

## Biomechanical evaluation of microbial cellulose (*Zoogloea sp.*) and expanded polytetrafluoroethylene membranes as implants in repair of produced abdominal wall defects in rats<sup>1</sup>

Avaliação biomecânica de membranas de celulose microbiana (*Zoogloea sp.*) e de politetrafluoretileno expandido como implantes no reparo de defeitos produzidos na parede abdominal em ratos

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### ABSTRACT

**Purpose:** To evaluate the Load of Rupture of implants of membranes of microbial cellulose (*Zoogloea sp.*) and extended polytetrafluoroethylene in sharp defects of abdominal wall of rats. Methods: Sixty Wistar male rats, with a mean weight of  $437,7g \pm 40,9$ , anesthetized by a mixture of ketamine (5mg/100g) and xylazine (2mg/100g), were submitted to a rectangular (2x3cm) excision of the abdominal wall, including fascia, muscle and peritoneum, and treated with membranes of microbial cellulose (MC) (MC Group- 30 animals) or extended polytetrafluoroethylene (ePTFE) (ePTFE Group- 30 animals). Each group was subdivided in 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups. Under anesthesia, animals were submitted to euthanasia at 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD for evaluation of Load of Rupture. **Results:** Load of Rupture levels were significantly elevated ( $p < 0,05$ ) among 14<sup>th</sup>, 28<sup>th</sup> and 60<sup>th</sup> postoperative days from each Group. When compared between groups, values of Load of Rupture were significantly larger ( $p < 0,05$ ) in ePTFE Group than in MC Group. **Conclusion:** Resistance to strength at implant/host interface was more pronounced in PTFEe Group than in MC Group.

**Key words:** Membranes, Artificial. Zoogloea. Polytetrafluoroethylene. Implants, Experimental. Abdominal wall. Tensile Strength. Rats.

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### RESUMO

**Objetivo:** Avaliar a Carga de Ruptura de implantes de membranas de celulose microbiana (*Zoogloea sp.*) e de politetrafluoretileno expandido em defeitos agudos produzidos na parede abdominal de ratos. **Métodos:** Sessenta ratos machos Wistar, com média de peso de  $437,7g \pm 40,9$ , anestesiados com uma mistura de cetamina (5mg/100g) e xilazina (2mg/100g), foram submetidos à excisão retangular (2x3cm) na parede ventral do abdômen, incluindo fâscia, músculo e peritônio. Subseqüentemente, foram tratados com implante de membranas de celulose microbiana (CM) (Grupo CM - 30 animais) ou de politetrafluoretileno expandido (PTFEe) (Grupo PTFEe - 30 animais). Cada grupo foi ainda subdividido nos Subgrupos 14<sup>o</sup> DPO, 28<sup>o</sup> DPO e 60<sup>o</sup> DPO. Os animais foram submetidos à eutanásia com doses letais de tiopental no 14<sup>o</sup> DPO, 28<sup>o</sup> DPO e 60<sup>o</sup> DPO, para avaliação da Carga de Ruptura na área do implante. **Resultados:** Os níveis da Carga de Ruptura foram significativamente elevados ( $p < 0,05$ ) entre os Subgrupos 14<sup>o</sup> DPO, 28<sup>o</sup> DPO e 60<sup>o</sup> DPO de cada grupo estudado. Quando comparados entre Grupos, os valores da Carga de Ruptura foram significativamente maiores ( $p < 0,05$ ) no Grupo PTFEe do que no Grupo CM. **Conclusão:** A interface implante/hospedeiro apresentou maior resistência a tração no Grupo PTFEe do que no Grupo CM.

**Descritores:** Membranas artificiais. Zoogloea. Politetrafluoretileno. Implantes experimentais. Parede abdominal. Resistência à tração. Ratos.

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1. Research performed at Laboratory of Experimental Surgery-Department of Surgery- Federal University of Pernambuco, Brazil.

## Introduction

The best method for the treatment of muscle-aponeurotic defects of the abdominal wall is the approach, without tension, of fibromuscular structures of the own patient's tissue.

