SURGICAL TECHNOLOGY INTERNATIONAL Online
The latest developments in surgical operative techniques and technnologies

Publication: ***Surgical Technology International XVI - Surgical Overview***

Article title: Hyaluronan-Based Antiadhesive Agents in Abdominal Surgery: Applications, Results, and Mechanisms of Action

Contents:

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Abstract

Postsurgical intra-abdominal adhesions cause significant morbidity and mortality, with small bowel obstruction being the most common complication. The urge to prevent adhesion formation has resulted in multiple experimental and clinical trials and the development of numerous antiadhesive agents. Through the years, hyaluronan-based antiadhesives have proved to be successful in the reduction of adhesion formation. Despite the obvious effectiveness of hyaluronan, there is still much debate on its clinical use and mechanisms of action. Various hyaluronan-containing products have been introduced and withdrawn from the market. The application of hyaluronan in combination with meshes for hernia repair appears to be a promising concept. Not all different applications of hyaluronan are well known and its use in patients with a malignancy or abdominal infection remains controversial. Here an overview is given on the effects of hyaluronan-based antiadhesive agents in abdominal surgery, its use in infectious conditions, and its oncologic repercussions. The most important mechanism of action appears to be the mechanical separation of damaged peritoneal surfaces. However, the biological effects of hyaluronan, such as modulation of cell proliferation and peritoneal biology, might also be of influence.

INTRODUCTION

Peritoneal trauma during abdominal surgery and abdominal infection can lead to intra-abdominal adhesion formation (Fig. 1). Adhesions, especially when excessive, can cause severe complications and are responsible for considerable morbidity and mortality. Adhesions are the main cause of intestinal obstruction in the developed world and account for approximately 70% of readmissions for small bowel obstruction.1 After conventional colorectal surgery, one out of five patients is readmitted for reasons directly or indirectly related to adhesions within 4 years after the operation.2 The relative risk of adhesion-related complications in this group is 29.7 per 100 initial procedures over 4 years time. Furthermore, adhesions account for 15% to 20% of cases of secondary infertility in women and are associated with chronic abdominal and pelvic pain.3,4 Relaparotomies are complicated by the presence of adhesions as well; procedures are longer and the risk of inadvertent enterotomy is approximately 20%, which in turn is associated with a higher incidence of postoperative complications, an increased risk of admission to intensive care units, and prolonged hospital stays.5 Complication rates may be even higher when adhesions are accompanied by abdominal infection and abscess formation. The high incidence of adhesion-related complications, their severity, and the obvious impact on the health care burden urge attention for the prevention of postsurgical adhesion formation.

Figure 1. (a) Intra-abdominal adhesion between small bowel loops. (b) Intra-abdominal adhesion between omentum and the abdominal wall.

Mechanical separation of adhesiogenic wound surfaces during the first phase of peritoneal healing — which takes 5 days to 7 days — is the most common concept of adhesion prevention. During the last decades, several mechanical barriers have been developed. Membranes of oxidized regenerated cellulose or expanded polytetrafluoroethylene have been demonstrated to decrease the incidence of adhesion formation.6–9 However, oxidized regenerated cellulose was less effective in the presence of blood. Expanded polytetrafluoroethylene may not be the ideal antiadhesive, as it is a permanent device; it remains in situ on the injured site where it is placed, prone for device-related complications.

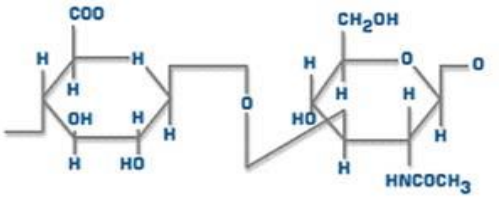

Figure 2. Hyaluronan is a polysaccharide made up of repeating disaccharide units of sodium glucuronate and N-acetyl-glucosamine linked by glycosidic bonds.

Hyaluronan (HA) is a polysaccharide made up of repeating disaccharide units of sodium glucuronate and N-acetyl-glucosamine linked by glycosidic bonds (Fig. 2). In 1934, Meyer and Palmer were the first to describe hyaluronic acid, a polysaccharide isolated from the vitreous fluid (hyalos) that contained uronic acid.10 Later, the name hyaluronan was introduced.11 The intraperitoneal application of HA derivates was considered a promising concept for the reduction of adhesions. Today HA is well known for its antiadhesive properties and has been studied extensively. Currently, HA-based agents are the most frequently used antiadhesive agents worldwide with an undisputed adhesion reducing effect. Use of HA under contaminated or infectious conditions and during oncologic procedures is described as well. However, its use under these conditions is still controversial. In this chapter, an overview is given on the role of HA-based antiadhesive agents in abdominal surgery, with attention for different application forms, mechanisms of action, results, and oncologic repercussions.

HYALURONAN-BASED ANTIADHESIVE AGENTS

1. Sodium Hyaluronan Carboxymethylcellulose Membrane

The hyaluronan-carboxymethylcellulose (CMC) antiadhesive barrier, known as Seprafilm® (Genzyme Biosurgery, Inc., Cambridge, MA, USA), is a sterile, bioresorbable membrane (Fig. 3).

