Antibodies against the Bile Salt Export Pump Associated with Bacterial Infection following Major Hepatectomy. *Biomed Res Int*, 2018, 6197152.

CHAPMAN, M. E. & WIDEMAN JR, R. F. 2006. Evaluation of the serotonin receptor blocker methiothepin in broilers injected intravenously with lipopolysaccharide and microparticles. *Poultry Science*, 85, 2222-2230.

CHETVERIKOV, P. E., DESNITSKIY, A. G. & NAVIA, D. 2015. Confocal microscopy refines generic concept of a problematic taxon: rediagnosis of the genus Neoprothrix and remarks on female anatomy of eriophyoids (Acari: Eriophyoidea). *Zootaxa*, 3919, 179-91.

CHITI, F. & DOBSON, C. M. 2006. Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem*, 75, 333-66.

CHIVA-BLANCH, G., SUADES, R., PADRÓ, T., VILAHUR, G., PEÑA, E., YBARRA, J., POU, J. M. & BADIMON, L. 2016. Microparticle Shedding by Erythrocytes, Monocytes and Vascular Smooth Muscular Cells Is Reduced by Aspirin in Diabetic Patients. *Revista Espanola de Cardiologia*, 69, 672-680.

CHU, A. J. 2006a. Role of tissue factor in thrombosis. Coagulation-inflammation-thrombosis circuit. *Front Biosci,* 11, 256-71.

CHU, A. J. 2006b. Tissue factor upregulation drives a thrombosis-inflammation circuit in relation to cardiovascular complications. *Cell Biochem Funct,* 24, 173-92.

CILIA LA CORTE, A. L., PHILIPPOU, H. & ARIËNS, R. A. S. 2011. Role of fibrin structure in thrombosis and vascular disease. *Adv Protein Chem Struct Biol,* 83, 75-127.

CINGOZ, G. S. & GUREL, E. 2016. Effects of salicylic acid on thermotolerance and cardenolide accumulation under high temperature stress in Digitalis trojana Ivanina. *Plant Physiology and Biochemistry,* 105, 145-149.

COATS, S. R., PHAM, T. T., BAINBRIDGE, B. W., REIFE, R. A. & DARVEAU, R. P. 2005. MD-2 mediates the ability of tetra-acylated and penta-acylated lipopolysaccharides to antagonize Escherichia coli lipopolysaccharide at the TLR4 signaling complex. *J Immunol,* 175, 4490-8.

COLLET, J.-P., LESTY, C., MONTALESCOT, G. & WEISEL, J. W. 2003. Dynamic changes of fibrin architecture during fibrin formation and intrinsic fibrinolysis of fibrin-rich clots. *Journal of Biological Chemistry,* 278**,** 21331-21335.

CONLON, R. L. D. A. P. J. April 1995. Prolonged Expression of Lipopolysaccharide (LPS)-InducedInflammatory Genes in Whole Blood Requires. Continual Exposure to LPS. *INFECTION AND IMMUNITY,* 63, 1362–1368.

COOPER, G. J. S., LEIGHTON, B., DIMITRIADIS, G. D., PARRYBILLINGS, M., KOWALCHUK, J. M., HOWLAND, K., ROTHBARD, J. B., WILLIS, A. C. & REID, K. B. M. 1988. Amylin Found in Amyloid Deposits in Human Type-2 Diabetes-Mellitus May Be a Hormone That Regulates Glycogen-Metabolism in Skeletal-Muscle. *Proc Natl Acad Sci,* 85, 7763-7766.

COOPER, G. J. S., WILLIS, A. C., CLARK, A., TURNER, R. C., SIM, R. B. & REID, K. B. M. 1987. Purification and characterization of a peptide from amyloid-rich pancreases of type

2 diabetic patients. *Proc Natl Acad Sci U S A,* 84, 8628-32.

CUAZ-PÉROLIN, C., BILLIET, L., BAUGÉ, E., COPIN, C., SCOTT-ALGARA, D., GENZE, F., BÜCHELE, B., SYROVETS, T., SIMMET, T. & ROUIS, M. 2008. Antiinflammatory and antiatherogenic effects of the NF-κB inhibitor acetyl-11-Keto-β-boswellic acid in LPS-challenged ApoE-/- mice. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 28, 272-277.

CURRY, N. S., DAVENPORT, R., PAVORD, S., MALLETT, S. V., KITCHEN, D., KLEIN, A. A., MAYBURY, H., COLLINS, P. W. & LAFFAN, M. 2018. The use of viscoelastic haemostatic assays in the management of major bleeding. *British Journal of Haematology,* 182**,** 789-806.

CZERKIES, M., BORZECKA, K., ZDIORUK, M. I., PŁÓCIENNIKOWSKA, A., SOBOTA, A. & KWIATKOWSKA, K. 2013. An interplay between scavenger receptor A and CD14 during activation of J774 cells by high concentrations of LPS. *Immunobiology,* 218, 1217-1226.

DAI, B., WEI, D., ZHENG, N. N.. 2018. Coccomyxa Gloeobotrydiformis Polysaccharide Inhibits Lipopolysaccharide-Induced Inflammation in RAW 264.7 Macrophages. *Cell Physiol Biochem,* 51, 2523-2535.

DANESH, J., LEWINGTON, S., THOMPSON, S. G.,., et al. 2005. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *Jama,* 294, 1799-809.

DE CASTRO, C., PARRILLI, M., HOLST, O. & MOLINARO, A. 2010. Microbe-associated molecular patterns in innate immunity. Extraction and chemical analysis of gram-negative bacterial lipopolysaccharides. *Methods in Enzymology.*

DE PUNDER, K. & PRUIMBOOM, L. 2015. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Front Immunol,* 6, 223.

DIACOVICH, L. & GORVEL, J. P. 2010. Bacterial manipulation of innate immunity to promote infection. *Nature Reviews Microbiology,* 8, 117-128.

DICKNEITE, G., HERWALD, H., KORTE, W., ALLANORE, Y., DENTON, C. P. & MATUCCI CERINIC, M. 2015. Coagulation factor XIII: a multifunctional transglutaminase with clinical potential in a range of conditions. *Thromb Haemost,* 113, 686-97.

DING, P. H., WANG, C. Y., DARVEAU, R. P. & JIN, L. 2013. Porphyromonas gingivalis LPS stimulates the expression of LPS-binding protein in human oral keratinocytes in vitro. *Innate Immunity,* 19, 66-75.

DRAXLER, D. F. & MEDCALF, R. L. 2015. The Fibrinolytic System—More Than Fibrinolysis? *Transfusion medicine reviews,* 29**,** 102-109.

DUBURCQ, T., TOURNOYS, A., GNEMMI, V., HUBERT, T., GMYR, V., PATTOU, F. & JOURDAIN, M. 2015. Impact of obesity on endotoxin-induced disseminated intravascular coagulation. *Shock,* 44**,** 341-347.

DUNN, E. J. & ARIËNS, R. A. S. 2004. Fibrinogen and fibrin clot structure in diabetes. *Herz,* 29, 470-9.

DUNN, E. J., ARIËNS, R. A. S. & GRANT, P. J. 2005. The influence of type 2 diabetes on fibrin structure and function. *Diabetologia,* 48, 1198-206.

DUNN, E. J., PHILIPPOU, H., ARIËNS, R. A. S. & GRANT, P. J. 2006. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. *Diabetologia,* 49, 1071-80.

EDISON, M., JEEVA, J. B. & SINGH, M. 2011. Digital analysis of changes by Plasmodium vivax malaria in erythrocytes. *Indian J Exp Biol,* 49, 11-5.

EISENBERG, D. & JUCKER, M. 2012. The amyloid state of proteins in human diseases. *Cell,* 148, 1188-203.

ELBLBESY, M. A., HEREBA, A. R. M. & SHAWKI, M. M. 2012. Effects of aspirin on rheological properties of erythrocytes in vitro. *International Journal of Biomedical Science,* 8, 188-193.

ELSING, C., ERNST, S., KAYALI, N., STREMMEL, W. & HARENBERG, S. 2011. Lipopolysaccharide binding protein, interleukin-6 and C-reactive protein in acute gastrointestinal infections: Value as biomarkers to reduce unnecessary antibiotic therapy. *Infection,* 39, 327-331.

ERENLER, A. K. & YARDAN, T. 2015. Presepsin (sCD14-ST) as a biomarker of sepsis in clinical practice and in emergency department: A mini review. *LaboratoriumsMedizin,* 39, 367-372.

FÄNDRICH, M., MEINHARDT, J. & GRIGORIEFF, N. 2009. Structural polymorphism of Alzheimer Abeta and other amyloid fibrils. *Prion,* 3, 89-93.

FARRELL, D. H. 2012. gamma' Fibrinogen as a novel marker of thrombotic disease. *Clin Chem Lab Med,* 50, 1903-9.

FREUDENBERG, M. A. & GALANOS, C. 1990. Bacterial lipopolysaccharides: structure, metabolism and mechanisms of action. *Int Rev Immunol,* 6, 207-21.

FUCHSBERGER, C., FLANNICK, J., TESLOVICH, T. M.,., et al. 2016. The genetic architecture of type 2 diabetes. *Nature,* 536, 41-7.

FUJIKAWA, S. & ENDOH, K. 2014. Cryo-scanning electron microscopy to study the freezing behavior of plant tissues. *Methods Mol Biol,* 1166, 99-116.

FUNDERBURG, N. T., STUBBLEFIELD PARK, S. R., SUNG, H. C., HARDY, G., CLAGETT, B., IGNATZ-HOOVER, J., HARDING, C. V., FU, P., KATZ, J. A., LEDERMAN, M. M. & LEVINE, A. D. 2013. Circulating CD4+ and CD8+ T cells are activated in inflammatory bowel disease and are associated with plasma markers of inflammation. *Immunology,* 140, 87-97.

GHANIM, H., ABUAYSHEH, S., SIA, C. L., KORZENIEWSKI, K., CHAUDHURI, A., FERNANDEZ-REAL, J. M. & DANDONA, P. 2009. Increase in plasma endotoxin concentrations and the expression of toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: Implications

for insulin resistance. *Diabetes Care,* 32, 2281-2287.

GLAROS, T. G., CHANG, S., GILLIAM, E. A., MAITRA, U., DENG, H. & LI, L. 2013. Causes and consequences of low grade endotoxemia and inflammatory diseases. *Front Biosci (Schol Ed),* 5, 754-65.

GOLDBLUM, S. E., BRANN, T. W., DING, X., PUGIN, J. & TOBIAS, P. S. 1994. Lipopolysaccharide (LPS)-binding protein and soluble CD14 function as accessory molecules for LPS-induced changes in endothelial barrier function, in vitro. *Journal of Clinical Investigation,* 93, 692-702.

GONZALEZ-QUINTELA, A., ALONSO, M., CAMPOS, J., VIZCAINO, L., LOIDI, L. & GUDE, F. 2013. Determinants of Serum Concentrations of Lipopolysaccharide-Binding Protein (LBP) in the Adult Population: The Role of Obesity. *PLoS ONE,* 8.

GROENNING, M. 2010. Binding mode of Thioflavin T and other molecular probes in the context of amyloid fibrils-current status. *Journal of Chemical Biology,* 3, 1-18.

GYAWALI, P., RICHARDS, R. S., BWITITI, P. T. & NWOSE, E. U. 2015. Association of abnormal erythrocyte morphology with oxidative stress and inflammation in metabolic syndrome. *Blood Cells Mol Dis,* 54, 360-3.

HAIMOVITZ-FRIEDMAN, A., CORDON-CARDO, C., BAYOUMY, S.,. 1997b. Lipopolysaccharide induces disseminated endothelial apoptosis requiring ceramide generation. *Journal of Experimental Medicine,* 186, 1831-1841.

HALABY, R., POPMA, C. J., COHEN, A., CHI,. 2015. D-Dimer elevation and adverse outcomes. *J Thromb Thrombolysis,* 39, 55-9.

HALDER, B., SINGH, S. & THAKUR, S. S. 2015. Withania somnifera Root Extract Has Potent Cytotoxic Effect against Human Malignant Melanoma Cells. *PLoS One,* 10, e0137498.

HARTE, A. L., DA SILVA, N. F., CREELY, S. J., 2010b. Elevated endotoxin levels in non-alcoholic fatty liver disease. *Journal of Inflammation,* 7.

HAYAT, M. A. 1974. *Principles and techniques of scanning electron microscopy. Biological*

applications., New York, Van Nostrand Reinhold Company.

HE, J., WANG, N., TSURUI, H., KATO, M., 2016. Noninvasive, label-free, three-dimensional imaging of melanoma with confocal photothermal microscopy: Differentiate malignant melanoma from benign tumor tissue. *Sci Rep,* 6, 30209.

HEPPNER, F. L., RANSOHOFF, R. M. & BECHER, B. 2015. Immune attack: The role of inflammation in Alzheimer disease. *Nature Reviews Neuroscience,* 16, 358-372.

HERCZENIK, E. & GEBBINK, M. F. B. G. 2008. Molecular and cellular aspects of protein misfolding and disease. *FASEB J,* 22, 2115-33.

HEUMANN, D., ADACHI, Y., LE ROY, D., OHNO, N., YADOMAE, T., GLAUSER, M. P. & CALANDRA, T. 2001. Role of plasma, lipopolysaccharide-binding protein, and CD14 in response of mouse peritoneal exudate macrophages to endotoxin. *Infection and Immunity,* 69, 378-385.

HOFFMAN, A., GOETZ, M., VIETH, M., GALLE, P. R., NEURATH, M. F. & KIESSLICH, R. 2006. Confocal laser endomicroscopy: technical status and current indications. *Endoscopy,* 38, 1275-83.

HOLST, O. 2007. The structures of core regions from enterobacterial lipopolysaccharides - An update. *FEMS Microbiology Letters,* 271, 3-11.

HÖPPENER, J. W. M. & LIPS, C. J. M. 2006. Role of islet amyloid in type 2 diabetes mellitus. *Int J Biochem Cell Biol,* 38, 726-36.

HOU, H., GE, Z., YING, P., DAI, J., SHI, D., XU, Z., CHEN, D. & JIANG, Q. 2012. Biomarkers of deep venous thrombosis. *J Thromb Thrombolysis,* 34, 335-46.

HURLEY, J. C., NOWAK, P., ÖHRMALM, L., GOGOS, C., ARMAGANIDIS, A. & GIAMARELLOS-BOURBOULIS, E. J. 2015. Endotoxemia as a Diagnostic Tool for Patients with Suspected Bacteremia Caused by Gram-Negative Organisms: a Meta-Analysis of 4 Decades of Studies. *Journal of Clinical Microbiology,* 53**,** 1183-1191.

ITZHAKI, R. F., LATHE, R., BALIN, B. J., BALL, M. J. 2016b. Microbes and Alzheimer's

Disease. *J Alzheimers Dis,* 51, 979-984.

JAIKARAN, E. T. A. S. & CLARK, A. 2001. Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology. *Biochim Biophys Acta,* 1537, 179-203.

JIANG, N., KIM, H. J., CHOZINSKI, T. J., AZPURUA, J. E., EATON, B. A., VAUGHAN, J. C. & PARRISH, J. Z. 2018. Superresolution imaging of Drosophila tissues using expansion microscopy. *Mol Biol Cell,* 29, 1413-1421.

JIANG, W., LEDERMAN, M. M., HUNT, P., SIEG, S. F., HALEY, K., RODRIGUEZ, B., LANDAY, A., MARTIN, J., SINCLAIR, E., ASHER, A. I., DEEKS, S. G., DOUEK, D. C. & BRENCHLEY, J. M. 2009. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis,* 199, 1177-85.

JIAO, B. H., FREUDENBERG, M. & GALANOS, C. 1989. Characterization of the lipid A component of genuine smooth-form lipopolysaccharide. *Eur J Biochem,* 180, 515-8.

JÖRNESKOG, G., EGBERG, N., FAGRELL, B., FATAH, K., HESSEL, B., JOHNSSON, H., BRISMAR, K. & BLOMBÄCK, M. 1996. Altered properties of the fibrin gel structure in patients with IDDM. *Diabetologia,* 39, 1519-23.

JUFFERMANS, N. P., VERBON, A., VAN DEVENTER, S. J., BUURMAN, W. A., VAN DEUTEKOM, H., SPEELMAN, P. & VAN DER POLL, T. 1998. Serum concentrations of lipopolysaccharide activity-modulating proteins during tuberculosis. *J Infect Dis,* 178, 1839-42.

HAGAR, J. A., POWELL, D. A., AACHOUI, Y., ERNST, R. K. & MIAO, E. A. 2013. Cytoplasmic LPS activates caspase-11: Implications in TLR4-independent endotoxic shock. *Science,* 341, 1250-1253.

HAIDINGER, M., WERZOWA, J., KAIN, R., ANTLANGER, M., HECKING, M., PFAFFENBERGER, S., MASCHERBAUER, J., GREMMEL, T., GILBERTSON, J. A., ROWCZENIO, D., WEICHHART, T., KOPECKY, C., HÖRL, W. H., HAWKINS, P. N.

& SÄEMANN, M. D. 2013. Hereditary amyloidosis caused by R554L fibrinogen Aalpha-chain mutation in a Spanish family and review of the literature. *Amyloid,* 20, 72-9.

KAYAGAKI, N., YAMAGUCHI, N., ABE, M., HIROSE, S., SHIRAI, T., OKUMURA, K. & YAGITA, H. 2002. Suppression of antibody production by TNF-related apoptosis-inducing ligand (TRAIL). *Cellular Immunology,* 219, 82-91.

KELL, D., POTGIETER, M. & PRETORIUS, E. 2015a. Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities shared by environmental, laboratory, and clinical microbiology. *F1000Res,* 4, 179.

KELL, D. B. & KENNY, L. C. 2016. A dormant microbial component in the development of pre-eclampsia. BioRxiv preprint. . *bioRxiv,* 057356.

KELL, D. B., POTGIETER, M. & PRETORIUS, E. 2015b. Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities in environmental, laboratory, and clinical microbiology. *F1000Review,* In Press.

KELL, D. B. & PRETORIUS, E. 2014a. Serum ferritin is an important disease marker, and is mainly a leakage product from damaged cells. *Metallomics,* 6, 748-773.

KELL, D. B. & PRETORIUS, E. 2014c. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics,* 4, 748-773.

KELL, D. B. & PRETORIUS, E. 2015a. On the translocation of bacteria and their lipopolysaccharides between blood and peripheral locations in chronic, inflammatory diseases: the central roles of LPS and LPS-induced cell death. *Integr Biol,* 7, 1339-1377.

KELL, D. B. & PRETORIUS, E. 2015h. The simultaneous occurrence of both hypercoagulability and hypofibrinolysis in blood and serum during systemic inflammation, and the roles of iron and fibrin(ogen). *Integrative biology : quantitative biosciences from nano to macro,* 7, 24-52.

KELL, D. B. & PRETORIUS, E. 2016b. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting.

bioRxiv preprint. *bioRxiv*, 054734.

KELL, D. B. & PRETORIUS, E. 2016c. To what extent are the terminal stages of sepsis, septic shock, SIRS, and multiple organ dysfunction syndrome actually driven by a toxic prion/amyloid form of fibrin? bioRxiv preprint. *bioRxiv*, 057851.

KELL, D. B. & PRETORIUS, E. 2017. Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. *Prog Biophys Mol Biol,* 123, 16-41.

KELLUM, J. A., KONG, L., FINK, M. P., WEISSFELD, L. A., YEALY, D. M., PINSKY, M. R., FINE, J., KRICHEVSKY, A., DELUDE, R. L. & ANGUS, D. C. 2007. Understanding the inflammatory cytokine response in pneumonia and sepsis: Results of the genetic and inflammatory markers of sepsis (GenIMS) study. *Archives of Internal Medicine,* 167, 1655-1663.

KETCHUM, P. A. & NOVITSKY, T. J. 2000. Assay of endotoxin by limulus amebocyte lysate. *Methods Mol Med,* 36, 3-12.

KIKUCHI, M., INAGAKI, T., NITTA, M., IMAIDA, K., SHINAGAWA, N., BANNO, S., KOMATSU, H., WAKITA, A. & UEDA, R. 1996. [Changes in erythrocyte structure and in platelets in elderly patients with disseminated intravascular coagulation]. *Nihon Ronen Igakkai Zasshi,* 33, 847-51.

KIM, E. H., SONG, S. H., KIM, G. S., KO, J. S., GWAK, M. S. & LEE, S. K. 2015. Evaluation of "flat-line" thromboelastography after reperfusion during liver transplantation. *Transplant Proc,* 47, 457-9.

KIM, J. I., LEE, C. J., JIN, M. S., LEE, C. H., PAIK, S. G., LEE, H. & LEE, J. O. 2005. Crystal structure of CD14 and its implications for lipopolysaccharide signaling. *J Biol Chem,* 280, 11347-51.

KIM, K. E., CHO, Y. S., BAEK, K. S., LI, L., BAEK, K. H., KIM, J. H., KIM, H. S. & SHEEN, Y. H. 2016. Lipopolysaccharide-binding protein plasma levels as a biomarker of obesity-

related insulin resistance in adolescents. *Korean J Pediatr,* 59**,** 231-8.

KIM, E. H., SONG, S. H., KIM, G. S., KO, J. S., GWAK, M. S. & LEE, S. K. 2015. Evaluation of "flat-line" thromboelastography after reperfusion during liver transplantation. *Transplant Proc,* 47**,** 457-9.

KISTENMACHER, T. J., MARZILLI, L. G. & ROSSI, M. 1976. Conformational properties of the osmium tetraoxide bispyridine ester of 1-methylthymine and a comment on the linearity of the trans O=Os=O group. *Bioinorg Chem,* 6**,** 347-64.

KITCHENS, R. L. & THOMPSON, P. A. 2003. Impact of sepsis-induced changes in plasma on LPS interactions with monocytes and plasma lipoproteins: Roles of soluble CD14, LBP, and acute phase lipoproteins. *Journal of Endotoxin Research,* 9, 113-118.

KLEIN, D. C. G., SKJESOL, A., KERS-REBEL, E. D., SHERSTOVA, T., SPORSHEIM, B., EGEBERG, K. W., STOKKE, B. T., ESPEVIK, T. & HUSEBYE, H. 2015. CD14, TLR4 and TRAM Show Different Trafficking Dynamics During LPS Stimulation. *Traffic,* 16, 677-690.

KNOWLES, T. P. J., VENDRUSCOLO, M. & DOBSON, C. M. 2014. The amyloid state and its association with protein misfolding diseases. *Nat Rev Mol Cell Biol,* 15, 384-396.

KOCH, L., HOFER, S., WEIGAND, M. A., FROMMHOLD, D. & POESCHL, J. 2009. Lipopolysaccharide-induced activation of coagulation in neonatal cord and adult blood monitored by thrombelastography. *Thromb Res,* 124, 463-7.

KOOHSHEKAN, B., DIVSALAR, A., SAIEDIFAR, M., SABOURY, A. A., GHALANDARI, B., GHOLAMIAN, A. & SEYEDARABI, A. 2016. Protective effects of aspirin on the function of bovine liver catalase: A spectroscopy and molecular docking study. *Journal of Molecular Liquids,* 218, 8-15.

KUZNETSOVA, I. M., SULATSKAYA, A. I., MASKEVICH, A. A., UVERSKY, V. N. & TUROVEROV, K. K. 2016. High Fluorescence Anisotropy of Thioflavin T in Aqueous Solution Resulting from Its Molecular Rotor Nature. *Analytical Chemistry,* 88, 718-724.

LAMPING, N., DETTMER, R., SCHRODER, N. W., PFEIL, D., HALLATSCHEK, W., BURGER, R. & SCHUMANN, R. R. 1998. LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. *J Clin Invest,* 101, 2065-71.

LANAS, A., CARRERA-LASFUENTES, P., ARGUEDAS, Y., GARCÍA, S., BUJANDA, L., CALVET, X., PONCE, J., PEREZ-AÍSA, T., CASTRO, M., MUÑOZ, M., SOSTRES, C. & GARCÍA-RODRÍGUEZ, L. A. 2015. Risk of upper and lower gastrointestinal bleeding in patients taking nonsteroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. *Clinical Gastroenterology and Hepatology,* 13, 906-912.

LANDSEM, A., FURE, H., CHRISTIANSEN, D., NIELSEN, E. W., OSTERUD, B., MOLLNES, T. E. & BREKKE, O. L. 2015. The key roles of complement and tissue factor in Escherichia coli-induced coagulation in human whole blood. *Clin Exp Immunol*.

LANDSEM, A., NIELSEN, E. W., FURE, H., CHRISTIANSEN, D., LUDVIKSEN, J. K., LAMBRIS, J. D., OSTERUD, B., MOLLNES, T. E. & BREKKE, O. L. 2013. C1-inhibitor efficiently inhibits Escherichia coli-induced tissue factor mRNA up-regulation, monocyte tissue factor expression and coagulation activation in human whole blood. *Clin Exp Immunol,* 173, 217-29.

LATTA, C. H., BROTHERS, H. M. & WILCOCK, D. M. 2014. Neuroinflammation in Alzheimer's disease; A source of heterogeneity and target for personalized therapy. *Neuroscience*.

LAUGERETTE, F., VORS, C., GELOEN, A., CHAUVIN, M. A., SOULAGE, C., LAMBERT-PORCHERON, S., PERETTI, N., ALLIGIER, M., BURCELIN, R., LAVILLE, M., VIDAL, H. & MICHALSKI, M. C. 2011. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. *J Nutr Biochem,* 22, 53-9.

LE ROY, D., DI PADOVA, F., TEES, R., LENGACHER, S., LANDMANN, R., GLAUSER, M. P., CALANDRA, T. & HEUMANN, D. 1999. Monoclonal antibodies to murine lipopolysaccharide (LPS)-binding protein (LBP) protect mice from lethal endotoxemia by

blocking either the binding of LPS to LBP or the presentation of LPS/LBP complexes to CD14. *Journal of Immunology,* 162, 7454-7460.

LEITHÄUSER, B., MROWIETZ, C., PARK, J. W. & JUNG, F. 2012. Influence of acetylsalicylic acid (Aspirin) on cutaneous microcirculation. *Clinical Hemorheology and Microcirculation,* 50, 25-34.

LEVENSON, C. W. 2003. Iron and Parkinson's disease: chelators to the rescue? *Nutr Rev,* 61, 311-3.

LEVINE III, H. 1997. Stopped-flow kinetics reveal multiple phases of thioflavin T binding to Alzheimer β(140) amyloid fibrils. *Archives of Biochemistry and Biophysics,* 342, 306-316.

LEVI, M. & VAN DER POLL, T. 2013. Disseminated intravascular coagulation: a review for the internist. *Internal and emergency medicine,* 8**,** 23-32.

LI, L., ZHANG, Y., LUO, H., HUANG, C., LI, S., LIU, A. & JIANG, Y. 2018. Systematic Identification and Analysis of Expression Profiles of mRNAs and IncRNAs in Macrophage Inflammatory Response. *Shock.*

LI, X., WANG, D., CHEN, Z., LU, E., WANG, Z., DUAN, J., TIAN, W., WANG, Y., YOU, L., ZOU, Y., CHENG, Y., ZHU, Q., WAN, X., XI, T., BIRNBAUMER, L. & YANG, Y. 2015. Gαi1 and Gαi3 regulate macrophage polarization by forming a complex containing CD14 and Gab1. *Proceedings of the National Academy of Sciences of the United States of America,* 112, 4731-4736.

LI, Z., SCOTT, M. J., FAN, E. K., LI, Y., LIU, J., XIAO, G., LI, S., BILLIAR, T. R., WILSON, M. A., JIANG, Y. & FAN, J. 2016. Tissue damage negatively regulates LPS-induced macrophage necroptosis. *Cell Death and Differentiation.*

LIH, E., JUNG, J. W., JOUNG, Y. K., AHN, D. J. & HAN, D. K. 2016. Synergistic effect of anti-platelet and anti-inflammation of drug-coated Co-Cr substrates for prevention of initial in-stent restenosis. *Colloids and Surfaces B: Biointerfaces,* 140, 353-360.

LIN, C. C., EZZELARAB, M., SHAPIRO, R., EKSER, B., LONG, C., HARA, H., ECHEVERRI, G., TORRES, C., WATANABE, H., AYARES, D., DORLING, A. & COOPER, D. K. C. 2010. Recipient tissue factor expression is associated with consumptive coagulopathy in pig-to-primate kidney xenotransplantation. *American Journal of Transplantation,* 10, 1556-1568.

LIPINSKI, B., PRETORIUS, E., OBERHOLZER, H. M. & VAN DER SPUY, W. J. 2012. Iron enhances generation of fibrin fibers in human blood: Implications for pathogenesis of stroke. *Microsc Res Tech,* 75, 1185-1190.

LIU, Y., WALTER, S., STAGI, M., CHERNY, D., LETIEMBRE, M., SCHULZ-SCHAEFFER, W., HEINE, H., PENKE, B., NEUMANN, H. & FASSBENDER, K. 2005. LPS receptor (CD14): A receptor for phagocytosis of Alzheimer's amyloid peptide. *Brain,* 128, 1778-1789.

LOOMES, K. M. 2011. Survival of an islet beta-cell in type-2 diabetes: curbing the effects of amyloid cytotoxicity. *Islets,* 3, 38-9.

LOPES, R. D., RAO, M., SIMON, D. N., THOMAS, L., ANSELL, J., FONAROW, G. C., GERSH, B. J., GO, A. S., HYLEK, E. M., KOWEY, P., PICCINI, J. P., SINGER, D. E., CHANG, P., PETERSON, E. D. & MAHAFFEY, K. W. 2016. Triple vs Dual Antithrombotic Therapy in Patients with Atrial Fibrillation and Coronary Artery Disease. *American Journal of Medicine,* 129, 592-599.

LORENZO, A., RAZZABONI, B., WEIR, G. C. & YANKNER, B. A. 1994. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. *Nature,* 368, 756-60.

LUO, G., CHENG, B. C., ZHAO, H., FU, X. Q., XIE, R., ZHANG, S. F., PAN, S. Y. & ZHANG, Y. 2018. Schisandra Chinensis Lignans Suppresses the Production of Inflammatory Mediators Regulated by NF-kappaB, AP-1, and IRF3 in Lipopolysaccharide-Stimulated RAW264.7 Cells. *Molecules,* 23.

MACKMAN, N. 2009. The role of tissue factor and factor VIIa in hemostasis. *Anesth Analg,* 108,

1447-52.

MAESHIMA, N. & FERNANDEZ, R. C. 2013. Recognition of lipid A variants by the TLR4-MD-2 receptor complex. *Front Cell Infect Microbiol,* 3, 3.

MAITRA, U., DENG, H., GLAROS, T., BAKER, B., CAPELLUTO, D. G., LI, Z. & LI, L. 2012. Molecular mechanisms responsible for the selective and low-grade induction of proinflammatory mediators in murine macrophages by lipopolysaccharide. *J Immunol,* 189, 1014-23.

MAITRA, U., GAN, L., CHANG, S. & LI, L. 2011. Low-dose endotoxin induces inflammation by selectively removing nuclear receptors and activating CCAAT/enhancer-binding protein delta. *J Immunol,* 186, 4467-73.

MARSHALL, J. C., WALKER, P. M., FOSTER, D. M., HARRIS, D., RIBEIRO, M., PAICE, J., ROMASCHIN, A. D. & DERZKO, A. N. 2002. Measurement of endotoxin activity in critically ill patients using whole blood neutrophil dependent chemiluminescence. *Critical Care,* 6, 342-348.

MASON, P. J., FREEDMAN, J. E. & JACOBS, A. K. 2004. Aspirin resistance: current concepts. *Rev Cardiovasc Med,* 5, 156-63.

MATTSBY-BALTZER, I., LINDGREN, K., LINDHOLM, B. & EDEBO, L. 1991. Endotoxin shedding by enterobacteria: free and cell-bound endotoxin differ in Limulus activity. *Infect Immun,* 59, 689-95.

MCBROOM, A. J., JOHNSON, A. P., VEMULAPALLI, S. & KUEHN, M. J. 2006. Outer membrane vesicle production by Escherichia coli is independent of membrane instability. *Journal of Bacteriology,* 188, 5385-5392.

MEANS, T. K., LIEN, E., YOSHIMURA, A., WANG, S., GOLENBOCK, D. T. & FENTON, M. J. 1999. The CD14 ligands lipoarabinomannan and lipopolysaccharide differ in their requirement for toll-like receptors. *Journal of Immunology,* 163, 6748-6755.

MEDVED, L. & NIEUWENHUIZEN, W. 2003. Molecular mechanisms of initiation of

fibrinolysis by fibrin. *Thrombosis and haemostasis,* 89**,** 409-419.

METZGER, M., KONRAD, A., SKANDARY, S., ASHRAF, I., MEIXNER, A. J. & BRECHT, M. 2016. Resolution enhancement for low-temperature scanning microscopy by cryo-immersion. *Opt Express,* 24, 13023-32.

MITCHELL, J., KIM, S. J., SEELMANN, A., VEIT, B., SHEPARD, B., IM, E. & RHEE, S. H. 2018. Src family kinase tyrosine phosphorylates Toll-like receptor 4 to dissociate MyD88 and Mal/Tirap, suppressing LPS-induced inflammatory responses. *Biochem Pharmacol,* 147, 119-127.

MOHAMMADI ARIA, M., ERTEN, A. & YALCIN, O. 2019. Technology Advancements in Blood Coagulation Measurements for Point-of-Care Diagnostic Testing. *Frontiers in Bioengineering and Biotechnology,* 7.

MONICI, M. 2005. Cell and tissue autofluorescence research and diagnostic applications. *Biotechnol Annu Rev,* 11**,** 227-56.

MONROE, D. M. & KEY, N. S. 2007. The tissue factor-factor VIIa complex: procoagulant activity, regulation, and multitasking. *J Thromb Haemost,* 5, 1097-105.

MORAES, J. A., FRONY, A. C., BARCELLOS-DE-SOUZA, P., MENEZES DA CUNHA, M., BRASIL BARBOSA CALCIA, T., BENJAMIM, C. F., BOISSON-VIDAL, C. & BARJA-FIDALGO, C. 2018. Downregulation of Microparticle Release and Pro-Inflammatory Properties of Activated Human Polymorphonuclear Neutrophils by LMW Fucoidan. *J Innate Immun,* 1-17.

MORAN, A. P., PRENDERGAST, M. M. & APPELMELK, B. J. 1996. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. *FEMS Immunology and Medical Microbiology,* 16, 105-115.

MORENO-NAVARRETE, J. M., ESCOTE, X., ORTEGA, F. 2013. A role for adipocyte-derived lipopolysaccharide-binding protein in inflammation- and obesity-associated adipose tissue dysfunction. *Diabetologia,* 56, 2524-37.

MOODY, D. B. & COTTON, R. N. 2017. Four pathways of CD1 antigen presentation to T cells. *Curr Opin Immunol,* 46, 127-133.

MORENO-NAVARRETE, J. M., MANCO, M., IBANEZ, J. Metabolic endotoxemia and saturated fat contribute to circulating NGAL concentrations in subjects with insulin resistance. *Int J Obes (Lond),* 34, 240-9.

MORRISON, D. C. & ULEVITCH, R. J. 1978. The effects of bacterial endotoxins on host mediation systems. *American Journal of Pathology,* 93, 527-617.

MORTENSEN, K. I., SUNG, J., SPUDICH, J. A. & FLYVBJERG, H. 2016. How to Measure Separations and Angles Between Intramolecular Fluorescent Markers. *Methods Enzymol,* 581, 147-185.

MÜLLER-LOENNIES, S., BRADE, L., MACKENZIE, C. R., DI PADOVA, F. E. & BRADE, H. 2003. Identification of a cross-reactive epitope widely present in lipopolysaccharide from enterobacteria and recognized by the cross-protective monoclonal antibody WN1 222-5. *Journal of Biological Chemistry,* 278, 25618-25627.

MUROI, M. & TANAMOTO, K. I. 2002. The polysaccharide portion plays an indispensable role in Salmonella lipopolysaccharide-induced activation of NF-κB through human toll-like receptor 4. *Infection and Immunity,* 70, 6043-6047.

MYUNG, J., PARK, S. J., LIM, J., KIM, Y. H., SHIN, S. & LIM, C. H. 2016. Effects of lipopolysaccharide on changes in red blood cells in a mice endotoxemia model. *Clin Hemorheol Microcirc,* 63**,** 305-312.

NARAYANAN, S. 1999. Multifunctional roles of thrombin. *Ann Clin Lab Sci,* 29**,** 275-80.

NEDELA, V., HRIB, J., HAVEL, L., HUDEC, J. & RUNSTUK, J. 2016. Imaging of Norway spruce early somatic embryos with the ESEM, Cryo-SEM and laser scanning microscope. *Micron,* 84, 67-71.

NI, H., ZOU, L., GUO, Q. & DING, X. 2017. Lateral resolution enhancement of confocal microscopy based on structured detection method with spatial light modulator. *Opt*

Express, 25, 2872-2882.

NELSON, N. & JUNGE, W. 2015. Structure and energy transfer in photosystems of oxygenic photosynthesis. *Annu Rev Biochem,* 84, 659-83.

NIELSEN, V. G. 2008. Beyond cell based models of coagulation: analyses of coagulation with clot "lifespan" resistance-time relationships. *Thromb Res,* 122, 145-52.

NIELSEN, V. G., GURLEY, W. Q., JR. & BURCH, T. M. 2004. The impact of factor XIII on coagulation kinetics and clot strength determined by thrombelastography. *Anesth Analg,* 99, 120-3.

NIELSEN, V. G., KIRKLIN H.K., HOOGENDOORN H, ELLIS T.C., HOLMAN W.L. 2007. Thromboelastographic method to quantify the contribution of factor XIII to coagulation kinetics. *Blood coagulation & Fibrinolysis: an international journal in haemostasis and thrombosis,* 18, 145-150.

NIELSEN, V. G. & PRETORIUS, E. 2014a. Iron-enhanced coagulation is attenuated by chelation A thrombelastographic and ultrastructural analysis. *Blood Coagul Fibrinolysis,* 25, 845-50.

NIEUWENHUIZEN, W. 2001. Fibrin-mediated plasminogen activation. *Annals of the New York Academy of Sciences,* 936**,** 237-246

NOORT, A. R., TAK, P. P. & TAS, S. W. 2015. Non-canonical NF-kB signaling in rheumatoid arthritis: Dr Jekyll and Mr Hyde? *Arthritis Research and Therapy,* 17.

NORRIS, L. A. 2003. Blood coagulation. *Best practice & research. Clinical obstetrics & gynaecology,* 17**,** 369-383.

NOVITSKY, T. J. 1998. Limitations of the Limulus amebocyte lysate test in demonstrating circulating lipopolysaccharides. *Ann N Y Acad Sci,* 851, 416-21.

NUNES, K. P., RIGSBY, C. S. & WEBB, R. C. 2010. RhoA/Rho-kinase and vascular diseases: what is the link? *Cell Mol Life Sci,* 67, 3823-36.

NWANESHIUDU, A., KUSCHAL, C., SAKAMOTO, F. H., ANDERSON, R. R.,

SCHWARZENBERGER, K. & YOUNG, R. C. 2012. Introduction to confocal microscopy. *J Invest Dermatol,* 132, e3.

NYMARK, M., PUSSINEN, P. J., TUOMAINEN, A. M., FORSBLOM, C., GROOP, P. H. & LEHTO, M. 2009. Serum lipopolysaccharide activity is associated with the progression of kidney disease in finnish patients with type 1 diabetes. *Diabetes Care,* 32, 1689-1693.

O'NEILL, L. A. J. 2014. Glycolytic reprogramming by TLRs in dendritic cells. *Nature Immunology,* 15, 314-315.

OHNO, T., HORESH, M. Y., MERRITT, K. A. & WAGAI, R. 2002. Calcium and pH effects on salicylic acid phytotoxicity. *Allelopathy Journal,* 9, 19-25.

OLANOW, C. W. & BRUNDIN, P. 2013. Parkinson's disease and alpha synuclein: is Parkinson's disease a prion-like disorder? *Mov Disord,* 28, 31-40.

OLUMUYIWA-AKEREDOLU, O. O., SOMA, P., BUYS, A. V., DEBUSHO, L. K. & PRETORIUS, E. 2017. Characterizing pathology in erythrocytes using morphological and biophysical membrane properties: Relation to impaired hemorheology and cardiovascular function in rheumatoid arthritis. *Biochim Biophys Acta Biomembr,* 1859, 2381-2391.

OPAL, S. M., PALARDY, J. E., MARRA, M. N., FISHER JR, C. J., MCKELLIGON, B. M. & SCOTT, R. W. 1994. Relative concentrations of endotoxin-binding proteins in body fluids during infection. *Lancet,* 344, 429-431.

OPAL, S. M., SCANNON, P. J., VINCENT, J. L., WHITE, M., CARROLL, S. F. 1999. Relationship between plasma levels of lipopolysaccharide (LPS) and LPS- binding protein in patients with severe sepsis and septic shock. *Journal of Infectious Diseases,* 180, 1584-1589.

OSNES, L. T., HAUG, K. B., JOO, G. B., WESTVIK, A. B., OVSTEBO, R. & KIERULF, P. 2000. Aspirin potentiates LPS-induced fibrin formation (FPA) and TNF-alpha-synthesis in whole blood. *Thromb Haemost,* 83**,** 868-73.

ØVSTEBØ, R., HELLUM, M., AASS, H. C. D., TRØSEID, A. M., BRANDTZAEG, P.,

MOLLNES, T. E. & HENRIKSSON, C. E. 2014. Microparticle-associated tissue factor activity is reduced by inhibition of the complement protein 5 in Neisseria meningitidis-exposed whole blood. *Innate Immunity,* 20, 552-560.

PAGE, M. J., THOMSON, G. J. A., NUNES, J. M. 2019. Serum amyloid A binds to fibrin(ogen), promoting fibrin amyloid formation. *Sci Rep,* 9**,** 3102.

PANT, S., LI, C., GONG, Z. & CHEN, N. 2017. Line-scan focal modulation microscopy. *J Biomed Opt,* 22, 50502.

PARIJA, S. C. 2009. *Textbook of Microbiology & Immunology,* India, India: Elsevier. .

PARK, B. S., SONG, D. H., KIM, H. M., CHOI, B. S., LEE, H. & LEE, J. O. 2009. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature*, 458, 1191-1195.

PATERSON, J. R., BAXTER, G., DREYER, J. S., HALKET, J. M., FLYNN, R. & LAWRENCE, J. R. 2008. Salicylic acid sans aspirin in animals and man: Persistence in fasting and biosynthesis from benzoic acid. *Journal of Agricultural and Food Chemistry,* 56, 11648-11652.

PERI, F., PIAZZA, M., CALABRESE, V., DAMORE, G. & CIGHETTI, R. 2010. Exploring the LPS/TLR4 signal pathway with small molecules. *Biochem Soc Trans,* 38, 1390-5.

PERNERSTORFER, T., STOHLAWETZ, P., HOLLENSTEIN, U., DZIRLO, L., EICHLER, H. G., KAPIOTIS, S., JILMA, B. & SPEISER, W. 1999. Endotoxin-induced activation of the coagulation cascade in humans: effect of acetylsalicylic acid and acetaminophen. *Arterioscler Thromb Vasc Biol,* 19**,** 2517-23.

PETERS, J. U., SCHNIDER, P., MATTEI, P. & KANSY, M. 2009. Pharmacological promiscuity: Dependence on compound properties and target specificity in a set of recent roche compounds. *ChemMedChem,* 4, 680-686.

PETKOVA, A. T., LEAPMAN, R. D., GUO, Z., YAU, W. M., MATTSON, M. P. & TYCKO,

R. 2005. Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science*, 307, 262-5.

PETRENKO, V. E., ANTIPOVA, M. L. & GURINA, D. L. 2016. Salicylic acid, acetylsalicylic acid, methyl salicylate, salicylamide, and sodium salicylate in supercritical carbon dioxide: Solute - Cosolvent hydrogen bonds formation. *Journal of Supercritical Fluids*, 116, 62-69.

PICKEN, M. M. 2010. Fibrinogen amyloidosis: the clot thickens! *Blood*, 115, 2985-6.

PIETERS, M., COVIC, N., LOOTS DU, T., VAN DER WESTHUIZEN, F. H., VAN ZYL, D. G., RHEEDER, P., JERLING, J. C. & WEISEL, J. W. 2006. The effect of glycaemic control on fibrin network structure of type 2 diabetic subjects. *Thromb Haemost*, 96, 623-9.

PILLAY, K. & GOVENDER, P. 2013. Amylin uncovered: a review on the polypeptide responsible for type II diabetes. *Biomed Res Int*, 2013, 826706.

