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Adhesions on polypropylene versus Sepramesh® meshes: an experimental study in rats.

Aderências em telas de polipropileno versus telas Sepramesh®: estudo experimental em ratos.

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ABSTRACT

Objective: to compare the formation of induced intraperitoneal adhesions in rats when using polypropylene and Sepramesh® meshes. **Methods:** we used 20 male Wistar rats, randomly grouped in two groups of ten animals each. We arranged two 10x20mm meshes intraperitoneally into each animal, one being the polypropylene (PP), and the other, Sepramesh®. In Group 1, the polypropylene mesh was positioned to the right, and the Sepramesh®, to the left. In Group 2, the meshes' layout was reversed. After 14 days of the procedure, we euthanized the animals and analyzed the incorporation and percentages of adhesions macroscopically in each mesh. We submitted the collected data to statistical analysis with a significance level of 5% ($p<0.05$). **Results:** all meshes showed adhesions. In the Sepramesh® ones, the percentage of surface covered by adhesions ranged from 2% to 86%, with a mean of 18.6±18.6%, while in the polypropylene meshes, it varied between 6% and 86%, with an average of 57.4%±34.9% ($p<0.05$). The preferred adhesion sites on both meshes were the edges. **Conclusion:** although no mesh was able to completely inhibit the development of adhesions, the Sepramesh® mesh presented less adhesions to the polypropylene mesh. The most common sites of adhesion formation were the edges of the prosthesis, which evidences the importance of the adequate fixation of the meshes.

Keywords: Tissue Adhesions. Surgical Mesh. Hernia, Ventral.

INTRODUCTION

Ventral or incisional hernias are protrusions of a portion of organs or tissues by a defect in the abdominal wall[1]. The development of hernias is a multifactorial process related to anatomical weaknesses, increased intra-abdominal pressure[2], surgeries and trauma[3]. Such hernias are rarely symptomatic, but present the risk of strangulation, resulting in severe complications if not treated[2].

Hernias are common debilitating conditions that affect more than one million Americans per year, with more than 350,000 surgeries annually[4] and 400,000 surgeries in Europe[5], making this the most common procedure in General Surgery[4,6,7]. In Brazil, although we do not have current data, it is estimated that, between 1993 and 1996, 500,000 herniorraphies were performed, with an estimated cost of R$ 100 million[8]. It is believed that over 20 million meshes be deployed per year worldwide[7].

The development of incisional hernias after abdominal surgeries is frequent[1], being the most common surgical complication in the United States[3], with incidence between 2% and 40%[1,5,8,9] and recurrence between 24% and 43%[5]. It is believed that a 1% reduction in the isolated recurrence rate would correspond to a savings of US$ 32 million annually[5].

The treatment of ventral hernias is essentially surgical[1,10], with several techniques described[10]. The correction aims at restoring the normal anatomy of the abdominal wall and preventing recurrence by providing biomechanical strength to the attenuated fascial structures[7]. The simple approximation and suture of the tissues was the method recommended during one century[11].

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From 1958, the proposal of the use of meshes for correction of hernias by Dr. Francis Usher[3] made this treatment model essential, reducing costs and recurrences[6,10,12-14]. The popularity of the method made the use of meshes the gold-standard treatment in herniorraphies[12], allowing the development of prostheses composed of different materials.

In the last decades, it has been recommended the laparoscopic implantation of meshes. This approach is preferred because it reduces hospitalization time and infections and allows the recognition of multiple herniations[14]. Via laparoscopy, the meshes are arranged intraperitoneally, being in direct contact with the abdominal structures. This technique allows the development of complications such as adhesions[3], fistulas and intestinal obstruction[10].

Adhesions are fibrous bands that connect intra-abdominal organs or tissues, typically formed after abdominal surgeries[15], representing an important clinical challenge[16]. They are a consequence of peritoneal irritation due to infections or surgical trauma, and can be considered a pathological part of the healing process[17]. Their prevalence after abdominal procedures is estimated between 63% and 97%[15,17,18]. The main complications related to adhesions are: intestinal obstruction, pelvic or abdominal pain, infertility, and difficulties in subsequent surgeries[17,18].