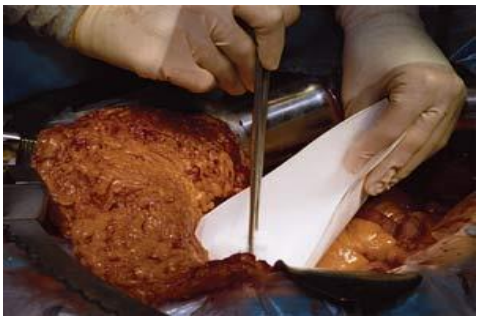

Figure 3. Application of the HA-CMC membrane in open surgery for digestive disease. A dry white Tyvek® sleeve, in which the transparant HA-CMC membrane is supplied, is used to position the membrane in the abdomen. The Tyvek® sleeve is removed once the hydrophilic HA-CMC membrane has been properly positioned. (Image kindly provided by Genzyme Biosurgery, nc.)

The US Food and Drug Administration (FDA) approval of the agent was obtained in 1996. The membrane is applied to injured peritoneal spots, thereby acting as a physical barrier to separate traumatized peritoneal layers during the first phase of peritoneal wound healing. It is composed of two polysaccharides — sodium HA and CMC — and turns into a gel within 24 hours to 48 hours, remaining at the site of placement in this gel form for up to 7 days.

Experimental studies concerning the use of this HA-CMC membrane are numerous. The report from Burns et al. in 1997 first describes its adhesion-reducing capacity in a rat cecal abrasion and sidewall injury model.12 Hellebrekers et al. studied various antiadhesive barriers in a rat model, with HA-CMC showing superior results.9 In another study by Kutlay et al., the HA-CMC membrane was more effective than heparin and aprotinin in a cecal abrasion model in rats.13 Any presumed fibrinolytic activity of the HA-CMC membrane could not be supported by experimental studies from Reijnen et al. and Tarhan et al.14,15

In a randomized clinical trial, Becker et al. studied the effect of the HA-CMC membrane in patients with ulcerative colitis or familial polyposis who were scheduled for ileal pouch-anal anastomosis with diverting-loop ileostomy.16 The included patients were randomized into two groups: in one group, the HA-CMC membrane was placed under the midline incision prior to closure; and in the other group, no antiadhesive agent was applied. Adhesions were evaluated laparoscopically at the time of ileostomy closure 8 weeks to 12 weeks later. The data of 175 patients were analyzed. Only 5 (6%) of 90 control patients were free of adhesions versus 43 (51%) of 85 patients treated with the HA-CMC membrane ($p < 0.001$). The mean percent of incision length involved with adhesions was also significantly greater in the control group (63% versus 23%, $p < 0.001$). Furthermore, the percentage of patients with dense adhesions was significantly higher in the control group (58% versus 15%, $p < 0.001$). The use of the HA-CMC membrane was not related to an increased incidence of adverse events.

In another randomized trial, Diamond studied the effect of the HA-CMC membrane in women undergoing uterine myomectomy.17 The 127 patients included in the trial were randomized to treatment with the HA-CMC membrane or

to no adjunctive antiadhesive treatment at the end of the procedure. Adhesions were assessed during second-look laparoscopy. The mean number of sites adherent to the uterine surface was significantly lower in the HA-CMC membrane treated group (4.98 sites ± 0.52 sites) compared with the no-treatment group (7.88 sites ± 0.48 sites), as were the mean uterine adhesion severity scores (1.94 ± 0.14 versus 2.43 ± 0.10; all values treatment versus no treatment, respectively), mean extent scores (1.23 ± 0.12 versus 1.68 ± 0.10), and the mean area of adhesions (13.2 cm2 ± 1.67 cm2 versus 18.7 cm2 ± 1.66 cm2). Again, the use of the HA-CMC membrane was not associated with an increase in postoperative complications.

Vrijland et al. assessed the applicability of the HA-CMC membrane in patients requiring a Hartmann's procedure for sigmoid diverticulitis or obstructed rectosigmoid.18 Patients were randomized to either HA-CMC membrane placement in the pelvis and under the midline incision or no additional antiadhesive treatment. Adhesions were evaluated laparoscopically at second-stage surgery for restoration of bowel continuity. In that study of 42 patients, although the incidence of adhesions did not differ significantly between groups, the severity of the adhesions was significantly reduced in the group treated with the HA-CMC membrane. There was no correlation between the use of the HA-CMC membrane and the incidence of postoperative complications.

In a worldwide trial focusing on the incidence of bowel obstruction, Fazio et al. recently reported on the efficacy of the HA-CMC membrane in patients who underwent intestinal resection.19 Patients were randomized to be treated with the HA-CMC membrane, which was applied on adhesiogenic areas, or no treatment. The mean follow-up time was 3.5 years. Although the overall rate of bowel obstruction was similar in both groups, the incidence of adhesive small-bowel obstruction requiring reoperation was significantly lower in the group treated with the HA-CMC membrane: 1.8% versus 3.4% ($p < 0.05$). Two other randomized studies have shown the value of the HA-CMC membrane in reducing peristomal adhesions, facilitating early closure, and in reducing postoperative adhesions in pediatric patients.20,21

In a safety study by Beck et al., the use of this membrane was not associated with an increased incidence of abdominal or pelvic abscess, pulmonary embolism, or foreign-body reaction.22 However, wrapping the suture or staple line of a bowel anastomosis with the HA-CMC membrane should be avoided, as this might increase the risk of detrimental sequelae associated with anastomotic leaks.

