Peritoneal Adhesion Formation and Modulation

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Index

Abstract

Author Statement

Chapter 1: Introduction

Chapter 2: Literature Review

Statement of authorship

Article; Abdominal Adhesion Prevention: Still a Sticky Subject?

Chapter 3: Challenge to Current Practice

Statement of authorship

Article; Abdominal adhesion prevention, time to change our everyday practice?

Chapter 4a: Experimental Work

Rat Model of adhesion formation

Statement of authorship

Article; Use of a modified chitosan – dextran gel to prevent peritoneal adhesions in a rat model.

Chapter 4b: Experimental Work

Porcine Model of adhesion formation

Statement of authorship

Article; Use of a Modified Chitosan – Dextran Gel to Prevent Peritoneal Adhesions in a Porcine Hemicolecotomy Model

Chapter 5: Conclusions

Chapter 6: Discussion

Acknowledgements
Abstract

This thesis examines the subject of peritoneal adhesion formation following surgery in the format of a Master of Surgery by publication. A comprehensive literature review examines all aspects of peritoneal adhesions from the basic science to the evidence supporting products aimed at adhesion reduction.

Subsequent experimental work utilises two animal models to form adhesions and test the ability of a novel gel product to reduce adhesion formation. The gel is a hybrid hydrogel consisting of modified chitosan and dextran. These two components are combined by a chemical cross linking reaction to form an inert gel that can be applied to the site of surgery. The gel confers several beneficial properties when used to prevent adhesions. Firstly it provides a physical separation of the injured peritoneal surfaces whilst also inhibiting the ingress of fibroblasts to the area. Secondary characteristics which promote haemostasis and inhibit bacterial growth enhance the gels adhesion reducing potential.

Initially the gel was trialled in a small animal model to test varying compositions and volumes of the gel. Two different surgical models of adhesion formation were utilised to provide a range of stimuli in the post operative period. Results from these experiments were encouraging, showing a statistically significant reduction in adhesion formation.
Following on from this initial study a large animal study was conceived to further evaluate the effectiveness of the gel in differing environments. The porcine model also allowed for a true bowel resection with anastomosis to test the safety of the gel when used in this scenario. Allied to this the gel was also trialled following adhesiolysis at the mid point of the study, while monitoring for sensitisation or toxicity to the gel. Infective complications and abscess formation proved to be a difficult hurdle to overcome in this model. As such, no significant reduction in adhesion reformation following adhesiolysis was observed. There were however some promising results with a reduction in adhesions to the wound noted with treatment at the time of laparoscopy, as well as a reduction in adhesions involving the bowel at the study end point.

The experimental work highlights the difficulties associated with peritoneal adhesion reduction. Overcoming the numerous stimuli to adhesion formation is not an easy task, and there remains no currently available treatment for the prevention of adhesions without certain caveats to its use. An effective product that could be used safely in practically all environments would certainly be a step forward in this branch of surgical research. It is plausible that a product such as the gel may be improved upon to show further benefit. However, long term studies will still be required to show a beneficial effect in long term outcome measures such as the incidence of small bowel obstruction.
Author declaration

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Abdominal adhesion prevention: still a sticky subject?
Lauder CI, Garcea G, Strickland A, Maddern GJ.

Abdominal adhesion prevention, time to change our everyday practice?
Lauder CI, Strickland A, Maddern GJ.

Use of a Modified Chitosan-Dextran Gel to Prevent Peritoneal Adhesions in a Rat Model.
Lauder CI, Garcea G, Strickland A, Maddern GJ.
J Surg Res. 2010 Sep 8

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Introduction

In laymen’s terms, adhesions can be described as bands of scar tissue that form as a response to surgery. Adhesions have been posing significant difficulties for practically all surgeons since the inception of surgery itself. The main aim of this thesis is to help understand the processes driving adhesion formation and explore ways in which their formation may be reduced. Given my general surgical background, the focus for this project has been peritoneal adhesions. The peritoneum lines the abdominal cavity, covering almost all of the organs within it and becoming involved in the process of adhesion formation when damaged. The mechanism for peritoneal damage includes not only surgical interventions but also infection, inflammation or damage from radiotherapy.

The strength and number of peritoneal adhesions varies considerably from one patient to the next with a large number of factors dictating their development. It is still not possible to predict patients at a greater risk of developing adhesions. Whilst certain procedures are more likely to result in adhesion formation, complications associated with surgery may also be the driving force for the adhesive response. As such, it should be regarded that each patient shares the same risk and treatment aimed at adhesion reduction used routinely.
Literature Review

Abdominal Adhesion Prevention: Still a Sticky Subject?

