Incorporation by host tissue of two biomaterials used as repair of defects produced in abdominal wall of rats[1]

Incorporação por tecido do hospedeiro de dois biomateriais usados como reparo de defeitos produzido em parede abdominal de ratos

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ABSTRACT

Purpose: Biomaterials may be used as treatment of great abdominal wall defects to avoid tension during repair. In the present research we intended to investigate incorporation type by host tissue of membranes of microbial cellulose (MC), produced by the bacteria *Zoogloea sp.*, and of polytetrafluoroethylene (ePTFE) in abdominal wall defects of rats. **Methods:** Sixty male rats Wistar, anesthetized by ketamine (5mg/100g) and xylazine (2mg/100g), were submitted to a rectangular excision (2x3cm) of the abdominal wall, including fascia, muscles and peritoneum and further treated with implants of microbial cellulose (MC Group - 30 animals) or expanded polytetrafluoroethylene (ePTFE Group- 30 animals). Each group was subdivided in 14th DPO, 28th DPO and 60th DPO Subgroups. **Results:** Incorporation of biomaterials was observed by wrapping and infiltration by host tissue. It has been found that wrapping associated to infiltration of host connective tissue in implants of ePTFE were present in 100% of the observed samples, and this may be responsible for increase resistance to traction. Inversely, wrapping without host tissue infiltration was seen in 100% of examined specimens of MC implants. **Conclusion:** Wrapping and host tissue infiltration is seen only in ePTFE implants.

Key-words: Microbial cellulose. *Zoogloea sp*. Expanded polytetrafluoroethylene. Incorporation. Abdominal defect. Rats.

RESUMO

Objetivo: Biomateriais podem ser usados como tratamento de grandes defeitos da parede abdominal para evitar tensão durante reparo. Na presente pesquisa pretendeu-se investigar o tipo de incorporação pelo tecido do hospedeiro de membranas de celulose microbiana (CM), produzidas pela bactérias *Zoogloea sp.*, e de politetrafluoretileno (PTFEe) em defeitos da parede abdominal de ratos. **Métodos:** Sessenta ratos machos Wistar, anestesiados através de cetamina (5mg/100g) e xilazina (2mg/100g), foram submetidos a uma excisão retangular (2x3cm) da parede abdominal, incluindo fascia, músculos e peritoneum e posteriormente tratadas com implantes de celulose microbiana (Groupo CM - 30 animais) ou politetrafluoretileno (Grupo PTFEe - 30 animais). Cada grupo foi subdividido em Subgrupos14º DPO, 28º DPO e 60º DPO. **Resultados:** Incorporação do biomaterial foi observada através de envoltório e infiltração pelo tecido do receptor. Foi encontrado que o envoltório associado à infiltração de tecido conjuntivo do hospedeiro em implantes de ePTFE estava presente em 100% das amostras observadas, podendo ser responsável por aumento da resistência à tração. Inversamente, envoltório sem infiltração de tecido do hospedeiro foi visto em 100% dos espécimes examinados nos implantes de CM. **Conclusões:** Pode-se ser concluído que o envoltório associado à infiltração de tecido do hospedeiro só é vista nos implantes de PTFEe.

Descritores: Celulose microbiana. *Zoogloea sp. P*olitetrafluoretileno expandido. Incorporação. Defeito abdominal. Ratos.

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

Introduction

In some instances where reconstruction of muscle-aponeurotics defects is affected by great distance among its edges or by lack of tissue with proper characteristics for an appropriate approach, synthetic and biological implants, or even muscular grafts, vascularized or not, can be used for tissue repair [1].

In Veterinary Medicine, few experimental reports for clinical applications were found on the use of biocelulose produced by *Acetobacter xylinum* such as; cuff protection in the reconstruction of peripheral nerves[2], healing of experimental wounds in mammary teats of bovine[3], experimental teguments wounds in equine[4] and swine[5], prophylaxis of the occurrence of membrane after laminectomy in dogs[6] and healing of experimental incisional lesions of the cornea in canine[7].