However, in some circumstances the repair can be affected by the great distance among the edges of the defect or by the lack of tissue with characteristics for an appropriate approach. In these cases, synthetic and biological prostheses, or even muscular grafts, vascularized or free, can be used for reconstruction<sup>1</sup>. The synthetic prostheses are of high cost and vascularized or free muscle grafts frequently constitute complex and prolonged surgeries that imply in creation of weak sites in surrounding areas of defect, that could need repair<sup>1,2</sup>.

Certain bacteria, algae and fungi produce cellulose<sup>3</sup>. In the last years, because of its good mechanical properties and biocompatibility, there was an increasing interest in bacterial cellulose, produced by the *Acetobacter xylinum* and its application in medicine. Important examples include products for temporary skin substitution and tissue replacement. These activities have been accompanied by the isolation of new bacterial strain producing biocellulose<sup>3</sup>.

In Northeast of Brazil, cellulose membrane was produced in the Estação de Cana-de-Açúcar de Carpina-UFRPE, by the microorganism *Zoogloea sp.*<sup>4</sup>.

Finally, it is not of our knowledge reports of previous use of cellulose membranes produced by the microorganism *Zoogloea sp.* or by other bacterial species, as repair of muscle-aponeurotic defects of the abdominal wall, in experimental or clinical scope.

Our purpose was to study comparatively the Load of Rupture of implants using membranes of bacterial cellulose and polytetrafluoroethylene in sharp defects of abdominal wall produced in the rat.

## Methods

The microbial cellulose membrane is constituted by an exopolysaccharide produced by the bacteria *Zoogloea sp.*, isolated by the Instituto de Antibióticos da Universidade Federal de Pernambuco, obtained in static culture, having molasses of the sugar-cane as culture medium. The non soluble content in water reaches 88% of the components of the gross membrane of exopolysaccharide<sup>4,5</sup>.

A double layer of this microbial cellulose (MC) was used. It was compressed and then evaporated on the air during the dehydration process, conserved in isopropyl alcohol moisturized at 20%, conditioned in polypropylene envelopes and sterilized in  $\gamma$  rays<sup>a</sup>. This process propitiates membranes with pores of mean diameter of 0,07 $\mu$ m (70nm)<sup>5</sup>.

The film of expanded polytetrafluorethyene (ePTFE)<sup>b</sup> was obtained from vascular prostheses of internal diameter of 8mm and wall thickness of 0,8mm, with pores of

25 $\mu$ m, sectioned in longitudinal direction, after removal of the external helical structure. Rectangles of 2x3cm were prepared, conditioned in polypropylene envelopes and submitted to the sterilization in  $\gamma$  rays.

Sixty male Wistar rats, with mean weight of 437,7g $\pm$ 40,9, were conditioned in appropriate cages, fed with specific food<sup>c</sup> and mineral water *ad libitum*, before they were submitted to the procedures considered in the project.

The animals were distributed in two groups: A) Microbial Cellulose Group (MC Group): composed of 30 animals submitted to a sharp defect of the ventral wall of the abdomen, including fáschia, muscle and peritoneum, being treated with membrane of microbial cellulose; B) Expanded Polytetrafluoroethylene Group (ePTFE Group): composed of 30 animals submitted to the same procedure of MC Group, being treated with membrane of expanded polytetrafluoroethylene;

Each group was subdivided in three subgroups of 10 rats, in agreement with the evaluation period, being denominated of 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups (Figure 1).

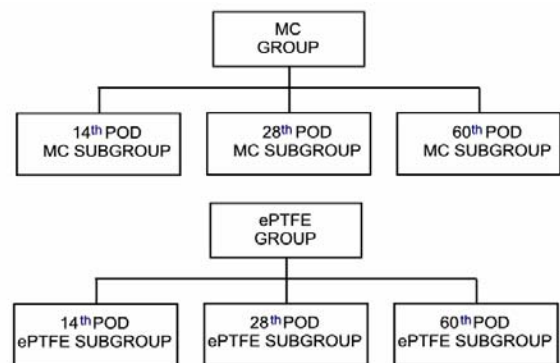


FIGURE 1 - Distribution of groups and subgroups

Anesthesia was accomplished with a mixture of cloridrate of ketamine (5mg/100g of weight) and cloridrate of xylazine (2mg/100g of weight), preceded by sulphate of atropine (0,044mg/kg)<sup>d</sup>, all administered by intramuscular route.