PŁÓCIENNIKOWSKA, A., HROMADA-JUDYCKA, A., BORZĘCKA, K. & KWIATKOWSKA, K. 2015. Co-operation of TLR4 and raft proteins in LPS-induced pro-inflammatory signaling. *Cellular and Molecular Life Sciences*, 72, 557-581.

POLANOWSKA-GRABOWSKA, R., GEANACOPOULOS, M. & GEAR, A. R. L. 1993. Platelet adhesion to collagen via the α2β1 integrin under arterial flow conditions causes rapid tyrosine phosphorylation of pp125(FAK). *Biochemical Journal*, 296, 543-547.

POLTORAK, A., HE, X., SMIRNOVA, I., LIU, M. Y., VAN HUFFEL, C., DU, X., BIRDWELL, D. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science*, 282, 2085-2088.

POLTORAK, A., RICCIARDI-CASTAGNOLI, P., CITTERIO, S. & BEUTLER, B. 2000. Physical contact between lipopolysaccharide and toll-like receptor 4 revealed by genetic complementation. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 2163-2167.

POIKONEN, K., LAJUNEN, T., SILVENNOINEN-KASSINEN, S., LEINONEN, M. & SAIKKU, P. 2009. Effects of CD14, TLR2, TLR4, LPB, and IL-6 gene polymorphisms on Chlamydia pneumoniae growth in human macrophages in vitro. *Scand J Immunol,* 70, 34-9.

POOLA, P. K. & JOHN, R. 2017. Label-free nanoscale characterization of red blood cell structure and dynamics using single-shot transport of intensity equation. *J Biomed Opt,* 22, 1-7.

PÖSCHL, J. M. B., LERAY, C., RUEF, P., CAZENAVE, J. P. & LINDERKAMP, O. 2003. Endotoxin binding to erythrocyte membrane and erythrocyte deformability in human sepsis and in vitro. *Critical Care Medicine,* 31, 924-928.

POTGIETER, M., BESTER, J., KELL, D. B. & PRETORIUS, E. 2015a. The dormant blood microbiome in chronic, inflammatory diseases. *FEMS Microbiol Rev,* pii: fuv013.

POTGIETER, M., BESTER, J., KELL, D. B. & PRETORIUS, E. 2015c. The dormant blood microbiome in chronic, inflammatory diseases. *FEMS Microbiol Rev,* 39, 567-91.

PRETORIUS, E. 2013. The adaptability of red blood cells. *Cardiovascular Diabetology,* 12.

PRETORIUS, E., AKEREDOLU, O.-O., SOMA, P. & KELL, D. B. 2016a. Major involvement of bacterial components in rheumatoid arthritis and its accompanying oxidative stress, systemic inflammation and hypercoagulability. *Exp Biol Med,* in press.

PRETORIUS, E., BESTER, J. & KELL, D. B. 2016b. A bacterial component to Alzheimer-type dementia seen via a systems biology approach that links iron dysregulation and inflammagen shedding to disease *J Alzheimers Dis,* 53, 1237-1256.

PRETORIUS, E., BESTER, J., VERMEULEN, N., ALUMMOOTTIL, S., SOMA, P., BUYS, A. V. & KELL, D. B. 2015. Poorly controlled type 2 diabetes is accompanied by significant morphological and ultrastructural changes in both erythrocytes and in thrombin-generated fibrin: implications for diagnostics. *Cardiovasc Diabetol,* 13, 30.

PRETORIUS, E., BESTER, J., VERMEULEN, N., LIPINSKI, B., GERICKE, G. S. & KELL, D. B. 2014b. Profound morphological changes in the erythrocytes and fibrin networks of

patients with hemochromatosis or with hyperferritinemia, and their normalization by iron chelators and other agents. *PLoS ONE,* 9.

PRETORIUS, E. & KELL, D. B. 2014. Diagnostic morphology: biophysical indicators for iron-driven inflammatory diseases. *Integrative Biol,* 6, 486-510.

PRETORIUS, E. & LIPINSKI, B. 2013. Differences in morphology of fibrin clots induced with thrombin and ferric ions and its pathophysiological consequences. *Heart Lung and Circulation,* 22, 447-449.

PRETORIUS, E. & LIPINSKI, B. 2013b. Thromboembolic ischemic stroke changes red blood cell morphology. *Cardiovasc Pathol,* 22, 241-2.

PRETORIUS, E., MBOTWE, S., BESTER, J., ROBINSON, C. J. & KELL, D. B. 2016e. Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide. *J R Soc Interface,* 123, 20160539.

PRETORIUS, E., MBOTWE, S. & KELL, D. B. 2017a. Lipopolysaccharide-binding protein (LBP) reverses the amyloid state of fibrin seen in plasma of type 2 diabetics with cardiovascular co-morbidities. *Sci Rep,* 7, 9680.

PRETORIUS, E., OBERHOLZER, H. M., VAN DER SPUY, W. J., SWANEPOEL, A. C. & SOMA, P. 2011a. Qualitative scanning electron microscopy analysis of fibrin networks and platelet abnormalities in diabetes. *Blood Coagul Fibrinol,* 22, 463-7.

PRETORIUS, E., OLUMUYIWA-AKEREDOLU, O. O., MBOTWE, S. & BESTER, J. 2016f. Erythrocytes and their role as health indicator: Using structure in a patient-orientated precision medicine approach. *Blood Rev,* 30, 263-74.

PRETORIUS, E., PAGE, M. J., ENGELBRECHT, L., ELLIS, G. C. & KELL, D. B. 2017b. Substantial fibrin amyloidogenesis in type 2 diabetes assessed using amyloid-selective fluorescent stains. *Cardiovasc Diabetol,* 16, 141.

PRETORIUS, E., PAGE, M. J., MBOTWE, S. & KELL, D. B. 2018. Lipopolysaccharide-binding

protein (LBP) can reverse the amyloid state of fibrin seen or induced in Parkinson's disease. *PLoS One,* 13, e0192121.

PRETORIUS, E., STEYN, H., ENGELBRECHT, M., SWANEPOEL, A. C. & OBERHOLZER, H. M. 2011b. Differences in fibrin fiber diameters in healthy individuals and thromboembolic ischemic stroke patients. *Blood Coagul Fibrinolysis,* 22, 696-700.

PRETORIUS, E., SWANEPOEL, A. C., BUYS, A. V., VERMEULEN, N., DUIM, W. & KELL, D. B. 2014d. Eryptosis as a marker of Parkinson's disease. *Aging-US,* 6, 788-818.

PRETORIUS, E., SWANEPOEL, A. C., DEVILLIERS, S. & BESTER, J. 2017c. Blood clot parameters: Thromboelastography and scanning electron microscopy in research and clinical practice. *Thromb Res,* 154, 59-63.

PRETORIUS, E., VERMEULEN, N. & BESTER, J. 2014e. Atypical erythrocytes and platelets in a patient with a pro-thrombin mutation. . *Platelets,* 25, 461-462. .

PRETORIUS, E., VERMEULEN, N., BESTER, J., DU PLOOY, J. L. & GERICKE, G. S. 2014f. The effect of iron overload on red blood cell morphology. *The Lancet,* 383, 722.

PRETORIUS, E., VERMEULEN, N., BESTER, J., LIPINSKI, B. & KELL, D. B. 2013b. A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: the use of scanning electron microscopy. *Toxicol Mech Methods,* 23, 352-9.

PRETORIUS, E., DU PLOOY, J., SOMA, P. & GASPARYAN, A. Y. 2014b. An ultrastructural analysis of platelets, erythrocytes, white blood cells, and fibrin network in systemic lupus erythematosus. *Rheumatol Int,* 34, 1005-9.

PROTOPOPOVA, A. D., BARINOV, N. A., ZAVYALOVA, E. G., KOPYLOV, A. M., SERGIENKO, V. I. & KLINOV, D. V. 2015. Visualization of fibrinogen alphaC regions and their arrangement during fibrin network formation by high-resolution AFM. *J Thromb Haemost,* 13, 570-9.

PUGIN, J., HEUMANN, I. D., TOMASZ, A., KRAVCHENKO, V. V., AKAMATSU, Y., NISHIJIMA, M., GLAUSER, M. P., TOBIAS, P. S. & ULEVITCH, R. J. 1994. CD14 is

a pattern recognition receptor. *Immunity,* 1, 509-16.

PUSSINEN, P. J., HAVULINNA, A. S., LEHTO, M., SUNDVALL, J. & SALOMAA, V. 2011. Endotoxemia Is Associated With an Increased Risk of Incident Diabetes. *Diabetes Care,* 34, 392-397.

QIAO, H., PHAN, C. M., WALTHER, H., SUBBARAMAN, L. N. & JONES, L. 2017. Depth Profile Assessment of the Early Phase Deposition of Lysozyme on Soft Contact Lens Materials Using a Novel In Vitro Eye Model. *Eye Contact Lens*.

RAMACHANDRAN, G. 2014. Gram-positive and gram-negative bacterial toxins in sepsis: A brief review. *Virulence,* 5, 196-201.

RAMBARAN, R. N. & SERPELL, L. C. 2008. Amyloid fibrils: abnormal protein assembly. *Prion,* 2, 112-7.

RAMCHAND, P., NYIRJESY, S., FRANGOS, S., DOERFLER, S., NAWALINSKI, K., QUATTRONE, F., JU, C., PATEL, H., DRISCOLL, N., MALONEY-WILENSKY, E., STEIN, S. C., LEVINE, J. M., KASNER, S. E. & KUMAR, M. A. 2016. Thromboelastography Parameter Predicts Outcome After Subarachnoid Hemorrhage: An Exploratory Analysis. *World Neurosurg,* 96, 215-221.

RAO, L. V. & PENDURTHI, U. R. 2005. Tissue factor-factor VIIa signaling. *Arterioscler Thromb Vasc Biol,* 25, 47-56.

RASKOB, G. E., ANGCHAISUKSIRI, P., BLANCO, A. N., BULLER, H., GALLUS, A., HUNT, B. J., HYLEK, E. M., KAKKAR, A., KONSTANTINIDES, S. V., MCCUMBER, M., OZAKI, Y., WENDELBOE, A. & WEITZ, J. I. 2014. Thrombosis: a major contributor to global disease burden. *Arterioscler Thromb Vasc Biol,* 34, 2363-71.

REED-GEAGHAN, E. G., SAVAGE, J. C., HISE, A. G. & LANDRETH, G. E. 2009. CD14 and toll-like receptors 2 and 4 are required for fibrillar Aβ-stimulated microglial activation. *Journal of Neuroscience,* 29, 11982-11992.

RITTIG, M. G., KAUFMANN, A., ROBINS, A., SHAW, B., SPRENGER, H., GEMSA, D.,

FOULONGNE, V., ROUOT, B. & DORNAND, J. 2003. Smooth and rough lipopolysaccharide phenotypes of Brucella induce different intracellular trafficking and cytokine/chemokine release in human monocytes. *Journal of Leukocyte Biology,* 74, 1045-1055.

SALVADO, M. D., DI GENNARO, A., LINDBOM, L., AGERBERTH, B. & HAEGGSTROM, J. Z. 2013. Cathelicidin LL-37 induces angiogenesis via PGE2-EP3 signaling in endothelial cells, in vivo inhibition by aspirin. *Arterioscler Thromb Vasc Biol,* 33, 1965-72.

SANDAHL, T. D., GRØNBÆK, H., MØLLER, H. J., STØY, S., THOMSEN, K. L., DIGE, A. K., AGNHOLT, J., HAMILTON-DUTOIT, S., THIEL, S. & VILSTRUP, H. 2014. Hepatic Macrophage Activation and the LPS Pathway in Patients With Alcoholic Hepatitis: A Prospective Cohort Study. *American Journal of Gastroenterology*.

SCHLICHTING, E., ASPELIN, T. & LYBERG, T. 1996. Interactions of endotoxin with human blood cells and serum proteins. *Scand J Clin Lab Invest,* 56, 167-76.

SCHRODER, N. W., OPITZ, B., LAMPING, N., MICHELSEN, K. S., ZAHRINGER, U., GOBEL, U. B. & SCHUMANN, R. R. 2000. Involvement of lipopolysaccharide binding protein, CD14, and Toll-like receptors in the initiation of innate immune responses by Treponema glycolipids. *J Immunol,* 165, 2683-93.

SCHROEDL, W., FUERLL, B., REINHOLD, P., KRUEGER, M. & SCHUETT, C. 2001. A novel acute phase marker in cattle: lipopolysaccharide binding protein (LBP). *J Endotoxin Res,* 7, 49-52.

SCHUMANN, R. R. & ZWEIGNER, J. 1999. A novel acute-phase marker: Lipopolysaccharide binding protein (LBP). *Clinical Chemistry and Laboratory Medicine,* 37, 271-274.

SERPELL, L. C. 2000. Alzheimer's amyloid fibrils: structure and assembly. *Biochim Biophys Acta,* 1502, 16-30.

SERPELL, L. C., BENSON, M., LIEPNIEKS, J. J. & FRASER, P. E. 2007. Structural analyses

of fibrinogen amyloid fibrils. *Amyloid,* 14, 199-203.

SHAO, H. C., HWANG, W. L. & CHEN, Y. C. 2012. Optimal multiresolution blending of confocal microscope images. *IEEE Trans Biomed Eng,* 59, 531-41.

SHI, J., ZHAO, Y., WANG, Y., GAO, W., DING, J., LI, P., HU, L. & SHAO, F. 2014. Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature,* 514, 187-192.

SIMMONS, J. & PITTET, J. F. 2015. The coagulopathy of acute sepsis. *Current Opinion in Anaesthesiology,* 28, 227-236.

SMITH, S. A. 2009. The cell-based model of coagulation. *J Vet Emerg Crit Care (San Antonio),* 19**,** 3-10.

SIPE, J. D., BENSON, M. D., BUXBAUM, J. N., IKEDA, S., MERLINI, G., SARAIVA, M. J. & WESTERMARK, P. 2014. Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. *Amyloid,* 21, 221-4.

SPENCER, C. G. C., FELMEDEN, D. C., BLANN, A. D. & LIP, G. Y. H. 2007. Haemorheological, platelet and endothelial indices in relation to global measures of cardiovascular risk in hypertensive patients: A substudy of the Anglo-Scandinavian Cardiac Outcomes Trial. *Journal of Internal Medicine,* 261, 82-90.

SRIAMORNSAK, P., THIRAWONG, N., CHEEWATANAKORNKOOL, K., BURAPAPADH, K. & SAE-NGOW, W. 2008. Cryo-scanning electron microscopy (cryo-SEM) as a tool for studying the ultrastructure during bead formation by ionotropic gelation of calcium pectinate. *International Journal of Pharmaceutics,* 352, 115-122.

STÅHL, A. L., SARTZ, L., NELSON, A., BÉKÁSSY, Z. D. & KARPMAN, D. 2009. Shiga toxin and lipopolysaccharide induce platelet-leukocyte aggregates and tissue factor release, a thrombotic mechanism in hemolytic uremic syndrome. *PLoS ONE,* 4.

STANGOU, A. J., BANNER, N. R., HENDRY, B. M., RELA, M., PORTMANN, B., WENDON, J., MONAGHAN, M., MACCARTHY, P., BUXTON-THOMAS, M., MATHIAS, C. J.,

LIEPNIEKS, J. J., O'GRADY, J., HEATON, N. D. & BENSON, M. D. 2010. Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. *Blood,* 115, 2998-3007.

STIEF, T. W. 2009. Coagulation activation by lipopolysaccharides. *Clinical and Applied Thrombosis/Hemostasis,* 15, 209-219.

STRUKOVA, S. 2006. Blood coagulation-dependent inflammation. Coagulation-dependent inflammation and inflammation-dependent thrombosis. *Front Biosci,* 11, 59-80.

STRYDOM, M. A., BESTER, J., MBOTWE, S. & PRETORIUS, E. 2016. The effect of physiological levels of South African puff adder (Bitis arietans) snake venom on blood cells: an in vitro model. *Sci Rep,* 6, 35988.

SU, W. & DING, X. 2015. Methods of Endotoxin Detection. *J Lab Autom,* 20, 354-64.

SWANEPOEL, A. C., NIELSEN, V. G. & PRETORIUS, E. 2015. Viscoelasticity and Ultrastructure in Coagulation and Inflammation: Two Diverse Techniques, One Conclusion. *Inflammation,* 38, 1707-26.

TAPPING, R. I. & TOBIAS, P. S. 1999. Cell surface binding of LBP-LPS complexes to a protein component distinct from CD14. *Innate Immunity,* 5, 52-55.

TERAWAKI, H., YOKOYAMA, K., YAMADA, Y., MARUYAMA, Y., IIDA, R., HANAOKA, K., YAMAMOTO, H., OBATA, T. & HOSOYA, T. 2010. Low-grade endotoxemia contributes to chronic inflammation in hemodialysis patients: examination with a novel lipopolysaccharide detection method. *Ther Apher Dial,* 14, 477-82.

TERZAKIS, J. A., SANTAGADA, E., HERNANDEZ, A. & TASKIN, M. 2005. Scanning electron microscopy of peripheral blood smears: comparison of normal blood with some common leukemias. *Ultrastruct Pathol,* 29**,** 19-28.

TERMEER, C., BENEDIX, F., SLEEMAN, J., FIEBER, C., VOITH, U., AHRENS, T., MIYAKE, K., FREUDENBERG, M., GALANOS, C. & SIMON, J. C. 2002. Oligosaccharides of hyaluronan activate dendritic cells via Toll-like receptor 4. *Journal*

of Experimental Medicine, 195, 99-111.

TIIROLA, T., SINISALO, J., NIEMINEN, M. S., SILVENNOINEN-KASSINEN, S., PALDANIUS, M., SAIKKU, P., JAUHIAINEN, M. & LEINONEN, M. 2007. Chlamydial lipopolysaccharide is present in serum during acute coronary syndrome and correlates with CRP levels. *Atherosclerosis,* 194, 403-407.

TIKHOMIROVA, I. A., OSLYAKOVA, A. O. & MIKHAILOVA, S. G. 2011. Microcirculation and blood rheology in patients with cerebrovascular disorders. *Clinical Hemorheology and Microcirculation,* 49, 295-305.

TIKHONOVA, T. N., ROVNYAGINA, N. R., ZHEREBKER, A. Y., SLUCHANKO, N. N., RUBEKINA, A. A., OREKHOV, A. S., NIKOLAEV, E. N., FADEEV, V. V., UVERSKY, V. N. & SHIRSHIN, E. A. 2018. Dissection of the deep-blue autofluorescence changes accompanying amyloid fibrillation. *Arch Biochem Biophys,* 651**,** 13-20.

TIPPING, K. W., VAN OOSTEN-HAWLE, P., HEWITT, E. W. & RADFORD, S. E. 2015. Amyloid Fibres: Inert End-Stage Aggregates or Key Players in Disease? *Trends Biochem Sci,* 40, 719-27.

THOMPSON, P. A., TOBIAS, P. S., VIRIYAKOSOL, S., KIRKLAND, T. N. & KITCHENS, R. L. 2003. Lipopolysaccharide (LPS)-binding protein inhibits responses to cell-bound LPS. *J Biol Chem,* 278, 28367-71

TOBIAS, P. S., SOLDAU, K., KLINE, L., LEE, J. D., KATO, K., MARTIN, T. P. & ULEVITCH, R. J. 1993. Cross-linking of lipopolysaccharide (LPS) to CD14 on THP-1 cells mediated by LPS-binding protein. *J Immunol,* 150, 3011-21.

TONKIN, A. M., BLANKENBERG, S., KIRBY, A., ZELLER, T.2015. Biomarkers in stable coronary heart disease, their modulation and cardiovascular risk: The LIPID biomarker study. *Int J Cardiol,* 201, 499-507.

TRACEY, K. J. & LOWRY, S. F. 1990. The role of cytokine mediators in septic shock. *Adv Surg,*

23, 21-56.

TRIANTAFILOU, M. & TRIANTAFILOU, K. 2002. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol,* 23, 301-4.

TSOUCALAS, G., CHEVALLIER, J., KARAMANOU, M., PAPAIOANNOU, T., SGANTZOS, M. & ANDROUTSOS, G. 2016. Historical hallmarks of anticoagulation and antiplatelet agents. *Current Pharmaceutical Design*, 22, 1857-1861.

TSUTSUMI-ISHII, Y., SHIMADA, K., DAIDA, H., TOMAN, R. & NAGAOKA, I. 2008. Low potency of Chlamydophila LPS to activate human mononuclear cells due to its reduced affinities for CD14 and LPS-binding protein. *International Immunology,* 20, 199-208.

TZENG, Y. L., DATTA, A., KUMAR KOLLI, V., CARLSON, R. W. & STEPHENS, D. S. 2002. Endotoxin of Neisseria meningitidis composed only of intact lipid A: Inactivation of the meningococcal 3-deoxy-D-manno-octulosonic acid transferase. *Journal of Bacteriology,* 184, 2379-2388.

UNDAS, A., BRUMMEL-ZIEDINS, K. E. & MANN, K. G. 2007. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. *Blood,* 109**,** 2285-92.

UNDAS, A., CELINSKA-LOWENHOFF, M., LOWENHOFF, T. & SZCZEKLIK, A. 2006. Statins, fenofibrate, and quinapril increase clot permeability and enhance fibrinolysis in patients with coronary artery disease. *J Thromb Haemost,* 4**,** 1029-36.

VERMA, A. & , H. 2017. Thromboelastography as a novel viscoelastic method for hemostasis monitoring: Its methodology, applications, and constraints. *Global Journal of Transfusion Medicine,* 2**,** 8-18.

VIRIYAKOSOL, S. & KIRKLAND, T. N. 1995. A region of human CD14 required for lipopolysaccharide binding. *J Biol Chem,* 270**,** 361-8.

WADA, H., MATSUMOTO, T. & HATADA, T. 2012. Diagnostic criteria and laboratory tests for disseminated intravascular coagulation. *Expert review of hematology,* 5**,** 643-652.

WALKER, J. B. & NESHEIM, M. E. 1999. The molecular weights, mass distribution, chain

composition, and structure of soluble fibrin degradation products released from a fibrin clot perfused with plasmin. *Journal of Biological Chemistry,* 274**,** 5201-5212.

WEISEL, J. 2007. Structure of fibrin: impact on clot stability. *Journal of Thrombosis and Haemostasis,* 5**,** 116-124.

WEISEL, J. & LITVINOV, R. 2008. The biochemical and physical process of fibrinolysis and effects of clot structure and stability on the lysis rate. *Cardiovascular & Hematological Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Cardiovascular & Hematological Agents),* 6**,** 161-180.

WEISEL, J. W. 2005. Fibrinogen and fibrin. *Advances in protein chemistry.* Elsevier.

WHITING, D. & DINARDO, J. A. 2014. TEG and ROTEM: Technology and clinical applications. *American Journal of Hematology,* 89**,** 228-232.

WRIGHT, M. J., SCHRODER, M. & NIELSON, A. J. 1981. Tissue fixation and staining by osmium tetraoxide: a possible role for alkaloids. *J Histochem Cytochem,* 29**,** 1347-8.

WRIGHT, S. D., TOBIAS, P. S., ULEVITCH, R. J. & RAMOS, R. A. 1989. Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *J Exp Med,* 170**,** 1231-41.

WU, L.-C., LIN, X. & SUN, H. 2012. Tanshinone IIA protects rabbits against LPS-induced disseminated intravascular coagulation (DIC). *Acta Pharmacologica Sinica,* 33**,** 1254.

YU, P.-X., ZHOU, Q.-J., ZHU, W.-W., WU, Y.-H., WU, L.-C., LIN, X., CHEN, M.-H. & QIU, B.-T. 2013. Effects of quercetin on LPS-induced disseminated intravascular coagulation (DIC) in rabbits. *Thrombosis research,* 131**,** e270-e273.

ZHANG, Y., NEOGI, T., CHEN, C., CHAISSON, C., HUNTER, D. J. & CHOI, H. 2014. Low-dose aspirin use and recurrent gout attacks. *Ann Rheum Dis,* 73**,** 385-90.

Table of Index

INFORMATION LEAFLET AND INFORMED CONSENT FORM\: Ethics number: 123/2015

Title of study:

The effect of aspirin and lipopolysaccharide binding protein on hypercoagulability induced by lipopolysaccharide

You act as a healthy participant blood donor for this study

Principal investigator: Sthembile Sibanda; Department of Physiology

INTRODUCTION

You are invited to participate in a research study where we are looking at blood cells, conducted by the Department of Physiology (School of Health Sciences) from the University of Pretoria. This information leaflet is to help you to decide if you would like to participate. Before you agree to take part in this study you should fully understand what is involved. The medical practitioner will explain the reason for the drawing of blood. One tube will be given to the research team at the University of Pretoria for research purposes, and the second blood tube will be sent to a Pathology laboratory to determine your iron levels.

PURPOSE OF STUDY

The researcher is investigating how your blood, consisting of red blood cells, platelets and fibrin, will be influenced by adding LPS (neurotoxins) to your blood. We will view your blood under a specialized microscope that can magnify up to 100 000x (called an electron microscope). The iron levels will be used to show high or low iron levels, this will give us an indication of any inflammatory diseases that could be present. That could influence the shape of the red blood cells, platelets and fibrin.

Who will draw the blood?

Dr Morne´ Strydom will draw 2 blood tubes from you.

PROCEDURES

Two tubes of blood will be drawn by Dr M Strydom protocol number 169/2016 was approved.
TUBE 1 and 2: Blood will be drawn in a citrate tube.

The first tube will be given to Dr M Strydom, all remains from his preparations will be used by Mrs Sthembile Sibanda, who will use it to study your blood cells and fibrin fibres, using specialized microscopes.

HAS THE TRIAL RECEIVED ETHICAL APPROVAL?

The Faculty of Health Sciences Research Ethics Committee, University of Pretoria, approved this protocol and the committee has granted written approval. The study has been structured in accordance with the Declaration of Helsinki, which deals with the recommendations guiding doctors in biomedical research involving human/subjects.

RESEARCH KNOWLEDGE OBTAINED IN THIS STUDY

The current laboratory study is not intended to benefit you but will add to our knowledge about your blood clotting patterns, red blood cell ultrastructure influenced by neurotoxins introduced to your blood.

MAY THE PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE OR SIDE EFFECTS?

Venipunctures (i.e. drawing blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in a bruise at the puncture site, or less commonly swelling of the vein, infection and bleeding from the site. For your protection, the procedures will be performed under sterile conditions by a medical practitioner, Dr Morne´ Strydom.

INSURANCE AND FINANCIAL ARRANGEMENTS

Neither you nor your medical scheme will be expected to pay for your participation in the study. No study-related injury is expected, as a Clinician takes the blood.

CONFIDENTIALITY

All information obtained during the course of this study is strictly confidential. Data that may be reported in scientific journals will not include any information, which identifies you as a patient in this study. In connection with this study, it might be important for domestic and foreign regulatory health authorities and the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, to be able to review the results obtained by from the investigation, pertaining to this study.

ADDITIONAL CONSIDERATIONS

Our research group has various on-going studies, including a study where we use control samples (ethics approval number 169/2016 E Pretorius).

All excess blood (plasma and whole blood) not used in the experiments discussed in this protocol will be stored in formaldehyde or at -80°C, under ethics approval 506/2014, in order not to recollect or waste healthy control samples.

INFORMED CONSENT

I hereby confirm that I have been informed by a doctor about the nature, conduct, benefits and risks of study. I have also received, read and understood the above written information (Patient Information Leaflet and Informed Consent) regarding research. I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report. I may, at any stage, without prejudice, withdraw my consent and participation in the study. I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

Healthy donor's name (Please print)

……

Patient's signature……..**Date**……..

Patient ID number:……..

Principal investigator's signature (Mrs Sthembile Sibanda)

……

INFORMATION FILLED IN BY Dr M. Strydom/Phlebotomist

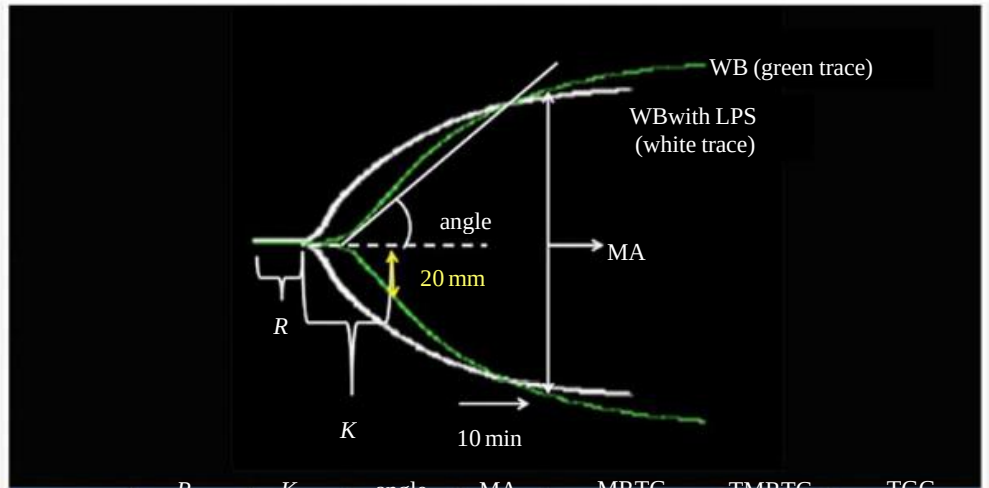
Age	
Gender	
Inflammatory condition?	
Does the donor smoke?	
Is the donor on the pill (hormones contraceptives) if female	
Any other chronic condition?	

Table showing the study population gender and age (in years)

Participant	Male (Age)	Female (Age)
1	31	
2		20
3		33
4		56
5	26	
6	23	
7		27
8	19	
9	49	
10	60	
11		58
12		56
14	45	
15	48	
16		21
17	30	
18		20
19	19	
20	21	
21		22
22	22	
23		23
24		50
25		23
26	65	
27	19	
28		24
29	19	
30		44
31	20	
32	***45***	
33	***48***	
34	***45***	
35	***34***	

36	***56***	
37		***46***
38		***45***
39	***35***	
40	***34***	
41		***48***
42	***45***	
43		***27***
44	***38***	
45		***34***
46	***27***	
47		***43***
48		***23***

Whole blood and LPS tracings reference with highlighted parameters



	R (min)	*K* (min)	angle (°)	MA (mm)	MRTG (dyn.cm $^{-2}$. s^{-1})	TMRTG (s)	TGG (dyn.cm $^{-2}$)
control whole blood	9.5	5.8	46	55.5	2.27	16.33	627.06
with added LPS	6.3	4.7	54.6	47.4	2.58	8.08	452.08

Figure shows TEG curves from a control whole blood sample with and without added LPS overlay. R, reaction time, first measurable clot formation; K, achievement of clot firmness; angle, kinetics of clot development; MA, maximum clot strength; MRTG, maximum rate of thrombus generation; TMRTG, time to maximum rate of thrombus generation; TTG, final clot strength. This is adapted from (Pretorius et al., 2016d)

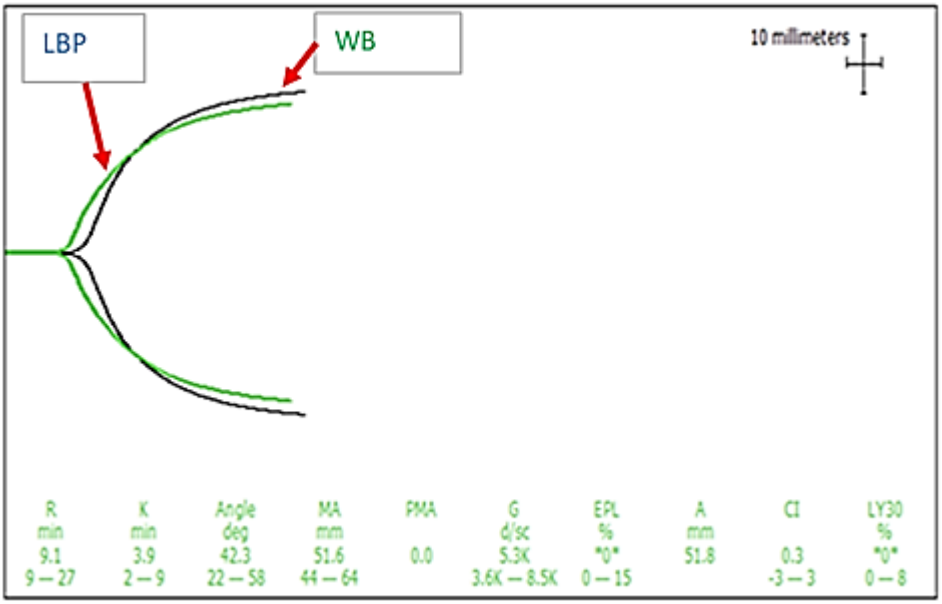
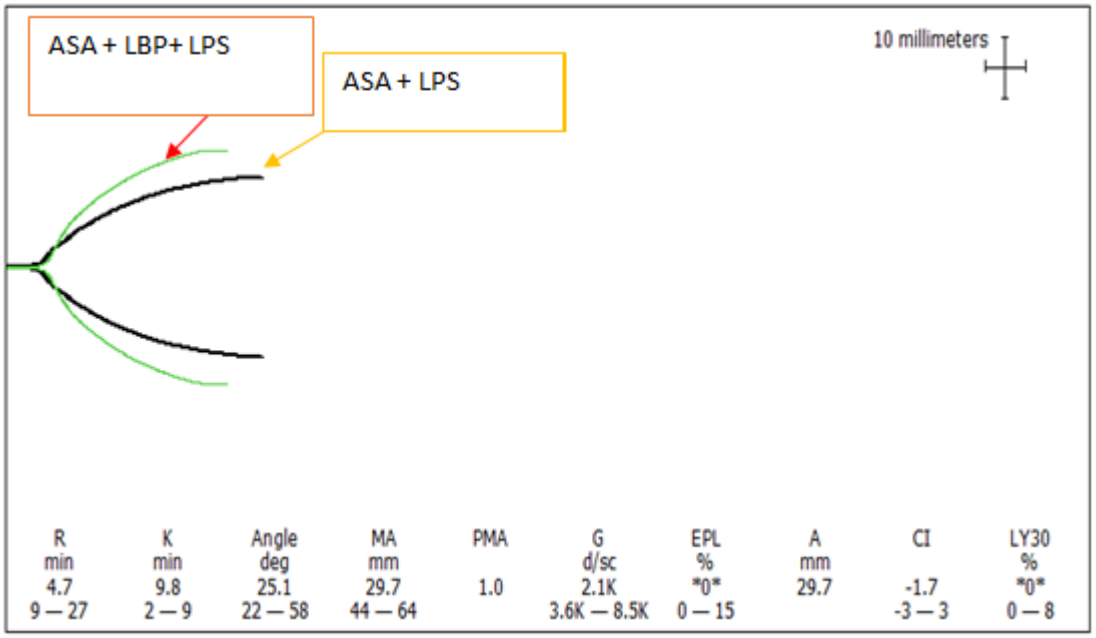


Figure above are TEG tracings. Showing the whole blood (WB) naïve (black curve) and the Lipopolysaccharide binding protein (LBP) green curve.



TEG tracings showing a rare case of hypocoaguable state causeed by Lipopolysaccharide (LPS) (black curve), after treatment with aspirin (ASA) and Lipopolysaccharide binding prtein (LBP) the curve slightly improves (green curve) , decrease the size and strenght of clot (TGG decreased)

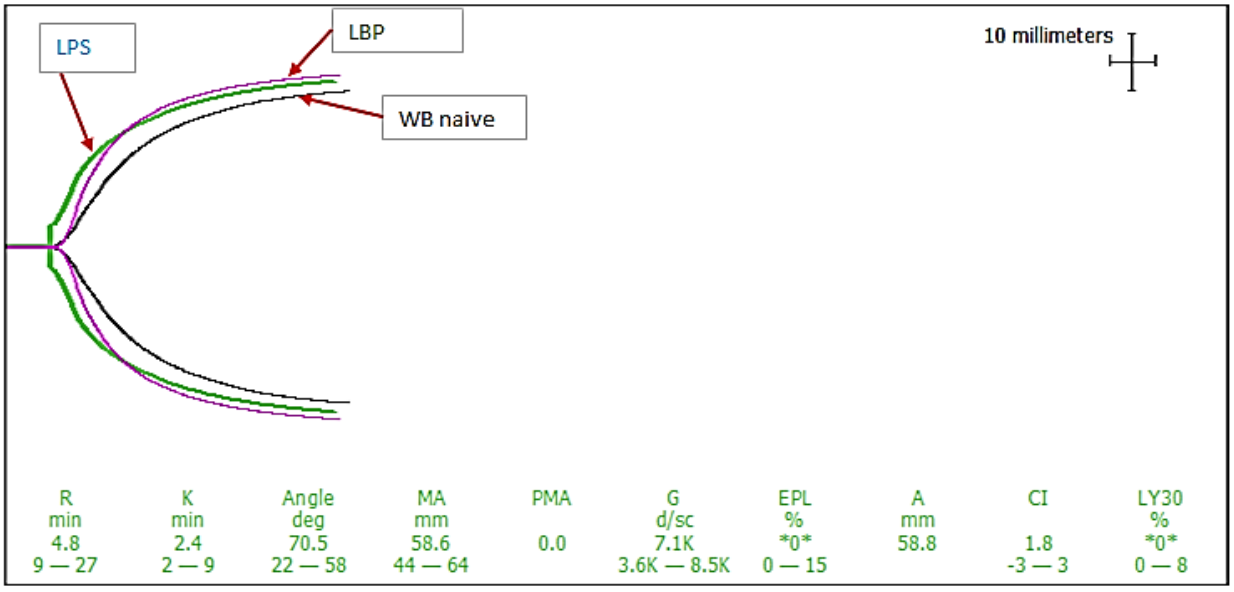


Figure above shows three TEG tracings showing cruves from whole blood mixed with Lipopolysaccharide (LPS) (green , curve), whole blood mixed with LBP final exposure concentration of 2ng.L^{-1} (pink), whole blood (WB) naïve (black curve)

Table of confocal images data Mean and standard deviation of light fluorescence intensity before and after treatments of Platelets poor plasma (PPP)

control		LPSBP		LPS		MIX (LPS and LPSBP)		Aspirin		Aspirin + LPSBP		Aspirin + LPS		Aspirin + Mix	
Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
3.110	5.543	2.457	4.471	3.424	6.143	2.725	4.858	2.442	4.624	3.212	5.161	5.286	6.621	4.864	6.830
1.734	4.545	2.996	4.771	3.601	6.387	3.263	4.469	2.951	4.703	4.463	6.212	2.533	4.864	6.443	8.622
2.588	4.968	2.950	4.784	3.216	5.473	2.198	4.524	3.535	6.806	6.768	6.826	4.215	5.786	6.140	8.820
2.047	5.025	3.364	4.804	5.139	7.509	2.024	4.518	2.342	4.508	1.549	4.511	3.295	5.090	6.155	8.533
3.241	6.808	3.326	4.869	3.629	6.392	1.426	4.148	5.816	5.209	1.870	4.573	4.530	5.806	3.930	5.443
1.840	4.088	3.023	4.575	4.536	13.191	1.854	4.259	6.055	5.635	2.027	4.653	3.968	7.350	7.879	6.673
3.488	4.972	1.866	4.251	2.186	4.831	3.252	5.438	7.247	5.778	1.606	4.366	3.810	5.707	5.933	6.371
5.441	5.393	2.851	4.555	3.147	5.218	2.998	4.871	1.506	4.292	2.368	5.127	5.250	5.939	5.349	5.484
1.659	4.573	1.462	4.282	3.252	6.113	2.340	4.736	2.632	4.653	1.782	5.109	2.301	5.209	1.233	4.193
2.137	4.574	1.868	4.485	8.698	19.518	2.787	4.761	3.894	5.179	1.584	4.985	2.515	5.267	1.547	4.321
1.583	4.597	2.060	4.550	8.430	19.246	2.160	4.438	7.104	7.739	1.124	4.434	1.978	4.943	1.298	4.289
2.711	5.220	2.361	6.763	9.123	13.571	3.365	6.085	5.700	7.229	1.917	4.766	2.056	4.971	1.159	4.240
4.610	5.810	2.497	4.766	13.777	22.546	7.100	8.514	5.813	7.177	1.289	4.432	2.834	6.042	3.288	7.153
4.408	5.730	2.492	4.649	2.191	4.528	4.233	7.257	2.068	5.083	1.538	5.701	2.874	5.909	2.189	6.407
3.394	5.267	2.784	6.692	4.594	7.128	2.210	6.475	3.544	5.857	2.745	4.318	2.190	7.249	4.086	7.185
3.216	4.946	1.495	4.596	4.852	7.854	2.882	6.790	2.937	5.591	2.654	4.599	3.835	7.501	2.158	6.287
4.030	8.058	1.144	4.497	3.183	6.927	2.266	4.947	2.769	5.712	3.612	5.065	2.195	6.718	2.141	4.742
2.934	7.692	1.181	4.504	2.624	6.261	2.654	6.479	4.188	6.805	2.582	4.042	1.513	5.469	3.882	5.825
2.876	4.801	1.622	3.946	2.002	4.865	2.180	5.614	2.534	5.591	1.971	3.931	7.328	9.684	6.806	7.821
2.195	4.920	1.407	3.747	2.361	6.146	1.838	3.764	3.030	5.809	2.843	4.039	4.069	7.056	7.508	7.935

3.064	4.630	1.546	3.789	1.491	4.680	1.568	3.726	2.581	5.545	2.950	4.322	2.905	5.531	1.461	4.699
0.776	3.641	2.250	4.014	2.231	5.165	1.950	3.673	1.529	4.087	1.661	3.743	2.652	7.749	1.865	4.853
0.808	3.689	2.696	4.278	4.483	6.118	2.774	6.070	2.145	4.209	3.115	4.240	2.879	7.296	2.058	5.216
0.882	3.714	1.480	3.782	3.668	7.945	2.611	4.206	1.867	3.955	3.198	4.691	2.622	7.506	1.697	4.151
2.230	4.142	1.180	3.644	2.946	7.135	2.753	4.430	1.476	3.755	3.554	4.942	3.381	5.081	2.844	4.514
2.353	4.149	1.112	3.627	4.347	7.667	2.595	4.228	1.811	4.093	5.106	5.940	4.144	5.663	2.192	4.190
1.754	3.918	1.605	3.651	2.109	5.177	2.745	4.413	1.417	5.872	5.282	6.200	2.301	4.451	2.382	3.996
1.984	3.945	2.290	3.997	2.997	6.952	2.962	4.690	1.091	3.706	3.743	5.164	2.509	4.939	1.748	3.863
1.339	3.705	2.405	4.097	3.548	6.911	3.155	4.197	2.528	6.313	2.859	4.863	2.985	6.207	2.080	4.820
2.717	4.204	1.757	3.779	2.080	5.698	3.977	4.694	3.009	5.022	1.516	3.715	2.085	5.626	3.019	4.341
1.930	3.843	2.866	4.252	2.556	6.135	3.650	4.654	3.668	4.806	1.284	3.494	2.720	4.966	2.967	4.386
2.174	4.041	2.027	4.841	2.950	4.502	2.129	3.822	4.039	5.344	2.458	3.790	3.696	7.441	2.248	4.062
2.329	4.074	2.433	4.793	2.509	4.939	3.009	7.961	2.164	4.098	2.844	4.900	5.184	6.212	2.321	4.657
0.750	4.254	2.091	4.974	2.985	6.207	2.856	6.350	2.425	4.812	2.164	4.302	4.450	4.721	2.273	4.326
0.650	4.172	2.072	4.900	2.085	5.626	3.147	7.503	3.132	5.221	2.163	3.908	4.302	4.975	2.395	4.500
1.347	4.310	1.060	3.695	2.720	4.966	3.136	5.118	3.093	5.057	2.216	3.859	4.609	5.873	1.508	4.581
1.341	4.270	2.055	3.768	3.696	7.441	3.221	5.312	2.169	4.164	2.058	3.960	7.327	16.708	2.860	3.716
2.246	4.857	0.836	3.822	2.189	3.893	3.715	5.446	2.783	4.553	2.765	4.402	3.282	8.444	1.855	4.878
1.473	4.377	2.110	3.875	3.357	4.862	3.013	4.751	3.989	5.122	2.759	4.134	4.750	10.582	2.537	5.204
1.379	6.637	2.188	3.830	3.404	5.171	2.987	4.803	1.353	3.797	1.857	3.911	2.820	3.953	4.088	6.346
1.558	3.918	3.872	4.914	3.814	5.572	2.584	4.701	4.009	5.026	2.210	4.004	3.659	8.962	5.297	4.354
1.956	3.746	3.044	4.042	8.166	13.893	2.472	5.026	3.655	4.749	3.206	4.474	4.724	10.108	3.165	3.692
2.115	3.855	2.765	5.005	5.163	7.757	2.609	4.024	4.374	5.139	1.485	5.972	1.885	4.517	4.396	4.165
2.777	4.449	0.984	4.259	5.700	7.635	2.205	4.207	3.749	5.242	2.181	4.451	2.505	6.122	5.908	4.774
3.136	5.027	1.279	4.320	1.689	4.472	3.947	4.893	1.765	4.314	2.676	4.890	4.022	9.028	2.424	5.236

