

## Ventral abdominal wall defect correction in rats with contaminated meshes<sup>1</sup>

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DOI: <http://dx.doi.org/10.1590/S0102-865020160100000007>

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### ABSTRACT

**PURPOSE:** To investigate whether there is a difference between Marlex<sup>®</sup> and Dynamesh PP-light Marlex<sup>®</sup> meshes, in the abdominal wall defect correction, on rats in contaminated surgical site.

**METHODS:** Twenty-eight Wistar rats were divided into two groups of 14, and four subgroups of seven animals. All subgroups underwent similar surgical procedure. One group received the mesh Marlex<sup>®</sup> and the other Dynamesh PP-light<sup>®</sup> for correction of the defect. Before implanting, the meshes went through a contamination process, on which was used standard solution containing 10 UFC of *Escherichia coli*. Fragments of the animal's abdominal wall received macroscopic, microscopic and microbiological analysis.

**RESULTS:** There was no statistical significance in the analysis of macroscopic variables. Accentuated inflammatory process was shown in all subgroups. The foreign body type reaction was mild in all subgroups, except Dynamesh<sup>®</sup>-14, which was moderate with no statistical significance. The microbiological analysis of the meshes was also similar between the subgroups.

**CONCLUSION:** There was no difference between the meshes of Marlex<sup>®</sup> and Dynamesh PP-light<sup>®</sup> in the ventral abdominal wall defect correction on rats in contaminated surgical site.

**Key words:** Incisional Hernia. Surgical Mesh. Materials Testing. Rats.

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## Introduction

The incisional hernias or abdominal wall eventrations are frequent complications of abdominal surgery by laparotomy, estimating its incidence in 2-8%. Among the main risk factors for its appearance, are wound infection and obesity, among others<sup>1-3</sup>.

The surgical treatment is usually laborious and has high recurrence rates, even with experienced hands. There are several techniques for repair, from aponeurotic flaps to tension-free techniques, using synthetic prosthesis<sup>4</sup>.

The development of polypropylene prosthesis revolutionized surgery for repair of the abdominal wall hernia. When compared to basic repairs, the tension-free techniques reduced recurrences and made possible the reconstruction of large ventral defects that were irreparable<sup>5</sup>.

Despite initial concerns about the possible rejection and infection, resulting from the use of prosthesis, there is evidence that tension-free hernioplasties using biomaterials, have significantly reduced recurrence and complication rates, making it accepted worldwide<sup>6</sup>.

The quality of synthetic meshes and surgical techniques has shown great developments in the past years. Materials such as polypropylene, polyglactin, polytetrafluoroethylene, woven polyester, polyvinylidene, among others, may be part, alone or in combination, of the composition of meshes currently used<sup>7-12</sup>. Besides the material, which forms the mesh, its density also gives it particular characteristics. High density or microporous meshes (pores smaller than 10 $\mu$ m) may increase the chance of infection and fistula formation<sup>13,14</sup>. However, the low density or macroporous meshes (pores larger than 75  $\mu$ m) prevents the development of infections<sup>15,16</sup>.

Often times the surgeon finds strangulated ventral hernias, urgently operated, with occlusion or with associated intestinal sub-occlusion, which favors the phenomenon of bacterial translocation. Considering several other clinical situations of possible peritoneal cavity contamination, such as those that occur directly through intestinal necrosis with perforations, fistulas and bacterial peritonitis, or in patients who underwent elective abdominal surgery with opening of the gastrointestinal tract and require associated herniorrhaphy, there is always the question as to whether or not use prosthesis, and what is the most appropriate prosthesis for this situation.

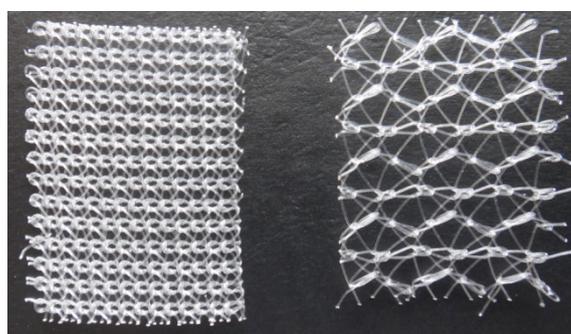
Thereby, this study aims to determine if there is a difference between the meshes of Marlex<sup>®</sup> and Dynamesh PP-light<sup>®</sup> in the ventral abdominal wall defect correction on rats in contaminated surgical site.

