Characteristics of the fibroplasia and collagen expression in the abdominal wall after implant of the polypropylene mesh and polypropylene/polyglecaprone mesh in rats[1]

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ABSTRACT

PURPOSE: To compare fibroplasia and the resistance of the abdominal wall when polypropylene meshes and polypropylene/poliglecaprone are used.

METHODS: Seventy-seven male Wistar rats were divided into three groups: Control Group (for resistance); Group E (polypropylene mesh); and Group U (polypropylene/poliglecaprone mesh). The animals in Groups E and U had a standard muscular and aponeurotic defect, with integral peritoneum, and correction with the mesh. Measurements were taken 4, 7, 14, 28 and 56 days after surgery. The resistance, and collagen density were studied.

RESULTS: Resistance on the 56th day was similar in both meshes. The gain in resistance described an ascending curve for the polypropylene mesh and was irregular in the case of the polypropylene/poliglecaprone. Fibroplasia showed a gain in type I and type III collagen in both groups ($p<0.001$). Collagen III stabilized in the 14th day and collagen I continued to ascend.

CONCLUSIONS: The gain in resistance of the polypropylene mesh is regular and ascending, whereas the polypropylene/poliglecaprone is not regular. The final resistance of both meshes is similar; the collagen density increases over time, and show the same inflammatory potential.

Key words: Surgical Mesh. Hernia, Ventral. Wound Healing. Collagen. Rats.

Introduction

An incisional hernia, ventral hernia or eventration consists of protruding viscera through an abnormal opening in the abdominal wall following a surgical procedure[1].

According to an American analysis, one in ten patients submitted to a laparotomy develops an incisional hernia[2-4]. It is estimated that approximately 50% of incisional hernias occur in the first two years following a surgery, and 74% in the first three years[5-7].

An incisional hernia forms due to defective healing following a laparotomy, the mechanism of which is of a multi factor nature. The collagen deposition and the environment of the healing of the wound are among the main mechanisms involved[8].

Following a median laparotomy, the rectus muscles maintain their origin and insertion, but the insertion of the lateral muscles is lost. Anatomically, the linea alba consists of a tendon which, when cut, induces muscular alterations of the abdominal wall[8]. When the insertion of the tendon is dislocated from the large muscles that support the abdomen and consequently their retraction, incisional hernias worsen, especially those of the median line. This is a result of atrophy, fatty degeneration and fibrosis of the lateral muscles, factors that hinder the reinsertion of the reinsertion of the tendon in the supporting muscle[8].

Repairs using tension-free techniques are currently recommended. The introduction of the polypropylene mesh by Usher *et al.*[9] was a great step towards definitive treatment of hernias and led to a significant reduction in recurrence rates.

Random studies have shown that 31% to 55% of treatments without the use of meshes result in a hernia relapse[10]. However, treatments with a mesh result in a relapse in 16% to 24% of cases[10-13].

An ideal mesh is one that has good resistance to traction, has no carcinogenic potential, is chemically inert (no potential for infection or delaying healing), is capable of developing an inflammatory response to the material and does not cause rejection. It is also important that it does no cause an allergy or hypersensitivity, has a low cost, enables sterilization, has the capacity to resist mechanical stress and can be incorporated by the host[14,15].

The most frequently used prostheses are inorganic. The most widely used is polypropylene[16]. Its intra peritoneal use is associated with adverse effects such as adhesion, chronic pain, bowel obstruction and fistulas[17-19].

Fibroplasia or wound healing is characterized by a harmonious and coordinated sequence of cellular and molecular events that interact to promote repair and reconstruction of the damaged tissue, the extension of which depends on local inflammatory activity[14,20]. It is a requirement for the reconstruction of a mechanically stable abdominal wall[17].

The polypropylene mesh is surrounded by dense fibrotic material, the consequence of the local reaction to the wound and the presence of the mesh. The strengthening of the abdominal wall with this mesh is the result of the resistance of the mesh itself and the surrounding fibrosis, and its biointegration results from the inflammatory infiltrate and connective tissue synthesis[21].

The combination of non-absorbable and absorbable material, such as the combination of polypropylene and poliglecaprone or even polyglactine could modify the inflammatory pattern and favor the deposition of connective material around the fibers[3,21].

In the first part of this study we studied the inflammatory reaction[22]. The aim of this study was to compare the use of a non-absorbable mesh of polypropylene and a partially absorbable mesh made of polypropylene and poliglecaprone filaments, evaluating the resistance of the abdominal wall and collagen expression both qualitatively and quantitatively.