Table of scanning electron microscope ecoefficiency of variance for all treatments and naïve platelet poor plasma using

Naïve PPP	PPP+ LPSBP	PPP +LPS	PPP +LPS+ LBP	PPP + Aspirin	PPP + Aspirin + LBP	PPP + Aspirin + LPS	PPP + Aspirin + LBP+ LPS
0.5081	0.5861	0.5130	0.5923	0.6585	0.6183	0.5326	0.5675
0.4976	0.5845	0.4287	0.5655	0.6412	0.6139	0.5600	0.6707
0.4956	0.5875	0.4370	0.5976	0.6484	0.6189	0.4847	0.6992
0.5491	0.5644	0.4279	0.5786	0.6258	0.6185	0.4681	0.6396
0.6608	0.6033	0.4335	0.5979	0.6415	0.6187	0.5383	0.6500
0.9299	1.1007	0.4655	1.0927	0.7894	0.6302	0.4591	0.5731
0.9168	1.0143	0.5033	0.8315	1.0100	0.6411	0.4337	0.5918
0.9195	0.9035	0.4967	0.8290	0.8304	0.5711	0.4446	0.5982
0.9001	0.8830	0.4878	0.7468	0.8563	0.6495	0.4318	0.6050
0.8892	0.8380	0.4824	57.987	0.7774	0.6042	0.4148	0.6266
0.8227	0.7190	0.5375	0.8084	0.6742	0.7689	0.4110	0.5600
0.9638	0.7403	0.5655	0.7571	0.5464	0.8435	0.4207	0.5662
0.7798	0.7892	0.5795	0.7682	0.6384	0.8263	0.4165	0.5420
0.8407	0.6612	0.4617	0.7782	0.6127	0.8013	0.4270	0.5680
0.9001	0.7260	0.4193	0.7471	0.6019	0.8044	0.7878	0.5313
0.8346	0.7240	0.6712	0.9324	0.8964	0.6790	0.8002	0.9129
0.7292	0.7191	0.6204	0.8884	0.9131	0.6657	0.7494	0.8720
0.7327	0.7184	0.5879	0.9473	0.9221	0.6197	0.6749	0.8357
0.7783	0.6496	0.5922	0.8754	0.8555	1.0901	0.7653	0.8235
0.8368	0.5990	0.5602	0.8715	0.8812	1.1157	0.5271	0.8467
0.7607	0.5453	0.5421	0.8461	0.8967	1.0376	0.6426	0.6864
0.6307	0.5669	0.5206	0.8316	0.8567	1.0537	0.5771	0.7023
0.7482	0.5744	0.5571	0.8687	0.8784	1.0855	0.5165	0.6139
0.7424	0.5924	0.7447	0.8004	0.8789	1.0409	0.6347	0.6592
0.7878	0.5858	0.5836	0.7583	0.8732	1.0431	0.4099	0.6003

0.6053	0.5425	0.4559	0.5812	0.7803	1.0141	0.4212	0.6860
0.5957	0.5043	0.4225	0.6034	0.7669	1.0501	0.4216	0.8053
0.6343	0.4830	0.4052	0.6195	0.8404	1.0454	0.4022	0.8007
0.6567	0.4966	0.4161	0.6045	0.7180	1.0668	0.4122	0.7389
0.6463	0.4855	0.4350	0.5691	0.8206	1.1031	0.5481	0.7219
0.6449	0.8314	0.5635	0.6144	0.7992	0.6018	0.5207	0.7704
0.6316	0.7678	0.5865	0.5525	0.8605	0.5984	0.5355	0.6988
0.6321	0.7917	0.5687	0.7712	0.9090	0.5997	0.5346	0.7677
0.6168	0.8556	0.5342	0.6053	0.8962	0.6040	0.5387	0.7602
0.6845	0.6684	0.5615	0.6456	0.8955	0.6170	0.5452	0.6712
0.6529	0.8176	0.5997	0.5504	0.9223	0.5201	0.5516	0.4298
0.6738	0.8060	0.5283	0.5644	0.5412	0.4486	0.5354	0.3935
0.6470	0.7753	0.5899	0.5642	0.5326	0.4612	0.5339	0.4068
0.6806	0.5987	0.6159	0.5695	0.5094	0.5240	0.5143	0.3959
0.7329	0.5970	0.5635	0.5815	0.5298	0.4984	0.5682	0.3838
0.5790	0.7976	0.5562	0.5814	0.4746	1.0992	0.5323	0.5326
0.5487	0.7857	0.5956	0.5861	0.4706	1.0851	0.5634	0.5531
0.6115	0.7847	0.5817	0.6463	0.4725	0.9821	0.5423	0.5478
0.5662	0.7903	0.4883	0.5846	0.5157	1.0750	0.5366	0.5385
0.5804	0.7711	0.4561	0.5996	0.4790	1.0635	0.4693	0.4734
0.6041	0.7060	0.5159	0.5408	0.8552	0.5914	0.4673	0.5296
0.5213	0.7279	0.4477	0.4904	0.9042	0.5606	0.4598	0.5523
0.5126	0.7473	0.4608	0.4839	0.9230	0.6162	0.5059	0.5816
0.5031	0.6523	0.4683	0.5309	0.9337	0.7593	0.4706	0.5844
0.4784	0.6571	0.4705	0.5178	0.9024	0.8325	0.6890	0.5740
0.4620	1.1108	0.4741	0.6771	0.8194	0.6946	0.5284	1.0430
0.6958	1.0213	0.4766	0.6593	0.8005	0.6840	0.5018	0.6858
0.6650	1.0278	0.4830	0.6318	0.7381	0.6635	0.4987	0.7484
0.6366	0.9786	0.4726	0.6213	0.8507	0.6881	0.5058	0.6256

0.6876	1.0520	0.4745	0.6433	0.6935	0.7268	0.7778	0.7451
0.6488	0.8917	0.5341	0.8998	0.6138	1.0775	0.5556	0.7803
0.7792	0.8953	0.5308	0.8645	0.6373	1.0627	0.5287	0.8153
0.7485	0.9256	0.5764	0.7772	0.5218	1.0644	0.7078	0.7279
0.7922	0.9185	0.6092	0.8855	0.4943	1.0536	0.6170	0.7495
0.6864	0.8274	0.5572	0.8605	0.5309	1.0062	0.4315	0.7383
0.7257	0.9097	0.6056	0.8643	1.0188	1.0675	0.4093	0.7274
0.7265	0.8373	0.4772	0.7733	0.9852	1.2029	0.4252	0.7690
0.7065	0.8610	0.6063	0.6925	1.0278	1.1477	0.4108	0.7038
0.7324	0.8949	0.6055	0.7774	0.9598	0.9971	0.4097	0.6749
0.7194	0.8650	0.5615	0.7227	1.0308	1.0808	0.6530	0.7314
0.6767	0.8750	0.4601	0.7485	0.9077	0.6269	0.6786	0.7798
0.6840	0.8881	0.5330	0.7628	0.8324	0.6821	0.7951	0.7700
0.6599	0.8850	0.4949	0.5668	0.8248	0.6795	0.6230	0.7150
0.6644	0.8542	0.4973	0.5668	0.7766	0.6636	0.6524	0.7892
0.6321	0.9337	0.5231	0.4622	0.8873	0.6950	0.6447	0.7754
0.6656	0.6669	0.6110	0.4445	0.7711	0.5307	0.6225	0.8502
0.7187	0.8424	0.5546	0.4789	0.7087	0.5509	0.6162	0.6794
0.7873	0.8769	0.5287	0.4884	0.7186	0.5228	0.5989	0.6854
0.7617	0.8541	0.5161	0.6927	0.7045	0.5454	0.5999	0.6577
0.7138	0.8350	0.5784	0.7093	0.7447	0.5509	0.5325	0.6572
0.7412	0.8190	0.5876	0.7347	0.8822	0.7751	0.5368	0.6734
0.6692	0.8426	0.6032	0.7336	0.8617	0.7592	0.4396	0.7055
0.7640	0.7599	0.6030	0.7365	0.8411	0.7948	0.4386	0.6992
0.7269	0.8297	0.6040	0.8104	0.8566	0.7986	0.4404	0.5710
0.8891	0.8755	0.5852	0.7919	0.8553	0.7795	0.5245	0.6789
0.7394	0.6865	0.4593	0.8146	0.8344	0.8804	0.4752	0.6563
0.5619	0.6832	0.4584	0.8108	0.8638	0.9035	0.5144	0.6477
0.5213	0.5723	0.4269	0.8131	0.8833	0.9002	0.4996	0.6200

0.5538	0.5826	0.4425	0.6319	0.8709	0.9015	0.5350	0.6326
0.5644	0.5998	0.4609	0.6431	0.8500	0.9046	0.4636	0.7344
0.5893	0.6139	0.6476	0.6009	0.8810	0.8151	0.6547	0.6956
0.8334	0.5955	0.7436	0.6012	0.8864	0.8968	0.4721	0.7961
0.7625	0.5832	0.5127	0.6005	0.8767	0.9542	0.4644	0.8006
0.8227	0.6005	0.4819	0.6222	0.8372	0.8456	0.4779	0.8116
0.6859	0.5778	0.4747	0.5804	0.8165	0.8476	0.5704	0.7237
0.7844	0.7563	0.8729	0.7514	0.8580	0.9191	0.8251	0.9055
0.9668	0.7655	0.8150	0.6449	0.8299	0.9463	0.8143	0.8779
0.9636	0.7626	0.8257	0.6150	0.8723	0.8923	0.8528	0.8987
0.9384	0.7737	0.8277	0.8863	0.8339	0.8850	0.8915	0.8998
0.8879	0.7696	0.7939	0.8676	0.9032	0.8949	0.5287	0.8535
0.9255	0.9345	0.6054	0.8954	0.9666	0.9388	0.4446	0.8912
0.8616	0.9441	0.5943	0.9081	0.9231	0.8497	0.4561	0.7995
0.8580	0.8905	0.5244	0.9051	0.9346	0.8254	0.4441	0.8297
0.8351	0.8798	0.5412	0.8165	0.9143	0.7935	0.4467	0.7841
0.8895	0.8995	0.5439	0.8382	0.8777	0.7678	0.7050	0.7735
0.8942	0.8689	0.4965	0.9165	0.6942	0.7914	0.7035	0.8797
0.6431	0.8804	0.4830	0.8121	0.7056	0.8093	0.7218	0.8099
0.6356	0.9074	0.4779	0.8582	0.8618	0.7996	0.5556	0.8013
0.6040	0.9057	0.4673	0.9203	0.9065	0.8223	0.5429	0.7953
0.7715	0.8942	0.4902	0.9000	0.8185	0.8108	0.7291	0.8307
0.6860	0.8583	0.7502	0.8191	0.8598	0.5892	0.7773	0.7414
0.8168	0.8407	0.7355	0.8738	0.8453	0.5956	0.6475	0.7868
0.7355	0.8428	0.6001	0.8835	0.8448	0.5960	0.6812	0.7320
0.7885	0.8416	0.6062	0.7845	0.8207	0.5046	0.5412	0.8511
0.7157	0.7887	0.5957	0.7813	0.7976	0.5695	0.7256	0.8123
0.7996	0.8421	0.5898	0.7444	0.9223	0.6151	0.5968	0.8026
0.7874	0.8361	0.5244	0.7702	0.9039	0.6197	0.6291	0.7877

0.8438	0.7439	0.5196	0.7083	0.9001	0.5958	0.6556	0.7929
0.8473	0.8656	0.5389	0.8952	0.9289	0.6169	0.6721	0.7605
0.7730	0.8542	0.5289	1.0105	0.9105	0.5964	0.4673	0.8291
0.8168	0.8020	0.4058	0.8636	0.4766	0.3927	0.4330	0.9479
0.8433	0.7431	0.3924	0.8872	0.4728	0.3876	0.4077	0.9393
0.8908	0.8076	0.4054	0.8318	0.4880	0.3722	0.4493	0.8782
0.8875	0.7730	0.4099	0.7108	0.5002	0.3696	0.4551	0.8606
0.8452	0.8034	0.4034	0.7364	0.4583	0.3894	0.4755	0.8488
0.8728	0.5723	0.5192	0.6762	0.6754	0.5639	0.4797	0.5689
0.4920	0.6124	0.4833	0.7051	0.6428	0.5735	0.4930	0.6330
0.5637	0.6878	0.4959	0.7264	0.6408	0.6283	0.4856	0.6355
0.6081	0.6532	0.4837	0.7471	0.6077	0.6144	0.4657	0.6057
0.6324	0.6760	0.4834	0.7119	0.6228	0.6015	0.7345	0.6218
0.6154	0.8136	0.7016	0.7616	0.9432	0.7563	0.7425	0.9196
0.6755	0.7793	0.6823	0.7450	0.7263	0.9366	0.7218	0.9632
0.6634	0.7438	0.6499	0.7168	0.7924	0.9052	0.6576	0.9020
0.6264	0.7523	0.6892	0.9438	0.8052	0.9235	0.6411	0.9722
0.9005	0.7569	0.7015	0.8699	0.8636	0.9101	0.7876	0.9618
0.8635	1.0150	0.6951	0.9245	0.9677	1.0124	0.5299	0.9563
1.0292	0.7493	0.4960	0.8730	1.0557	1.0677	0.6006	0.9791
0.9861	0.8372	0.5569	0.8918	1.0208	0.9879	0.5533	0.9787
0.9033	0.9820	0.5470	0.6748	0.9604	0.9478	0.5143	0.9737
0.9460	0.8538	0.6384	0.6670	0.9286	0.9448	0.6875	0.9558
0.9907	0.5799	0.5020	0.6188	0.7350	0.5512	0.5634	0.5832
0.5677	0.5714	0.4952	0.6006	0.7181	0.5874	0.5755	0.5568
0.5469	0.5765	0.5017	0.6210	0.7123	0.6658	0.6119	0.6802
0.5946	0.5599	0.4874	0.6267	0.6802	0.5974	0.6291	0.6712
0.6067	0.5752	0.4836	0.6841	0.6650	0.6288	0.6544	0.6929
0.6135	0.6645	0.6993	0.6255	0.7378	0.7801	0.6805	0.7140

0.6602	0.6522	0.6676	0.6155	0.7022	0.6938	0.6593	0.7126
0.6636	0.6571	0.6616	0.6508	0.6704	0.7045	0.6530	0.7307
0.6543	0.6902	0.6729	0.6308	0.6872	0.6739	0.6593	0.6595
0.6930	0.7461	0.6629	0.5812	0.7246	0.7011	0.9095	0.6825
0.6753	0.6223	0.5029	0.6082	0.6604	0.7779	0.7356	0.7933
0.6304	0.6863	0.5129	0.6239	0.6422	0.7765	0.6768	0.8350
0.6307	0.6620	0.5590	0.5917	0.6260	0.8233	0.5445	0.8098

Tables of all measured fibres thickness (width) in nm of all participants (2019.10)

SV02 naïve		SV02 LPSBP		SV02 LPS		SV02 LPS and LPSBP		SV02 Aspirin		SV02 aspirin + LPSBP		SV02 Aspirin + LPS		SV02 Aspirin + LPS+LPSBP	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,007		128,007		128,01		128,007		128,007		128,007		128,007		128,007
14,67	114,58	11,392	89,00	116,619	911,04	18,57	145,08	22,784	177,99	10,75	83,98	219,13	1711,86	24,585	192,06
14,727	115,05	17,385	135,81	106,75	833,94	20,83	162,70	17,537	137,00	18,95	148,04	298,991	2335,74	13,333	104,16
25,368	198,18	12	93,74	70,604	551,56	16,22	126,72	14,667	114,58	14,907	116,45	66,239	517,46	12,293	96,03
16,865	131,75	13,132	102,59	31,805	248,46	13,92	108,74	10,667	83,33	12	93,74	197,981	1546,64	21,868	170,83
11,314	88,39	14,907	116,45	132,812	1037,54	12,074	94,32	12,293	96,03	16,055	125,42	306,275	2392,64	12,649	98,81
12,579	98,27	14,422	112,67	209,605	1637,45	17,385	135,81	16,865	131,75	12,074	94,32	96,093	750,69	16,055	125,42
10,75	83,98	16,055	125,42	453,774	3544,92	13,728	107,24	9,333	72,91	13,597	106,22	140,159	1094,93	18,856	147,30
16,971	132,58	13,132	102,59	85,093	664,75	12,649	98,81	11,47	89,60	14,667	114,58	89,612	700,06	11,47	89,60
12,649	98,81	17,889	139,75	104,307	814,85	12,293	96,03	17,385	135,81	17,333	135,41	505,771	3951,12	13,597	106,22
17,333	135,41	14,667	114,58	209,338	1635,36	15,202	118,76	16,275	127,14	10,667	83,33	148,486	1159,98	8,537	66,69
16,111	125,86	11,314	88,39	95,452	745,68	18,135	141,67	13,4	104,68	8,537	66,69	300,855	2350,30	12,074	94,32
13,728	107,24	17,938	140,13	192,042	1500,25	13,728	107,24	14,907	116,45	14,422	112,67	130,162	1016,84	16,221	126,72
13,333	104,16	16,111	125,86	333,84	2607,98	15,202	118,76	9,428	73,65	12,074	94,32	124,458	972,27	9,615	75,11
13,4	104,68	15,085	117,85	113,529	886,90	12	93,74	10,414	81,35	16,221	126,72	28,347	221,45	15,549	121,47
10,667	83,33	16,707	130,52	258,753	2021,40	10,154	79,32	14,422	112,67	17,385	135,81	39,576	309,17	12,293	96,03
16,971	132,58	13,4	104,68	109,731	857,23	15,549	121,47	14,907	116,45	20,742	162,04	86,214	673,51	11,314	88,39
12,293	96,03	10,75	83,98	69,346	541,74	21,499	167,95	7,18	56,09	8,11	63,36	307,503	2402,24	16,707	130,52
12,293	96,03	13,132	102,59	80,144	626,09	18,135	141,67	14,422	112,67	15,202	118,76	348,61	2723,37	19,23	150,23
14,36	112,18	15,606	121,92	153,246	1197,17	14,907	116,45	16,865	131,75	13,597	106,22	370,669	2895,69	18,95	148,04
16,707	130,52	16,055	125,42	73,37	573,17	12,293	96,03	16,275	127,14	9,707	75,83	203,341	1588,51	6,799	53,11
12,649	98,81	12,293	96,03	91,467	714,55	20,177	157,62	11,314	88,39	14,727	115,05	205,078	1602,08	10,995	85,89
15,606	121,92	11,314	88,39	282,67	2208,24	14,667	114,58	10,154	79,32	20,352	158,99	294,646	2301,80	5,963	46,58
9,333	72,91	19,821	154,84	242,798	1896,76	17,789	138,97	10,75	83,98	16,055	125,42	607,523	4746,01	13,4	104,68
17,385	135,81	11,47	89,60	310,701	2427,22	16,707	130,52	15,202	118,76	12,649	98,81	413,191	3227,88	18,523	144,70
10,667	83,33	12,293	96,03	231,992	1812,34	16,111	125,86	10,154	79,32	12,293	96,03	518,386	4049,67	12,293	96,03
15,202	118,76	8	62,50	192,037	1500,21	22,191	173,36	14,727	115,05	11,392	89,00	311,27	2431,66	14,907	116,45
11,314	88,39	11,392	89,00	149,696	1169,44	25,157	196,53	13,4	104,68	12	93,74	149,429	1167,35	15,202	118,76
5,497	42,94	14,907	116,45	239,659	1872,23	17,075	133,39	16,055	125,42	13,597	106,22	75,672	591,16	17,385	135,81
13,597	106,22	17,333	135,41	233,15	1821,38	12,293	96,03	13,728	107,24	17,385	135,81	855,299	6681,66	18,135	141,67
12,649	98,81	14,907	116,45	252,208	1970,27	8,944	69,87	12	93,74	13,4	104,68	302,811	2365,58	17,385	135,81
14,174	110,73	16	124,99	224,396	1753,00	14,727	115,05	16,492	128,84	6,667	52,08	424,103	3313,12	8,944	69,87
16,055	125,42	16,492	128,84	164,265	1283,25	11,314	88,39	18,571	145,08	14,667	114,58	282,642	2208,02	13,132	102,59
19,23	150,23	18,667	145,83	52,273	408,36	17,537	137,00	13,132	102,59	10,667	83,33	928,308	7252,01	13,333	104,16
16,111	125,86	15,549	121,47	205,338	1604,12	15,202	118,76	17,789	138,97	12,293	96,03	561,39	4385,62	14,174	110,73
13,132	102,59	16,055	125,42	88,974	695,07	17,385	135,81	9,428	73,65	16	124,99	86,667	677,05	10,75	83,98
12,579	98,27	13,92	108,74	334,092	2609,95	16,111	125,86	10,995	85,89	17,333	135,41	211,698	1653,80	13,597	106,22
13,92	108,74	15,202	118,76	132,061	1031,67	14,907	116,45	9,428	73,65	14,727	115,05	183,734	1435,34	9,333	72,91
10,414	81,35	17,075	133,39	136,815	1068,81	25,751	201,17	18,856	147,30	12,074	94,32	40,354	315,25	9,707	75,83
13,333	104,16	15,549	121,47	76,269	595,82	26,8	209,36	8,11	63,36	16,221	126,72	181,891	1420,95	10,667	83,33
14,907	116,45	12	93,74	314,107	2453,83	21,499	167,95	12,579	98,27	14,667	114,58	92,856	725,40	6,667	52,08
18,95	148,04	12,649	98,81	97,406	760,94	15,606	121,92	14,727	115,05	11,47	89,60	140,937	1101,01	13,728	107,24
15,202	118,76	13,597	106,22	629,956	4921,26	19,821	154,84	15,202	118,76	16,865	131,75	69,449	542,54	14,727	115,05
14,36	112,18	8,537	66,69	428,732	3349,29	13,333	104,16	13,92	108,74	12,579	98,27	983,715	7684,85	15,202	118,76
9,333	72,91	13,132	102,59	341,484	2667,70	9,707	75,83	12,293	96,03	14,907	116,45	288,151	2251,06	25,889	202,25
13,597	106,22	17,333	135,41	79,387	620,18	13,199	103,11	4,807	37,55	18,135	141,67	188,486	1472,47	16,055	125,42
16,492	128,84	17,938	140,13	350,634	2739,18	17,385	135,81	8,433	65,88	14,422	112,67	181,5	1417,89	15,202	118,76
17,938	140,13	15,202	118,76	189,601	1481,18	14,174	110,73	23,017	179,81	13,597	106,22	108,288	845,95	10,75	83,98
16,275	127,14	13,4	104,68	170,854	1334,72	20,827	162,70	14,174	110,73	12,293	96,03	545,392	4260,64	17,333	135,41
14,907	116,45	15,606	121,92	446,891	3491,15	13,333	104,16	14,907	116,45	11,392	89,00	252,314	1971,10	10,154	79,32
11,314	88,39	16,055	125,42	116,031	906,44	15,085	117,85	8,944	69,87	17,333	135,41	155,886	1217,79	17,889	139,75
Average	**110,81**		**113,24**		**1532,12**		**126,44**		**105,43**		**109,24**		**2132,62**		**109,90**
Median	**109,74**		**116,45**		**1308,99**		**120,11**		**107,99**		**109,44**		**1595,30**		**106,22**
SD	**24,56**		**19,93**		**970,12**		**31,14**		**29,10**		**23,47**		**1723,01**		**33,06**

SV04 naïve		SV04 LPSBP		SV04 LPS		SV04 LPSBP + LPS		SV04 Aspirin		SV04 Asprirn + LPSBP		SV04 Aspirin +LPS		SV04 Aspirin +LPSBP+ LPS	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
14,174	110,73	5,333	41,66	97,068	758,30	28	218,74	9,333	72,91	12,649	98,81	264,286	2064,62	23,324	182,21
18,667	145,83	16,275	127,14	213,371	1666,87	26,466	206,75	13,728	107,24	6,799	53,11	389,107	3039,73	21,868	170,83
15,202	118,76	6,667	52,08	91,263	712,95	18,856	147,30	15,202	118,76	4	31,25	279,775	2185,62	18,135	141,67
21,333	166,65	13,597	106,22	207,508	1621,07	14,36	112,18	12	93,74	15,606	121,92	372,761	2912,04	8	62,50
18,95	148,04	12,579	98,27	129,601	1012,45	16,055	125,42	10,75	83,98	17,789	138,97	354,12	2766,41	11,392	89,00
13,199	103,11	11,392	89,00	86,861	678,56	5,333	41,66	16	124,99	12,074	94,32	364,51	2847,58	11,926	93,17
14,667	114,58	8,944	69,87	264,215	2064,07	8,944	69,87	16,492	128,84	17,889	139,75	323,209	2524,93	20	156,24
23,627	184,58	14,667	114,58	120,34	940,10	17,075	133,39	12	93,74	8,11	63,36	468,378	3659,00	9,333	72,91
9,615	75,11	16,707	130,52	98,432	768,96	17,938	140,13	6,667	52,08	17,889	139,75	432,103	3375,62	13,4	104,68
16,055	125,42	15,606	121,92	130,49	1019,40	10,75	83,98	9,428	73,65	13,333	104,16	578,607	4520,12	7,18	56,09
19,23	150,23	28,284	220,96	117,878	920,87	34,667	270,82	8,433	65,88	13,4	104,68	593,973	4640,16	88,534	691,63
16	124,99	10,75	83,98	102,935	804,14	17,333	135,41	4	31,25	17,333	135,41	785,062	6132,96	69,908	546,13
15,085	117,85	14,907	116,45	69,602	543,74	22,823	178,29	9,333	72,91	9,428	73,65	509,419	3979,62	12,579	98,27
20,827	162,70	17,333	135,41	63,386	495,18	15,202	118,76	21,868	170,83	20,044	156,59	558,667	4364,35	14,422	112,67
17,537	137,00	15,202	118,76	160,139	1251,02	11,926	93,17	14,727	115,05	6,667	52,08	370,676	2895,75	17,385	135,81
9,615	75,11	16,055	125,42	54,667	427,06	13,4	104,68	14,667	114,58	16,055	125,42	809,338	6322,61	13,132	102,59
18,95	148,04	11,926	93,17	98,892	772,55	29,242	228,44	13,333	104,16	17,333	135,41	313,336	2447,80	16,055	125,42
26,667	208,32	15,202	118,76	196,977	1538,80	19,09	149,13	12,579	98,27	13,4	104,68	852,176	6657,26	13,597	106,22
21,082	164,69	17,333	135,41	282,67	2208,24	16,055	125,42	24,585	192,06	10,667	83,33	472,092	3688,02	18,523	144,70
10,995	85,89	7,775	60,74	56,143	438,59	17,075	133,39	26,965	210,65	6,667	52,08	549,333	4291,43	4,216	32,94
4,216	32,94	19,821	154,84	586,668	4583,09	16	124,99	16,055	125,42	7,775	60,74	127,757	998,05	11,926	93,17
13,4	104,68	13,333	104,16	123,144	962,01	19,09	149,13	14,36	112,18	6,799	53,11	302,667	2364,46	15,202	118,76
11,314	88,39	15,202	118,76	236	1843,65	12,074	94,32	15,549	121,47	8,537	66,69	384,28	3002,02	16,221	126,72
13,92	108,74	16,055	125,42	48,295	377,28	11,392	89,00	32,441	253,43	13,4	104,68	423,492	3308,35	14,727	115,05
19,09	149,13	10,75	83,98	317,359	2479,23	15,202	118,76	6,667	52,08	7,18	56,09	416,722	3255,46	13,333	104,16
13,92	108,74	12,074	94,32	350,624	2739,10	15,085	117,85	12,293	96,03	10,75	83,98	612,459	4784,57	8,11	63,36
17,537	137,00	9,333	72,91	150,955	1179,27	17,889	139,75	12,579	98,27	7,18	56,09	735,908	5748,97	8	62,50
13,597	106,22	11,392	89,00	67,145	524,54	8,433	65,88	12,293	96,03	21,082	164,69	442,516	3456,97	7,18	56,09
17,333	135,41	12	93,74	22,94	179,21	28,628	223,64	13,199	103,11	13,728	107,24	688,437	5378,12	15,085	117,85
18,135	141,67	5,657	44,19	230,671	1802,02	15,202	118,76	12,074	94,32	17,075	133,39	303,251	2369,02	16,111	125,86
13,333	104,16	14,422	112,67	431,038	3367,30	18,523	144,70	20,309	158,66	9,707	75,83	373,928	2921,15	20	156,24
18,571	145,08	10,75	83,98	64,512	503,97	2,981	23,29	14,422	112,67	15,549	121,47	323,344	2525,99	14,174	110,73
20,177	157,62	10,75	83,98	201,968	1577,79	10,414	81,35	21,705	169,56	9,333	72,91	694	5421,58	12,293	96,03
16,111	125,86	13,199	103,11	126,842	990,90	10,667	83,33	22,706	177,38	16,865	131,75	391,528	3058,65	12,074	94,32
17,889	139,75	16,865	131,75	229,349	1791,69	12,293	96,03	10,667	83,33	9,615	75,11	490,047	3828,28	11,392	89,00
19,09	149,13	9,615	75,11	237,786	1857,60	15,549	121,47	18,714	146,20	8,944	69,87	701,223	5478,01	15,549	121,47
21,499	167,95	15,202	118,76	433,342	3385,30	26,667	208,32	6,799	53,11	6,799	53,11	287,568	2246,50	16,055	125,42
14,36	112,18	10,75	83,98	244,477	1909,87	10,154	79,32	12,074	94,32	12,293	96,03	306,869	2397,28	15,202	118,76
19,09	149,13	8,11	63,36	79,308	619,56	10,995	85,89	17,938	140,13	14,422	112,67	296,591	2316,99	8,11	63,36
24	187,49	15,549	121,47	330,758	2583,91	7,775	60,74	11,392	89,00	11,392	89,00	296,591	2316,99	11,47	89,60
16,492	128,84	15,202	118,76	115,762	904,34	14,667	114,58	14,422	112,67	6,667	52,08	520	4062,28	23,627	184,58
16,221	126,72	6,799	53,11	88,654	692,57	14,667	114,58	15,606	121,92	10,995	85,89	362,667	2833,18	10,995	85,89
18,95	148,04	17,333	135,41	113,333	885,37	11,926	93,17	13,92	108,74	10,414	81,35	260,031	2031,38	7,18	56,09
17,889	139,75	8,537	66,69	128,34	1002,60	14,907	116,45	19,821	154,84	12,074	94,32	369,102	2883,45	9,707	75,83
17,789	138,97	13,4	104,68	116,428	909,54	9,428	73,65	10,667	83,33	6,799	53,11	486,762	3802,62	4	31,25
17,537	137,00	29,242	228,44	44,02	343,89	7,775	60,74	13,333	104,16	12	93,74	331,354	2588,56	9,428	73,65
14,907	116,45	18,856	147,30	158,689	1239,69	12,293	96,03	17,333	135,41	7,542	58,92	275,591	2152,94	16,111	125,86
21,082	164,69	5,497	42,94	318,803	2490,51	16,221	126,72	20,352	158,99	16	124,99	393,388	3073,18	15,202	118,76
9,428	73,65	8,944	69,87	318,893	2491,22	18,667	145,83	12,074	94,32	5,497	42,94	361,587	2824,74	7,18	56,09
11,314	88,39	16,055	125,42	116,03	906,44	15,085	117,85	8,944	69,87	17,33	135,41	155,886	1217,79	17,889	139,75
Average	**129,63**		**104,25**		**1355,33**		**122,06**		**112,93**		**92,32**		**3399,26**		**124,44**
Median	**136,20**		**104,42**		**996,75**		**118,30**		**105,70**		**91,37**		**3020,88**		**105,45**
SD	**32,57**		**37,89**		**920,06**		**49,57**		**42,39**		**33,68**		**1295,06**		**108,98**

SV05 naïve		SV05 LPSBP		SV05 LPS		SV05 LPSBP + LPS		SV05 Aspirin		SV05 Aspirin + LPSBP		SV05 Aspirin+ LPS		SV05 Aspirin + LPS+ LPSBP	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
30,696	239,80	16	124,99	177,414	1385,97	11,47	89,60	16,055	125,42	5,333	41,66	90,872	709,90	27,455	214,48
15,606	121,92	13,333	104,16	109,723	857,16	15,202	118,76	8	62,50	13,4	104,68	192,185	1501,36	9,615	75,11
13,199	103,11	8,537	66,69	314,898	2460,01	13,4	104,68	12,074	94,32	8,433	65,88	134,197	1048,36	8,537	66,69
11,47	89,60	10,414	81,35	248,805	1943,68	12,293	96,03	17,537	137,00	16,055	125,42	175,525	1371,21	6,667	52,08
25,889	202,25	10,995	85,89	86,708	677,37	7,18	56,09	4,807	37,55	8	62,50	454,438	3550,10	15,202	118,76
12,579	98,27	6,667	52,08	112,071	875,51	9,428	73,65	12,579	98,27	11,47	89,60	280,308	2189,79	9,707	75,83
18,95	148,04	21,082	164,69	94,281	736,53	10,995	85,89	14,422	112,67	7,775	60,74	634,567	4957,28	16,055	125,42
9,428	73,65	9,333	72,91	187,583	1465,41	8,537	66,69	5,497	42,94	9,333	72,91	773,216	6040,42	20,044	156,59
19,23	150,23	4	31,25	287,784	2248,19	18,95	148,04	9,428	73,65	19,09	149,13	306,777	2396,56	15,202	118,76
22,351	174,61	5,657	44,19	9,615	75,11	13,333	104,16	12,293	96,03	8,11	63,36	642,668	5020,57	8	62,50
12,649	98,81	12,649	98,81	350,973	2741,83	10,75	83,98	14,907	116,45	12,649	98,81	154,69	1208,45	7,18	56,09
12,293	96,03	14,727	115,05	345,336	2697,79	17,789	138,97	18,667	145,83	11,392	89,00	362,777	2834,04	18,135	141,67
21,705	169,56	5,333	41,66	85,925	671,25	9,707	75,83	5,333	41,66	21,082	164,69	60,472	472,41	35,226	275,19
28,503	222,67	11,392	89,00	288,003	2249,90	19,821	154,84	10,75	83,98	24,585	192,06	100,036	781,49	16,707	130,52
10,154	79,32	8,944	69,87	188,302	1471,03	11,926	93,17	10,995	85,89	115,293	900,68	64,677	505,26	31,693	247,59
7,18	56,09	14,727	115,05	305,336	2385,31	12,293	96,03	7,775	60,74	22,191	173,36	246,042	1922,10	8,944	69,87
22,823	178,29	20,827	162,70	176	1374,92	6,667	52,08	11,926	93,17	8,537	66,69	164,022	1281,35	10,995	85,89
12,579	98,27	12,293	96,03	186,743	1458,85	9,615	75,11	22,667	177,08	13,4	104,68	367,973	2874,63	18,135	141,67
17,075	133,39	11,926	93,17	314,059	2453,45	10,154	79,32	11,926	93,17	73,43	573,64	475,701	3716,21	17,333	135,41
4	31,25	12,579	98,27	421,335	3291,50	6,667	52,08	19,414	151,66	10,995	85,89	156,006	1218,73	9,333	72,91
17,938	140,13	20,699	161,70	196,959	1538,66	8,944	69,87	16,275	127,14	16,971	132,58	157,271	1228,61	14,422	112,67
9,428	73,65	5,657	44,19	247,9	1936,61	10,154	79,32	11,392	89,00	13,333	104,16	64,139	501,06	6,667	52,08
13,333	104,16	9,333	72,91	184,391	1440,48	1,886	14,73	9,615	75,11	58,682	458,43	103,185	806,09	8,944	69,87
17,385	135,81	13,92	108,74	304,292	2377,15	17,537	137,00	11,926	93,17	21,499	167,95	138,262	1080,11	17,537	137,00
8,11	63,36	9,615	75,11	122,674	958,34	14,907	116,45	17,938	140,13	32,796	256,20	97,114	758,66	4,807	37,55
4,216	32,94	5,657	44,19	127,736	997,88	17,333	135,41	8,944	69,87	20,396	159,34	88,01	687,54	14,907	116,45
21,082	164,69	8,11	63,36	183,094	1430,34	4,807	37,55	13,597	106,22	47,479	370,91	133,56	1043,38	11,392	89,00
12,293	96,03	11,926	93,17	147,38	1151,34	5,333	41,66	9,333	72,91	29,605	231,28	187,64	1465,86	29,242	228,44
2,981	23,29	17,385	135,81	191,379	1495,07	16,707	130,52	8,537	66,69	11,47	89,60	472,041	3687,62	26,998	210,91
22,94	179,21	5,963	46,58	147,85	1155,01	15,202	118,76	12,074	94,32	6,799	53,11	147,012	1148,47	9,428	73,65
10,154	79,32	13,597	106,22	363,651	2840,87	10,667	83,33	9,428	73,65	20,309	158,66	172,557	1348,03	15,549	121,47
9,428	73,65	12,293	96,03	308,312	2408,56	5,963	46,58	21,99	171,79	5,333	41,66	127,513	996,14	24,148	188,65
5,963	46,58	19,414	151,66	219,64	1715,84	11,926	93,17	13,4	104,68	11,314	88,39	100,788	787,36	9,428	73,65
6,799	53,11	2,981	23,29	209,095	1633,47	8,537	66,69	13,597	106,22	46,438	362,78	432,403	3377,96	5,963	46,58
10,414	81,35	10,414	81,35	384,234	3001,66	10,667	83,33	16	124,99	2,981	23,29	94,3	736,68	9,428	73,65
10,75	83,98	10,154	79,32	238,938	1866,60	14,667	114,58	10,75	83,98	16,707	130,52	158,437	1237,72	14,907	116,45
41,074	320,87	9,333	72,91	47,647	372,22	12	93,74	6,799	53,11	6,799	53,11	138,32	1080,57	16,707	130,52
20	156,24	45,607	356,29	142,436	1112,72	8,11	63,36	16,055	125,42	5,497	42,94	474,314	3705,38	24,037	187,78
6,799	53,11	6,667	52,08	108,008	843,77	11,392	89,00	10,667	83,33	54,732	427,57	154,068	1203,59	189,784	1482,61
7,18	56,09	22,627	176,76	167,226	1306,38	11,47	89,60	15,606	121,92	8,537	66,69	118,457	925,39	16,055	125,42
18,571	145,08	9,428	73,65	109,341	854,18	2,667	20,83	12,649	98,81	17,333	135,41	79,151	618,33	7,775	60,74
9,615	75,11	7,18	56,09	65,388	510,82	16,865	131,75	8,11	63,36	15,202	118,76	89,343	697,95	13,597	106,22
12,293	96,03	12,293	96,03	143,883	1124,02	11,926	93,17	6,667	52,08	13,132	102,59	236,267	1845,73	20,044	156,59
15,085	117,85	5,657	44,19	205,774	1607,52	19,09	149,13	5,497	42,94	12,293	96,03	140,025	1093,89	9,615	75,11
22,706	177,38	20,699	161,70	133,34	1041,66	5,963	46,58	14,422	112,67	10,667	83,33	318,901	2491,28	11,926	93,17
16,055	125,42	7,775	60,74	158,672	1239,56	15,549	121,47	7,775	60,74	10,995	85,89	186,671	1458,29	14,36	112,18
20	156,24	26,667	208,32	117,341	916,68	8,11	63,36	13,92	108,74	94,751	740,20	86,667	677,05	76,513	597,73
31,468	245,83	9,615	75,11	121,333	947,86	17,333	135,41	18,714	146,20	29,814	232,91	324,011	2531,20	21,705	169,56
18,714	146,20	18,523	144,70	43,41	339,12	15,085	117,85	13,333	104,16	17,889	139,75	362,777	2834,04	7,775	60,74
13,92	108,74	15,606	121,92	113,333	885,37	14,422	112,67	15,202	118,76	22,191	173,36	90,872	709,90	27,455	214,48
Average	**119,53**		**97,84**		**1491,41**		**90,84**		**96,44**		**172,26**		**1767,28**		**154,88**
Median	**103,63**		**87,44**		**1408,16**		**89,60**		**94,32**		**104,68**		**1223,67**		**116,45**
SD	**60,91**		**55,86**		**753,88**		**33,93**		**33,46**		**176,41**		**1313,69**		**210,93**

