(54) Title: PREVENTION OF ABSCESS FORMATION

(57) Abstract

A method for the prevention or reduction in the severity of abscess formation in a human subject involves the use of a dilute solution of a polyanionic polysaccharide, preferably a solution of hyaluronic acid or carboxymethyl cellulose, which is applied to tissue at the site of a surgical procedure.
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PREVENTION OF ABSCESS FORMATION

Background of the Invention

This invention relates to the use of dilute solutions of polyanionic polysaccharides for the treatment of a subject in order to prevent or reduce the likelihood of abscess formation. The polyanionic polysaccharide solution is applied to the tissue surfaces which are exposed during a surgical procedure performed on the subject.

The numbers in parenthesis, as used herein, designate references which are listed and described in detail in the “Reference” section immediately preceding the claims.

The term “polyanionic polysaccharide,” also as used herein, is intended to designate polymeric polysaccharides and their derivatives containing anionic groups at physiological pH. These include, but are not limited to, hyaluronic acid (“HA”), carboxymethyl cellulose (“CMC”), derivatives of HA, crosslinked HA, derivatives of CMC, crosslinked CMC, chondroitin sulphates, derivatives of chondroitin sulphates, crosslinked chondroitin sulphates or mixtures thereof. See U.S. Patent No. 4,582,865; U.S. Patent No. 4,937,270; and U.S. Patent No. 5,017,229.

Routine abdominal surgery can result in bacterial contamination in the intra-abdominal area of the body which is exposed during surgery. Depending on the type of bacteria encountered, this type of contamination can result in intra-abdominal sepsis and abscess formation. Abscesses, if left untreated, may cause fever, prolonged hospitalization, and possibly mortality.

Intra-abdominal infections may also result from a perforated bowel or appendicitis (secondary peritonitis). Patients suffering from these conditions generally undergo laparotomy to eliminate the infectious focus, accompanied by debridement and intra-operative lavage.

Intra-abdominal infection is typically accompanied by fibrin deposition in the abdominal cavity. This fibrin deposition may lead to both adhesion formation and abscess formation. Both adhesion formation and abscess formation have significant clinical relevance. Intra-abdominal abscesses are a significant cause of morbidity and mortality in patients with generalized peritonitis. Adhesions are the main cause of intestinal obstruction in the developed world. Furthermore, adhesions are responsible for 15% to 20% of cases of infertility, and are associated with chronic abdominal and pelvic pain (1, 2, 3).
Various pharmaceutical agents have been investigated for use in the prevention of adhesions. Such agents include dextran, corticosteroids, phosphatidyl choline, phospholipase inhibitors, non-steroidal anti-inflammatory drugs, heparin and tissue plasminogen activator (tPA) (4). Those pharmaceutical agents which have been shown to interfere with coagulation (the formation of fibrin) and fibrinolysis (the dissolution of fibrin) include, respectively, heparin and tPA, both of which have also been studied for the prevention of intra-abdominal abscess formation. However, relevant clinical experience with such agents is limited, largely due to the fear of bleeding complications.

Other pharmaceutical agents which have been investigated for inducing protection against abscess formation include polysaccharide A. These agents are described in U.S. Patent Nos. 5,679,654 and 5,700,787. The abscess formation is generally caused by polymicrobials originating in the bowel. Anaerobic bacteria, including *Bacteroides fragilis*, play a key role in abscess formation.

The ability of hyaluronic acid solutions to prevent the formation of post-surgical adhesions in a non-infectious environment is well known (8, 9, 10, 16, 17). Recently, modified versions of hyaluronic acid have been shown to be successful in preventing postsurgical adhesions in both experimental and clinical studies (5 to 11). It is believed that hyaluronic acid solutions can reduce postoperative peritoneal adhesions by reducing serosal trauma by precoating the peritoneal surfaces, which would otherwise become damaged during surgery or trauma (39). A similar benefit could be derived by coating the tissues following trauma. As a consequence, peritoneal surfaces do not adhere to each other by fibrinous deposits, and may heal without adhesion formation.

