Do antiseptic coated sutures reduce colo-colonic anastomotic leaks?

CF Elsenbroek

MBChB, Dept of Surgery, University of Pretoria, South Africa

M Brand

MBChB, FCS, MMed, PhD, Cert Gastro-Enterology (Surg), Dept of Surgery, University of Pretoria, South Africa

Abstract

Background: Colorectal anastomotic leaks remain one of the most significant complications following colorectal surgery. Various interventions to reduce anastomotic leaks have been investigated, however few have resulted in a significant improvement. To date antiseptic coated monofilament sutures for sutured bowel anastomoses have not been assessed, hence this study was undertaken to investigate whether or not triclosan impregnated polydioxanone suture material (PDS) results in fewer anastomotic leaks.

Methods: A rabbit colo-colonic anastomotic model was developed to compare the tensile strength and local inflammatory response between triclosan coated PDS and uncoated PDS.

Results: Of the 42 anastomoses there were 4 (9.5%) leaks. Of the remaining 38 anastomoses neither the leak pressures, degree of bowel wall inflammation or fibrosis were statistically different ($p= 0.11$; 0.813 and 0.658 respectively) when comparing the two suture materials.

Conclusions: In an animal model, triclosan coated PDS is as safe as uncoated PDS in performing colo-colonic anastomosis.

Abbreviations

(CAL) Colorectal anastomotic leak

(AEC) Animal ethics committee

(PDS) Polydioxanone suture

(DNA) Deoxyribonucleic acid

(RNA) Ribonucleic acid

(PCR) Polymerase chain reaction

(IBM) International Business Machines

(SPSS) Statistical Package for the Social Sciences

(SAS) Statistical Analysis System

(RCT) Randomised control trial

(Cm H_2O) Centimetres water pressure

Introduction

A colorectal anastomotic leak (CAL) may be defined as local bowel dehiscence following a colo-colonic or colorectal anastomosis. The prevalence of CAL has been reported to be between 1 and 19% (1) with an associated increased morbidity and mortality rate. Overall mortality rate ranges between 10 and 16%. (2) Furthermore, a study from 2007 and 2009 in the United Kingdom demonstrated that the additional cost of health care to treat CAL complications ranged between £1,1 million and £3,5 million annually. (3)

Physiological considerations for anastomotic healing include factors associated with poor wound healing such as diabetes mellitus, current steroid use and organ dysfunction; increased intraluminal pressures and the presence or absence of specific anaerobic and aerobic bacteria. Primary healing at the anastomotic site follows three phases: acute inflammation (under influence of matrix metalloproteinases rapid collagen degradation lasting 4 days,), proliferation (increase in collagen production) and maturation (crosslinking of collagen fibres). Type 1 collagen is the predominant connective tissue type, being most prevalent in the submucosal layer and is the most important component lending strength to the anastomotic site. It is thus imperative for early anastomotic strength that the suture or staple bridges this early phase of wound reconstitution. (4) It also follows that any attenuation of the acute inflammatory response will add to collagen retention at the wound margins. The inhibition of matrix metalloproteinases at systemic level have been investigated in animal models, showing a 48% increase in breaking strength at anastomotic site. (4) One study using doxycycline (metalloproteinase inhibitor) coated sutures for colorectal anastomosis in a rat model showed a 17% increase in breaking strength at the anastomotic line. (5)

The two anastomotic techniques most commonly used in colorectal surgery are either stapled or hand sewn anastomoses. A systematic review of nine randomised control trials of emergency colorectal surgery in 2012 demonstrated no advantage in mortality, hospital stay, infection rates or need for reoperation when comparing these two methods. The only statistically significant differences were an increased time to perform anastomosis with hand suturing and increased stricture formation with stapling. (6)