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Digestive Surgery 2010: Accepted Paper

The literature review explores the complexity of adhesion formation, from the basic science to the evidence base of products aimed at adhesion reduction. There is a huge body of research on the subject that can be overwhelming for surgical trainees and experienced surgeons alike. The review provides a comprehensive overview of the subject with the hope that it could be used as a resource for medical practitioners looking to expand their understanding of this fascinating subject.
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**NOTE:**
This publication is included on pages 4-15 in the print copy of the thesis held in the University of Adelaide Library.
Challenge to Current Practice

Abdominal adhesion prevention, time to change our everyday practice?

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ANZ Journal of Surgery 2010: Accepted Paper

Having performed the literature review it became evident that although numerous studies had been performed, little had been achieved in the widespread uptake of adhesion reduction strategies. A paper was therefore written challenging the current practices of general surgeons regarding their use of adhesion reduction regimens.

This is of particular importance given the wealth of studies and a recent Cochrane systematic review recommending the use of certain adhesion reducing agents. Despite these guidelines, few surgeons adopt these practices in their everyday activities. Clearly this has a direct effect on the potential outcomes of patients surgical procedures. Hopefully by continued publication on the subject the perception of adhesion prevention may change.
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Experimental Work

The basis for the experimental work in this project was the investigation of a novel product to reduce adhesion formation. This product is in the form of a gel that can be applied into the abdominal cavity after a surgical procedure.

The gel was first used in our institution by the ENT department having being developed in conjunction with the biochemists at the University of Otago. It can be classified as a hybrid gel since there are number of mechanisms to its mode of action. The two constituents of the gel are dextran and chitosan. Dextran is a complex glucan which has been used in a variety of medical applications. For the formation of the gel the dextran is oxidised to allow chemical cross linkage with chitosan to from a hydrogel. Chitosan is a product of chitin formed by the process of deacetylation. Chitin is another long chain polysaccharide, which in this preparation is obtained from the exoskeleton of squid.

With respect to the mechanisms of adhesion prevention, there are four key areas where the gel acts. Firstly, the simplest function the gel is to provide physical separation of the injured peritoneal surfaces in the initial period following surgery. Allied to this the second major mode of action is the inhibition of fibroblast proliferation. This is an important step in the potential transformation of an immature adhesion that may be dealt with by the body’s fibrinolytic system, to a mature fibrous adhesion which will persist.
The other beneficial properties of the gel stem from the chitosan component. As chitosan is haemostatic, it can help to stem minor post operative bleeding which could have precipitated adhesion formation. The final advantage relates to the antibacterial properties, with chitosan helping reduce growth of a number of organisms that may otherwise proliferate at the surgical site.

Initial studies using the gel after sinus surgery showed it to be effective in reducing post operative adhesions as well as aiding haemostasis. It seemed a logical progression that the gel to be trialled within the abdomen for potential adhesion reduction. However, there were several factors to be addressed when considering the use of the gel in the abdomen as opposed to the nasal cavity.

Whilst the composition of the gel proved to be non-toxic in ENT studies the gel would be used at a different dose within the abdomen and also subject to differing conditions within the abdomen. The gel will also be contained within the abdomen indefinitely, therefore relying on the body’s ability to metabolise the constituents completely. Asides from these issues regarding the environment of the peritoneal cavity, the gel would also be in contact with the area of the surgical procedure. This is of particular relevance when considering any anastomoses as a detrimental effect on the healing of this union could have dire consequences. Finally, the mode of application may necessitate modifying the gels properties to aid placement within the abdomen, either at the time of open surgery or at laparoscopy. To investigate these principals, studies were conceived to test the gel in a variety of settings.
Rat Model of Adhesion Formation

Use of a modified chitosan – dextran gel to prevent peritoneal adhesions in a rat model.

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Journal of Surgical Research 2010: Accepted Paper

With this rat model two methods of peritoneal damage were performed to assess the gels effectiveness and safety. Only a limited number of studies have been conducted previously using formulations containing chitosan to prevent abdominal adhesions. A review of the literature was therefore performed to identify experiments with relevant methodology. There are many means by which damage may occur to the peritoneal surface during surgery, and little consistency between models in the research setting. The model of a caecal abrasion was identified as giving repeatable results that had been used to validate the use of adhesion reducing products.

In addition a further surgical procedure was selected to simulate the conditions of a bowel anastomosis. This involved creating a full thickness injury to the bowel with subsequent repair. Initially it was hoped that a true
bowl anastomosis could be performed. However owing to the small calibre of
the bowel and the time constraints for each procedure, it became apparent
that this would not be possible. An enterotomy was felt to be a valid
alternative to the anastomosis which could be performed in a short space of
time with consistent results that would not introduce any unnecessary bias to
the study.

