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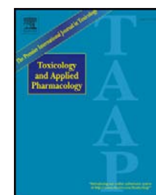

In vivo uptake and acute immune response to orally administered chitosan and PEG coated PLGA nanoparticles

B. Semete [a,⁎,1], L.I.J. Booysen [a,b,1], L. Kalombo [a], J.D. Venter [c], L. Katata [a], B. Ramalapa [a], J.A. Verschoor [d], H. Swai [a]

[a] Council for Scientific and Industrial Research, Polymers and Composites, P O Box 395 Pretoria, 0001, South Africa
[b] Department of Pharmaceutics, North-West University, Potchefstroom Campus, Potchefstroom, 2520, South Africa
[c] South African Medical Research Council, TB laboratory, Pretoria, 0001, South Africa
[d] Department of Biochemistry, University of Pretoria, Pretoria, 0001, South Africa

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ABSTRACT

Nanoparticulate drug delivery systems offer great promise in addressing challenges of drug toxicity, poor bioavailability and non-specificity for a number of drugs. Much progress has been reported for nano drug delivery systems for intravenous administration, however very little is known about the effects of orally administered nanoparticles. Furthermore, the development of nanoparticulate systems necessitates a thorough understanding of the biological response post exposure. This study aimed to elucidate the *in vivo* uptake of chitosan and polyethylene glycol (PEG) coated Poly, DL, lactic-*co*-glycolic Acid (PLGA) nanoparticles and the immunological response within 24 h of oral and peritoneal administration. These PLGA nanoparticles were administered orally and peritoneally to female Balb/C mice, they were taken up by macrophages of the peritoneum. When these particles were fluorescently labelled, intracellular localisation was observed. The expression of pro-inflammatory cytokines IL-2, IL-6, IL-12p70 and TNF-α in plasma and peritoneal lavage was found to remain at low concentration in PLGA nanoparticles treated mice as well as ZnO nanoparticles during the 24 hour period. However, these were significantly increased in lipopolysaccharide (LPS) treated mice. Of these pro-inflammatory cytokines, IL-6 and IL-12p70 were produced at the highest concentration in the positive control group. The anti-inflammatory cytokines IL-10 and chemokines INF-γ, IL-4, IL-5 remained at normal levels in PLGA treated mice. IL-10 and INF-γ were significantly increased in LPS treated mice. MCP-1 was found to be significantly produced in all groups in the first hours, except the saline treated mice. These results provide the first report to detail the induction of cytokine production by PLGA nanoparticles engineered for oral applications.

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Introduction

Nanoparticles have to date been extensively used for various applications including drug delivery (Liversidge and Cundy, 1995; Duncan, 2005), tissue engineering (Langer, 2000) and imaging (Bruchez, 2005). Their physiochemical properties including their small size and large surface area have led to these advances. In drug delivery, they have been reported to significantly improve the bioavailability of drugs and minimise drug toxicity (Bawarski et al., 2008; Farokhzad and Langer, 2006; Langer, 2000), thus leading to more efficient therapies.

In drug delivery, the nano size range of particles is the 'holy grail' of efficient drug delivery, facilitating efficient uptake of the drugs via various uptake mechanisms (Jones et al., 2003). Intracellular uptake of the drugs is not very efficient with conventional formulations, albeit its necessity, primarily for drugs against intracellular microorganism. This shortfall is addressed by nanoparticulate drug delivery systems, where increased intracellular concentrations of drugs are observed when the drugs were nanoencapsulated (Kisich et al., 2007). The first cellular targets for nanoparticles are macrophages and dendritic cells (DC), which are professional antigen presenting cells that are at the fore front of the body's defence system. After engulfing foreign material, they mature to become active antigen presenting cells expressing specific maturation markers such as CD11c and MOMA-2 and others (Noti and Reinemann, 1995). In addition, when these cells are activated, they produce cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, IL-18 and tumor necrosis factor alpha (TNF-α) and chemokines that attract other inflammatory cells to the site of inflammation (Anderson et al., 2008).

Since nanoparticles are foreign, their uptake may result in the release of the pro-inflammatory cytokines (Chang, 2010; Lee et al., 2009). The immunogenicity of synthetic polymers highly depended

⁎ Corresponding author. Fax: +27 12 841 3553.
E-mail address: Bsemete@csir.co.za (B. Semete).
[1] These authors contributed equally to this work.

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on their size, shape, composition, surfactant properties, electrical charge and on the inherent ability of the host to recognise them. Furthermore, the oxidative potential of nanoparticles is another important parameter for evaluating their inflammatory or immunological responses. Synthetic polymers used in biological applications, such a drug delivery and tissue engineering, must therefore be biocompatible and biodegradable, i.e. their introduction into the body must not provoke a hazardous reaction (Kim et al., 2007; Rihova, 2002). Various groups are thus proposing studies that will measure the cell viability, inflammatory effects and biomedical effects of nanomaterials (Kim et al., 2007).

In this study we investigated the *in vivo* uptake of chitosan and polyethylene glycol (PEG) coated PLGA (referred to in this manuscript as PLGA nanoparticles) nanoparticles post oral administration. These particles are currently being explored for delivery of various compounds including antibiotics for the treatment of tuberculosis (TB). Furthermore, we analysed the *in vivo* immunological response to the uptake of these particles. This is the first study to analyse the uptake of PLGA nanoparticles *in vivo* and in conjunction evaluate the subsequent immune reaction by analysing the concentration profile of the secreted pro- and anti-inflammatory cytokines.