The polypropylene mesh is the most commonly used surgical mesh for hernia repair[3,10]. Polypropylene is a non-absorbable polymer widely used due to its flexibility, low cost, resistance to biological degradation, infection and mechanical stress, stimulation of cell growth, and acceptable inflammatory response[2,10,12]. However, when placed in contact with intraperitoneal structures, it promotes adhesions.

The composite meshes were developed in order to reduce the formation of adhesions when inserted intraperitoneally. They combine more than one material, forming a prosthesis with two distinct surfaces: a visceral microporous surface to prevent adhesions, and a macroporous parietal surface to favor the incorporation[19]. The Sepramesh® is a polypropylene visceral face mesh covered by an absorbable barrier of sodium hyaluronate and carboxymethylcellulose[20], which has been widely indicated for intra-abdominal corrections due to effectively preventing the adhesion formation[14,16,18].

This study aims at comparing adhesion formation with the polypropylene mesh and with the Sepramesh®.

METHODS

This project was submitted to the Ethics Committee for the Use of Animals of the Biological Sciences Sector (CEUA-BIO) of the Federal University of Paraná (UFPR), which, once approved, received the registration number 23075.177495/ 2017-42.

The sample consisted of 20 male rats (*Rattus norvegicus albinus*, *rodentia Mammalia*) of the Wistar lineage, with age between 100 and 120 days, and weighing between 360g and 480g, mean 413.25±34.58g. The animals were housed in the Laboratory of the Discipline of Surgical Technique and Experimental Surgery of UFPR, with a temperature of 20±2°C and with changes of air of the environment and luminosity according to cycles of light and dark of 12 hours. The animals were kept in polypropylene boxes, suitable for the species, containing white wood (changed daily), in number of five animals per box. They received water and standard commercial chow for the species *ad libitum*.

We randomly divided the sample into two groups, with ten rats each. We inserted both meshes into each animal on the intraperitoneal surface of the abdominal wall, so that each rat was its own control. In Group 1, the polypropylene (PP) mesh was placed on the peritoneal surface to the right of the median incision, and the Sepramesh®, on the left. In Group 2, the meshes' layout was inverted, leaving the polypropylene mesh on the left and the Sepramesh® on the right.

Before the surgical procedure, we kept the rats in quarantine for two weeks for setting in the laboratory. Anesthesia was performed with intramuscular ketamine hydrochloride (50mg/kg) and xylazine (20mg/kg), supplemented with inhalation induction with isoflurane 1% to 1.5% in a mask associated with 100% oxygen. We made a median, xifopubic, incision of four centimeters. We positioned the 10x20mm meshes in the intraperitoneal plane according to the corresponding group of the animal, and fixed them with 5.0 polypropylene suture with simple stitches at the vertices of the mesh, with knots facing the aponeurotic plane, minimizing the amount of intraperitoneal foreign body. We then performed the synthesis of the wall, made in two planes, the first in the peritoneum-muscle-aponeurosis and the second in the skin, with continuous synthesis with 4.0 monofilament nylon. We used dipyrone intramuscular injection (10mg/kg) for analgesia.

After 14 days of the procedure, we held euthanasia under anesthesia, according to the protocol described in the CONCEA Euthanasia Practice Guidelines (2013) and the Brazilian Good Practice Guide on Euthanasia in animals of the Federal Medical Veterinary Council (2013). Anesthetic induction was performed with inhaled isoflurane and intravenously administered sodium thiopental solution (10mg/kg), followed by venous puncture of the tail vein with administration of 10% potassium chloride (5mg/kg).

For the measurement, we opened the abdominal cavity with a U-shaped incision that, when folded, allowed the evaluation of the meshes and the adhesion *status* (Figure 1). We analyzed the integration of the meshes to the abdominal wall and the presence of adhesions. We included adhesions to the meshes, and excluded those to the laparotomy closure and/or to fixation points, sites which tend to form adhesions independently of the prosthesis material. We carried out photographic documentation of all abdominal cavities.

