Peritoneal healing after fibrin glue application: a comparative study in a rat model

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The influence of fibrin glue on adhesion formation and peritoneal healing is evaluated in a prospective, randomized, controlled study. In all, 20 Wistar rats underwent microsurgical suturing of two silicone sheets, one covered with a fibrin glue barrier, to the anterior peritoneum. Each animal thus served as its own control. After 10 days, adhesions and peritoneal healing were evaluated by a blinded observer through a second-look laparotomy. Adhesions were scored using a modification of the classification of Diamond. Tissue around the silicone sheet was examined histologically and by scanning electron microscopy to evaluate the inflammatory reaction and peritoneal healing (ingrowth of blood vessels and quality of peritoneal cells). Adhesion scores for treated and control sides were (mean ± SD) 2.89 ± 4.68 and 6.79 ± 9.09 (P = 0.181) respectively, and the percentage of the sheet covered by peritoneum was 26.25 ± 31.50 and 29.21 ± 40.21 (P = 0.226) respectively. Using the paired Wilcoxon rank test, the P values for the ingrowth of blood vessels and peritoneal healing evaluated by histology and scanning electron microscopy were 0.842, 0.692 and 0.695 respectively. We conclude that although the mean adhesion score was reduced by >50% by fibrin glue, there is no statistically significant difference concerning adhesion formation or peritoneal healing with the use of fibrin glue.

Key words: adhesions/angiogenesis/fibrin glue/mesothelium/peritoneum

Introduction

During abdominal oncological or pelvic surgery, large deperitonealized areas are present at the end of the surgical procedure and are exposed to the risk of adhesion formation. Following a laparotomy, the overall incidence of adhesion formation ranges from 53 to 91% (Doody et al., 1989). Bowel obstruction, ureteral obstruction, infertility and chronic pelvic pain are frequent complications of intra-abdominal adhesions. The development of peritoneal adhesions also interferes with the intraperitoneal (i.p.) distribution of fluids, and limits the use of i.p. chemotherapy and radioactive colloids. Therefore, several attempts have been made to prevent adhesion formation. The results remain very disappointing.

Increasingly, surgeons are using laparoscopy to perform surgical procedures that previously called for the use of a laparotomy incision. Although the use of the laparoscopic technique reduces the formation of de-novo adhesions in experimental and clinical conditions (Luciano et al., 1989), adhesions still occur after laparoscopy (Operative Laparoscopic Study Group, 1991). In this context, the handling characteristics of barrier materials assume great importance.

Fibrin glue, a two-component glue, has the advantage of being liquid at the moment of application. Shortly after the mixing is accomplished, and the two components meet in the abdominal cavity, they become a highly polymerized solid fibri film, for which fibroblasts have a particular chemotaxis (Harmand et al., 1994). This application characteristic has the advantage that the gel is easily applied through laparoscopy. Fibrin glue is made by mixing a source of highly concentrated human fibrinogen with bovine thrombin, calcium and Factor XIII. In several fields of surgery fibrin glue is used for haemostasis; in fertility surgery it is used for tubal anastomosis (Adamyan et al., 1991).

Different studies in animal models demonstrate fibrin glue to be highly effective in preventing adhesion formation (Lindenberg and Lauritze, 1984; De Virgilio et al., 1990). However, this result could not be reproduced by Tulandi (1991). In a study performed by Caballero and Tulandi (1992), fibrin glue indeed reduced adhesion formation, but no more than Ringer's lactate.

A theoretically valid treatment in adhesion prevention should increase peritoneal neoangiogenesis and the repair of peritoneal lesions, but at the same time should decrease vascularization of the adhesion (Diamond and Decherney, 1987; Bigatti et al., 1995). Peritoneal healing should be in one single plane, and not bind surrounding tissue (Rafferty, 1973).

In the model used for this experiment, based on the work of Bigatti et al. (1995), we studied the effect of fibrin glue on the inflammatory reaction, neovascularization and peritoneal healing on the surface of an implanted silicone sheet, where healing can only take place from the margins and not from the base of the wound (Guo et al., 1993). We also evaluated whether fibrin glue can affect de-novo adhesion formation. Because the incidence of spontaneous adhesion is ~80% in the model described by Bigatti et al. (1995), changes in adhesion formation, positive or negative, can be evaluated.