Materials and methods

Preparation of PLGA particles

Poly, DL, lactic-*co*-glycolic Acid (PLGA) 50:50 (Mw: 45,000–75,000), nanoparticles were prepared using a modified double emulsion solvent evaporation technique (Lamprecht et al., 1999). An aqueous phosphate buffer solution (PBS) pH 7.4 was emulsified for a short period with a solution of 100 mg PLGA dissolved in 8 ml of ethyl acetate (EA), by means of a high speed homogeniser (Silverson L4R) with a speed varying between 3000 and 5000 rpm. This water-in-oil (w/o) emulsion obtained was transferred into a specific volume of an aqueous solution of 1% w/v of the polyvinyl alcohol (PVA) (Mw: 13,000–23,000, partially hydrolysed (87–89%)) as a stabiliser. The mixture was further emulsified for 5 min by homogenisation at 5000 or 8000 rpm. These methods were carried out aseptically using a laminar airflow chamber. The double emulsion (w/o/w) obtained was directly fed into a bench top Buchi mini-spray dryer (Model B-290) and spray dried at a temperature ranging between 95 and 110 degrees Celsius (°C), with an atomizing pressure varying between 6 and 7 bars.

1% PEG was used in the formulation as an excipient to increase the *in vivo* residence time of nanoparticles in the blood stream (Torchilin and Trubetskoy, 1995). In order to enhance the uptake in the gastrointestinal tract, a mucoadhesive and positively charged ligand, chitosan was added in the formulation as recommended in previous reports (Cui et al., 2006; Takeuchi et al., 2005). 3% (volume/volume) chitosan was added to the formulation. Rhodamine 6G (Sigma, South Africa) labelled PLGA nanoparticles were prepared using the same method, where Rhodamine 6G was added in the aqueous phase of the emulsion.

Particle characterisation

Particle size, zeta potential and composition. Particle size and size distribution of PLGA and ZnO particles as well as polystyrene beads were measured by Dynamic Laser Scattering (DLS) or Photon Correlation Spectroscopy (PCS) using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK). For each sample 1–3 mg of nanoparticles were suspended in filtered water (0.2 μm filter), then vortexed and/or sonicated for a few minutes. Each sample was measured in triplicate. The zeta potential was also determined using the same instrument. Surface morphology of PLGA nanoparticles was studied by scanning electron microscopy (LEO 1525 Field Emission SEM). The chitosan content in the PLGA particles was characterised via Fourier Transformed Infrared (FT-IR) using the PerkinElmer Spectrum 100 FT-IR Spectrometer.

Test for pyrogens in the particles. The PryoDetect System supplied by Biotest AG (Germany) was used for the analysis of pyrogen content in the PLGA, polystyrene and ZnO nanoparticles, according to the manufactures' instructions. Briefly, the particles were mixed with sterile cryo blood (provided with the kit) in a cell culture plate in triplicate and kept in a CO_2 incubator at 37 °C for 24 h. The test detects for IL-1B produced by blood monocytes in the presence of pyrogens. For the detection of IL-1B, the nanoparticle–blood mixture was transferred into an ELISA microplate coated with antibody specific for IL-1B and incubated for 2 h, then washed. IL-1B molecules present in the supernatant would then bind to the immobilised antibody. A horseradish peroxidase (HRP) labelled anti-human polyclonal antibody specific for IL-1B was added and incubated for 1 h and thereafter washed. A substrate provided with the kit was added and incubated at room temperature for 20 min resulting in a colour reaction and a stop solution added thereafter. The plate was then analysed at 450 nm on the BIO-TEK ELx800 plate reader. The standard curve was generated using a different concentration of the endotoxin standard provided with the kit. The data was analysed using the Combistats software programme and presented in Endotoxin Units per ml (EU/ml).

Animals. Unchallenged, healthy Balb/C male mice weighing 20–25 g were selected and housed under standard environment conditions at ambient temperature of 25 °C, and supplied with food and water *ad libitum*. Ethics approval was obtained from this study from the MRC Ethics Committee for Research on Animals (ECRA), Tygerberg, Cape Town, South Africa.

In vivo particle uptake. To evaluate particle uptake, saline was administered via the oral and intraperitoneal (i.p) routes respectively to mice as a negative control (Group 1) and 4% Brewers thioglycolate broth as a positive control (Group 2). A volume of 0.2 ml of 20 mg/ml Rhodamine 6G labelled nanoparticles was administered via the oral route once daily over five days (Group 3) and another group via the intraperitoneal route once only over the period of five days (Group 4). PLGA nanoparticles that were not fluorescently labelled were also administered at the same concentration to another group in a similar manner (Group 5).