Risk factors shown to increase CAL are divided into pre-operative factors such as male gender, American Anaesthesiology Score of 2 and above, comorbidities, kidney disease and previous radiotherapy and intraoperative factors including surgical technique, operative time longer than four hours, pre-existing contamination and excess blood loss. Surgical technique is considered a crucial risk factor and relates to the experience of the surgeon, where emphasis is placed on tension free anastomosis, adequate tissue apposition and good tissue perfusion; regardless of anastomotic method used. (1) However, the evidence shows that even well perfused, tension free anastomoses may develop CAL and that experienced surgeons fail to predict which anastomoses are at risk of leaking. (7) Reversible factors which increase the risk of CAL include smoking, obesity, heavy alcohol intake, current immunosuppressant drug use and hypoalbuminaemia. (1) The mechanism by which these risk factors contribute to CAL has been poorly studied and shows wide variance in retrospective studies. (8) An intervention that has been shown to decrease CAL rate is preoperative intestinal bowel decontamination (using poorly absorbed oral antibiotics) together with simultaneously systemic antibiotics. (9) However, this is not ideal as this method could promote bacterial resistance.

The presence of bacteria at sites of wound healing delay the resolution of the acute inflammatory response by perpetuating innate immune responses, (10) however the nature of bowel healing following anastomosis and bacterial influence is poorly understood. (8) The assumed role of collagenase producing bacteria in reducing anastomotic healing is

controversial, considering the persistence of these organisms at anastomotic sites in studies following mechanical and antibiotic bowel preparation. (11) The previously mentioned study (9) inferred that colonic luminal bacterial decontamination with oral and systemic antibiotics with resulting alteration of enteric flora improved anastomotic healing. (8) Recent studies using bacterial DNA analysis of bowel harvested following anastomosis, demonstrate prevalence of certain families of bacteria (lachnospiraceae, with unknown virulence) at leaking anastomotic sites when compared to control groups. It must still be established whether or not these organisms directly or indirectly, if at all, contribute to the development of a CAL. (12) Metagenomics, used to analyse gastro-intestinal tract microbial RNA and DNA, has shown that there is a much wider variety in microbes than previously thought. It does not require cultivated clonal cultures but rather through PCR and shotgun sequencing may identify and match bacterial DNA to genetic databases, as opposed to traditional culture techniques which fail to identify organisms as a result of the difficulty in culturing them. (13)

Triclosan (5-chloro-2-{2,4-dichlorophenoxy} phenol) is a general antibacterial and antifungal agent present in many detergents, cosmetic soaps and tooth paste. It's mechanism of action is by inhibiting the enzyme enoyl-acyl carrier protein reductase essential for the synthesis of fatty acid in bacterial cell membranes. (14) In vitro studies demonstrate how triclosan coated sutures display antimicrobial activity sufficient to prevent colonisation by *S. epidermidis* and *S. aureus.* (15) This resulted in the development of 'antimicrobial' sutures. The first, Vicryl Plus (polyglactin 910 suture coated with triclosan) was approved by the Food and Drug Administration USA in 2002. (16) Since its introduction it has been widely studied in reducing post-surgical abdominal wound sepsis. A recent meta-analysis of 21 randomised control trials including 6462 patients demonstrated a relative wound infection risk reduction of 15%. (17) To date triclosan coated suture materials have not been studied in a colo-colonic anastomosis model. Hence the need for this study in an attempt to reduce colo-colonic leak rates.

Methods

Fourteen adult male New Zealand White rabbits were used in the study. One rabbit was used in a pilot study to confirm study method and feasibility. The 13 remaining animals were divided into two groups, each animal had three colo-colonic anastomoses performed. Animals were assigned to groups using a non-randomised numerical system as they arrived in theatre. The designated number determined the experimental arm assigned to (odd vs even numbers). All 14 animals were included in the study analysis. The first proximal anastomosis was performed 15cm distal to the ileocecal valve with uncoated 3-0 PDS, the second at 30cm distal to the ileocecal with coated 3-0 PDS, these anastomoses were used for histological analysis. The third anastomosis 75cm distal to the ileocecal was used to perform a leak test. Even numbered rabbits had this anastomosis sown with coated 3-0 PDS anastomosis (Illustration A), uneven numbered rabbits with uncoated 3-0 PDS, except for rabbits number 12, 13 and 14 who all had their third anastomosis sown with uncoated 3-0 PDS to make up for numbers lost due to premature death in the plain PDS arm. All anastomoses were created using a single layered, hand sewn continuous Lembert suture.