2. Glycerol Sodium Hyaluronan Carboxymethylcellulose Membrane

The HA-CMC membrane has been modified by adding glycerol (G). G-HA-CMC membrane, also known as Seprafilm II® (Genzyme Biosurgery, Inc.) was developed to improve the handling characteristics of the HA-CMC membrane. The HA-CMC membrane is known to be brittle and sometimes it can be technically difficult to apply; contact of the membrane with an area other than the desired one can lead to a sticky mass, which is hard to replace. Moreover, the HA-CMC membrane is difficult to use in laparoscopic procedures due to its characteristics. The addition of glycerol made the membrane easier to apply. However, only a few studies evaluated its efficacy and safety.

Kayaoglu et al. studied the use of the G-HA-CMC membrane in a rat adhesion model under clean conditions and during peritonitis.23 Surprisingly, the use of the membrane did not reduce adhesion formation under clean circumstances and even caused increased adhesion formation in bacterial peritonitis.

Recently, a clinical trial by Cohen et al. reported on the use of the G-HA-CMC membrane in patients undergoing restorative proctocolectomy and ileal pouch-anal anastomosis with diverting loop ileostomy.24 Indications for surgery were ulcerative colitis and familial polyposis. The evaluation of adhesions was performed during ileostomy closure by means of laparoscopy. A significant reduction in incidence, extent, and severity of adhesions was observed in patients treated with the G-HA-CMC membrane. However, an increased incidence of infectious complications was noted, possibly due to the glycerol application. Further production and marketing was then stopped by the manufacturers.

3. Hyaluronan Solution

The use of solutions of HA to prevent adhesions was already described in the early 1990s. In this group of agents, Sepracoat® (Genzyme Biosurgery, Inc.) is probably one of the best known products. It is a sterile-filtered, nonpyrogenic 0.4% solution of sodium HA in phosphate buffered saline.

Solutions containing HA have been studied extensively for their adhesion-reducing capacity. Burns et al. performed one of the most extensive experimental studies on this topic.25 They described the adhesion-reducing capacity of different concentrations of HA solution in a rat cecal abrasion model. The use of the HA solution resulted in a reduced severity of adhesions, with 0.4% HA solution being most successful.

Only one clinical prospective randomized trial has been published to our knowledge describing the use of 0.4% HA solution for the reduction of postsurgical adhesion formation. Diamond assessed the use of 0.4% HA solution in women who underwent various gynecologic procedures via laparotomy.26 During the procedure, the peritoneum was repeatedly coated with the 0.4% HA solution or with phosphate-buffered saline, acting as a placebo. After 40 days, adhesions were assessed during second-look laparoscopy. The data of 277 women were available for safety evaluations and of 245 women for efficacy studies. The group treated with the 0.4% HA solution had a significantly lower incidence of adhesions when compared with the placebo group. The proportion of sites involved was lower (0.23 ± 0.02 versus 0.30 ± 0.02, respectively) and the percentage of patients without de novo adhesions was higher (13.1% versus 4.6%, respectively). Furthermore, the use of the 0.4% HA solution resulted in a significantly reduced adhesion extent and severity. In that study, no obvious adverse effects of 0.4% HA solution were described.

Nevertheless, in 1997 the FDA panel voted that Sepracoat® was not yet approvable due to the lack of proven clinical effectiveness. Further studies and evaluation were suggested. However, based upon market dynamics at the time the

manufacturers chose not to pursue US market approval. This led to the complete withdrawal of the product from all markets due to business economics (personal communication).

4. 0.5% Ferric Hyaluronan Gel

In the abdominal cavity, an HA solution has a very short residence time and may disappear within hours after placement. Therefore, modifications of the HA solution have been brought to the market to increase its viscosity and prolong its residence time. Crosslinking of HA with ferric ions has resulted in the production of 0.5% ferric HA gel (Gynecare Intergel®, Ethicon, Somerville, NJ, USA), a sterile, nonpyrogenic, viscous solution. Initially, this gel was brought to the market under the name of Lubricoat®.