SV06 naïve		SV06 LPSBP		SV06 LPS		SV06 LPSBP + LPS		SV06 Aspirin		SV06 Aspirin + LPSBP		SV06 Aspirin + LPS		SV06 Aspirin + LPSBP+LPS	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
17,075	133,39	11,314	88,39	49,549	387,08	20	156,24	16	124,99	19,09	149,13	81,464	636,40	22,784	177,99
14,667	114,58	15,202	118,76	91,234	712,73	28,253	220,71	8,433	65,88	10,75	83,98	78,531	613,49	10,154	79,32
14,667	114,58	18,714	146,20	48	374,98	14,36	112,18	8,537	66,69	12,293	96,03	73,345	572,98	17,537	137,00
12,293	96,03	17,333	135,41	100,39	784,25	8,433	65,88	14,667	114,58	21,333	166,65	61,867	483,31	8	62,50
16,707	130,52	8,11	63,36	70,402	549,99	19,821	154,84	18,856	147,30	4,216	32,94	74,093	578,82	12	93,74
18,667	145,83	9,333	72,91	177,629	1387,65	17,537	137,00	10,75	83,98	6,667	52,08	66,667	520,81	16,275	127,14
13,333	104,16	10,667	83,33	96,922	757,16	8,433	65,88	19,821	154,84	10,154	79,32	72,111	563,34	12,293	96,03
6,799	53,11	8,11	63,36	94,695	739,76	8	62,50	21,705	169,56	7,775	60,74	158,285	1236,53	11,314	88,39
10,75	83,98	10,667	83,33	80,011	625,05	21,375	166,98	14,36	112,18	19,09	149,13	74,774	584,14	13,597	106,22
21,333	166,65	15,549	121,47	131,791	1029,56	5,497	42,94	12,293	96,03	17,537	137,00	102,771	802,85	29,273	228,68
29,454	230,10	10,154	79,32	329,983	2577,85	16,111	125,86	10,75	83,98	5,657	44,19	37,759	294,98	14,422	112,67
11,392	89,00	13,4	104,68	86,42	675,12	12,579	98,27	17,075	133,39	10,667	83,33	103,09	805,35	18,667	145,83
18,856	147,30	12,293	96,03	52,392	409,29	10,75	83,98	7,18	56,09	12,074	94,32	80,554	629,29	5,657	44,19
1,886	14,73	7,775	60,74	207,461	1620,70	5,333	41,66	17,333	135,41	18,856	147,30	276,524	2160,23	18,135	141,67
4,216	32,94	12,579	98,27	76,756	599,62	10,154	79,32	16,055	125,42	12,579	98,27	211,008	1648,41	24	187,49
12,649	98,81	10,667	83,33	221,975	1734,08	18,135	141,67	21,082	164,69	13,333	104,16	95,35	744,88	10,75	83,98
15,202	118,76	10,414	81,35	97,662	762,94	7,18	56,09	21,333	166,65	9,333	72,91	163,392	1276,43	37,547	293,32
13,4	104,68	9,707	75,83	66,104	516,41	18,571	145,08	11,314	88,39	17,537	137,00	44,502	347,65	12,074	94,32
12,293	96,03	11,314	88,39	68,052	531,63	21,499	167,95	14,174	110,73	10,667	83,33	66,999	523,40	5,497	42,94
10,995	85,89	10,414	81,35	70,073	547,42	4,807	37,55	17,938	140,13	4,807	37,55	190,181	1485,71	15,202	118,76
5,497	42,94	6,799	53,11	123,454	964,43	18,856	147,30	11,392	89,00	8,537	66,69	182,788	1427,95	10,995	85,89
9,428	73,65	9,428	73,65	189,694	1481,90	11,47	89,60	14,907	116,45	16,055	125,42	132,168	1032,51	19,09	149,13
7,542	58,92	7,542	58,92	112,23	876,75	14,174	110,73	7,18	56,09	16,492	128,84	41,526	324,40	20,699	161,70
13,92	108,74	10,154	79,32	53,35	416,77	10,75	83,98	11,926	93,17	11,47	89,60	161,988	1265,46	9,707	75,83
8,11	63,36	22,94	179,21	231,9	1811,62	7,775	60,74	13,728	107,24	5,333	41,66	82,71	646,14	13,333	104,16
14,667	114,58	10,995	85,89	143,914	1124,27	17,075	133,39	10,75	83,98	10,154	79,32	76,012	593,81	10,75	83,98
10,667	83,33	20,699	161,70	143,065	1117,63	21,333	166,65	15,549	121,47	23,286	181,91	105,367	823,13	7,18	56,09
4	31,25	9,428	73,65	125,822	982,93	5,657	44,19	11,47	89,60	5,333	41,66	66,72	521,22	15,549	121,47
13,4	104,68	12,293	96,03	93,343	729,20	16	124,99	25,051	195,70	15,606	121,92	377,124	2946,12	14,667	114,58
5,497	42,94	9,615	75,11	171,508	1339,83	18,667	145,83	12,293	96,03	16,492	128,84	170,687	1333,42	15,606	121,92
11,392	89,00	11,47	89,60	166,8	1303,05	8,11	63,36	16	124,99	17,385	135,81	94,901	741,37	27,455	214,48
6,799	53,11	10,75	83,98	262,802	2053,03	7,775	60,74	22,706	177,38	17,333	135,41	94,929	741,59	18,667	145,83
14,174	110,73	8	62,50	81,344	635,47	13,333	104,16	13,333	104,16	9,428	73,65	22,351	174,61	10,154	79,32
16,055	125,42	13,132	102,59	94,817	740,72	15,202	118,76	8,11	63,36	11,926	93,17	20,177	157,62	16,221	126,72
7,775	60,74	12,074	94,32	155,795	1217,08	8,537	66,69	17,385	135,81	8,11	63,36	36,222	282,97	13,4	104,68
10,414	81,35	12,293	96,03	93,343	729,20	10,75	83,98	19,09	149,13	16,111	125,86	49,495	386,66	27,358	213,72
14,36	112,18	10,667	83,33	26,466	206,75	13,728	107,24	12,649	98,81	12,579	98,27	77,437	604,94	13,333	104,16
6,667	52,08	14,667	114,58	96,83	756,44	15,202	118,76	17,537	137,00	12	93,74	33,413	261,02	10,995	85,89
5,657	44,19	11,314	88,39	205,177	1602,86	32,277	252,15	18,667	145,83	8,433	65,88	44,322	346,25	21,499	167,95
13,92	108,74	12,649	98,81	72,049	562,85	15,606	121,92	6,667	52,08	17,537	137,00	29,242	228,44	6,667	52,08
7,542	58,92	9,615	75,11	46,514	363,37	8,11	63,36	15,085	117,85	6,667	52,08	40,814	318,84	16,275	127,14
11,314	88,39	10,414	81,35	138,98	1085,72	13,728	107,24	21,499	167,95	21,705	169,56	35,876	280,27	10,75	83,98
12	93,74	11,926	93,17	77,379	604,49	9,615	75,11	21,705	169,56	18,523	144,70	31,127	243,17	13,4	104,68
4	31,25	12,649	98,81	100,39	784,25	15,549	121,47	17,537	137,00	34,897	272,62	31,383	245,17	11,392	89,00
16,492	128,84	14,422	112,67	139,306	1088,27	8	62,50	5,333	41,66	19,09	149,13	31,127	243,17	16,275	127,14
6,799	53,11	19,23	150,23	142,311	1111,74	14,174	110,73	14,36	112,18	11,314	88,39	39,395	307,76	16,971	132,58
17,889	139,75	16,865	131,75	117,091	914,72	13,728	107,24	13,92	108,74	18,714	146,20	20,396	159,34	22,94	179,21
10,154	79,32	12,293	96,03	77,746	607,36	20,044	156,59	8,433	65,88	4,807	37,55	123,598	965,56	16,111	125,86
15,085	117,85	16,221	126,72	207,469	1620,76	54,291	424,13	10,75	83,98	4,216	32,94	32,931	257,26	22,627	176,76
17,333	135,41	14,907	116,45	63,344	494,85	11,314	88,39	10,667	83,33	10,667	83,33	36,099	282,01	16,865	131,75
Average	92,99		95,18		933,03		113,69		113,33		102,28		693,02		122,20
Median	94,89		88,39		760,05		107,24		112,18		94,03		575,90		116,67
SD	**40,42**		**26,96**		**488,71**		**63,90**		**36,91**		**47,27**		**545,96**		**49,85**

SV08N naïve		SV08 LPSBP		SV08 LPS		SV08 LPS+LPSBP		SV08 Aspirin		SV08 Aspirin + LPSBP		SV08 Aspirin + LPS		SV08 Aspirin + LPSBP +LPS	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
11,392	89,00	10,75	83,98	39,576	309,17	11,314	88,39	11,392	89,00	13,728	107,24	176,101	1375,71	21,375	166,98
4,216	32,94	14,174	110,73	143,883	1124,02	16,275	127,14	19,23	150,23	9,333	72,91	79,554	621,48	21,99	171,79
17,075	133,39	8,433	65,88	12,074	94,32	14,907	116,45	18,667	145,83	17,385	135,81	176,01	1375,00	24	187,49
19,23	150,23	16,971	132,58	48,461	378,58	20,309	158,66	18,571	145,08	10,667	83,33	129,106	1008,59	14,727	115,05
20,827	162,70	9,615	75,11	14,667	114,58	22,823	178,29	13,333	104,16	17,385	135,81	97,224	759,52	15,202	118,76
14,667	114,58	8,11	63,36	53,93	421,31	8,537	66,69	17,385	135,81	16,492	128,84	20,177	157,62	15,549	121,47
7,775	60,74	13,333	104,16	82,03	640,82	17,333	135,41	9,615	75,11	10,667	83,33	9,428	73,65	18,856	147,30
6,799	53,11	9,615	75,11	103,846	811,25	12	93,74	17,537	137,00	17,333	135,41	13,333	104,16	11,314	88,39
14,422	112,67	4,216	32,94	40,2	314,05	10,667	83,33	28,127	219,73	13,4	104,68	4,216	32,94	13,4	104,68
14,36	112,18	18,135	141,67	20	156,24	13,333	104,16	12,293	96,03	14,907	116,45	5,497	42,94	13,92	108,74
17,385	135,81	9,428	73,65	32,111	250,85	7,775	60,74	19,821	154,84	10,995	85,89	7,18	56,09	12,293	96,03
18,667	145,83	17,385	135,81	102,667	802,04	15,549	121,47	19,09	149,13	13,92	108,74	66,946	522,99	25,298	197,63
17,385	135,81	10,75	83,98	42,1	328,89	8,537	66,69	20,309	158,66	16,055	125,42	10,414	81,35	6,667	52,08
2,981	23,29	12	93,74	57,766	451,27	22,823	178,29	14,36	112,18	14,667	114,58	37,357	291,84	11,314	88,39
18,95	148,04	17,537	137,00	29,333	229,15	14,174	110,73	21,082	164,69	18,856	147,30	39,033	304,93	15,202	118,76
13,132	102,59	6,667	52,08	77,952	608,97	11,392	89,00	5,497	42,94	11,47	89,60	40	312,48	20,87	163,04
12	93,74	18,571	145,08	36	281,23	13,4	104,68	11,926	93,17	8,537	66,69	132,269	1033,30	17,333	135,41
24	187,49	15,202	118,76	28,032	218,99	11,926	93,17	14,667	114,58	13,597	106,22	33,652	262,89	18,856	147,30
18,135	141,67	20,044	156,59	28,503	222,67	5,657	44,19	17,075	133,39	9,428	73,65	34,072	266,17	15,202	118,76
12,293	96,03	14,727	115,05	52	406,23	11,392	89,00	9,615	75,11	12,074	94,32	39,033	304,93	10,995	85,89
9,333	72,91	10,414	81,35	25,368	198,18	10,667	83,33	19,23	150,23	14,422	112,67	33,652	262,89	14,174	110,73
13,597	106,22	13,4	104,68	72,111	563,34	24,037	187,78	12,579	98,27	19,23	150,23	393,342	3072,82	11,392	89,00
16,492	128,84	11,314	88,39	49,978	390,43	10,995	85,89	19,23	150,23	19,414	151,66	26,297	205,43	12,579	98,27
24,585	192,06	9,615	75,11	64,36	502,79	12,074	94,32	10,414	81,35	8,944	69,87	30,17	235,69	12,293	96,03
12,649	98,81	16,111	125,86	89,958	702,76	13,333	104,16	14,727	115,05	14,907	116,45	18,95	148,04	14,36	112,18
15,202	118,76	17,075	133,39	116,375	909,13	12	93,74	7,542	58,92	13,92	108,74	68,104	532,03	16,055	125,42
8,11	63,36	11,926	93,17	41,761	326,24	10,75	83,98	17,075	133,39	27,455	214,48	20,87	163,04	18,856	147,30
13,132	102,59	17,889	139,75	52,068	406,76	9,428	73,65	12,579	98,27	16,221	126,72	76,187	595,18	20,396	159,34
19,414	151,66	13,728	107,24	23,589	184,28	10,667	83,33	10,75	83,98	12,074	94,32	28,284	220,96	11,47	89,60
15,202	118,76	8,11	63,36	32,028	250,21	14,667	114,58	7,18	56,09	20,699	161,70	219,203	1712,43	21,705	169,56
17,889	139,75	10,667	83,33	31,241	244,06	14,727	115,05	8	62,50	11,47	89,60	17,537	137,00	15,202	118,76
15,202	118,76	8,433	65,88	49,978	390,43	21,705	169,56	12,293	96,03	13,333	104,16	111,363	869,98	13,199	103,11
17,385	135,81	4,807	37,55	37,547	293,32	8,944	69,87	7,542	58,92	11,926	93,17	21,499	167,95	6,667	52,08
14,907	116,45	7,18	56,09	105,662	825,44	5,963	46,58	8,433	65,88	10,75	83,98	57,95	452,71	16,275	127,14
12	93,74	13,132	102,59	121,399	948,38	12,074	94,32	16,865	131,75	14,727	115,05	33,44	261,24	16,971	132,58
10,995	85,89	16,275	127,14	147,88	1155,25	6,667	52,08	13,597	106,22	23,589	184,28	37,428	292,39	14,907	116,45
8,944	69,87	12,579	98,27	20,352	158,99	18,135	141,67	10,75	83,98	16,221	126,72	54,144	422,98	11,47	89,60
16	124,99	14,422	112,67	26,667	208,32	24,148	188,65	17,075	133,39	13,728	107,24	121,801	951,52	18,523	144,70
8,537	66,69	10,995	85,89	41,161	321,55	14,36	112,18	12,293	96,03	11,314	88,39	132,397	1034,29	14,36	112,18
17,333	135,41	16,055	125,42	134,528	1050,94	18,523	144,70	23,627	184,58	17,385	135,81	44,502	347,65	8,537	66,69
10,995	85,89	21,705	169,56	78,937	616,66	7,775	60,74	7,542	58,92	10,414	81,35	52,273	408,36	8,944	69,87
4,807	37,55	13,333	104,16	157,503	1230,42	16,055	125,42	16	124,99	16,055	125,42	166,603	1301,51	10,154	79,32
11,47	89,60	15,606	121,92	184,294	1439,72	11,926	93,17	10,995	85,89	10,414	81,35	59,029	461,14	12,293	96,03
14,174	110,73	6,799	53,11	241,79	1888,88	10,995	85,89	14,422	112,67	10,154	79,32	71,005	554,70	17,385	135,81
10,995	85,89	12,293	96,03	203,93	1593,12	5,963	46,58	15,549	121,47	16,055	125,42	31,013	242,28	14,174	110,73
17,789	138,97	12,293	96,03	22,351	174,61	5,963	46,58	19,821	154,84	13,199	103,11	256,766	2005,87	20	156,24
12,074	94,32	13,199	103,11	33,993	265,56	8,537	66,69	12,074	94,32	19,09	149,13	57,069	445,83	16,221	126,72
9,428	73,65	16,971	132,58	64,222	501,71	20,177	157,62	11,926	93,17	20	156,24	71,765	560,63	12,649	98,81
7,542	58,92	18,135	141,67	104,995	820,23	13,199	103,11	14,907	116,45	17,385	135,81	156,268	1220,78	12,293	96,03
12	93,74	10,414	81,35	48,166	376,28	17,333	135,41	17,789	138,97	10,75	83,98	16	124,99	7,775	60,74
Average	106,85		100,08		542,05		103,42		112,87		113,45		568,02		116,50
Median	108,47		100,43		390,43		94,03		112,42		108,74		330,07		113,61
SD	**37,07**		**31,58**		**408,24**		**38,35**		**37,00**		**30,75**		**584,34**		**33,68**

SV09 naïve		SV09 LPSBP		SV09 LPS		SV09 LPSBP+ LPS		SV09 Aspirin		SV09 Aspirin + LPSBP		SV09 Aspirin + LPS		SV09 Aspirin +LPS+LPSBP	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
19,686	153,79	10,995	85,89	70,679	552,15	39,373	307,58	31,213	243,84	15,202	118,76	88,363	690,30	36,222	282,97
16,055	125,42	15,202	118,76	49,549	387,08	22,706	177,38	13,333	104,16	14,36	112,18	72,049	562,85	23,286	181,91
18,667	145,83	20,742	162,04	168,28	1314,62	27,745	216,75	14,727	115,05	21,375	166,98	60,809	475,04	8,433	65,88
17,385	135,81	10,414	81,35	50,596	395,26	34,692	271,02	20,742	162,04	33,44	261,24	74,964	585,62	21,868	170,83
22,823	178,29	9,428	73,65	76,012	593,81	23,324	182,21	21,499	167,95	12	93,74	77,746	607,36	15,606	121,92
8,433	65,88	17,333	135,41	64,498	503,86	29,273	228,68	5,333	41,66	8,11	63,36	166,88	1303,68	10,154	79,32
27,841	217,50	16,111	125,86	50,049	390,99	35,578	277,94	16,055	125,42	9,428	73,65	143,716	1122,72	15,606	121,92
26,566	207,54	10,667	83,33	308,003	2406,14	17,789	138,97	29,814	232,91	5,657	44,19	46,686	364,71	24,585	192,06
25,298	197,63	15,606	121,92	20,177	157,62	21,333	166,65	8,433	65,88	11,926	93,17	252,792	1974,83	25,298	197,63
18,856	147,30	14,36	112,18	17,385	135,81	10,667	83,33	22,667	177,08	20	156,24	77,299	603,87	16,275	127,14
13,132	102,59	16,492	128,84	48,461	378,58	28,844	225,33	12,074	94,32	16,865	131,75	166,88	1303,68	34,998	273,41
12,649	98,81	18,856	147,30	43,04	336,23	16,492	128,84	22,667	177,08	10,75	83,98	143,716	1122,72	16,865	131,75
25,473	199,00	10,75	83,98	111,754	873,03	23,324	182,21	17,789	138,97	13,597	106,22	46,686	364,71	24,037	187,78
33,333	260,40	13,597	106,22	40,814	318,84	21,868	170,83	28,032	218,99	12,293	96,03	252,792	1974,83	9,333	72,91
12,649	98,81	12,293	96,03	69,449	542,54	27,487	214,73	8,433	65,88	12,074	94,32	77,299	603,87	13,333	104,16
20,699	161,70	14,667	114,58	80,277	627,13	15,549	121,47	10,667	83,33	19,23	150,23	462,943	3616,54	29,364	229,39
21,333	166,65	16,275	127,14	48,074	375,56	18,667	145,83	12,579	98,27	9,615	75,11	62,154	485,55	33,731	263,51
17,537	137,00	21,375	166,98	256,014	2000,00	19,09	149,13	15,549	121,47	40,552	316,80	246,008	1921,83	18,95	148,04
13,132	102,59	26,667	208,32	246,796	1927,99	16,707	130,52	17,938	140,13	16	124,99	70,029	547,07	17,385	135,81
17,789	138,97	13,597	106,22	389,518	3042,94	17,789	138,97	14,907	116,45	12,649	98,81	200,09	1563,12	16	124,99
10,75	83,98	19,23	150,23	187,735	1466,60	9,428	73,65	17,938	140,13	14,174	110,73	66,88	522,47	15,085	117,85
18,667	145,83	10,75	83,98	177,939	1390,07	7,18	56,09	23,017	179,81	8,433	65,88	72,012	562,56	22,823	178,29
21,499	167,95	20	156,24	42,374	331,03	29,333	229,15	11,314	88,39	20	156,24	112,159	876,19	13,132	102,59
18,856	147,30	19,09	149,13	116,969	913,77	14,907	116,45	13,333	104,16	12,649	98,81	104,732	818,17	17,789	138,97
12,649	98,81	18,714	146,20	82,903	647,64	16,707	130,52	10,75	83,98	16,971	132,58	138,114	1078,96	21,705	169,56
8,944	69,87	14,727	115,05	141,34	1104,16	17,385	135,81	21,375	166,98	15,202	118,76	70,364	549,69	12,293	96,03
12,649	98,81	13,333	104,16	48,534	379,15	26,667	208,32	16,055	125,42	10,75	83,98	79,744	622,97	18,135	141,67
16,055	125,42	9,428	73,65	124,029	968,92	16,492	128,84	19,821	154,84	9,333	72,91	66,198	517,14	16	124,99
12,579	98,27	16,055	125,42	44,322	346,25	48,074	375,56	10,154	79,32	10,75	83,98	80,62	629,81	14,667	114,58
17,333	135,41	17,385	135,81	22,667	177,08	29,963	234,07	23,324	182,21	2,667	20,83	96,24	751,83	19,821	154,84
18,95	148,04	9,615	75,11	180,079	1406,79	4,807	37,55	27,195	212,45	9,615	75,11	26,566	207,54	29,242	228,44
16,221	126,72	17,385	135,81	65,551	512,09	18,95	148,04	12,579	98,27	6,799	53,11	48,185	376,42	25,647	200,36
26,7	208,58	20	156,24	131,101	1024,17	34,897	272,62	10,154	79,32	13,132	102,59	54,975	429,47	16	124,99
12,293	96,03	15,549	121,47	31,383	245,17	24,037	187,78	13,597	106,22	13,597	106,22	29,12	227,49	21,082	164,69
20,177	157,62	11,47	89,60	32	249,99	28,127	219,73	23,851	186,33	17,385	135,81	43,594	340,56	18,135	141,67
13,333	104,16	28,783	224,85	378,688	2958,34	15,202	118,76	13,597	106,22	16,055	125,42	62,723	490,00	18,667	145,83
10,667	83,33	12,579	98,27	60,133	469,76	13,92	108,74	15,606	121,92	9,707	75,83	69,538	543,24	25,647	200,36
14,422	112,67	29,12	227,49	44,02	343,89	19,23	150,23	19,686	153,79	25,368	198,18	45,509	355,52	23,851	186,33
7,775	60,74	23,286	181,91	97,224	759,52	15,202	118,76	12,293	96,03	18,667	145,83	62,254	486,33	18,135	141,67
10,154	79,32	17,385	135,81	173,113	1352,37	16,492	128,84	14,907	116,45	24,513	191,50	127,311	994,56	14,667	114,58
12,293	96,03	28	218,74	387,523	3027,36	14,422	112,67	20,177	157,62	16	124,99	57,689	450,67	21,499	167,95
6,667	52,08	14,907	116,45	194,913	1522,67	23,286	181,91	17,385	135,81	20,827	162,70	96,977	757,59	13,597	106,22
14,907	116,45	26,667	208,32	112,293	877,24	13,199	103,11	19,23	150,23	10,667	83,33	134,693	1052,23	17,385	135,81
17,537	137,00	18,95	148,04	109,122	852,47	11,392	89,00	10,414	81,35	17,537	137,00	153,981	1202,91	25,051	195,70
10,75	83,98	16	124,99	197,63	1543,90	14,422	112,67	12	93,74	19,23	150,23	47,14	368,26	16,275	127,14
16,221	126,72	19,821	154,84	112,388	877,98	20	156,24	13,132	102,59	7,775	60,74	79,577	621,66	18,571	145,08
10,667	83,33	22,706	177,38	33,333	260,40	17,075	133,39	28,127	219,73	10,414	81,35	109,366	854,38	16,111	125,86
8,11	63,36	11,926	93,17	68,261	533,26	21,99	171,79	22,071	172,42	13,4	104,68	61,333	479,14	28,032	218,99
22,667	177,08	18,571	145,08	54,406	425,02	16,055	125,42	25,612	200,08	17,385	135,81	88,01	687,54	25,612	200,08
21,99	171,79	14,907	116,45	81,114	633,67	12,293	96,03	34,409	268,81	13,333	104,16	78,949	616,76	21,082	164,69
Average	129,96		131,52		897,22		162,40		137,11		115,22		805,34		155,78
Median	126,72		125,64		610,47		146,93		125,42		105,45		605,61		143,37
SD	**45,93**		**40,00**		**748,94**		**66,25**		**51,19**		**51,65**		**588,48**		**49,10**

SV011 Naïve		SV011 LPSBP		SV011 LPS		SV011 LPS+LPSBP		SV011 Aspirin		SV011 Aspirin +LPSBP		SV011 Aspirin + LPS		Sv011 Aspirin + LPS+ LPSBP	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
14,422	112,67	18,856	147,30	126,842	990,90	24,801	193,75	13,333	104,16	21,375	166,98	95,824	748,58	9,428	73,65
29,515	230,57	19,09	149,13	158,015	1234,42	16,111	125,86	16,865	131,75	5,963	46,58	169,92	1327,43	15,549	121,47
13,4	104,68	12,649	98,81	114,643	895,60	11,392	89,00	14,667	114,58	11,47	89,60	129,443	1011,22	30,696	239,80
11,47	89,60	20,309	158,66	42,353	330,86	10,154	79,32	5,657	44,19	12,293	96,03	81,104	633,59	17,938	140,13
15,085	117,85	10,75	83,98	99,563	777,79	8,11	63,36	17,938	140,13	12,293	96,03	90,165	704,38	15,202	118,76
19,821	154,84	20,396	159,34	112,704	880,45	18,856	147,30	8,11	63,36	26,264	205,18	88,974	695,07	30,434	237,75
9,333	72,91	8,944	69,87	155,703	1216,36	6,667	52,08	17,385	135,81	10,995	85,89	169,711	1325,79	26,998	210,91
16,707	130,52	17,789	138,97	123,274	963,03	16,055	125,42	22,706	177,38	14,174	110,73	106,842	834,66	10,75	83,98
16,275	127,14	14,667	114,58	81,508	636,75	18,95	148,04	9,428	73,65	11,47	89,60	158,044	1234,65	17,537	137,00
16,971	132,58	15,549	121,47	78,712	614,90	16,055	125,42	24,148	188,65	7,775	60,74	86,163	673,11	10,414	81,35
8,537	66,69	18,523	144,70	67,264	525,47	12	93,74	10,75	83,98	11,314	88,39	157,491	1230,33	16,111	125,86
22,706	177,38	11,314	88,39	97,443	761,23	10,414	81,35	25,889	202,25	24,037	187,78	200,204	1564,01	14,907	116,45
13,333	104,16	13,333	104,16	71,616	559,47	8,537	66,69	18,667	145,83	12,293	96,03	92,415	721,95	9,615	75,11
13,132	102,59	14,36	112,18	37,19	290,53	24,037	187,78	13,132	102,59	13,4	104,68	105,494	824,13	13,92	108,74
20,044	156,59	16,221	126,72	50,684	395,95	13,333	104,16	8	62,50	15,202	118,76	176,182	1376,35	16,865	131,75
15,606	121,92	13,132	102,59	165,339	1291,64	10,414	81,35	14,36	112,18	16,492	128,84	333,973	2609,02	16,221	126,72
14,907	116,45	16,111	125,86	46,838	365,90	10,75	83,98	11,392	89,00	19,09	149,13	120,628	942,35	20,044	156,59
16,275	127,14	28,783	224,85	93,333	729,12	16,971	132,58	11,47	89,60	8,537	66,69	88,944	694,84	7,542	58,92
13,132	102,59	17,333	135,41	83,501	652,32	9,333	72,91	9,428	73,65	9,333	72,91	118,088	922,51	21,705	169,56
19,09	149,13	21,375	166,98	69,793	545,23	19,23	150,23	13,333	104,16	25,889	202,25	79,911	624,27	16,055	125,42
12,293	96,03	7,775	60,74	127,513	996,14	19,09	149,13	16,111	125,86	18,523	144,70	169,081	1320,87	18,714	146,20
20	156,24	23,286	181,91	127,36	994,95	14,422	112,67	11,47	89,60	14,174	110,73	215,262	1681,64	14,907	116,45
12,293	96,03	20,044	156,59	54,975	429,47	44,181	345,15	18,523	144,70	14,667	114,58	71,678	559,95	18,856	147,30
16,055	125,42	16,865	131,75	102,84	803,39	10,75	83,98	14,667	114,58	36,025	281,43	157,045	1226,85	20,699	161,70
16,865	131,75	14,727	115,05	60,472	472,41	10,414	81,35	28,032	218,99	20	156,24	138,474	1081,77	18,135	141,67
20,87	163,04	20	156,24	63,246	494,08	20,699	161,70	16,055	125,42	19,821	154,84	142,142	1110,42	9,707	75,83
24,037	187,78	11,926	93,17	276,013	2156,23	12,579	98,27	15,606	121,92	21,333	166,65	198,953	1554,24	18,523	144,70
16,111	125,86	24,513	191,50	160,804	1256,21	16,492	128,84	8,944	69,87	16,492	128,84	101,544	793,27	9,428	73,65
28,032	218,99	26,8	209,36	20,699	161,70	13,199	103,11	20	156,24	15,085	117,85	99,849	780,03	14,907	116,45
18,523	144,70	20,352	158,99	81,867	639,55	14,174	110,73	17,385	135,81	11,392	89,00	60,369	471,61	11,926	93,17
16,111	125,86	16,865	131,75	101,893	796,00	13,333	104,16	13,92	108,74	14,422	112,67	78,768	615,34	10,75	83,98
14,907	116,45	16	124,99	93,114	727,41	13,333	104,16	14,727	115,05	16	124,99	40,617	317,30	16,221	126,72
28	218,74	24,477	191,22	51,294	400,71	19,821	154,84	17,789	138,97	17,385	135,81	105,468	823,92	22,94	179,21
15,202	118,76	12,649	98,81	37,047	289,41	11,392	89,00	13,728	107,24	11,926	93,17	127,01	992,21	21,499	167,95
16	124,99	16,275	127,14	62,254	486,33	19,09	149,13	9,333	72,91	17,385	135,81	136,652	1067,54	20,699	161,70
16,707	130,52	16,221	126,72	92,164	719,99	24,585	192,06	11,47	89,60	18,135	141,67	145,009	1132,82	16	124,99
22,667	177,08	17,385	135,81	49,026	382,99	16,055	125,42	4	31,25	19,414	151,66	216,132	1688,44	25,298	197,63
16,221	126,72	30,463	237,98	33,993	265,56	10,75	83,98	25,333	197,90	16,055	125,42	83,16	649,65	12,074	94,32
21,333	166,65	19,821	154,84	156,915	1225,83	12	93,74	10,414	81,35	8,433	65,88	151,144	1180,75	14,907	116,45
17,938	140,13	8,944	69,87	76,373	596,63	31,213	243,84	8,433	65,88	20,352	158,99	87,686	685,01	17,385	135,81
17,889	139,75	17,333	135,41	51,88	405,29	9,615	75,11	14,667	114,58	18,714	146,20	141,358	1104,30	22,191	173,36
16,707	130,52	28,628	223,64	100,036	781,49	13,92	108,74	13,92	108,74	17,385	135,81	97,37	760,66	18,95	148,04
11,926	93,17	11,392	89,00	109,179	852,91	18,856	147,30	11,314	88,39	10,75	83,98	177,288	1384,99	16,111	125,86
16,055	125,42	9,428	73,65	107,629	840,81	11,314	88,39	15,606	121,92	10,414	81,35	174,239	1361,17	20,396	159,34
21,333	166,65	9,707	75,83	76	593,72	15,202	118,76	14,174	110,73	22,706	177,38	74,679	583,40	14,907	116,45
17,385	135,81	9,615	75,11	124,715	974,28	20,827	162,70	23,017	179,81	20,827	162,70	121,363	948,10	25,889	202,25
19,09	149,13	28,032	218,99	190,834	1490,81	9,428	73,65	11,926	93,17	17,385	135,81	202,776	1584,10	15,549	121,47
15,202	118,76	13,728	107,24	224,063	1750,40	17,385	135,81	19,821	154,84	18,95	148,04	134,091	1047,53	18,135	141,67
18,714	146,20	5,497	42,94	153,426	1198,58	21,499	167,95	10,995	85,89	18,714	146,20	72,197	564,01	20,309	158,66
18,135	141,67	18,856	147,30	118,419	925,10	16,492	128,84	13,92	108,74	13,597	106,22	193,375	1510,66	22,667	177,08
Average	134,74		132,35		775,33		122,54		114,36		125,95		1025,62		135,40
Median	128,83		129,45		728,27		111,70		109,74		125,21		970,15		129,24
SD	33,99		45,46		395,57		50,86		40,66		42,98		414,62		41,00

SV012 Naïve		SV012 LPSBP		SV012 LPS		SV012 LPS+ LPSBP		SV012 Aspirin		SV012 Aspirin +LPSBP		SV012 Aspirin + LPS		SV012 Aspirin + LPSBP+ LPS	
Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)	Length	Scale bar (nm)
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
22,823	178,29	18,667	145,83	77,517	605,57	18,667	145,83	17,385	135,81	12,293	96,03	84,433	659,60	25,473	199,00
5,497	42,94	15,202	118,76	161,421	1261,03	32,111	250,85	12,579	98,27	13,132	102,59	59,524	465,01	18,523	144,70
14,36	112,18	16,707	130,52	66,667	520,81	19,09	149,13	11,47	89,60	19,686	153,79	32,796	256,20	49,351	385,53
14,174	110,73	14,36	112,18	108,033	843,96	13,333	104,16	12,293	96,03	29,273	228,68	42,541	332,33	10,995	85,89
14,174	110,73	5,497	42,94	141,641	1106,51	20	156,24	17,938	140,13	26,833	209,62	68,832	537,72	22,071	172,42
5,497	42,94	10,75	83,98	73,636	575,25	14,907	116,45	12,649	98,81	13,333	104,16	84,042	656,54	14,36	112,18
18,135	141,67	15,202	118,76	86,831	678,33	14,422	112,67	8,537	66,69	10,667	83,33	226,231	1767,33	28,032	218,99
17,537	137,00	17,889	139,75	93,343	729,20	18,856	147,30	26,833	209,62	13,333	104,16	722,943	5647,68	18,667	145,83
5,497	42,94	17,537	137,00	59,584	465,47	13,199	103,11	13,333	104,16	16,221	126,72	276,116	2157,04	13,92	108,74
5,497	42,94	15,202	118,76	65,605	512,51	17,537	137,00	20,87	163,04	13,132	102,59	193,939	1515,07	13,199	103,11
9,615	75,11	14,422	112,67	38,482	300,62	17,938	140,13	8,11	63,36	12,293	96,03	200,018	1562,56	8,537	66,69
12,293	96,03	8	62,50	72,443	565,93	16,971	132,58	14,667	114,58	14,667	114,58	97,479	761,51	11,392	89,00
8,537	66,69	14,667	114,58	130,183	1017,00	13,92	108,74	13,728	107,24	14,727	115,05	184,391	1440,48	21,499	167,95
12,293	96,03	2,667	20,83	158,807	1240,61	19,821	154,84	10,414	81,35	14,422	112,67	242,831	1897,01	17,333	135,41
19,09	149,13	10,995	85,89	181,73	1419,69	17,889	139,75	13,728	107,24	27,487	214,73	127,234	993,96	16,111	125,86
17,938	140,13	13,199	103,11	81,235	634,61	13,92	108,74	13,333	104,16	20,044	156,59	106,667	833,29	18,571	145,08
14,907	116,45	9,615	75,11	112,507	878,91	22,667	177,08	9,707	75,83	9,333	72,91	130,673	1020,83	33,572	262,27
12,293	96,03	8,944	69,87	60,237	470,58	17,789	138,97	14,36	112,18	25,647	200,36	158,717	1239,91	23,851	186,33
12,649	98,81	16,275	127,14	190,181	1485,71	16,055	125,42	9,707	75,83	5,963	46,58	104,452	815,99	16,492	128,84
14,727	115,05	14,907	116,45	65,997	515,57	30,696	239,80	3,771	29,46	18,856	147,30	100,885	788,12	14,727	115,05
20,742	162,04	13,4	104,68	194,136	1516,60	19,821	154,84	13,597	106,22	18,667	145,83	90,755	708,98	16,865	131,75
18,714	146,20	10,414	81,35	483,463	3776,85	11,392	89,00	6,799	53,11	8,433	65,88	233,368	1823,09	25,051	195,70
18,856	147,30	11,926	93,17	225,349	1760,44	16,221	126,72	11,47	89,60	14,667	114,58	75,07	586,45	20,742	162,04
14,667	114,58	8,537	66,69	284,013	2218,73	11,926	93,17	11,926	93,17	9,707	75,83	184,8	1443,67	25,473	199,00
9,707	75,83	18,856	147,30	273,415	2135,94	23,851	186,33	12,074	94,32	11,47	89,60	214,969	1679,35	12,649	98,81
13,728	107,24	17,385	135,81	608	4749,74	14,174	110,73	18,523	144,70	23,589	184,28	188,472	1472,36	19,23	150,23
14,667	114,58	7,18	56,09	230,682	1802,10	13,4	104,68	14,907	116,45	16,275	127,14	281,737	2200,95	17,889	139,75
12,293	96,03	13,92	108,74	289,361	2260,51	16,865	131,75	10,667	83,33	17,333	135,41	239,325	1869,62	13,4	104,68
9,707	75,83	7,775	60,74	173,845	1358,09	17,075	133,39	12,579	98,27	10,75	83,98	311,307	2431,95	20,177	157,62
11,926	93,17	4,807	37,55	87,168	680,96	22,784	177,99	21,333	166,65	24,513	191,50	90,136	704,15	10,75	83,98
13,92	108,74	10,154	79,32	141,421	1104,79	15,202	118,76	16,055	125,42	8,433	65,88	250,667	1958,23	14,727	115,05
5,963	46,58	6,799	53,11	123,389	963,92	10,154	79,32	9,615	75,11	12,293	96,03	173,891	1358,45	14,667	114,58
11,47	89,60	9,333	72,91	42,667	333,32	16,492	128,84	19,23	150,23	18,714	146,20	71,765	560,63	14,667	114,58
10,75	83,98	16,111	125,86	66,68	520,91	22,706	177,38	15,202	118,76	16,055	125,42	84,095	656,96	13,4	104,68
8,433	65,88	8,537	66,69	132,027	1031,40	11,926	93,17	13,333	104,16	13,597	106,22	77,379	604,49	20,044	156,59
23,739	185,45	7,542	58,92	153,339	1197,90	18,95	148,04	10,667	83,33	21,499	167,95	81,726	638,45	17,385	135,81
10,995	85,89	16,707	130,52	144,056	1125,38	26,8	209,36	10,995	85,89	10,154	79,32	141,012	1101,60	30,696	239,80
14,727	115,05	6,667	52,08	123,259	962,91	21,705	169,56	8	62,50	14,907	116,45	57,889	452,23	28,844	225,33
16,221	126,72	21,705	169,56	220,488	1722,47	15,549	121,47	12,074	94,32	9,428	73,65	173,564	1355,89	22,667	177,08
17,537	137,00	6,799	53,11	193,039	1508,03	14,667	114,58	10,995	85,89	12	93,74	83,48	652,15	34,998	273,41
13,92	108,74	10,75	83,98	251,463	1964,45	16	124,99	17,938	140,13	12,074	94,32	87,818	686,04	19,686	153,79
13,333	104,16	13,132	102,59	190,709	1489,83	18,714	146,20	18,135	141,67	21,082	164,69	112,23	876,75	26,566	207,54
10,667	83,33	12,649	98,81	78,768	615,34	19,23	150,23	20	156,24	10,75	83,98	189,037	1476,77	12,293	96,03
9,428	73,65	10,154	79,32	117,523	918,10	10,667	83,33	16,221	126,72	21,333	166,65	95,796	748,37	12,579	98,27
11,926	93,17	14,667	114,58	91,758	716,82	20,309	158,66	14,422	112,67	20	156,24	167,199	1306,17	11,47	89,60
12,293	96,03	14,907	116,45	287,487	2245,87	15,606	121,92	14,907	116,45	18,135	141,67	144,8	1131,19	17,385	135,81
21,333	166,65	11,926	93,17	354,195	2767,00	15,202	118,76	20,309	158,66	12	93,74	225,396	1760,81	19,09	149,13
16	124,99	13,199	103,11	428,299	3345,90	16,492	128,84	10,75	83,98	11,926	93,17	221,333	1729,07	13,597	106,22
13,333	104,16	5,497	42,94	236,241	1845,53	13,199	103,11	17,537	137,00	5,333	41,66	120,104	938,26	20,044	156,59
17,385	135,81	7,18	56,09	60,369	471,61	19,09	149,13	15,202	118,76	14,667	114,58	83,149	649,57	12,074	94,32
Average	105,61		93,64		1298,79		136,86		107,94		120,48		1218,22		149,33
Median	105,70		95,99		1068,10		132,16		104,16		113,62		1007,39		137,78
SD	**34,72**		**33,64**		**911,06**		**35,52**		**33,22**		**43,77**		**842,59**		**58,50**

SV017 Naïve		SV017 LPSBP		SV017 LPS		SV017 LPSBP + LPS		SV017 Aspirin		SV017 Aspirin + LPSBP		SV017 Aspirin + LPS		SV017 Aspirin + LPSBP + LPS	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
25,65	200,36	8,94	69,87	502,82	3928,08	16,49	128,84	14,36	112,18	13,60	106,22	199,88	1561,51	13,40	104,68
16,22	126,72	11,47	89,60	496,12	3875,69	9,43	73,65	13,92	108,74	12,58	98,27	285,20	2228,02	13,33	104,16
5,96	46,58	10,75	83,98	340,00	2656,13	8,94	69,87	25,61	200,08	11,93	93,17	70,26	548,90	10,41	81,35
8,94	69,87	14,17	110,73	117,31	916,44	10,41	81,35	20,83	162,70	12,07	94,32	292,71	2286,66	17,54	137,00
11,39	89,00	9,62	75,11	200,84	1569,00	5,33	41,66	10,75	83,98	16,22	126,72	144,31	1127,34	7,54	58,92
17,39	135,81	15,20	118,76	52,29	408,49	12,00	93,74	13,33	104,16	11,93	93,17	204,10	1594,44	5,96	46,58
14,36	112,18	19,23	150,23	46,69	364,71	10,75	83,98	5,33	41,66	14,73	115,05	72,75	568,32	12,65	98,81
14,67	114,58	9,33	72,91	57,35	448,01	8,94	69,87	13,60	106,22	10,75	83,98	197,16	1540,21	16,06	125,42
5,96	46,58	20,00	156,24	58,73	458,78	13,73	107,24	16,71	130,52	11,31	88,39	42,85	334,78	9,43	73,65
13,92	108,74	13,13	102,59	49,41	385,96	18,95	148,04	11,31	88,39	17,54	137,00	46,44	362,78	19,41	151,66
7,18	56,09	11,00	85,89	32,03	250,21	8,00	62,50	13,60	106,22	16,87	131,75	86,92	679,05	13,13	102,59
13,40	104,68	10,75	83,98	308,01	2406,21	20,00	156,24	15,09	117,85	16,06	125,42	84,55	660,50	13,33	104,16
8,00	62,50	10,15	79,32	115,97	905,96	10,41	81,35	13,73	107,24	7,54	58,92	38,67	302,07	20,40	159,34
9,43	73,65	14,36	112,18	260,01	2031,25	14,91	116,45	16,28	127,14	18,86	147,30	301,34	2354,06	11,00	85,89
13,33	104,16	8,11	63,36	300,58	2348,15	8,43	65,88	14,91	116,45	17,39	135,81	177,41	1385,97	17,79	138,97
8,94	69,87	8,94	69,87	238,67	1864,51	9,43	73,65	16,06	125,42	20,00	156,24	177,13	1383,78	18,86	147,30
9,33	72,91	16,00	124,99	202,95	1585,44	15,20	118,76	16,87	131,75	13,92	108,74	184,94	1444,80	9,33	72,91
9,43	73,65	12,00	93,74	98,70	771,08	12,00	93,74	15,55	121,47	10,75	83,98	313,05	2445,55	18,86	147,30
9,62	75,11	9,62	75,11	185,92	1452,44	11,00	85,89	16,22	126,72	9,43	73,65	54,41	425,02	11,47	89,60
9,71	75,83	6,67	52,08	51,23	400,17	11,39	89,00	17,89	139,75	12,29	96,03	207,46	1620,70	19,41	151,66
14,67	114,58	6,80	53,11	65,97	515,36	8,43	65,88	14,36	112,18	12,00	93,74	140,41	1096,91	15,55	121,47
9,71	75,83	8,43	65,88	199,92	1561,79	10,75	83,98	18,14	141,67	19,41	151,66	41,42	323,57	15,20	118,76
15,55	121,47	14,67	114,58	120,60	942,13	10,67	83,33	12,29	96,03	20,83	162,70	102,75	802,65	14,73	115,05
16,28	127,14	14,17	110,73	131,56	1027,77	11,31	88,39	16,71	130,52	24,00	187,49	41,33	322,90	16,71	130,52
17,39	135,81	12,07	94,32	155,28	1213,02	15,20	118,76	8,00	62,50	12,29	96,03	113,53	886,90	17,89	139,75
15,20	118,76	13,33	104,16	169,34	1322,89	11,00	85,89	14,17	110,73	18,57	145,08	52,61	411,01	26,67	208,32
16,22	126,72	9,62	75,11	96,08	750,61	12,00	93,74	10,41	81,35	11,00	85,89	77,33	604,13	12,00	93,74
21,38	166,98	12,29	96,03	65,02	507,94	17,33	135,41	9,43	73,65	16,06	125,42	422,02	3296,82	14,36	112,18
10,75	83,98	13,33	104,16	53,68	419,37	16,11	125,86	14,91	116,45	15,61	121,92	85,77	670,04	18,14	141,67
4,81	37,55	11,39	89,00	78,03	609,58	13,60	106,22	28,03	218,99	20,00	156,24	95,79	748,30	12,58	98,27
7,18	56,09	7,18	56,09	65,12	508,68	8,54	66,69	16,11	125,86	8,54	66,69	175,84	1373,70	11,39	89,00
16,06	125,42	12,29	96,03	34,77	271,62	16,71	130,52	9,62	75,11	14,36	112,18	52,83	412,72	11,00	85,89
12,29	96,03	13,13	102,59	518,67	4051,87	7,78	60,74	16,49	128,84	13,73	107,24	65,59	512,40	15,61	121,92
17,89	139,75	15,20	118,76	213,35	1666,71	8,11	63,36	32,03	250,21	13,33	104,16	148,22	1157,87	18,71	146,20
14,67	114,58	12,58	98,27	114,15	891,78	10,41	81,35	13,60	106,22	12,00	93,74	215,89	1686,51	14,36	112,18
15,20	118,76	8,43	65,88	162,45	1269,09	10,67	83,33	13,60	106,22	12,58	98,27	106,42	831,33	12,58	98,27
4,00	31,25	10,15	79,32	187,64	1465,86	10,75	83,98	13,20	103,11	13,33	104,16	163,85	1279,99	11,31	88,39
11,00	85,89	14,73	115,05	64,06	500,41	11,39	89,00	19,82	154,84	8,94	69,87	144,91	1132,06	5,66	44,19
6,67	52,08	12,29	96,03	257,35	2010,41	11,47	89,60	14,73	115,05	17,08	133,39	335,71	2622,58	17,54	137,00
11,47	89,60	17,33	135,41	165,38	1291,98	16,22	126,72	11,39	89,00	18,86	147,30	211,16	1649,63	12,07	94,32
9,43	73,65	12,00	93,74	762,70	5958,24	14,17	110,73	9,43	73,65	12,65	98,81	197,63	1543,87	15,61	121,92
8,43	65,88	21,54	168,28	669,34	5228,89	10,41	81,35	11,93	93,17	8,11	63,36	155,00	1210,87	12,29	96,03
9,43	73,65	8,54	66,69	90,17	704,38	10,15	79,32	11,31	88,39	13,92	108,74	76,27	595,82	12,00	93,74
13,33	104,16	8,94	69,87	143,61	1121,90	13,92	108,74	14,73	115,05	8,94	69,87	65,61	512,51	14,36	112,18
18,71	146,20	17,33	135,41	235,05	1836,19	11,93	93,17	12,00	93,74	16,22	126,72	102,68	802,10	13,33	104,16
9,71	75,83	15,09	117,85	42,10	328,89	14,73	115,05	6,67	52,08	10,75	83,98	34,28	267,80	10,41	81,35
14,42	112,67	21,38	166,98	38,83	303,32	10,75	83,98	6,67	52,08	12,65	98,81	174,57	1363,71	10,67	83,33
10,41	81,35	12,29	96,03	694,67	5426,83	13,13	102,59	11,39	89,00	17,79	138,97	194,65	1520,64	10,67	83,33
17,08	133,39	12,65	98,81	244,00	1906,18	6,67	52,08	13,33	104,16	11,47	89,60	58,45	456,65	12,65	98,81
16,49	128,84	16,87	131,75	333,33	2604,02	14,91	116,45	13,40	104,68	17,08	133,39	46,82	365,75	12,29	96,03
Average	96,66		97,73		1544,89		92,96		112,46		110,59		1106,32		109,00
Median	92,82		96,03		1167,46		87,14		107,99		105,19		991,90		104,16
SD	**34,72**		**28,25**		**1391,06**		**24,84**		**38,03**		**28,87**		**705,59**		**30,66**