## Methods

This study took place at the Experimental Surgery Laboratory of the Faculdade Evangélica do Paraná, after approval by the Ethics Committee on Animal Research.

The sample consisted of 28 Wistar rats (*Rattus norvegicus albinus*) males, adults, weighing between 280-358g, and the groups identified by picric acid staining at certain points, keeping the rats on day and night cycles of 12 h at room temperature of 24°C. Throughout the experiment, they received proper feed for the species and had free access to water.

For the experiment were used meshes of Marlex<sup>®</sup> and DynaMesh – PP light<sup>®</sup> (Figure 1).



**FIGURE 1** – To the left, meshes of Marlex<sup>®</sup> and to the right, Dynamesh PP-light<sup>®</sup>

The mesh of Marlex<sup>®</sup> consists of polypropylene monofilament classified as microporous (pores of 0.6 mm) and heavy grammage (95 g/m<sup>2</sup>). As Amid P<sup>17</sup> defined meshes with larger pores than 75 microns are considered macroporous. These can be considered large pores when the pores are larger than 1.5 mm or 150 microns. Then the meshes used in the study are macroporous with large pores (DynaMesh PP light) and small pores (Marlex). The DynaMesh–PP light<sup>®</sup> is composed of polypropylene monofilament, classified as macroporous (pores of 2.6 mm) and low grammage (36 g/m<sup>2</sup>). The meshes were cutted into pieces of 2.0x2.5cm, and contaminated with standard solution of *Escherichia coli*. The strains were cultivated in BHI broth; batch YF209, sterile, at 36° C. The solution was prepared 24 h before the experiment and reached a final concentration of 10<sup>9</sup> UFC.

The 28 animals were split randomly into two groups of 14. The groups were given names related to the used meshes. The Marlex<sup>®</sup> group was subdivided in two subgroups of seven animals. In the Marlex<sup>®</sup>-7 subgroup, the euthanasia occurred on the 7<sup>th</sup> day after surgery and in the Marlex<sup>®</sup>-14 subgroup, the euthanasia on the 14<sup>th</sup> day. The Dynamesh<sup>®</sup> group was divided into two groups

of seven. In the Dynamesh®-7 subgroup, the euthanasia occurred on the 7<sup>th</sup> postoperative day and in the Dynamesh®-14 subgroup, on the 14<sup>th</sup> day.

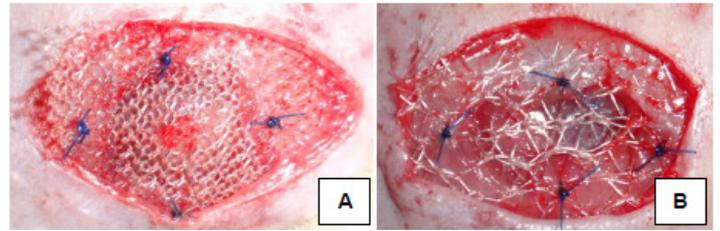
A digital scale weighed the animals and then forwarded them to the anesthesia. Each rat received a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally. The animals received anesthesia and considered ready for the surgical procedure as soon as they lost ocular and caudal reflexes.

The surgical procedures began with sub-xiphoid midline incision of approximately 3 cm length, followed by muscle-aponeurotic resection of 1.0x1.5 cm on the upper left quadrant of the abdominal wall without opening of the peritoneum (Figure 2).



**FIGURE 2** – Muscle-aponeurotic resection in the upper left quadrant.

Cutted meshes were submerged in the standard solution of *Escherichia coli*, stored in a Becker and applied in the muscular plane, with four separate points of polypropylene 4-0 (Figure 3). Then, the skin was closed with continuous suture, using polypropylene thread 4-0.



**FIGURE 3** – Meshes fixed in the muscular plane: A) Marlex® and B) Dynamesh PP-light®.

The animals were kept warm, until complete recovery from anesthesia, with free access to water and food. The sedation in postoperative occurred with the subcutaneous administration of tramadol (15 mg/kg), in daily doses during the first three days. Daily evaluation was done by checking the disposition, dietary compliance, motor activity and possible complications with the surgical wound.