The first clinical application of membranes of biocelulose produced by microorganism *Zoogloea sp.*, in gross state, was done in UFRPE to treat natural cutaneous wounds of dogs. The results suggested control of the infection, accelerated growth of granulation tissue and abbreviation of healing time, when compared with the conventional treatment (antiseptic + cicatricial ointments) [8,9]. In this work, the authors, possibly took advantage of the beneficial properties of sugar, main constituent of the wrapping of gross membranes (sugar-cane molasses), on wound healing. Studies of biocompatibility and citotoxity of the microbial cellulose produced by *Zoogloea sp.* were previously done, and authorize the accomplishment of experimental research for clinical application [10,11,12,13].

The use of cellulose membranes produced by the *Zoogloea sp.* or by other bacterial species, as repair of muscle-aponeurotics defects of the abdominal wall of animals, in experimental or clinical scope, has not been reported.

The present work has the objective to compare the use of membrane of microbial cellulose (MC) produced by the *Zoogloea sp.* and synthetic membrane of expanded polytetrafluoroethylene (ePTFE), as repair of produced defect in the abdominal wall of rats, through observation of incorporation of implanted material by host tissue.

Methods

Materials used as implants

The exopolyssacharide pellicle was produced by bacteria *Zoogloea sp.*, isolated by the Institute of Antibiotics of the Federal University of Pernambuco, in static culture, having sugar-cane molasses as nutritious medium[14]. During the treatment process, the membrane was purified in solution of sodium hypochlorite (NaOCl) followed by several rinse sessions, mechanical compression and evaporation at the air, conditioned in polypropylene envelope immerged in solution of isopropyl alcohol moisturized at 20%, and finally sterilized in g rays *.

The membrane of expanded polytetrafuoroethylene (ePTFE) was obtained from vascular prostheses with internal diameter of 8mm and wall thickness of 0,8mm, with pores size of 25µm, cutting in the longitudinal direction, after removal of the external helical structure. Rectangles of 2x3cm were prepared, then conditioned in polypropylene envelopes and submitted to the sterilization in g rays.

Groups and Subgroups

The animals were distributed in two groups:

Microbial Cellulose Group (MC Group): composed of 30 animals that were submitted to a muscle-aponeurotic defect on ventral wall of abdomen and treated with membrane of microbial cellulose;

Expanded Polytetrafuoroethylene Group (ePTFE Group): composed of 30 animals that were submitted to a muscle-aponeurotic defect on ventral wall of abdomen and treated with membrane of expanded polytetrafluoroethylene;

Each group was subdivided in three Subgroups of 10 rats, in agreement with the postoperative day (POD) observation, being denominated of 14th POD Subgroup, 28th POD Subgroup and 60th POD Subgroup.

Animals

Sixty male Wistar rats, with mean weight of 437, 7g±40, 9, were housed in appropriate cages, fed with proper ration and mineral water *ad libitum*.

Anesthetic and surgical procedure

The animals were anesthetized with a mixture of ketamine (5mg/100g)† and xylazine (2mg/100g)‡ by intramuscular route, for accomplishment of a middle abdominal incision (5cm), proceeded by a rectangular excision (2x3cm) including fascia, muscles and peritoneum and then treated with implants of membranes of microbial cellulose or expanded polytetrafluoroethylene. At the days programmed for evaluations, under intraperitonial administration of sodium thiopental§ and, subsequently lethal doses of this barbiturate, the animals were submitted to the euthanasia for accomplishment of the histological exams.

Biopsy and stains

A segment corresponding to the implant/host interface, with 0, 5 cm of width, embracing the cranial extension of the sample, free from suture, was excised and immerged in buffered solution of formalin at 10%. After fixation of the samples, they were included in paraffin,

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* Department of Nuclear Energy, Federal University of Pernambuco
† Ketalar 50mg. Cristalia Laboratories of Brazil
‡ Rompum 20mg. Bayer Laboratories of Brazil
§ Thiopentax, Cristalia Laboratories of Brazil

sectioned and stained by hematoxylin-eosin (H-E) and Tricromic of Masson.

Histological observations

Observations were made on the presence of Wrapping without Infiltration in the Implants and Wrapping with Infiltration in the Implants, at 14th POD, 28th POD and 60th POD Subgroups, in rats of the MC and ePTFE Groups.

