Porcine peritoneum as source of biocompatible collagen in mice

Peritônio suíno como fonte de colágeno biocompatível em camundongos

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ABSTRACT
Purpose: To investigate the biocompatibility and biodegradability of a membrane made from porcine peritoneum. Methods: The membrane (5x5 mm) was inserted in the subcutaneous tissue on the back of 15 mice, which were killed after 1, 3 and 9 weeks (ISO 10993-6). The cellular components of the inflammatory response and degradation of the membrane were analyzed in hematoxylin-eosin-stained histological sections. Results: After one week, mononuclear cells were observed inside the membrane. After three weeks, the material was almost completely absorbed. After nine weeks, there was no presence of material and there were signs of tissue remodeling. There was neither a foreign body reaction nor signs of tissue necrosis. Conclusion: The collagen membrane derived from porcine peritoneum is biocompatible and bioabsorbable when implanted in the subcutaneous tissue of mice.
Key words: Materials Testing. Biocompatible Materials. Subcutaneous Tissue. Peritoneum. Mice

RESUMO
Objetivo: Investigar a biocompatibilidade e biodegradabilidade de uma membrana feita de peritônio suíno. Métodos: A membrana (5x5 mm) foi inserida no tecido subcutâneo dorsal de 15 camundongos, que foram mortos após uma, três e nove semanas (ISO 10993-6). Os componentes celulares da resposta inflamatória e a degradação da membrana foram analisados em cortes corados em hematoxilina-eosina. Resultados: Após uma semana, células mononucleares foram observadas dentro da membrana. Após três semanas, o material foi quase completamente absorvido. Após nove semanas, não houve presença do material e houve sinais de remodelação tecidual. Não houve reação de corpo estranho nem sinais de necrose tecidual. Conclusão: A membrana colágena derivada de peritônio suíno é biocompatível e bioabsorvível quando implantada no tecido subcutâneo do camundongo.

Introduction

Tissue regeneration has been a significant challenge for clinical and dental medicine. The use of absorbable and non-absorbable membranes is meant to guide tissue repair so as to favor its regeneration despite the healing processes. These membranes are supposed to prevent fibroblast migration and invasion, exclude inhibiting factors, concentrate factors that stimulate growth on the site, have stimulating properties, and be rigid, but not to the point of hindering them from being handled.

Porcine peritoneum is a suitable tissue for the production of membranes that will be used in Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR), since its structure, which consists mainly of collagen, results in a biocompatible and absorbable material, when processed properly.

Collagen has been considered superior to other materials for working actively on clot formation and promoting fibroblast chemotaxis. However, the enzymatic activity of macrophages and polymorphonuclear leukocytes results in fast biodegradation of native collagen, thus allowing undesired cell types to invade the graft area. Several techniques have been used in order to extend the biodegradation time of collagen-based membranes. Physical and chemical treatments have been investigated with the purpose of controlling the degradation of the collagen membrane.

Despite its degradability, its potential for bladder reconstruction, tissue engineering of the small intestine, reconstruction of the flexor tendon sheath and for periodontal healing has been investigated.
Recently, a collagen membrane produced from cortical bovine bone tissue promoted the complete regeneration of critical defects in rat skulls, demonstrating the potential this type of biomesh has for regeneration processes\textsuperscript{10}.

The objective of this study is to evaluate tissue response and biodegradation of a membrane derived from acellular porcine peritoneum.

**Methods**

Porcine peritoneum membrane (PPM) was obtained in slaughterhouses certified by the Federal Inspection Service and that have a tracking system. The material was processed by means of mechanical cleaning, chemical treatment, lyophilization (freeze-drying) and sterilization. The lyophilized membranes were cut into 5 mm x 5 mm sections, individually packed and sterilized by gamma radiation (25K Gy).

The study used 15 Balb/c 60-day-old mice (around 75g) obtained from the UFF Animals Center, which throughout the entire experimental period were kept under proper hygiene and care conditions, according to Brazilian College of Animal Experimentation (COBEA) standards and as approved by the Committee on Animal Research and Ethics (Protocol N. 0035-8). The animals received food and water *ad libitum*.

The animals were sedated with a mixture of ketamine hydrochloride (Dopalen\textsuperscript{®}) and xylazine hydrochloride (Anasedan\textsuperscript{®}), in a 1:1 (v/v) proportion, which was applied by intramuscular route at 0.5 ml per kg of body weight. After the region submitted to the surgical procedure underwent trichotomy and antisepsis, the dorsal region of each animal received a 1.5cm-incision for the material implant. A PPM was implanted in the animals' subcutaneous tissue. The animals were killed 1, 3 and 9 weeks later through cervical dislocation. Whenever possible, palpation of the subcutaneous tissue allowed to locate the membrane, which was removed involved in granulation tissue with a safety margin of approximately 2 mm.

The histological sections containing the collected material were fixed in 10% buffered formalin solution, pH 7.2, and processed for inclusion in paraffin wax. 5µm-thick sections were stained with hematoxylin and eosin (HE) and evaluated in regards to type and intensity of the inflammatory infiltrate, presence and integrity of the membrane.

**Results**

In the first 24 hours following the surgery, all animals were clinically healthy, presenting a slight edema in the dorsal region, which is compatible with the surgical procedure that had been performed; they did not present any signs of necrosis, suppuration or infection. Seven days after surgery, the material was easy to locate through palpation of the animals dorsal region; this fact was not observed at the other points of the essay. After nine weeks, it was not possible to locate any signs indicating the presence of the membrane, even after extensive divulsion of the subcutaneous tissue.