The euthanasia occurred on programmed days and carried out through poisoning by carbon dioxide in a closed chamber.

Immediately after euthanasia, was evaluated the surgical wound for the presence or absence of hematoma, seroma, skin necrosis or wound dehiscence. Next, was performed antisepsis of the abdominal wall with topical PVPI. The instruments used were sterile. Then, was made an incision in “C”, from the upper left quadrant to the left iliac fossa, folding up the skin and subcutaneous tissue of the ventral wall, widely exposing the surgical site. Next, evaluation for the presence of collections, signs of infection and intra-abdominal adhesions was done (Figure 4).



**FIGURE 4** – Incision in “C” exposing the surgical site.

To quantify the macroscopic analysis, was used the graduation 0=absent, 1=present, in the following items: skin necrosis; dehiscence in the surgical wound; seroma; hematoma at the surgical site; infection at the surgical site; and intra-abdominal adhesions.

Resection of a specimen, involving the complete operative area, was realized in all and with a cross section, they were divided into two equal parts. The cranial half was stored in

sterile bottle with saline solution of 0.9% and sent for culture; the caudal, fixed in a cardboard template avoiding retraction into the bottle of formaldehyde (10% formalin) and sent for microscopic analysis. All vials received proper identification.

For microscopic analysis, the slides were processed in the usual way and stained with hematoxylin-eosin. In analysis, the slides were evaluated as following: acute inflammatory process or polymorphonuclear, and chronic or monomorphonuclear, and foreign body reaction or gigantocellular.

The cell count occurred by means of blind evaluation, where the pathologist had no knowledge of which subgroup he was evaluating. Based on the average of cells in the five largest power fields, ranked inflammation and gigantocellular in mild, moderate or marked on each animal (Figure 5).

Intensity of the inflammatory process	Average of cells/field 400x
Mild	Up to 5 neutrophils or lymphocytes
Moderate	6 – 20 neutrophils or lymphocytes
Marked	>20 neutrophils or lymphocytes

Intensity of gigantocellular reaction	Average of cells/field 400x
Mild	Up to 3 giant cells
Moderate	4 – 7 giant cells
Marked	>7 giant cells

FIGURE 5 – Classification of the inflammatory and gigantocellular processes.

For microbiological analysis of the surgical specimen, was held culture in blood and MacConkey agar and considering positive if there was growth of *Escherichia coli* at any concentration after 48 h in culture medium.

Statistical analysis

The results of quantitative variables were described by medians, minimum and maximum values, and the qualitative as frequencies and percentages. For comparing two groups in relation to qualitative variables considered the Fisher exact test and for the quantitative, non-parametric Mann-Whitney. Values of  $p < 0.05$  showed statistically significance. Analyzed the data with the computer program IBM SPSS Statistics v.20.

Results

The surgical procedures passed appropriately. The

surgical average time was 10 min for each animal. There was an accidental opening of the parietal peritoneum in one animal from the Marlex®-7 subgroup. In this animal, it was opted to perform the procedure in the upper right quadrant.

The animals had good postoperative evolution. One of the Marlex®-7 subgroup died in the immediate postoperative period.

Macroscopic analysis

The Figure 6 illustrates the macroscopic appearance of the normal evolution of the healing process in the Marlex®-14 and Dynamesh®-14 subgroups.

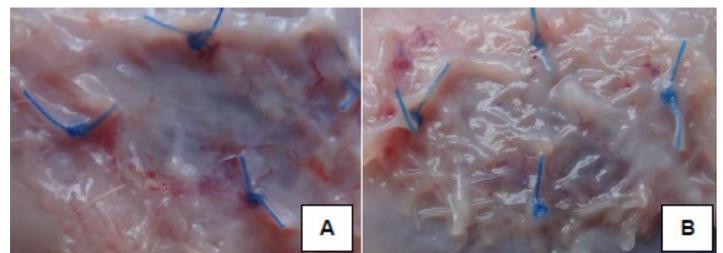


FIGURE 6 – Normal evolution of the healing process: A) Marlex®-14; B) 14-Dynamesh®.