This specific dose of PLGA was selected as it corresponds to the concentration of PLGA particles used in our research group for the administration of PLGA encapsulated anti-TB drugs, at a drug dose

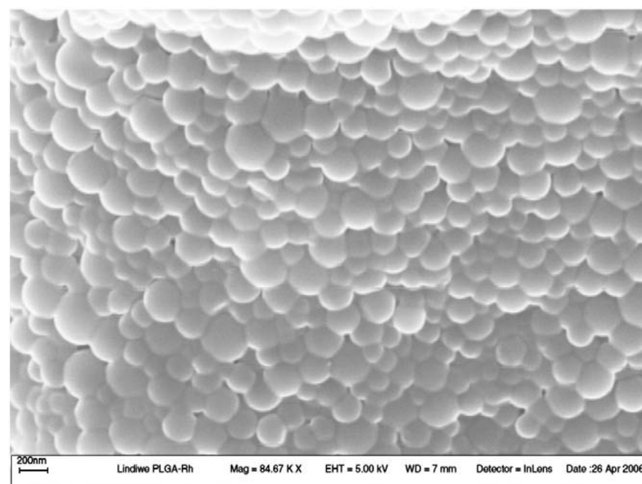

Fig. 1. SEM image of Rhodamine labelled PLGA nanoparticles.

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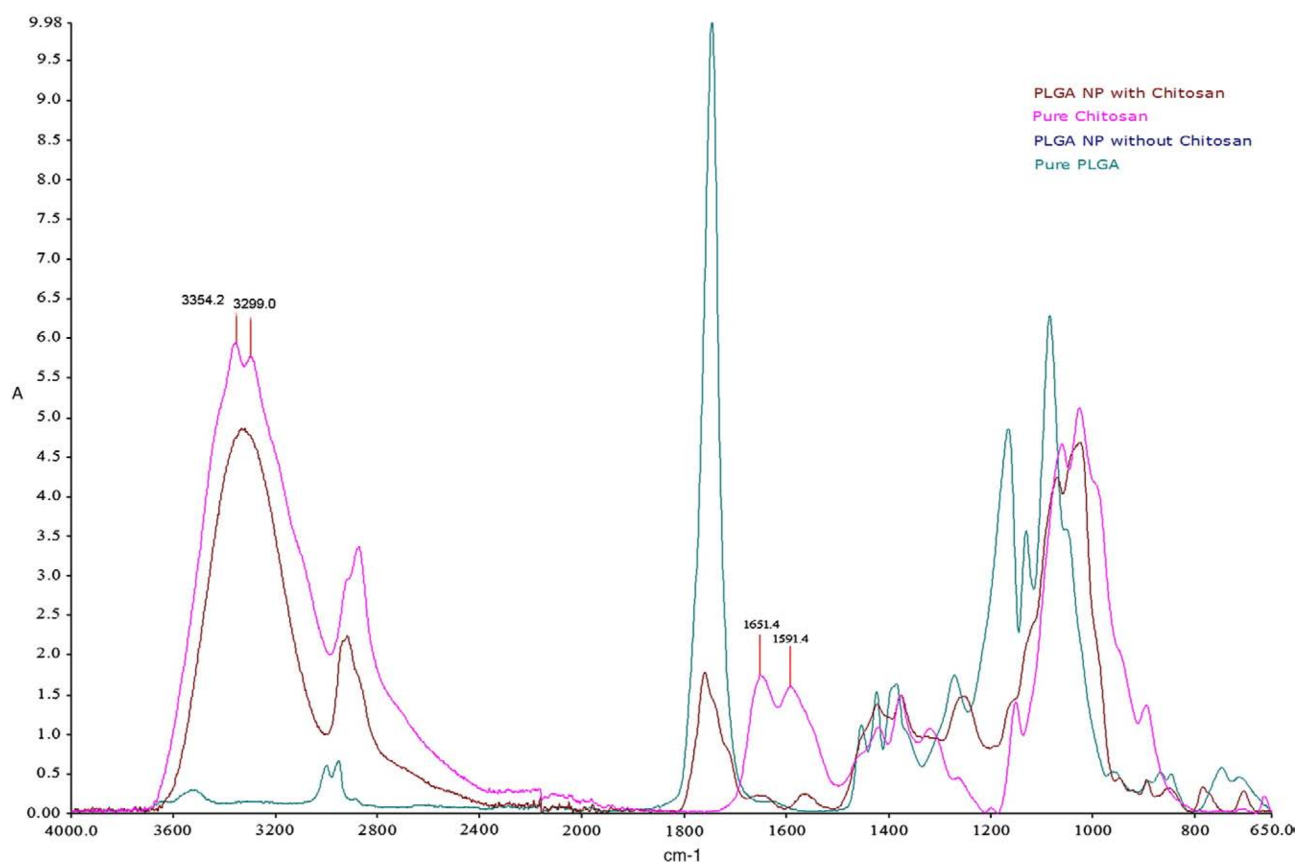

Fig. 2. FT-IR image of PLGA, chitosan and chitosan coated PLGA nanoparticles. The area under the curve for the peaks at 3354.2 and 3299.0 cm^{-1} as they are characteristic of the OH stretch was utilised for quantification of the chitosan content in the nanoparticles. The peaks at 1651.4 and 1591.4 cm^{-1} indicate the un-reacted NH bending of pure chitosan. A = absorbance.

which has proven to be efficacious (unpublished data). Thus, since this work forms part of that study, we maintained the same dose. Fluorescein-5-isothiocyanate (FITC) labelled polystyrene beads (0.2 µm, Sigma South Africa) were used as a control for uptake because they have a homogenous size distribution and they are well studied (Brown et al., 2001).