All rabbits underwent laparotomy with a ketamine based general anaesthesia during which the three anastomoses were performed following clean transection at each designated point in the colon. All anastomoses were performed under the senior researcher's supervision, this served to control for surgeon experience in limiting technical errors. Care was taken not to injure the colonic mesentery thus avoiding potential iatrogenic ischaemia at the anastomotic lines. The abdominal wall was repaired using Nylon 2-0 sutures. The rabbits were allowed to recover from anaesthesia and kept in a postoperative recovery area for the duration of the study.

Initial observations including vital signs, clinical signs of adequacy in pain management and tender abdomen were documented and managed appropriately by the veterinary nursing staff.

Signs of bowel ischaemia or perforation were used to determine the need for early euthanasia, at which time the animal was returned to theatre for a general anaesthetic, an exploratory laparotomy to determine the source of the acute abdomen. If there was anastomotic leak, the leak site was noted and the animal euthanised. Otherwise the animals underwent a second laparotomy 10 days after the first. The decision to analyse the anastomoses on day 10, was made based on the inference that fibrosis (ie healing with collagen deposition) would be maximal after the first week of the colo-colonic anastomosis. This will allow us to compare the tensile strength and degree of fibrosis between the triclosan coated and uncoated PDS anastomosis suture lines.

The rabbits were housed in a conventional unit with filtered air. They were single housed in floor pens with straw bedding and enrichment (hide, balls, wooden toys and cardboard). They had a light cycle of 12 hours light and 12 hours dark, temperature of 20 +/-2ºC. They were provided with EPOL brand rabbit pellets, fresh fruit and vegetables daily, reverse osmosis water, fresh grass species and lucerne ad libitum daily. Animals were assessed twice daily for any adverse reactions or abnormal behaviour. When there were incidents or sick animals the veterinarian was immediately notified as well as the researcher. Following discussion, if needed, the animals were euthanised.

During the second laparotomy the surgeon resected 2mm on either side of the first two proximal anastomotic lines, these were stored in formalin and sent for histological examination with immunohistochemical staining to determine the extent of inflammation and fibrosis. Inflammation was graded from minimal to severe using the simplified Geboes score (18) grade, serving to gauge the degree of foreign body tissue reaction to the suture materials. The scoring system uses the presence of plasma cells, eosinophils, neutrophils and extent of ulceration of surface epithelium to gauge the severity of inflammation, and is scored from 0 to 4 (Table 1). Fibrosis, a surrogate marker for healing, was graded from minimal to severe, based on the

number of fibroblasts observed and thickness of collagen layers laid down during healing (Minimal: few cell layers thick; Mild: multiple cell layers thick; Moderate: interlacing bundles; Severe: multiple interlacing bundles). The third (distal) anastomoses were used for pressure tests. All procedures including the pressure analysis were performed by the same surgeon to prevent inter-operator variation. These were performed by clamping the bowel segment 5cm either side of the anastomosis and injecting normal saline with blue dye into the bowel lumen and documenting the intraluminal pressure at which it perforated by using a pressure transducer. (19) The rabbits were euthanised once the samples and pressure tests were completed.

Ethics approval was obtained from the University of Pretoria Animal Ethics Committee (H005-18). The sample size was determined by the Animal Ethics Committee in consultation with a statistician to allow for adequate statistical strength, while preventing unnecessary animal suffering and death. The use of permutation testing allowed for a minimum of 5 subjects a side in the pressure analysis and sufficed for a pilot study of this nature. The histology specimens were doubled per animal as each served as their own internal control.

The primary outcome was to determine if triclosan coated PDS leak more or less then plain PDS. The secondary outcome measured and compared the extent of inflammation and fibrosis at anastomotic sites.