Hyaluronic acid is a biocompatible, non-toxic, high molecular weight polyanionic polysaccharide consisting of repeating units of alternating N-acetyl-glucosamine and D-glucuronic acid. Hyaluronic acid is found in all tissues and body fluids of vertebrates (12). Hyaluronic acid is known to have a stabilizing effect on extracellular matrices. Furthermore, hyaluronic acid has been shown to interact with cell surfaces to modify cell behavior.

Carboxymethyl cellulose, a polyanionic polysaccharide, is a derivatized form of cellulose in which the glucosidic hydroxyl groups have been carboxymethylated, rendering the polymer water soluble. Carboxymethyl cellulose is used in food products (it is currently listed in the CFR as Generally Recognized As Safe (“GRAS”)), and as a moisturizing agent in topical eye products.

It has been demonstrated that a significant reduction in intra-abdominal adhesion formation following colectomy occurs using a hyaluronic acid/carboxymethylcellulose
-3-

bioresorbable membrane (5). Such membranes are manufactured by the Genzyme Corporation, and sold under the trademark Seprafilm®. In addition, the use of a hyaluronic acid solution has been found to cause a reduction in de novo adhesion formation following gynecological surgery (6). Such solutions are also manufactured by Genzyme Corporation, and are sold in Europe under the trademark Sepracoat®.

The anti-adhesive effect of Sepracoat® has not been previously assessed in an infectious environment, nor has their influence on intra-abdominal abscess formation been studied in any detail. It is an objective of this invention to make such an assessment with respect to polyanionic polysaccharide compositions, and to utilize the results to provide a method of preventing or reducing the formation of abscesses.

Summary of the Invention

It has now been discovered that a dilute solution of a polyanionic polysaccharide is effective in preventing or reducing the formation of abscesses which may result from a surgical procedure performed on a subject with established peritonitis. Preferably, the dilute polyanionic polysaccharide solution is a 0.2% to 2.0% by weight solution which is applied to tissue surfaces at the site of the surgical procedure which have been exposed as a result of surgery. This can be accomplished by applying the polyanionic polysaccharide solution to the tissue surfaces either prior to, during or immediately following surgery.

While the polyanionic polysaccharide solution can be applied to the tissue surfaces at any time after the tissue has been exposed, it is preferable to apply it to the tissue surfaces following surgery so as not to interfere with the surgical procedure. The polyanionic polysaccharide solution can, for instance, be used as an intra-operative lavage solution following surgical elimination of the infectious site and debridement of the surrounding tissue. The lavage solution can be left in the abdomen prior to closure which may serve to prevent residual infection or abscess formation. Alternatively, the solution can be supplied to the patient, to treat residual infection, by using a surgical drain, by intraperitoneal injection, by an indwelling catheter, or by using a similar device which may be present in the patient as a result of the surgical procedure. The solution can also be supplied by means of a dialysis port, if available.

Typically, the method of this invention can be used when abdominal surgery is performed on a human patient. This method has been found to be effective in preventing or ameliorating abscesses due to the activity of the following bacteria: Bacteroides fragilis,
Proteus sp., Escherichia coli, coliform gram negative bacteria, anaerobe gram negative rod bacteria, Enterococci and Staphylococci.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although any method and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder, including published patent applications, and issued or granted patents, are incorporated by reference herein. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only, and are not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and form the claims.

**Brief Description of the Drawings**

Figure 1 is a graph showing the relative severity of adhesions for one and three weeks following cecal ligation and puncture. In the graph, the points represent the level of adhesions in individual animals, and the bars indicate median adhesion levels.

Figure 2 is a diagram showing the pathway for adhesion and abscess formation in the cecal ligation and puncture model, and possible methods of action of hyaluronic acid and carboxymethyl cellulose.