Peritoneal exudate cells (PECS) were collected from Balb/C mice subsequent to intraperitoneal or oral administration of the particles. Mice were sacrificed via cervical dislocation, and the PECS harvested by lavaging the peritoneal cavity with RPMI media. The harvested cells were counted using a haemocytometer and viability analysed via Trypan blue exclusion. The cells (1×10^6 cell/well) were cultured on 6-well plates in RPMI 1640 with 1% non-essential amino acid, 1% glutamine, 10% foetal bovine serum (FBS), Penicillin, (100 U/ml) and Streptomycin (100 µg/ml) for 2–3 h.

Fluorescently labelled monoclonal anti-mouse antibodies specific for phagocytic macrophages and dendritic cells, CD11c-Phycoerythrin (PE), and MOMA-FITC supplied by Beckman Coulter™ were utilised for the distinction of macrophage cells from the rest of the PECS population. The cells were incubated with the antibodies for 1 h. Extracellular particles and excess antibodies were washed off. The uptake of the particle by macrophages was analysed via fluorescence activated cell sorting (FACS) on the Beckman Coulter™ FC-500. Experiments were conducted in triplicate and repeated twice.

Cytokine production assay. To determine the acute immune response to nanoparticle exposure, particles were orally administered to mice. The supernatants from the peritoneal lavage as well as plasma were collected at 1, 2, 6, 8 and 24 h after exposure and utilised for determination of cytokine content. Lipopolysacharride (LPS, derived from *Salmonella enterica* serotype enteridis, was purchased from Sigma Aldrich, South Africa) at 20 mg/kg was used as a positive control of an inflammatory response due to its known ability to activate antigen presenting cells (Lee et al., 2009). Polystyrene beads that were not fluorescently labelled and of a similar size range to PLGA nanoparticles were used as a negative control. PLGA nanoparticles in a suspension of 20 mg/ml were administered in a volume of 0.2 ml. The same concentration was used for polystyrene beads. Spherical Zinc Oxide nanoparticles (ZnO nanopowder was supplied by Sigma Aldrich South Africa with an average particle size of 100 nm and a range of 50–150 nm) which have been reported to result in *in vitro* toxicity in cell lines (Lee et al., 2009) were included to represent metal based nanoparticles. Although the ZnO nanoparticle size was provided by the supplier, it was also analysed via DLS using the Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK). Using ZnO nanoparticles would provide a comparison of biodegradable and biocompatible polymer based (PLGA) nanoparticles and metal based nanoparticles. ZnO nanoparticles were administered at 4 mg/0.2 ml saline (i.e. 20 mg/ml). The Mouse TH1/TH2 Kit (BD Biosciences) was utilised on the BD FACSarray™ for the detection of IL-4, IL-2, TNF-α and Interferon γ (INF-γ). IL-5, IL-6, IL-10, monocyte chemotactic protein (MCP-1) and IL-12p70 were analysed via the Mouse Inflammation Kit from BD Biosciences (Morgan et al., 2004). Cytokine concentrations were determined by the reference standard curves based on standards that were supplied with the kits. The cytokine data were analysed using the FCAP array™ v1.0 software and expressed as picograms per millilitre (pg/ml) of the mean of the triplicate and repeats.

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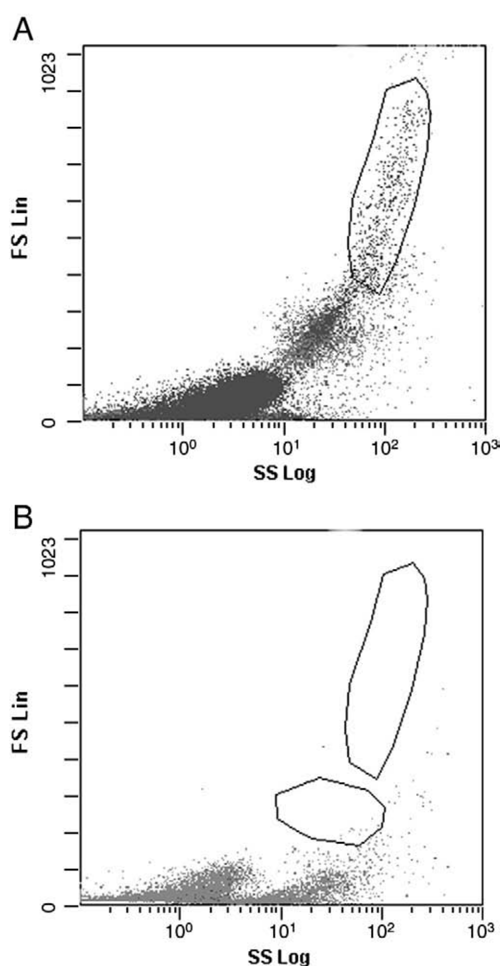

Fig. 3. FS/SS plot of peritoneal exudate cells after FACS analysis. The x-axis indicates the side scatter and the y-axis indicates the forward scatter. A = peritoneal exudate cells subsequent to thioglycolate administration to mice. B = peritoneal exudate cells subsequent to saline administration to mice.

Results

Particle characterisation

Various parameters were optimized to obtain an average particle size for PLGA ranging between 250 and 350 nm and an average polydispersity index (PDI) of 0.2. The use of a stabiliser, i.e. PVA, led to well distributed and uniform PLGA nanoparticles with an average size around 300 nm, characterised by a very smooth surface as depicted by the SEM image in Fig. 1. The particles had a zeta potential of −11 mV. It has been reported that small size (less than 500 nm) and a spherical shape give rise to an enhanced efficiency of cell internalization (Jani, 1990) and that spherical particles possess the right curvature allowing attachment onto the cells (Trewyn et al., 2008). It is generally accepted that spherical particles offer maximum volume for drug incorporation.