Statistical analysis of anastomotic leak pressures was performed using non-parametric tests to compare the control and intervention groups using IBM SPSS Statistics 26 and SAS 9.4 software. Differences between the two groups were determined using the Independent-samples Median test and reporting Fisher's exact significance level, permutation testing for the means and the Mann-Whitney U test for the distributions. Histology results were analysed using a Chi-square test. The degree of fibrosis and the extent of inflammation were compared between

the intervention and control groups. The standardized grading of resected specimens was based on categorical data sets, measured from minimum to severe, for both fibrosis and inflammation.

The level of significance was specified at $\alpha = 0,05$ (5%). The null hypothesis was rejected if a p-value of $< 0,05$ was obtained.

Results

Of the 42 performed there were 4 (9.5%) leaks, one at a triclosan coated PDS anastomosis and three at uncoated PDS anastomoses resulting in the premature deaths of 4 rabbits (Table 2). These were excluded from analysis. No other adverse events (i.e. allergy/toxicity) were noted during the study.

The rabbit's mean pre-operative weight was 3.43kg (Range 3.09-3.84kg). All rabbits lost approximately 0,3kg of their pre-operative weight (Appendix 1).

There were 9 anastomoses available for pressure analysis, 5 were triclosan coated PDS with a mean leak pressure of 9,6 cmH_2O (Range 7-15 cmH_2O) and 4 uncoated PDS with a mean leak pressure of 5,25 cmH_2O (Range 2-8cmH_2O) (Appendix 2). No statistical difference was found between the two groups with regards to the means and medians of the leak pressures (permutation test p=0,164 and Fisher's exact p =1.00 from the median test)(Figure 1).

There were 18 anastomoses available for histological analysis (9 triclosan coated and 9 uncoated). All histological analysis was performed by the same pathologist. Each animal served as their own control. Each anastomosis was histologically analyzed and graded for both inflammation and fibrosis as previously described. Both the inflammation and fibrosis group values were plotted on a line graphs. (Figure 2 and Figure 3). Chi-squared tests were performed, confirming the observed similarity between the two groups with inflammation (p =0.813) and fibrosis (p=0.658). Individual inflammation and fibrosis grades tables in Appendix 3.

Discussion

The standard definition of a clinically significant anastomotic leak is an anastomotic breakdown leading to local and/or systemic complications in the operated individual which can be proven radiologically or at repeat surgery. This is in contrast to an insignificant leak which has no clinical significance but is found incidentally on imaging or repeat surgery for other indications. The real area of interest is in identifying and preventing clinically significant anastomotic leaks.

In spite of accounting for known risk factors associated with colorectal leak, this complication remains common in both elective and emergency anastomotic surgery (1). Whether altering local colonic bacteria may potentially decrease the incidence of colorectal leak remains unknown. In this study the mechanical strength as well as inflammatory response of triclosan coated PDS was compared to plain PDS in a murine model.

The primary outcome was to determine whether triclosan coated PDS leaked less than uncoated PDS in colo-colonic anastomoses. The maximum pressure at which an anastomosis leaks under increasing intraluminal pressure was used as a surrogate for determining leak pressures in this laboratory model. There was moderate evidence of a statistical difference between the distribution of the two groups (Mann-Whitney U test p =0,111). This suggests a higher leak pressure in triclosan coated PDS compared to uncoated PDS, however, the power of this test is low due to small sample size. In this study, the tensile strength between triclosan coated PDS and uncoated PDS suture (in a colo-colonic anastomosis rabbit model) is comparable.

The secondary outcome of comparing inflammation, a surrogate marker for tissue injury, and fibrosis, a surrogate marker for healing, between the two groups using histological grading systems was not statistically significant. In theory, if there are less proinflammatory bacteria at the suture line, there should be a milder inflammatory response (as what can be expected from

the antibacterial effects of triclosan), this should also lead to improved anastomosis healing by limiting the presence of collagenase producing bacteria, hampering collagen deposition and effective fibrosis. The distribution of data was similar between both groups with a predominance of a mild inflammatory response and severe fibrosis at the suture lines. This confirms the similarity between both suture types in provoking a similar acute immune reaction and does not add to the theory that alteration of bacteria at the suture line alters the local inflammatory response or tensile strength.