**Detailed Description of the Invention**

It has now been found that dilute solutions of a polyanionic polysaccharide reduce the incidence of intra-abdominal abscess formation in a subject when used in the presence of generalized bacterial peritonitis, and that such reduction does not adversely affect the morbidity and mortality of the subject. Preferably the polyanionic polysaccharide solution is a Sepracoat® hyaluronic acid solution (a 0.4% by weight solution) or carboxymethyl cellulose solution (a <2% by weight solution).

Chemically modified versions of HA and CMC, including both derivatized versions as well as crosslinked versions, can also be used in the practice of this invention. Derivatized versions are prepared by reacting the HA or CMC with an activating agent, such as a
carbodiimide. Crosslinked versions of HA and CMC are prepared by reacting the HA or CMC with a suitable crosslinking agent, such as divinyl sulfone.

In order to more readily appreciate the various features and advantages of this invention, it is useful to compare the activity and function of the polyanionic polysaccharide solution of this invention with a control, such as saline, and other pharmaceutically active compositions, such as methylhydroxypropylcellulose and a hyaluronic acid/carboxymethyl cellulose film (Seprafilm®).

The amount of polyanionic polysaccharide solution used in the method of this invention should be sufficient to evenly coat the surfaces of the affected tissue. It is preferred to use an overabundance of polyanionic polysaccharide solution as compared to an inadequate amount. It is believed that the use of an overabundance of polyanionic polysaccharide solution may have a "floating" effect on intra-abdominal organs, which may also result in the prevention of adhesions. In contrast, the use of large amounts of a saline solution does not have a measurable influence on adhesion and abscess formation. This result is consistent given the substantial difference in viscosity and the absorption ability between, for instance, hyaluronic acid and saline solutions. Hyaluronic acid in the abdominal cavity is believed to be absorbed by the diaphragmatic stomata in a manner similar to peritoneal fluid. The hyaluronic acid is biocompatible and is subsequently degraded in the same manner as endogenous hyaluronic acid, mainly in the lymph and blood, but also in the liver (33). Saline, however, is absorbed by the whole peritoneum.

The use of a 0.4% by weight solution of hyaluronic acid can also be compared and contrasted with the use of a methylhydroxypropylcellulose gel and a liquefied Seprafilm® hyaluronic acid/carboxymethyl cellulose bioresorbable membrane. It has recently been shown that a methylhydroxypropylcellulose gel did not reduce adhesion and abscess formation in rats with intra-abdominal infections (30). Moreover, the Seprafilm® bioresorbable membrane, which liquefies after approximately 24 hours under normal conditions, did not seem to reduce adhesion and abscess formation. The lack of effectiveness of the Seprafilm® membrane in comparison to the Sepracoat® solution is somewhat surprising. The failure of Seprafilm® to reduce adhesions in an infectious environment is, however, in accordance with the findings of Medina et al. in a rabbit model of incomplete colon anastomosis (34). These results may indicate that the Sepracoat® solution is unique in its ability to prevent or reduce adhesions. It should be noted that the Seprafilm® bioresorbable membrane was only placed at the site of the surgery, i.e., the cecum resection site and under the midline incision, whereas the peritoneal injury was more generalized (18). Thus, the Seprafilm® therapy is essentially local
compared to the use of the Sepracoat® solution which is more generalized. This may be due to adhesion and abscess formation in other parts of the abdominal cavity when Sepraﬁlm® is used. However, abscess formation was noted at the sites where the Sepraﬁlm® was located. The Sepraﬁlm® membrane may have acted as a foreign substance which, in the presence of bacteria, increases the inﬂammatory reaction. Carboxymethyl cellulose, a component of Sepraﬁlm®, has been reported to reduce adhesion formation in two animal models (16, 35). However, a study of injured parietal peritoneum in a rabbit model of peritoneal damage has shown the opposite (36).

The early reduction of ﬁbrinous adhesions in intra-abdominal infection results in bacteremia and subsequent mortality as earlier described (30). Increased mortality associated with the hyaluronic acid solution was not found, probably as a result of antibiotic treatment. However, an antibiotic effect of the hyaluronic acid solution itself was not ruled out. Recently, it has been suggested that hyaluronic acid confers resistance to phagocytosis of gram positive bacteria (37).