Based on the area under the curve from the peak at 3354.2 and 3299.0 cm^{-1} generated from the FT-IR image of the particles as depicted in Fig. 2, coating with chitosan was efficient, with approximately 2.8% chitosan on the surface of the particles from the initial 3% which was added in the formulation. 1% PEG was included in the preparation of PLGA nanoparticles. Since PEG has a similar composition to PVA which is also in the formulation, characterisation thereof would not be accurate. It was thus presupposed based on the loss chitosan in the formulation that the particles were coated with 0.9% PEG. ZnO nanoparticles presented with an average size of 110 nm with a range of 50–150 nm. Polystyrene beads had an average size of 200 nm, with a PDI of 0.1.

Pyrogen test of the particles

The PLGA particles presented with average EU/ml of 0.38 ± 0.18 at 0.5 mg/ml, 0.58 ± 0.12 at 2× dilution and 0.90 ± 0.11 at 4× dilution. ZnO nanoparticles presented with average EU/ml of 0.398 ± 0.08 at 0.5 mg/ml, at 2× dilution 0.247 ± 0.11 and at 4× dilution 0.258 ± 0.09. Polystyrene beads at 0.5 ml/ml had an average EU/ml of 0.4 ± 0.15, at 2× dilution 0.27 ± 0.21 and at 4× dilution 0.21 ± 0.18. Based on the pyrogen test data no pyrogens were present in either of the particles analysed since the average EU/ml detected was below the contaminant limit concentration (CLC) of 2.63 EU/ml as per the protocol provided with the kit. This kit detects for both Gram negative and positive bacteria components as well as yeast and moulds. Thus, it can be said based on these results that the particles were free of any these contaminants.

In vivo particle uptake

Various studies have been reported where particle uptake was observed *in vitro* (Yoshida et al., 2006; Kisich et al., 2007). However, this specific study presents data illustrating *in vivo* particle uptake, by macrophage cells of the peritoneal exudates cells. Initially macrophages were characterised from the subpopulation of PECS as indicated in Fig. 3A using thioglycolate broth induced macrophage proliferation as a positive control. Anti-CD11c and MOMA-2 antibodies were used to characterise these cells. In general macrophages are large in size and also granular, thus they were detected in the higher populations of the forward and side scatter as observed in Fig. 3A. In the saline treated group no activated macrophages were detected in the gated channel as depicted in Fig. 3B.

When FITC labelled polystyrene beads were administered intraperitoneally and orally to mice and the peritoneal exudate cells analysed for particle uptake, an increase in fluorescence intensity in the gated channel for macrophages was observed as indicated in Fig. 4. This result complements data from Olivier et al. (2004) where the *in vitro* uptake of polystyrene beads was observed. The high forward scattering (FS) indicates the large size of the cells and the increase in the FL3 indicates the intensity of the fluorescent label, FITC. Thus, indicating that the macrophage cell population has taken up the fluorescent beads.

The analysis of PECS subsequent to intraperitoneal and oral administration of PLGA nanoparticles indicated that peritoneal macrophages did take up the particle as indicated in Fig. 5 compared

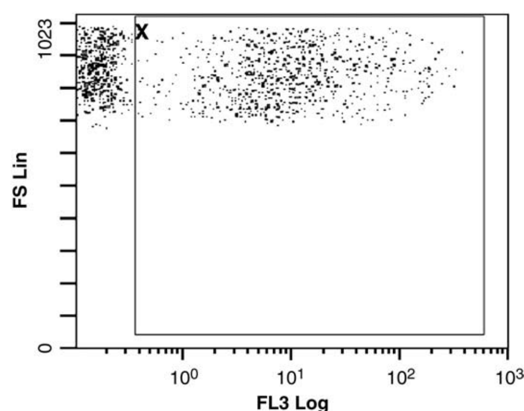

Fig. 4. FACS data of PECS subsequent to administration of polystyrene beads to mice. The x-axis indicates the fluorescent label for FITC and the y-axis indicates the forward scatter.

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Fig. 5. Uptake of PLGA nanoparticles measured by FACS. A = negative control, where mice were treated with saline only. B = indicates positive control where mice were treated with thioglycolate. C = indicates macrophage cells subsequent to intraperitoneal administration and D = indicates post oral administration of PLGA particles.

to the negative control. A positive signal for both anti-CD11c and anti MOMA-2 was observed in the G2 quadrants of the positive control and the experimental group.

To further confirm that uptake of the particles by macrophages of the peritoneal did occur and that the macrophage population detected did not merely consist of activated monocytes that had not phagocytosed the nanoparticles, Rhodamine 6G labelled particles were administered intraperitoneally to mice. The detection was specific for the Rhodamine 6G fluorescence at excitation 525 nm and emission 555 nm, as depicted in Fig. 6 FS indicates the large cells and FL3 indicates the Rhodamine 6G fluorescence intensity. Thus similar to the FITC labelled polystyrene beads, these results illustrate that macrophages (high FS) have taken up the Rhodamine 6G labelled PLGA particles as indicated by the increase in FL3.