The possibility that triclosan coated PDS does not alter pathognomonic bacteria sufficiently to affect the local immune response, or that bacteria at the suture line do not necessarily play a significant role in local wound healing is still present. These theories should be tested using direct bacterial analysis using both bacterial culture and metagenomic methods in a larger randomised anastomotic trial.

The main limitation of this study was the small study group and that it was not randomised, however, we were limited by ensuring an ethically appropriate study using a minimum number of animals that would yield a statistically assessable result.

We have demonstrated that triclosan coated PDS is at least as safe as uncoated PDS in colo-colonic anastomoses. There was no significant difference in leak pressures, inflammation or fibrosis but these outcomes should be confirmed in a larger study with greater statistical power.

Table 1: *The simplified Geboes score (18)*

Grade 0 No inflammation	No abnormalities, including architectural or mononuclear cell infiltrate
Grade 1 Basal Plasma cells	Mild to marked increase
Grade 2a Eosinophils in lamina propria	Mild to marked increase
Grade 2b Neutrophils in lamina propria	Mild to marked increase
Grade 3 Neutrophils in epithelium	Presence of neutrophils in epithelium extending into crypts
Grade 4 Epithelium injury	Ulceration or crypt destruction

Table 2: *Anastomotic leaks data*

Rabbit number	Day of leak	Site of leak (anastomosis)
3	2	Proximal (uncoated)
9	4	Middle (coated)
11	3	Distal (uncoated)
14	2	Proximal (uncoated)

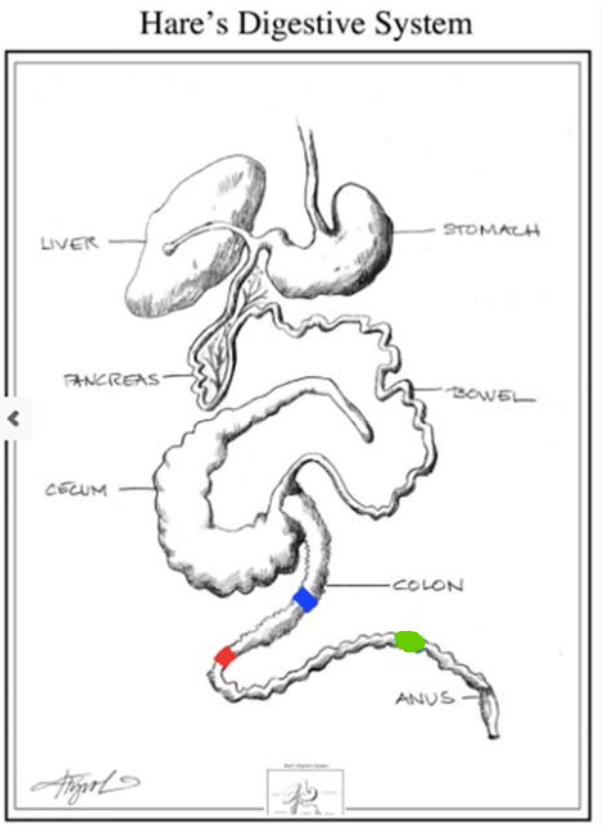

Illustration A (20)

Blue: 1st Anastomosis (Plain PDS 3-0)

Red: 2nd Anastomosis (Triclosan Coated PDS 3-0)

Green: 3rd Anastomosis (Either Triclosan coated or plain PDS, depending on rabbit number, see methods)