The study was initially performed with sixty animals, demonstrating
encouraging results. After analysis and presentation of the findings it was felt
necessary to further validate the study with additional controls as well as
trialling differing compositions of the gel. An extension to the project was
therefore arranged to involve a further 20 animals. The findings of the study
provided encouraging evidence of the effectiveness of the gel at preventing
adhesions. However, there were some criticisms of the study that came to
light after peer review. Readers wanted to see the gel applied to a true
anastomosis. The paranoia of surgeons brought on by the application of any
product around an anastomosis can seemingly only be relieved by direct
replication of the circumstances in clinical practice. As such the need for a
large animal model was indentified. This was not the sole reason for selecting
a large animal model since there are several other advantages. It provides the
opportunity to test the gel in a volume that would be equivalent to that
potentially used in a human trial, with an animal whose physiology is
remarkably similar to humans.
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Lauder, C.I.W., Garcea, G., Strickland, A. & Maddern, G.J. (2010) Use of a Modified Chitosan-Dextran Gel to Prevent Peritoneal Adhesions in a Rat Model
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Porcine Model of Adhesion Formation

Use of a Modified Chitosan – Dextran Gel to Prevent Peritoneal Adhesions in a Porcine Hemicolecotomy Model

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The second phase of the experimental work was designed utilising a porcine model. Compared to the rat, the volume of literature using a porcine model in adhesion studies is considerably less. Formation of a model was therefore more difficult to validate and design. No directly comparable study was identified where an adhesion reduction agent was used following surgery. However, given the information and experience gained from the rat based study it was possible to formulate the study design to encompass the needs of the project.

Important aspects included in the study were the formation of a true bowel anastomosis with resection of a segment of the bowel. In order to prepare for this, an opportunity to perform a dissection of the abdomen of an animal being sacrificed by another study group was utilised. This provided important
experience with the anatomy of the pig intestine to reduce the time taken for the surgical procedures during the study. Following this dissection, the decision was made to perform an ileocolic resection, with complete removal of the caecum (a sizable portion of bowel in the pig). This allowed the formation of a small to large bowel anastomosis without removing a portion of bowel that would significantly affect the animals gut physiology.

In order to maximise the potential data collection during the study an intervention at the mid point of the study was conceived. This would allow assessment of the gels ability to prevent adhesion reformation following adhesiolysis as well as providing a chance to take blood samples without causing distress to the animals. Monitoring of toxicity by haematological and biochemical tests could also be reviewed at three separate time points to examine for any trends across the study. This mid point procedure was also designed to assess for sensitisation to the gel, with application at two sequential time points. Any allergic reaction that could result at the time of a second application of the gel could be noted and treated accordingly if required.

Planning for the laparoscopic procedure was designed by a translational approach from an equivalent procedure in a human patient. Utilising the laparoscopic approach for adhesiolysis enabled identical access for surgical procedures throughout the abdomen in each animal. The inherent benefits of reduced recovery time and post operative pain associated with laparoscopic surgery were also of considerable importance. Standardised port placement
between animals ensured a consistent view of the abdomen. This minimised any variation of the video footage for independent observer evaluation.

After consultation with the veterinarian, it was concluded that in contrast to humans, muscle relaxants would not be necessary to achieve a pneumoperitoneum. This is due to the Ketamine sedative that is used to facilitate anaesthesia. Accessing the peritoneal cavity was performed in a manner such that damage to underlying bowel was minimised and adhesions formed were not displaced prior to grading.

The approach to adhesiolysis was again very similar to that used in human patients. An exception to this would be that in order to maintain continuity of treatment between animals it was felt that the procedure should remain laparoscopic unless a truly insurmountable problem occurred. In order to achieve this complex laparoscopic procedures, including the laparoscopic suturing of damaged bowel was undertaken. There were some instances where correlating the video from the adhesiolysis with the findings at autopsy would indicate that there may well have been greater damage to the bowel than appreciated at the time of the laparoscopic procedure. It is likely that under these circumstances in a human patient the decision would have been made to convert the procedure to an open laparotomy. However, as already stated, in order to achieve continuity between animals the procedures remained laparoscopic. Certainly if this aspect of the study were to be repeated, a more fastidious approach to assessing damage to bowel would be performed to try and prevent abscess formation which clouded the results of
the gels ability to reduced adhesion reformation from laparoscopy to euthanasia.
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Use of a Modified Chitosan – Dextran Gel to Prevent Peritoneal
Adhesions in a Porcine Hemicolecotomy Model

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ABSTRACT

Background

The prevention of peritoneal adhesions following abdominal surgery remains an ongoing challenge, with the ideal product for adhesion reduction still elusive. This study examines the outcome of application of a modified Chitosan-Dextran (CD) gel within the intraperitoneal cavity of a porcine model to assess its effect on adhesion formation. This is a unique synthetic gel, its active ingredients being succinyl chitosan and dextran aldehyde.