Results

Microbial Cellulose Group (type of incorporation)

The observation of the specimens stained by H-E and Tricromic of Masson, in14th DPO, 28th DPO and 60th DPO Subgroups of MC Group revealed a type of incorporation characterized by presence of wrapping without infiltration in implants in 100% of the sample readings (Table 1, Figure 1).

TABLE 1 - Type of incorporation: Incidence of Wrapping without Infiltration in Implants and Wrapping with Infiltration in Implants, obtained in 14th DPO, 28th DPO and 60th DPO Subgroups, in rats submitted to muscle-aponeurotics defects, treated with double layer of membrane of Microbial Cellulose (MC) (0,2 mm).

*= YES; **= NO; ***= Sample technically inappropriate for reading due to imperfections during the inclusion in paraffin

Exp. N°	MC 14th DPO SUBGROUP		Exp. N°	MC 28th DPO SUBGROUP		Exp. N°	MC 60th DPO SUBGROUP		Total
	Type of Incorporation			Type of Incorporation			Type of Incorporation		
	Wrapping without Infiltration in Implants	Wrapping with Infiltration in Implants		Wrapping without Infiltration in Implants	Wrapping with Infiltration in Implants		Wrapping without Infiltration in Implants	Wrapping with Infiltration in Implants	
1	-***	-	1	Y	N	1	Y	N	
2	Y*	N**	2	Y	N	2	Y	N	
3	Y	N	3	Y	N	3	Y	N	
4	Y	N	4	Y	N	4	Y	N	
5	Y	N	5	Y	N	5	Y	N	
6	Y	N	6	Y	N	6	Y	N	
7	Y	N	7	Y	N	7	Y	N	
8	Y	N	8	Y	N	8	Y	N	
9	Y	N	9	Y	N	9	Y	N	
10	Y	N	10	Y	N	10	Y	N	
Total by Attribute	09 (100%)	09 (100%)		10 (100%)	10 (100%)		10 (100%)	10 (100%)	
Total by Subgroup	18 (31,04%)			20 (34,48%)			20 (34,48%)		58 (100%)

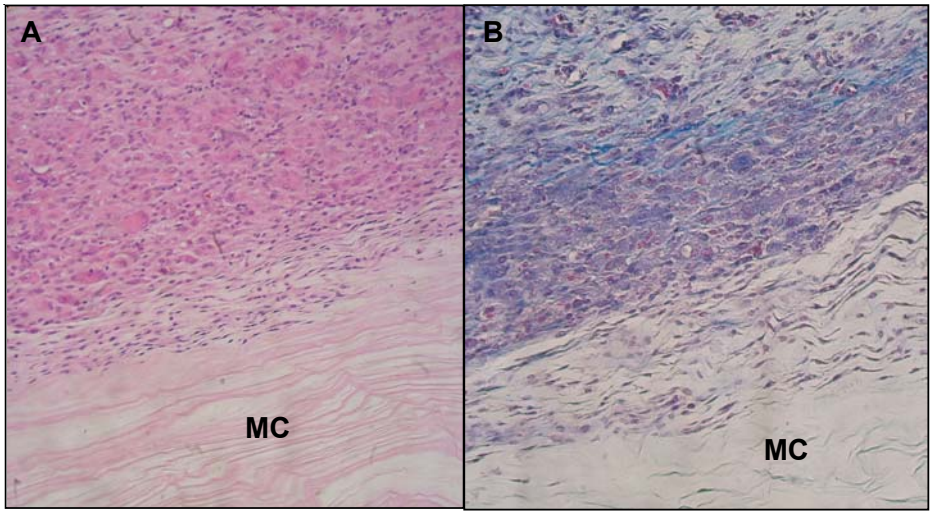

FIGURE 1 - Micrograph of interface area between implants of Microbial Cellulose (MC) and the host. Note membrane of MC with wrapping but without tissue infiltration. Stain: A) hematoxylin and eosin; B) Trichromic of Masson. Magnification 220 x in

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Expanded Polytetrafluoroethylene Group (type of incorporation)

Using the same stains, in 14th DPO, 28th DPO and 60th DPO Subgroups of the ePTFE Group, the type of incorporation was represented by wrapping with infiltration in implants in 100% of the occasions (Table 2, Figure 2).