**1 week.** At this point, it was observed that the material was not much integrated in the subcutaneous tissue, but was involved in vascularized granulation tissue. The microscopic analysis showed first signs of membrane degradation through the increase of inrafibrilar gaps, although a dense and compact region remained (Figure 1). There was an intensive inflammatory infiltrate with predominance of mononuclear cells that are compatible with the macrophages observed permeating PPM fibers. In this period, polymorphonuclear leukocytes were only occasionally observed (Figure 1).

**FIGURE 1** - Photomicrography of week 1 of the experiment. PPM presented a dense and compact region (black arrows) and an area of free fibers (tip of black arrow) permeated by intense inflammatory infiltrate, mainly mononuclear (tip of blue arrow) with areas of edema (asterisk). HE stains. Zoom: 40x. PPM: porcine peritoneum membrane
3 weeks. The macroscopic analysis did not detect PPM in 3 of the 5 animals used at this point, but a fibrous tissue remained in the area of its likely location. The microscopic analysis showed an almost complete degradation of the material in 3 animals (Figure 2). However, in 2 animals there was still spacing between membrane fibers, edema areas and an intense mononuclear inflammatory infiltrate, mainly of macrophages, and some occasional multinuclear giant cells. Fibroblastic proliferation was intense and indicated tissue remodeling.

9 weeks. At this point of the essay, none of the animals presented presence of PPM. The conjunctive tissue was loosely organized and there were still some congested vessels, suggesting tissue remodeling, as well as the presence of skin annexes, such as the sweat duct (Figure 3).

FIGURE 2 - Photomicrographies of week 3 of the experiment. A) Presence of intense inflammatory infiltrate, rich in mononuclear cells involving the fibers of the PPM that is quite disorganized (tip of black arrow). B) Intense presence of fibroblasts (arrow) and macrophages (tip of red arrow) in granulation tissue. HE stains. Zoom: 40x (A) and 100x (B). PPM: porcine peritoneum membrane

FIGURE 3 - Photomicrographies of week 9 of the experiment. A) Absence of PPM. B) Loose conjunctive tissue presenting congested vessels (arrow) and sweat duct (asterisk). HE stains. Zoom: 10x (A) and 40x (B). PPM: porcine peritoneum membrane
Discussion

The objective of this study was to analyze the biocompatibility and biodegradability of a membrane processed from porcine peritoneum. Results have shown that the processed membrane is biocompatible and reabsorbable after a period of three weeks.

Since it is absorbable, PPM does not have to be removed after being implanted. This constitutes an advantage, particularly in implantodontics, periodontology and dental surgeries, since non-reabsorbable membranes, such as those made of polytetrafluorethylene, require a second surgical procedure to remove them, which may cause post-surgical complications, as well as discomfort for the patient.

For guided bone regeneration applications in dentistry, the membrane has to remain whole from the structural and functional point of view for, at least, six weeks in humans so to allow for the migration of osteogenic cells and promote the regeneration of the lost original tissue; this period is critical for the regeneration process. Studies emphasize that membranes with a very short reabsorption time, i.e. less than three weeks, should be avoided. Also, when used as a barrier, the dissolution of the reabsorbable membrane should occur in a time that is predictable and compatible with bone regeneration.

The chemical composition of a biodegradable membrane determines not only its stability, but also the inflammatory and immune response. The main characteristic of the peritoneum is that it consists mostly of collagen, the most common protein in the animal kingdom and the main constituent of bones, skin and conjunctive tissues of all mammals. Collagen has been largely used in the production of biomaterials and as vehicle for its biocompatibility and minimum immunological response. The use of this abundant source associated with the proper processing of these tissues may represent a significant source of new materials, which may be used in tissue engineering or in regeneration techniques.

Membrane derived from porcine type I and III collagen (Bioguide®), implanted in mice subcutaneous tissue, presents excellent tissue integration, followed by fast vascularization by the end of one month, when a full biodegradation with a discrete reaction to a foreign body occurs. This fast degradation may be explained by the fact that this membrane has pores that favor its degradation, because they improve its interaction with the tissue. In this study, the degradation of the evaluated membrane occurred after three weeks by a mononuclear inflammatory infiltrate and some occasional multinuclear giant cells.

The existence of crossed links does not necessarily mean that the material will be degraded faster. Tissue response to Bioguide®, which has crossed links, promotes a faster degradation when compared to TutoDent® (without crossed links), possibly because the latter is more compact, thus hindering cell penetration. However, it has been shown that implants of type I bovine collagen membranes without crossed links promote the reabsorption of biomaterial in 42 days.

In this study, the biodegradation process of the porcine collagen membrane in the subcutaneous tissue of mice was guided by mononuclear macrophage-like cells in week 1, but was amplified by the fibroblastic proliferation as of week three. A comparative study on rat subcutaneous tissue using membranes made of cortical bovine bones treated or not with tetracycline observed great similarity between both groups in regards to their inflammatory infiltrate; both groups presented a primary invasion of PMN and lymphocytes, which was quickly replaced by a mononuclear infiltrate and giant cells. Another study, conducted on membrane from bovine pericardium, did not present giant cells as it occurs with bovine bioprosthesis.

Conclusion

The membrane from porcine peritoneum collagen is biocompatible and bioabsorbable in three weeks when implanted in the subcutaneous tissue of mice.

References

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