Under aseptic conditions, a middle incision of five cm was accomplished in the skin and subcutaneous layers of the abdominal ventral area of the animals. These plans were dissected from the aponeurotic layer of the corresponding area, followed by placement of superficial retractors, to allow the creation of an area for the production of muscle-apneurotic sharp defect.

Through a mold made of RX film, measuring 2cm of width for 3cm of length, the defect was produced, centered in the middle line of abdomen, 1 cm below the xifoid appendix. A full thickness wall involving fascia, musculature and peritoneum were excised. In the MC Group, membrane of microbial cellulose was sutured at the level of the muscle-aponeurotic defect with polypropilene<sup>e</sup> 4-0 and continuous suture, anchored at the four angles of the rectangle. In the ePTFE Group the same surgical procedures adopted in the precedent group were accomplished (Figure 2). Then the skin was closed with thread of nylon<sup>f</sup> 4-0 through an interrupted suture.

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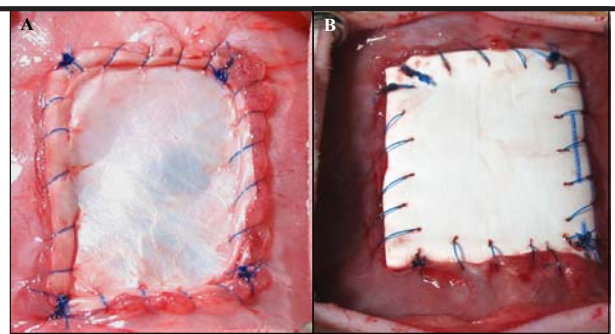
<sup>b</sup> GoreTex®, W. L. Gore & Associates, Inc., Newark, DE.

<sup>c</sup> Labina, Laboratório Nestle/Purina/PetCare Company, São Lourenço da Mata PE.

<sup>d</sup> Protocol for anesthesia in rodents adopted at the Núcleo de Cirurgia Experimental

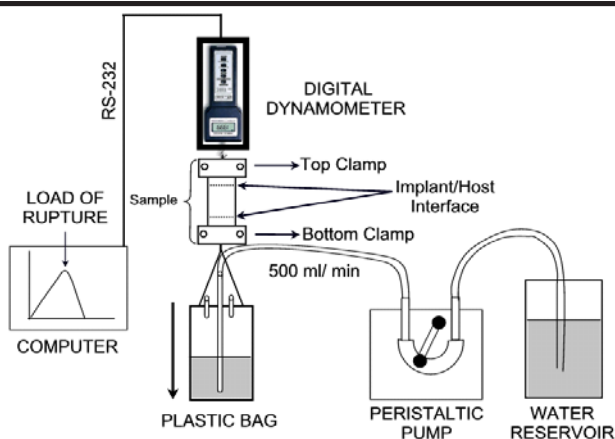
<sup>e</sup> Prolene®, Ethicon, Johnson & Johnson Comércio e Distribuição Ltda.

<sup>f</sup> Mononylon®, Ethicon, Johnson & Johnson Comércio e Distribuição Ltda.



**FIGURE 2** - Suture placed on microbial cellulose (A) and polytetrafluoroethylene (B) membranes

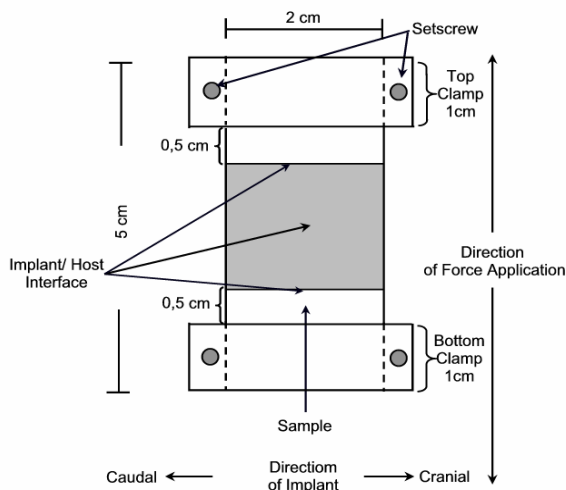
A device for the biomechanical measurement of Load of Rupture at the implant/host interface was built in the Núcleo de Cirurgia Experimental (Figure 3).