TABLE 2 - Type of incorporation: Incidence of Wrapping without Infiltration in Implants and Wrapping with Infiltration in Implants , obtained in 14th DPO, 28th DPO and 60th DPO Subgroups, in rats submitted to muscle-aponeurotics defects, treated with membrane of expanded polytetrafluoroethylene (ePTFE) (0,8 mm).

*= YES; **= NO; ***= Sample technically inappropriate for reading due to imperfections during the inclusion in paraffin

	ePTFE 14ª DPO SUBGROUP			ePTFE 28ª DPO SUBGROUP			ePTFE 60ª DPO SUBGROUP		Total
Exp. Nº	Type of Incorporation: Wrapping without Infiltration in Implants	Wrapping with Infiltration in Implants	Exp. Nº	Type of Incorporation: Wrapping without Infiltration in Implants	Wrapping with Infiltration in Implants	Exp. Nº	Type of Incorporation: Wrapping without Infiltration in Implants	Wrapping with Infiltration in Implants	
1	N**	Y*	1	Y	N	1	Y	N	
2	N	Y	2	-	-	2	Y	N	
3	N	Y	3	Y	N	3	Y	N	
4	-	-***	4	Y	N	4	Y	N	
5	N	Y	5	Y	N	5	Y	N	
6	N	Y	6	Y	N	6	Y	N	
7	N	Y	7	Y	N	7	Y	N	
8	N	Y	8	Y	N	8	Y	N	
9	N	Y	9	Y	N	9	Y	N	
10	N	Y	10	Y	N	10	Y	N	
Total by Attribute	09 (100%)	09 (100%)		09 (100%)	09 (100%)		10 (100%)	10 (100%)	
Total by Subgroup	18 (32,14%)			18 (32,14%)			20 (35,72%)		56 (100%)

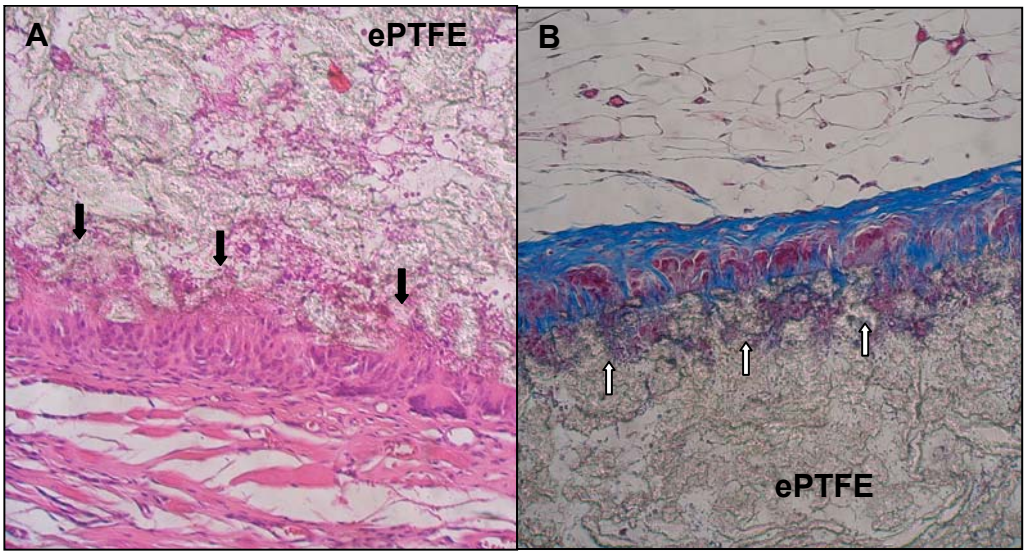

FIGURE 2 - Micrograph of interface area between implants of membrane of expanded polytetrafluoroethylene (ePTFE) and the host. Note the membrane of ePTFE with wrapping and tissue infiltration (black arrows) and presence of collagen fibers inside of the membrane (white arrows).Stain: A) hematoxylin and eosin; B) Tricromic of Masson. Magnification 220 x in A and B.