Few experimental studies on the use of 0.5% ferric HA gel have been published. In 1997, Johns et al. reported on an experimental study on various test formulations applied as peritoneal instillates in a sidewall and uterine horn model in rabbits.27 Adhesion formation was assessed at 7 days and at 14 days. HA that was not ionically crosslinked was ineffective in reducing adhesions in these models even when applied at high viscosity, whereas the ionically crosslinked formulations of HA with trivalent iron were highly effective. Efficacy improved with increased levels of ionic crosslinking. In contrast, in more recent experimental studies the use of 0.5% ferric HA gel has shown no convincing adhesion-reducing effects in a laparoscopic adhesion model in rabbits and in rats.28,29

Thornton et al. assessed the safety and efficacy of 0.5% ferric HA gel in reducing adhesions in patients undergoing abdominal surgery by laparotomy with a planned second-look laparos-copy.30 Women desirous of fertility, aged from 24 to 41, were randomized to receive either 300 ml of 0.5% ferric HA gel or lactated Ringer's solution at the completion of the procedure. Treated patients had significantly fewer intra-abdominal adhesions and the adhesions that did form in this group were significantly less extensive and less severe. Lundorff et al., who randomized patients undergoing a laparotomy for various gynecological procedures including myomectomy, ovariectomy, salpingostomy and adhesiolysis, performed a similar study.31 Women were aged 18 to 46 and adhesions were evaluated at 24 sites at second-look laparoscopy 6 weeks to 12 weeks later. The ferric HA group had significantly fewer adhesions and again the adhesions that formed despite the use of ferric HA were significantly less extensive and less severe compared with controls. The American Fertility Society score for adnexal adhesions was reduced by 69% in the treatment group compared to controls. In a larger clinical trial by Johns et al., 281 women were randomized with 143 women in the treatment arm and 138 in the control arm.32 Results were similar to the results in the other two studies. The American Fertility Society score for adnexal adhesions was now reduced by 59%. In all three studies, no obvious adverse effects of the use of the ferric HA solution were noted. However, a recently published trial conducted by Tang et al. examining the efficacy and safety of 0.5% ferric HA gel in colorectal resections had to be suspended because of high morbidity in the treatment group, mainly due to anastomotic dehiscence and prolonged postoperative ileus.33

In 2003, the manufacturers withdrew Intergel® from the market. The product was intended to be used in conventional gynecological surgery to reduce postsurgical adhesions as an adjunct to good surgical technique. The voluntary withdrawal was conducted to complete an assessment of information obtained during post-marketing experience with the device, including adverse events associated with off-label use in laparoscopy and laparoscopic procedures such as hysterectomies. The post-market reports included late-onset postoperative pain and repeat surgeries following the onset of pain, noninfectious foreign body reactions, and tissue adherence (information provided by the FDA and manufacturers).

5. Hyaluronan Carboxymethylcellulose Gel

As described above, the HA-CMC membrane proved to be effective in adhesion reduction. However, as mentioned its handling characteristics may not be optimal, especially in laparoscopic surgery, where application of the membrane can be difficult. Laparoscopic use of a solution or gel is much easier. HA-CMC gel (Sepragel®, Genzyme Biosurgery, Inc.) was developed as an alternative to the HA-CMC membrane.

In 1996, Burns et al. described the use of HA-CMC gel for the prevention of adhesions in adhesion models in rats and rabbits.34 Treatment with the gel resulted in an increased number of animals without adhesions by 70% in a rat cecal abrasion model and over 90% in a rabbit sidewall defect-bowel abrasion model. Leach et al. showed a reduction of adhesions using the HA-CMC gel in a rabbit uterine horn model.35 The uterine horn model was shown to be adhesiogenic, with 29 (70%) of 42 untreated uterine horns found to have adhesions. After treatment with HA-CMC gel, 22 (55%) of 40 uterine horns were free of adhesions compared with 12 (30%) of 42 uterine horns in controls. Postsurgical adhesion formation was significantly reduced in animals treated with HA-CMC gel when compared with controls ($p < 0.05$). Separate analysis for extent, severity, and density showed significant differences for adhesion severity and density ($p < 0.05$). Clinical trials studying the safety and efficacy of HA-CMC gel have not yet been performed to our knowledge. HA-CMC gel has not been submitted for regulatory review in the United States or the European Union and is therefore not available for clinical use. According to the manufacturers, this is a clinical development project that is still under investigation (personal communication).

6. Auto-Crosslinked Hyaluronan Gel

Another novelty in the field of HA-based instillates was the development of auto-crosslinked HA gel (Hyalobarrier® gel, Fidia Advanced Biopolymers, Abano Terme, Italy). This highly viscous gel was obtained by means of an internal crosslinking reaction of pure HA in the absence of any chemical substance foreign to the native HA structure. The commercially offered auto-crosslinked HA gel had a concentration of 4% and a prolonged residence time in the