SV018 naïve		SV018 LPSBP		SV018 LPS		SV018 LPSBP+ LPS		SV018 Aspirin		SV018 Aspirin + LPSBP		SV018 Aspirin + LPS		SV018 Aspirin + LPs + LPSBP	
Length	Scale bar nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
18,14	141,67	17,79	138,97	162,93	1272,85	20,31	158,66	18,52	144,70	17,39	135,81	168,68	1317,71	21,71	169,56
10,67	83,33	22,67	177,08	144,40	1128,02	26,26	205,18	11,93	93,17	13,60	106,22	21,50	167,95	9,71	75,83
23,63	184,58	17,39	135,81	123,14	962,01	20,83	162,70	15,20	118,76	22,67	177,08	42,35	330,86	6,67	52,08
9,43	73,65	16,06	125,42	30,70	239,80	33,41	261,02	11,47	89,60	18,86	147,30	20,70	161,70	10,41	81,35
12,58	98,27	15,09	117,85	23,59	184,28	20,00	156,24	13,13	102,59	20,35	158,99	125,68	981,82	7,54	58,92
20,04	156,59	11,47	89,60	28,63	223,64	21,71	169,56	21,38	166,98	9,62	75,11	25,37	198,18	9,71	75,83
14,91	116,45	9,71	75,83	34,69	271,02	33,33	260,40	13,92	108,74	17,39	135,81	29,27	228,68	16,06	125,42
12,65	98,81	18,67	145,83	35,20	274,99	31,69	247,59	14,73	115,05	13,33	104,16	21,71	169,56	17,89	139,75
16,11	125,86	9,71	75,83	25,61	200,08	12,29	96,03	10,15	79,32	14,17	110,73	24,15	188,65	10,67	83,33
17,33	135,41	6,80	53,11	28,13	219,73	13,40	104,68	7,54	58,92	18,14	141,67	30,93	241,60	14,73	115,05
24,04	187,78	5,33	41,66	351,95	2749,47	18,86	147,30	11,00	85,89	11,00	85,89	24,80	193,75	23,74	185,45
7,18	56,09	10,75	83,98	258,89	2022,44	29,12	227,49	8,54	66,69	18,86	147,30	40,49	316,28	6,80	53,11
13,33	104,16	9,62	75,11	21,99	171,79	17,94	140,13	13,92	108,74	15,09	117,85	30,23	236,15	5,96	46,58
10,41	81,35	23,63	184,58	46,67	364,57	23,63	184,58	9,62	75,11	16,06	125,42	37,36	291,84	20,87	163,04
18,95	148,04	20,00	156,24	40,79	318,67	15,09	117,85	9,62	75,11	10,41	81,35	23,29	181,91	5,96	46,58
12,58	98,27	10,75	83,98	52,15	407,43	16,22	126,72	19,09	149,13	13,73	107,24	26,70	208,58	11,93	93,17
11,93	93,17	11,00	85,89	462,67	3614,40	22,82	178,29	22,71	177,38	16,49	128,84	28,00	218,74	19,41	151,66
16,06	125,42	13,92	108,74	644,01	5031,02	20,00	156,24	13,40	104,68	11,00	85,89	38,69	302,25	12,58	98,27
15,20	118,76	11,39	89,00	686,69	5364,45	10,15	79,32	12,29	96,03	13,20	103,11	33,99	265,56	14,91	116,45
17,33	135,41	12,58	98,27	336,02	2625,04	13,33	104,16	13,92	108,74	29,36	229,39	44,82	350,14	9,33	72,91
16,22	126,72	18,67	145,83	61,46	480,16	12,58	98,27	11,47	89,60	14,67	114,58	21,50	167,95	11,47	89,60
11,31	88,39	16,06	125,42	50,67	395,81	22,19	173,36	20,31	158,66	7,18	56,09	32,80	256,20	7,78	60,74
12,29	96,03	14,17	110,73	43,04	336,23	19,23	150,23	17,33	135,41	9,43	73,65	27,00	210,91	7,18	56,09
21,54	168,28	18,86	147,30	36,78	287,34	10,41	81,35	8,11	63,36	10,41	81,35	96,37	752,85	20,18	157,62
8,94	69,87	11,47	89,60	17,54	137,00	17,08	133,39	12,29	96,03	19,41	151,66	105,87	827,08	15,61	121,92
21,87	170,83	8,94	69,87	31,81	248,46	14,91	116,45	15,20	118,76	11,31	88,39	24,04	187,78	23,29	181,91
17,79	138,97	23,74	185,45	126,84	990,90	15,09	117,85	13,33	104,16	12,29	96,03	14,36	112,18	8,94	69,87
18,95	148,04	13,33	104,16	422,69	3302,05	18,14	141,67	15,55	121,47	9,62	75,11	49,78	388,90	9,62	75,11
19,23	150,23	12,58	98,27	106,75	833,94	16,71	130,52	11,47	89,60	21,08	164,69	44,82	350,14	11,47	89,60
20,00	156,24	16,06	125,42	52,02	406,36	16,06	125,42	17,94	140,13	23,32	182,21	58,80	459,37	16,22	126,72
13,33	104,16	10,75	83,98	133,36	1041,82	9,62	75,11	14,42	112,67	17,79	138,97	110,67	864,54	17,54	137,00
17,39	135,81	9,62	75,11	224,00	1749,94	16,06	125,42	8,94	69,87	19,41	151,66	30,23	236,15	19,09	149,13
15,20	118,76	26,97	210,65	258,99	2023,22	20,04	156,59	16,11	125,86	22,19	173,36	29,24	228,44	18,67	145,83
10,41	81,35	14,17	110,73	434,70	3395,87	10,75	83,98	22,78	177,99	9,62	75,11	22,82	178,29	8,11	63,36
18,14	141,67	18,86	147,30	240,91	1881,98	18,86	147,30	13,60	106,22	17,54	137,00	30,78	240,47	9,33	72,91
24,33	190,08	24,80	193,75	229,40	1792,05	10,15	79,32	12,29	96,03	20,31	158,66	35,23	275,19	14,73	115,05
14,91	116,45	19,09	149,13	297,34	2322,81	9,43	73,65	10,67	83,33	18,14	141,67	307,45	2401,81	11,47	89,60
14,36	112,18	17,89	139,75	340,44	2659,56	11,39	89,00	16,22	126,72	11,47	89,60	217,64	1700,22	8,11	63,36
10,41	81,35	20,83	162,70	326,67	2551,95	8,94	69,87	21,87	170,83	21,71	169,56	40,55	316,80	11,93	93,17
16,22	126,72	13,33	104,16	162,67	1270,81	13,20	103,11	18,95	148,04	15,20	118,76	69,15	540,24	10,75	83,98
15,09	117,85	19,09	149,13	47,18	368,56	8,43	65,88	21,54	168,28	13,33	104,16	62,25	486,33	13,92	108,74
19,23	150,23	15,09	117,85	77,90	608,52	11,47	89,60	12,65	98,81	7,18	56,09	24,59	192,06	13,92	108,74
18,52	144,70	11,47	89,60	73,96	577,79	13,92	108,74	6,67	52,08	18,14	141,67	25,47	199,00	18,71	146,20
7,54	58,92	17,79	138,97	97,34	760,44	10,41	81,35	8,00	62,50	9,62	75,11	35,78	279,49	6,67	52,08
18,14	141,67	17,94	140,13	43,68	341,20	12,65	98,81	13,60	106,22	19,23	150,23	33,99	265,56	18,86	147,30
12,07	94,32	13,33	104,16	155,64	1215,83	11,00	85,89	10,75	83,98	14,17	110,73	15,61	121,92	8,54	66,69
14,36	112,18	18,67	145,83	172,54	1347,87	9,43	73,65	5,50	42,94	10,75	83,98	24,15	188,65	8,00	62,50
16,22	126,72	16,11	125,86	96,23	751,76	23,02	179,81	11,00	85,89	21,08	164,69	29,81	232,91	20,18	157,62
13,92	108,74	14,91	116,45	282,96	2210,50	8,11	63,36	14,91	116,45	17,89	139,75	9,43	73,65	14,67	114,58
20,00	156,24	12,58	98,27	274,70	2145,95	12,29	96,03	10,75	83,98	29,27	228,68	10,41	81,35	13,40	104,68
Average	**121,94**		**118,97**		**1321,65**		**129,98**		**107,23**		**123,97**		**387,37**		**102,32**
Median	**122,09**		**117,15**		**897,97**		**121,63**		**104,42**		**122,09**		**238,31**		**93,17**
SD	**32,73**		**37,21**		**1264,91**		**50,65**		**33,65**		**40,08**		**424,64**		**39,12**

SV019 naïve		SV019 LPSBP		SV019 LPS		SV019 LPSBP+ LPS		SV019 Aspirin		SV019 Aspirin + LPSBP		SV019 Aspirin + LPS		SV019 Aspirin + LPSBP+LPS	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
8,54	66,69	18,86	147,30	137,34	1072,91	12,07	94,32	21,50	167,95	14,67	114,58	75,85	592,53	17,39	135,81
7,78	60,74	8,54	66,69	116,27	908,29	23,29	181,91	22,71	177,38	16,06	125,42	269,66	2106,63	16,06	125,42
6,67	52,08	15,20	118,76	105,00	820,23	16,00	124,99	10,75	83,98	12,29	96,03	150,67	1177,07	39,64	309,69
14,36	112,18	7,78	60,74	156,84	1225,25	10,67	83,33	13,20	103,11	11,47	89,60	44,56	348,12	25,75	201,17
32,06	250,42	8,00	62,50	127,03	992,38	1,89	14,73	10,41	81,35	13,13	102,59	205,81	1607,76	12,29	96,03
21,54	168,28	9,33	72,91	108,46	847,30	8,94	69,87	6,67	52,08	10,75	83,98	86,59	676,41	18,71	146,20
23,59	184,28	13,33	104,16	158,47	1237,94	14,67	114,58	26,67	208,32	22,78	177,99	85,34	666,71	28,28	220,96
16,11	125,86	11,00	85,89	153,51	1199,25	9,62	75,11	36,39	284,30	11,93	93,17	500,18	3907,43	34,67	270,82
16,71	130,52	13,92	108,74	89,44	698,74	11,31	88,39	8,43	65,88	16,06	125,42	161,42	1261,03	20,04	156,59
12,58	98,27	5,50	42,94	170,54	1332,29	10,75	83,98	19,09	149,13	23,74	185,45	101,47	792,72	17,33	135,41
24,51	191,50	15,61	121,92	179,38	1401,35	10,75	83,98	18,86	147,30	12,65	98,81	55,12	430,60	9,62	75,11
16,97	132,58	11,31	88,39	183,42	1432,92	14,91	116,45	4,81	37,55	13,33	104,16	98,99	773,32	11,93	93,17
18,86	147,30	10,75	83,98	165,65	1294,03	16,49	128,84	6,67	52,08	17,79	138,97	85,84	670,60	34,18	266,99
17,39	135,81	13,13	102,59	104,35	815,19	8,11	63,36	21,08	164,69	16,22	126,72	110,50	863,22	20,74	162,04
21,71	169,56	15,61	121,92	78,98	617,01	8,11	63,36	17,89	139,75	13,33	104,16	122,73	958,79	11,93	93,17
23,29	181,91	14,42	112,67	52,75	412,06	12,07	94,32	15,20	118,76	20,04	156,59	79,25	619,12	13,20	103,11
29,81	232,91	13,13	102,59	106,27	830,22	11,31	88,39	17,33	135,41	12,29	96,03	85,38	666,96	23,29	181,91
18,57	145,08	8,11	63,36	164,89	1288,10	9,43	73,65	12,58	98,27	11,39	89,00	96,00	749,96	15,55	121,47
21,08	164,69	17,39	135,81	208,62	1629,78	8,43	65,88	18,14	141,67	11,39	89,00	53,68	419,37	17,33	135,41
17,33	135,41	13,40	104,68	147,18	1149,79	12,29	96,03	16,11	125,86	25,75	201,17	201,98	1577,89	15,61	121,92
13,92	108,74	8,94	69,87	119,57	934,09	12,00	93,74	9,71	75,83	32,25	251,93	269,32	2103,97	13,73	107,24
16,06	125,42	17,39	135,81	104,21	814,12	20,70	161,70	16,49	128,84	22,82	178,29	343,49	2683,33	14,91	116,45
7,18	56,09	10,41	81,35	118,13	922,87	9,71	75,83	12,07	94,32	8,94	69,87	253,02	1976,59	14,17	110,73
20,00	156,24	13,73	107,24	212,00	1656,16	12,65	98,81	16,00	124,99	4,81	37,55	309,62	2418,78	33,99	265,56
13,60	106,22	14,17	110,73	126,19	985,80	10,75	83,98	22,67	177,08	22,63	176,76	61,35	479,26	22,71	177,38
8,43	65,88	18,86	147,30	106,74	833,88	16,00	124,99	16,71	130,52	18,71	146,20	185,03	1445,48	34,77	271,62
12,07	94,32	14,36	112,18	179,09	1399,06	10,75	83,98	15,61	121,92	27,49	214,73	157,11	1227,38	20,83	162,70
14,36	112,18	15,20	118,76	212,89	1663,09	13,40	104,68	20,04	156,59	16,06	125,42	134,25	1048,77	62,72	490,00
12,58	98,27	13,40	104,68	189,50	1480,40	10,41	81,35	22,78	177,99	20,04	156,59	87,81	685,96	22,71	177,38
19,09	149,13	12,29	96,03	106,75	833,94	19,23	150,23	23,74	185,45	20,83	162,70	347,17	2712,11	31,81	248,46
22,35	174,61	16,06	125,42	130,23	1017,37	7,18	56,09	14,67	114,58	20,04	156,59	104,01	812,53	21,71	169,56
30,23	236,15	10,41	81,35	64,01	500,08	3,77	29,46	21,71	169,56	13,92	108,74	81,51	636,75	17,94	140,13
7,18	56,09	28,00	218,74	195,16	1524,64	12,65	98,81	20,18	157,62	12,65	98,81	207,22	1618,83	22,67	177,08
9,71	75,83	15,61	121,92	87,81	685,96	12,65	98,81	16,97	132,58	11,93	93,17	236,00	1843,65	31,24	244,06
13,40	104,68	21,99	171,79	61,39	479,59	18,52	144,70	13,92	108,74	21,38	166,98	91,23	712,73	34,90	272,62
16,71	130,52	17,79	138,97	169,59	1324,85	10,75	83,98	20,87	163,04	8,43	65,88	107,67	841,13	6,67	52,08
10,41	81,35	9,43	73,65	226,77	1771,50	9,43	73,65	12,07	94,32	13,60	106,22	66,31	517,99	105,03	820,49
14,91	116,45	13,13	102,59	76,27	595,82	13,92	108,74	8,11	63,36	11,00	85,89	44,70	349,21	119,05	930,01
12,00	93,74	15,20	118,76	77,57	606,01	6,80	53,11	16,28	127,14	17,54	137,00	105,84	826,81	70,57	551,27
20,70	161,70	18,67	145,83	32,93	257,26	14,91	116,45	15,61	121,92	15,20	118,76	69,77	545,03	49,50	386,66
12,65	98,81	14,73	115,05	29,61	231,28	13,13	102,59	38,69	302,25	17,33	135,41	249,88	1952,07	35,00	273,41
10,15	79,32	11,93	93,17	22,78	177,99	11,00	85,89	17,54	137,00	9,33	72,91	91,39	713,94	5,96	46,58
6,67	52,08	16,00	124,99	17,89	139,75	17,79	138,97	18,86	147,30	17,39	135,81	81,10	633,59	16,06	125,42
13,60	106,22	17,39	135,81	170,36	1330,86	9,43	73,65	18,14	141,67	20,87	163,04	90,82	709,52	28,25	220,71
24,04	187,78	13,60	106,22	114,18	891,96	10,75	83,98	18,71	146,20	19,09	149,13	127,12	993,09	32,99	257,68
13,13	102,59	17,39	135,81	57,40	448,37	13,33	104,16	12,65	98,81	17,08	133,39	174,99	1367,05	24,04	187,78
17,94	140,13	13,33	104,16	86,21	673,51	11,00	85,89	11,31	88,39	18,67	145,83	100,32	783,70	13,33	104,16
11,31	88,39	11,47	89,60	59,06	461,37	18,14	141,67	5,50	42,94	27,49	214,73	148,89	1163,11	11,93	93,17
9,33	72,91	4,81	37,55	99,56	777,79	10,75	83,98	11,00	85,89	34,67	270,82	201,34	1572,87	37,90	296,08
11,00	85,89	8,11	63,36	65,35	510,50	12,29	96,03	12,58	98,27	19,09	149,13	121,36	948,10	16,00	124,99
Average	**124,08**		**105,94**		**942,70**		**94,51**		**129,00**		**132,54**		**1143,79**		**215,08**
Median	**114,32**		**105,45**		**900,13**		**88,39**		**127,99**		**126,07**		**833,97**		**166,13**
SD	**48,77**		**32,91**		**421,13**		**31,10**		**52,10**		**47,36**		**728,96**		**169,76**

SV020 Naïve		SV020 LPSBP		SV020 LPS		SV020 LPSBP + LPS		SV020 Aspirin		SV020 Aspirin + LPSBP		SV020 Aspirin + LPS		SV020 Aspirin + LPS + LPSBP	
Length	Scal bar nm	Length	Scal bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
13,33	104,16	16,28	127,14	39,03	304,93	19,69	153,79	20,00	156,24	18,52	144,70	22,71	177,38	11,93	93,17
12,00	93,74	5,66	44,19	13,60	106,22	11,47	89,60	11,47	89,60	13,73	107,24	15,20	118,76	21,33	166,65
13,33	104,16	24,15	188,65	17,89	139,75	24,15	188,65	16,06	125,42	4,81	37,55	24,04	187,78	21,08	164,69
12,00	93,74	32,28	252,15	23,85	186,33	20,00	156,24	20,70	161,70	6,67	52,08	50,60	395,26	18,52	144,70
15,20	118,76	20,04	156,59	166,70	1302,30	23,59	184,28	18,86	147,30	17,79	138,97	20,87	163,04	21,54	168,28
18,52	144,70	11,93	93,17	16,71	130,52	45,35	354,30	11,47	89,60	18,52	144,70	18,86	147,30	12,29	96,03
18,86	147,30	16,06	125,42	27,75	216,75	28,03	218,99	16,00	124,99	17,39	135,81	11,93	93,17	14,36	112,18
10,75	83,98	12,00	93,74	44,02	343,89	18,52	144,70	9,33	72,91	20,18	157,62	117,29	916,26	14,91	116,45
21,08	164,69	13,40	104,68	13,13	102,59	23,32	182,21	11,47	89,60	19,41	151,66	20,70	161,70	13,33	104,16
16,11	125,86	9,71	75,83	179,38	1401,35	15,61	121,92	14,67	114,58	14,42	112,67	110,35	862,09	10,75	83,98
17,33	135,41	11,00	85,89	104,00	812,46	24,00	187,49	26,30	205,43	7,78	60,74	87,72	685,25	16,28	127,14
16,22	126,72	16,97	132,58	17,39	135,81	21,71	169,56	16,06	125,42	11,31	88,39	30,08	234,99	20,87	163,04
27,75	216,75	6,67	52,08	24,51	191,50	17,33	135,41	17,89	139,75	18,86	147,30	41,07	320,87	11,00	85,89
20,70	161,70	12,07	94,32	24,04	187,78	21,08	164,69	22,63	176,76	17,33	135,41	38,87	303,68	11,39	89,00
11,39	89,00	20,70	161,70	24,04	187,78	17,33	135,41	23,63	184,58	15,20	118,76	36,88	288,09	16,71	130,52
16,97	132,58	18,14	141,67	33,33	260,40	21,50	167,95	13,33	104,16	11,39	89,00	31,47	245,83	20,87	163,04
16,06	125,42	12,29	96,03	209,68	1638,01	16,06	125,42	12,29	96,03	18,86	147,30	53,13	415,08	15,20	118,76
8,00	62,50	13,33	104,16	50,68	395,95	12,00	93,74	14,17	110,73	16,28	127,14	27,49	214,73	22,35	174,61
17,33	135,41	12,58	98,27	69,45	542,54	21,54	168,28	11,47	89,60	9,43	73,65	63,81	498,45	29,61	231,28
19,09	149,13	10,75	83,98	21,33	166,65	27,46	214,48	14,73	115,05	16,06	125,42	28,28	220,96	13,40	104,68
15,20	118,76	13,33	104,16	6,67	52,08	22,71	177,38	14,91	116,45	19,82	154,84	26,70	208,58	24,04	187,78
12,07	94,32	15,20	118,76	19,69	153,79	17,39	135,81	16,87	131,75	16,11	125,86	54,68	427,19	13,92	108,74
16,00	124,99	20,40	159,34	21,50	167,95	12,07	94,32	18,57	145,08	22,35	174,61	29,52	230,57	8,94	69,87
13,60	106,22	14,73	115,05	30,67	239,57	21,08	164,69	25,33	197,90	16,49	128,84	20,35	158,99	23,29	181,91
4,81	37,55	17,54	137,00	114,40	893,66	14,17	110,73	29,52	230,57	16,49	128,84	41,87	327,08	10,15	79,32
12,29	96,03	21,71	169,56	153,91	1202,37	19,09	149,13	22,07	172,42	9,71	75,83	61,39	479,59	12,00	93,74
13,40	104,68	12,07	94,32	108,80	849,91	10,15	79,32	20,00	156,24	17,94	140,13	60,24	470,58	8,94	69,87
23,74	185,45	18,52	144,70	120,12	938,37	34,69	271,02	18,71	146,20	12,00	93,74	70,68	552,15	10,75	83,98
19,23	150,23	22,19	173,36	113,40	885,92	23,02	179,81	25,37	198,18	11,00	85,89	132,49	1035,03	10,15	79,32
9,33	72,91	21,08	164,69	73,38	573,27	28,00	218,74	11,39	89,00	23,32	182,21	29,36	229,39	19,69	153,79
28,78	224,85	16,28	127,14	120,60	942,13	31,47	245,83	17,08	133,39	17,33	135,41	48,09	375,71	12,07	94,32
12,29	96,03	12,65	98,81	106,07	828,65	18,71	146,20	16,06	125,42	15,20	118,76	172,33	1346,25	22,82	178,29
12,07	94,32	8,43	65,88	28,28	220,96	28,63	223,64	12,58	98,27	8,43	65,88	109,95	858,93	16,71	130,52
16,28	127,14	18,14	141,67	20,83	162,70	26,26	205,18	13,40	104,68	4,81	37,55	21,33	166,65	16,06	125,42
14,91	116,45	18,14	141,67	15,55	121,47	19,69	153,79	18,52	144,70	20,31	158,66	77,33	604,13	20,35	158,99
18,71	146,20	13,13	102,59	21,38	166,98	14,17	110,73	13,60	106,22	12,29	96,03	177,17	1384,09	19,09	149,13
13,13	102,59	9,62	75,11	36,10	282,01	29,24	228,44	7,18	56,09	5,66	44,19	75,25	587,84	19,82	154,84
18,14	141,67	12,07	94,32	22,67	177,08	7,78	60,74	16,87	131,75	13,13	102,59	123,71	966,40	12,65	98,81
19,23	150,23	9,62	75,11	51,93	405,70	17,33	135,41	29,96	234,07	12,29	96,03	28,00	218,74	21,71	169,56
13,40	104,68	12,58	98,27	17,08	133,39	18,52	144,70	16,00	124,99	13,33	104,16	109,25	853,48	17,33	135,41
8,00	62,50	17,54	137,00	15,09	117,85	11,47	89,60	19,09	149,13	14,36	112,18	132,65	1036,29	12,00	93,74
17,54	137,00	6,67	52,08	19,41	151,66	16,06	125,42	21,38	166,98	12,07	94,32	142,52	1113,35	16,00	124,99
19,41	151,66	9,62	75,11	19,82	154,84	20,00	156,24	18,67	145,83	23,59	184,28	111,65	872,22	25,47	199,00
11,00	85,89	22,78	177,99	19,69	153,79	19,69	153,79	13,60	106,22	22,19	173,36	102,26	798,85	16,87	131,75
13,92	108,74	8,94	69,87	32,03	250,21	16,97	132,58	17,39	135,81	7,78	60,74	31,01	242,28	16,11	125,86
21,33	166,65	14,42	112,67	70,98	554,50	11,47	89,60	19,41	151,66	11,93	93,17	72,65	567,55	12,65	98,81
17,08	133,39	11,93	93,17	19,69	153,79	18,86	147,30	10,15	79,32	21,33	166,65	113,20	884,33	16,06	125,42
11,93	93,17	29,61	231,28	32,03	250,21	20,04	156,59	6,80	53,11	21,71	169,56	97,33	760,37	14,67	114,58
17,54	137,00	28,25	220,71	23,29	181,91	16,87	131,75	13,13	102,59	12,58	98,27	46,67	364,57	12,00	93,74
21,08	164,69	17,08	133,39	71,28	556,84	14,36	112,18	7,78	60,74	11,31	88,39	184,02	1437,57	8,54	66,69
Average	123,03		120,34		413,54		157,76		130,29		115,74		524,19		126,33
Median	125,21		108,67		218,85		153,79		125,42		118,76		405,17		125,21
SD	**36,44**		**45,43**		**389,08**		**52,96**		**41,78**		**38,81**		**365,43**		**38,26**

SV0024 naïve		SV024 LPSBP		SV024 LPS		SV024 LPSBP+ LPS		SV024 Aspirin		SV024 Aspirin + LPSBP		SV024 Aspirin + LPS		SV024 Aspirin + LPSBP+ LPS	
Length	Scale bar nm	Length	Scale bar nm	Lentgh	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
16,28	127,14	26,97	210,65	272,47	2128,56	12,58	98,27	17,94	140,13	13,13	102,59	29,61	231,28	20,00	156,24
13,13	102,59	16,28	127,14	326,67	2551,95	11,31	88,39	8,94	69,87	14,73	115,05	21,08	164,69	18,57	145,08
22,71	177,38	15,20	118,76	267,10	2086,60	13,33	104,16	17,94	140,13	16,06	125,42	24,00	187,49	20,00	156,24
13,73	107,24	18,71	146,20	183,57	1434,02	16,06	125,42	14,91	116,45	13,33	104,16	37,64	294,06	12,58	98,27
14,42	112,67	24,91	194,59	125,48	980,27	18,95	148,04	11,93	93,17	8,54	66,69	62,89	491,32	11,00	85,89
17,08	133,39	11,00	85,89	323,42	2526,56	15,09	117,85	20,00	156,24	22,19	173,36	80,90	631,96	11,93	93,17
11,47	89,60	17,39	135,81	325,36	2541,72	8,43	65,88	12,29	96,03	21,87	170,83	19,82	154,84	22,35	174,61
7,78	60,74	10,67	83,33	255,17	1993,40	16,28	127,14	11,39	89,00	6,67	52,08	16,22	126,72	22,19	173,36
11,93	93,17	11,00	85,89	218,70	1708,52	18,86	147,30	17,39	135,81	14,17	110,73	21,71	169,56	8,94	69,87
24,15	188,65	16,06	125,42	180,65	1411,22	10,75	83,98	16,22	126,72	16,06	125,42	9,62	75,11	16,28	127,14
13,13	102,59	17,54	137,00	44,80	349,99	12,29	96,03	13,60	106,22	10,75	83,98	131,49	1027,19	16,71	130,52
10,41	81,35	25,37	198,18	79,34	619,83	8,43	65,88	11,93	93,17	15,61	121,92	47,35	369,88	21,08	164,69
13,33	104,16	8,11	63,36	45,41	354,76	15,61	121,92	25,37	198,18	14,67	114,58	28,28	220,96	8,94	69,87
13,60	106,22	11,39	89,00	58,80	459,37	13,13	102,59	16,71	130,52	16,06	125,42	29,33	229,15	14,17	110,73
14,17	110,73	16,71	130,52	57,33	447,89	12,58	98,27	16,06	125,42	17,94	140,13	11,93	93,17	10,15	79,32
11,93	93,17	17,39	135,81	35,08	274,01	7,18	56,09	10,75	83,98	11,93	93,17	8,43	65,88	10,67	83,33
7,18	56,09	17,08	133,39	31,10	242,94	8,11	63,36	13,73	107,24	18,71	146,20	13,92	108,74	16,22	126,72
18,67	145,83	28,63	223,64	20,18	157,62	13,60	106,22	28,50	222,67	17,39	135,81	20,00	156,24	7,54	58,92
16,06	125,42	34,77	271,62	122,78	959,19	14,91	116,45	12,65	98,81	19,09	149,13	11,93	93,17	8,94	69,87
20,74	162,04	19,09	149,13	91,47	714,55	16,22	126,72	9,62	75,11	16,97	132,58	56,51	441,43	13,33	104,16
17,89	139,75	23,29	181,91	129,42	1011,01	7,78	60,74	8,11	63,36	8,11	63,36	27,49	214,73	7,78	60,74
10,75	83,98	12,00	93,74	26,57	207,54	19,09	149,13	9,33	72,91	12,29	96,03	24,59	192,06	11,39	89,00
31,21	243,84	14,17	110,73	118,04	922,11	9,71	75,83	20,40	159,34	15,61	121,92	16,00	124,99	8,43	65,88
15,61	121,92	9,71	75,83	308,49	2409,92	10,41	81,35	21,87	170,83	9,43	73,65	10,15	79,32	12,65	98,81
25,20	196,89	19,82	154,84	41,87	327,08	18,14	141,67	8,11	63,36	9,62	75,11	5,33	41,66	13,60	106,22
12,68	99,06	16,22	126,72	83,19	649,90	22,07	172,42	18,57	145,08	5,66	44,19	27,49	214,73	16,22	126,72
17,94	140,13	5,50	42,94	124,69	974,11	14,67	114,58	11,47	89,60	11,00	85,89	18,14	141,67	9,33	72,91
24,00	187,49	16,11	125,86	100,58	785,71	10,67	83,33	22,82	178,29	6,67	52,08	21,87	170,83	19,69	153,79
17,94	140,13	10,67	83,33	511,11	3992,84	3,77	29,46	16,71	130,52	9,43	73,65	28,25	220,71	15,20	118,76
11,93	93,17	16,00	124,99	477,43	3729,68	10,41	81,35	14,67	114,58	4,22	32,94	60,06	469,19	10,75	83,98
11,00	85,89	22,71	177,38	89,69	700,67	17,33	135,41	18,67	145,83	9,62	75,11	98,75	771,43	13,33	104,16
20,04	156,59	20,35	158,99	407,32	3182,03	16,71	130,52	12,29	96,03	17,39	135,81	33,33	260,40	7,78	60,74
13,33	104,16	21,38	166,98	201,61	1575,00	13,20	103,11	16,97	132,58	17,79	138,97	181,57	1418,46	18,95	148,04
17,94	140,13	13,33	104,16	214,66	1676,96	20,04	156,59	20,83	162,70	18,67	145,83	26,97	210,65	11,31	88,39
8,54	66,69	10,67	83,33	212,60	1660,87	13,13	102,59	21,38	166,98	22,63	176,76	80,72	630,58	8,54	66,69
22,71	177,38	9,62	75,11	174,69	1364,67	20,35	158,99	26,97	210,65	17,79	138,97	17,39	135,81	11,39	89,00
7,18	56,09	13,33	104,16	234,47	1831,72	12,65	98,81	18,57	145,08	18,71	146,20	36,69	286,59	15,20	118,76
10,15	79,32	10,67	83,33	268,86	2100,36	18,14	141,67	16,06	125,42	14,36	112,18	24,00	187,49	14,36	112,18
8,11	63,36	11,00	85,89	37,14	290,16	16,06	125,42	9,33	72,91	13,92	108,74	46,90	366,35	17,39	135,81
17,33	135,41	24,51	191,50	16,49	128,84	17,33	135,41	20,00	156,24	10,75	83,98	28,03	218,99	13,13	102,59
10,15	79,32	18,95	148,04	40,35	315,25	20,40	159,34	23,59	184,28	17,39	135,81	27,49	214,73	17,33	135,41
8,43	65,88	14,42	112,67	250,79	1959,22	33,36	260,61	16,06	125,42	20,83	162,70	20,00	156,24	13,92	108,74
10,75	83,98	12,07	94,32	138,11	1078,91	19,09	149,13	20,04	156,59	8,94	69,87	21,50	167,95	13,92	108,74
22,71	177,38	9,71	75,83	159,43	1245,46	12,29	96,03	8,11	63,36	11,47	89,60	30,78	240,47	33,44	261,24
19,09	149,13	12,07	94,32	193,04	1508,03	15,55	121,47	13,20	103,11	8,11	63,36	45,41	354,76	23,85	186,33
12,65	98,81	20,87	163,04	178,85	1397,16	12,29	96,03	15,20	118,76	12,07	94,32	12,29	96,03	12,29	96,03
5,66	44,19	10,75	83,98	47,29	369,44	14,91	116,45	18,14	141,67	12,29	96,03	13,92	108,74	17,39	135,81
11,31	88,39	8,54	66,69	62,04	484,65	11,47	89,60	15,20	118,76	4,81	37,55	21,08	164,69	24,59	192,06
20,00	156,24	9,33	72,91	268,62	2098,50	23,32	182,21	17,94	140,13	10,67	83,33	16,22	126,72	18,14	141,67
8,94	69,87	10,75	83,98	117,47	917,68	8,94	69,87	14,17	110,73	6,80	53,11	20,00	156,24	20,87	163,04
Average	116,09		123,86		1296,77		113,58		125,40		105,13		269,52		116,20
Median	105,19		121,88		1044,96		110,40		125,42		106,45		189,77		108,74
SD	**43,27**		**47,53**		**939,63**		**39,13**		**38,60**		**37,20**		**251,86**		**41,26**

SV025 Naïve		SV025 LPSBP		SV025 LPS		SV025 LPS+ LPSBP		SV025 Aspirin		SV025 Aspirin + LPSBP		SV025 Aspirin + LPS		SV025 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
13,33	104,16	18,95	148,04	40,49	316,28	14,91	116,45	14,42	112,67	30,70	239,80	73,44	573,73	12,29	96,03
25,37	198,18	10,67	83,33	41,48	324,08	12,29	96,03	18,14	141,67	18,57	145,08	85,77	670,04	13,33	104,16
8,11	63,36	17,54	137,00	35,00	273,41	20,31	158,66	16,00	124,99	20,74	162,04	295,47	2308,26	27,20	212,45
7,54	58,92	16,71	130,52	59,54	465,12	13,60	106,22	21,54	168,28	13,33	104,16	79,21	618,77	15,20	118,76
13,73	107,24	24,04	187,78	164,27	1283,25	10,67	83,33	10,67	83,33	16,87	131,75	124,52	972,77	25,65	200,36
13,60	106,22	16,00	124,99	270,07	2109,77	13,20	103,11	20,00	156,24	27,84	217,50	104,15	813,59	10,75	83,98
14,42	112,67	20,40	159,34	270,13	2110,26	6,67	52,08	13,92	108,74	14,17	110,73	104,31	814,85	20,18	157,62
12,58	98,27	13,92	108,74	214,68	1677,12	7,18	56,09	20,18	157,62	14,42	112,67	307,22	2400,00	16,06	125,42
12,29	96,03	17,89	139,75	102,81	803,12	4,22	32,94	10,67	83,33	28,63	223,64	158,62	1239,12	25,47	199,00
11,39	89,00	11,31	88,39	161,42	1261,03	9,71	75,83	20,00	156,24	18,52	144,70	134,67	1052,08	16,49	128,84
8,00	62,50	8,43	65,88	310,68	2427,04	11,47	89,60	37,92	296,27	19,09	149,13	63,25	494,08	14,17	110,73
9,43	73,65	22,19	173,36	342,68	2677,02	9,33	72,91	9,62	75,11	20,31	158,66	47,18	368,56	17,33	135,41
17,33	135,41	7,18	56,09	235,08	1836,45	10,41	81,35	16,11	125,86	29,27	228,68	43,41	339,12	13,60	106,22
16,11	125,86	14,42	112,67	32,22	251,71	10,41	81,35	16,87	131,75	22,67	177,08	55,20	431,23	19,41	151,66
12,58	98,27	5,96	46,58	30,70	239,80	9,71	75,83	26,20	204,65	16,49	128,84	54,29	424,13	24,59	192,06
10,67	83,33	15,09	117,85	21,71	169,56	8,94	69,87	30,78	240,47	14,17	110,73	89,64	700,28	28,00	218,74
20,87	163,04	16,87	131,75	31,47	245,83	7,78	60,74	21,71	169,56	29,36	229,39	66,67	520,81	21,71	169,56
13,92	108,74	11,47	89,60	32,44	253,43	44,00	343,73	21,08	164,69	13,20	103,11	77,33	604,13	17,33	135,41
8,00	62,50	14,91	116,45	16,28	127,14	9,71	75,83	13,92	108,74	13,20	103,11	106,44	831,52	10,15	79,32
10,67	83,33	14,36	112,18	19,09	149,13	17,54	137,00	20,31	158,66	28,03	218,99	52,75	412,06	25,05	195,70
8,00	62,50	9,43	73,65	19,41	151,66	6,67	52,08	29,27	228,68	14,91	116,45	52,02	406,36	19,41	151,66
12,29	96,03	41,66	325,41	100,42	784,46	14,91	116,45	10,75	83,98	21,38	166,98	43,08	336,55	25,30	197,63
7,18	56,09	16,00	124,99	197,34	1541,62	8,43	65,88	20,00	156,24	14,17	110,73	60,02	468,84	16,28	127,14
21,71	169,56	13,73	107,24	239,48	1870,84	16,22	126,72	30,78	240,47	14,73	115,05	66,79	521,74	22,71	177,38
10,67	83,33	13,20	103,11	30,93	241,60	16,06	125,42	16,06	125,42	12,65	98,81	66,88	522,47	14,42	112,67
6,67	52,08	8,94	69,87	56,58	442,04	9,62	75,11	15,09	117,85	12,65	98,81	59,70	466,40	7,78	60,74
12,29	96,03	17,39	135,81	121,62	950,10	13,92	108,74	25,37	198,18	6,67	52,08	158,96	1241,79	19,23	150,23
12,65	98,81	14,36	112,18	90,23	704,91	11,00	85,89	23,29	181,91	6,67	52,08	220,49	1722,47	25,30	197,63
21,38	166,98	6,67	52,08	116,43	909,54	8,94	69,87	16,71	130,52	21,08	164,69	54,98	429,47	13,33	104,16
11,39	89,00	13,40	104,68	68,30	533,56	13,13	102,59	15,61	121,92	24,48	191,22	77,30	603,87	16,28	127,14
19,82	154,84	16,06	125,42	190,93	1491,57	12,65	98,81	10,75	83,98	22,71	177,38	213,24	1665,82	22,94	179,21
18,71	146,20	9,33	72,91	30,41	237,53	7,78	60,74	20,04	156,59	16,11	125,86	175,65	1372,16	18,86	147,30
20,18	157,62	13,33	104,16	18,86	147,30	13,33	104,16	35,83	279,88	19,82	154,84	117,24	915,90	11,31	88,39
19,82	154,84	14,67	114,58	17,94	140,13	20,40	159,34	15,20	118,76	19,23	150,23	52,02	406,36	14,67	114,58
9,71	75,83	8,94	69,87	24,51	191,50	12,07	94,32	18,14	141,67	13,13	102,59	65,35	510,50	25,65	200,36
14,42	112,67	21,08	164,69	25,16	196,53	8,54	66,69	21,08	164,69	11,39	89,00	193,63	1512,63	5,96	46,58
17,08	133,39	19,41	151,66	26,70	208,58	12,07	94,32	19,09	149,13	12,07	94,32	189,80	1482,75	14,42	112,67
18,14	141,67	14,42	112,67	26,57	207,54	10,67	83,33	14,73	115,05	9,33	72,91	90,68	708,37	10,75	83,98
16,11	125,86	12,00	93,74	84,10	656,96	14,17	110,73	18,52	144,70	9,71	75,83	129,44	1011,22	20,35	158,99
11,93	93,17	17,39	135,81	146,67	1145,77	13,60	106,22	22,94	179,21	8,54	66,69	118,19	923,34	8,43	65,88
14,91	116,45	12,29	96,03	174,75	1365,14	6,80	53,11	24,15	188,65	14,42	112,67	70,68	552,15	26,20	204,65
13,73	107,24	16,06	125,42	306,64	2395,45	9,71	75,83	14,67	114,58	13,60	106,22	134,94	1054,14	22,82	178,29
9,62	75,11	17,89	139,75	70,72	552,45	15,09	117,85	27,84	217,50	14,73	115,05	77,90	608,52	13,33	104,16
17,89	139,75	8,43	65,88	13,92	108,74	12,07	94,32	20,00	156,24	18,14	141,67	173,52	1355,54	6,67	52,08
14,67	114,58	12,00	93,74	275,12	2149,28	10,41	81,35	14,73	115,05	25,47	199,00	235,61	1840,59	12,00	93,74
20,35	158,99	17,54	137,00	120,36	940,28	11,93	93,17	18,95	148,04	21,38	166,98	189,80	1482,75	23,02	179,81
10,41	81,35	14,42	112,67	34,69	271,02	16,06	125,42	25,05	195,70	16,00	124,99	197,32	1541,48	19,82	154,84
9,62	75,11	22,78	177,99	42,75	333,97	16,06	125,42	20,00	156,24	9,43	73,65	89,44	698,74	21,99	171,79
12,65	98,81	19,09	149,13	30,67	239,57	7,78	60,74	18,14	141,67	14,17	110,73	201,58	1574,73	20,00	156,24
11,00	85,89	12,29	96,03	20,70	161,70	11,47	89,60	21,71	169,56	14,42	112,67	139,54	1090,07	21,08	164,69
Average	107,01		117,57		805,44		95,86		153,82		134,80		912,34		139,72
Median	98,81		112,67		453,58		89,60		152,69		120,72		704,32		141,36
SD	**34,76**		**44,64**		**750,52**		**44,83**		**48,36**		**47,92**		**519,84**		**45,85**