Necrosis

One animal of the Marlex®-7 subgroup evolved with necrosis of the entire surgical wound extension, beyond an underlying abscess. One animal of the Dynamesh®-7 subgroup showed a necrotic spot in the pelvic region, associated with an intra-cavity abscess in this topography. There was no statistical significance in subsection necrosis in both groups (Figure 7).

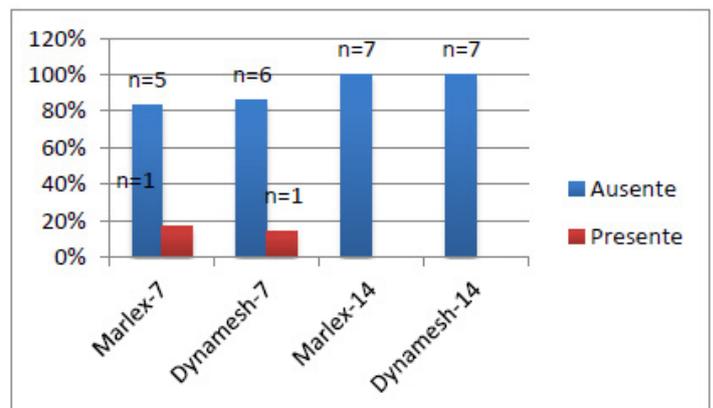
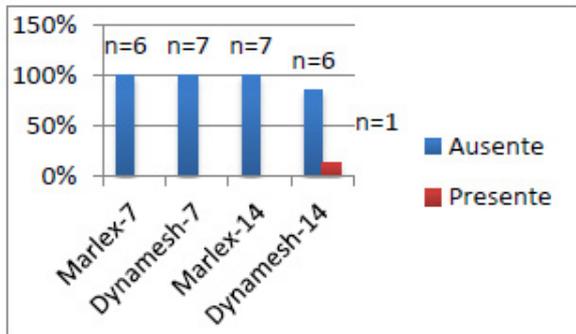


FIGURE 7 – Details of necrosis in the subgroups (p=1).

*Dehiscence of surgical wound*

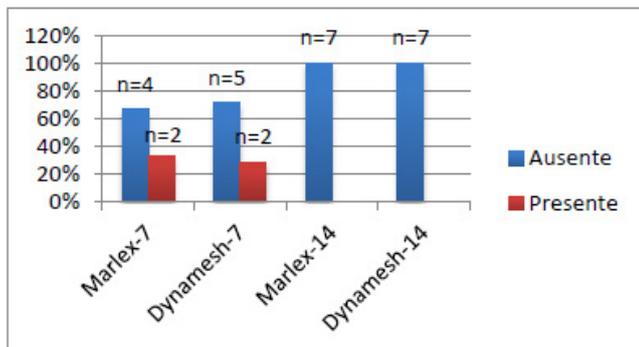
One animal from the Dynamesh®-14 subgroup evolved with partial dehiscence with wound associated to the abscess at the surgical site. There was no statistical significance in the subsection wound dehiscence (Figure 8).



**FIGURE 8** - Dehiscence of surgical wound in the subgroups (p=1).

*Seroma*

Two animals from the Marlex®-7 subgroup presented seroma at the implantation site of the surgical mesh. There was no statistical significance in the subsection seroma in both subgroups (Figure 9).



**FIGURE 9** - Seroma in the subgroups (p=1).

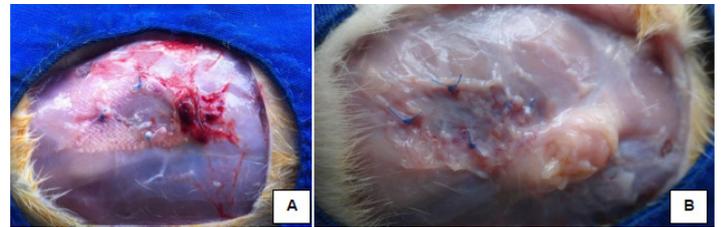
*Hematoma at the surgical site*

No animal showed hematoma.