The mechanism of operation of the method of this invention has not been completely elucidated at present. It is known that intra-abdominal infection is a potent stimulus of peritoneal injury which inevitably leads to adhesion formation (16). The precoating effect of hyaluronic acid does not explain the reduction of adhesions and abscesses, and other mechanisms of action may be involved. It has been suggested that the use of hyaluronic acid decreases inﬂammation, interferes with ﬁbrin formation, and accelerates the healing of peritoneal tissue. This is illustrated diagrammatically in Figure 2 (19,20,21). Inflammation is considered pivotal in adhesion and abscess formation (18,22,23,24). Hyaluronic acid has been reported to inhibit the release of proteases from peritoneal leukocytes, the release of oxygen radicals from macrophages, and the scavenging of free oxygen radicals (19,25,26).

Macrophages carry a hyaluronate-CD44 receptor on their membrane which is known to modulate cytokine response (27,28).

It is known that intra-abdominal infection markedly impairs ﬁbrinolysis which is reﬂected by the high concentrations of plasminogen activating inhibitors in peritoneal tissue and ﬂuid (23,24,29). Reducing plasminogen activating inhibitor activity is important to facilitate ﬁbrin degradation and the subsequent reduction in adhesion and abscess formation (30). It has also been found that there is a signiﬁcantly lower plasminogen activating inhibitor activity in abdominal ﬂuid six hours after the use of a hyaluronic acid solution as compared with normal saline. This ﬁnding supports the thesis of a beneﬁcial effect of hyaluronic acid on ﬁbrinolysis (20).
Hyaluronic acid is known to accelerate the healing of various tissues, including the peritoneum, without excessive growth of connective tissue (20,21). Stimulation of mesothelial recovery also seems to protect against adhesion formation (31,32). Such a mechanism of action seems unlikely since hyaluronic acid solution disappears from the abdominal cavity within 24 hours after use, before peritoneal healing takes place.

EXAMPLE

These examples involve the use of an animal model of generalized bacterial peritonitis to study and compare the effect of the use of a Seprafilm® bioresorbable membrane, dilute hyaluronic acid solutions (including Sepracoat®), and a dilute carboxymethyl cellulose solution.

Design of the study

Two separate studies were conducted consisting of 72 and 192 male Wistar rats (Harlan Nederland, Zeist, the Netherlands). These rats, each weighing about 250-325 grams, were allowed to become accustomed to laboratory conditions for one week prior to experimental use. The animals were housed at 21°C with a day-night cycle of 12 hours. They had free access to water and standard rodent chow (Hope Farms BV, Woerden, the Netherlands). The study protocol was approved by the Animal Ethics Review Committee of the Faculty of Medicine, the University of Nijmegen.

In all rats a bacterial peritonitis was induced by performing a cecal ligation and puncture procedure, according to Wicherman et al. (14).

The animals were fasted for 12 hours before the first operation. On the first day (day 0), rats were weighed and anaesthetized with a fluothane (Zeneca, Cheshire, United Kingdom) -nitrous oxide-oxygen mixture. Before the operation, the abdomen of the animal was shaved and disinfected with 70% alcohol. Using a three-centimeter midline laparotomy, the cecum was dissected without damaging the vascularization, and was filled backwards with feces. Thereafter, the cecum was ligated just distal of the ileocecal valve, with a 3.0 polyglactin suture (Vicryl®, Ethicon, Norderstedt, Germany), and at the antimesenterial site the cecum was punctured once with a 19 gauge needle. The abdominal wall was closed in two layers with a 3.0 polyglactin suture. Immediately after operation, rats received one single dose of 6 mg/kg body weight gentamicin (Centrafarm Services BV, Etten-Leur, the Netherlands) intramuscularly and 0.1 mg/kg body weight buprenorfine (Temgesic®, Reckitt & Colman...
Products Ltd., Amstelveen, the Netherlands) subcutaneously. All animals were resuscitated
with 10 ml normal saline administered subcutaneously.