abdominal cavity was claimed by the manufacturers.
De Iaco et al. described the use of auto-crosslinked HA gel in a laparoscopic adhesion model in rabbits.36 Use of auto-crosslinked HA gel resulted in a significant reduction of adhesion formation: 35% of the treated animals had severe adhesions versus 66% in the control group. Furthermore the mean adhesion score was significant lower in the treated group. In a subsequent study, auto-crosslinked HA gel appeared to be effective in reducing adhesions in the presence of inadequate hemostasis as well.37 In a rat model of laparotomy and uterine horn injury, Koçak et al. managed to reduce adhesions using auto-crosslinked HA gel.38 Belluco et al. used an adhesion model in rabbits with different concentrations of the gel.39 Again, the adhesion-reducing effect was obvious, with the 4% concentration being the most efficient.
The only available clinical data to our knowledge come from studies in women undergoing abdominal surgery for gynecological indications; Acunzo et al. used the auto-crosslinked HA gel in women following hysteroscopic intrauterine adhesiolysis.40 After 3 months, a significantly lower rate of intrauterine adhesions was observed in the auto-crosslinked HA treated group (n = 43) compared with controls (n = 41; 14% versus 32%, $p < 0.05$). Patients in the treated group showed significantly lower adhesion scores at follow-up in comparison with those in the control group (2.0 ± 0.0 versus 5.3 ± 0.2, $p < 0.001$). When an intrauterine adhesion staging was performed according to the American Fertility Society, all auto-crosslinked HA gel treated patients had mild (Stage I) adhesions. Among control patients, only 25% had mild adhesions and 75% had moderate (Stage II) adhesions. The same research group has performed a comparable study in women with a single surgically remediable intrauterine lesion.41 Again, a significantly lower rate of intrauterine adhesions was observed in the auto-crosslinked HA gel treated group (n = 67) compared with controls (n = 65; 10% versus 26%, $p < 0.05$). The mean adhesion score was significantly lower as well (2.42 ± 0.78 versus 3.83 ± 0.98, $p < 0.05$). The adhesion severity according to the American Fertility Society demonstrated a significantly decreased adhesion severity in the treated group (86% mild adhesions, 14% moderate adhesions) compared with controls (24% mild, 76% moderate). No adverse gel-related effects were detected in the auto-crosslinked HA gel treated group.
In a recent study by Pellicano et al., the gel proved useful for reducing the incidence of postsurgical adhesions after laparoscopic myomectomy.42 Auto-crosslinked HA gel-treated patients (n = 18) had a significantly lower rate of postsurgical adhesions in comparison with controls (n = 18; 28% versus 78%, $p < 0.01$). The rate of adhesions was significantly higher ($p < 0.05$) in patients treated with interrupted "figure-eight" sutures than in subjects treated with subserous sutures. Four out of nine patients (44%) in the auto-crosslinked HA gel-treated group and eight out of nine patients (89%) in the control group who were treated with so-called interrupted "figure-eight" sutures developed postoperative adhesions, whereas one out of nine (11%) auto-crosslinked HA gel-treated patients and six out of nine (67%) control patients who were treated with subserous sutures developed postoperative adhesions. No side effects of the applied gel were reported.
Currently, the auto-crosslinked HA gel is also no longer available for clinical use. It was withdrawn from the market after it failed to show its effectiveness in adhesion reduction on a large scale after clinical research (personal communication).

7. Other Hyaluronan-Based Products

A few other crosslinked HA products have been described in recent years. Haney et al. studied a barrier composed of chemically crosslinked HA (Incert®, Anika Therapeutics, Woburn, MA, USA).43 The product was used in a murine uterine horn model. Fewer adhesions were present when excision injuries were separated by the barrier (43% versus 88%), whereas the number of adhesions was unchanged after electrocautery injuries (54% versus 65%, N.S.). Despite human pilot trials to test the safety and effectiveness of this product for prevention of adhesions after spinal surgery, no trials studying the abdominal use are available to our knowledge. Until now, the barrier has not been approved by the FDA for use in the United States.
Jackson et al. focused on the use of a paclitaxel-loaded crosslinked HA film — HA crosslinked with water-soluble carbodiimide and containing 10% glycerol and 1% or 5% paclitaxel.44 In a rat cecal abrasion model, both 1% and 5% paclitaxel film effectively reduced the formation of adhesions, with the 5% paclitaxel film being the most effective. However, this product led to excess fluid in the abdominal cavity at necropsy. No further studies have been reported at this time.
Li et al. described the development of a crosslinked HA hydrogel that contained a covalently bound derivative of the antiproliferative drug mitomycin C (MMC).45 The hydrogel was tested with 0.5% MMC and 2% MMC in vitro and in vivo. In vitro HA film loaded with 0.5% MMC inhibited proliferation when incubated with human T31 tracheal scar fibroblasts, whereas the 2% MMC films were cytotoxic. In vivo, the MMC films were implanted intra-abdominally in rats; the HA-MMC films reduced the thickness of fibrous tissue formed surrounding it. In a second study by the same group, HA-MMC films and gels were evaluated in a uterine horn model in rats.46 Both films and gels were tested in several concentrations and were highly efficient in reducing adhesions. The results of the HA-MMC films were dose-dependent. The efficacy of the gels was highly correlated to the concentration, with the HA 0.625% MMC gel being the most effective. The use of this novelty is not yet described inclinical studies to our knowledge.