*Surgical site infection*

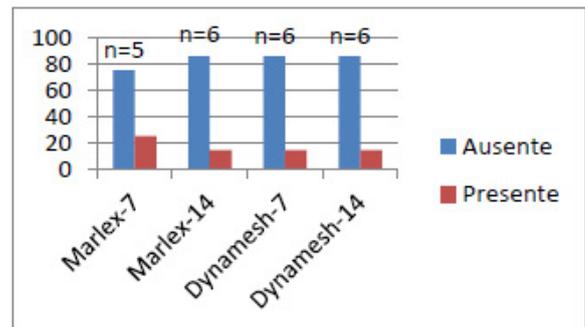
Abscess in the abdominal wall was present in one animal from the Marlex® 7-subgroup and one from the Marlex®-14

subgroup (Figure 10). One animal from the Dynamesh®-7 subgroup showed an intra-cavity abscess in the pelvic area and one from the Dynamesh-14 subgroup presented an abscess in the abdominal wall.



**FIGURE 10** – Presence of abdominal wall abscess on animals from the Marlex® subgroup-14 (A) and Dynamesh-14® (B).

There was no statistical significance in the subsection surgical site infection in both subgroups (Figure 11).



**FIGURE 11** – Infection in the subgroups (p=1).

*Intraperitoneal adhesions*

No animal showed intraperitoneal adhesions.

*Microscopic analysis*

In the Marlex® 7-subgroup, the average polymorphonuclear cells was 50.83 and the median 31.8. The average mononuclear cells was 40.09 and median of 39.1. The average foreign body giant cells was 3.23 with a median of 2.6. It characterized as inflammation of sharp intensity, with mild foreign body reaction (Figure 12A).

In the Dynamesh®-7 subgroup, the average of polymorphonuclear cells was 40.85 with a median of 32.8. The average of mononuclear cells was 53.82 with a median of 53.4. The average of foreign body giant cells was 1.54, with a median of 1.8. Characterized as severe inflammation and mild foreign body

reaction (Figure 12B). The analysis of inflammatory cells showed no statistical difference between the subgroups of seven days.

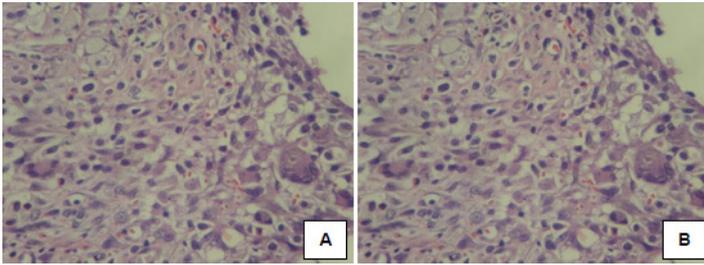


FIGURE 12 – Severe inflammatory process in the subgroups Marlex®-7 (A) and Dynamesh®-7(B).

In the Marlex® subgroup-14, the average polymorphonuclear cells was 24.48 and the median 24.6. The average mononuclear cells was 53.74 and the median 59. The average of foreign body giant cells was 3.17 with a median of 3.0. Characterized as severe inflammatory process with mild foreign body reaction (Figure 13A).

In the Dynamesh-14 subgroup, the average polymorphonuclear cells was 20.88 and the median 21.8 cells. The average of mononuclear cells was 56.02 and the median was 53.4 cells. The average of foreign body giant cells was 4.37 with a median of 3.0 characterized as marked inflammation with moderate foreign body reaction (Figure 13B). The analysis of inflammatory cells showed no statistical difference between the subgroups.

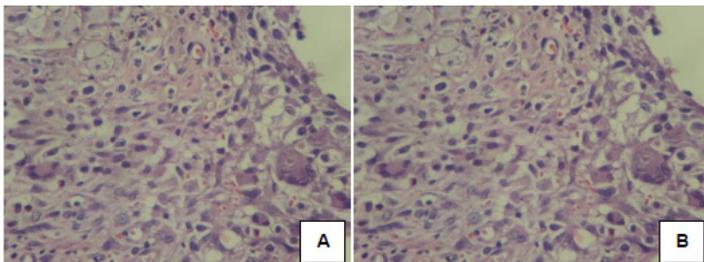


FIGURE 13 – Severe inflammatory process in the subgroup Marlex®-14 (A) and Dynamesh®-14 (B).