On day one, the animals were weighed and the abdomen was reopened under
anesthesia, peritoneal fluid samples were taken and collected in a BBL™ Port-A-Cul™
envelope (Becton Dickinson, Cockeysville, USA) for microbiological examination. The
abdominal cavity was rinsed with 10 ml of normal saline, and the cecum was resected.

In a first independent study (38), before closure of the abdomen, the animals were
randomly assigned to receive normal saline (8 ml, group 1, n=24), Seprafilm® bioresorbable
membrane (Genzyme Corporation, Cambridge, MA, USA) (4x4 cm wrapped around the cecal
resection site and 2x3 cm under the midline incision, group 2, n=24) or Sepracoat® (Genzyme
Corporation, Cambridge, MA, USA) (8 ml, instilled throughout the whole abdominal cavity,
group 3, n=24). Seprafilm® is a membrane formed from hyaluronic acid and carboxymethyl
cellulose, and Sepracoat® is a 0.4% hyaluronic acid solution. After one and three weeks,
respectively, half of the animals in each group were weighed and killed by CO₂ asphyxiation.

In a second independent study, before closure of the abdomen, the animals were
randomly assigned to one of sixteen groups, each containing 12 animals. Four groups received
0.4% hyaluronic acid, receiving 1 ml, 2 ml, 4 ml and 8 ml, respectively. One group received 8
ml of 0.2% hyaluronic acid. Four groups received 1% hyaluronic acid, receiving 1 ml, 2 ml, 4
ml, and 8 ml, respectively. Four groups received carboxymethyl cellulose (approximately
1.7%), receiving 1 ml, 2 ml, 4 ml, and 8 ml, respectively. Two groups received phosphate
buffered saline (PBS), receiving 4 ml and 8 ml, respectively. One group served as a surgical
control, which received no solution prior to closure. After one week, the animals in each
group were weighed and killed by CO₂ asphyxiation.

Adhesions were scored in a blinded manner by one observer according to Zuhlke,
whereby grade zero means no adhesions and grade 4 means firm, extensive adhesions. Grade
4 adhesions are only dissectable with sharp instruments and organ damage almost is
unavoidable (15). The sites of the adhesions scored, were the midline, the upper abdomen
(liver), the area between bowel loops, the parietal peritoneum and the omentum.

If present, abscesses were noted and their size was taken. An abscess was defined as a
walled-off collection containing purulent material. Samples were taken from the abscesses for
microbiological examination.
**Bacterial cultures**

Samples of peritoneal fluid and abscesses were cultured semi-quantitatively in aerobic and anaerobic conditions. Colombia III agar with 5% sheep blood (Becton & Dickinson, Etten-Leur, the Netherlands), Levine Eosin Methylene Blue agar (Oxoid, Haarlem, the Netherlands) and Fastidious Anaerobic agar (Tapley, Bury, United Kingdom) with or without kanamycin, were used for cultures.

After 24 and 48 hours of incubation at 37°C, bacteria were identified according to standard procedures.

**Analysis of Results**

The data obtained following the above procedure were analyzed using routine statistical analysis. All tests were two tailed, p<0.05 was considered significant.

Following cecal ligation and puncture, all rats were found to have symptoms of intra-abdominal sepsis. The rats demonstrated apathetic behavior, and had ocular exudates, pilo-erection and diarrhea. These symptoms were resolved within two days following the relaparotomy and removal of the necrotic, perforated cecum and peritoneal lavage. Three of the 72 (4%) animals died in the first independent study, and 54 of 192 (28%) died in the second independent study. There was no significant difference in the mortality rate among the groups in either the first independent study (Fisher exact test), Table 1, or the second independent study (Chi-Square Test), Table 2. None of the rats died due to bleeding.

