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Bubble plastic use in rats hernioplasty: Uso do plástico bolha em hernioplastias de ratos

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ABSTRACT

Purpose: Despite the high frequency of hernioplasties worldwide, their complications and recurrences are still a challenge to be overcome. The search for prostheses that aim to promote the correction of hernia defects has been a challenge. For this purpose, the materials used in hernioplasties must be biocompatible, promote the formation of little or no peritoneal adhesion, possess compatible texture and flexibility, providing the necessary resistance to protect the viscera and allow the movement of the abdomen.

Methods: The aim of the present study was to evaluate the effectiveness of bubble plastic (low density polyethylene, LDPE) as a material for the correction of hernia in the abdominal wall. For this, twenty male rats (Rattus norvegicus, Wistar variety) were used and divided into four groups of five animals. The animals were evaluated at 7, 15, 30 and 90 days after surgery according to clinical, thermographic and morphological parameters (macroscopic and microscopic).

Results: The results showed that the bubble plastic induced inflammatory reaction in the initial period (7 day), followed by a reduction (30 day) to increase considerably at 90 days after the operation.

Conclusion: So, bubble plastic can be used for temporary implants (up to 30 days).

1. Introduction

The studies on the use of synthetic and biological meshes as materials to replace tissue structures when damage has increased are important for improving surgical treatment and techniques [1].

The biomaterials must be accessible, cheap, easy to sterilize, non-carcinogenic, non-allergenic, and, above all, be tolerated on the implantation site [2].

Despite, the high frequency of hernioplasties per year, their complications and recurrences still make them a challenge for modern

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surgery [3].

The public Unified Health System (SUS), from Brazil, estimates that 280.000 surgeries for correcting hernias are performed for year [4].

Studies carried out in 2018 and 2019, in the USA and Denmark respectively, analyzing data from 103,000 patients, found the formation of incisional hernias in an average of 15% of patients after open surgery [5,6].

Currently, several types of meshes are available for hernioplasties. All objectivate be incorporated into the tissue, it related meshes from biological sourced (human, bovine, porcine, the skin of fish and amphibians) [7–10], unabsorbable synthetic materials, like the base polypropylene meshes [11].

One of the most common complications of using meshes is the adhesion of the viscera to the implant surface [12]. Studies using polypropylene meshes in abdominal hernioplastys show complications in the postoperative period, like edema, seroma, infections, hematomas, chronic pain and adhesions in different structures, and even recurrence [13–15].

Extensive studies related to use of polypropylene and derivates of polypropylene as meshes for hernia repairs, but even is still related to their complications. Considering the base in Principe of 3 Rs [16], thinking about reducing the numbers of animals, avoid unnecessary pain and suffering, the comparison of the results was based with what is published in literature [13–15][.]

Thus, the search for materials that prevent the formation of adhesions has been a constant in surgical practice. The bullous laminar Low Density Polyethylene (LDPE) (Bubble Plastic) routinely used to wrap household appliances, can be a new alternative for herniorrhaphies or corrections of birth defects, being applied with the bubbles facing inwards, it reduces by approximately 45% the surface of contact of the viscera with the implant and consequently reduces the possibility of formation of abdominal adhesions. Considering that there is little information on the use of low-density polyethylene (LDPE) (bubble plastic) in hernioplasties, the present study intends to evaluate the effectiveness and local inflammatory reaction of the implant using LDPE to correct defects in the abdominal wall of *Rattus norvegicus*, Wistar variety.

2. Methods

Twenty male young adult rats (*Rattus norvegicus*, Wistar variety), with an average weight of 300 g, were used in this study. The animals were kept in captivity in the bioterium of the Centro Universitário Serra dos Órgãos (UNIFESO), Teresópolis, RJ and were maintained with *ad libtum* feed and water. This study was approved by the Ethics Committee of UNIFESO (registration nº 435/2015).

Commercially available Bubble plastic (low-density polyethylene, LDPE) was purchased and disinfected by immersion in a 2% chlorhexidine aqueous solution for 30 min and washed in a 0.9% sterile saline solution before use.