Materials and methods

A total of 20 female Wistar rats, weighing 200–250 g, were allowed only water 24 h prior to the intervention. They were anaesthetized...
The rats were placed in the supine position and the abdomen shaved and disinfected with 1% iodine alcohol. A mid-line laparotomy was performed using a clean but not strictly aseptic operative technique. Eversion of the abdominal wall was carried out using a steel spatula, exposing the anterior peritoneum without touching it. In the exposed area, a 0.5 cm square piece of silastic (Silastic®; Dow Corning Corporation Medical Products, Midland, MI, USA), 0.2 mm thick, was fixed 1 cm lateral to the epigastric artery, with two separate angular nylon 9/0 stitches. On one side (control side) the silicone plate was left uncovered, while on the contralateral (treated) side a thin layer of fibrin glue (Tissucol®; Immuno, Vienna, Austria) was applied just before the silastic was sutured to the peritoneum. The silastic on the treated side was oriented in such a way that the surface covered with fibrin glue faced the abdominal cavity. The side of treatment was chosen at random. Each animal served as its own control.

The operation was performed using a Zeiss OPMI 6 or 7 microscope (Zeiss Belgium, Zaventem, Belgium), fitted with a 200 mm focal length lens, ×12 eyepieces and 160 mm binocular tubes. This electrically foot-controlled zoom microscope provided a magnification between ×8 and ×25. The open-abdomen time was registered for each animal.

After application of the silicone plates, the abdomen was rinsed with saline and was closed in two layers, with separate vicryl 3/0 stitches for the musculoperitoneum and a vicryl 3/0 running suture for the skin. The animals were then maintained for 10 days with food and water ad libitum.

After 10 days, the rats were anaesthetized as described for the first procedure, and a second-look operation was performed. The abdominal wall was everted, exposing the peritoneal area where the silastic had been fixed. The adhesions were scored by a modified classification of Diamond et al. (1987) (Table I). For each side a score of the extent, type and strength of the adhesions was made, and the total adhesion score was calculated as follows: Total adhesion score = Extent × (Type + Strength).

In a subset of nine animals, after lysis of the adhesions, the silastic and the tissue covering the silastic were rinsed with saline to remove blood and red blood cells. On the side of each patch of silastic, a graduated grid with an accuracy of 1/10 mm was placed and photographs were taken at prefixed magnifications of ×5 and ×12.5.

In addition, a 2×2 cm piece of parietal peritoneum and underlying muscle around the silastic was resected with complete removal of the tissue flap. Each specimen was divided in two. One half of the silastic was fixed in 10% formalin, cut into 5 μm sections and stained with haematoxylin–eosin for histology. The other half of the specimen was used for scanning electron microscopy. After fixation in 10% glutaraldehyde, they were rinsed in saline and incubated for 15 min in ethanol solutions of increasing concentration (25, 50, 70, 80, 90, 95 and 100%). The specimens were dried in CO₂ (critical point determination) and sealed with gold dust. At the end of the experiment the animals were killed with an intracardiac injection of 0.3 ml T61® (Hoechst, Brussels, Belgium).

### Table I. Adhesion scores found on second-look laparotomy and scores for peritoneal healing

<table>
<thead>
<tr>
<th>Adhesion formation</th>
<th>Extent</th>
<th>Type</th>
<th>Strength</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated side</td>
<td>0.58 ± 0.02</td>
<td>1.00 ± 1.49</td>
<td>0.05 ± 1.35</td>
<td>2.89 ± 4.68</td>
</tr>
<tr>
<td>Control side</td>
<td>1.21 ± 1.58</td>
<td>1.58 ± 1.74</td>
<td>1.05 ± 1.18</td>
<td>6.79 ± 9.09</td>
</tr>
<tr>
<td>P value</td>
<td>0.45</td>
<td>0.25</td>
<td>0.37</td>
<td>0.181</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage of sheet covered</th>
<th>Vascularity</th>
<th>Histology</th>
<th>Scanning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated side</td>
<td>26.25 ± 31.50</td>
<td>0.75 ± 3.34</td>
<td>1.71 ± 0.86</td>
</tr>
<tr>
<td>Control side</td>
<td>46.25 ± 3.34</td>
<td>3.87 ± 6.35</td>
<td>2.00 ± 0.87</td>
</tr>
<tr>
<td>P value</td>
<td>0.226</td>
<td>0.842</td>
<td>0.629</td>
</tr>
</tbody>
</table>

In inflammatory reaction: histology

At magnifications of ×10 and ×100 the inflammatory reaction was scored as mild (score 1), moderate (score 2) or severe (score 3), according to Guo et al. (1993).