**Table 1**

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<td>Seprafilm®</td>
<td>23/24</td>
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<td>CMC</td>
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<td>0.2% to 0.4% HA</td>
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Adhesions

In the first independent study, one week after cecal ligation and puncture, the number of rats in group 3 with grade 3 and 4 adhesions (six out of eleven; 55%) was significantly less (p<0.05; Fisher’s exact test) than that of control rats (eleven out of twelve; 92%). There was no significant difference in the occurrence of grade 3 and 4 adhesions between the rats in group 2 (nine out of twelve; 75%) and control rats. The most frequent sites of adhesions were the omentum, the lateral peritoneum, between bowel loops and the midline incision, in that order. Three weeks after cecal ligation and puncture, 67% of control rats and 55% of rats in group 2 had grade 3 and 4 adhesions, whereas in group 3 only 18% of rats had grade 3 adhesions and none had grade 4 adhesions (p<0.05; Fisher’s exact test). The median severity of adhesions after one and three weeks is shown in Figure 1. The median severity of adhesions in rats of group 3 was significantly lower at one week (2, range 1-4) (p<0.01; Mann Whitney-U test) and at three weeks (2, range 1-3) (p<0.01; Mann Whitney-U test) in comparison with control rats (4, range 2-4 and 4, range 1-4, respectively). There was no difference in the median severity after one week or three weeks between group 2 (4, range 2-4 and 3, range 1-3, respectively) and the control rats. Involvement of the omentum in adhesion formation was analyzed separately. At week one, the median severity of omental adhesions was significantly lower in group 3 compared with control animals (p<0.01; Mann Whitney-U test). There was no difference in the appearance of omental adhesions between group 1 and 2.

At three weeks no grade 3 and/or 4 adhesions of the omentum were observed in rats treated with Sepracoat® solution, while in 33% of the control rats and 33% of rats in group 2 grade 3 and/or 4 adhesion of the omentum were found. The difference did not reach statistical significance (p=0.06; Mann Whitney-U test).
The results of the second study are summarized in Table 3 below. All of these evaluations were conducted at day 7. The combined control groups (no solution and PBS) had a mean adhesion score of 10.6. The combined CMC groups had a mean adhesion score of 5.2. This was significantly different from the controls (p<0.001; Mann Whitney-U test). The lower concentrations of hyaluronic acid (0.2% and 0.4%) were combined. These combined groups had a mean adhesion score of 6.5. This was also significantly different from the controls (p<0.005; Mann Whitney-U test). The combined 1% hyaluronic acid groups had a mean adhesion score of 8.5; this did not reach statistical significance compared to the controls (p = 0.17; Mann Whitney-U test).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN ADHESION SCORE</th>
<th>INCIDENCE OF ADHESION-FREE ANIMALS</th>
<th>STATISTICAL SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (PBS and no solution)</td>
<td>10.6</td>
<td>1/29</td>
<td>N/A</td>
</tr>
<tr>
<td>CMC</td>
<td>5.2</td>
<td>9/33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.2% to 0.4% HA</td>
<td>6.5</td>
<td>4/39</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>1% HA</td>
<td>8.5</td>
<td>2/37</td>
<td>0.17</td>
</tr>
</tbody>
</table>

**Table 3**

Abscesses were predominantly located at the cecal resection site.

In the first study, at day seven, 55% of rats in group 3 had abscesses, whereas this percentage was 83% in groups 1 and 2, and in group 3, abscesses larger than 2 cm were not observed (p<0.02; Fisher’s exact test). At day 21, no abscesses were found in group 3, while 33% of control rats and 45% of the rats in group 2 had an intra-abdominal abscess. This difference did not reach statistical significance (p=0.09; Fisher’s exact test).

The results of the second study are summarized in Table 4 below. All of these evaluations were conducted at day 7. The combined control groups (no solution and PBS) had an abscess rate of 83%. The combined CMC groups had an abscess rate of 36%. This was significantly different from the controls (p<0.001; Chi-Square test). The lower concentrations of hyaluronic acid (0.2% and 0.4%) were combined. These combined groups had an abscess
rate of 33%. This was also significantly different from the controls (p<0.001; Chi-Square test). The combined 1% hyaluronic acid groups had an abscess rate of 62%; this did not reach statistical significance compared to the controls (p = 0.067; Chi-Square test).

