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Hyaluronic acid on collagen membranes: An experimental study in rats

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ABSTRACT

Objective: The aim of this study was to evaluate the effect of hyaluronic acid (HA) in the structure and degradation patterns of BioGide® and OsseoGuard™ collagen membranes. HA mediates inflammation and acts in cell migration, adhesion, and differentiation, benefitting tissue remodeling and vascularization. These are desirable effects in guided regeneration procedures, but it is still unknown whether HA alters the barrier properties of absorbable membranes.
Design: Bone defects were created in the calvaria of rats, which were treated with HA gel 1% (HA group) or simply filled with blood clot (control group), and covered with BioGide® or OsseoGuard™. The animals were euthanized after 1, 30, and 60 days, and their calvarias were processed for histological analysis.
Results: BioGide®, in both HA and control groups, showed vascularization, intense cell colonization, bone formation, and tissue integration at 30 and 60 days. In contrast, Osseoguard™ presented minimal cellular colonization, and inflammatory reaction associated to foreign body reaction in both time points and groups. The HA group of BioGide® showed higher cell colonization (574.9 ± 137.6) than the control group (269.1 ± 70.83) at 60 days ($p < 0.05$). Despite this finding, the structure and degradation pattern were similar for BioGide® and Osseoguard™ in the HA and control groups.
Conclusion: The results suggest that HA did not interfere with tissue integration and structural degradation of BioGide® and Osseoguard™ membranes.

1. Introduction

The regeneration of tissues lost due to disease is the ultimate goal of periodontal treatment (Karring, Nyman, Gottlow, & Laurell, 1993). In particular, infrabony periodontal defects are a frequent clinical challenge (Vanden Bogaerde, 2009). Likewise, lack of bone tissue in implant sites is a recurrent problem that must be corrected prior to implant placement so that it can be adequately stabilized (Esposito et al., 2009). Both obstacles may be addressed by regeneration surgical techniques. In this regard, guided tissue regeneration (GTR) and guided bone regeneration (GBR) have been thoroughly tested and are currently common procedures (Pellegrini, Pagni, & Rasperini, 2013). In these surgical techniques, barrier membranes with or without bone graft or bone substitutes are applied to the surgical site to improve tissue regeneration outcome. These membranes act as a protective barrier against soft-tissue invasion. They separate cells with bone regenerative potential, like osteoblasts and desmodontal fibroblasts, from rapidly proliferating epithelial and connective tissue cells (Rothamel et al., 2014). There are currently two types of barrier membranes: non-absorbable and bioabsorbable (Bunyaratavej & Wang, 2001). Collagen membranes are bioabsorbable and equivalent to non-absorbable membranes with regard to some surgical outcomes like probing depth reduction, clinical attachment gain, and percent of bone fill (Corinaldesi, Lizio, Badiali, Morselli-Labate, & Marchetti, 2011 ; Karring et al., 1993; Merli et al., 2014). Collagen resorption occurs via biodegradation and renders unnecessary the 2-stage surgical protocol (Hämmerle & Jung, 2003), which is an advantage over non-absorbable membranes. The biodegradation process occurs through infiltration and colonization by fibroblasts and blood vessels, leading to the collagen scaffold remodeling and replacement with new extracellular matrix (Rothamel et al., 2005; Rothamel et al., 2014; Willershausen et al., 2014). It has been

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established that the biodegradation rate of collagen membranes influences its effectiveness as a barrier, and is altered by its composition, structure, and three dimensional surface (Ferreira, Gentile, Chiono, & Ciardelli, 2012; Gielkens et al., 2008; Karring et al., 1993; Rothamel et al., 2014). This rate may also be influenced by other materials, and the degradation process must be slow enough to achieve tissue regeneration before membrane disintegration (Liu & Kerns, 2014). Thus, new adjuvant treatments that could accelerate the healing process are most welcome, as long as they do not jeopardize the proper biodegradation profile of the collagen membranes.

Hyaluronic acid (HA), a natural linear glycosaminoglycan, is biocompatible, biodegradable (Fraser, Laurent, & Laurent, 1997), and has been suggested to display anti-inflammatory and bacteriostatic action (Campo et al., 2012; Pirnazar et al., 1999). Furthermore, HA has important tissue healing properties, including the induction of angiogenesis, and the promotion of cell migration, adhesion, and proliferation (Burd et al., 1991; Jiang, Liang, & Noble, 2007; Toole, 2001). Due to its desirable effects, HA-based gels have been used in various medical specialties (Robert, 2015), and recent studies (Fawzy El-Sayed, Dahaba, Aboul-Ela, & Darhous, 2012) have analyzed its application in dentistry. Some authors suggest that HA may promote a clinical attachment level gain when used as an adjuvant to treat infrabony periodontal defects (Briguglio, Briguglio, Briguglio, Cafiero, & Isola, 2013; Fawzy El-Sayed et al., 2012; Vanden Bogaerde, 2009). It is worth noting that another study found that the application of HA gel accelerates the healing process in tooth sockets of rats (Mendes et al., 2008). It stimulates the expression of osteogenic proteins such as BMP-2 and osteopontin by local cells, thus indicating a osteoinductive potential of HA application. In addition, the presence of HA in the blood clot favors its invasion by mesenchymal cells, which are involved in bone tissue repair (Aslan, Simsek, & Dayi, 2006; Toole, 2001). Hence, HA is a promising candidate as an adjuvant in bone regeneration clinical procedures. Nevertheless, it may affect the membrane collagen degradation profile. That is to say, HA may hinder the membrane's role as a mechanical barrier and as a substrate for colonization by regenerative cells. Therefore, the aim of this study was to evaluate whether HA application influences collagen membrane degradation in rats. This study focused on two collagen membranes ubiquitously used in regenerative techniques, a porcine type I and III collagen membrane (BioGide®) (BG) and a bovine type I collagen membrane (OsseoGuard™) (OG).