Materials and Methods

Twenty female domestic pigs were randomised to undergo surgery alone or to receive CD gel at the time of surgery. The surgical procedures comprised of laparotomy and ileocaecal resection with ileo-colic anastomosis. At postoperative day 21, a laparoscopy was performed, and adhesions graded using a predetermined adhesion measurement score. Adhesiolysis was then performed and CD gel applied to all animals. After a further 21 days animals were euthanized and adhesions graded using the same scoring regimen.

Results

Adhesions involving the wound were significantly reduced following application of the gel at the time of open surgery (P=0.01). Following adhesiolysis and further application of the gel a decrease in adhesion scores
involving the bowel was noted (P=0.03). No significant adverse outcomes were observed with application of the gel, specifically no anastomotic leak occurred.

Conclusions

Chitosan-Dextran gel is a well tolerated hydrogel with beneficial properties which have been designed in an effort to reduce postoperative peritoneal adhesion formation. The observed reduction of adhesion scores following the application of the gel is encouraging and should stimulate further development of this product. The lack of adverse outcomes following application of CD gel supports its safe use around a bowel anastomosis.
INTRODUCTION

Peritoneal adhesions following surgery continue to provide a challenge to treatment, with no approach providing a complete solution to this dilemma. Adhesions pose numerous problems when further surgery is considered. The incidence of inadvertent enterotomy at the time of adhesiolysis has been demonstrated to occur in up to 19% of patients undergoing further surgery.\(^1\) Given that there is approximately a 1 in 20 risk of a direct adhesion-related readmission following colonic surgery after 5 years\(^2\), a large number of patients will potentially be exposed to this risk.

Any product aimed at reducing this risk must also be cost effective. A study examining the economics of adhesion reduction showed that an adhesion reduction product costing €300 per patient will not return the initial costs per patient within the first year even if it results in a 100% reduction in readmissions. However, the product would return the initial costs if a reduction in re-admissions of 60% could be demonstrated after 3 years\(^3\). Currently no commercially available product has been able to fulfil this requirement. Whilst reducing the cost of treatment is an important consideration, patient safety must be paramount. A problem experienced with the use of some adhesion reduction strategies has been the increased risk of enteric leak following their use around bowel anastomoses\(^4,5\). This study examines the safety of applying a Chitosan-Dextran (CD) gel around the site of a bowel anastomosis to reduce adhesion formation. A further aspect of the study is assessment of the gels ability to prevent adhesion reformation after adhesiolysis.
MATERIALS AND METHODS

Materials

Modified Chitosan and Dextran was supplied from the Department of Chemistry, University of Otago, Dunedin, New Zealand within sealed containers. A detailed description of the synthesis and structure of the components is described by Liu et al\textsuperscript{6}. The dextran powder was sterilised by Steritech (NSW) using Gamma irradiation at a dosage of 25 kGray. The chitosan was supplied as a solution with sterility achieved by autoclave. Dextran was dissolved in 10ml sterile water, with the addition of bicarbonate to increase the speed of dissolution. Combination of the Chitosan and Dextran solutions was performed immediately before application, with a mixing time of around one minute to provide a gel of the correct viscosity. The cross-linking time and physical properties of the gel can be controlled over a wide range through changes in the synthesis conditions which impact on the level of cross-linking functionalities in the polymer\textsuperscript{6}.

The volume of the gel applied in each animal was 40mls. This was an appropriate volume to achieve adequate coverage of the damaged peritoneal surfaces given the size of the animals. It is also an amount that is inexpensive to produce, and provides a comparative application volume to similar products already in use.
Animals

The study was approved by the Adelaide University and the South Australia Institute of Medical and Veterinary Science ethics committees with the care of the animals performed under the established guidelines set by these institutions. Twenty juvenile female domestic pigs were supplied from the University of Adelaide Laboratory Animal Services for use in the study from around 12 weeks of age with a typical weight around 30kg. Animals were housed at a constant room temperature with a 12 hour light and dark cycle. Standard porcine diet was provided twice daily with water ad libitum. The animals welfare was monitored with the use of a standard observation chart by experienced animal technicians.

Surgical Procedures

Surgical procedures were performed in a maximum group size of 3 animals per day to ensure close monitoring during the immediate period post operatively. Food was withheld for the morning of surgery.

Animals were initially sedated with an intramuscular (IM) injection of Ketamine at a dose of 10mg/kg. Anaesthesia was then achieved using a face mask to deliver 2-3% Isofluorane. The airway was then secured with an endotracheal tube to allow continuous delivery of Isofluorane. Cannulation of an ear vein
was performed to allow phlebotomy as well as administration of 1000ml of
normal saline. A single dose of long acting antibiotic (Betamox 450mg) was
also administered IM as well as buprenorphine at a dose of 0.1mg/kg.