### Scanning electron microscopy

Scanning electron microscopy was performed for a qualitative evaluation of the 'peritoneal' surface. According to Guo et al. (1993), a score was calculated as follows: silastic not covered = 0; only fibrin and inflammatory cells = 1; occasional fibroblasts and peritoneal cells = 2; good peritoneal covering = 3; complete healed peritoneum with microvilli present = 4.

### Results

One rat died during the first operation because of an anaesthetic complication and was excluded from the study. The mean ± SD open-abdomen time for application of the silastics was 18.60 ± 2.52 min (range 15-25).

### Presence of adhesions

On the treated side adhesions were present in seven of the 19 rats. On the control side adhesions were found in nine of the 19 rats (P > 0.1). The adhesion scores are listed in Table I. Although the mean adhesion score on the control side was...
almost twice as high as on the fibrin glue side, this difference was not statistically significant (paired Wilcoxon rank test).

**Peritoneal healing**

*Tissue regeneration and neovascularization*

The percentage of the silastic covered by 'peritoneum' and the ingrowth of blood vessels are given in Table I.

Regarding tissue regeneration, the difference between the control side and the fibrin glue side was not statistically significant ($P = 0.226$). Concerning the ingrowth of blood vessels, one animal had to be excluded because of a technical problem with fixation, and the statistics were performed on the remaining eight animals. There was no statistically significant difference between the treated and control sides ($P = 0.842$).

**Inflammatory reaction**

The scores for the inflammatory reaction were as listed in Table I. No score could be given in slides of four rats (nos 2, 4, 5 and 7) because no submesothelial tissue was visible on the slides. For the remaining five animals, no statistically significant difference in the inflammatory reaction could be determined between the two sides ($P = 0.629$).

**Scanning electron microscopy**

As a pilot study prior to this work we examined the peritoneum of a non-operated rat so as to be able to use this as a standard (Figure 1).

The scores given by scanning electron microscopy are listed in Table I. One rat (no. 7) was excluded because of a fixation defect. No statistically significant difference was observed between the treated and control sides ($P = 0.695$). Completely healed peritoneum was not seen in any animal on either side (Figure 2).

**Discussion**

Previously, several studies have been performed estimating the effect of different barrier methods, and of fibrin glue in particular, on adhesion formation. The contribution of this
study lies in the fact that, besides the evaluation of the effect of a fibrin glue barrier on adhesion formation, the effect on tissue healing is also evaluated. With this rigorous and well standardized model, the revascularization of the tissue covering the silastic was possible only from the edges, and only in the presence of fibrin glue could peritonealization occur over the silicone sheets.

Concerning adhesion formation on this non-traumatized peritoneum, the study design was such that a negative as well as a positive effect could be evaluated. The study was based on the model developed by Bigatti et al. (1995), who reported a spontaneous adhesion incidence of 80% after 10 days postoperatively. However, we noticed a 'spontaneous' adhesion incidence of only ~50% on the control sides. This is probably because of the even more atraumatic operation technique used, conducted by an experienced microsurgeon, and underscores the importance of gentle tissue handling in adhesion prevention.

Although different studies have demonstrated a beneficial effect of fibrin glue after more extensive surgery, no significant effect could be seen on adhesion formation when traumatic microsurgical techniques were used.

With the use of fibrin glue, peritonealization appears to be satisfactory. The peritonealization and neovascularization processes were not inhibited and there was no additional inflammatory reaction.

There was a trend towards a reduction of both vascular ingrowth and de-novo adhesion formation, as in healing peritoneal tissue. However, as stated above, this difference was not statistically significant. This does not exclude the possibility of a clinical benefit. As there were no adverse side-effects on adhesion formation and tissue repair, fibrin glue can be safely used for haemostasis and suture-free anastomoses.

Human fibrinogen is used for the manufacture of fibrin glue, raising the possibility of disease transmission. However, the solvent/detergent virus inactivation method for human fibrinogen is used. With this technique not a single case of transmission of hepatitis B virus, hepatitis C virus or human immunodeficiency virus has been reported (Horowitz et al., 1993).

Further investigations on larger animals and humans undergoing laparoscopic intervention should be performed to evaluate the ultimate efficacy of fibrin glue on adhesion prevention, neoangiogenesis and peritoneal healing and to evaluate the particular factors that play a role in this phenomenon.

From this study we conclude that peritoneal healing and neoangiogenesis are not influenced by the fibrin glue; nor does fibrin glue alter the inflammatory reaction induced by an i.p. silastic foreign body. Although the mean adhesion score was reduced by 50%, there was no statistically significant difference in adhesion incidence.

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References


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