For anesthetic induction, 90 mg/kg ketamine and 2 mg/kg xylazine was used intraperitoneally before the start of surgery; for analgesia, 30 mg/kg of tramadol was used subcutaneously [17]. When necessary, anesthetic maintenance was performed with



Fig. 1. Photography of the sequence of making the gap in the abdominal wall. A, first incision: longitudinal in the linea alba and approximately 3 cm; B, second incision: transverse in the linea alba with approximately 1 cm. C, third incision: longitudinal and parallel to the linea alba. D, fourth incision: flap removal.

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isoflurane in inhalation anesthesia and an open circuit mask ¹⁷.

For implant placement (LDPE) to replace part of the abdominal wall, an incision was made in the midline of the skin of the xiphopubic region with dissection of the subcutaneous tissue (Fig. 1 A). From the midline and towards the left side of the abdominal wall, a 1×3 cm gap was made in the entire thickness of the abdominal wall, including muscle aponeurosis, muscles and peritoneum (Fig. 1 B - D).

Based on the dimensions of the defect, LDPE prosthesis was placed with its bullous surface facing the peritoneal cavity in order to reduce the contact surface of the prosthesis with the abdominal viscera. The implantation of LDPE was performed according to modified method described by Ricciardi et al. [17] the fragments were sutured to musculature of the remaining abdominal wall on its four sides with simple continuous suture using a nylon thread 4-0 (Fig. 2). The subcutaneous tissue and skin were closed in a single plane with simple stitches and using nylon thread 4-0.

After surgery, the animals were separately placed in a warm environment for recovery. After fully awakening from anesthesia, the animals were transferred to their cages, and commercial feed and water were offered *ad libtum*.

Afterwards, the animals were placed in cages with 5 animals each. The animals were euthanized at 7, 15, 30 and 90 days after implant placement surgery.

2.1. Clinical evaluation

Postoperative evaluations were performed at 7, 15, 30 and 90 days after implant surgery, comparing the mean weight of the animals and the clinical evaluation of surgical wound.

For this, each animal was weighed before the anesthetic-surgical procedure for implant placement and on the day of euthanasia. For the statistical analysis of the variation in weight between animals, Student's "t" test was used. In addition, the surgical lesion was evaluated during the postoperative period for the presence of edema, seroma, hematoma, serous secretion, abscess, fistula, suture dehiscence and necrosis.

Skin suture dehiscence was evaluated based on the classification proposed by Aramayo and coworkers [17] where Grade 0: absent, Grade 1: partial suture dehiscence without prosthesis exposure, Grade 2: total suture dehiscence without prosthesis exposure, Grade 3: partial or total suture dehiscence with prosthesis exposure, and Grade 4: suture dehiscence with evisceration.

To verify the measures of inflammatory process scores, suture decency and presence and degree of adhesions, the Kruskal-Wallis (KW) Nonparametric Tests were applied for comparison between groups. If there was a significant difference between the groups with the KW test, the Mann-Whitney test was applied.

2.2. Infrared thermography evaluation

Based on the fact that every living organism produces heat and emits infrared radiation directly proportional to its temperature, thermography consists of the immediate and non-aggressive assessment of the mapping of this radiation load, expressing the gradual variation of the gradient in the color pattern in a thermogram. When detecting infrared radiation, numerous changes related to changes in blood flow can be identified, making it possible to recognize vascular, neurological and muscle functional changes. Consequently, thermography aids in the assessment of repair after a surgical procedure, as well as in the diagnosis of neovascularization in the surgical bed after the use of implants [18].

In this study, thermography was used to observe the inflammatory process at the site of the prosthesis, and to measure the size of the prosthesis, and tissue contraction.

The measurement of skin temperature variation was performed with the aid of a thermograph brand Flir®, model T420, Danderyd, Sweden, with a resolution of 320×240 , with thermal sensitivity of 0.045 °C and emissivity of 0.99.

The entire procedure was performed by the same observer and in an acclimatized room with a temperature between 21 and 24 °C, where the animals were acclimated for 1 h before the thermography. The thermograph was positioned at a vertical distance of 1 m from



Fig. 2. Photograph showing placement of the prosthesis.

the animals, kept under physical restraint and without anesthesia; they were kept in the supine position with the abdomen shaved. To avoid interference from the table temperature, the animals were kept on a surface with thermal insulation; thermographic imaging was done in the skin region.