Discussion

Incorporation of microbial cellulose implants

The membrane of microbial cellulose (MC) produced by the *Zoogloae sp.* has been considered as non porous material and incorporation of implant is achieve by capsulation (wrapping) [13]. In a different way of ePTFE the cellulose produced by the *Zoogloae sp.* did not permitted invasion of fibroblasts in experimental repair of arteries and veins (angioplasties)[15], as seen in the present report (Table 1, Figure 1)

Studies on biocompatibility through inclusion of MC, produced by the *Acetobacter xylinum*, in the abdominal musculature of rats, emphasizes the evolutionary histological composition of the wrapping (capsulation), from acute inflammatory phase (neutrofils) until the regenerative phase (fibroblasts and collagen synthesis), in a period of 28 days, without reference of infiltration of host tissue in the implant[16]. These authors also report the presence of giant cells with progressive character until 28th DPO.

However, in another study accomplished with microbial cellulose produced by *Acetobacter xylinum subsp. sucrofermentans*, in static culture, the obtained pellicles consisted of a flexible porous net of nanofibrils, not joined; whose capacity to retain water was 99%. The net of fibrils was examined by scanning electronic microscopy (SEM) and described as denser in the interface between the culture medium and the air (compact side) and more porous on the opposite side (porous side). To the end of 12 weeks, on the porous side, the fibroblasts were completely integrated inside the structure of the membrane of MC, having synthesized collagen[17]. The same authors used a film of MC with retention 99% of water, allowing in this way infiltration of cells and proliferation of smooth muscular tissue in the spaces among fibrils, in a process of tissue engineering for construction of blood vessels. Through SEM, cells could be seen on the porous side, moving away the nanofibrils when the migration took place inside of the net of MC fibrils. The maximum infiltration depth into the MC observed after 1 week was of 20 µm. An infiltration depth into CM of up to 40 µm could be seen after two weeks in culture. To facilitate the growth of cells inside of the MC, they mention a technique to create wider spaces in the membrane, with the use of paraffin spheres, followed by removal of the same ones with solution of NaOH[18].

In the sense to obtain a product that allows better incorporation with host tissue, multiperforated cellulose membrane is now being tested at the Núcleo de Cirurgia Experimental.

Incorporation of ePTFE implants

Reports on incorporation of ePTFE implants used as implants revealed a fibrous tissue firmly adherent to the surface of this material (wrapping). Fibroblasts were found in its interstice. The presence of these cells in the empty spaces of the structure of the polymer propitiates the synthesis of collagen, resulting in a stable and resistant repair[19]. These results are in agreement with our findings (Table 2, Figure 2).The depth reached by the infiltration of connective tissue within the ePTFE was evaluated in about 200 µm, in a period of five months[19]. In experimental angioplasties, the tissue invasion observed within the ePTFE patches has been described and occurred because they present an appropriated porous size (25 µm), which allow cellular migration[15]. The infiltration of cells and tissue is a process limited by the pore size of ePTFE, not being observed tissue infiltration in pores under 10 µm in diameter[20].

Wrapping associated to infiltration of recipient tissue seen in ePTFE implants, as compared with microbial cellulose (MC) may be responsible for increased resistance to traction at the interface implant/host [21].

Conclusion

Incorporation composed by wrapping associated to host tissue infiltration is seen only in ePTFE implants.

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Acknowledgment

We thank Professor Sílvio Romero Marques who gently provided ePTFE material.

This work was support by Brazilian Government scholarship from CAPES (Coordenação de Aperfeiçoamento de Nível Superior).

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Conflict of interest: none
Financial support: In acknowledgment

Received: September 28, 2007
Review: November 21, 2007
Accepted: December 12, 2007

How to cite this article

Falcão SC, Evêncio Neto J, Coelho ARC. Incorporation by host tissue of two biomaterials used as repair of defects produced in abdominal wall of rats. Acta Cir Bras. [serial on the Internet] 2008 Jan-Feb;23(1). Available from URL: http://www.scielo.br/acb

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