**FIGURE 3** - Device for measurement of Load of Rupture

The applied load to the tissue sample was provided by a system of distilled water pumping, with a constant flow of 500 ml/min, into a plastic bag, which weight exerts traction in the implant/host sample.

Sample was positioned in the top and bottom clamps and tightened (Figure 4).



**FIGURE 4**-Sample positioned for biomechanical evaluation

Top clamp was attached to a Digital Dynamometer® and the bottom clamp was connected to

the plastic bag. The plastic bag, moved down by an increment of constant weight, applied parallel to the sample. The dynamometer expressed the Load of Rupture in Kgf (scale precision:  $\pm 0.5\%$ ), (resolution: 0.01 kg). A serial interface RS-232 was connected between the dynamometer and a computer and a dedicated software displays results as curves and tables.

Animals were submitted to euthanasia with lethal doses of thiopental<sup>h</sup> to obtain samples.

The sample was composed by implant and part of the abdominal musculature, free from suture, measuring 5x2 cm, including the segment inserted within clamps (1 cm in each end), so that the implant/tissue rectangle between clamps measured 3x2 cm (Figure 4).

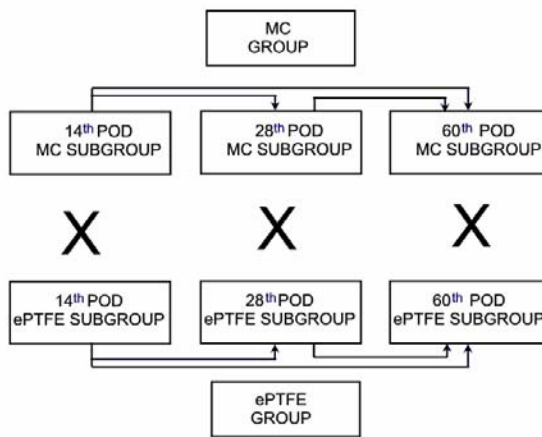
For validation of biomechanical test, only ruptures occurring in the implant/host interface were considered, not being computed results where the rupture occurs out of these structures, (i.e. abdominal musculature).

The test of differences of means (two tailed Student's "t" test), with probability of error of  $p < 0,05$  were applied between the averages of obtained values of Load of Rupture (in Kgf):

A) in 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of the Microbial Cellulose Group (Group MC);

B) in 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of the Expanded Polytetrafluoroethylene Group (ePTFE Group);

C) in 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of each Group, in corresponding periods (Figure 5). Observations were made on the presence infiltration within the implants of microbial cellulose and expanded polytetrafluoroethylene.