The abdomen was shaved and prepared with alcohol and iodine solution.
After drying, a 15cm laparotomy was performed to gain access to the
abdominal cavity. The position of the incision was standardised to be 5cm
above the umbilicus and 10cm below.

Briefly, the caecum was identified and the mesentery divided over ties to allow
resection of the caecum and a segment of colon with the ileocaecal valve and
a short segment of the terminal ileum. The distal colonic resection margin was
simultaneously closed and divided using a surgical stapling device. A hand
sewn end to side anastomosis was then performed using multiple
monofilament polyglyconate synthetic absorbable sutures. A leak test was
performed by insufflation of the bowel with direct needle puncture adjacent to
the anastomosis while the proximal and distal portions of bowel were closed
with soft bowel clamps.

The abdominal wall fascia was closed with an 0 polyglactin suture. Prior to the
placement of the final abdominal closure suture, the animal was randomly
assigned to treatment or control group. In the treatment groups, 40ml of gel
was applied directly to the anastomosis as well as around the abdominal
cavity using a syringe and a sterile plastic cannula. Skin closure was
performed with a 3/0 braided absorbable subcuticular suture. Local
anaesthetic (0.5% bupivicaine at a dose of 2.5mg/kg) was infiltrated to the entire length of the wound. The time taken for the procedures was kept to a minimum, typically around 50 minutes.

Animals were observed continuously until recovered from anaesthetic and ambulatory. Further analgesia in the form of IM buprenorphine was given at 12 hourly intervals for the first 24-48 hours depending on the animals' observations with respect to movement or discomfort. Food was provided the morning following surgery as a slurry of the usual feed mixed with water for 48 hours.

For the laparoscopic procedures, the pre operative regimen was repeated to anaesthetise the animals. Formation of a pneumoperitoneum was achieved by an open cut down to obtain access to the abdominal cavity. A 10mm port was then inserted and the abdomen inflated with CO2. The incision for this first port was placed in the left lower quadrant, lateral to the previous midline scar to avoid inadvertent enterotomy. Two further 5mm operating ports were placed either side of the first port under direct vision. A laparoscopy was then performed to assess adhesion formation. All adhesions that had formed were taken down with blunt dissection where possible, or sharp dissection if necessary. Once adhesiolysis was complete 40ml of gel was applied under direct vision around the abdominal cavity, attempting to cover any sites of adhesiolysis. This was achieved using a syringe and a sterile plastic cannula passed through either 5mm port. Procedures were videotaped to allow
independent observers blinded to the treatment arms to score adhesion formation.

The post operative protocol following laparoscopy differed slightly in that animals were given a normal diet the same day as the procedure.

**Evaluation of adhesion formation**

On the twenty first day after laparoscopy the animals were euthanized using a standard protocol. Animals were initially sedated with an intramuscular (IM) injection of Ketamine at an increased dose of 20mg/kg. Anaesthesia was then achieved using a face mask to deliver 2-3% Isofluorane to allow cannulation of an ear vein. After phlebotomy a lethal 50ml dose of phenobarbitone was administered. A laparotomy was then undertaken to assess adhesion formation. The initial incision was performed at a position remote to the original laparotomy scar to prevent disturbing any adhesions between the abdominal viscera and abdominal wall. The abdominal wall was then opened fully under direct vision to examine the extent of adhesions formed.

Any adhesions found at laparotomy were initially graded by the principal investigator using a scoring system developed to detail the full spectrum of adhesive response seen following surgery. This included describing the sites of all adhesions as well as the nature of adhesions found. Each site (with the exception of the area of the anastomosis) was assigned two potential areas for scoring. These primary or secondary locations were assigned on the basis
of the density of adhesions, with the primary site having the greatest density. Secondary scores were only generated when a site was affected by more than one adhesion.

In order to allow blinded independent observers to utilise the system effectively, descriptions of adhesion strength were kept simple, either filmy (translucent) or dense (opaque), receiving a score of one or two respectively. This avoided descriptions used in other systems where the score was influenced by the type of dissection required to lyse the adhesion being examined. In doing so we hope to have achieved a more objective measure for observers viewing video or photographic images. The scoring system is shown in Table 1. Examples of the differing grades of adhesion formation are shown in Figure 1.