2.3. Visceral adhesion formation

After euthanasia, a "U"-shaped incision was made along the abdominal wall and the rate of implant adhesion to the viscera was evaluated according to Kist and coworkers [19] and classified into different degrees [20]: Grade 0 - absence of adhesions; Grade 1 - reduced number of adhesions (\leq 3), fibrinous and easily undone by manipulation without injuring the viscera; Grade 2 - firm adhesions (>3), resistant to manipulation between the abdominal wall and the organ; Grade 3 - firm adhesions, resistant to manipulation between intestinal loops but not involving the abdominal wall; Grade 4 - firm adhesions, resistant to manipulation between intestinal loops and between the loops and the abdominal wall with enteric fistula.

2.4. Light microscopy analysis

The abdominal wall from each animal, including the implant covered by the skin, was removed and fixed in 10% neutral buffered formalin solution for 72 h. After rapid washing in water, 5 mm fragments were processed according to the standard histological technique for paraffin embedding. Five microtome sections were stained with hematoxylin-eosin ²¹, Mallory's Trichrome [21] and picrosirius red with observation under brightfield and polarized light microscopy [22].

For immunohistochemistry, paraffin sections were de-waxed, hydrated and washed in 0.1 M phosphate buffered (PB). The endogenous peroxidase was blocked using a 3% hydrogen peroxide solution for 30 min. After that, sections were immersed in 1% bovine serum albumin (B4287; Sigma) in PB in a humid chamber for 30 min at room temperature. The sections were incubated overnight at 4 °C with anti-human muscle actin (Dako, cat. no. M0635; 1:50). After rinsing in PB, the sections were incubated with biotinylated secondary antibodies diluted to 1:200 for 30 min, then with ABC complex (diluted to 1:200) for 30 min (both from PK 6200, Vector Lab. Inc.). The sections were washed in PB and revealed with a 3'3-diaminobenzidine tetrahydrochloride (DAB) solution containing 0.1% hydrogen peroxide. After rinsing in distilled water, the sections were counterstained with hematoxylin, and permanent preparations are made as usual for the standard technique. As control procedure, the incubation with the primary antibody was omitted.



Fig. 3. Infrared image (A, C) and temperature gradient (B, D) of the abdomen.: Hernioplasty at 7 days (A, B) and at 90 days (C, D). Note the image corresponding to the prosthesis (in yellow tones; black arrow) and to an inflammatory process (in green; white arrow). (Personal File, 2016).

3. Results

Bubble plastic of LDPE revealed to be a material that is easy to handle, flexible, inelastic, fragile to rupture, breaking easily when passing the needle and suture thread. The simple and continuous suture pattern with 4.0 nylon for fixing the prosthesis to the abdominal wall proved to be satisfactory since the prostheses were well fixed, did not move and no animal had peritonitis. In addition, no prosthesis was rejected; the use of antibiotic therapy or administration of anti-inflammatory drugs was also not necessary.

Regarding the weight, the animals at 7 days post-surgery showed weight loss, followed by weight gain at 15 and 30 days and significant gain at 90 days. No animal exhibited seroma, hematoma, abscess, fistula, necrosis, eventration or evisceration. Never-theless, two animals showed moderate edema at 7 days and skin suture dehiscence was observed in one animal at 30 days and two animals at 90 days. In addition, suture dehiscence without prosthesis exposure was observed in one animal at 30 days. Partial or total descent with prosthesis exposure was observed in one animal at 90 days. No animal presented evisceration.

Through infrared thermography, the temperature of the rats ranged from 37.5 to 38 °C in all regions of the abdomen that did not undergo surgical intervention, but only the trichotomy (Fig. 3C). In the region of surgery, the temperature was lower when compared to another region of the abdomen of the rat, ranging from 33.5 to 34.5 °C. In the region of the protheses, the temperature was around 35 °C (Fig. 3 A, C). In addition, the prosthesis size remained unchanged. At 7 days, the prosthesis represented 11.8% (Fig. 3 A, B), at 15 days 10,5%, at 30 days 12.3% and at 90 days 12.6% on average of the analyzed area (Fig. 3 C, D).

Abdominal adhesions of the prosthesis with the omentum, right and left testicles were observed in all animals and in all periods (Fig. 4 A, B); however, the adhesions were easily detached.