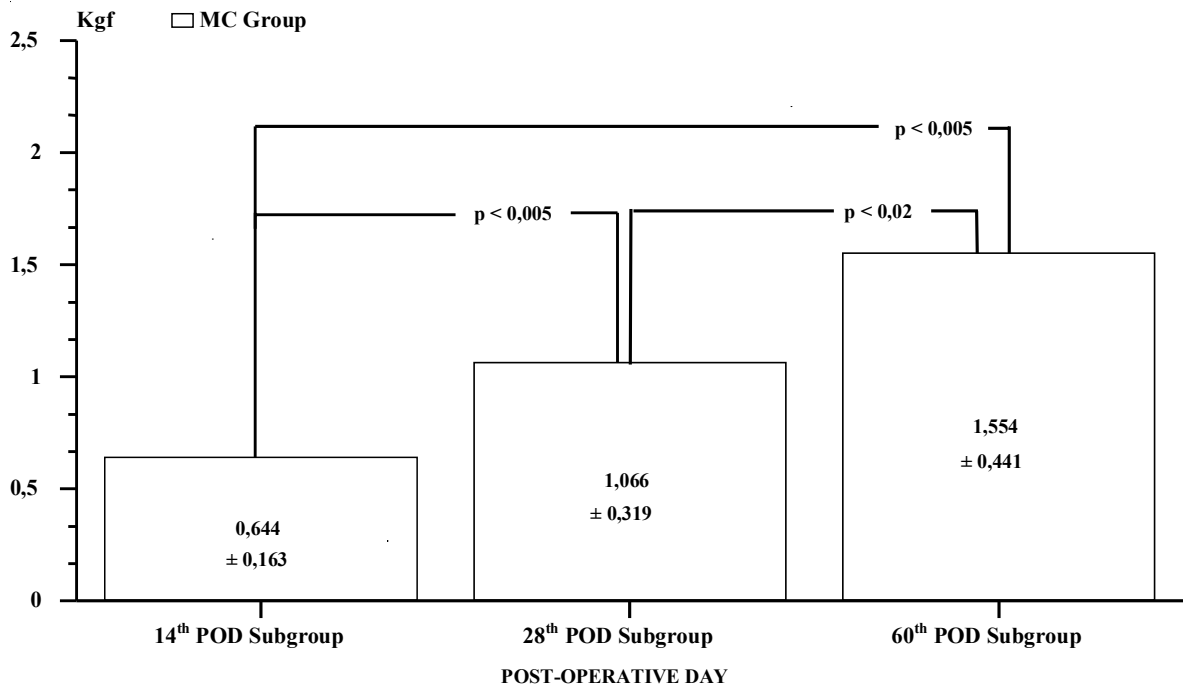
**FIGURE 5** - Diagram showing statistical analysis programmed for Load of Rupture

## Results

Comparative analysis accomplished among the averages of the values of the Load of Rupture obtained in the animals of 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of MC Group are shown in graphic form (Figure 6).

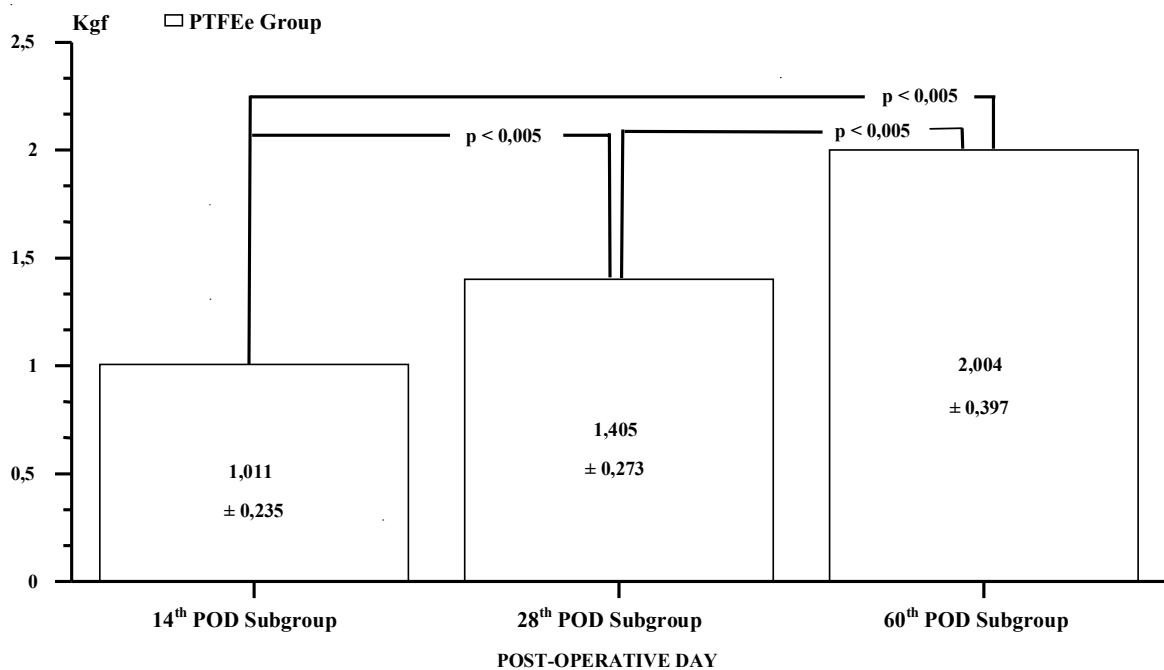
® Lutron FG-20 kg- Rs232- Taiwan, Impac Instrumentos de Medição - BR.