Methods

The study was evaluated by the Ethics Committee on the Use of Animals in Biological Sciences, Universidade Federal do Paraná (UFPR), allotted process number 23075.038578/2012-11, approved on 30/11/2012 - R.O. 11/2012, as number 659 and in compliance with Federal Law 11.794, which establishes the procedures for the scientific use of animals.

Seventy-seven male Wistar rats (*Rattus norvegicus albinus, Rodentia mammalia)* aged 140 days and weighing 422.54 ± 64.19 g from the Central *Vivarium* of UFPR were used. They were housed in groups of five in polypropylene boxes of appropriate dimensions for the species. The dark/light cycle was 12 hours, the temperature was 20 ± 2^{0}C and the humidity was that of the environment. They had free access to water and standard commercial food. The sample was divided into three groups at random: the control group (GC, n=7), the polypropylene group (GE, n=35) and the polypropylene/poliglecaprone group (GU, n=35). GE and GU were subdivided into five groups of seven in accordance with the time of evaluation (4, 7, 14, 28 and 56 days) following surgery.

For GE a 180 µ (Marlex®, Cirúrgica Brasil) polypropylene mesh was used. This mesh is non-absorbable and is of high density, with average pores of 0.8mm and an estimated weight of 100g/m^2.

For GU, a mixed mesh of polypropylene and poliglecaprone was used (Ultrapro®, Johnson & Johnson International, C/O European Logistic Centre, Belgium). This is a low density, partly absorbable mesh with pores greater than 3.0mm and an estimated weight of 28g/m^2.

Under general anesthetic, assisted by a veterinary surgeon, a median incision of approximately four centimeters was made in the skin and subcutaneous mesh. A muscular-aponeurotic fragment with a diameter of 1.5 cm was removed from the abdominal wall. The parietal peritoneum was preserved intact. The defect was corrected by inserting the mesh, according to the group to which each animal belonged, in a pre-peritoneal situation. The mesh was attached with eight separate stitches using polypropylene monofilament thread 4.0, followed by a skin synthesis, with the application of a continuous suture of nylon monofilament 4.0.

Having recovered from the anesthetic and the administration of an intramuscular analgesic (dipyrone 10mg/kg), the animals were returned to their boxes, where they remained until the day of their euthanasia, under the same environmental and feeding conditions they had experienced prior to the surgery.

Euthanasia was performed using a lethal dose of intra-peritoneal thionembutal (120mg/kg), in accordance with the guidelines of Resolution 1000 of the Brazilian Federal Council of Veterinary medicine. For the necropsy, a new laparotomy was performed. A macroscopic analysis evaluated the presence of secretions and the integrity or otherwise of the corrected region. A segment was removed from the abdominal wall measuring 6.0 cm in width and 4.0 cm in length with the implanted mesh and divided transversally. The cranial half was retained in a physiological solution and enabled an immediate evaluation of the resistance and the caudal half was set in formaldehyde 10% for the histological study.

The resistance of the abdominal wall was analyzed using an EMIC DL-500 MF® extensometer and MTest EMIC® software. The values of resistance for GC, GE and GU were obtained. The resistance of the walls of the control group was obtained without intervention and this served to compare the gain in resistance of the corrected walls with that of normal walls.

The process continued with the histological technique. To identify the collagen, Picrosirius Red staining was used. The images were captured by a Sony CCD101 camera transmitted to a Trinitron Sony® color monitor, frozen and digitalized using an oculus TCX®. The images were analyzed using Image-Plus® 4.5 for Windows® from MediaCybernetics on a microcomputer. In each cut, five fields enlarged 400 times were analyzed. The presence of collagen I and collagen III was analyzed.

For the statistical analysis, the median, standard deviation and minimum and maximum values were used. To evaluate these tests between the groups at different times, the parametric Mann-Whitney test was used, and for the intra-group evaluation the Kruskal-Wallis was used. To analyze frequency, Fisher's exact test was used. A $p \leq 0.05$ or 5% was established as the level for rejection the null hypothesis. The program used for the statistical analysis was Statsoft, Inc (2008). Statistica (data analysis software system), version 8.0.

Results

There was a loss of information on six animals due to postoperative occurrences. Thirty-four animals of the GE group remained and thirty from GU.

No macroscopic abscesses were found and the mesh syntheses were intact at the time of evaluation. There were no intracavity adhesions.