HERNIA REPAIR AND HYALURONAN CONTAINING MESHES

Stimulated by the adhesion-reducing properties of HA in abdominal surgery, other applications have recently been explored, including its use in hernia repair. Adhesion reduction after abdominal wall reconstruction using prosthetic meshes is challenging. Incisional hernia after abdominal surgery is a common complication and tension-free repair is considered a prerequisite for successful treatment. This requires the use of a prosthetic mesh. To allow optimal ingrowth and to prevent reherniation, the mesh should be macroporous and nonsoluble. Polypropylene is the most commonly used biomaterial for this purpose. However, a major drawback is the propensity of adhesion formation at the peritoneal side of this mesh, introducing the risk of bowel obstruction, fistula formation, and inadvertent enterotomy at relaparotomy. Experimental studies have shown a successful reduction of mesh-related adhesions using a combination of polypropylene mesh with a separate HA-CMC membrane.47–49 To improve the handling characteristics, a polypropylene mesh was covered on one side with sodium HA-CMC (Sepramesh®, Genzyme Biosurgery, Inc.). Greenawalt et al. were the first to describe results of this antiadhesive mesh.50

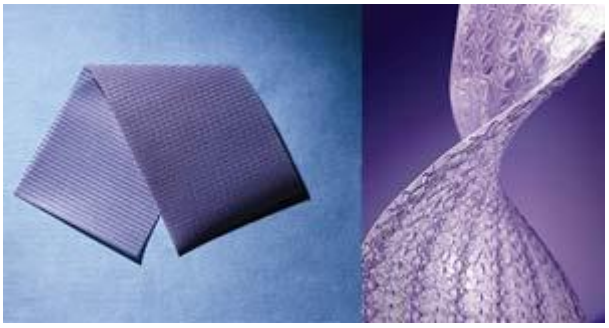

Figure 4. Polypropylene mesh co-knitted with polyglycolic acid fibers, coated with a HA-CMC polyethylene glycol based hydrogel.
(Image kindly provided by Genzyme Biosurgery, Inc.)

The use of the composite mesh resulted in a reduction of mesh-related adhesions compared with a polypropylene mesh or polypropylene/ePTFE mesh in a rabbit model of incisional hernia. Van 't Riet et al. performed experiments in a rat incisional hernia model, comparing several antiadhesive meshes or combinations of mesh with antiadhesive agents.51 The composite HA-CMC mesh was the most effective in adhesion reduction. This superior behavior with regard to adhesion reduction compared with other meshes was confirmed by a recent experimental study by Sikkink et al.52 Adhesion reduction was obtained without compromising reherniation and infection rates.

Figure 5. Handling characteristics of the polypropylene polyglycolic acid HA-CMC polyethylene glycol mesh: flexible and well-suited for laparoscopic procedures.
(Image kindly provided by Genzyme Biosurgery, Inc.)

Felemovicius et al. studied the use of the polypropylene HA-CMC mesh and polypropylene HA-CMC mesh combined with an additional HA-CMC membrane in a rat hernia model.53 Polypropylene HA-CMC mesh reduced adhesions by roughly three-quarters compared to polypropylene mesh. The combination of the polypropylene HA-CMC mesh with the membrane nearly eliminated adhesion formation. Building on these successes, human studies should now further enlighten the clinical value of the polypropylene HA-CMC mesh.
Recently, the polypropylene HA-CMC mesh has been further developed into a polypropylene mesh co-knitted with polyglycolic acid (PGA) fibers (Sepramesh® IP, Genzyme Biosurgery, Inc.) (Fig. 4). The PGA surface is coated with a bioresorbable, chemically modified sodium HA, CMC, and polyethylene glycol-based hydrogel. The co-knitting of polyglycolic acid in this mesh provides for a stronger bond between the permanent polypropylene mesh and the temporary adhesion barrier component as compared to the previous generation. A literature search does not reveal any clinical or preclinical data on this second generation product at this time, although the manufacturers claim the same level of benefit as the first generation product with substantially better handling characteristics and easier use in laparoscopic procedures (Fig. 5).

THE USE OF HYALURONAN IN INFECTIOUS CONDITIONS

The use of HA-based antiadhesives under infectious conditions remains controversial. The majority of the available data at this time results from experimental studies and presents contradicting outcomes. Reijnen et al. described the use of the HA-CMC membrane in a peritonitis model in rats.54 Although the contrary was hypothesized, the HA-CMC

membrane did not reduce adhesions under these conditions. Tzianabos et al. stated that adhesion reduction devices might even potentiate intra-abdominal infection.55 In a rat model, they used HA-CMC and G-HA-CMC membranes after the insertion of a bacterial inoculum in the peritoneal cavity. Both membranes did not increase abscess rates. Mortality data were only available for HA-CMC membrane, which did not increase mortality. In this study, the effect of the membranes on adhesion formation was not evaluated. Ghellai et al. described use of the HA-CMC membrane after cecal ligation and puncture or cecal ligation alone in rats.56 Again, the HA-CMC membrane did not reduce the number or tenacity of adhesions. A trend toward increased abscess formation was associated with the use of the HA-CMC membrane in the cecal ligation group. Again using a cecal ligation and puncture model in rats, Tüzüner et al. found no differences between the control and HA-CMC membrane-treated groups regarding mortality, abdominal abscess formation, and median adhesion scores.57 On the contrary, the use of the HA-CMC membrane led to significantly less dense adhesions. The increased adhesion formation after use of the G-HA-CMC membrane in bacterial peritonitis in the study of Kayaoglu et al. was already mentioned earlier in this chapter.23 The only clinical prospective study that we are aware of describing the use of HA-CMC membrane in patients suffering from peritonitis is the above mentioned study of Vrijland et al.18 In this study, 81% of the patients treated with the HA-CMC membrane were diagnosed to have sigmoid diverticulitis with signs of peritonitis. Although the number of patients in this study was small, it is interesting to note that although the incidence of adhesions did not differ between groups, the severity of adhesions was reduced by the HA-CMC membrane.