<sup>h</sup>Thiopentax, Laboratório Cristália-BR



**FIGURE 6** - Graph showing results of statistical analysis between mean values of Load of Rupture (Kgf) obtained in subgroups of MC group

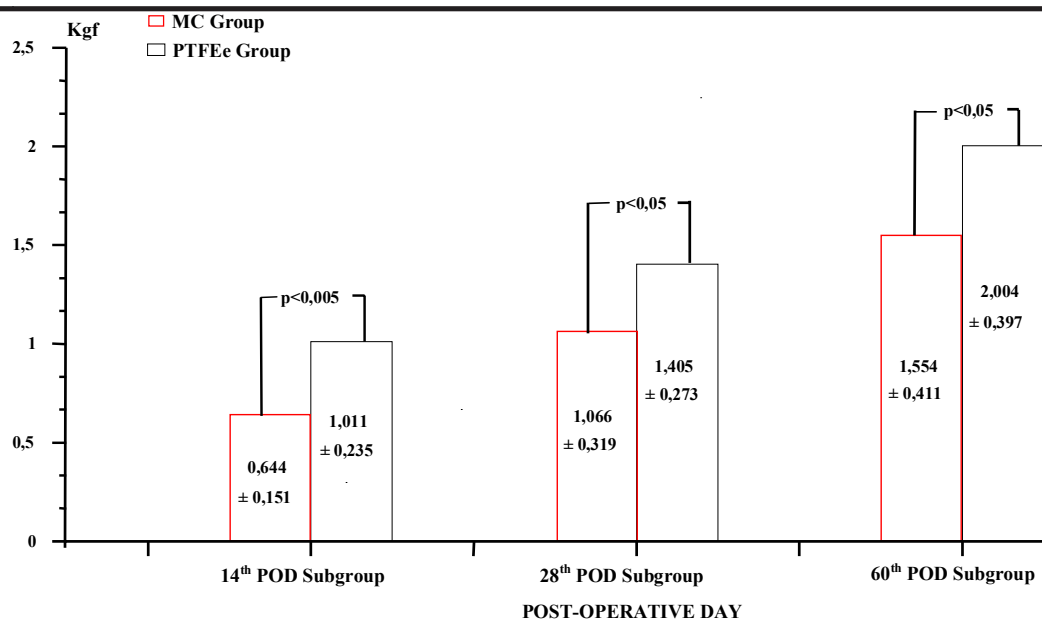
Comparative analysis accomplished among the averages of the values of the Load of Rupture obtained in the animals of 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of ePTFE Group are shown in graphic form (Figure 7).



**FIGURE 7** - Graph showing results of statistical analysis between mean values of Load of Rupture (Kgf) obtained in subgroups of ePTFE group

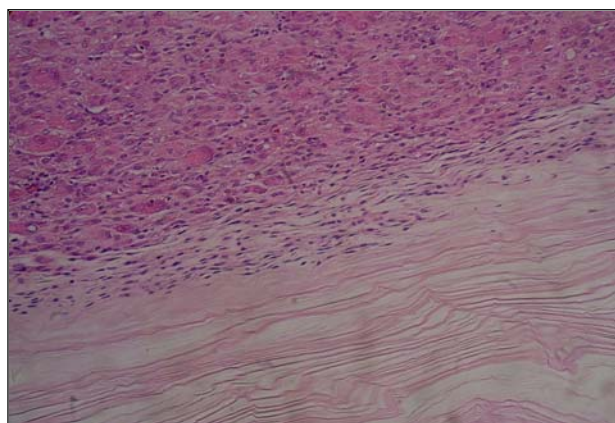
Comparative analysis accomplished between the averages of the values of the Load of Rupture obtained in the animals of 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of MC Group and those obtained in the animals of 14<sup>th</sup> POD, 28<sup>th</sup> POD and 60<sup>th</sup> POD Subgroups of ePTFE Group, in corresponding periods, are shown in graphic form (Figure 8).



