Prevention of peritoneal adhesion using a bacterial cellulose hydrogel, in experimental study[1]

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ABSTRACT

PURPOSE: To analyze the effectiveness of bacterial cellulose hydrogel as a barrier in preventing postoperative peritoneal adhesion in rat model.

METHODS: Experimental study with 45 Wistar rats (*Rattus norvegicus*) that were divided into three groups for the following treatments: A. Saline, B. Oxidized Regenerated Cellulose (ORC) barrier, and C Bacterial Cellulose Hydrogel (BCH) barrier. After 45 days of the surgery the adhesions were classified and graded according to the qualitative score. The histological parameters were evaluated using a modified semi-quantitative scale to rate the extent of fibrosis, inflammatory reaction and vascular proliferation.

RESULTS: Compared with the saline group (A), the treatments with ORC barrier (B) and BHC barrier (C) resulted in a smaller number of adhesions (p=0.019 and p=0.003 on *Fisher's exact test*, respectively). Data from inflammation and neovascularization showed no statistically significant difference between the groups BHC and ORC (p=0.426 and 0.446 on *chi-square test*, respectively).

CONCLUSION: Bacterial cellulose hydrogel is effective as a bio-re-absorbable barrier for preventing postoperative peritoneal adhesions.

Key words: Cell Adhesion. Hydrogel. Animal Experimentation. Rats.

Introduction

Adhesions are a frequent postoperative complication that may result in small-bowel obstruction, infertility, chronic abdominal pain, and high risk of complications in a second operation. However, the formation of adhesions continues to pose a challenge and is a major cause of morbidity and mortality rates[1].

Both general surgeons and gynecologists[2] are involved in increasing knowledge of the pathophysiology and the study of new mechanisms of adhesion prevention. Towards this, we evaluated different mechanical barriers as an efficient method for preventing postoperative intra-abdominal adhesions and reduce their complications[3]. Significant advances have been made in surgical techniques for preventing adhesions and in the development of products to prevent adhesion[4].

To prevent peritoneal adhesion, we used a bacterial cellulose hydrogel (BCH), 0.8% cellulose in 99.2% water as a mechanical barrier. This bacterial cellulose[5] has been previously used with satisfactory results in various areas of experimental surgery such as in the scarring of cutaneous wounds[6] dressing used after hypospadias surgery[7], as well as in the grafting used in vascular surgery[8,9]. The biocompatibility of the bacterial cellulose suggests that it does not induce inflammatory reaction and might be used as a sealant to prevent adhesions following gynecological and abdominal surgery.

The mechanical separation of the peritoneal surfaces of the pelvic organs plays an important role in the post-operative scarring process and in reducing the risk of future adhesions[10]. Liquid and solid barriers are used to prevent post-operative adhesions[11-14].

The aim of this study was to evaluate the protection effect of the bacterial cellulose hydrogel (BCH) as a mechanical barrier to reduce inflammatory reactions, fibrosis and the adhesion formation in the intra-abdominal structures after surgical incision on the uterine horn of rats, comparing the results with the use of an oxidized regenerated cellulose (ORC) barrier and using a saline group.

Methods

This research was approved by the Institutional Animal Bioethics Committee of the Center Biological Sciences of UFPE, protocol number: 23076.027396/2009.09, December, 14, 2011.

Forty-five female Wistar rats (*Rattus norvegicus albinus*), age 180 days. The rats were randomly allocated into three groups of 15 animals: A. saline, B. Oxidized Regenerated Cellulose – ORC barrier, and C. Bacterial Cellulose Hydrogel – BCH barrier. They were housed with cycles of night and day with standard food and water *ad libitum*.

The surgical procedure was carried out under anesthesia with the intramuscular application of ketamine chloral hydrate (5mg/100g of body weight) and xylazine chloral hydrate (2mg/100g of body weight). Intramuscular atropine sulfate was used as a pre-anesthetic (0.44mg/kg).

Synthesis of bacterial cellulose hydrogel

Bacterial cellulose hydrogel (BCH) was produced from sugars of sugar cane in the laboratory of biopolymers at the Experimental Station of Sugarcane, Federal Rural University of Pernambuco, Brazil[5]. The hydrogel was obtained by hydration of microcrystalline bacterial cellulose at a ratio of 0.8% cellulose in 99.2% water and sterilization by gamma ray.

Surgical procedure: induction of adhesions and evaluation

The uterine horns were exposed and a 1.5 cm linear incision was made by electro-cautery on the anterior surface of the left horn. Hemorrhage points were coagulated, and the incision was closed with Prolene 7-0 in continuous stitches.

The wound was then covered with a barrier of ORC film, or 5 mL of saline, or hydrogel of bacterial cellulose according to the respective group. The abdominal wall was closed using continuous prolene 5.0 fascia sutures and the skin was closed with Nylon 5.0 thread (Mononylon®). Antibiotic prophylaxis was not provided and the animals were checked daily for complications control.

On the 45th day after surgery, the rats were anesthetized in the same way and a U-shaped abdominal incision was used to approach the peritoneal cavity to investigate the formation and intensity of adhesions.

The peritoneal cavity was photographed using a Sony® DSC - s650 camera fixed at a standard distance. Photos were used for later analysis of the extent of site-specific adhesions using AutoCAD. The operated segment of the uterus was then removed and fixed in 10% formaldehyde for histological analysis. The animals were euthanized by an overdose of sodium thiopental.