In the above mentioned experimental study of Reijnen et al. showing no beneficial effect of the HA-CMC membrane, the 0.4% HA solution proved to reduce the incidence of adhesions and abscesses in a peritonitis model.54 The median severity of adhesions was significant lower using the HA solution at Day 7 and at Day 21 postoperatively. At Day 21, none of the rats treated with the HA solution had an intra-abdominal abscess, in contrast to 4 out of 12 (33%) rats in the control group. These results were confirmed by a second, larger study on this topic showing high volumes of 0.2% and 0.4% HA to be most effective, which may suggest a "hydroflotation" effect of the HA solution.58 These superior results of HA solution compared to the HA-CMC membrane under infectious conditions were confirmed by the quoted study of Tüzüner et al.57 Unfortunately, withdrawal of Sepracoat® from the market has prohibited further clinical studies.

There are no clinical data available on the use of ferric HA gel under contaminated and infectious conditions. The study of Tzianabos et al. describes the use of a preparation of ferric HA gel in an intra-abdominal model of infection in rats.55 This study focused on a potential propagation of the device on intra-abdominal infection. The use of ferric HA gels potentiated bacterial peritonitis and led to significantly increased mortality rates of 90% to 100%, compared to 49% in controls. This could be explained by the known increase in virulence of bacterial species by iron. Furthermore, some virulent strains of human pathogens are able to use iron for the survival and replication of the organism.

Sikkink et al. studied the use of auto-crosslinked HA gel in a rat peritonitis model.59 Bacterial peritonitis was induced using a cecal ligation and puncture model, and the animals were randomized to receive 4% auto-crosslinked HA gel or phosphate buffered saline. Different amounts of the gel were used and the effects on adhesion and abscess formation were evaluated at different time points. In this study, a trend toward increased mortality due to fecal peritonitis with subsequent sepsis in the auto-crosslinked HA gel treated groups was observed. There were no significant differences in median total adhesion scores and abscess rates between groups.

ONCOLOGIC REPERCUSSIONS OF HYALURONAN

Little is known at this time about the use of HA during oncologic surgical procedures. Nevertheless, experimental studies have documented a critical role for HA in tumor growth and metastasis, as it interacts with cell behavior in various ways. The physical properties of HA contribute to tissue biomechanics. Furthermore, it acts as a template for the assembly of other pericellular macromolecules and interacts directly with cell surface receptors that transduce intracellular signals. Consequently, HA may promote anchorage, independent growth, and invasiveness of cancer cells as described by Toole.60,61

Haverlag et al. studied the influence of HA solution on tumor cell adhesion.62 In vitro, mesothelial cells were cultured in monolayers and the effect of HA solution on adhesion of tumor cells evaluated. The use of HA solution showed an inhibitory effect on tumor adhesion. In a uterine abrasion model in rats, HA solution tended to increase tumor load. In a laparotomy model, the mean total tumor scores did not differ significantly. Underwood et al. examined the effect of the HA-CMC membrane on tumor cell implantation at surgical wound and laparoscopic trocar sites in a hamster model.63 It was concluded that the HA-CMC membrane neither had a protective nor an adverse effect on tumor implantation or growth. On the contrary, Tan et al. demonstrated that the HA solution significantly increased tumor cell proliferation and motility in vitro.64 In vivo, a significantly higher total tumor nodule count was noted when using the HA solution in a rat model. Hubbard et al. examined the effect of the HA-CMC membrane on cancer cell growth and metastasis in a mouse model.65 The HA-CMC membrane did not affect tumor metastasis. However, the placement of the membrane on nontraumatized peritoneum led to increased local tumor growth. It was concluded that not the HA but the traumatic placement of the membrane was responsible for local increased tumor growth. Pucciarelli et al.

studied the effect of auto-crosslinked HA gel, native HA, and HA-CMC membrane on experimental intraperitoneal tumor implantation in mice.66 Human HT29 colorectal cells were used, and the antiadhesives showed no negative impact on survival or tumor implantation. In an experimental laparoscopic study in mice by Sasaki et al., a protective effect of the HA-CMC membrane on port site metastasis was suggested.67 Sikkink et al. recently studied the influence of the G-HA-CMC membrane on intraperitoneal tumor implantation and growth in a mouse and rat model of peritoneal trauma.68 No major effects were found. However, a uniform conclusion cannot be drawn from these contradictory experimental results.

Human studies are even more scarce. Kusunoki et al. already described the use of the HA-CMC membrane in 1999 for the reconstruction of the pelvic floor after abdominoperineal rectal excision for oncologic reasons in three patients.69 No remarks were made about the safety of HA in these cases. In a retrospective study in patients with colorectal excisions and short follow-up, Oikonomakis et al. found no adverse effects of the HA-CMC membrane.70 More recently, Kusunoki et al. showed in a prospective randomized study that the use of the HA-CMC membrane had no adverse effects in a group of patients with rectal carcinoma who were treated with radiation therapy, two-stage surgery, and chemotherapy.71 The median follow-up period was 43.6 months. The treatment with the HA-CMC membrane reduced adhesions in this chemoradiated group. Meanwhile, no other prospective randomized human studies have been performed to our knowledge.