SV026 Naïve		SV026 LPSBP		SV026 LPS		SV026 LPSBP+ LPS		SV026 Aspirin		SV026 Aspirin + LPSBP		SV026 Aspirin + LPS		SV026 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
13,40	104,68	17,33	135,41	149,84	1170,55	21,33	166,65	26,47	206,75	24,04	187,78	132,64	1036,18	14,91	116,45
10,15	79,32	6,67	52,08	78,26	611,37	13,33	104,16	11,47	89,60	17,89	139,75	96,76	755,86	13,33	104,16
4,81	37,55	11,93	93,17	69,54	543,24	22,94	179,21	12,65	98,81	16,87	131,75	108,29	845,95	18,86	147,30
10,67	83,33	14,91	116,45	46,65	364,42	16,06	125,42	21,99	171,79	16,06	125,42	53,93	421,31	15,09	117,85
7,78	60,74	7,78	60,74	45,51	355,52	20,18	157,62	13,60	106,22	22,67	177,08	152,54	1191,63	15,61	121,92
10,15	79,32	17,08	133,39	82,29	642,85	20,35	158,99	13,33	104,16	13,13	102,59	83,76	654,31	19,82	154,84
13,73	107,24	11,39	89,00	68,53	535,39	12,00	93,74	21,87	170,83	14,73	115,05	61,42	479,82	12,00	93,74
7,18	56,09	13,33	104,16	156,92	1225,83	18,14	141,67	9,43	73,65	18,14	141,67	244,52	1910,24	12,29	96,03
7,18	56,09	14,91	116,45	35,08	274,01	17,89	139,75	36,03	281,43	12,29	96,03	166,05	1297,17	10,75	83,98
16,71	130,52	11,39	89,00	97,33	760,37	12,29	96,03	10,75	83,98	8,94	69,87	89,13	696,32	24,04	187,78
13,33	104,16	13,40	104,68	48,90	382,00	15,09	117,85	14,73	115,05	18,57	145,08	105,87	827,08	18,14	141,67
27,00	210,91	14,17	110,73	41,87	327,08	21,54	168,28	25,30	197,63	15,20	118,76	183,31	1432,02	17,33	135,41
17,39	135,81	17,54	137,00	34,69	271,02	12,29	96,03	20,31	158,66	16,06	125,42	316,66	2473,79	19,23	150,23
13,20	103,11	22,82	178,29	32,69	255,35	15,61	121,92	20,40	159,34	18,14	141,67	96,71	755,51	13,40	104,68
7,18	56,09	11,00	85,89	73,95	577,69	16,00	124,99	18,67	145,83	18,95	148,04	109,37	854,38	11,39	89,00
16,06	125,42	15,20	118,76	96,98	757,59	16,71	130,52	23,63	184,58	8,54	66,69	84,93	663,45	10,67	83,33
12,58	98,27	21,08	164,69	32,69	255,35	26,83	209,62	12,07	94,32	18,57	145,08	220,00	1718,66	13,20	103,11
10,15	79,32	3,77	29,46	54,42	425,15	10,41	81,35	20,83	162,70	15,20	118,76	238,34	1861,95	11,39	89,00
5,96	46,58	16,28	127,14	32,06	250,42	22,67	177,08	14,91	116,45	16,00	124,99	138,59	1082,68	14,17	110,73
19,09	149,13	9,71	75,83	37,33	291,65	12,00	93,74	11,39	89,00	15,20	118,76	155,77	1216,90	24,59	192,06
16,22	126,72	7,78	60,74	29,61	231,28	22,07	172,42	25,75	201,17	5,50	42,94	154,17	1204,36	13,13	102,59
13,33	104,16	14,73	115,05	116,61	910,97	9,33	72,91	10,41	81,35	10,41	81,35	220,63	1723,54	13,13	102,59
5,66	44,19	16,22	126,72	108,43	847,04	12,29	96,03	9,43	73,65	18,14	141,67	126,73	990,02	17,33	135,41
12,29	96,03	12,00	93,74	37,33	291,65	12,29	96,03	13,73	107,24	8,94	69,87	266,26	2080,01	11,47	89,60
13,73	107,24	12,29	96,03	55,51	433,62	12,29	96,03	9,43	73,65	15,09	117,85	183,06	1430,08	23,29	181,91
16,00	124,99	6,80	53,11	56,65	442,53	9,43	73,65	24,51	191,50	18,67	145,83	119,33	932,22	29,61	231,28
13,73	107,24	21,71	169,56	23,29	181,91	19,23	150,23	19,09	149,13	16,22	126,72	177,95	1390,15	16,11	125,86
11,93	93,17	11,47	89,60	68,83	537,72	11,31	88,39	16,06	125,42	9,62	75,11	94,68	739,62	33,76	263,71
17,39	135,81	13,20	103,11	71,88	561,50	11,39	89,00	14,73	115,05	12,07	94,32	77,03	601,80	26,70	208,58
15,09	117,85	17,89	139,75	48,31	377,43	13,92	108,74	10,75	83,98	13,33	104,16	177,89	1389,68	12,29	96,03
24,04	187,78	11,93	93,17	36,61	286,02	19,82	154,84	20,83	162,70	12,29	96,03	145,43	1136,12	22,67	177,08
15,20	118,76	17,39	135,81	82,80	646,81	6,67	52,08	6,67	52,08	16,06	125,42	130,67	1020,83	22,78	177,99
10,41	81,35	8,54	66,69	114,95	897,96	15,09	117,85	12,07	94,32	13,33	104,16	302,74	2365,03	17,79	138,97
11,47	89,60	17,08	133,39	68,33	533,77	20,31	158,66	10,75	83,98	18,67	145,83	232,60	1817,10	16,97	132,58
5,50	42,94	8,94	69,87	60,46	472,30	31,69	247,59	14,73	115,05	12,58	98,27	189,26	1478,54	20,00	156,24
12,58	98,27	10,67	83,33	78,71	614,90	13,33	104,16	9,62	75,11	8,54	66,69	175,36	1369,94	12,07	94,32
11,31	88,39	6,67	52,08	48,33	377,57	26,67	208,32	15,61	121,92	7,54	58,92	134,83	1053,26	10,75	83,98
10,67	83,33	4,81	37,55	33,65	262,89	9,62	75,11	11,39	89,00	10,15	79,32	66,96	523,09	15,55	121,47
9,62	75,11	5,96	46,58	114,27	892,69	13,13	102,59	22,67	177,08	11,93	93,17	84,75	662,06	11,31	88,39
10,75	83,98	7,78	60,74	49,35	385,53	13,13	102,59	12,07	94,32	13,33	104,16	35,83	279,88	10,75	83,98
17,39	135,81	17,33	135,41	73,77	576,28	24,04	187,78	25,65	200,36	17,79	138,97	45,57	355,98	21,87	170,83
6,67	52,08	11,39	89,00	73,76	576,19	17,79	138,97	21,50	167,95	15,55	121,47	62,03	484,54	24,04	187,78
13,40	104,68	13,13	102,59	182,91	1428,87	16,71	130,52	16,49	128,84	9,71	75,83	31,47	245,83	13,33	104,16
14,36	112,18	2,98	23,29	80,18	626,36	20,18	157,62	14,36	112,18	14,67	114,58	117,82	920,39	12,29	96,03
12,07	94,32	13,60	106,22	50,12	391,54	12,29	96,03	16,06	125,42	37,71	294,61	113,46	886,35	17,89	139,75
11,93	93,17	20,04	156,59	29,33	229,15	16,00	124,99	8,94	69,87	7,18	56,09	252,24	1970,52	13,40	104,68
10,75	83,98	14,42	112,67	75,80	592,16	11,00	85,89	15,20	118,76	10,41	81,35	206,41	1612,47	9,33	72,91
18,57	145,08	14,91	116,45	48,33	377,57	15,09	117,85	5,50	42,94	12,07	94,32	286,58	2238,76	32,11	250,85
10,41	81,35	20,04	156,59	73,38	573,27	14,73	115,05	10,75	83,98	17,39	135,81	263,45	2058,12	22,67	177,08
8,94	69,87	16,22	126,72	32,25	251,93	17,39	135,81	11,47	89,60	17,08	133,39	181,51	1417,97	14,42	112,67
Average	97,66		101,49		521,73		126,85		124,37		115,17		1171,07		132,65
Median	95,18		103,63		457,42		119,88		115,05		118,30		1067,97		119,66
SD	**34,91**		**37,00**		**272,03**		**40,36**		**48,51**		**40,73**		**570,24**		**45,88**

SV027 Naïve		SV027 LPBP		SV027 LPS		SV027 LPS +LPSBP		SV027 Aspirin		SV027 Aspirin + LPSBP		SV027 Aspirin + LPS		SV027 Aspirin + LPS+LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
12,07	94,32	12,29	96,03	179,19	1399,83	13,60	106,22	24,80	193,75	38,46	300,44	147,52	1152,43	13,60	106,22
14,73	115,05	16,11	125,86	160,83	1256,38	15,61	121,92	12,58	98,27	32,06	250,42	161,99	1265,46	14,42	112,67
25,65	200,36	21,50	167,95	50,19	392,10	15,20	118,76	25,33	197,90	38,46	300,44	63,61	496,93	11,47	89,60
17,33	135,41	12,00	93,74	255,03	1992,28	16,22	126,72	11,00	85,89	30,41	237,53	71,47	558,31	15,09	117,85
11,47	89,60	12,00	93,74	280,37	2190,28	22,19	173,36	17,39	135,81	27,49	214,73	111,51	871,10	6,67	52,08
14,17	110,73	16,06	125,42	94,75	740,20	15,55	121,47	24,04	187,78	15,61	121,92	58,73	458,78	17,89	139,75
9,43	73,65	13,13	102,59	141,21	1103,12	18,86	147,30	12,00	93,74	28,54	222,92	40,02	312,65	4,22	32,94
20,04	156,59	19,82	154,84	102,56	801,23	8,00	62,50	28,25	220,71	18,14	141,67	30,08	234,99	9,43	73,65
12,58	98,27	14,91	116,45	144,25	1126,91	15,61	121,92	8,43	65,88	13,92	108,74	25,89	202,25	10,75	83,98
17,79	138,97	21,50	167,95	425,39	3323,15	20,18	157,62	31,24	244,06	36,39	284,30	45,51	355,52	9,43	73,65
18,86	147,30	20,04	156,59	423,02	3304,68	11,39	89,00	6,67	52,08	29,61	231,28	87,49	683,50	9,71	75,83
15,20	118,76	18,95	148,04	433,35	3385,38	12,65	98,81	25,65	200,36	28,35	221,45	20,40	159,34	13,33	104,16
9,71	75,83	9,71	75,83	88,49	691,32	11,39	89,00	23,85	186,33	8,94	69,87	58,80	459,37	14,73	115,05
18,57	145,08	20,74	162,04	288,00	2249,88	12,07	94,32	17,08	133,39	24,59	192,06	128,35	1002,66	5,33	41,66
18,67	145,83	18,71	146,20	321,36	2510,47	16,49	128,84	22,94	179,21	32,47	253,65	66,79	521,74	14,73	115,05
7,54	58,92	9,62	75,11	176,05	1375,28	12,00	93,74	17,08	133,39	25,89	202,25	23,59	184,28	12,65	98,81
19,69	153,79	15,20	118,76	158,04	1234,65	12,65	98,81	18,14	141,67	13,73	107,24	33,01	257,89	7,78	60,74
14,42	112,67	11,39	89,00	212,33	1658,75	18,57	145,08	29,81	232,91	14,36	112,18	98,68	770,86	12,00	93,74
17,54	137,00	10,67	83,33	54,81	428,20	24,15	188,65	12,58	98,27	28,28	220,96	75,09	586,64	14,91	116,45
15,61	121,92	9,62	75,11	137,86	1076,95	18,71	146,20	21,08	164,69	25,61	200,08	65,59	512,40	11,93	93,17
10,15	79,32	10,67	83,33	94,36	737,12	22,82	178,29	13,73	107,24	8,94	69,87	48,00	374,98	10,67	83,33
12,58	98,27	7,54	58,92	65,78	513,89	20,00	156,24	15,61	121,92	6,67	52,08	137,70	1075,74	10,67	83,33
11,47	89,60	18,67	145,83	152,09	1188,17	17,94	140,13	21,08	164,69	16,87	131,75	32,47	253,65	14,67	114,58
18,86	147,30	15,61	121,92	145,77	1138,79	21,08	164,69	16,06	125,42	12,07	94,32	40,11	313,35	14,73	115,05
18,14	141,67	16,00	124,99	147,27	1150,49	15,55	121,47	26,30	205,43	10,67	83,33	40,00	312,48	17,33	135,41
12,07	94,32	17,54	137,00	29,61	231,28	13,92	108,74	21,71	169,56	14,36	112,18	116,00	906,20	18,14	141,67
9,33	72,91	18,14	141,67	159,58	1246,63	14,42	112,67	20,83	162,70	16,06	125,42	24,80	193,75	12,65	98,81
18,71	146,20	17,39	135,81	150,67	1177,02	13,13	102,59	16,06	125,42	16,06	125,42	43,08	336,55	12,00	93,74
7,54	58,92	14,17	110,73	156,95	1226,10	18,95	148,04	11,47	89,60	26,70	208,58	78,03	609,58	15,20	118,76
5,96	46,58	10,15	79,32	329,40	2573,30	16,11	125,86	8,43	65,88	9,62	75,11	108,01	843,77	18,95	148,04
10,41	81,35	12,65	98,81	265,33	2072,80	18,95	148,04	25,65	200,36	20,18	157,62	115,38	901,34	13,33	104,16
7,78	60,74	8,54	66,69	269,33	2104,05	14,36	112,18	22,67	177,08	14,17	110,73	77,38	604,49	11,47	89,60
13,92	108,74	10,75	83,98	217,34	1697,85	24,80	193,75	9,62	75,11	10,15	79,32	63,36	494,96	13,20	103,11
12,65	98,81	13,33	104,16	284,31	2221,07	14,17	110,73	23,85	186,33	14,42	112,67	49,55	387,08	11,00	85,89
13,33	104,16	9,43	73,65	268,21	2095,29	14,91	116,45	19,09	149,13	18,86	147,30	49,48	386,52	16,06	125,42
18,86	147,30	11,93	93,17	68,00	531,22	18,52	144,70	16,00	124,99	13,40	104,68	128,00	999,95	10,67	83,33
23,74	185,45	10,75	83,98	126,35	987,06	12,58	98,27	7,78	60,74	9,33	72,91	63,81	498,45	9,71	75,83
13,60	106,22	14,91	116,45	38,83	303,32	14,36	112,18	15,20	118,76	12,07	94,32	24,59	192,06	15,55	121,47
16,11	125,86	19,23	150,23	34,90	272,62	22,19	173,36	15,20	118,76	18,71	146,20	22,71	177,38	12,07	94,32
9,62	75,11	16,28	127,14	28,03	218,99	17,39	135,81	24,04	187,78	14,91	116,45	105,33	822,87	14,17	110,73
17,39	135,81	21,71	169,56	30,78	240,47	7,18	56,09	17,39	135,81	21,08	164,69	33,12	258,73	8,94	69,87
12,29	96,03	18,14	141,67	42,94	335,43	14,17	110,73	17,89	139,75	21,71	169,56	78,23	611,10	14,91	116,45
10,15	79,32	30,78	240,47	65,39	510,82	11,39	89,00	25,65	200,36	12,65	98,81	42,10	328,89	9,33	72,91
8,43	65,88	6,80	53,11	47,80	373,39	14,73	115,05	14,42	112,67	20,31	158,66	66,57	520,07	11,00	85,89
13,60	106,22	13,40	104,68	46,44	362,78	10,67	83,33	14,17	110,73	18,57	145,08	42,67	333,32	20,70	161,70
6,67	52,08	28,28	220,96	47,48	370,91	11,93	93,17	12,29	96,03	13,33	104,16	58,49	456,89	9,43	73,65
18,57	145,08	13,40	104,68	41,85	326,90	17,89	139,75	15,61	121,92	11,93	93,17	119,76	935,60	7,18	56,09
11,39	89,00	14,42	112,67	36,10	282,01	11,00	85,89	13,92	108,74	13,60	106,22	100,60	785,92	9,62	75,11
16,22	126,72	15,09	117,85	122,06	953,57	19,09	149,13	17,89	139,75	9,62	75,11	50,68	395,95	6,67	52,08
24,04	187,78	14,91	116,45	224,57	1754,36	16,00	124,99	11,47	89,60	9,43	73,65	121,80	951,52	4,00	31,25
Average	111,66		117,89		1228,77		123,63		140,77		148,07		546,20		94,37
Median	107,48		116,45		1132,85		121,47		134,60		125,42		495,94		93,74
SD	**36,40**		**37,96**		**866,50**		**30,93**		**47,94**		**66,23**		**291,61**		**28,83**

SV029 naïve		SV029 LPSBP		SV029 LPS		SV029 LPS + LPSBP		SV029 Aspirin		SV029 Aspirin + LPSBP		SV029 Aspirin +LPS		SV029 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
9,33	72,91	16,06	125,42	35,58	277,94	12,00	93,74	13,20	103,11	11,39	89,00	140,41	1096,91	10,75	83,98
21,08	164,69	8,43	65,88	41,07	320,87	12,00	93,74	9,43	73,65	10,15	79,32	143,83	1123,59	15,55	121,47
16,97	132,58	3,77	29,46	39,58	309,17	13,40	104,68	28,25	220,71	10,15	79,32	25,47	199,00	16,00	124,99
13,33	104,16	24,04	187,78	30,70	239,80	15,61	121,92	14,17	110,73	7,78	60,74	31,81	248,46	8,54	66,69
12,58	98,27	14,42	112,67	71,88	561,50	18,71	146,20	11,00	85,89	24,04	187,78	54,91	428,96	15,55	121,47
17,89	139,75	19,09	149,13	28,78	224,85	10,15	79,32	10,41	81,35	17,33	135,41	57,35	448,01	9,71	75,83
11,47	89,60	22,63	176,76	24,48	191,22	4,22	32,94	20,74	162,04	16,49	128,84	49,82	389,17	10,15	79,32
9,62	75,11	29,12	227,49	37,55	293,32	13,40	104,68	28,28	220,96	13,40	104,68	60,65	473,79	9,71	75,83
21,08	164,69	13,33	104,16	53,81	420,40	51,78	404,49	7,78	60,74	13,33	104,16	78,98	617,01	17,94	140,13
9,43	73,65	21,08	164,69	19,23	150,23	13,33	104,16	10,67	83,33	13,20	103,11	126,19	985,80	8,54	66,69
5,96	46,58	7,18	56,09	32,06	250,42	8,43	65,88	17,39	135,81	28,25	220,71	60,53	472,87	15,55	121,47
6,80	53,11	12,00	93,74	74,35	580,79	8,00	62,50	17,08	133,39	20,87	163,04	32,03	250,21	5,96	46,58
8,00	62,50	22,35	174,61	29,81	232,91	5,50	42,94	10,15	79,32	16,71	130,52	46,23	361,12	7,54	58,92
13,33	104,16	27,20	212,45	28,03	218,99	9,62	75,11	11,39	89,00	9,43	73,65	36,61	286,02	6,67	52,08
5,33	41,66	21,71	169,56	39,78	310,74	5,96	46,58	9,62	75,11	11,39	89,00	99,80	779,61	11,93	93,17
12,29	96,03	9,33	72,91	22,71	177,38	4,00	31,25	18,57	145,08	14,17	110,73	168,76	1318,37	7,54	58,92
21,71	169,56	13,60	106,22	29,96	234,07	6,67	52,08	10,41	81,35	9,33	72,91	141,56	1105,88	21,71	169,56
13,33	104,16	10,67	83,33	50,95	398,00	21,08	164,69	4,22	32,94	12,29	96,03	136,03	1062,65	7,78	60,74
13,92	108,74	9,43	73,65	65,10	508,58	12,07	94,32	8,11	63,36	11,47	89,60	116,03	906,44	12,29	96,03
19,41	151,66	17,79	138,97	30,70	239,80	4,81	37,55	10,41	81,35	11,93	93,17	128,06	1000,43	10,67	83,33
18,71	146,20	11,00	85,89	36,61	286,02	18,14	141,67	17,39	135,81	16,71	130,52	39,64	309,69	13,92	108,74
5,66	44,19	12,07	94,32	78,26	611,37	16,06	125,42	21,08	164,69	18,52	144,70	104,31	814,85	9,62	75,11
18,14	141,67	17,94	140,13	164,32	1283,67	21,99	171,79	17,79	138,97	7,18	56,09	47,14	368,26	13,13	102,59
10,75	83,98	28,35	221,45	65,66	512,93	18,71	146,20	9,43	73,65	25,37	198,18	47,14	368,26	14,17	110,73
23,63	184,58	19,69	153,79	61,35	479,26	18,14	141,67	18,86	147,30	14,17	110,73	23,85	186,33	10,41	81,35
6,67	52,08	14,73	115,05	32,28	252,15	15,09	117,85	9,33	72,91	14,17	110,73	119,50	933,57	9,43	73,65
13,40	104,68	22,63	176,76	54,72	427,44	18,14	141,67	11,47	89,60	11,47	89,60	92,86	725,40	20,00	156,24
20,04	156,59	4,22	32,94	49,17	384,13	19,41	151,66	12,00	93,74	8,94	69,87	162,47	1269,23	11,93	93,17
25,33	197,90	25,61	200,08	45,61	356,29	17,33	135,41	5,66	44,19	26,70	208,58	88,04	687,77	12,65	98,81
9,33	72,91	16,11	125,86	83,69	653,82	10,75	83,98	7,78	60,74	11,31	88,39	65,06	508,26	12,29	96,03
28,03	218,99	1,89	14,73	34,90	272,62	14,67	114,58	21,08	164,69	11,00	85,89	49,35	385,53	13,40	104,68
16,49	128,84	4,81	37,55	122,12	954,03	14,91	116,45	16,00	124,99	21,50	167,95	285,62	2231,31	20,00	156,24
23,85	186,33	23,29	181,91	33,44	261,24	18,57	145,08	18,71	146,20	28,25	220,71	289,29	2259,93	8,54	66,69
11,31	88,39	28,00	218,74	64,44	503,43	9,71	75,83	11,39	89,00	17,39	135,81	33,73	263,51	17,79	138,97
21,71	169,56	13,60	106,22	20,40	159,34	17,79	138,97	20,35	158,99	12,29	96,03	37,55	293,32	19,09	149,13
22,78	177,99	9,43	73,65	81,28	634,96	7,78	60,74	17,39	135,81	30,78	240,47	17,54	137,00	6,80	53,11
20,40	159,34	17,08	133,39	107,27	838,02	16,11	125,86	7,78	60,74	18,57	145,08	47,14	368,26	16,11	125,86
9,43	73,65	7,78	60,74	44,18	345,15	16,22	126,72	16,06	125,42	8,94	69,87	22,78	177,99	7,18	56,09
14,73	115,05	25,65	200,36	41,42	323,57	9,62	75,11	9,43	73,65	14,91	116,45	35,78	279,49	21,87	170,83
20,74	162,04	12,07	94,32	39,78	310,74	13,73	107,24	13,60	106,22	17,54	137,00	21,54	168,28	7,78	60,74
18,95	148,04	8,54	66,69	32,93	257,26	13,13	102,59	14,91	116,45	8,11	63,36	49,78	388,90	16,28	127,14
5,50	42,94	13,13	102,59	52,75	412,06	15,20	118,76	18,57	145,08	15,55	121,47	132,97	1038,74	19,41	151,66
22,82	178,29	13,92	108,74	48,53	379,15	6,67	52,08	8,54	66,69	10,75	83,98	154,40	1206,20	11,31	88,39
19,23	150,23	7,78	60,74	43,41	339,12	13,13	102,59	6,80	53,11	24,04	187,78	104,69	817,85	18,57	145,08
10,75	83,98	10,75	83,98	41,07	320,87	13,60	106,22	4,22	32,94	15,20	118,76	212,40	1659,27	11,00	85,89
24,04	187,78	19,82	154,84	77,16	602,79	6,80	53,11	7,78	60,74	16,11	125,86	215,49	1683,42	10,41	81,35
12,07	94,32	21,71	169,56	42,85	334,78	22,71	177,38	13,33	104,16	7,18	56,09	78,26	611,37	13,20	103,11
19,09	149,13	10,15	79,32	49,35	385,53	18,71	146,20	13,40	104,68	25,30	197,63	363,16	2837,02	13,13	102,59
11,47	89,60	11,47	89,60	32,11	250,85	16,00	124,99	21,50	167,95	18,71	146,20	192,71	1505,48	10,41	81,35
10,15	79,32	6,67	52,08	26,47	206,75	14,91	116,45	9,43	73,65	18,14	141,67	32,11	250,85	11,47	89,60
Average	118,44		119,22		389,61		110,06		105,04		121,54		765,79		98,64
Median	106,71		107,48		322,22		106,73		91,67		110,73		559,81		93,17
SD	**47,56**		**55,91**		**212,87**		**57,06**		**43,87**		**47,22**		**598,62**		**33,23**

SV030 Naïve		SV030 LPSBP		SV030 LPS		SV030 LPSBP + LPS		SV030 Aspirin		SV030 Aspirin + LPSBP		SV030 Aspirin + LPS		SV030 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale Bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
16,49	128,84	10,75	83,98	99,75	779,26	13,60	106,22	36,10	282,01	17,39	135,81	123,885	967,80	46,44	362,78
22,71	177,38	12,00	93,74	123,26	962,91	12,00	93,74	16,06	125,42	13,20	103,11	36,782	287,34	16,06	125,42
17,33	135,41	18,95	148,04	56,02	437,60	10,75	83,98	16,28	127,14	14,17	110,73	76,571	598,18	30,70	239,80
19,41	151,66	15,20	118,76	24,59	192,06	17,39	135,81	16,28	127,14	22,19	173,36	29,963	234,07	27,84	217,50
18,57	145,08	18,71	146,20	22,67	177,08	5,96	46,58	10,75	83,98	20,00	156,24	37,047	289,41	22,94	179,21
8,43	65,88	14,42	112,67	58,55	457,36	10,15	79,32	18,14	141,67	16,22	126,72	75,057	586,35	20,70	161,70
17,33	135,41	15,20	118,76	54,42	425,15	14,67	114,58	18,71	146,20	7,78	60,74	130,340	1018,23	9,71	75,83
24,04	187,78	6,67	52,08	17,89	139,75	15,09	117,85	26,67	208,32	20,35	158,99	81,279	634,96	13,92	108,74
22,71	177,38	16,49	128,84	46,67	364,57	10,75	83,98	9,62	75,11	13,60	106,22	54,975	429,47	12,00	93,74
17,33	135,41	18,86	147,30	95,18	743,57	18,71	146,20	21,38	166,98	15,61	121,92	32,551	254,29	13,33	104,16
18,14	141,67	8,94	69,87	60,30	471,04	7,54	58,92	16,06	125,42	10,67	83,33	40,486	316,28	17,89	139,75
15,20	118,76	14,91	116,45	33,01	257,89	13,60	106,22	12,07	94,32	18,52	144,70	45,353	354,30	6,80	53,11
13,40	104,68	15,09	117,85	95,82	748,58	20,83	162,70	16,71	130,52	20,04	156,59	59,703	466,40	14,91	116,45
22,19	173,36	6,67	52,08	74,60	582,74	18,52	144,70	10,67	83,33	17,79	138,97	69,231	540,84	10,75	83,98
22,35	174,61	20,04	156,59	46,90	366,35	8,94	69,87	20,00	156,24	17,94	140,13	98,568	770,02	16,00	124,99
12,00	93,74	16,11	125,86	43,00	335,91	9,71	75,83	15,61	121,92	14,67	114,58	294,187	2298,21	34,18	266,99
8,54	66,69	22,35	174,61	57,77	451,27	19,09	149,13	16,06	125,42	12,07	94,32	24,037	187,78	22,82	178,29
16,11	125,86	12,29	96,03	58,68	458,43	9,33	72,91	14,17	110,73	13,92	108,74	45,412	354,76	19,82	154,84
20,00	156,24	8,43	65,88	128,56	1004,33	10,75	83,98	5,96	46,58	17,54	137,00	46,284	361,57	33,44	261,24
20,31	158,66	18,67	145,83	74,54	582,28	12,29	96,03	18,71	146,20	19,41	151,66	112,704	880,45	36,69	286,59
18,86	147,30	12,29	96,03	49,78	388,90	9,62	75,11	11,47	89,60	14,91	116,45	75,813	592,26	25,37	198,18
22,82	178,29	22,67	177,08	61,98	484,21	10,15	79,32	18,71	146,20	13,40	104,68	57,689	450,67	30,23	236,15
20,40	159,34	9,71	75,83	66,96	523,09	14,91	116,45	15,20	118,76	13,13	102,59	108,583	848,26	8,94	69,87
17,94	140,13	20,31	158,66	119,26	931,64	14,17	110,73	16,06	125,42	15,20	118,76	40,022	312,65	9,43	73,65
31,24	244,06	21,54	168,28	88,97	695,07	12,07	94,32	18,71	146,20	12,58	98,27	28,503	222,67	27,49	214,73
21,50	167,95	14,36	112,18	76,57	598,18	8,11	63,36	21,54	168,28	10,41	81,35	71,118	555,58	36,42	284,49
30,70	239,80	25,33	197,90	101,62	793,88	8,11	63,36	19,09	149,13	20,18	157,62	33,652	262,89	14,73	115,05
13,33	104,16	9,33	72,91	36,03	281,43	13,13	102,59	21,08	164,69	18,14	141,67	75,425	589,23	23,85	186,33
9,43	73,65	15,61	121,92	58,68	458,43	16,06	125,42	24,04	187,78	21,99	171,79	55,458	433,24	12,29	96,03
13,33	104,16	9,62	75,11	37,71	294,61	14,73	115,05	12,07	94,32	21,33	166,65	68,986	538,92	10,41	81,35
19,41	151,66	15,61	121,92	65,22	509,53	21,08	164,69	17,33	135,41	16,06	125,42	107,373	838,81	13,73	107,24
23,02	179,81	14,73	115,05	54,86	428,58	14,42	112,67	9,43	73,65	22,82	178,29	58,545	457,36	14,91	116,45
5,66	44,19	9,62	75,11	52,39	409,29	14,42	112,67	19,82	154,84	22,78	177,99	51,502	402,34	12,29	96,03
18,86	147,30	21,50	167,95	28,28	220,96	10,75	83,98	28,25	220,71	17,89	139,75	26,800	209,36	5,33	41,66
19,09	149,13	13,73	107,24	28,84	225,33	16,22	126,72	19,09	149,13	4,22	32,94	26,998	210,91	38,76	302,79
3,77	29,46	11,00	85,89	46,97	366,93	10,75	83,98	20,35	158,99	12,29	96,03	31,127	243,17	13,33	104,16
12,07	94,32	17,94	140,13	43,08	336,55	9,62	75,11	8,94	69,87	14,42	112,67	44,562	348,12	13,40	104,68
9,43	73,65	11,00	85,89	24,51	191,50	22,67	177,08	18,57	145,08	10,15	79,32	78,937	616,66	17,08	133,39
14,91	116,45	16,11	125,86	30,17	235,69	16,06	125,42	16,87	131,75	15,20	118,76	26,965	210,65	5,50	42,94
10,75	83,98	26,26	205,18	87,65	684,70	15,09	117,85	17,39	135,81	8,11	63,36	44,502	347,65	6,67	52,08
13,13	102,59	26,67	208,32	99,04	773,67	17,39	135,81	20,83	162,70	16,28	127,14	17,889	139,75	8,00	62,50
17,54	137,00	19,09	149,13	124,58	973,22	14,17	110,73	12,29	96,03	17,33	135,41	60,722	474,36	15,55	121,47
9,71	75,83	9,71	75,83	47,80	373,39	18,95	148,04	20,04	156,59	11,00	85,89	22,940	179,21	23,32	182,21
22,19	173,36	6,67	52,08	28,63	223,64	12,58	98,27	20,40	159,34	19,82	154,84	15,085	117,85	14,42	112,67
20,40	159,34	12,58	98,27	28,84	225,33	14,42	112,67	17,33	135,41	10,41	81,35	29,364	229,39	13,33	104,16
14,91	116,45	12,29	96,03	42,67	333,32	13,73	107,24	26,70	208,58	5,50	42,94	38,667	302,07	11,00	85,89
29,36	229,39	11,47	89,60	48,07	375,56	14,42	112,67	14,17	110,73	11,39	89,00	43,594	340,56	15,61	121,92
21,08	164,69	9,71	75,83	70,36	549,69	11,00	85,89	6,67	52,08	13,60	106,22	48,166	376,28	26,70	208,58
10,41	81,35	24,00	187,49	57,40	448,37	17,79	138,97	18,67	145,83	21,50	167,95	33,993	265,56	32,25	251,93
18,14	141,67	8,94	69,87	169,47	1323,87	16,22	126,72	9,71	75,83	8,43	65,88	38,827	303,32	27,49	214,73
Average	135,30		117,20		491,93		106,63		133,87		119,30		465,01		149,17
Median	140,90		115,75		442,99		108,99		135,41		118,76		358,17		121,69
SD	**46,43**		**41,63**		**254,52**		**29,62**		**43,79**		**35,62**		**343,49**		**76,91**

SV013 naïve		SV013 LPSBP		SV013 LPS		SV013 LPS+ LPSBP		SV013 Aspirin		SV013 Aspirin + LPSBP		SV013 Aspirin + LPS		SV013 Aspirin + LPS+LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
32,00	249,99	29,81	232,91	57,84	451,87	14,91	116,45	27,00	210,91	26,70	208,58	87,07	680,17	21,71	169,56
37,55	293,32	24,00	187,49	77,07	602,07	10,15	79,32	13,73	107,24	18,86	147,30	83,96	655,89	5,50	42,94
17,39	135,81	15,20	118,76	39,64	309,69	10,67	83,33	18,71	146,20	9,71	75,83	40,00	312,48	21,54	168,28
10,67	83,33	19,09	149,13	116,43	909,54	17,54	137,00	10,75	83,98	18,71	146,20	50,26	392,65	13,33	104,16
17,08	133,39	30,93	241,60	57,35	448,01	10,41	81,35	17,89	139,75	18,95	148,04	50,77	396,63	20,00	156,24
14,36	112,18	29,36	229,39	86,21	673,51	10,75	83,98	10,15	79,32	19,82	154,84	87,69	685,01	26,47	206,75
20,18	157,62	18,67	145,83	58,49	456,89	11,00	85,89	12,58	98,27	11,93	93,17	173,35	1354,25	35,50	277,35
15,20	118,76	25,05	195,70	52,07	406,76	21,54	168,28	13,73	107,24	22,67	177,08	22,78	177,99	8,43	65,88
17,33	135,41	8,54	66,69	36,97	288,84	8,43	65,88	14,42	112,67	24,51	191,50	35,50	277,35	40,90	319,52
16,06	125,42	7,18	56,09	28,00	218,74	8,54	66,69	16,22	126,72	20,40	159,34	88,94	694,84	36,61	286,02
17,39	135,81	7,78	60,74	46,67	364,57	10,75	83,98	17,89	139,75	18,86	147,30	53,67	419,24	20,18	157,62
13,40	104,68	17,33	135,41	37,43	292,39	24,33	190,08	12,65	98,81	25,65	200,36	87,81	685,96	25,47	199,00
14,67	114,58	14,91	116,45	46,05	359,77	14,73	115,05	16,87	131,75	12,29	96,03	67,99	531,12	40,20	314,05
14,73	115,05	20,83	162,70	46,21	360,97	10,15	79,32	13,40	104,68	18,86	147,30	60,00	468,72	26,83	209,62
16,28	127,14	13,40	104,68	41,85	326,90	8,54	66,69	21,50	167,95	20,00	156,24	132,42	1034,51	18,71	146,20
21,38	166,98	9,43	73,65	33,12	258,73	12,29	96,03	18,86	147,30	21,50	167,95	148,38	1159,19	31,10	242,94
16,11	125,86	19,82	154,84	33,99	265,56	18,71	146,20	35,78	279,49	27,49	214,73	160,00	1249,93	40,00	312,48
23,02	179,81	12,58	98,27	26,57	207,54	10,67	83,33	17,89	139,75	21,71	169,56	69,15	540,24	30,08	234,99
17,39	135,81	11,00	85,89	56,25	439,45	12,00	93,74	4,81	37,55	22,82	178,29	129,89	1014,70	21,87	170,83
20,35	158,99	15,09	117,85	89,44	698,74	9,43	73,65	16,22	126,72	36,78	287,34	144,03	1125,13	10,75	83,98
14,42	112,67	20,00	156,24	62,20	485,89	18,67	145,83	18,86	147,30	19,09	149,13	194,74	1521,32	18,67	145,83
14,42	112,67	4,81	37,55	70,57	551,27	16,00	124,99	6,67	52,08	9,62	75,11	248,09	1938,10	25,61	200,08
14,73	115,05	12,29	96,03	50,60	395,26	14,17	110,73	13,40	104,68	17,33	135,41	333,69	2606,77	17,54	137,00
19,82	154,84	12,65	98,81	43,37	338,80	16,87	131,75	19,82	154,84	13,92	108,74	133,13	1040,04	39,64	309,69
14,36	112,18	16,06	125,42	59,06	461,37	10,75	83,98	9,43	73,65	19,69	153,79	108,01	843,77	36,10	282,01
16,49	128,84	21,33	166,65	153,12	1196,22	19,82	154,84	12,58	98,27	22,07	172,42	62,72	490,00	28,28	220,96
17,33	135,41	15,61	121,92	35,50	277,35	17,39	135,81	13,73	107,24	30,70	239,80	189,38	1479,42	23,63	184,58
13,33	104,16	8,54	66,69	17,94	140,13	6,80	53,11	12,58	98,27	5,96	46,58	130,91	1022,69	12,58	98,27
10,75	83,98	12,58	98,27	33,12	258,73	12,29	96,03	15,20	118,76	11,93	93,17	222,68	1739,62	10,75	83,98
13,40	104,68	19,09	149,13	59,18	462,32	10,67	83,33	32,25	251,93	15,61	121,92	224,02	1750,03	14,36	112,18
17,39	135,81	16,06	125,42	66,96	523,09	11,93	93,17	18,71	146,20	14,67	114,58	101,47	792,72	22,71	177,38
19,82	154,84	13,33	104,16	23,85	186,33	18,14	141,67	16,28	127,14	20,40	159,34	59,76	466,87	13,73	107,24
14,42	112,67	8,94	69,87	29,45	230,10	13,40	104,68	29,24	228,44	12,65	98,81	44,02	343,89	9,43	73,65
14,73	115,05	34,69	271,02	50,77	396,63	15,55	121,47	14,91	116,45	19,09	149,13	140,23	1095,47	16,71	130,52
16,22	126,72	19,82	154,84	57,70	450,79	11,00	85,89	15,20	118,76	28,35	221,45	40,55	316,80	7,78	60,74
24,04	187,78	18,57	145,08	89,49	699,12	25,37	198,18	13,33	104,16	28,03	218,99	51,86	405,16	12,00	93,74
16,22	126,72	25,37	198,18	37,76	294,98	17,39	135,81	18,95	148,04	19,09	149,13	44,32	346,25	14,73	115,05
15,55	121,47	20,35	158,99	111,47	870,79	18,14	141,67	13,20	103,11	8,00	62,50	41,48	324,08	30,70	239,80
21,08	164,69	21,54	168,28	59,03	461,14	13,33	104,16	14,17	110,73	12,29	96,03	30,23	236,15	30,67	239,57
13,33	104,16	18,52	144,70	76,51	597,73	5,96	46,58	16,71	130,52	15,20	118,76	55,70	435,12	18,95	148,04
18,57	145,08	16,28	127,14	33,99	265,56	12,00	93,74	17,79	138,97	14,67	114,58	71,77	560,63	13,73	107,24
17,54	137,00	7,18	56,09	67,74	529,17	18,67	145,83	12,07	94,32	19,23	150,23	28,25	220,71	22,71	177,38
11,00	85,89	25,33	197,90	50,95	398,00	16,06	125,42	14,73	115,05	16,06	125,42	24,15	188,65	14,67	114,58
16,22	126,72	13,60	106,22	137,36	1073,06	22,71	177,38	21,50	167,95	10,41	81,35	110,96	866,79	20,83	162,70
16,22	126,72	18,67	145,83	26,67	208,32	11,47	89,60	22,78	177,99	16,49	128,84	92,27	720,82	10,75	83,98
14,42	112,67	11,00	85,89	32,93	257,26	15,55	121,47	17,94	140,13	17,54	137,00	48,30	377,28	12,00	93,74
17,33	135,41	13,73	107,24	28,54	222,92	17,79	138,97	19,09	149,13	22,78	177,99	60,37	471,61	19,23	150,23
12,65	98,81	15,20	118,76	25,61	200,08	6,80	53,11	9,43	73,65	13,92	108,74	125,43	979,83	9,71	75,83
12,29	96,03	5,66	44,19	33,65	262,89	13,92	108,74	10,75	83,98	12,29	96,03	154,56	1207,41	21,99	171,79
14,73	115,05	14,36	112,18	25,05	195,70	12,58	98,27	27,49	214,73	14,91	116,45	29,81	232,91	15,09	117,85
Average	132,07		129,86		424,63		108,85		129,65		143,77		777,54		165,68
Median	126,72		123,67		379,91		101,21		122,74		147,30		668,03		156,93
SD	**36,93**		**52,67**		**226,15**		**35,42**		**46,80**		**47,97**		**521,16**		**74,48**

SV031naive		SV031 LPSBP		SV031 LPS		SV031 LPS+ LPSBP		SV031 Aspirin		SV031 Aspirin + LPSBP		SV031 Aspirin + LPS		SV031 Aspirin + LPS +LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
21,87	170,83	22,78	177,99	105,64	825,24	16,87	131,75	13,33	104,16	12,07	94,32	51,03	398,68	22,94	179,21
18,52	144,70	15,55	121,47	57,89	452,23	17,94	140,13	8,94	69,87	20,35	158,99	67,42	526,71	21,50	167,95
20,70	161,70	22,71	177,38	113,43	886,10	13,92	108,74	4,00	31,25	25,37	198,18	16,71	130,52	33,44	261,24
14,17	110,73	16,49	128,84	188,53	1472,84	18,71	146,20	5,33	41,66	17,08	133,39	104,04	812,79	16,87	131,75
17,08	133,39	9,62	75,11	70,97	554,41	18,52	144,70	17,89	139,75	19,23	150,23	60,37	471,61	15,61	121,92
9,43	73,65	24,04	187,78	54,86	428,58	12,00	93,74	12,65	98,81	15,55	121,47	63,23	493,97	12,29	96,03
13,33	104,16	12,65	98,81	20,04	156,59	12,29	96,03	15,20	118,76	20,70	161,70	135,38	1057,58	19,69	153,79
11,39	89,00	10,67	83,33	46,67	364,57	13,13	102,59	15,55	121,47	12,65	98,81	14,42	112,67	29,61	231,28
8,94	69,87	21,38	166,98	28,72	224,37	23,29	181,91	6,80	53,11	18,57	145,08	25,61	200,08	16,97	132,58
7,18	56,09	13,60	106,22	44,32	346,25	10,15	79,32	5,66	44,19	20,00	156,24	67,99	531,12	27,46	214,48
24,04	187,78	23,29	181,91	29,45	230,10	15,20	118,76	17,54	137,00	14,91	116,45	211,87	1655,14	25,65	200,36
9,43	73,65	18,14	141,67	40,55	316,80	17,33	135,41	8,94	69,87	13,33	104,16	36,27	283,35	18,86	147,30
13,33	104,16	13,40	104,68	284,01	2218,73	16,22	126,72	7,54	58,92	13,40	104,68	43,59	340,56	28,63	223,64
15,61	121,92	7,18	56,09	70,78	552,94	14,42	112,67	17,54	137,00	9,62	75,11	76,09	594,45	18,86	147,30
14,91	116,45	18,71	146,20	24,59	192,06	8,54	66,69	12,07	94,32	16,06	125,42	60,24	470,58	11,00	85,89
12,00	93,74	13,40	104,68	277,35	2166,65	19,82	154,84	17,33	135,41	16,00	124,99	71,28	556,84	20,18	157,62
9,62	75,11	19,82	154,84	79,39	620,18	11,00	85,89	20,70	161,70	17,89	139,75	116,19	907,69	32,11	250,85
25,33	197,90	14,36	112,18	498,67	3895,68	12,29	96,03	13,33	104,16	22,71	177,38	127,28	994,34	28,28	220,96
16,49	128,84	12,65	98,81	473,52	3699,18	9,33	72,91	15,09	117,85	18,14	141,67	91,02	711,05	22,71	177,38
15,20	118,76	11,31	88,39	52,02	406,36	11,39	89,00	14,91	116,45	12,65	98,81	64,01	500,08	16,87	131,75
13,92	108,74	21,87	170,83	78,50	613,22	10,75	83,98	13,40	104,68	12,00	93,74	103,86	811,39	12,29	96,03
6,80	53,11	18,71	146,20	109,33	854,12	14,67	114,58	23,59	184,28	20,00	156,24	151,84	1186,15	17,94	140,13
20,40	159,34	16,00	124,99	76,29	596,00	20,18	157,62	14,42	112,67	16,06	125,42	169,71	1325,76	18,52	144,70
19,41	151,66	15,55	121,47	140,01	1093,74	14,73	115,05	17,39	135,81	16,11	125,86	81,73	638,45	24,51	191,50
13,13	102,59	12,65	98,81	76,42	597,00	22,07	172,42	14,91	116,45	13,33	104,16	157,91	1233,60	16,49	128,84
12,58	98,27	18,71	146,20	69,05	539,43	7,78	60,74	13,13	102,59	14,67	114,58	132,54	1035,40	19,41	151,66
13,13	102,59	16,11	125,86	112,46	878,54	12,29	96,03	14,42	112,67	7,78	60,74	118,07	922,34	20,00	156,24
18,14	141,67	11,47	89,60	171,86	1342,54	13,73	107,24	13,13	102,59	9,43	73,65	100,22	782,94	10,15	79,32
10,67	83,33	21,71	169,56	105,22	822,02	13,73	107,24	24,04	187,78	10,15	79,32	70,60	551,56	14,42	112,67
9,62	75,11	24,51	191,50	93,42	729,80	10,41	81,35	13,73	107,24	17,33	135,41	47,35	369,88	10,67	83,33
9,43	73,65	20,83	162,70	63,36	494,96	9,43	73,65	9,33	72,91	12,00	93,74	43,08	336,55	10,75	83,98
16,00	124,99	11,39	89,00	88,38	690,45	14,36	112,18	8,94	69,87	25,65	200,36	55,84	436,23	20,04	156,59
13,73	107,24	10,41	81,35	128,84	1006,49	25,05	195,70	14,17	110,73	16,28	127,14	198,21	1548,46	12,65	98,81
17,54	137,00	14,73	115,05	159,43	1245,46	25,75	201,17	5,66	44,19	15,55	121,47	213,21	1665,60	21,99	171,79
14,67	114,58	10,15	79,32	121,52	949,29	11,00	85,89	14,73	115,05	9,71	75,83	59,58	465,47	20,40	159,34
11,39	89,00	16,28	127,14	146,72	1146,20	13,13	102,59	21,50	167,95	18,14	141,67	192,68	1505,19	21,33	166,65
14,67	114,58	26,80	209,36	73,77	576,28	13,60	106,22	18,86	147,30	13,33	104,16	113,99	890,50	21,33	166,65
17,33	135,41	14,42	112,67	145,72	1138,41	13,13	102,59	5,96	46,58	17,33	135,41	76,65	598,81	20,00	156,24
15,61	121,92	24,91	194,59	178,67	1395,80	13,40	104,68	13,73	107,24	14,67	114,58	114,14	891,65	16,06	125,42
16,49	128,84	27,75	216,75	102,67	802,04	13,73	107,24	13,33	104,16	14,42	112,67	155,41	1214,09	9,71	75,83
8,43	65,88	24,80	193,75	116,12	907,16	14,36	112,18	10,15	79,32	10,41	81,35	120,27	939,53	11,39	89,00
14,73	115,05	21,08	164,69	133,36	1041,82	9,43	73,65	22,19	173,36	17,08	133,39	124,68	974,00	13,92	108,74
17,89	139,75	17,94	140,13	118,09	922,51	7,78	60,74	14,42	112,67	20,40	159,34	60,30	471,04	18,67	145,83
24,04	187,78	18,52	144,70	118,67	927,04	11,93	93,17	11,39	89,00	19,41	151,66	125,57	980,94	10,75	83,98
16,28	127,14	17,39	135,81	130,84	1022,11	13,73	107,24	9,62	75,11	17,39	135,81	81,43	636,15	14,91	116,45
12,07	94,32	16,28	127,14	170,85	1334,72	12,00	93,74	8,94	69,87	17,39	135,81	163,54	1277,58	15,55	121,47
18,71	146,20	22,67	177,08	105,37	823,13	14,91	116,45	9,33	72,91	9,62	75,11	101,97	796,61	5,96	46,58
12,65	98,81	33,99	265,56	81,51	636,75	21,87	170,83	9,43	73,65	13,13	102,59	147,57	1152,80	17,54	137,00
16,06	125,42	18,52	144,70	140,43	1097,06	26,30	205,43	10,15	79,32	15,20	118,76	171,09	1336,55	14,91	116,45
12,58	98,27	30,78	240,47	148,60	1160,87	16,87	131,75	6,80	53,11	13,40	104,68	66,67	520,81	18,14	141,67
Average	115,09		140,41		947,90		114,75		101,74		122,43		784,92		143,72
Median	114,58		137,97		824,19		107,24		104,16		123,23		746,99		143,19
SD	**33,84**		**45,00**		**730,43**		**35,74**		**38,06**		**31,22**		**398,95**		**47,23**