**Statistical analysis and power calculations**

Power calculations were performed by the University of Adelaide Statistical Support Services to establish the number of animals needed for the study to achieve a power of 90%. This was calculated using the standard deviations of results from previously published work in which an alternative anti-adhesion substance was evaluated in a porcine model\textsuperscript{7,8}. It was considered that a significant effect following treatment had occurred if the two arms of the study differed by an average of two points on the adhesion scores. Statistical analysis was performed using R statistical language (R Development Core Team (2008)). R: A language and environment for statistical computing. R
Foundation for Statistical Computing, Vienna, Austria. Animals were randomised with respect to both surgical procedure as well as treatment or control arms.

RESULTS

All animals survived the study with no significant adverse outcomes throughout both periods of recovery from surgical procedures. One animal in the treatment group developed a skin rash following initial surgery which settled but reappeared after laparoscopy. After test doses of medications were administered, the reaction was isolated to a sensitivity to Ketamine.

An animal in the control group developed an asymptomatic midline incisional hernia that was associated with a wound infection. Another animal in the control group required repeated doses of antibiotics after initial surgery due to repeated episodes of sepsis. There were no intra abdominal findings significantly different from the remaining control animals at the study end point. At autopsy the animal was found to have extensive adhesions between the myocardium and pericardium which was thought to be as a consequence of pericarditis.

Inter rating analysis of scores between the principal investigator and two blinded observers was performed using Cohen's kappa coefficient. This demonstrated substantial agreement between observers for both video and photographic images.
Experiment One – Adhesion formation by laparotomy and ileocaecal resection treated with Chitosan-Dextran gel versus control

The open bowel resection provided a major stimulus for adhesion formation. Significantly fewer adhesions were noted around the laparotomy wound in animals treated with the CD gel (P=0.01 Wilcoxon signed-rank test). Other sites of adhesion formation did not show a statistically significant difference between groups. Adhesiolysis was possible in all animals, and was performed entirely laparoscopically by a combination of blunt and sharp dissection. Inadvertent enterotomies were made in two animals during the laparoscopic procedure. The first in an animal in the treatment group that had extensive adhesions secondary to an intra abdominal abscess (not associated with the anastomosis). During blunt dissection of adhesions, a small tear developed in the colonic wall. The second instance, also in the treatment group, occurred during insertion of the first port where the tip of the introducer made a small perforation of the colon during insertion (not relating to a site of adhesion). Both defects were successfully repaired with laparoscopic sutures.
Experiment Two – Adhesion reformation following adhesiolysis treated with Chitosan-Dextran gel versus control.

When comparing animals initially treated with CD gel to the control group at the end point of the study, there was a statistically significant reduction in adhesions involving the bowel (P=0.03 Wilcoxon signed-rank test). The previously noted variation in the incidence of wound adhesions between groups was no longer observed. This is as expected given the adhesiolysis procedure, and application of gel to all animals. Analysis of the distribution of adhesions between time points demonstrated that animals noted to have greater adhesion scores at laparoscopy were more likely to exhibit a greater score at the study end point. (Figure 2 regression plot). There was no significant differences among adhesion scores when comparing groups from the time of the laparoscopy to the study endpoint. This is again in keeping with use of the CD gel in both groups after adhesiolysis.
DISCUSSION

Chitosan remains a relatively new component of adhesion reducing agents. There are many benefits to its use, aiding haemostasis\textsuperscript{9,10}, exhibiting antimicrobial properties\textsuperscript{11,12} as well as possessing the ability to prevent fibroblast ingress\textsuperscript{13,14}. The composition of the gel has been discussed in detail in the experimental work preceding this model\textsuperscript{15}. The formulation of the gel was not changed for this model although it can be manipulated easily to modify its physical properties to allow for different application environments found at open or laparoscopic surgery.

There are a number of ways in which the data can be analysed in this study. As well as looking at overall adhesion scores between groups, further analyses were performed between subgroups of adhesion sites. This gave the opportunity to relate site to clinical importance. Adhesions involving the wound are more likely to cause problems at future surgery and are therefore of particular interest. This is also true for adhesions involving the bowel since adhesions of this nature may precipitate a bowel obstruction. Omental adhesions can result in both bowel obstruction and difficulty at future operation, although their importance will vary depending on the exact site. The adhesions noted to occur from the spleen and liver are less likely to produce symptoms given the relatively immobile nature of these organs. The only significant clinical setting would be if a splenectomy or hepatic resection were considered. Allied to this, the adhesions noted around the liver and spleen in this study were sparse and predominantly filmy in nature. The use of
a true bowel anastomosis in the study is an important aspect in affirming the safety of CD gel when used in this context.