At 7 days, no complete interaction between the mouse skin and the implant was visualized through the histological analysis. However, mononuclear cells such as mast cells, lymphocytes, plasma cells and giant multinucleated cells were visualized in the connective tissue around the implant (Fig. 5 A). The occurrence of these cells indicates a marked area of inflammatory reaction, where neutrophils represented about 20% (Fig. 5 B–C). Eosinophils were observed in two animals. In addition, areas of edema and congestion were identified in the dermis and/or in the region of contact of the implant with the abdominal cavity in all animals (Fig. 5 A), in addition to an increase in blood vessels, suggestive of angiogenesis (Fig. 5 D). However, hemorrhagic focus in the implant region was detected in only two animals.

At 15 days, a wide space (lacunae) between the skin and the implant is observed, in addition to an intense inflammatory reaction in the connective tissue around the implant (Fig. 6 B). Lacunae is likely due to removal of the plastic implant during histological procedure for paraffin embedding. Although the inflammatory reaction was mild in two animals, moderate in two others and accentuated in only one animal, neutrophils were not visualized. However, eosinophils frequently occurred in areas of tissue congestion facing the abdominal cavity in three animals, and one animal exhibited tissue fibrosis adjacent to the implant (Fig. 6 B). Blood vessels occurred more frequently around the implant, indicating angiogenesis. In addition, tissue hemorrhage area was not identified.

At 30 days, lacuna is still visualized and the connective tissue of the dermis with inflammatory tissue forms projections that insinuate between the indentations of the bubble plastic (Fig. 7 A). In this group, four animals showed a slight inflammatory reaction while in one animal the reaction was moderate. In addition, macrophages, multinucleated giant cells, mast cells, lymphocytes, plasma cells were observed; however, neutrophils were not visualized and eosinophils were less frequent (Fig. 7 A). Slight tissue fibrosis was identified in some animals while all animals showed edema in the region of the dermis facing the abdominal cavity.

Blood vessels were more frequent in the connective tissue of the dermis around the lacunae, indicating angiogenic activity (Fig. 7 B). Furthermore, only one animal revealed a hemorrhagic area in the connective tissue close to the abdominal cavity.

At 90 days, slight inflammatory reaction in the connective tissue of the dermis was detected in one animal while the reaction was more intense in 4 animals. However, the inflammatory reaction was less prominent in comparison to previous groups. In connective





Fig. 4. A and B: Abdominal adhesions with internal structures (omentum and testicular ligaments).



Fig. 5. Photomicrographs of the abdominal wall of animals at 7 days. A: Note the negative space of the prosthesis (\star). Around the implant, it is possible to observe an inflammatory area (\star) with a predominance of mononuclear cells; HE. B: Notice giant cells (arrowhead); HE. C: Note inflammatory infiltrate (\star), congestion (arrow) and adhesion (\star); HE. D: Note vessels (arrows) suggestive of neovascularization; immunohistochemistry for alpha-actin. (Personal File, 2016).



Fig. 6. Photomicrographs of the abdominal wall of animals at 15 days. A: Note fibrous material (stained in red) around lacunae (★), picrosirius red stain. B: Note inflammatory reaction (*), congestion area (arrow head) and fibrosis focus (arrow), HE. (Personal File, 2016).

tissue mast cells, lymphocytes, and plasma cells occurred; neutrophils and eosinophils were not visualized. Macrophages were visualized in all animals, although multinucleated giant cells were only observed in 3 animals. An increase in fibrous elements around the implant was identified in all animals (Fig. 8A–B). In addition, hemorrhagic areas were visualized in three animals, among the fibrous elements, while edema occurred in only one animal.

4. Discussion

In this study, the LDPE implant proved to be effective, protecting the abdominal viscera, since no animal presented recurrence of



Fig. 7. Photomicrographs of the abdominal wall of animals at 30 days. A: Note projection (arrows) of the connective tissue with inflammatory mononuclear cells (*) to lacunae of bubble plastic, HE. B: New blood vessels (angiogenesis) (arrow head) interspersed with edema in connective tissue facing de abdominal cavity with adhesions (*), HE. (Personal File, 2016).