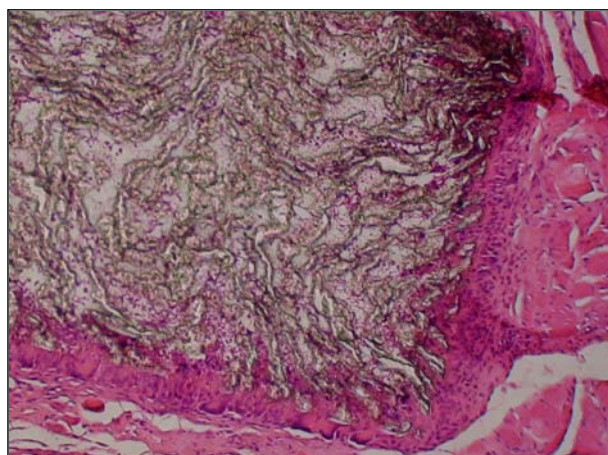


**FIGURE 8** - Graph showing results results of statistical analysis between mean values of Load of Rupture (Kgf) obtained in MC Group compared to those obtained in ePTFE Group, in corresponding periods

Infiltration of host tissue within implants was observed only in ePTFE Group (Figures 9 and 10)



**FIGURE 9** - Cellulose/host interface without infiltration of host tissue. Rat n° 38, 60<sup>th</sup> POD.



**FIGURE 10** - ePTFE/host interface with infiltration of host tissue. Rat n° 24, 60<sup>th</sup> POD.

## Discussion

The use of microbial cellulose (*Acetobacter xilynum*) has been suggested in different areas of medicine as: substitute of blood vessels and linfatics<sup>6,7</sup>; substitute of hollow internal organs as ureter, trachea and digestive tract<sup>6,7,8</sup>; cuff for reconstruction of nerves<sup>7</sup>; substitute of duramater<sup>9,10</sup>; substitute of the abdominal wall, skin, subcutaneous tissue, articulation, cartilage and reinforcement of areas of decreased resistance in the abdominal wall, esophagus and intestinal tube<sup>8</sup>; threads<sup>11</sup>; agent for increases soft tissue, reconstruction of the pelvic floor, suspension of bladder, repair of hernias and patch for inguinal hernias<sup>9</sup>.

In humans, microbial cellulose (*Acetobacter xilynum*) has been used on lesions of tegument (serious burns, skin graft (in the donor and receiving areas), facial peeling, infectious dermolysis, abrasion of tattoos, chronic ulcers, Hanseníase of the distal members<sup>12,13</sup>). Experimental studies for clinical application have been accomplished with membrane produced by the *Acetobacter xilynum* in several conditions as: protective cover for reconstruction of nerves<sup>14</sup>; duraplasty<sup>15</sup>; healing of epithelial lesions of cornea<sup>16</sup>; healing of duodenal lesions<sup>17</sup>; substitute of blood vessels<sup>18</sup>; cuffs for of reconstruction of micronerves<sup>18</sup>; reconstruction of the retroperitoneum<sup>19</sup>; and technical training in microsurgery<sup>18</sup>.

With the creation of a research group named *biopolímero da cana de açúcar* at the Federal University of Pernambuco, studies of biocompatibility of the microbial cellulose produced by *Zoogloea sp*<sup>20,21,22,23</sup>. The satisfied results authorize the accomplishment of experimental research for clinical application. Thus, studies were performed on: repair of stomach and bladder lesions<sup>24,25</sup>; substitution and patch of blood vessels<sup>26</sup>; repair of tympanic lesions<sup>27</sup>; suburethral slings for treatment of urinary incontinence<sup>28</sup>; urethroplasty<sup>29</sup>; reconstruction of *tunica*

*albuginea* of the penis<sup>30</sup>; treatment of vesico-ureteral reflux<sup>31</sup>; and dressing after surgical correction of hypospadias<sup>32</sup>.