Comparing the Marlex®-7 and Marlex®-14 subgroups, it was noticed that the number of polymorphonuclear leukocytes and giant cells decreased while the mononuclear increased, featuring chronicity of the process (Figure 14A).

The same cell behavior appeared when comparing the Dynamesh®-7 subgroups Dynamesh®-14 (Figure 14B).

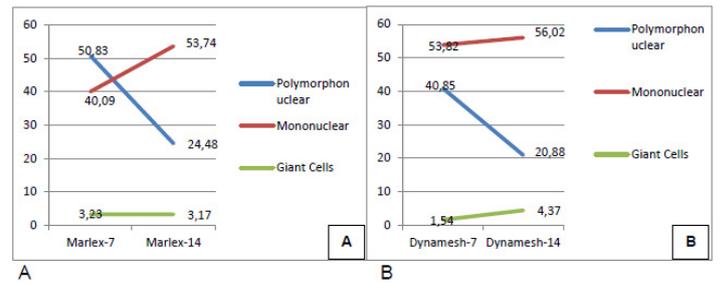


FIGURE 14 – Evolutionary cellularity in the subgroups Marlex®-7 and Marlex®-14 (A) and Dynamesh®-7 with Dynamesh®-14(B).

### Microbiological analysis

Three animals from the Marlex® 7-subgroup and three from the Dynamesh®-7 subgroup presented positive cultures for *Escherichia coli*. There was no statistical difference between the subgroups. Three animals from the Marlex®-14 subgroup and two from the Dynamesh®-14 subgroup presented positive cultures for *Escherichia coli*. There was no statistical difference between the subgroups.

### Discussion

The chosen animal for the research is the rat, due to its widely usage in studies involving meshes and repair of abdominal wall defects<sup>8,17,18</sup>.

There is no consensus in literature on the ideal prosthesis for use in contaminated environment. In most situations, one should choose low-density meshes with large pores and minimal surface area. Ideally, it should consist of a monofilament. If the mesh is placed in the peritoneal cavity, it needs a hybrid with a mesh of absorbable surfaces<sup>6,19,20</sup>.

The development of the polypropylene prosthetic revolutionized surgery for abdominal wall defects correction. The reduction in density of polypropylene, with the creation of lightweight meshes, theoretically reduced the foreign body reaction, causing less mesh contraction and providing better mesh incorporation in the abdominal wall, resulting in improved physiology of the abdominal wall<sup>21-23</sup>. Utiyama *et al.*<sup>24</sup> already showed no difference between polypropylene (high-density) and Ultrapro(r) (low-density) meshes at 21 days after surgery in extraperitoneal use in rats, comparing inflammatory response, mesh shortening, adhesions or complications.

This research focuses on experimental study of contaminated meshes in abdominal wall defect correction. This

scenario simulates emergencies with incarcerated and strangulated hernias, in addition to elective situations where it is required to open the gastrointestinal tract, such as the paracolostomy hernia repair. The mesh used in the study was the polypropylene, chosen by being the most widespread and used both globally and in our field<sup>25</sup>.

The *Escherichia coli* is the most widely used bacteria in experimental studies that worked with contamination. Most studies use standard solutions containing this microorganism<sup>25-27</sup>. In 1989, Deitch *et al.*<sup>28</sup> in a clinical study of patients operated in emergency situations, with or without intestinal occlusion, noted that 59% had bacterial translocation and the more involved bacteria was *Escherichia coli*.

The option to use *Escherichia coli* for research, found that in cases of intestinal obstruction in strangulated hernias, the possibility of bacterial translocation can occur and this is the bacteria most often involved in these cases<sup>28,29</sup> and also is in the incidental openings of the human gastrointestinal tract.

The average time of the surgical procedure was approximately 10 min per animal. The most delicate moment of the surgery was the blunt dissection of the abdominal wall muscles, so that there was no violation of the parietal peritoneum, since the used meshes could not get in contact with the intra-abdominal viscera. Performing this blunt dissection with the aid of a swab was performed without difficulties.