Mr L naïve		Mr L LPSBP		Mr L LPS		Mr L LPS+LPSBP		Mr L Aspirin		Mr L Aspirin + LPSBP		Mr L Aspirin + LPS		MrL Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scae bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
10,67	83,33	13,20	103,11	73,96	577,79	17,39	135,81	13,60	106,22	22,82	178,29	382,40	2987,37	14,73	115,05
14,17	110,73	11,47	89,60	192,91	1507,03	9,43	73,65	14,17	110,73	11,31	88,39	135,35	1057,37	15,20	118,76
20,00	156,24	23,32	182,21	42,69	333,47	16,28	127,14	12,29	96,03	10,15	79,32	115,48	902,12	17,54	137,00
5,33	41,66	10,15	79,32	98,13	766,56	14,73	115,05	12,29	96,03	13,20	103,11	108,92	850,88	15,55	121,47
9,43	73,65	9,62	75,11	40,11	313,35	12,07	94,32	18,67	145,83	18,86	147,30	182,02	1421,98	16,22	126,72
10,15	79,32	13,60	106,22	106,14	829,17	12,07	94,32	13,33	104,16	23,32	182,21	238,94	1866,58	17,94	140,13
8,54	66,69	12,07	94,32	51,10	399,22	20,00	156,24	18,86	147,30	18,71	146,20	420,65	3286,11	16,06	125,42
12,00	93,74	15,20	118,76	25,65	200,36	16,06	125,42	14,91	116,45	6,67	52,08	60,60	473,44	17,39	135,81
17,33	135,41	22,78	177,99	24,04	187,78	8,94	69,87	20,70	161,70	17,33	135,41	45,12	352,46	18,67	145,83
16,49	128,84	10,75	83,98	20,00	156,24	14,42	112,67	19,82	154,84	11,00	85,89	52,93	413,51	13,33	104,16
8,11	63,36	14,17	110,73	28,00	218,74	12,58	98,27	6,67	52,08	16,97	132,58	16,28	127,14	15,20	118,76
9,71	75,83	13,33	104,16	37,14	290,16	21,33	166,65	10,41	81,35	14,73	115,05	17,54	137,00	19,23	150,23
18,71	146,20	14,42	112,67	27,49	214,73	18,57	145,08	7,78	60,74	13,92	108,74	112,08	875,57	16,06	125,42
12,29	96,03	6,67	52,08	24,59	192,06	14,91	116,45	15,61	121,92	16,22	126,72	170,11	1328,90	13,92	108,74
12,00	93,74	5,50	42,94	20,31	158,66	12,00	93,74	14,17	110,73	8,54	66,69	168,19	1313,91	16,06	125,42
10,75	83,98	14,91	116,45	66,79	521,74	16,71	130,52	11,39	89,00	8,94	69,87	84,68	661,49	30,67	239,57
14,17	110,73	11,39	89,00	70,36	549,69	15,20	118,76	11,00	85,89	22,63	176,76	33,94	265,15	18,86	147,30
11,31	88,39	18,86	147,30	31,69	247,59	19,23	150,23	12,65	98,81	12,00	93,74	29,96	234,07	26,47	206,75
6,67	52,08	16,06	125,42	43,41	339,12	10,67	83,33	5,96	46,58	27,36	213,72	54,28	424,00	12,00	93,74
5,33	41,66	14,17	110,73	148,21	1157,83	9,43	73,65	22,71	177,38	13,92	108,74	17,94	140,13	10,75	83,98
16,06	125,42	8,11	63,36	20,00	156,24	6,67	52,08	13,73	107,24	13,33	104,16	46,57	363,82	18,14	141,67
22,71	177,38	17,54	137,00	41,18	321,72	11,93	93,17	18,71	146,20	15,61	121,92	25,65	200,36	12,65	98,81
9,43	73,65	18,14	141,67	49,82	389,17	14,42	112,67	21,99	171,79	17,39	135,81	106,58	832,57	21,87	170,83
5,96	46,58	16,06	125,42	95,59	746,77	13,33	104,16	22,35	174,61	18,86	147,30	355,46	2776,88	5,33	41,66
10,41	81,35	13,60	106,22	46,97	366,93	8,43	65,88	14,42	112,67	9,43	73,65	47,74	372,95	8,00	62,50
12,29	96,03	18,95	148,04	113,20	884,33	9,43	73,65	11,93	93,17	6,80	53,11	26,70	208,58	18,67	145,83
18,95	148,04	20,70	161,70	91,39	713,94	11,39	89,00	28,00	218,74	11,39	89,00	52,88	413,11	20,74	162,04
16,11	125,86	11,00	85,89	173,52	1355,57	5,96	46,58	16,22	126,72	10,15	79,32	44,32	346,25	17,39	135,81
8,00	62,50	16,00	124,99	148,57	1160,63	9,33	72,91	5,96	46,58	26,67	208,32	71,62	559,47	9,62	75,11
25,05	195,70	12,29	96,03	191,79	1498,25	6,80	53,11	16,11	125,86	20,00	156,24	68,88	538,12	13,13	102,59
29,96	234,07	14,67	114,58	272,94	2132,25	5,66	44,19	23,29	181,91	17,33	135,41	33,12	258,73	16,22	126,72
14,91	116,45	9,43	73,65	231,94	1811,92	10,67	83,33	18,86	147,30	18,57	145,08	61,07	477,10	16,71	130,52
22,67	177,08	16,28	127,14	224,50	1753,77	16,22	126,72	16,71	130,52	20,35	158,99	216,72	1693,06	17,08	133,39
13,33	104,16	5,96	46,58	194,14	1516,60	17,89	139,75	11,47	89,60	11,31	88,39	174,71	1364,86	21,50	167,95
14,67	114,58	15,20	118,76	50,60	395,26	13,33	104,16	9,71	75,83	14,67	114,58	190,26	1486,33	17,33	135,41
18,86	147,30	14,73	115,05	137,75	1076,14	18,57	145,08	10,15	79,32	15,20	118,76	33,12	258,73	16,11	125,86
13,33	104,16	10,75	83,98	94,90	741,37	18,67	145,83	9,62	75,11	12,29	96,03	17,33	135,41	13,20	103,11
17,79	138,97	20,04	156,59	24,04	187,78	17,54	137,00	7,78	60,74	17,89	139,75	29,36	229,39	17,33	135,41
15,55	121,47	11,39	89,00	28,28	220,96	15,09	117,85	16,06	125,42	16,71	130,52	14,17	110,73	12,65	98,81
12,00	93,74	20,00	156,24	27,20	212,45	12,29	96,03	11,39	89,00	14,91	116,45	57,47	448,98	16,97	132,58
5,96	46,58	9,71	75,83	12,29	96,03	13,73	107,24	16,28	127,14	14,91	116,45	27,46	214,48	10,75	83,98
12,00	93,74	13,73	107,24	30,67	239,57	15,55	121,47	11,31	88,39	16,06	125,42	20,40	159,34	17,94	140,13
13,33	104,16	16,06	125,42	22,71	177,38	16,28	127,14	10,15	79,32	20,70	161,70	110,41	862,52	18,95	148,04
14,73	115,05	11,00	85,89	36,42	284,49	10,41	81,35	16,87	131,75	11,31	88,39	67,70	528,87	12,29	96,03
17,54	137,00	10,67	83,33	41,87	327,08	17,89	139,75	16,28	127,14	10,67	83,33	28,50	222,67	12,58	98,27
11,31	88,39	15,20	118,76	193,82	1514,10	14,42	112,67	8,43	65,88	10,15	79,32	74,14	579,19	10,75	83,98
19,09	149,13	9,43	73,65	118,94	929,14	16,22	126,72	13,33	104,16	29,36	229,39	30,70	239,80	16,87	131,75
6,67	52,08	28,78	224,85	137,81	1076,54	14,17	110,73	24,80	193,75	16,06	125,42	16,00	124,99	13,73	107,24
16,06	125,42	24,15	188,65	334,04	2609,54	8,00	62,50	13,60	106,22	24,15	188,65	62,15	485,55	18,14	141,67
12,00	93,74	6,80	53,11	243,89	1905,26	14,42	112,67	10,75	83,98	9,71	75,83	20,74	162,04	9,62	75,11
Average	106,23		110,62		719,25		105,73		113,00		121,95		715,48		124,06
Median	100,10		108,99		397,24		111,70		106,73		117,61		436,49		126,29
SD	**41,03**		**37,97**		**618,52**		**30,43**		**39,33**		**41,54**		**741,17**		**33,93**

Mr P naïve		Mr P LPSBP		Mr P LPS		Mr P LPSBP+ LPS		Mr P Aspirin		Mr P Aspirin + LPSBP		Mr P Aspirin + LPS		Mr P Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
14,17	110,73	18,14	141,67	181,93	1421,22	14,91	116,45	9,43	73,65	21,54	168,28	131,47	1027,03	16,71	130,52
11,93	93,17	11,93	93,17	70,45	550,38	18,14	141,67	13,92	108,74	16,00	124,99	92,88	725,55	19,41	151,66
9,62	75,11	9,71	75,83	105,75	826,10	16,11	125,86	13,73	107,24	21,99	171,79	226,13	1766,50	12,29	96,03
13,60	106,22	14,91	116,45	91,50	714,77	20,40	159,34	11,39	89,00	12,65	98,81	189,78	1482,61	15,20	118,76
16,06	125,42	20,70	161,70	20,35	158,99	10,41	81,35	9,71	75,83	17,94	140,13	209,55	1637,05	17,94	140,13
16,00	124,99	13,33	104,16	112,96	882,48	13,33	104,16	18,86	147,30	15,20	118,76	53,33	416,64	16,71	130,52
11,93	93,17	22,71	177,38	88,65	692,57	12,29	96,03	12,65	98,81	12,00	93,74	122,73	958,79	19,09	149,13
11,39	89,00	14,17	110,73	35,90	280,46	15,20	118,76	17,54	137,00	15,61	121,92	177,77	1388,78	18,67	145,83
8,94	69,87	14,17	110,73	232,00	1812,43	15,20	118,76	17,39	135,81	13,33	104,16	54,47	425,53	25,89	202,25
9,62	75,11	5,66	44,19	236,96	1851,16	16,28	127,14	20,04	156,59	14,17	110,73	76,00	593,72	11,39	89,00
17,08	133,39	11,39	89,00	63,39	495,18	14,67	114,58	16,22	126,72	20,00	156,24	99,00	773,39	16,87	131,75
6,80	53,11	16,49	128,84	81,87	639,55	17,33	135,41	8,43	65,88	11,47	89,60	84,26	658,28	12,58	98,27
10,75	83,98	13,33	104,16	204,50	1597,57	25,16	196,53	14,36	112,18	13,33	104,16	155,48	1214,58	28,54	222,92
8,00	62,50	9,33	72,91	76,93	600,98	8,94	69,87	11,31	88,39	13,33	104,16	156,01	1218,73	13,33	104,16
11,31	88,39	11,00	85,89	32,22	251,71	13,33	104,16	15,55	121,47	14,91	116,45	139,54	1090,07	9,43	73,65
11,31	88,39	10,75	83,98	26,70	208,58	17,39	135,81	15,09	117,85	14,17	110,73	185,45	1448,77	20,31	158,66
8,94	69,87	12,58	98,27	43,53	340,08	22,82	178,29	12,58	98,27	18,67	145,83	193,79	1513,93	16,11	125,86
13,33	104,16	14,36	112,18	33,41	261,02	13,60	106,22	8,94	69,87	11,93	93,17	142,67	1114,57	22,82	178,29
16,28	127,14	8,11	63,36	166,80	1303,05	12,58	98,27	16,06	125,42	22,35	174,61	66,95	522,99	10,15	79,32
19,23	150,23	10,67	83,33	126,04	984,64	19,41	151,66	18,57	145,08	16,06	125,42	69,65	544,13	12,65	98,81
14,91	116,45	14,73	115,05	232,03	1812,67	6,67	52,08	12,58	98,27	17,54	137,00	89,89	702,22	18,57	145,08
11,93	93,17	11,93	93,17	188,01	1468,71	9,43	73,65	15,20	118,76	11,93	93,17	167,99	1312,34	12,29	96,03
13,92	108,74	11,93	93,17	81,38	635,72	12,00	93,74	8,94	69,87	20,83	162,70	133,33	1041,61	13,60	106,22
10,75	83,98	14,91	116,45	39,64	309,69	16,11	125,86	10,41	81,35	17,08	133,39	87,65	684,70	21,33	166,65
8,54	66,69	10,75	83,98	57,35	448,01	9,62	75,11	20,83	162,70	11,39	89,00	106,98	835,70	17,54	137,00
15,20	118,76	16,28	127,14	148,02	1156,37	17,39	135,81	14,91	116,45	9,43	73,65	95,42	745,46	18,67	145,83
24,91	194,59	12,29	96,03	106,68	833,35	14,67	114,58	13,33	104,16	19,09	149,13	121,63	950,15	21,08	164,69
10,75	83,98	19,09	149,13	106,80	834,33	13,92	108,74	15,20	118,76	8,94	69,87	149,43	1167,35	22,63	176,76
10,15	79,32	17,08	133,39	64,35	502,68	10,75	83,98	13,13	102,59	10,75	83,98	76,51	597,73	18,52	144,70
16,49	128,84	17,94	140,13	119,74	935,42	14,67	114,58	17,39	135,81	8,54	66,69	141,39	1104,55	17,54	137,00
14,36	112,18	11,93	93,17	104,14	813,53	10,41	81,35	18,86	147,30	12,65	98,81	167,58	1309,15	8,43	65,88
17,79	138,97	17,08	133,39	100,57	785,64	10,41	81,35	13,92	108,74	15,20	118,76	206,46	1612,85	12,29	96,03
11,47	89,60	18,52	144,70	31,24	244,06	8,00	62,50	27,49	214,73	13,92	108,74	34,90	272,62	14,73	115,05
14,73	115,05	15,20	118,76	61,70	481,97	16,22	126,72	19,23	150,23	16,28	127,14	123,43	964,26	14,17	110,73
13,40	104,68	13,92	108,74	89,37	698,19	8,00	62,50	9,71	75,83	10,15	79,32	95,82	748,58	18,52	144,70
16,11	125,86	14,17	110,73	34,69	271,02	11,39	89,00	11,39	89,00	33,76	263,71	193,07	1508,28	18,14	141,67
23,59	184,28	21,50	167,95	124,99	976,45	15,20	118,76	6,67	52,08	20,18	157,62	91,21	712,57	32,00	249,99
13,33	104,16	9,62	75,11	80,99	632,73	8,43	65,88	12,58	98,27	9,71	75,83	217,30	1697,57	11,93	93,17
19,23	150,23	13,92	108,74	24,59	192,06	24,51	191,50	18,52	144,70	14,42	112,67	283,24	2212,65	15,20	118,76
17,54	137,00	19,09	149,13	135,35	1057,37	16,06	125,42	13,60	106,22	11,39	89,00	185,35	1447,99	10,75	83,98
6,67	52,08	5,33	41,66	38,18	298,27	14,67	114,58	24,04	187,78	18,14	141,67	141,53	1105,67	13,40	104,68
10,75	83,98	6,67	52,08	19,82	154,84	8,43	65,88	20,83	162,70	17,79	138,97	233,43	1823,56	11,39	89,00
17,39	135,81	7,18	56,09	47,70	372,66	21,50	167,95	20,04	156,59	14,91	116,45	150,69	1177,20	13,92	108,74
17,33	135,41	14,91	116,45	83,58	652,90	7,78	60,74	16,06	125,42	11,39	89,00	256,59	2004,47	9,33	72,91
7,78	60,74	13,73	107,24	101,76	794,97	8,11	63,36	12,58	98,27	11,93	93,17	297,33	2322,79	17,54	137,00
17,33	135,41	21,54	168,28	45,82	357,96	8,43	65,88	11,00	85,89	16,97	132,58	257,34	2010,34	14,73	115,05
6,67	52,08	9,71	75,83	106,74	833,88	13,73	107,24	21,54	168,28	13,73	107,24	128,34	1002,60	13,33	104,16
10,75	83,98	9,43	73,65	55,46	433,24	22,94	179,21	21,08	164,69	10,15	79,32	117,40	917,15	20,35	158,99
9,43	73,65	10,15	79,32	194,40	1518,64	7,78	60,74	12,00	93,74	13,92	108,74	165,47	1292,65	17,08	133,39
15,20	118,76	11,00	85,89	113,33	885,37	6,67	52,08	15,20	118,76	16,00	124,99	125,26	978,50	12,00	93,74
Average	103,85		105,45		745,31		108,02		117,18		117,92		1135,70		127,66
Median	104,16		105,70		672,74		107,99		114,32		111,70		1097,31		128,19
SD	**32,02**		**32,24**		**460,53**		**37,27**		**34,06**		**34,86**		**480,08**		**37,77**

SV021 naïve		SV021 LPSBP		SV021 LPS		SV021 LPS + LPSBP		SV021 Aspirin		SV021 Aspirin + LPSBP		SV021 Aspirin+ LPS		sv021 Aspirin+ LPS+LPSBP	
Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
10,75	83,98	16,06	125,42	20,83	162,70	19,09	149,13	13,60	106,22	12,00	93,74	105,05	820,69	20,04	156,59
8,54	66,69	8,11	63,36	26,30	205,43	10,67	83,33	13,92	108,74	16,00	124,99	333,88	2608,29	11,93	93,17
16,22	126,72	10,75	83,98	16,28	127,14	18,86	147,30	19,82	154,84	14,67	114,58	68,73	536,91	11,47	89,60
13,33	104,16	5,96	46,58	140,80	1099,97	6,80	53,11	13,60	106,22	21,99	171,79	70,42	550,09	14,73	115,05
9,43	73,65	8,11	63,36	78,30	611,72	16,28	127,14	11,93	93,17	10,75	83,98	167,35	1307,37	14,91	116,45
6,67	52,08	9,62	75,11	23,29	181,91	17,54	137,00	24,59	192,06	8,54	66,69	44,50	347,65	19,09	149,13
12,29	96,03	13,33	104,16	44,18	345,15	32,47	253,65	13,73	107,24	7,18	56,09	75,63	590,79	5,96	46,58
7,54	58,92	14,67	114,58	97,42	761,01	18,14	141,67	12,29	96,03	9,33	72,91	319,63	2496,93	18,14	141,67
11,93	93,17	9,71	75,83	95,18	743,57	25,33	197,90	15,61	121,92	25,61	200,08	66,20	517,14	13,33	104,16
16,71	130,52	8,94	69,87	185,57	1449,67	15,20	118,76	14,91	116,45	17,79	138,97	43,68	341,20	13,92	108,74
2,98	23,29	11,00	85,89	97,26	759,80	20,00	156,24	12,65	98,81	14,67	114,58	37,05	289,41	9,33	72,91
12,29	96,03	9,62	75,11	100,14	782,32	16,00	124,99	20,87	163,04	16,97	132,58	56,02	437,60	9,43	73,65
15,61	121,92	22,19	173,36	118,67	927,09	12,65	98,81	22,19	173,36	16,49	128,84	74,49	581,91	14,17	110,73
14,17	110,73	13,33	104,16	45,12	352,46	20,00	156,24	10,75	83,98	25,16	196,53	46,67	364,57	14,17	110,73
9,33	72,91	14,36	112,18	13,13	102,59	16,22	126,72	11,47	89,60	8,43	65,88	97,56	762,15	13,33	104,16
13,40	104,68	20,00	156,24	64,06	500,41	8,94	69,87	12,65	98,81	15,20	118,76	55,97	437,23	18,71	146,20
12,07	94,32	15,20	118,76	22,71	177,38	18,14	141,67	13,92	108,74	14,17	110,73	67,74	529,17	15,20	118,76
11,31	88,39	13,40	104,68	46,28	361,57	16,06	125,42	21,08	164,69	12,29	96,03	50,40	393,75	14,17	110,73
7,18	56,09	18,14	141,67	110,64	864,29	12,58	98,27	10,75	83,98	9,62	75,11	41,40	323,40	7,78	60,74
14,67	114,58	26,57	207,54	96,59	754,58	15,55	121,47	18,52	144,70	10,67	83,33	182,67	1427,05	13,73	107,24
12,29	96,03	16,97	132,58	132,06	1031,67	13,60	106,22	7,78	60,74	13,40	104,68	154,63	1208,00	7,78	60,74
7,78	60,74	8,94	69,87	70,72	552,45	10,41	81,35	14,17	110,73	8,00	62,50	86,82	678,24	9,33	72,91
7,54	58,92	19,23	150,23	31,13	243,17	13,92	108,74	36,00	281,23	14,17	110,73	33,73	263,51	15,55	121,47
17,54	137,00	10,15	79,32	41,42	323,57	12,58	98,27	16,22	126,72	12,29	96,03	40,02	312,65	12,58	98,27
17,39	135,81	15,20	118,76	56,02	437,60	16,49	128,84	8,94	69,87	11,93	93,17	50,68	395,95	13,33	104,16
14,42	112,67	13,13	102,59	77,38	604,49	17,33	135,41	16,22	126,72	10,75	83,98	43,04	336,23	18,57	145,08
7,78	60,74	15,20	118,76	112,13	875,94	15,20	118,76	10,67	83,33	11,00	85,89	55,83	436,11	6,80	53,11
16,87	131,75	14,91	116,45	9,33	72,91	14,73	115,05	13,33	104,16	13,33	104,16	29,45	230,10	8,11	63,36
10,41	81,35	17,94	140,13	40,11	313,35	15,20	118,76	13,13	102,59	14,91	116,45	42,37	331,03	14,42	112,67
17,54	137,00	11,39	89,00	17,33	135,41	13,33	104,16	19,09	149,13	12,00	93,74	37,57	293,51	12,00	93,74
10,15	79,32	14,73	115,05	18,52	144,70	7,54	58,92	16,22	126,72	17,79	138,97	43,68	341,20	17,39	135,81
8,94	69,87	12,29	96,03	266,88	2084,89	12,65	98,81	23,29	181,91	13,73	107,24	21,71	169,56	12,58	98,27
10,41	81,35	18,71	146,20	91,15	712,04	9,43	73,65	15,09	117,85	12,58	98,27	26,70	208,58	16,11	125,86
9,33	72,91	16,06	125,42	82,65	645,63	11,93	93,17	21,54	168,28	17,89	139,75	22,67	177,08	24,04	187,78
14,91	116,45	10,75	83,98	77,95	608,97	11,93	93,17	17,39	135,81	15,09	117,85	13,73	107,24	11,00	85,89
13,92	108,74	12,07	94,32	20,00	156,24	22,67	177,08	14,17	110,73	17,08	133,39	23,85	186,33	19,09	149,13
12,29	96,03	13,20	103,11	86,68	677,13	17,54	137,00	14,73	115,05	13,13	102,59	31,13	243,17	17,39	135,81
16,06	125,42	12,29	96,03	79,70	622,61	8,43	65,88	6,67	52,08	16,49	128,84	44,72	349,36	19,09	149,13
8,54	66,69	19,09	149,13	22,82	178,29	22,71	177,38	20,00	156,24	20,00	156,24	45,41	354,76	12,65	98,81
9,62	75,11	21,50	167,95	101,34	791,69	18,14	141,67	16,06	125,42	11,31	88,39	61,17	477,90	16,06	125,42
15,20	118,76	17,33	135,41	35,30	275,78	18,14	141,67	17,33	135,41	20,31	158,66	55,97	437,23	25,33	197,90
12,07	94,32	8,54	66,69	37,55	293,32	20,18	157,62	9,71	75,83	8,54	66,69	90,91	710,20	14,67	114,58
14,91	116,45	18,57	145,08	32,03	250,21	23,63	184,58	10,67	83,33	11,31	88,39	27,49	214,73	14,42	112,67
8,54	66,69	9,62	75,11	48,19	376,42	21,33	166,65	13,20	103,11	5,33	41,66	50,47	394,30	16,87	131,75
24,48	191,22	13,33	104,16	75,43	589,23	20,74	162,04	8,43	65,88	8,94	69,87	119,41	932,86	16,06	125,42
4,22	32,94	20,00	156,24	43,18	337,36	22,35	174,61	14,36	112,18	18,14	141,67	134,00	1046,80	20,00	156,24
10,15	79,32	15,09	117,85	56,73	443,14	6,80	53,11	14,36	112,18	13,60	106,22	133,10	1039,79	18,86	147,30
16,06	125,42	13,33	104,16	82,46	644,20	17,39	135,81	12,07	94,32	10,75	83,98	78,71	614,90	16,97	132,58
18,52	144,70	13,73	107,24	54,14	422,98	16,00	124,99	12,29	96,03	9,62	75,11	66,00	515,57	15,09	117,85
11,00	85,89	16,00	124,99	28,03	218,99	16,22	126,72	12,29	96,03	9,62	75,11	32,11	250,85	10,15	79,32
Average	94,57		110,67		526,70		125,78		117,85		106,33		578,42		113,40
Median	94,32		105,96		440,37		126,07		109,74		103,37		416,03		112,67
SD	**31,68**		**33,04**		**376,15**		**39,32**		**39,42**		**34,09**		**504,66**		**32,52**

SV022 naïve		SV022 LPSBP		SV022 LPS		SV022 LPS+LPSBP		SV022 Aspirin		SV022 Aspirin +LPSBP		SV022 Aspirin + LPS		SV022 Aspirin + LPS+LPSBP	
Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm	Length	nm
	128,01		128,01		128,01		128,01		128,01		128,01		128,01		128,01
22,07	172,42	35,43	276,77	88,49	691,32	17,79	138,97	20,18	157,62	14,42	112,67	160,58	1254,44	15,20	118,76
15,20	118,76	10,15	79,32	49,82	389,17	20,35	158,99	25,37	198,18	13,40	104,68	120,36	940,28	13,60	106,22
8,54	66,69	17,79	138,97	22,67	177,08	12,29	96,03	24,04	187,78	15,55	121,47	40,11	313,35	16,28	127,14
13,13	102,59	31,81	248,46	39,03	304,93	12,29	96,03	14,36	112,18	9,43	73,65	64,80	506,23	26,47	206,75
14,67	114,58	27,49	214,73	41,42	323,57	9,62	75,11	20,70	161,70	15,20	118,76	40,49	316,28	27,00	210,91
5,66	44,19	19,09	149,13	74,77	584,14	26,70	208,58	20,04	156,59	12,29	96,03	43,98	343,57	28,25	220,71
8,43	65,88	24,04	187,78	71,38	557,63	28,25	220,71	11,00	85,89	11,31	88,39	52,93	413,51	20,35	158,99
22,07	172,42	17,79	138,97	36,03	281,43	16,71	130,52	17,39	135,81	16,87	131,75	51,52	402,47	11,47	89,60
9,43	73,65	5,33	41,66	190,90	1491,36	14,42	112,67	20,87	163,04	19,23	150,23	59,40	464,07	11,47	89,60
20,00	156,24	29,45	230,10	93,99	734,24	11,39	89,00	17,54	137,00	20,31	158,66	5,96	46,58	14,17	110,73
10,75	83,98	10,75	83,98	76,37	596,63	26,70	208,58	23,74	185,45	15,55	121,47	56,85	444,12	11,47	89,60
12,29	96,03	35,90	280,46	38,67	302,07	9,71	75,83	7,54	58,92	15,09	117,85	262,52	2050,81	20,04	156,59
12,29	96,03	16,11	125,86	48,00	374,98	21,50	167,95	16,22	126,72	13,33	104,16	202,20	1579,58	18,52	144,70
8,94	69,87	12,07	94,32	28,25	220,71	11,39	89,00	13,20	103,11	14,17	110,73	195,71	1528,86	11,47	89,60
14,91	116,45	50,21	392,23	26,57	207,54	13,40	104,68	13,33	104,16	16,49	128,84	49,50	386,66	17,39	135,81
14,17	110,73	16,06	125,42	112,23	876,75	11,93	93,17	8,94	69,87	13,13	102,59	172,83	1350,12	15,20	118,76
18,86	147,30	11,93	93,17	176,69	1380,32	20,18	157,62	13,33	104,16	14,91	116,45	340,09	2656,84	10,15	79,32
15,61	121,92	22,07	172,42	374,15	2922,92	11,39	89,00	16,06	125,42	18,14	141,67	118,70	927,27	14,17	110,73
17,08	133,39	10,75	83,98	51,23	400,17	10,15	79,32	12,29	96,03	14,17	110,73	111,06	867,61	15,20	118,76
8,43	65,88	17,89	139,75	257,47	2011,38	14,91	116,45	17,54	137,00	26,47	206,75	54,00	421,82	17,39	135,81
14,17	110,73	23,29	181,91	81,33	635,38	19,69	153,79	11,31	88,39	16,97	132,58	60,30	471,04	9,71	75,83
14,91	116,45	16,06	125,42	117,86	920,69	19,82	154,84	19,23	150,23	6,67	52,08	29,61	231,28	16,28	127,14
22,07	172,42	11,00	85,89	157,59	1231,08	17,94	140,13	19,82	154,84	26,30	205,43	22,07	172,42	11,39	89,00
16,71	130,52	11,00	85,89	154,03	1203,32	25,89	202,25	10,41	81,35	15,55	121,47	152,64	1192,45	22,19	173,36
18,14	141,67	13,92	108,74	104,42	815,72	11,39	89,00	11,39	89,00	11,93	93,17	24,59	192,06	16,11	125,86
16,71	130,52	10,75	83,98	148,05	1156,61	14,36	112,18	16,00	124,99	14,91	116,45	21,08	164,69	14,91	116,45
10,15	79,32	13,13	102,59	100,81	787,57	7,78	60,74	17,33	135,41	14,91	116,45	110,96	866,79	7,18	56,09
16,97	132,58	17,33	135,41	317,92	2483,60	11,00	85,89	21,38	166,98	18,71	146,20	133,76	1044,94	22,07	172,42
11,00	85,89	26,30	205,43	313,70	2450,64	18,95	148,04	10,41	81,35	14,91	116,45	49,35	385,53	11,47	89,60
16,97	132,58	17,39	135,81	266,54	2082,23	21,50	167,95	10,75	83,98	7,78	60,74	41,68	325,58	12,00	93,74
27,49	214,73	21,87	170,83	172,74	1349,48	17,89	139,75	11,93	93,17	16,71	130,52	70,67	552,06	21,54	168,28
10,67	83,33	18,67	145,83	69,86	545,73	8,54	66,69	9,62	75,11	16,71	130,52	81,10	633,59	16,06	125,42
10,67	83,33	11,39	89,00	54,68	427,19	14,17	110,73	16,06	125,42	27,00	210,91	39,49	308,47	16,28	127,14
15,09	117,85	10,67	83,33	66,88	522,47	13,73	107,24	12,29	96,03	14,73	115,05	235,83	1842,35	15,61	121,92
5,96	46,58	16,71	130,52	102,81	803,12	16,28	127,14	10,41	81,35	30,46	237,98	146,09	1141,27	21,08	164,69
12,29	96,03	9,62	75,11	124,54	972,95	18,86	147,30	8,54	66,69	20,00	156,24	130,98	1023,22	14,91	116,45
18,86	147,30	22,07	172,42	71,08	555,29	13,13	102,59	12,00	93,74	14,91	116,45	157,68	1231,79	28,13	219,73
21,33	166,65	9,62	75,11	211,13	1649,33	6,67	52,08	19,69	153,79	23,29	181,91	91,57	715,38	27,46	214,48
10,41	81,35	12,29	96,03	161,09	1258,41	6,67	52,08	21,33	166,65	8,94	69,87	125,54	980,72	17,08	133,39
7,18	56,09	5,66	44,19	137,50	1074,12	10,75	83,98	16,28	127,14	19,41	151,66	108,07	844,28	16,06	125,42
16,28	127,14	9,62	75,11	89,37	698,19	14,17	110,73	19,69	153,79	14,91	116,45	251,10	1961,57	18,52	144,70
10,75	83,98	22,07	172,42	91,02	711,05	33,44	261,24	19,09	149,13	14,73	115,05	10,15	79,32	22,67	177,08
15,20	118,76	8,43	65,88	98,43	768,96	28,54	222,92	14,91	116,45	25,89	202,25	16,28	127,14	26,80	209,36
16,87	131,75	10,75	83,98	151,27	1181,76	13,20	103,11	12,07	94,32	14,73	115,05	24,80	193,75	14,91	116,45
18,57	145,08	15,20	118,76	126,44	987,77	10,41	81,35	13,20	103,11	7,54	58,92	21,50	167,95	21,08	164,69
15,20	118,76	16,06	125,42	60,30	471,04	8,94	69,87	23,29	181,91	23,02	179,81	22,07	172,42	15,20	118,76
20,70	161,70	21,50	167,95	20,87	163,04	11,39	89,00	16,06	125,42	14,17	110,73	15,61	121,92	30,93	241,60
17,33	135,41	15,55	121,47	43,18	337,36	17,39	135,81	19,09	149,13	9,71	75,83	9,71	75,83	20,70	161,70
18,14	141,67	12,29	96,03	253,34	1979,09	17,39	135,81	17,39	135,81	21,71	169,56	109,46	855,13	17,89	139,75
19,82	154,84	13,73	107,24	264,05	2062,81	5,66	44,19	13,73	107,24	16,06	125,42	68,30	533,56	33,57	262,27
Average	115,48		135,30		942,29		121,33		123,17		126,97		716,46		139,84
Median	118,30		125,42		751,60		110,73		125,42		117,15		488,63		127,14
SD	**37,06**		**66,70**		**667,49**		**49,00**		**35,34**		**40,34**		**590,96**		**46,25**

sv028 naïve		SV028 LPSBP		SV028 LPS		SV028 LPS+ LPSBP		SV028 Aspirin		SV028 Aspirin + LPSBP		SV028 Aspirin + LPS		SV028 Aspirin + LPS + LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
24,04	187,78	23,32	182,21	190,40	1487,43	21,99	171,79	12,29	96,03	19,41	151,66	57,84	451,87	25,47	199,00
16,22	126,72	12,65	98,81	263,73	2060,29	11,47	89,60	7,18	56,09	8,94	69,87	56,02	437,60	16,97	132,58
16,22	126,72	20,83	162,70	222,17	1735,62	14,36	112,18	22,19	173,36	22,71	177,38	52,75	412,06	16,00	124,99
16,06	125,42	16,28	127,14	320,09	2500,60	9,71	75,83	16,28	127,14	22,82	178,29	72,76	568,41	33,44	261,24
16,11	125,86	18,52	144,70	125,40	979,61	12,29	96,03	22,78	177,99	26,97	210,65	32,03	250,21	10,67	83,33
21,08	164,69	29,61	231,28	111,37	870,04	16,11	125,86	20,87	163,04	35,50	277,35	37,95	296,44	18,57	145,08
16,87	131,75	16,06	125,42	54,00	421,82	16,11	125,86	17,33	135,41	15,20	118,76	47,29	369,44	13,60	106,22
22,19	173,36	16,28	127,14	27,84	217,50	18,14	141,67	8,00	62,50	8,54	66,69	104,31	814,85	24,33	190,08
24,04	187,78	21,87	170,83	35,30	275,78	12,00	93,74	5,33	41,66	16,71	130,52	29,61	231,28	14,91	116,45
14,91	116,45	28,13	219,73	88,36	690,30	24,51	191,50	20,70	161,70	15,20	118,76	29,45	230,10	17,54	137,00
12,58	98,27	17,39	135,81	128,06	1000,43	14,91	116,45	14,36	112,18	13,40	104,68	56,02	437,60	9,71	75,83
12,65	98,81	29,52	230,57	144,03	1125,13	21,38	166,98	15,09	117,85	13,33	104,16	35,08	274,01	22,67	177,08
37,55	293,32	12,07	94,32	60,53	472,87	23,29	181,91	20,31	158,66	32,99	257,68	38,48	300,62	33,36	260,61
24,51	191,50	13,40	104,68	83,92	655,55	14,17	110,73	24,59	192,06	21,33	166,65	44,82	350,14	13,33	104,16
14,91	116,45	15,20	118,76	48,07	375,56	25,33	197,90	16,97	132,58	21,71	169,56	97,48	761,51	24,51	191,50
26,70	208,58	16,28	127,14	39,64	309,69	15,20	118,76	5,66	44,19	28,03	218,99	32,55	254,29	20,31	158,66
20,04	156,59	15,61	121,92	145,34	1135,40	19,23	150,23	13,20	103,11	13,33	104,16	81,10	633,59	28,35	221,45
25,30	197,63	20,83	162,70	43,08	336,55	37,14	290,16	20,70	161,70	25,47	199,00	56,60	442,16	20,00	156,24
21,38	166,98	15,20	118,76	69,60	543,74	14,36	112,18	19,23	150,23	11,47	89,60	51,23	400,17	16,28	127,14
25,89	202,25	17,33	135,41	48,17	376,28	20,87	163,04	16,00	124,99	30,78	240,47	59,04	461,26	11,47	89,60
13,33	104,16	19,82	154,84	86,46	675,44	24,15	188,65	20,00	156,24	13,33	104,16	83,96	655,89	17,33	135,41
29,24	228,44	18,52	144,70	66,57	520,07	16,71	130,52	12,29	96,03	29,61	231,28	44,32	346,25	25,47	199,00
28,13	219,73	15,20	118,76	132,01	1031,25	10,41	81,35	21,08	164,69	21,87	170,83	105,14	821,35	15,09	117,85
18,71	146,20	16,71	130,52	68,14	532,35	12,29	96,03	14,36	112,18	17,54	137,00	75,52	589,96	19,41	151,66
15,55	121,47	10,75	83,98	75,47	589,59	19,09	149,13	19,69	153,79	20,31	158,66	37,90	296,08	21,38	166,98
15,61	121,92	13,73	107,24	22,67	177,08	14,91	116,45	28,13	219,73	24,15	188,65	81,43	636,15	18,14	141,67
10,15	79,32	8,94	69,87	104,12	813,39	13,60	106,22	16,06	125,42	12,29	96,03	81,17	634,10	19,09	149,13
20,40	159,34	20,04	156,59	97,55	762,08	16,28	127,14	25,75	201,17	25,65	200,36	79,22	618,86	13,20	103,11
20,31	158,66	19,41	151,66	80,11	625,83	20,31	158,66	17,39	135,81	10,75	83,98	59,93	468,15	24,33	190,08
11,39	89,00	17,39	135,81	59,58	465,47	9,62	75,11	6,67	52,08	22,19	173,36	87,69	685,01	11,31	88,39
31,24	244,06	21,08	164,69	37,19	290,53	16,06	125,42	25,65	200,36	14,73	115,05	59,40	464,07	17,39	135,81
15,20	118,76	19,82	154,84	140,48	1097,46	16,22	126,72	10,15	79,32	12,07	94,32	61,33	479,14	27,49	214,73
19,41	151,66	17,54	137,00	70,52	550,88	12,29	96,03	16,71	130,52	13,13	102,59	42,37	331,03	36,78	287,34
9,33	72,91	13,60	106,22	59,18	462,32	16,97	132,58	11,47	89,60	14,91	116,45	54,73	427,57	17,79	138,97
25,47	199,00	18,14	141,67	85,34	666,71	18,67	145,83	9,71	75,83	10,15	79,32	71,02	554,80	21,38	166,98
22,67	177,08	15,09	117,85	148,02	1156,37	19,09	149,13	14,91	116,45	10,75	83,98	54,73	427,57	18,86	147,30
59,40	464,07	17,89	139,75	96,23	751,76	19,09	149,13	13,13	102,59	15,55	121,47	34,77	271,62	11,93	93,17
21,71	169,56	21,50	167,95	29,52	230,57	13,33	104,16	13,40	104,68	94,44	737,78	41,18	321,72	17,39	135,81
27,84	217,50	19,82	154,84	161,53	1261,90	21,71	169,56	16,11	125,86	33,44	261,24	156,32	1221,18	12,65	98,81
27,00	210,91	9,71	75,83	155,95	1218,32	21,71	169,56	12,07	94,32	21,87	170,83	27,00	210,91	32,11	250,85
23,63	184,58	12,58	98,27	91,08	711,51	16,06	125,42	13,92	108,74	13,92	108,74	47,35	369,88	25,30	197,63
31,69	247,59	13,33	104,16	42,35	330,86	21,50	167,95	14,36	112,18	28,03	218,99	27,49	214,73	21,71	169,56
30,46	237,98	23,63	184,58	27,49	214,73	20,04	156,59	26,70	208,58	21,50	167,95	38,67	302,07	19,82	154,84
23,63	184,58	23,74	185,45	174,34	1361,93	11,93	93,17	13,33	104,16	18,95	148,04	59,40	464,07	13,13	102,59
27,49	214,73	22,71	177,38	56,25	439,45	17,79	138,97	10,15	79,32	29,36	229,39	76,92	600,89	6,67	52,08
19,09	149,13	14,17	110,73	93,49	730,32	26,97	210,65	11,93	93,17	12,29	96,03	75,07	586,45	10,67	83,33
19,82	154,84	19,09	149,13	137,66	1075,43	23,74	185,45	32,25	251,93	24,00	187,49	47,25	369,14	21,71	169,56
10,75	83,98	14,17	110,73	78,68	614,64	16,06	125,42	4,81	37,55	13,20	103,11	28,78	224,85	28,54	222,92
9,71	75,83	17,33	135,41	95,34	744,80	16,28	127,14	22,63	176,76	16,06	125,42	41,74	326,08	24,59	192,06
13,92	108,74	9,62	75,11	89,33	697,88	6,67	52,08	18,71	146,20	24,59	192,06	72,79	568,61	19,09	149,13
Average	165,85		137,71		767,42		136,25		126,96		163,19		457,32		153,30
Median	159,00		135,61		671,08		127,14		125,21		149,85		432,59		148,22
SD	**66,62**		**36,66**		**477,50**		**41,67**		**48,45**		**99,52**		**195,54**		**52,30**