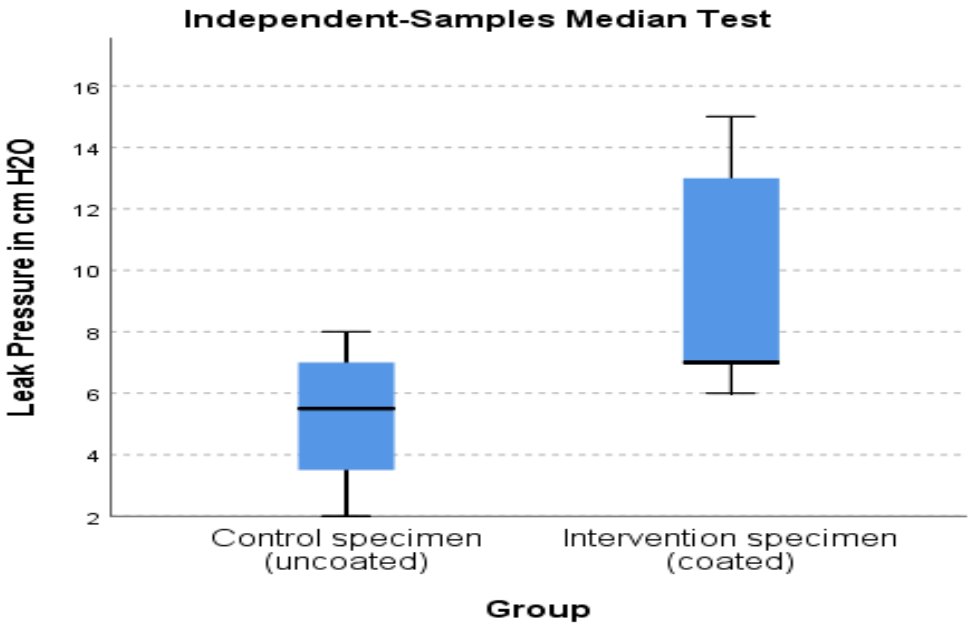

Figure 1: Box plot of leak pressures uncoated PDS versus triclosan coated PDS

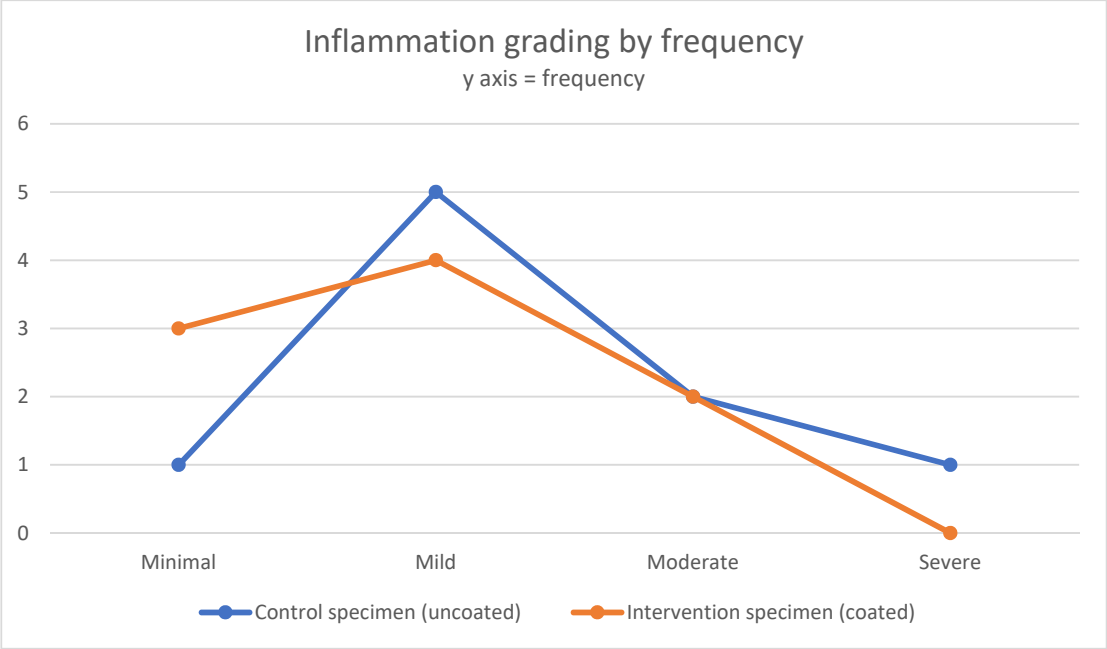

Figure 2: Suture material induced inflammation

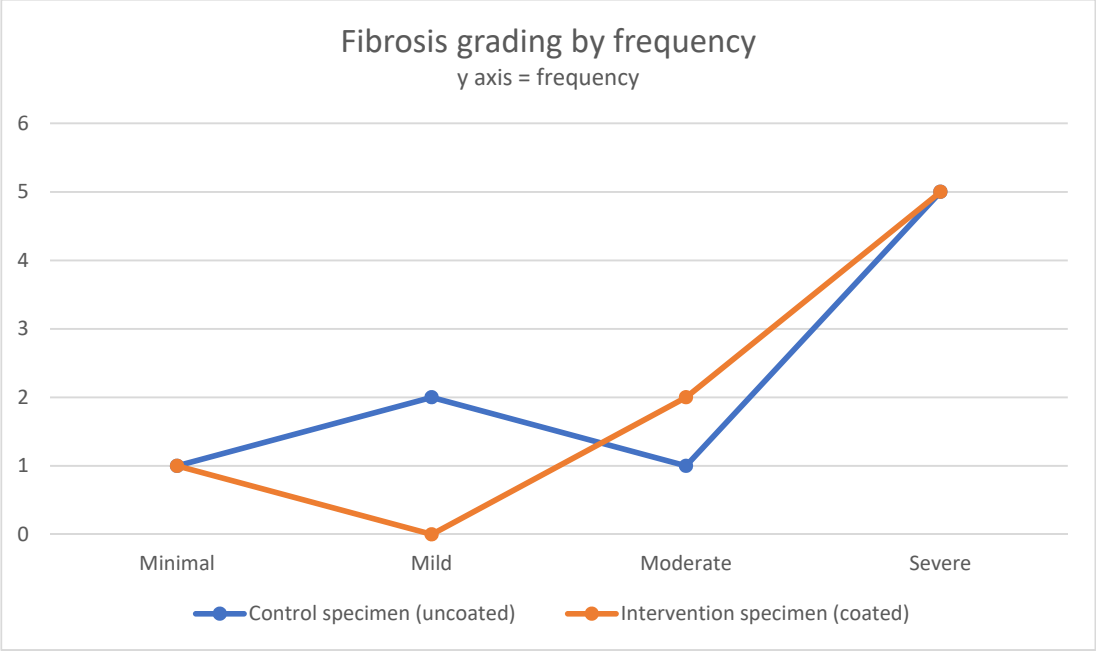

Figure 3: Suture material induced fibrosis

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Appendix 1: Main study rabbit weight table (kg)

Rabbit Number	Starting Weight Kg	End Weight Kg	Net Weight Loss Kg
2	3,84	3,38	0,46
3	3,45	3,4	0,05
4	3,35	3,11	0,24
5	3,58	3,1	0,48
6	3,74	3,22	0,52
7	3,45	3,18	0,27
8	3,58	3,21	0,37
9	3,17	2,73	0,44
10	3,43	3,2	0,23
11	3,38	3,25	0,13
12	3,09	3,05	0,04
13	3,4	2,85	0,55
14	3,19	3,01	0,18

Kg: kilograms

Appendix 2: Leak pressures (cm H_2O)

Rabbit number	**Leak pressure cmH_2O**
5 (uncoated)	6
7 (uncoated)	8
12 (uncoated)	5
13 (uncoated)	2
2 (coated)	7
4 (coated)	7
6 (coated)	6
8 (coated)	13
10 (coated)	15

cmH_2O: centimetres water

Appendix 3

Histology grading for uncoated PDS anastomosis per rabbit

Rabbit Number	Inflammation	Fibrosis
2	2	2
4	2	4
5	4	4
6	2	4
7	2	2
8	3	4
10	3	4
12	2	3
13	1	1

Histology grading for triclosan coated anastomosis per rabbit

Rabbit Number	Inflammation	Fibrosis
2	2	3
4	3	4
5	2	4
6	2	4
7	2	0
8	3	4
10	1	4
12	1	1
13	1	3