Table 4

<table>
<thead>
<tr>
<th>GROUP</th>
<th>INCIDENCE OF ABSCESS</th>
<th>% ABSCESS</th>
<th>STATISTICAL SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (PBS and no solution)</td>
<td>24/29</td>
<td>83%</td>
<td>N/A</td>
</tr>
<tr>
<td>CMC</td>
<td>12/33</td>
<td>36%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.2% to 0.4% HA</td>
<td>13/39</td>
<td>33%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1% HA</td>
<td>23/37</td>
<td>62%</td>
<td>0.067</td>
</tr>
</tbody>
</table>

**Bacterial cultures**

Bacterial cultures taken at the day of cecal resection revealed a mixed aerobic and anaerobic flora of *Proteus* sp., *Escherichia coli*, coliform gram negatives, anaerobe gram negative rods, *Enterococci* and *Staphylococci* in concentrations of $10^5$-$10^8$ colony forming units/ml (cfu/ml). A similar flora was found in cultures of abscesses present at day seven.

The predominant bacteria found in abscesses at day 21 were *Proteus* sp. and *E. coli*. The concentrations tended to be lower than those at day seven.
REFERENCES


From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. The use of particular terms and features to describe the invention is intended for illustration purposes only, and should not be construed as limiting the scope of the invention.
CLAIMS

What is claimed is:

1. A method for preventing or reducing the severity of abscess formation resulting from a surgical procedure performed on a subject comprising contacting tissue in proximity to the site of the surgical procedure with a dilute solution of a polyanionic polysaccharide.

2. The method of claim 1 wherein the polyanionic polysaccharide is hyaluronic acid.

3. The method of claim 2 wherein the hyaluronic acid is derivatized.

4. The method of claim 2 wherein the hyaluronic acid is crosslinked.

5. The method of claim 1 wherein the polyanionic polysaccharide is carboxymethyl cellulose.

6. The method of claim 5 wherein the carboxymethyl cellulose is derivatized.

7. The method of claim 5 wherein the carboxymethyl cellulose is crosslinked.

8. The method of claim 1 wherein the subject is a human patient.

9. The method of claim 1 wherein the treatment comprises contacting the tissue site with the solution prior to surgery.

10. The method of claim 1 wherein the treatment comprises contacting the tissue with the solution during surgery.

11. The method of claim 1 wherein the treatment comprises contacting the tissue with the solution following surgery.

12. The method of claim 1 wherein the solution is a 0.2% to 2.0% by weight solution.
13. The method of claim 1 wherein the surgery is abdominal surgery.

14. The method of claim 1 wherein the surgery is gynecological surgery.

15. The method of claim 1 wherein the site of the surgical procedure is contaminated with bacteria.

16. The method of claim 15 wherein the bacteria are selected from the group consisting of *Bacteroides fragilis, Proteus sp.*, *Escherichia coli, Enterococci, Staphylococci*, coliforme gram negative bacteria and anaerobe gram negative rod bacteria.

17. The method of claim 1 wherein the dilute solution is used as a lavage on a human patient during or following the surgical procedure.

18. The method of claim 17 wherein the surgical procedure results in a perforated bowel.

19. The method of claim 17 wherein the surgical procedure is an appendectomy.

20. The method of claim 17 wherein the solution is left in the abdominal cavity of the patient following closure of the surgical site.

21. The method of claim 17 wherein the dilute solution is supplied to the patient using a surgical drain.

22. The method of claim 17 wherein the dilute solution is supplied to the patient using an intraperitoneal injection.

23. The method of claim 17 wherein the dilute solution is supplied to the patient using a dialysis port.

24. The method of claim 17 wherein the dilute solution is supplied to the patient using an indwelling catheter.
FIG. 1
FIG. 2