2. Material and methods

2.1. Animals

The animal selection, management, and surgery protocol were approved by the Animal Care and Use Committee of The Pontifical Catholic University of Minas Gerais, Brazil. This study included thirty-two male albino rats of the Wistar strain (Rattus norvegicus), six months old, and weighing 300–400 g. During the experiment, the animals had ad libitum access to filtered water and rodent chow (NUV-Lab – Purina, Brazil).

2.2. Evaluated membranes

According to the manufacturer, BioGide® (BG; Geistlich Pharma AG, Wolhusen, Switzerland) is a pure collagen type I and III membrane of porcine origin. This collagen membrane is processed into a bilayered structure; one side of the structure is compact and smooth, constituting a low-porosity compact layer, while the other side is a more porous, three-dimensional spongy layer.

As described by the manufacturer, OsseoGuard™ (OG; Collagen Matrix Inc/Biomet 3i, Palm Beach Gardens, FL, USA) is a pure collagen type I membrane of bovine origin. This membrane has a pore size that allows it to be occlusive to gingival and epithelial cells, while still permeable to essential nutrients and gases.

2.3. Scanning electron microscopy (SEM) analysis of the membranes

A morphometric ultrastructural analysis of the membranes was conducted prior to the in vivo experiment. Sections of the membranes, measuring $8 \times 8 \times 8$ mm, were mounted onto aluminum and sputter coated with gold, under vacuum. The upper and lower surfaces of each specimen were then viewed and photographed under SEM (JSM – 6510LV, Jeol Ltd, Tokyo, Japan).

2.4. Surgical procedures

The animals were anesthetized by intramuscular injection of 0.1 mL/100 g ketamine hydrochloride 10% (Cetamin®, Syntec, Brazil) and 0.1 mL/100 g xylazine hydrochloride 2% (Xilazin®, Syntec, Brazil) on the inner face of the animal thigh. The frontal part of the skin covering the scalp was shaved. Following disinfection with 2% chlorhexidine digluconate, a midline linear incision was performed. Full thickness flaps were reflected, including skin and mucoperiostal tissues, to fully expose the calvarial bone (open flap debridement). Two bone defects were then drilled on each parietal bone region of each rat (Fig. 1A) using a trephine bur (d = 3 mm), operated at 900 rpm under continuous irrigation with chilled sterile saline solution, avoiding dural perforations. Half of the animals (n = 16) had the defects naturally filled with blood clot (control group). In the other animals, HA gel 1% (Farmanostra, São Paulo, Brazil) was injected directly into the defects using a small needle (26G) (HA group). The two different membranes, OG or BG, were then used to cover up the bone defect regions in both groups. As shown in Fig. 1B, the membranes were applied with the rougher side (lower surface) facing the bone defect, when applicable. Each kind of membrane was used in half of the specimens in each group. In the HA group, the membranes were soaked in HA gel 1% for 30 min before placement. In the control group, the membranes were hydrated with sterile saline solution for 30 min before implantation. For complete coverage of the bone defect regions, the membranes were prepared with a dimension of 12×6 mm. The soft tissues and periosteum were repositioned and the dermal tissues were sutured. The postoperative healing was uneventful in all rats.

2.5. Histological procedures

The rats were euthanized after 1 (4 animals), 30 (14 animals), and 60 days (14 animals), for histologic evaluation. Their eyes and jaws were removed to facilitate fixation and processing of the calvarias. Block sections containing the calvarias and surrounding tissues were explanted and fixed in 10% formalin for 48 h. After fixation, the block sections were demineralized in 10% ethylenediamine tetraacetic acid (EDTA) pH 7.3, dehydrated through graded ethanol solutions, embedded in paraffin, and serially sectioned at 4 μm in the sagittal plane (Fig. 1C). The sections obtained were stained with hematoxylin eosin (HE) for histological analysis.

An experienced researcher, blinded to the experimental conditions, performed the histological analysis. Only histological slides of the central region of the defects were included in the study. The slides were evaluated using a binocular light microscope (Olympus BX51, Olympus, Hamburg, Germany). The structure and degradation of the collagen membranes were analyzed first. In addition, the following parameters were evaluated: inflammatory cell infiltration and foreign body reaction (i.e., presence of