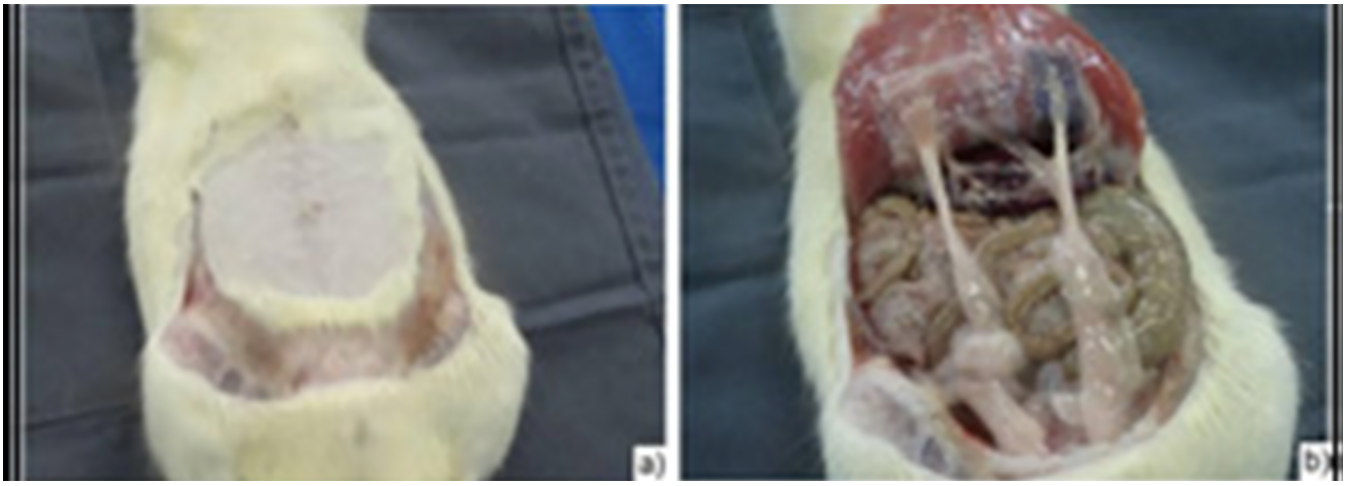

Figure 1. *a) U-shaped incision for access to the abdominal cavity; b) Abdominal wall reflected to allow evaluation of the adhesions formed in each mesh.*

For evaluation of the adhesion area, we designed a mold of the same size (10x20mm) on graph paper. For greater accuracy, we resected visceral adhesions for analysis of the portions covered by the meshes. From the projections on the graph paper, we calculated the commitment percentages of each mesh. We considered the meshes fixed to the peritoneum as incorporated, while those supported only by the attachment sutures, as unincorporated.

The results were submitted to statistical analysis by the Mann-Whitney test for means evaluation and the Fisher's test for the frequency, adopting $p<0.05$ or 5% as a level for rejection of the null hypothesis.

RESULTS

There were no postoperative complications or deaths. We excluded an animal from Group 1 from the statistical analysis because it presented bias in the placement of the mesh. All meshes presented with adhesions. Regarding incorporation, six polypropylene meshes and nine Sepramesh® ones were not incorporated into the parietal peritoneum, being fixed only by the sutures ($p=0.2574$).

In Group 1, the percentage of meshes covered by adhesions on the right side (polypropylene) variedor between 6% and 100% of surfaces, with an average of 67.61%±32.39%; in the left side (Sepramesh®), the percentage of covered meshes ranged from 7% to 86%, with a mean of 24.33%±24.18% ($p<0.05$) (Table 1).

In Group 2, the percentage of meshes covered by adhesions on the right side (Sepramesh®) varied from 2% to 32.50%, with a mean of 13.45%±10.53%; in the left side (polypropylene), it ranged from 14% to 100%, with a mean of 48.15%±36.14% ($p<0.05$) (Table 2).

There was no significant difference for both meshes when comparing the insertion sides, regardless of the group. The polypropylene mesh showed more adhesions when implanted on the right, mean 67.61%±32.39%, than on the left,

Table 1. *Percentage of mesh surface covered by adhesions in Group 1.*

Animal	Area with adhesions	
	Right side Polypropylene (%)	Left side Sepramesh® (%)
Rat 1	59.0	7.0
Rat 2	68.5	19.5
Rat 3*		
Rat 4	40.0	11.0
Rat 5	100.0	23.0
Rat 6	100.0	16.0
Rat 7	85.0	24.0
Rat 8	50.0	7.0
Rat 9	100.0	86.0
Rat 10	6.0	25.5
Average	67.61	24.33
Standard deviation (SD)	65.8	19.5
%SD	32.39	24.18
Median	47.9	99.38

** Animal excluded; Mann-Withney test, p<0.05.*

mean 48.15%±36.14%. The Sepramesh® had a larger area covered by adhesions when implanted on the left, with mean 24.33%±24.18%, than on the right, mean 13.45%±10.53%. There was greater adhesion involvement on the edges of the meshes (Figure 2).