Assessment of adhesion formation

The adhesion score was graded according to the qualitative numeric system developed by Wallwiener *et al*.[15] from 0 (absent)

to 3 (very severe), where 0 and 1 represent adhesions that are not clinically significant, whereas grade 2 and 3 adhesions may give rise to obstruction of the intestines. For the purpose of analysis, these ratings were further grouped as severe (2-3) or slight (0-1).

Histological evaluation

Slices of four micrometers of the segment of the uterus were colored with H&E and Masson's trichrome. These histological preparations were evaluated using a modified semi-quantitative scale (0-3), the extent of fibrosis, the inflammatory reaction and vascular proliferation[16].

Statistical analysis

The normality of variables was confirmed using the Kolmogorov-Smirnov normality test. Fisher's Test was used to compare qualitative variables. Parametric (ANOVA) and non-parametric (Kruskal-Wallis) methods were used for comparisons of qualitative variables. Multiple comparisons were carried out using Tamhane´s T2 test (ANOVA Post Hoc Tests). The *GraphPad Prism 4.0* software was used for statistical analysis. The significance level for rejecting the null hypothesis was 5% ($p \leq 0.05$).

Results

The animals responded well to the surgical procedure. One rat in the saline group died immediately after surgery, probably due to an anesthetic complication. Bodyweight changes followed a similar course in the various groups in all three experiments.

The number of animals with adhesion, severity of adhesion and absolute cross-sectional areas of adhesions were evaluated in groups A - Saline, B - ORC and C – BCH. They are shown in Table 1.

The severity of peritoneal adhesions (%) in groups B and C showed a statistically significant difference compared to group A ($p=0.019$ and 0.003 respectively). Between the groups B and C there was no statistically significant difference ($p=0.4621$, Fisher's Test). The comparison of the area of adhesions using Tamhane's T2 test, revealed that there was a significant difference between the means for groups B ($p<0.001$) and C ($p<0.001$) when compared with group A (Table 1).

Relative to inflammatory reaction and vascular proliferation, there was no statistically significant difference between the groups ($p=0.426$ and 0.446, respectively). Histopathological study of all cases did not reveal ORC or BCH residue in the peritoneal cavity.

TABLE 1 - Severity of adhesion per group.

Groups	N	With adhesions	% with adhesions	With severe adhesions	% with severe adhesions	Multidimensional Adhesion Score	Adhesions (cm2)
Saline	14	14	100	11	78.57	8.07±2.84	1.04±0.40
ORC	15	10	66,7 [a]	5	33.33 [a]	3.27±2.82 [a]	0.37±0.32 [a]
BCH	15	7	46,7 [a]	3	20.00 [a]	2.00±2.45 [a]	0.21±0.26 [a]

Note: Values represent mean ± SD. $p>0.05$. a ≠group A (saline). ORC, Oxidized Regenerated Cellulose; BCH, Bacterial Cellulose Hydrogel.

Discussion

Previous work has shown that, in combination with a good surgical technique, the ORC absorbable barrier prevents severe adhesions in 61% of cases and is twice as effective as surgery carried out without the use of anti-adhesion agents[17]. After laparoscopic surgery for ovarian cysts, 47% of the patients in whom this barrier was used were free of adhesions, compared to 25% in the control groups[18]. ORC is the anti-adhesion barrier that has been used for a long time in randomized clinical trials as the product that reduces the degree of adhesion severity. In controlled clinical studies and meta-analyses, the ORC absorbable barrier after open and laparoscopic surgery has been shown to be an effective anti-adhesive both for the first manifestation and recurrence of adhesions[19,20].

Thus this product was chosen to serve as the standard for comparison with BCH. Our results show that the use of ORC prevented the formation of severe adhesions in 66.67% of the rats; the BCH in 80% of the rats.

In this study, a multidimensional score was used, the percentage of animals free of adhesions, type, tenacity and extent of adhesions to determine the effectiveness of ORC and BCH as a barrier in comparison with a saline group. The experiments showed that the formation of adhesions was significantly lower in animals treated with ORC or BCH compared to those treated with saline. Despite the fact that bacterial cellulose hydrogel demonstrated an effective result, it failed to demonstrate statistical superiority over ORC (Table 1).

Histological evaluation showed that there exists a relation between fibrosis and the treatment groups. In the case of inflammatory reaction and vascular proliferation, there was no statistically significant difference between these groups (p=0.426 and 0.446, respectively). The hydrogel did not induce specific inflammatory reactions, causing a lower fibrotic response compared to the non-treatment and ORC barrier groups. The effectiveness of the BCH may be due to the various particularities of this material, especially its high tolerance and adequate persistence in the peritoneal cavity during scarring.

Bacterial cellulose hydrogel may be an alternative way of preventing adhesions. BCH stays for an adequate time in the peritoneal cavity as a protective barrier, as it is made up of sugars. Other devices based on bacterial cellulose, raw material of the hydrogel, have already been used and shown to be effective in other areas of medicine in experimental studies involving animals[6-9].

The high viscosity of bacterial cellulose hydrogel allows it to slip on the organs and spaces of the peritoneal cavity to form a thin layer on the visceral and parietal peritoneum. Different from solid products such as films, BCH can be molded into different shapes to fit different spaces in specific clinical situations.

In this study, after 45 days, the hydrogel was biodegraded and BCH residue was no longer found in the peritoneal cavity. This versatility gives it reach both on smooth and convex tissue surfaces, enabling it to be used in a wide variety of surgical scenarios.

Conclusion

Bacterial cellulose hydrogel is effective as a bio-re-absorbable barrier and is capable of preventing postoperative peritoneal adhesions in rat model.

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