The median resistance of the walls in GC was 2.42 ± 0.41 N, varying between 1.65 and 2.82 N. On the fourth day there was a median resistance of GU corresponded to 66.53% for the wall of GC and that of GE to 69.83% of the resistance of GC (p=0.931). Both groups showed a gain in resistance over time (Figure 1). On the 56th day, the median resistance of the GU walls reached a level of 93.40%, compared with the resistance of GC; the walls of GE were 92.15% of the GC value (p=0.876). The gain in resistance was more regular in GE and more irregular in GU.

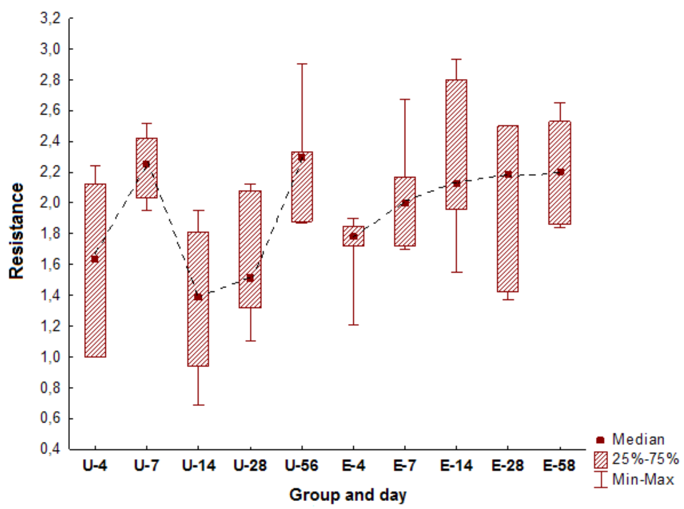

FIGURE 1 - Medians, quartiles, minimums and maximums in GE and GU at the five times under study.

The median total collagen in five fields showed a similar gradual gain of this protein in both groups (Figure 2). In the initial evaluations, more type III collagen was found. This quantity of collagen grew significantly until the 14th day, when the gain curve stabilized. The type I collagen, found in minimal quantities at first, showed a growth in gain throughout the observation period in both groups ($p<0.001$). In the early stages of the process, the septa showed little collagen, which was disordered and predominantly type III. As time passed, the septa became thicker and there was an increase in type I collagen. At first, the disposition of the collagen fibers was disordered, gaining in order and thickness, especially when evaluated on the 56th day.

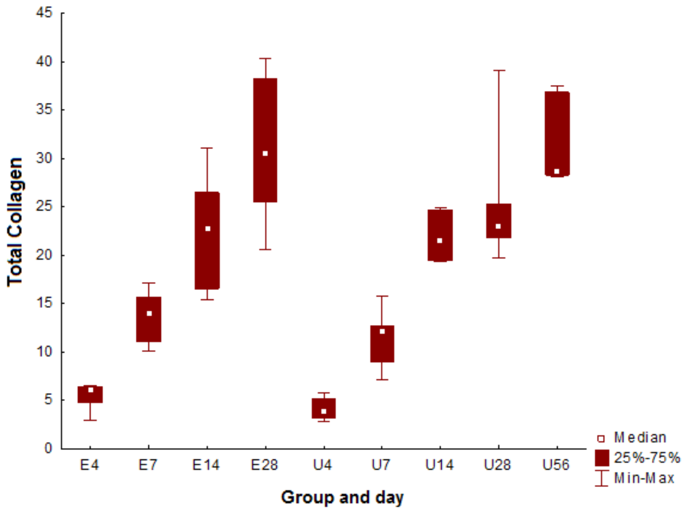

FIGURE 2 - Graph showing the collagen gain in groups GE and GU at the five times under study.
NOTE: Comparative Mann-Whitney Test between the groups
4th day: p=0.093; 7th day: p=0.394; 14th day: p=0.628; 28th day: p=0.699; 56th day: p=0.202

Discussion

The treatment of incisional hernias, especially in large proportions, is a challenge to any surgeon. Many biological and synthetic materials have been used with high, medium and low weights and with macro, medium and micro pores without any consensus being reached.

Although there have been descriptions of possible infection, hematomas, seromas and screen encapsulation, many authors have not reported such complications[15,19,23]. In the sample described in this study, no such situation was observed.