When analyzing the polypropylene mesh, regardless of the group and the side where it was applied, we verified that the percentage of surface covered by adhesions ranged from 6% to 100%, with an average of 57.37%±34,92%. For the Sepramesh®, the percentage covered by adhesions varied between 2% and 86%, with an average of 18,61%±18,61% ($p<0.05$) (Figure 3). Participated in the adhesions the omentum (67%), the fat of the spermatic cord (40%), the liver (12.5%), the mesentery (7.5%) and loops of small intestine (2.5%) (Figures 4 and 5).

Table 2. *Percentage of the mesh surface covered by adhesions in Group 2.*

Animal	Area with adhesions	
	Right side Sepramesh® (%)	Left side Polyproylene (%)
Rat 1	15.0	24.5
Rat 2	11.0	97.0
Rat 3	32.5	45.0
Rat 4	15.0	100.0
Rat 5	8.5	100.0
Rat 6	31.0	25.0
Rat 7	9.0	14.0
Rat 8	7.0	15.0
Rat 9	3.5	30.0
Rat 10	2.0	31.0
Average	13.45	48.15
Standard deviation (SD)	10	30.5
%SD	10.53	36.14
Median	78.29	75.06

Mann-Withney test, $p<0.05$.

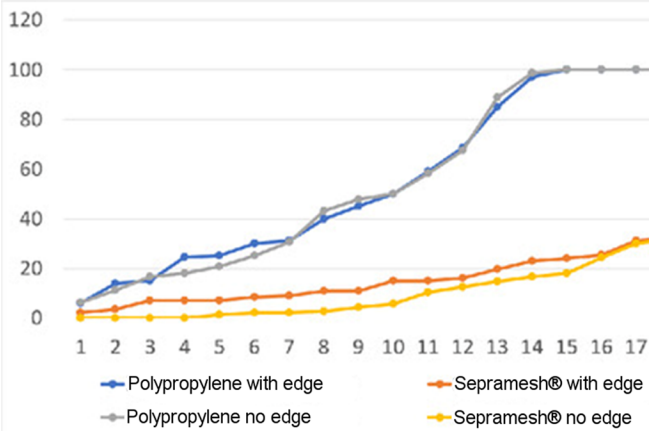

Figure 2. *Percentage of the surface affected in both meshes, with and without edges, in ascending order and in an individualized way.*