From this data, it can be suggested that PLGA particles are taken up *in vivo* by macrophages, and thus intracellular delivery can be achieved. The markers used, i.e. anti-CD11c and MOMA-2 allowed for the detection of macrophages within a population of peritoneal excudate cells. These markers thus represent phagocytotic macrophages which will thus function as antigen presenting cells. This hypothesis was further determined by analysing the cytokine production profile in PLGA treated mice. The particle uptake was further indicated by a population of macrophages that were positive for the Rhodamine 6G labelled particles. Although uptake of particles by macrophages has previously been reported (Ahsan et al., 2002; Olivier et al., 2004), much of the research has been conducted *in vitro.* With this approach, we were able to illustrate that subsequent to intraperitoneal and oral administration of PLGA nanoparticles of the reported size range, particle uptake by the macrophages of the peritoneum was observed. In our previous study (Semete et al., in

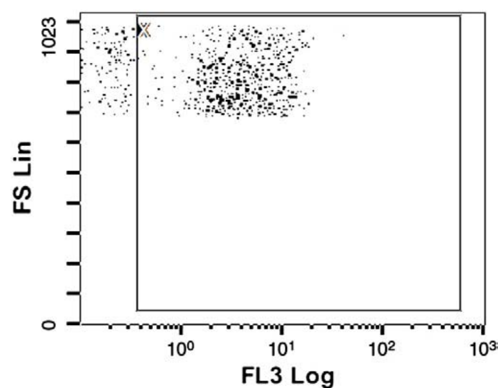

Fig. 6. Uptake of Rhodamine 6G labelled particles analysed by FACS. Data of PECS subsequent to i.p administration of Rhodamine 6G labelled nanoparticles to mice. The x-axis indicates the fluorescent label for Rhodamine and the y-axis indicates the forward scatter.

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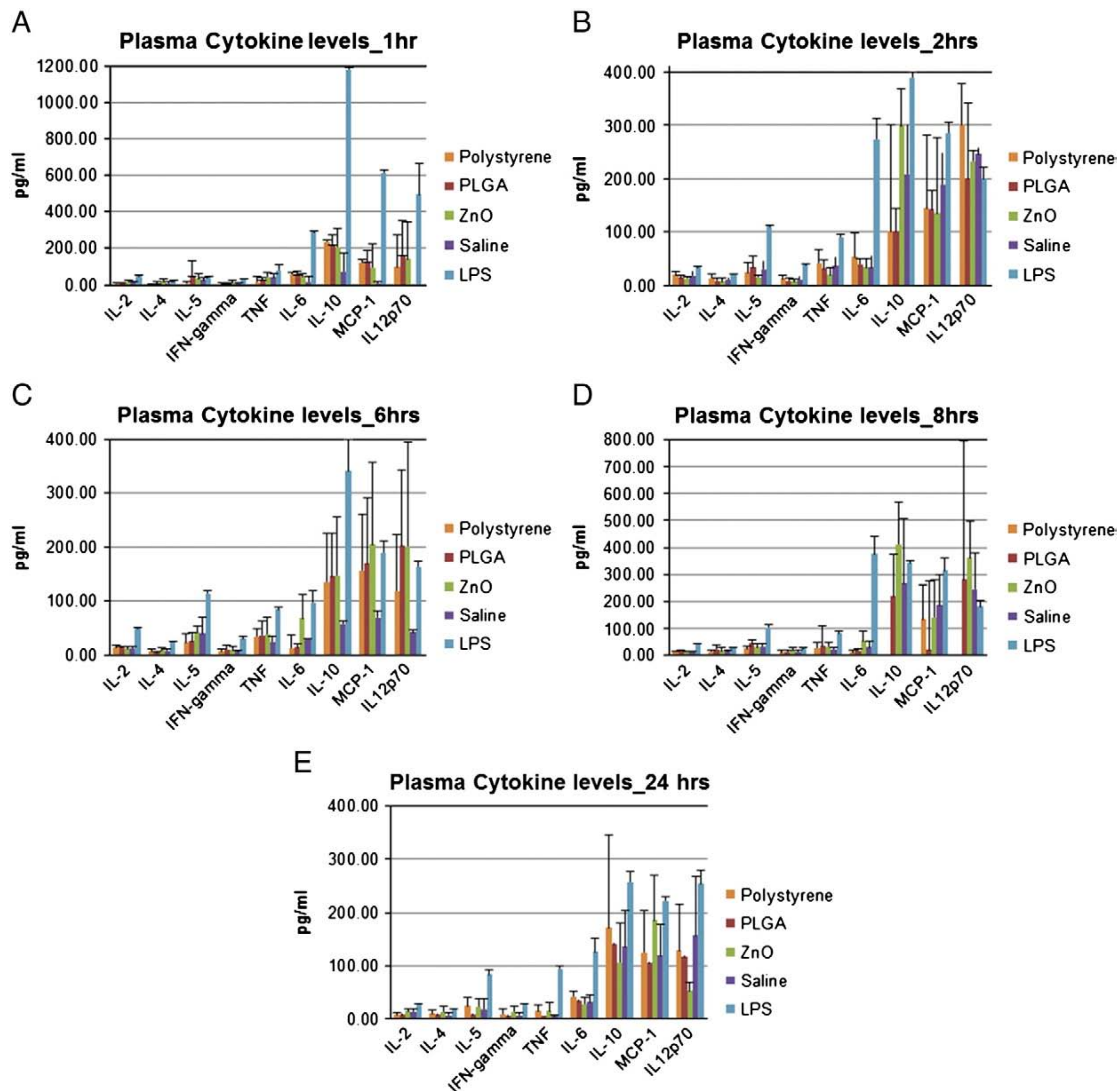

Fig. 7. Cytokine production profile in plasma post oral administration of particles.

press) we illustrated how oral administration of the particles resulted in biodistribution of these particles to various tissues, thus indicating that these particles migrate from the gastrointestinal tract.