Thus far, manufacturers have not promoted the use of HA-based agents in patients with malignancies. The product information for 0.5% ferric HA (Intergel®) clearly states that the product was not studied and is not recommended for use in patients with cancer. The same accounts for other products, such as HA solution (Sepracoat®), HA-CMC membrane (Seprafilm®), G-HA-CMC membrane (Seprafilm II®), polypropylene HA-CMC mesh (Sepramesh®), and polypropylene polyglycolic acid HA-CMC polyethylene glycol mesh (Sepramesh® IP).

MECHANISMS OF ACTION OF HYALURONAN

HA-based antiadhesive agents have proved to be effective in reducing intra-abdominal adhesions in both experimental and clinical studies. Although the use of these products is widespread, there is still much debate on the exact mechanisms of action. Mechanical separation of injured peritoneal surfaces is probably the most important. Once these surfaces have healed after a period of approximately five days, no de novo adhesions will form. HA-CMC membranes create a mechanical barrier between the adhesiogenic wound surfaces. This allows the peritoneal lining to heal without adherence to the adjacent structures. HA solutions create a medium in which the bowel floats (the hydroflotation hypothesis), thus separating the intestines and thereby allowing adhesion-free repair of the mesothelial lining. The mechanical mechanism of the action of gels is probably somewhere between acting as a barrier and creating hydroflotation.

Apart from these mechanical mechanisms, biological mechanisms of action may be involved as well. Present in virtually all tissues and body fluids of vertebrates and with a profound role in cell biology, HA might improve peritoneal healing and enhance fibrinolysis.72

The relation between HA synthesis and cell proliferation has been studied thoroughly. The concentration of HA in healing tissues is high.73,74 Furthermore, HA synthesis facilitates cell detachment, mitosis, and locomotion.75–79 An HA-rich environment provides an open, hydrated matrix that facilitates cell migration.80,81 Exogenous HA may be beneficial in wound healing.82,83 Rapid, undisturbed restoration of the mesothelial lining of the traumatized peritoneum after surgery or infection should prevent adhesions, as stated previously. This concept is supported by the adhesion-reducing effect of intraperitoneal seeding of mesothelial cells or mesenchymal stem cells.84,85 Several studies have shown that HA increases the proliferation rate of mesothelial cells in vitro.86,87 This process implies that part of the adhesion-reducing capacity of HA may partly be explained by its ability to improve peritoneal healing due to the stimulation of mesothelial cell proliferation.

Abdominal surgery and peritonitis are known to disturb the equilibrium between the coagulation cascade and the fibrinolytic system, resulting in decreased peritoneal fibrinolytic capacity and subsequently enhanced adhesion formation. The influence of HA on peritoneal fibrinolysis despite several studies is not completely clear. HA increases the fibrinolytic response of human peritoneal mesothelial cells, mainly by decreasing PAI-1 transcription and release but also by increasing the intracellular tPA concentration in vitro.88 However, in vivo HA-based membrane and solution did not affect tissue tPA antigen or its activity in rat peritoneal biopsies after colonic surgery, with and without peritonitis.14 Reijnen et al. discussed several explanations for these contradicting results in an earlier review.89 However, a more recent in vitro study by Sikkink et al. did not show a significant effect of HA on the production of tPA by mesothelial cells either.90 This study showed that cells of the monocyte-macrophage system modulate the fibrinolytic capacity of lipopolysaccharide-treated human peritoneal mesothelial cells by increasing PAI-1 and tPA. HA solution decreased PAI-1 production by mesothelial cells, an effect that was ameliorated by the presence of monocyte-like cells. Two other studies did not add clarity to this subject, as the use of HA showed no effect on tPA and PAI levels in one and even resulted in a decrease of tPA in the other.15,91 Perhaps sequential biopsies from the peritoneum in human studies could further enlighten the role of HA in fibrinolysis, although the harvesting of

successive biopsies would not be feasible in humans. Other sampling techniques, including the use of a peritoneal chamber or microdialysis, might overcome this in the near future.92

CONCLUSIONS

HA-based antiadhesive agents have proven to be effective reducers of postsurgical adhesions in both experimental and clinical studies. Unfortunately, several products have been withdrawn form the market before larger clinical trials could be initiated. The application of HA in composite meshes for the repair of abdominal wall hernia appears to be worthwhile as well. Data on the use of HA under infectious conditions are scarce and do not allow conclusions about its preventive potential and clinical safety under these conditions. However, the use of HA solution appears to be beneficial in experimental models. Data on oncologic repercussions using HA are also scarce and contradictory, troubling conclusions on the use of HA-based agents in patients with a malignancy. Widespread use of HA should therefore be restricted to noninfectious and benign conditions. Further elucidation of the involved mechanisms and the clinical application of HA in patients with peritonitis and patients with abdominal malignancies are challenges for future trials.

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