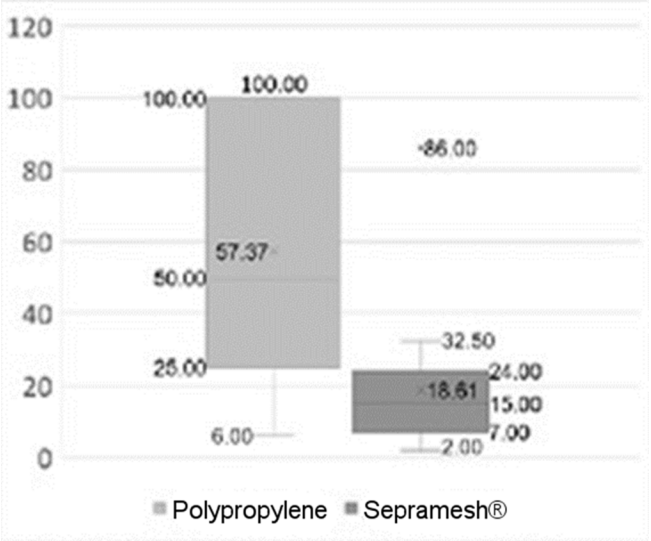

Figure 3. *Percentage of involvement in each mesh.*

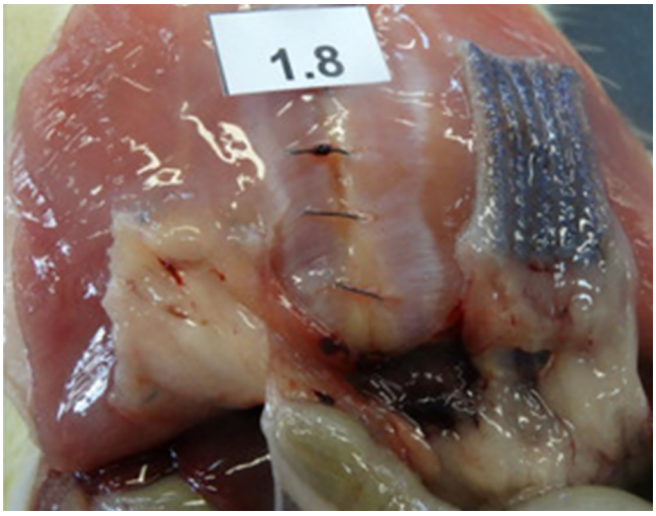

Figure 4. *Image showing the polypropylene mesh, placed to the right, completely covered by adhesions, and the Sepramesh®, placed to the left, partially covered by adhesions.*

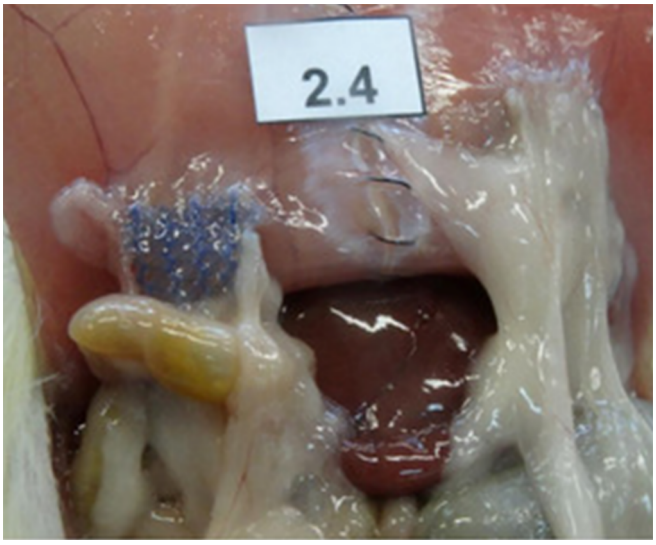

Figure 5. *Image showing the polypropylene mesh, placed to the left, completely covered by adhesions, and the Sepramesh®, placed to the right, partially covered by adhesions.*

DISCUSSION

In the hernias field study, several models were developed, using rats, rabbits, primates, sheep, dogs and pigs[4,21]. Although there are several limitations on the use of animals as experimental models, be it in attempting to replicate the exact pathophysiology of hernia, be it in the difficulty in representing the increase in intra-abdominal pressure due to the upright posture[2,4], they have several advantages[4]. They are the ability to conduct studies that are not possible in humans, the greater control over the design of the experiment (age, time, induced injury), the results consistency greater than those obtained with human cohorts, and the lower costs[4].

The most frequently used animal as a model for studies of adhesion formation on meshes is the rat[10,18]. Such a model allows direct exposure of abdominal viscera with contact to the mesh[18]. Although there are still doubts in the literature regarding the possibility of extrapolating to human data obtained with small animals[20] due to the anatomical differences[22], the similarities regarding the inflammatory process and biocompatibility make them an adequate model for assessing adhesions[21].

In the present study, we analyzed the formation of intra-abdominal adhesions in Sepramesh® and polypropylene meshes. We implanted bothmeshes in all animals, the differentiation between the two groups being defined by the side on which each mesh was positioned. Such methodology allowed to reduce the bias of the biocompatibility response, since each animal acted as its own control. The positioning of the meshes on both sides of the animal's body aimed at verifying if the weight of the different abdominal viscera, arranged asymmetrically, could influence the formation of adhesions. These were evaluated 14 days after the implant, according to the indication in the literature that there is no formation of new adhesions after the seventh day[16,18,23,24].