Experimentally, in Veterinary Medicine, the reports consulted about use of biocelulose produced by the *Acetobacter xylinum* refer to few applications for: conduit for isolation in reconstruction of peripheral nerves<sup>33</sup>; healing of experimental wounds of bovine mammary teats<sup>34</sup>; healing of experimental tegument wounds in equine<sup>35</sup> and swine<sup>36</sup>; prophylaxis of the formation of membrane post laminectomy in dogs<sup>37</sup> and healing of incisional experimental lesions of the cornea in dogs<sup>38</sup>. Clinically, gross (brute) membrane of bacterial cellulose produced by the *Zoogloea sp.* was firstly used in healing of natural wounds of dogs<sup>39,40</sup>.

In this review, no reports (experimental or clinical) on the use of microbial cellulose membrane were found as repair of defects of the abdominal wall in humans or animals.

Increases of resistance in the subgroups of the MC Group (Figure 6) and ePTFE Group (Figure 7) along the time, may be related to the evolution of the healing process, predominantly inflammatory at the early phase and with prevalence of fibroblasts and collagen fibers in the remodeling process, allowing the biomaterial to be incorporating more firmly by the host, already mentioned in biocompatibility studies<sup>41,42</sup>.

Elevation of resistance in ePTFE Group as compared to MC Group, in corresponding periods (Figure 8) is indicative of better integration between polymeric synthetic material and host tissue.

The most important factor in the response to the implants by the host is the structure of the material, more specifically its porous structure. Materials that have reticular or macroporous structure, as polypropylene mesh, seem to produce better integration with organism of the receiver and better resistance to the traction in the zone of repair<sup>43</sup>.

The microbial cellulose is characterized by a structure of microfibrils net. The cellulose membrane produced by the *Zoogloea sp.* presents small pores (empty spaces among the fibrils) with diameters around 0,07 $\mu$ m (70nm)<sup>5</sup>. To have an idea of the size of these pores, it is enough to compare them with a thrombocyte (platelet), one of the smallest components of the blood, with a diameter of about 3 $\mu$ m<sup>18</sup>. The microporosity of the microbial cellulose doesn't allow ingrowth of host in cellulose structure (Figure 9).

The size of pores of the microbial exopolysaccharide, resultant of the application of method of manual compression and exposition to the air for dehydration of the membrane, is reduced. Such pores, defined as spaces among the structural elements of the MC, enter in collapse due to superficial tension, as a consequence of liquid removal<sup>44</sup>.

The expanded polytetrafluoroethylene (ePTFE) presents a microscopic structure composed of nodules interconnected each other by fibrils. The interior of the fibrils net is constituted by empty spaces with dimensions sufficiently large (up to 80  $\mu$ m), to allow infiltration of tissue from host<sup>45</sup>. The porosity of ePTFE is an important characteristic in synthetic materials for implant that allows

the infiltration of connective tissue into the material, propitiating better integration between the implant and the organism<sup>46</sup>. In the present work it was used implants of ePTFE with pore diameters of 25 $\mu$ m, therefore, dimension enough to allow the penetration within implants with cellular elements and host tissue (Figure 10), starting from 14<sup>th</sup> POD. Reports indicate that starting of this process can be observed from 7<sup>th</sup> POP<sup>42</sup>.

The multiperforated cellulose membrane, partially dehydrate membrane and the gel form (by ultrasonic fragmentation of the membrane) interposed between sheets of cellulose (sandwich) are being tested at Núcleo de Cirurgia Experimental associated with Laboratório de Cana-de-Açúcar da Universidade Federal Rural de Pernambuco, in the sense to obtain a product that allows better integration with host tissue.

## Conclusion

In the experimentation conditions, and with a probability of error of 5%, the implant/host interface presented larger resistance in ePTFE Group as compared with MC Group.

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