Analysis of adhesion scores between initial treatment and control groups at the end point of the study effectively examines the scenario of a single or double application of gel following a hemicolecotmy. This situation mirrors a surgical setting where a patient undergoing a bowel resection without the use of an adhesion prevention product is later subject to a laparoscopy because of adhesional symptoms. It is typically only at this point that an adhesion reducing agent is used. A reduction noted in the incidence of adhesions involving the bowel in animals treated with CD gel when compared to control, can be explained by the gel acting predominantly at the time of open surgery. This finding would support the use of an adhesion reducing agent such as CD gel at the time of the primary operative procedure instead of during attempts at preventing adhesion reformation.

A difficulty encountered in the study was the potential for unrecognised enterotomy during laparoscopic adhesiolysis. Small serosal tears in the bowel are often difficult to assess laparoscopically and may progress with time to a full thickness defect in the bowel wall. Certainly, in four animals it would seem plausible that abscesses involving the small bowel found distant to the site of the anastomosis were as a result of trauma caused during adhesiolysis. Upon reviewing the video from these laparoscopic procedures, it was possible to identify one animal where an area of trauma to the small bowel that would, in retrospect, have been reinforced with sutures. While treatment of these areas
with CD gel did not appear to have any detrimental effect, it was also unable to curtail the resultant adhesive response. It is difficult to imagine that any treatment to reduce adhesion formation used in this context would be successful.

It is generally accepted that laparoscopic adhesiolysis represents a high risk of enterotomy with resulting complications\textsuperscript{16} however no animal within this subgroup developed signs of peritonitis. Given that inadvertent enterotomy in the clinical setting is has been reported as being associated with a mortality rate as high as 3.6\textsuperscript{17} it is encouraging that no deaths or serious morbidity were observed in this study.

The observation that animals having greater adhesion scores at laparoscopy were more likely to have greater scores at the study end point can also be explained by the trauma associated with adhesiolysis. The idea of atraumatic adhesiolysis is a fallacy since the procedure itself is a surgical intervention. Most clinicians will warn patients prior to any procedure for adhesions that it is likely that they may recur as a consequence of the surgery. Logic would dictate that the greater the amount of dissection required to deal with the formed adhesions, the greater the peritoneal damage. Whilst this may not be on a scale of the reaction to an open bowel resection, there is still a significant area of damaged peritoneum with the potential to form adhesions. Allied to this, bacterial translocation from damaged bowel wall may precipitate infective complications such as abscess formation.
There remains a very real risk that patients undergoing surgery for adhesion related disorders may actually develop more prolific adhesions after adhesiolysis. Of some encouragement in this study, there was no significant increase in adhesion scores following adhesiolysis, and not all animals at risk of proportionally greater adhesion scores from one time point to the next followed this pattern. There was also no apparent small bowel obstruction in any animal, which would be the one of the potentially most serious risks associated with the adhesions observed.

Another potential explanation for the observed distribution of adhesion scores following laparoscopy is that the adhesiolysis for the control group was essentially incomplete, owing to the greater number of adhesions requiring lysis. The situation is confounded by the difficulty of a laparoscopic procedure to fully identify all adhesions. Either way, the gel was clearly able to reduce adhesions at the time of the open operation, but it is likely that some adhesions were more readily seen (and therefore scored) at the study end point.

A problem noted with the assessment of adhesion formation relating to the surgical wounds is the incidence of wound infection amongst the animals. In an animal such as the pig achieving wound sterility in the post operative period is a practical impossibility. There will always be wound infections, it is regrettable that they are frequently associated with underlying adhesions which can introduce some bias to the results. Fortunately, there was no significant variation in the incidence of wound infections between groups.
There has been some debate as to the best way of treating symptomatic adhesions in the clinical setting. Some advocate the use of the open surgery to treat adhesions\textsuperscript{18} as opposed to the laparoscopic approach. There are certain theoretical benefits of the laparoscopic approach\textsuperscript{19} which should confer some benefit, however it is unlikely that any single study can absolutely prove one approach’s superiority over the other. It is likely that without compelling evidence against the laparoscopic approach it will expand to become the predominant method given the increasing popularity of laparoscopic surgery. The laparoscopic approach was utilised in this study in order to minimise the discomfort caused to the animals, as well as testing the suitability of the CD gel for application by the laparoscopic route.

**CONCLUSION**

Adhesion reduction poses a unique challenge to any new approach. On one hand, the concept of a complete resolution of adhesion formation could have a massive impact on surgical work load and expenditure. On the other, a product resulting in complications associated with its use will reinforce the most prevalent practice, which is to use nothing. There are also cost implications to the use of any adhesion reduction agent. An overly expensive treatment will not be of benefit to the majority of patients suffering from adhesional complications.