The evaluation of adhesion formation in meshes for correction of abdominal hernias frequently includes incidence, extension and quality[18], assessing the percentage of surface of the covered mesh and type of adhesion (omental and/or visceral)[20]. The surface coated by adhesionis an objective parameter, which allows quantification by

involving a two-dimensional plane and representing a defined site of adhesion after its formation[18]. In this study, we evaluated adhesions directly by macroscopy and calculation of the affected surface.

The peritoneal lesion due to surgery, infection or irritation initiates an inflammatory process, with formation of fibrin by the activation of the coagulation cascade[16]. This process is usually self-limited, with degradation of fibrin byfibrinolysis. With surgical trauma, however, the balance between coagulation and fibrinolysis is altered, favoring the formation of fibrin[16,17], which creates a substratefor the deposition of fibro-collagenous extra-cellular matrix[16], resulting in the formation of a dense fiber in about five days[25]. Generally, if fibrinolysis does not occur within a week after the peritoneal lesion, the fibrin matrix organizes, forming a definite adhesion[17].

Among the factors predisposing to the formation of adhesions are trauma (surgical), foreign body reaction, infection and ischemia[8]. Prevention of peritoneal adhesions formation is achieved by reducing the influence of these factors and can be grouped into four categories: Halsted principles, surgical technique, mechanical barriers and chemical agents[17].

Surgical approach can play an important role in the development of adhesions[17]. As a rule of thumb, the general surgical principles and the surgical technique seek the minimum of peritoneal injury[17]. However, in many cases, direct contact between the meshes and the intra-abdominal organs cannot be avoided, which would facilitate the formation of adhesions[26,27]. Moreover, with the advent of laparoscopy and its increasingly frequent indication[20,28], the meshes are arranged intraperitoneally, getting in direct contact with abdominal structures. Therefore, the search for meshes with lower adhesion indexes gained strength.

An ideal mesh should be safe, biodegradable, chemically stable, do not induce inflammation, immunogenic reaction or carcinogenesis, provide easy sterilization and application, resist traction, allow incorporation, present low cost and hamper infections and adhesion formation[3,17,27]. However, it is unlikely that a single biomaterial and displays all ideal parameters[4]. Factors such as structure, biocompatibility, traction strength, elasticity, resistance, porosity and weight, degradation, filaments and anisotropy influence the formation of adhesions and inflammatory reaction[6,10]. The choice of meshes used in this study (polypropylene and Sepramesh®) is justified by the preference for the use of polypropylene by health professionals and by the current demand for composite meshes that can be introduced intraperitoneally[24].

The polypropylene mesh with high weight (80 to 100 g/m^2) and medium-sized pores (0.8mm) is the most commonly used surgical mesh for hernia repair[3,10,25,29]. Polypropylene is a non-absorbable polymer used due to being flexible, having low cost, resisting biological degradation, infections and mechanical stress, stimulating cell growth and presenting an acceptable inflammatory response, allowing incorporation[2,10,12,25]. However, these same characteristics that favor its wide use promote adhesion formation[2,23,30,31]. In the present study, we found adhesions in all implanted polypropylene meshes, with a higher percentage of adhesions on the right than on the left, though with no statistically significant difference. However, six of the 20 polypropylene meshes did not incorporate into the abdominal wall.

The Sepramesh® is a macroporous, low-weight, composite-type mesh, with visceral face covered with a sodium hyaluronate and carboxymethylcellulose absorbable barrier[20], which

has been extensively indicated for intra-abdominal correction by effectively preventing adhesion formation[14,16,18]. It was developed in order to allow its visceral face to be in contact with the viscera of the abdominal cavity, while its portion of uncoated polypropylene is incorporated into the parietal peritoneum[2]. Its mechanism of action is to promote a physical and chemical barrier to the formation of adhesions during the first week, being later reabsorbed, leaving a regular synthetic mesh after this period.