Cytokine production

To determine whether PLGA nanoparticles elicit an acute immunological response during the activation of monocytes to macrophages followed by the uptake of the particles, the secretions of pro-inflammatory cytokines IL-2, IL-6, IL-12 and TNF-α together with anti-inflammatory cytokines IL-10 and chemokines INF-γ, IL-4, IL-5, and MCP-1 were analysed. The mice were treated as described. At the PLGA dose administered, i.e. 4 mg/0.2 ml saline, the expression of pro-inflammatory cytokines was found to be within similar production ranges to that of saline, polystyrene nanoparticles in both plasma and peritoneal lavage as depicted in Figs. 7 and 8 respectively. This observation was maintained over the time frame of analysis, i.e. 24 h. ZnO nanoparticles also presented with the same observation at the dose administered. However, LPS treated mice presented with increased production of these cytokines within the first hour of analysis, typical of an immune active substance. This was most prominent with IL-10: A 6 fold increase in the expression of IL-10 was observed within the first hour of LPS exposure. This may follow from the fact that the mode of administration was oral and IL-10 is a prominent immunoregulator in the intestinal tract. The production of IL-10 decreased with time in plasma, although it was maintained quite high in the peritoneal lavage for a longer period of time.

The concentration of MCP-1 in the polystyrene, ZnO and PLGA nanoparticles was variable compared to saline treated mice in the first hours in both the plasma and lavage, but did not maintain an enduring response over 24 h like LPS induced. The variable response could relate to the monocytes that were transiently activated to macrophages resulting from particle uptake and possibly some chemotaxis of cells such as the neutrophils, known to be attracted to sites where phagocytosis activity occurs. A study by Semete et al. (in press), indicated PLGA particle localisation occurring in various tissues including the liver, thus this variable MCP-1 secretion could be as a function of a localised chemotaxis at the tissues where particles are observed. LPS treated mice consistently presented with much higher