SV01 naive		SV01 LPSBP		SV01 LPS		SV01 LPSBP +LPS		SV01 Aspirin		SV01 Aspirin + LPSBP		SV01 Aspirin + LPS		SV01 Aspirin + LPS+LPSBBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
13,60	106,22	14,91	116,45	27,20	212,45	22,82	178,29	20,00	156,24	29,27	228,68	37,36	291,84	13,92	108,74
13,13	102,59	15,55	121,47	21,99	171,79	14,67	114,58	11,39	89,00	21,50	167,95	27,49	214,73	20,00	156,24
13,92	108,74	21,08	164,69	14,36	112,18	22,63	176,76	21,54	168,28	17,94	140,13	51,86	405,16	16,87	131,75
15,55	121,47	16,11	125,86	15,55	121,47	20,83	162,70	10,15	79,32	22,94	179,21	27,00	210,91	11,47	89,60
14,17	110,73	15,20	118,76	79,70	622,61	13,33	104,16	17,33	135,41	18,14	141,67	26,70	208,58	18,95	148,04
22,67	177,08	11,31	88,39	16,06	125,42	20,40	159,34	8,94	69,87	8,54	66,69	43,27	338,00	14,73	115,05
12,65	98,81	18,14	141,67	30,70	239,80	20,70	161,70	20,35	158,99	9,33	72,91	20,40	159,34	16,49	128,84
10,75	83,98	17,94	140,13	18,14	141,67	16,22	126,72	14,36	112,18	17,89	139,75	77,57	606,01	21,54	168,28
20,00	156,24	10,41	81,35	29,36	229,39	20,35	158,99	17,33	135,41	17,94	140,13	65,06	508,26	19,82	154,84
8,54	66,69	11,31	88,39	22,67	177,08	12,07	94,32	21,50	167,95	18,14	141,67	18,95	148,04	18,14	141,67
9,43	73,65	4,81	37,55	20,35	158,99	11,00	85,89	19,82	154,84	13,60	106,22	69,65	544,13	13,33	104,16
10,75	83,98	15,20	118,76	32,11	250,85	8,43	65,88	12,65	98,81	12,00	93,74	43,59	340,56	17,54	137,00
12,65	98,81	9,71	75,83	17,39	135,81	14,91	116,45	19,09	149,13	8,54	66,69	34,69	271,02	16,06	125,42
12,58	98,27	7,54	58,92	14,91	116,45	12,65	98,81	8,54	66,69	13,60	106,22	50,19	392,10	16,06	125,42
16,06	125,42	15,61	121,92	15,20	118,76	18,14	141,67	6,80	53,11	18,95	148,04	23,29	181,91	18,71	146,20
12,00	93,74	10,41	81,35	85,80	670,28	21,71	169,56	12,29	96,03	33,36	260,61	25,16	196,53	26,67	208,32
9,62	75,11	17,39	135,81	108,53	847,81	14,36	112,18	20,74	162,04	9,62	75,11	23,02	179,81	13,20	103,11
16,71	130,52	19,41	151,66	11,47	89,60	13,33	104,16	14,91	116,45	15,61	121,92	18,14	141,67	14,42	112,67
12,29	96,03	5,66	44,19	18,86	147,30	20,35	158,99	13,73	107,24	13,60	106,22	29,96	234,07	13,40	104,68
15,20	118,76	14,73	115,05	16,22	126,72	16,22	126,72	10,15	79,32	23,29	181,91	103,56	808,98	12,58	98,27
12,00	93,74	9,62	75,11	16,00	124,99	19,82	154,84	22,71	177,38	15,61	121,92	37,55	293,32	11,39	89,00
10,15	79,32	14,73	115,05	13,60	106,22	18,71	146,20	10,75	83,98	16,06	125,42	37,36	291,84	17,33	135,41
13,13	102,59	15,20	118,76	14,17	110,73	25,65	200,36	16,00	124,99	8,54	66,69	34,90	272,62	18,52	144,70
25,30	197,63	8,11	63,36	22,67	177,08	10,15	79,32	21,71	169,56	14,36	112,18	24,33	190,08	16,49	128,84
14,17	110,73	13,13	102,59	11,00	85,89	18,57	145,08	8,54	66,69	13,73	107,24	49,82	389,17	19,69	153,79
11,31	88,39	17,54	137,00	7,54	58,92	13,60	106,22	14,17	110,73	13,60	106,22	93,19	728,01	6,80	53,11
11,39	89,00	10,15	79,32	16,28	127,14	14,36	112,18	9,43	73,65	19,09	149,13	30,70	239,80	5,50	42,94
16,22	126,72	21,08	164,69	17,39	135,81	24,80	193,75	18,14	141,67	14,91	116,45	64,44	503,43	24,33	190,08
11,47	89,60	18,71	146,20	9,71	75,83	13,92	108,74	8,43	65,88	9,43	73,65	84,09	656,88	11,39	89,00
10,75	83,98	18,57	145,08	11,93	93,17	16,49	128,84	10,75	83,98	8,94	69,87	50,74	396,36	16,22	126,72
18,14	141,67	15,09	117,85	16,49	128,84	14,67	114,58	12,07	94,32	12,65	98,81	69,79	545,23	16,11	125,86
7,78	60,74	11,00	85,89	11,47	89,60	12,07	94,32	19,23	150,23	16,06	125,42	108,21	845,31	25,89	202,25
12,29	96,03	15,20	118,76	19,82	154,84	8,11	63,36	23,59	184,28	14,42	112,67	129,40	1010,84	7,54	58,92
7,78	60,74	9,71	75,83	15,20	118,76	20,18	157,62	12,29	96,03	12,00	93,74	25,89	202,25	10,15	79,32
16,22	126,72	13,13	102,59	15,55	121,47	11,47	89,60	17,54	137,00	12,07	94,32	21,50	167,95	16,97	132,58
10,15	79,32	12,58	98,27	10,15	79,32	17,33	135,41	18,14	141,67	24,80	193,75	64,13	500,95	19,23	150,23
16,28	127,14	10,75	83,98	13,92	108,74	14,91	116,45	9,71	75,83	10,15	79,32	61,19	478,01	6,80	53,11
20,31	158,66	5,50	42,94	12,65	98,81	6,67	52,08	19,09	149,13	10,67	83,33	36,03	281,43	11,39	89,00
7,54	58,92	9,62	75,11	17,79	138,97	12,65	98,81	7,54	58,92	13,60	106,22	92,09	719,39	9,71	75,83
13,40	104,68	9,33	72,91	12,65	98,81	8,43	65,88	7,18	56,09	11,93	93,17	29,24	228,44	13,60	106,22
9,43	73,65	10,75	83,98	12,58	98,27	23,29	181,91	19,82	154,84	10,75	83,98	154,67	1208,31	11,31	88,39
9,71	75,83	22,78	177,99	10,75	83,98	15,61	121,92	15,61	121,92	18,71	146,20	39,03	304,93	15,55	121,47
21,50	167,95	11,00	85,89	8,43	65,88	17,54	137,00	12,00	93,74	20,04	156,59	20,18	157,62	13,33	104,16
13,33	104,16	12,58	98,27	12,58	98,27	6,67	52,08	12,29	96,03	18,71	146,20	41,33	322,90	7,18	56,09
20,04	156,59	10,15	79,32	12,07	94,32	17,08	133,39	8,00	62,50	10,67	83,33	21,33	166,65	18,95	148,04
14,17	110,73	17,94	140,13	19,09	149,13	16,11	125,86	10,75	83,98	17,33	135,41	44,18	345,15	9,71	75,83
20,70	161,70	8,43	65,88	10,67	83,33	11,00	85,89	14,17	110,73	12,29	96,03	52,61	411,01	12,29	96,03
16,06	125,42	17,94	140,13	11,39	89,00	12,00	93,74	12,07	94,32	15,55	121,47	22,19	173,36	13,20	103,11
12,29	96,03	14,36	112,18	12,29	96,03	15,20	118,76	10,15	79,32	20,18	157,62	57,47	448,98	16,71	130,52
13,92	108,74	11,47	89,60	8,94	69,87	11,93	93,17	13,13	102,59	16,06	125,42	38,76	302,79	14,91	116,45
Average	107,68		104,46		159,57		122,51		111,97		121,35		383,29		117,51
Median	102,59		102,59		120,11		117,61		104,92		114,56		303,86		118,96
SD	**31,58**		**33,25**		**149,67**		**36,65**		**37,30**		**41,13**		**235,28**		**36,83**

SV03 naïve		SV03 LPSBP		SV03 LPS		SV03 LPSBP + LPS		SV03 Aspirin		SV03 Aspirin + LPSBP		SV03 Aspirin + LPS		SV03 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
18,14	141,67	30,70	239,80	37,57	293,51	13,92	108,74	8,94	69,87	26,30	205,43	166,05	1297,17	16,71	130,52
6,67	52,08	13,60	106,22	62,72	490,00	10,41	81,35	18,71	146,20	17,39	135,81	113,29	885,06	23,59	184,28
17,39	135,81	26,70	208,58	76,37	596,63	14,67	114,58	25,47	199,00	21,87	170,83	89,37	698,19	13,33	104,16
22,71	177,38	17,39	135,81	71,75	560,54	21,08	164,69	16,00	124,99	17,94	140,13	50,68	395,95	16,97	132,58
24,15	188,65	18,86	147,30	60,65	473,79	7,18	56,09	12,65	98,81	15,20	118,76	137,88	1077,10	20,00	156,24
18,14	141,67	9,71	75,83	29,81	232,91	18,67	145,83	16,49	128,84	25,61	200,08	113,53	886,90	14,17	110,73
24,15	188,65	4,22	32,94	170,67	1333,26	9,62	75,11	19,69	153,79	22,82	178,29	45,61	356,29	17,79	138,97
26,20	204,65	10,15	79,32	40,02	312,65	10,75	83,98	9,33	72,91	7,78	60,74	80,22	626,70	14,36	112,18
22,07	172,42	15,09	117,85	79,70	622,61	11,47	89,60	17,39	135,81	26,20	204,65	116,88	913,06	17,39	135,81
27,00	210,91	22,35	174,61	65,10	508,58	17,89	139,75	21,71	169,56	15,20	118,76	145,63	1137,70	20,83	162,70
15,09	117,85	18,14	141,67	53,93	421,31	20,04	156,59	27,00	210,91	21,08	164,69	161,44	1261,20	14,91	116,45
17,89	139,75	16,49	128,84	45,41	354,76	14,42	112,67	15,20	118,76	21,38	166,98	81,46	636,40	13,60	106,22
24,00	187,49	17,94	140,13	33,33	260,40	16,22	126,72	16,06	125,42	18,95	148,04	80,85	631,61	21,08	164,69
18,67	145,83	23,32	182,21	47,65	372,22	11,31	88,39	21,33	166,65	8,43	65,88	58,45	456,65	14,91	116,45
18,71	146,20	13,13	102,59	67,74	529,17	9,33	72,91	6,67	52,08	6,80	53,11	61,77	482,53	5,66	44,19
19,82	154,84	10,41	81,35	29,24	228,44	5,33	41,66	16,11	125,86	16,22	126,72	17,94	140,13	19,23	150,23
9,62	75,11	10,75	83,98	30,41	237,53	11,93	93,17	15,55	121,47	16,06	125,42	46,84	365,90	31,81	248,46
20,35	158,99	11,47	89,60	92,07	719,24	11,47	89,60	18,86	147,30	27,20	212,45	45,98	359,17	17,94	140,13
28,13	219,73	18,95	148,04	39,49	308,47	5,96	46,58	18,14	141,67	11,39	89,00	26,20	204,65	11,93	93,17
18,95	148,04	41,36	323,07	66,20	517,14	8,00	62,50	23,85	186,33	18,86	147,30	114,71	896,15	15,20	118,76
16,28	127,14	13,20	103,11	28,03	218,99	16,06	125,42	13,92	108,74	11,00	85,89	94,15	735,50	8,94	69,87
17,08	133,39	9,33	72,91	74,86	584,79	21,33	166,65	12,29	96,03	17,94	140,13	120,36	940,28	18,52	144,70
11,93	93,17	17,39	135,81	63,18	493,53	15,20	118,76	16,49	128,84	12,29	96,03	121,80	951,52	26,30	205,43
20,74	162,04	23,63	184,58	46,84	365,90	13,40	104,68	15,20	118,76	24,04	187,78	157,64	1231,48	17,39	135,81
33,94	265,15	13,33	104,16	103,56	808,98	18,71	146,20	19,09	149,13	5,96	46,58	99,58	777,93	14,91	116,45
15,09	117,85	8,11	63,36	230,65	1801,83	13,33	104,16	10,67	83,33	13,40	104,68	64,55	504,29	12,00	93,74
30,41	237,53	17,33	135,41	59,40	464,07	8,43	65,88	17,94	140,13	22,07	172,42	26,30	205,43	24,48	191,22
28,50	222,67	9,43	73,65	150,19	1173,33	12,65	98,81	12,29	96,03	24,80	193,75	139,42	1089,12	12,29	96,03
24,33	190,08	12,29	96,03	67,99	531,12	13,13	102,59	9,43	73,65	22,71	177,38	68,83	537,72	12,29	96,03
29,81	232,91	19,82	154,84	238,98	1866,92	14,91	116,45	19,82	154,84	22,82	178,29	94,28	736,53	11,39	89,00
12,29	96,03	21,50	167,95	149,10	1164,79	9,62	75,11	18,52	144,70	16,49	128,84	135,61	1059,43	17,79	138,97
21,71	169,56	20,40	159,34	96,76	755,86	11,39	89,00	26,47	206,75	12,29	96,03	48,19	376,42	25,33	197,90
18,86	147,30	21,71	169,56	75,90	592,90	13,73	107,24	19,09	149,13	29,27	228,68	64,22	501,71	15,20	118,76
11,31	88,39	17,33	135,41	54,68	427,19	13,33	104,16	8,11	63,36	15,61	121,92	78,03	609,58	15,09	117,85
17,54	137,00	17,33	135,41	71,01	554,70	17,33	135,41	24,51	191,50	22,63	176,76	130,34	1018,23	20,40	159,34
12,07	94,32	9,43	73,65	64,01	500,08	10,15	79,32	11,00	85,89	16,71	130,52	129,33	1010,36	9,71	75,83
6,80	53,11	6,67	52,08	39,22	306,35	10,75	83,98	11,93	93,17	13,33	104,16	86,67	677,05	16,97	132,58
26,40	206,23	30,78	240,47	35,43	276,77	11,39	89,00	13,73	107,24	10,75	83,98	72,94	569,84	19,23	150,23
15,20	118,76	23,02	179,81	85,84	670,60	12,29	96,03	21,50	167,95	14,17	110,73	66,67	520,81	25,89	202,25
22,67	177,08	28,03	218,99	34,77	271,62	13,60	106,22	22,63	176,76	19,69	153,79	102,77	802,85	12,29	96,03
21,08	164,69	9,62	75,11	78,26	611,37	14,42	112,67	10,15	79,32	28,78	224,85	77,16	602,79	17,33	135,41
24,33	190,08	9,71	75,83	97,42	761,01	10,75	83,98	20,40	159,34	11,47	89,60	81,10	633,59	23,85	186,33
13,60	106,22	16,11	125,86	134,36	1049,60	16,28	127,14	24,59	192,06	9,33	72,91	69,60	543,74	14,67	114,58
11,00	85,89	10,41	81,35	75,52	589,96	21,87	170,83	18,71	146,20	18,71	146,20	58,49	456,89	12,65	98,81
21,38	166,98	11,39	89,00	155,96	1218,37	7,78	60,74	23,02	179,81	20,18	157,62	137,55	1074,53	24,33	190,08
6,80	53,11	16,97	132,58	78,77	615,34	15,20	118,76	16,49	128,84	24,33	190,08	99,64	778,36	18,57	145,08
16,97	132,58	12,29	96,03	148,17	1157,50	16,71	130,52	17,08	133,39	14,36	112,18	133,92	1046,18	27,49	214,73
8,11	63,36	26,70	208,58	30,43	237,75	16,28	127,14	15,61	121,92	11,93	93,17	88,05	687,85	15,09	117,85
7,18	56,09	5,96	46,58	96,04	750,25	18,52	144,70	21,54	168,28	25,16	196,53	92,15	719,91	35,58	277,94
24,91	194,59	19,82	154,84	69,30	541,34	8,54	66,69	17,39	135,81	6,67	52,08	48,17	376,28	18,52	144,70
Average	147,82		129,64		614,72		104,18		133,54		137,73		709,69		138,41
Median	146,75		130,71		530,15		104,16		134,60		137,97		682,45		133,99
SD	**52,38**		**57,39**		**376,36**		**31,34**		**39,20**		**48,71**		**291,11**		**45,22**

SV07 naïve		SV07 LPSBP		SV07 LPS		SV07 LPS+LPSBP		SV07 Aspirin		SV07 Aspirin + LPSBP		SV07 Aspirin + LPS		SV07 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
8,00	62,50	7,18	56,09	162,40	1268,71	12,29	96,03	21,71	169,56	17,54	137,00	85,50	667,93	27,75	216,75
5,33	41,66	15,61	121,92	53,93	421,31	19,41	151,66	8,11	63,36	17,33	135,41	46,67	364,57	8,43	65,88
17,33	135,41	15,55	121,47	44,00	343,73	13,33	104,16	13,33	104,16	16,06	125,42	78,85	615,96	9,43	73,65
18,86	147,30	26,57	207,54	82,76	646,55	28,13	219,73	8,94	69,87	16,06	125,42	66,68	520,91	7,54	58,92
22,71	177,38	10,75	83,98	54,29	424,13	12,65	98,81	8,54	66,69	13,33	104,16	30,46	237,98	12,07	94,32
24,04	187,78	26,40	206,23	28,63	223,64	25,47	199,00	17,33	135,41	37,19	290,53	68,14	532,35	21,50	167,95
16,87	131,75	17,39	135,81	14,36	112,18	38,48	300,62	14,67	114,58	13,73	107,24	35,00	273,41	24,15	188,65
28,28	220,96	16,71	130,52	11,93	93,17	34,07	266,17	15,20	118,76	16,06	125,42	43,74	341,68	21,38	166,98
28,28	220,96	8,43	65,88	26,97	210,65	34,67	270,82	26,30	205,43	15,20	118,76	56,60	442,16	6,80	53,11
16,71	130,52	13,33	104,16	137,44	1073,67	21,50	167,95	13,13	102,59	16,28	127,14	42,16	329,39	5,66	44,19
16,28	127,14	23,85	186,33	113,34	885,43	13,33	104,16	18,57	145,08	19,82	154,84	62,68	489,67	17,79	138,97
22,82	178,29	14,42	112,67	36,10	282,01	15,55	121,47	12,58	98,27	12,07	94,32	21,87	170,83	9,71	75,83
10,75	83,98	17,79	138,97	73,35	572,98	15,09	117,85	21,08	164,69	21,08	164,69	70,60	551,56	11,39	89,00
14,36	112,18	14,42	112,67	37,43	292,39	40,11	313,35	16,71	130,52	20,31	158,66	45,51	355,52	13,40	104,68
17,39	135,81	22,82	178,29	67,30	525,78	13,33	104,16	17,39	135,81	21,87	170,83	65,35	510,50	18,52	144,70
20,35	158,99	19,23	150,23	105,34	822,94	15,55	121,47	19,09	149,13	16,06	125,42	112,23	876,75	13,33	104,16
17,39	135,81	16,71	130,52	113,37	885,62	16,00	124,99	24,04	187,78	16,00	124,99	68,05	531,63	11,31	88,39
21,50	167,95	9,62	75,11	33,57	262,27	26,83	209,62	9,33	72,91	21,38	166,98	64,68	505,26	18,14	141,67
17,39	135,81	18,14	141,67	44,72	349,36	12,58	98,27	15,20	118,76	17,39	135,81	56,02	437,60	25,37	198,18
9,62	75,11	13,40	104,68	85,19	665,49	12,07	94,32	18,71	146,20	26,70	208,58	32,03	250,21	16,71	130,52
18,67	145,83	19,82	154,84	78,97	616,93	7,78	60,74	16,22	126,72	20,00	156,24	39,64	309,69	9,62	75,11
18,67	145,83	11,47	89,60	69,30	541,34	21,50	167,95	21,08	164,69	24,04	187,78	58,80	459,37	8,43	65,88
19,09	149,13	19,09	149,13	78,85	615,96	10,75	83,98	14,17	110,73	15,55	121,47	51,05	398,81	9,62	75,11
13,20	103,11	17,89	139,75	41,76	326,24	10,15	79,32	23,29	181,91	18,67	145,83	132,03	1031,40	17,33	135,41
18,86	147,30	13,20	103,11	36,61	286,02	14,17	110,73	18,14	141,67	8,94	69,87	66,31	517,99	13,20	103,11
16,06	125,42	17,39	135,81	21,08	164,69	10,15	79,32	15,20	118,76	17,39	135,81	80,71	630,50	10,15	79,32
22,19	173,36	13,73	107,24	29,81	232,91	11,47	89,60	17,39	135,81	12,29	96,03	44,08	344,36	14,91	116,45
21,33	166,65	17,39	135,81	28,78	224,85	17,08	133,39	20,35	158,99	11,31	88,39	67,30	525,78	12,29	96,03
10,75	83,98	16,87	131,75	44,08	344,36	12,65	98,81	15,09	117,85	24,00	187,49	39,24	306,52	8,94	69,87
8,00	62,50	17,94	140,13	61,46	480,16	17,79	138,97	13,60	106,22	10,75	83,98	37,71	294,61	19,41	151,66
20,04	156,59	11,00	85,89	81,87	639,55	13,60	106,22	13,40	104,68	21,71	169,56	33,57	262,27	18,86	147,30
17,79	138,97	13,13	102,59	46,44	362,78	15,20	118,76	25,05	195,70	16,49	128,84	43,43	339,29	24,51	191,50
14,73	115,05	14,67	114,58	43,00	335,91	17,08	133,39	13,33	104,16	17,08	133,39	57,33	447,89	14,91	116,45
13,92	108,74	12,58	98,27	30,41	237,53	16,71	130,52	11,47	89,60	13,40	104,68	45,51	355,52	16,22	126,72
11,47	89,60	19,82	154,84	75,85	592,53	20,87	163,04	20,18	157,62	16,06	125,42	27,49	214,73	19,82	154,84
23,29	181,91	16,06	125,42	92,01	718,79	11,39	89,00	7,54	58,92	23,29	181,91	60,24	470,58	16,00	124,99
13,13	102,59	19,23	150,23	194,66	1520,68	13,33	104,16	18,86	147,30	13,73	107,24	32,11	250,85	18,57	145,08
18,52	144,70	13,40	104,68	69,45	542,54	13,20	103,11	22,67	177,08	12,29	96,03	21,08	164,69	19,82	154,84
23,29	181,91	13,60	106,22	80,28	627,13	13,33	104,16	24,04	187,78	24,04	187,78	73,53	574,40	12,29	96,03
17,89	139,75	7,78	60,74	58,09	453,79	16,06	125,42	14,91	116,45	8,11	63,36	53,48	417,81	8,94	69,87
11,00	85,89	24,51	191,50	89,34	697,95	14,91	116,45	16,49	128,84	35,90	280,46	36,78	287,34	15,61	121,92
14,17	110,73	10,15	79,32	38,69	302,25	16,06	125,42	27,49	214,73	20,18	157,62	122,91	960,15	13,73	107,24
17,08	133,39	20,70	161,70	66,68	520,91	8,94	69,87	10,15	79,32	17,39	135,81	132,97	1038,74	23,85	186,33
11,47	89,60	21,50	167,95	206,74	1615,03	24,15	188,65	14,17	110,73	16,06	125,42	73,95	577,69	12,29	96,03
9,62	75,11	18,71	146,20	86,67	677,05	12,07	94,32	13,33	104,16	14,17	110,73	125,40	979,61	13,92	108,74
17,39	135,81	10,67	83,33	47,18	368,56	12,58	98,27	12,00	93,74	15,09	117,85	23,74	185,45	12,07	94,32
16,71	130,52	24,59	192,06	75,25	587,84	13,92	108,74	9,43	73,65	23,29	181,91	30,70	239,80	24,04	187,78
22,71	177,38	30,23	236,15	20,18	157,62	13,73	107,24	17,33	135,41	18,86	147,30	24,04	187,78	15,20	118,76
28,03	218,99	18,14	141,67	44,32	346,25	16,00	124,99	17,54	137,00	4,81	37,55	23,74	185,45	12,29	96,03
13,40	104,68	20,70	161,70	61,39	479,59	18,14	141,67	7,54	58,92	21,38	166,98	33,44	261,24	13,33	104,16
Average	133,85		130,34		519,59		133,62		126,77		138,69		444,52		117,27
Median	135,81		131,13		466,69		117,15		122,74		131,11		408,31		107,99
SD	**41,26**		**39,73**		**325,78**		**57,33**		**39,41**		**46,12**		**223,93**		**42,05**

sv010 naïve		SV010 LPSBP		SV010 LPS		SV010 LPS+ LPSBP		SV010 Aspirin		SV010 Aspirin + LPSBP		SV010 Aspirin + LPS		SV010 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
10,75	83,98	14,91	116,45	15,20	118,76	18,52	144,70	16,22	126,72	18,71	146,20	161,36	1260,52	17,33	135,41
6,67	52,08	21,71	169,56	74,60	582,74	9,33	72,91	18,14	141,67	17,33	135,41	186,67	1458,29	13,60	106,22
19,82	154,84	16,49	128,84	63,57	496,60	10,75	83,98	15,09	117,85	13,40	104,68	26,67	208,32	9,43	73,65
19,82	154,84	9,71	75,83	34,90	272,62	36,03	281,43	12,29	96,03	19,82	154,84	89,37	698,19	10,75	83,98
11,93	93,17	13,40	104,68	125,60	981,22	19,23	150,23	14,42	112,67	20,00	156,24	24,04	187,78	17,08	133,39
12,58	98,27	13,33	104,16	76,51	597,73	15,20	118,76	15,55	121,47	17,39	135,81	198,74	1552,56	10,67	83,33
12,58	98,27	7,18	56,09	51,03	398,68	9,33	72,91	10,67	83,33	14,73	115,05	201,27	1572,31	23,63	184,58
17,94	140,13	20,87	163,04	102,05	797,22	18,57	145,08	17,33	135,41	13,20	103,11	124,52	972,77	10,75	83,98
16,00	124,99	19,69	153,79	142,32	1111,79	13,60	106,22	12,29	96,03	13,33	104,16	272,49	2128,68	12,29	96,03
26,47	206,75	12,00	93,74	60,00	468,72	12,29	96,03	14,67	114,58	17,79	138,97	224,95	1757,36	20,35	158,99
19,23	150,23	14,91	116,45	130,20	1017,16	11,39	89,00	5,96	46,58	16,22	126,72	208,23	1626,68	10,75	83,98
13,33	104,16	20,00	156,24	108,43	847,04	6,67	52,08	13,33	104,16	9,62	75,11	41,07	320,87	13,33	104,16
15,09	117,85	19,82	154,84	61,35	479,26	10,67	83,33	25,37	198,18	17,89	139,75	63,61	496,93	23,85	186,33
20,31	158,66	22,19	173,36	91,50	714,77	12,65	98,81	12,58	98,27	16,06	125,42	28,84	225,33	14,73	115,05
17,39	135,81	14,42	112,67	88,81	693,82	17,39	135,81	14,17	110,73	9,33	72,91	88,64	692,49	25,47	199,00
32,28	252,15	17,54	137,00	107,50	839,77	9,62	75,11	12,65	98,81	14,67	114,58	28,35	221,45	16,11	125,86
12,07	94,32	14,73	115,05	81,95	640,23	11,00	85,89	15,61	121,92	10,41	81,35	67,26	525,47	12,00	93,74
19,82	154,84	16,06	125,42	57,89	452,23	13,92	108,74	15,09	117,85	13,33	104,16	70,98	554,50	12,07	94,32
14,91	116,45	12,58	98,27	51,10	399,22	18,71	146,20	10,75	83,98	10,15	79,32	35,08	274,01	15,55	121,47
17,94	140,13	13,33	104,16	78,53	613,49	10,75	83,98	16,22	126,72	11,39	89,00	25,47	199,00	20,70	161,70
18,86	147,30	15,20	118,76	78,71	614,90	8,11	63,36	20,87	163,04	13,92	108,74	47,29	369,44	10,67	83,33
13,20	103,11	15,20	118,76	66,36	518,40	8,54	66,69	18,71	146,20	6,67	52,08	18,86	147,30	13,33	104,16
16,00	124,99	17,94	140,13	44,50	347,65	9,71	75,83	7,78	60,74	12,58	98,27	26,97	210,65	14,73	115,05
9,71	75,83	18,57	145,08	68,33	533,77	16,87	131,75	13,33	104,16	16,06	125,42	25,33	197,90	12,00	93,74
15,55	121,47	24,80	193,75	130,12	1016,51	17,54	137,00	16,00	124,99	25,65	200,36	69,54	543,24	7,78	60,74
16,49	128,84	11,00	85,89	101,98	796,68	14,91	116,45	8,94	69,87	8,11	63,36	57,89	452,23	13,33	104,16
13,33	104,16	19,82	154,84	109,34	854,18	12,07	94,32	12,65	98,81	12,29	96,03	394,67	3083,18	37,95	296,44
11,93	93,17	4,81	37,55	98,32	768,04	22,19	173,36	20,18	157,62	12,65	98,81	586,68	4583,19	16,06	125,42
28,25	220,71	17,39	135,81	111,94	874,45	13,33	104,16	11,47	89,60	13,60	106,22	164,01	1281,22	10,75	83,98
16,28	127,14	19,09	149,13	80,19	626,44	16,06	125,42	10,75	83,98	17,79	138,97	91,00	710,89	14,36	112,18
4,81	37,55	13,60	106,22	72,90	569,47	20,00	156,24	18,52	144,70	17,33	135,41	28,13	219,73	19,69	153,79
10,67	83,33	17,39	135,81	115,05	898,75	13,92	108,74	10,75	83,98	17,39	135,81	31,69	247,59	19,41	151,66
10,75	83,98	23,29	181,91	140,63	1098,64	9,43	73,65	13,60	106,22	14,17	110,73	24,51	191,50	12,07	94,32
14,73	115,05	5,96	46,58	95,34	744,80	8,11	63,36	13,73	107,24	9,62	75,11	31,24	244,06	22,94	179,21
14,91	116,45	23,29	181,91	98,00	765,57	14,91	116,45	11,93	93,17	17,79	138,97	25,05	195,70	20,35	158,99
14,73	115,05	16,06	125,42	114,18	891,96	14,42	112,67	10,15	79,32	9,43	73,65	26,70	208,58	8,94	69,87
11,93	93,17	17,33	135,41	102,45	800,35	13,33	104,16	16,22	126,72	13,40	104,68	97,42	761,01	11,93	93,17
21,08	164,69	8,43	65,88	166,97	1304,38	6,80	53,11	11,31	88,39	15,20	118,76	28,28	220,96	25,33	197,90
9,62	75,11	13,33	104,16	110,96	866,79	15,61	121,92	16,00	124,99	18,95	148,04	114,67	895,84	18,52	144,70
13,33	104,16	8,11	63,36	104,00	812,46	8,11	63,36	14,67	114,58	17,39	135,81	90,69	708,45	19,23	150,23
17,89	139,75	22,71	177,38	134,69	1052,23	14,36	112,18	17,39	135,81	11,47	89,60	81,10	633,59	24,04	187,78
18,71	146,20	10,15	79,32	115,78	904,47	10,75	83,98	12,65	98,81	12,29	96,03	27,75	216,75	9,43	73,65
12,29	96,03	22,71	177,38	120,33	939,99	19,23	150,23	13,60	106,22	8,54	66,69	95,04	742,48	16,49	128,84
10,41	81,35	16,28	127,14	110,74	865,10	7,78	60,74	11,39	89,00	6,67	52,08	44,82	350,14	16,71	130,52
21,08	164,69	13,73	107,24	70,00	546,82	11,31	88,39	8,54	66,69	25,47	199,00	128,35	1002,66	16,49	128,84
13,40	104,68	11,31	88,39	93,57	730,98	16,87	131,75	9,71	75,83	10,75	83,98	188,56	1473,06	24,04	187,78
9,71	75,83	8,43	65,88	78,71	614,90	16,06	125,42	17,39	135,81	16,00	124,99	332,09	2594,28	17,54	137,00
20,00	156,24	5,96	46,58	90,69	708,45	8,00	62,50	7,78	60,74	14,17	110,73	185,35	1447,99	14,91	116,45
12,29	96,03	6,80	53,11	105,14	821,35	13,20	103,11	12,58	98,27	16,06	125,42	173,46	1355,09	13,40	104,68
8,11	63,36	17,39	135,81	102,88	803,73	14,73	115,05	12,65	98,81	17,79	138,97	169,10	1321,00	13,33	104,16
Aveargе	119,83		118,89		724,31		106,53		107,75		113,11		870,86		125,96
Median	115,75		118,76		755,19		104,16		105,19		110,73		594,05		115,75
SD	**40,85**		**40,05**		**233,02**		**39,42**		**28,54**		**32,39**		**862,00**		**44,30**

SV016naive		SV016 LPSBP		SV016 LPS		SV016 LPS+ LPSBP		SV016 Aspirin		SV016 Aspirin + LPSBP		SV016 Aspirin + LPS		SV016 Aspirin + LPS+ LPSBP	
Length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	length	Scale bar nm	length	Scale bar nm	Length	Scale bar nm	length	Scale bar nm	length	Scale bar nm
	128,01		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**		**128,01**
19,09	149,13	12,29	96,03	21,38	166,98	19,09	149,13	14,42	112,67	12,00	93,74	17,33	135,41	20,35	158,99
15,20	118,76	13,13	102,59	16,11	125,86	20,00	156,24	7,54	58,92	16,49	128,84	20,40	159,34	13,20	103,11
15,09	117,85	20,83	162,70	19,82	154,84	17,94	140,13	12,29	96,03	9,71	75,83	62,89	491,32	15,20	118,76
12,07	94,32	24,04	187,78	13,60	106,22	16,06	125,42	21,08	164,69	13,33	104,16	314,73	2458,66	26,26	205,18
14,73	115,05	14,42	112,67	16,00	124,99	10,75	83,98	21,50	167,95	14,73	115,05	46,69	364,71	18,86	147,30
8,11	63,36	21,33	166,65	22,71	177,38	10,15	79,32	20,00	156,24	31,69	247,59	115,09	899,05	12,00	93,74
12,07	94,32	18,14	141,67	12,07	94,32	17,33	135,41	13,60	106,22	15,55	121,47	16,06	125,42	9,71	75,83
25,16	196,53	21,54	168,28	19,09	149,13	11,47	89,60	8,00	62,50	19,82	154,84	10,75	83,98	11,39	89,00
24,51	191,50	23,29	181,91	27,36	213,72	21,08	164,69	15,20	118,76	15,20	118,76	9,71	75,83	13,13	102,59
9,33	72,91	24,59	192,06	14,17	110,73	24,51	191,50	19,82	154,84	18,67	145,83	12,29	96,03	8,54	66,69
9,43	73,65	18,14	141,67	16,87	131,75	27,49	214,73	13,40	104,68	19,69	153,79	10,67	83,33	10,15	79,32
16,06	125,42	23,59	184,28	13,20	103,11	20,18	157,62	10,67	83,33	14,17	110,73	7,18	56,09	10,15	79,32
7,18	56,09	10,67	83,33	21,38	166,98	9,71	75,83	16,22	126,72	14,73	115,05	3,77	29,46	9,71	75,83
19,69	153,79	16,97	132,58	20,70	161,70	31,38	245,17	16,06	125,42	27,00	210,91	10,75	83,98	11,00	85,89
9,43	73,65	9,43	73,65	18,86	147,30	24,59	192,06	5,96	46,58	23,74	185,45	6,67	52,08	18,14	141,67
16,06	125,42	18,67	145,83	7,18	56,09	9,33	72,91	11,39	89,00	20,04	156,59	9,33	72,91	18,14	141,67
18,52	144,70	34,90	272,62	17,33	135,41	14,36	112,18	10,15	79,32	19,41	151,66	8,00	62,50	12,58	98,27
25,47	199,00	17,54	137,00	4,81	37,55	16,06	125,42	8,43	65,88	13,13	102,59	1,89	14,73	10,41	81,35
12,65	98,81	12,07	94,32	13,40	104,68	19,23	150,23	11,00	85,89	16,06	125,42	17,39	135,81	11,93	93,17
17,89	139,75	17,08	133,39	10,15	79,32	9,43	73,65	11,31	88,39	20,31	158,66	12,00	93,74	14,73	115,05
16,06	125,42	15,20	118,76	18,57	145,08	24,59	192,06	11,00	85,89	27,49	214,73	8,00	62,50	20,35	158,99
20,87	163,04	19,23	150,23	9,62	75,11	9,43	73,65	17,08	133,39	13,20	103,11	8,43	65,88	20,87	163,04
14,17	110,73	13,33	104,16	9,71	75,83	18,71	146,20	14,67	114,58	17,79	138,97	8,43	65,88	12,58	98,27
25,65	200,36	11,00	85,89	11,39	89,00	25,89	202,25	10,67	83,33	24,48	191,22	5,33	41,66	18,95	148,04
17,54	137,00	13,33	104,16	7,18	56,09	21,87	170,83	16,87	131,75	11,93	93,17	2,67	20,83	18,14	141,67
13,33	104,16	12,65	98,81	21,71	169,56	15,20	118,76	15,55	121,47	14,17	110,73	8,00	62,50	16,71	130,52
9,43	73,65	11,39	89,00	21,71	169,56	27,00	210,91	3,77	29,46	13,13	102,59	4,00	31,25	18,67	145,83
18,14	141,67	18,14	141,67	14,36	112,18	13,13	102,59	9,62	75,11	16,06	125,42	10,67	83,33	10,15	79,32
18,71	146,20	12,29	96,03	20,35	158,99	18,86	147,30	9,43	73,65	8,00	62,50	12,29	96,03	9,71	75,83
25,65	200,36	14,42	112,67	7,78	60,74	14,17	110,73	14,67	114,58	17,39	135,81	5,50	42,94	11,47	89,60
16,28	127,14	20,35	158,99	22,94	179,21	15,55	121,47	11,00	85,89	16,28	127,14	3,77	29,46	9,62	75,11
17,33	135,41	16,28	127,14	11,31	88,39	18,14	141,67	9,71	75,83	29,61	231,28	2,98	23,29	12,07	94,32
18,71	146,20	8,00	62,50	20,00	156,24	17,79	138,97	22,35	174,61	6,80	53,11	1,89	14,73	5,50	42,94
22,71	177,38	12,29	96,03	6,67	52,08	18,95	148,04	26,80	209,36	12,29	96,03	1,33	10,41	14,73	115,05
18,14	141,67	7,18	56,09	15,20	118,76	21,71	169,56	17,94	140,13	41,18	321,72	5,66	44,19	13,33	104,16
26,97	210,65	12,29	96,03	12,29	96,03	19,82	154,84	10,41	81,35	17,54	137,00	6,80	53,11	13,92	108,74
22,67	177,08	12,58	98,27	10,75	83,98	7,18	56,09	17,08	133,39	24,59	192,06	8,43	65,88	13,73	107,24
25,16	196,53	14,36	112,18	12,07	94,32	10,15	79,32	7,18	56,09	13,60	106,22	6,67	52,08	16,06	125,42
25,47	199,00	20,87	163,04	15,61	121,92	21,08	164,69	25,33	197,90	8,54	66,69	2,98	23,29	14,42	112,67
14,73	115,05	23,29	181,91	16,49	128,84	16,06	125,42	16,00	124,99	13,60	106,22	7,54	58,92	8,11	63,36
14,42	112,67	17,33	135,41	6,80	53,11	16,97	132,58	19,69	153,79	17,33	135,41	11,47	89,60	11,00	85,89
21,08	164,69	12,29	96,03	9,43	73,65	16,06	125,42	16,71	130,52	13,73	107,24	2,98	23,29	12,07	94,32
20,70	161,70	8,11	63,36	9,43	73,65	21,38	166,98	13,13	102,59	14,17	110,73	7,18	56,09	14,17	110,73
31,01	242,28	18,67	145,83	11,00	85,89	13,33	104,16	28,13	219,73	16,00	124,99	4,00	31,25	13,33	104,16
25,65	200,36	10,41	81,35	10,15	79,32	18,57	145,08	10,41	81,35	19,23	150,23	4,81	37,55	13,60	106,22
17,08	133,39	12,58	98,27	10,15	79,32	10,67	83,33	17,08	133,39	11,47	89,60	14,73	115,05	8,54	66,69
13,33	104,16	20,40	159,34	5,96	46,58	13,40	104,68	12,65	98,81	9,43	73,65	8,54	66,69	18,71	146,20
15,20	118,76	19,82	154,84	11,00	85,89	10,41	81,35	12,58	98,27	19,09	149,13	11,93	93,17	19,69	153,79
11,39	89,00	25,65	200,36	21,33	166,65	21,38	166,98	10,15	79,32	17,08	133,39	16,06	125,42	6,80	53,11
15,20	118,76	14,91	116,45	9,46	73,87	12,29	96,03	12,29	96,03	21,50	167,95	8,00	62,50	12,65	98,81
Average	137,57		128,32		111,96		134,34		110,61		134,70		144,46		107,06
Median	134,40		122,95		105,45		137,19		103,63		125,42		64,19		102,85
SD	**43,82**		**42,51**		**42,62**		**43,11**		**41,14**		**49,98**		**362,49**		**32,91**

Ethics Approval Amendment Certificate 2019

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

17 May 2019

Approval Certificate
Amendment

Ethics Reference No.: 213/2015
Title: The effect of aspirin and lipopolysaccharide binding protein on hypercoagulability induced by lipopolysaccharide.

Dear Mrs SV Sibanda

The **Amendment** as supported by documents received between and for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of .

Please note the following about your ethics approval:

- Please remember to use your protocol number (213/2015) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Ethics Annual Renewal Certificate 2019

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

17 May 2019

Approval Certificate
Annual Renewal

Ethics Reference No.: 213/2015
Title: The effect of aspirin and lipopolysaccharide binding protein on hypercoagulability induced by lipopolysaccharide.

Dear Mrs SV Sibanda

The **Annual Renewal** as supported by documents received between and for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of .

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on .
- Please remember to use your protocol number (213/2015) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

Ethics Approval Certificate 2015

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

20/08/2015

Approval Certificate
New Application

Ethics Reference No.: 213/2015

Title: The effect of the neurotoxin, lipopolysaccharide on the coagulability and red blood cell ultrastructure of blood from healthy individuals

Dear Ms Sthembile Mbotwe

The **New Application** as supported by documents specified in your cover letter dated 6/07/2015 for your research received on the 11/08/2015, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 12/08/2015.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**213/2015**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of 6 monthly written Progress Reports, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed.
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

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Ethics new approval certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 03/14/2020.

6 February 2020

Approval Certificate
Amendment

Ethics Reference No.: 213/2015

Title: The effect of aspirin and lipopolysaccharide binding protein on hypercoagulability induced by lipopolysaccharide

Dear Mrs SV Sibanda (Mbotwe)

The **Amendment** as supported by documents received between 12/4/2019 and 15/4/2019 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 29 May 2019. This Amendment entails the extension of the original MSc study into a PhD study with a title as above. This certificate also confirms that ethics approval for this study is valid from 20/8/2015 until 30/4/2020.

Please note the following about your ethics approval:

- Please remember to use your protocol number (213/2015) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Professor Werdie (CW) Van Staden
MBChB MMed(Psych) MD FCPsych(SA) FTCL UPL
Chairperson: Faculty of Health Sciences Research Ethics Committee
University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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