The resistance of a wound may be influenced by a set of factors including interactions between cells, the extracellular matrix and cytokines. The classic tissue reaction to the placing of the prosthesis is characterized by an intense inflammatory response that results in disordered deposition of collagen around the prosthesis and in the interstice of its fibers. The fibroblast reaction can cause mesh encapsulation. During the second week of healing, the fibroblasts in the wound perform the function of myofibroblasts, contributing to the definitive organization of the connective tissue and the contraction of the wound, factors that influence the final resistance of the wound[24].

The complete incorporation of the mesh is an important requirement for obtaining a good attachment[15]. The degree of infiltration of cells such as macrophages, fibroblasts, collagen and new vessels, depends on the size of the pores of the prosthesis, and the healing process requires an adequate pore size between 75 and 100 μm [25]. Strengthening the abdominal wall with the use of a polypropylene mesh is achieved by increasing resistance to traction and is the result of the presence of the mesh and the fibrotic reaction induced by the mesh with the infiltration of inflammatory cells and the deposition of the tissue favoring the biointegration of the mesh[15,26].

Non-absorbable synthetic meshes based on polymers (polypropylene, polyester) provide adequate supply of inflammatory cells and fibrosis, and are useful in maintaining the tensile resistance of the tissue[27]. This enables the prosthesis to attach to the tissues that surround it.

Regarding weight, meshes with a low molecular weight (under $40 g/m^2$) have better biocompatibility and this reduces complaints of postoperative pain and discomfort. These prostheses tend to be macroporous and composed of polymers such as polypropylene interwoven with absorbable material such as polyglactine or poliglecaprone. The combination of absorbable and non-absorbable materials can reduce complaints. Macro pores and the partial reabsorption of the mesh would enable the accumulation of fat instead of fibrotic material between the areas of granuloma formulation, allowing the tissue greater elasticity. However, some studies have shown that due to these factors, there has been an increase in the rate of relapse of hernias corrected with this type of mesh[28].

Utrabo *et al.*[19], in a study similar to the present study, showed that walls corrected with polypropylene and polypropylene/poliglecaprone, evaluated after 30 days, had similar resistance ($p=0.4702$). However, evaluation after sixty days showed greater resistance in the walls corrected with polypropylene/poliglecaprone ($p=0.0046$).

Altmel *et al.*[29] evaluated after 7 and 21 days and observed that the walls gained resistance but the polypropylene/poliglecaprone apparently gained less resistance.

In the present study, evaluations were made after 4, 7, 14, 28 and 56 days. On the fourth day, the resistance was compatible with 66.53% of the normal wall when repaired with polypropylene/poliglecaprone and 69.83% when repaired with polypropylene (p=0.931). After 56 days, the resistance of both groups had attained just over 90% of the resistance of a normal wall, with no significant difference between them.

There is no consensus regarding gain in resistance with the polypropylene/poliglecaprone mesh. In the constitution of this mesh there is an absorbable substance and over time it could lead the walls corrected by it to be less resistant. According to information from the manufacturer and Junge *et al.*[30], this part of the mesh would be reabsorbed in up to 84 days. Perhaps longer term observation could answer these questions. However, it should be remembered that the biological cycle of a rat is faster than that of a human and, thus, 56 days could be adequate time.

An analysis of the resistance curve showed that the walls corrected with polypropylene saw a progressive gain, stabilizing on the 14th day, while the curve for polypropylene/poliglecaprone showed a gain until the seventh day and on the fourteenth day an important loss was observed (p=0.008), with the gains returning on the 28th day and becoming similar to the polypropylene group on the 56th day. In other words, the polypropylene mesh had a regular gain curve whereas the polypropylene/poliglecaprone did not. This irregularity may be due to the inflammatory reaction and even the absorbable part of the mesh, although the study by Junge *et al.*[30] describes the first histological signs of the reabsorption of the poliglecaprone filaments after 56 days. However, these authors did not measure resistance. It should be considered that the inflammatory process would influence resistance.

Median total collagen density in the experiment showed a similar gradual gain of this protein in both groups over time, with the values always a little higher, but not significantly so, in GE. Utrabo *et al.*[19] claimed, in qualitative terms, that polypropylene meshes led to the formation of more fibrous tissue. The study by Junge *et al.*[30] showed that there was no difference in collagen levels when a polypropylene or polypropylene/poliglecaprone mesh was used.

Conclusions

The resistance offered by the polypropylene mesh has a regular and ascending gain as the process evolves, while that of the polypropylene/poliglecaprone is not regular. The final resistance of both meshes is similar. The collagen density increases over time and is similar for both meshes.

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