#### *Macroscopic evaluation*

Barbuto *et al.*<sup>30</sup> studied the polypropylene meshes behavior in rats with and without induced peritonitis. It identified 50% of wound dehiscence in rats with peritonitis and 60% of those without peritonitis, with no statistical difference. This study observed incidence of 14.3% of wound dehiscence, which occurred in the Dynamesh<sup>®</sup>-14 subgroup, a result similar to the study of found 12.5% of dehiscence of the surgical wound in Ultrapro-7 subgroup and 12.5% of wound dehiscence in the Proceed-28 subgroup.

The occurrence of seroma in the surgical site was early complication, as was present in 33.4% of the animals of the Marlex<sup>®</sup>-7 subgroup, 28.6% of Dynamesh<sup>®</sup>-7 and no animal of the 14 days subgroups. Our incidence of seroma was similar to that found by Klinge *et al.*<sup>22</sup>, 36% in the heavyweight meshes group and 20% in the lightweight. Greca *et al.*<sup>21</sup> in study comparing meshes of heavyweight and lightweight, in dogs, observed a rate of 20% of seroma in both meshes.

Was not found the formation of hematomas in any animal, as well as Utrabo *et al.*<sup>32</sup>. Pundek *et al.*<sup>31</sup> had 12.5% incidence of hematoma in the Proceed-15 subgroup. Isa *et al.*<sup>3</sup> found 11% of hematoma in the Ultrapro-7 subgroup and 12.5% in the Proceed-7 subgroup.

Exactly one animal from each of the four subgroups of this study developed an abscess at the surgical site. Pundek *et al.*<sup>31</sup> and Utrabo *et al.*<sup>32</sup> did not show any cases of surgical site infection, as their paper did not involve any source of contamination of the surgical site. In disagreement with literature - since the rat is very resistant to infections - Isa *et al.*<sup>3</sup> showed high rates of early infection at the surgical site; the first author, with 55.6% of infection and the second, with 66.7% in the subgroups of seven days.

No animals in this study presented intra-peritoneal adhesions. We only compared this result with Utrabo *et al.*<sup>32</sup> because it was the only one to preserve the peritoneum, not allowing the prosthesis to come into direct contact with the intra-abdominal viscera. The author found 18.75% of adhesions in the 30 days subgroup and 6.25% in 60 days, with no statistical significance.

#### *Microscopic evaluation*

In this study, was found no statistically significant difference in the evaluation of inflammatory response among Marlex<sup>®</sup>-7 subgroups and Dynamesh<sup>®</sup>-7, or between Marlex<sup>®</sup>-14 and Dynamesh<sup>®</sup>-14<sup>21</sup>.

Other studies with polypropylene meshes concluded that the higher the mesh grammage, greater the inflammatory response and surgical complications, determining a better biocompatibility of lightweight meshes<sup>13,22,23</sup>.

In disagreement, Weyhe *et al.*<sup>19</sup> in an experimental study in rats found worst biocompatibility of low-density meshes in comparison with high-density.

#### *Microbiological evaluation*

This study found positive cultures in 50% of the animals of the Marlex<sup>®</sup>-7 subgroup and 42.9% of the Dynamesh<sup>®</sup>-7 subgroup. In Marlex<sup>®</sup>-14 subgroup, the positive cultures was 42.9%, and Dynamesh<sup>®</sup>-14, 28.6%. However, at different times, Sebben *et al.*<sup>25</sup> obtained positive meshes cultures of 83% when the reoperation was in 24h, 33% when in 48h and 17% in 72h. As an overall result, his group showed 44% of positive meshes cultures.

## Conclusion

There was no difference between the meshes of Marlex® and Dynamesh PP-light® in the ventral abdominal wall defect correction on rats with contaminated meshes with standard solution of *Escherichia coli*.

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Received: Jun 20, 2016

Review: Aug 22, 2016

Accepted: Sep 21, 2016

Conflict of interest: none

Financial source: none

<sup>1</sup>Research performed at Medical Research Institute (IPEM), Postgraduate Program in Principles of Surgery, Faculdade Evangélica do Paraná (FEPAR), Curitiba-PR, Brazil. Part of Master degree thesis, Postgraduate Program in Principles of Surgery. Tutor: Nicolau Gregori Czecko.