The continued prevalence of adhesions despite the application of the gel certainly merits improvements to the gels action. Changes to the composition
of the gel may improve upon the results, whilst variation of the volume applied may also improve upon the effect. Owing to the complexity of the study design, comparison of treatment to control groups can be harder to define. However, the study succeeds in showing the gel to be safe and without an obvious risk of sensitisation when applied on more than one occasion. The development of a reproducible method of bowel resection and anastomosis in this study provides a useful tool for the evaluation of future adhesion reduction strategies.
Table 1. Adhesion scoring scheme

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Figure 1.

a. Profuse adhesions with concomitant wound infection, b. Complete resolution of adhesion formation in an animal treated with a double application of CD gel, c. Dense colonic adhesions, d. A persistent band adhesion involving the colon
Figure 2.
Regression plot of adhesion scores for treatment (T) and control (C) groups from the time of laparoscopic scoring (Adhesions1) to study end point (Adhesions2).
References


18. Prushik SG, Stucchi AF, Matteotti R, Aarons CB, Reed KL, Gower AC, Becker JM. Open adhesiolysis is more effective in reducing adhesion
reformation than laparoscopic adhesiolysis in an experimental model.


Conclusions

Analysis of the results from the initial rat based study provided encouraging data to support the effectiveness of the CD gel at preventing adhesion formation. There were some limitations to this initial study with the gel not always achieving a complete resolution of the adhesive response. However, a statistically significant reduction in adhesion formation was noted with the use of the gel. There was a more marked effect of the gel following minor trauma to the bowel. This would suggest that in the models where there is more peritoneal damage and bacterial soiling, the effect of the gel is overwhelmed. These findings were more pronounced in the porcine model where additional septic complications were noted, particularly wound infections. The associated inflammation proved to be a potent stimuli for adhesion formation, particularly given the ongoing nature of wound issues. Another scenario when adhesions persisted was in the presence of retained suture material. Whilst absorbable suture material is typically used for bowel anastomoses, there are still events such as the control of intra operative bleeding, where a non absorbable suture may be used. Although the adhesions associated with suture material were sparse when treated with CD gel, they remain a difficult entity to avoid.

The formulation of the CD gel is still not finalised so there remains the potential for improvement. When variants of the gel were trialled in the rat model it was evident that if the gel formation became too solid, it would evoke a foreign body reaction and actually precipitate adhesion formation. For
abdominal applications, the gel needs to be in a viscous liquid form. It may be that repeat studies are required to again test the gel in vivo. If the gel is at a stage where commercialisation is considered, it may be appropriate to perform studies where the control is another commercially available product. Having a direct comparison with a product already in the market place would certainly be an important stage for the development for the gel. Granted there is no other product currently available that has a mechanism of action the same as the CD gel. However, regardless of mechanism the gel is unlikely to be successful if it cannot at least be shown to be as effective as the currently available product. This is with the caveat that the CD gel may still be superior if it can be used in any circumstance without side effect.
Discussion

Adhesion formation is a very complex process and even with an understanding all of the mechanisms driving the process, it may still not be possible to prevent symptomatic adhesions from forming. The genes responsible for adhesion formation are highly conserved, with significant overlay from the processes of normal wound healing.

Many of the barriers to successful adhesion reduction are associated with surgical complications or persistent inflammation. Despite attempts to minimise the occurrence of these events inevitably they will occur. Problems such as wound infections, bleeding or intra abdominal collections provide a profound stimulus for adhesion formation, which may be prolonged. In this environment, damage limitation may be the only achievable result. Having conceded this point is not to say that there have been no advances in preventing adhesions. The wealth of research and promising new treatments provide hope that a cure to the problem may still be achievable. With the more widespread acceptance of these treatments it may be that more capable preventative measures will significantly improve patient outcomes.

Adhesion reducing products themselves may become more adept at alleviating problems such as bleeding or infection as well as separating the injured peritoneal surfaces following surgery. The CD gel used in the experimental models possesses these beneficial properties, however the results would suggest that these mechanisms may be saturated in the post
operative period. Whether we can potentiate the mode of action to become more effective remains to be seen. It should be remembered that simply reducing the number of adhesions may not have the desired outcome of limiting symptomatic adhesions. Completely abolishing adhesion formation is the only approach that can guarantee this result.

The results so far are optimistic for the future, but not yet revolutionary. Encouragingly the gel did not appear to have any deleterious effect on wound healing, an important factor in the development of any new agent. The process by which a product progresses towards clinical use must always be performed cautiously as any agent that is subsequently found to have any detrimental effect on patient outcome gives further doubt to those clinicians already reluctant to change their practice. Hopefully, if the perception of adhesion reduction agents becomes more positive then their uptake may increase throughout the world of surgery. Large volume, multicentre randomised controlled trials will be needed to facilitate this change.
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I would like to thank everyone involved in the production of this thesis for their ongoing support and input which has enabled me to finish this project. Without the help of my family it would simply not have been possible.

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