Fig. 8. Photomicrographs of the abdominal wall of animals at 90 days. A: Note fibrous area (arrow head) around lacunae, HE staining. B: Fibrous tissue facing abdominal cavity. Mallory's trichrome method. (Personal File, 2016).

herniation or evisceration in any post-surgical period. In all animals, abdominal movement was normal, as none of them moved with difficulty or showed significant weight loss. At 7 days, weight loss was possibly due to recent surgical trauma and not due to the reaction to the prosthesis.

The dehiscence detected in 10% of the animals at 30 and 90 days after surgery was only cutaneous and not associated with the presence of purulent secretion, abscess, evisceration or peritonitis. These findings suggest that the cause of the dehiscence may not have been inflammation, but the result of trauma to the suture region. Simple continuous suture with 4.0 nylon thread was satisfactory according noticed by Martinez and coworkers [2]. However, Gianlupi and Trindade [3] reported 12.5% of hernia recurrences when the sutures were made with interrupted "Wolf" stitches.

Through infrared thermography it was verified that the body temperature remained constant (37.5–38 °C), with a slight reduction in the area of the trichotomy. In the region of the surgical scar, the temperature was lower (33.5–34.5 °C) although in the prostheses region the temperature was a little higher (35 °C). The temperatures of the prostheses also remained constant.

Thermography has been used in several studies, such as exploring changes in temperature during the walk [23].

Thermographic images are also used to analyze changes in the thermal pattern in graft areas in order to help identify angiogenesis sites being effective in evaluating the healing process [18]. In addition, the effect of temperature on healing is directly related to the formation of new vessels during the healing process [24]. The higher temperature in the region of prosthesis can be related to the high number of circulating cells, because in a way the organism is recognizing a new tissue, as well as responding to it. So increased local angiogenesis will lead to increased temperature at the implant site.

Histological analysis also allowed us to verify that the intensity of the inflammatory reaction varied along the experimental period. Initially (7 and 15 days) there is a marked reduction in the inflammatory process, which increases after 30 days to show a slight reduction at 90 days. These findings are in agreement with the studies by Smart and coworkers [25]. The authors comment that, given the foreign body type in slow absorption or non-absorbable prostheses, the inflammatory process may be longer (chronic).

In this study, the occurrence of neutrophils at 7 days suggests that the inflammatory process is still in the initial phase. After this

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period, the inflammation is no longer acute and becomes chronic due to the occurrence of lymphocytes, plasma cells and mast cells as indicated by Aramayo and coworkers [17]. For Aramayo and coworkers [11] the initial increase in eosinophils followed by a reduction, so that they are no longer observed at the end, are suggestive of the establishment of an acute inflammatory process and/or hypersensitivity reaction. In addition, the occurrence of macrophages and multinucleated giant cells may suggest that a chronic inflammatory process has been established, indicating that the inflammation has not yet fully solved and that the healing process is still ongoing [5,17].

Although bubble plastic (LDPE) is in the veterinary clinical-surgical routine, the results revealed that, despite allowing the support of the abdominal viscera and normal abdominal movement, the prosthesis of bubble plastic did not integrate with the muscle tissue, causing an intense inflammatory reaction up to 90 days. Purchio [26] points out that a biomaterial can remain implanted for at least 1 year. Thus, despite the fact that bubble plastic is promising, new studies with longer periods of implantation (365 days) must be carried out, like Purchio [26] points.

5. Conclusions

Bullous laminar LDPE induced an intense inflammatory reaction at 90 days post-surgery. The treatment allowed for normal abdominal movement in the animals. The biomaterial may be considered appropriate for implants for temporary studies involving a few days, with a maximum limit for its use for longer periods. The tested material shows promise according to the analysis methods used. Although this study complements the use of an unusual material as a biomaterial for hernioplasty, further studies with longer periods of implantation and new tests should be performed.

Author contributions

Siria Jorge has conceived and designed the experiment, performed the anesthetic, surgical, clinical, thermographic and macroscopic analyzes of the rats, and analyzed and interpreted the data, and wrote the paper.

Marcelo Abidu has conceived and designed the experiments, performed the thermographic and statistical analysis, and analyzed and interpreted the data.

Lycia Gitirana has conceived and designed the experiments, and performed the histological analyses, and contributed reagents, materials and analysis data.

Carolina Seabra da Costa has assisted the surgical and anesthetic procediments, analyzed and interpreted the data, performed the theoretical structuring and formatting of the article, and wrote the paper.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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