Several studies have compared the formation of adhesions between the Sepramesh® and polypropylene meshes and between Sepramesh® and other composite meshes[18,20,23,27]. In an experimental study in rats, Gaertner *et al.*[18] demonstrated that Sepramesh® had less adhesions when compared to 14 other meshes for the correction of ventral hernias. Greenawalt *et al.*[23], in a study in rabbits, indicated Sepramesh® as forming less adhesions when compared with polypropylene and polytetrafluoroethylene meshes. The use of Seprafilm® alone (Genzyme Corp, Cambridge, MA), an integral membrane of sodium hyaluronate/carboxymethylcellulose, shows similar results in the literature[23,25,32,33]. In our study, we observed adhesions on all Sepramesh® implanted meshes, with a higher percentage of adhesions on the left side than on the right one, though with no statistically significant difference. However, after 14 days, nine of the 20 Sepramesh® meshes were not incorporated into the abdominal wall.

The results of the present study corroborate the ones obtained in the literature regarding the formation of intra-abdominal adhesions. All meshes (polypropylene and Sepramesh®) induced them. Sepramesh® promoted a significant reduction of adhesion formation when compared with the polypropylene mesh ($p<0.05$). There was no difference in adhesion formation for both meshes when evaluated separately for the insertion side ($p<0.05$), indicating that the weight and disposition of the different abdominal cavity viscera may not have a direct influence on adhesion formation. There was no difference in the incorporation into the abdominal wall between the two meshes ($p=0.2574$).

Regardless of the mesh type, the preferred sites for adhesion formation were the edges and attachment points, a situation clearly evident in the Sepramesh® meshes. This data is in agreement with the literature[16,18,23,33] and can be explained by the exposure of polypropylene to the edge of the mesh, a region not covered by the sodium hyaluronate barrier, allowing direct contact with the abdominal viscera. In addition, the fixation points of the mesh were polypropylene sutures, and every stitch produces a region of ischemia, considered an inducing factor of adhesions. Interestingly, when we disregarded the adhesions formed on the meshes' edges, we no longer verified the presence of adhesions on all Sepramesh® meshes. After this correction, four Sepramesh® meshes displayed no adhesion. Moreover, when suppressing the adhesions at the edges, the average surface area covered in the Sepramesh® was reduced by 4.7% (from 18.61%±18.61% to 13.9%±20.5%), while the polypropylene meshes showed a reduction of only 0.4% (from 57.37%±34.92% to 56.99%±35.96%), further reinforcing the results obtained.

In medical practice, composite meshes are often cut by surgeons to achieve size and shape ideal for correction of defects, a procedure that may facilitate the appearance of adhesions[33]. In this sense, within the context of adhesion prevention and since the edges of the meshes are its main site

of formation, the surgical technique should direct its main attention to an adequate fixation of the meshes, minimizing factors that are conducive to its development.

In rats, the Sepramesh® meshes form less adhesions when compared with polypropylene ones, and that the fixation of the meshes is an important factor in the development of adhesions.

R E S U M O

Objetivo: *comparar a formação de aderências intraperitoneais, induzidas em ratos, quando utilizadas as telas de polipropileno e Sepramesh®.* **Métodos:** *foram utilizados 20 ratos Wistar, machos, agrupados randomicamente em dois grupos de dez animais cada. Duas telas de dimensão 10x20mm foram dispostas intraperitonealmente em cada animal, uma de polipropileno (PP) e a outra Sepramesh®. No Grupo 1, a tela de polipropileno foi posicionada à direita e a tela Sepramesh® à esquerda. No Grupo 2, a disposição das telas foi invertida. Após 14 dias do procedimento, os animais foram eutanasiados e a incorporação e a porcentagem de aderências, em cada tela, analisadas macroscopicamente. Os dados coletados foram submetidos à análise estatística com nível de significância adotado de p<0,05.* **Resultados:** *todas as telas apresentaram aderências. Nas telas Sepramesh®, a porcentagem de superfície coberta por aderências variou entre 2% e 86%, com média de 18,6±18,6%, enquanto que, nas telas de polipropileno, variou entre 6% e 86%, com média de 57,4%±34,9% (p<0,05). Os sítios preferenciais de formação de aderências, em ambas as telas, foram as bordas.* **Conclusão:** *embora nenhuma tela tenha sido capaz de inibir completamente o desenvolvimento de aderências, a tela Sepramesh® apresentou menos aderências em relação à tela de polipropileno. A preferência da formação de aderências nas bordas das próteses evidencia a importância da fixação adequada das telas.*

Descritores: *Aderências Teciduais. Telas Cirúrgicas. Hérnia Ventral.*

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