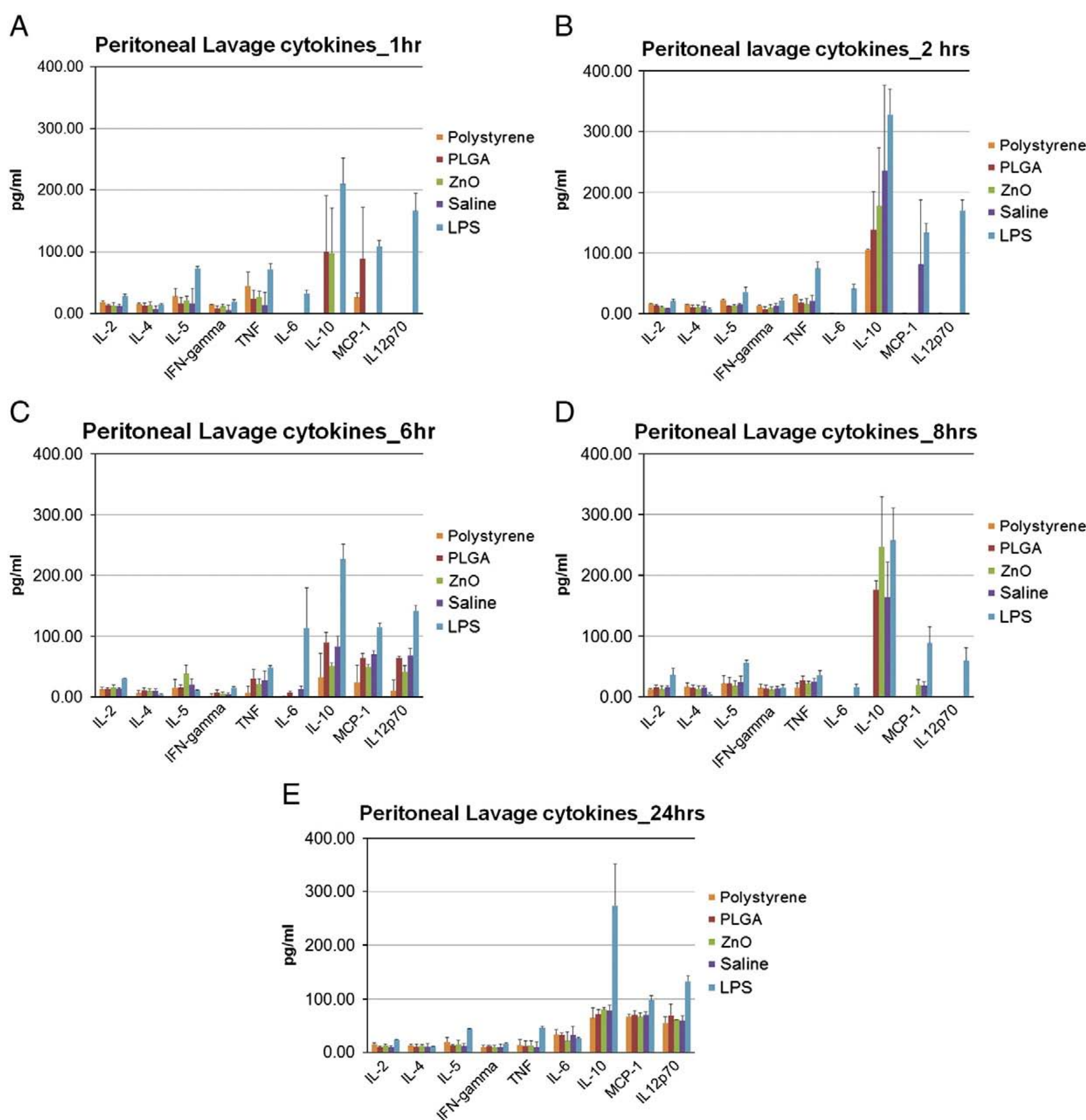

Fig. 8. Cytokine production profile in peritoneal lavage post oral administration of particles.

concentrations of MCP-1 sustained over the full 24 h of observation. IL-12 responded strongly and sustainable to LPS in especially the peritoneal lavage, compared to the PLGA and polystyrene particles. In plasma this effect was less visible. This strong inflammatory cytokine is secreted by all phagocytosing cells, but strongly in the case of inflammatory agents like LPS. The low, transient IL-12 response by the particles confirms the absence of any significant inflammatory activity associated with them.

Low levels of IL-4 were detected in both plasma and lavage throughout the time of analysis in all the treated groups. This argues against an allergic type response by any of the particles as well as LPS. The concentrations of IL-4 observed in the LPS treated mice were not as high as those reported by Lee et al. (2009). However, although Lee et al. (2009) administered LPS at the same concentration as in this study, it was by injection and not oral administration and therefore not strictly comparable.

The expression of TNF-α, IL-6 and IL-5 in LPS treated mice was significantly higher than that of PLGA, polystyrene and saline treated mice, both in the plasma and in the peritoneal lavage. The difference was also noted in nanoparticles treated mice suggesting that at the concentrations used i.e. 4 mg/0.2 ml, even ZnO based nanoparticles do not cause immunological response, thus suggestive of a dose dependent effect. A summary of the cytokines release profile for all the groups is presented in Table 1. Combined, all these results argue against an immunological contra-indication for the oral administration of PLGA particles in mice.

Discussion

At this specific size and composition of the nanoparticles, it can be concluded that chitosan and PEG coated PLGA nanoparticles are efficiently taken up by macrophage cells of the peritoneal exudate cells. Particle uptake by blood monocytes or other cells of the immune systems such as dendritic cells cannot be excluded. This observation indicates that intracellular delivery of compounds against intracellular pathogens will be feasible. The size of the particles, (low negative) zeta potential and their physicochemical properties all facilitated the observed intracellular uptake.

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Table 1
Summary of cytokine production profile across the groups.

Cytokine	LPS (20 mg/kg)	ZnO (20 mg/ml)	Polystyrene (20 mg/ml)	PLGA (20 mg/ml)	Saline
IL-2	↑(Small increase)	↔	↔	↔	↔
IL-4	↑(Small increase)	↔	↔	↔	↔
IL-5	↑(Small increase)	↔	↔	↔	↔
INF-γ	↑(Small increase)	↔	↔	↔	↔
TNF-α	↑(Significant increase)	↔	↔	↔	↔
IL-6	↑(Significant increase)	↔	↔	↔	↔
IL-10	↑(Significant increase)	↑	↑	↑	↔
MCP-1	↑(Significant increase)	↑	↑	↑	↔
IL-12p70	↑(Significant increase)	↔	↔	↔	↔

↑ = increase; ↔ = remain at similar levels to saline group.

There are a large number of *in vitro* studies on nanoparticulate cytotoxicity with various types of engineered nanoparticles (Suh et al., 2009). Some reports indicate the possibility of ultrafine particles acting as adjuvants to enhance inflammatory responses and improve release of pro-inflammatory mediators by macrophages. However, more information is needed about the potential effects of biodegradable and biocompatible nanoparticles versus those of metal oxide particles *in vivo* to determine whether the observed *in vitro* cytotoxicity and inflammatory response is physiologically relevant (Cho et al., 2009; Palomäki et al., 2010). The inability to elicit a significant effect of the immune response does not mean that PLGA particles are completely inert from the immunological point of view. As polyesters in nature, PLGA undergoes hydrolysis and enzymatic degradation upon implantation into the body, forming biologically compatible moieties that can be metabolised (lactic acid and glycolic acid) and are eventually removed from the body by the citric acid cycle (Campbell, 1995). These PLGA biodegradation products are formed at a very slow rate, and hence they do not affect the normal cell function.

Olivier et al. (2004) observed some toxicity when polystyrene beads were analysed *in vitro* in J774.2 macrophages and L929 fibroblasts. A composition dependent effect was also observed where, as reported in our previous study, high doses of PLGA did not lead to any tissue lesions as compared to the same high dose of ZnO nanoparticles that led to toxicity in mice. This study illustrates the safety of using PLGA nanoparticles for drug delivery applications and further complements our previous study (Semete et al., in press) where using various other parameters, the safety of PLGA nanoparticles was illustrated.

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