Biodegradation of three different collagen membranes: A histological study

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ABSTRACT

Aim The aim of this study was to evaluate in vivo the absorption rate of three membranes: bovine collagen (GenDerm, Baumer, Brazil), collagen (Genoss, Dentium, Korea) and porcine dermis membrane (Kytinon M.O.R., Asmopul, Argentina). The additional aims were to evaluate blood vessel penetration and fiber collagen penetration in the early wound healing process.

Materials and Methods Four dogs were used for the study and the membranes were applied by subperiosteal placement and analyzed after 15, 30, 60 and 90 days.

Results The absorption rate of <35% at 15 days was similar for all the membranes. At 30 days, rates between 35% and 65% were observed. After 60 days, only the bovine collagen membrane (GenDerm, Baumer SA, Brazil) was found to have achieved >65% absorption. This bovine collagen membrane was completely absorbed after 90 days. At this time point absorption of the bovine collagen (Collagen®, Dentium, Korea) was >65%, while absorption of the porcine dermis (Kytinon®, Asmopul, Argentina) was found to be between just 35% and 65%.

Conclusion Blood vessel penetration and fiber collagen penetration in the three membranes showed significant differences, with faster vascularization and collagen penetration in the more rapidly absorbed membranes.

INTRODUCTION

Guided bone regeneration, a common procedure in implant surgery (1), uses barrier membranes, excluding epithelial and connective tissues, to enable bone progenitor cell proliferation and differentiation into the isolated area (2, 3). Both bio-absorbable and non-absorbable barrier membranes are effective (1). Absorbable membranes with comparable clinical outcomes (4, 5) have become more popular in bone-regeneration procedures. Successful regeneration is possible if cell exclusion and space maintenance for the clot are maintained throughout the required period. Ramseier et al. (6) underline the key importance of the stability of the blood clot and the wound in the early healing phase. The required duration of a membrane before its resorption can vary between 3 and 12 months, depending on the dimensions of the bone defect (6, 8, 10). Polimeni et al. (9) conclude that: "Current scientific evidence points to the presence of cells originating from the periodontal ligament, wound stability, space provision, and primary intention healing, as fundamental biological and clinical factors that must be met to obtain periodontal regeneration". Absorbable membranes may be prepared from dura mater (7), polylactic acid (10, 11), polyglycolic acid, polyurethane (10) and collagen. The ability of collagen to promote progenitor cell adhesion, chemotaxis, homeostasis and physiological degradation, together with its ease of manipulation and low immunogenicity, make it an ideal material (12) for barrier preparation (12). Types I and III collagen of bovine or porcine origin (12, 13) are the main components of most commercially available collagen membranes.

Collagen has several advantages compared to other absorbable membranes, including the aforementioned unique biological properties and the fact that there is no need for a second surgical procedure to effect retrieval (6). One issue concerning collagen membranes is whether they act as an intact barrier long enough to have predictable outcomes in periodontal and other oral surgical procedures (11). In some clinical cases, rapid resorption is advantageous; in other cases longer resorption times will offer better results (23). While they require the combined use of a bone graft, collagen membranes have many potential applications in periodontal regenerative surgery and other oral surgical procedures, thanks to their ease of handling and resorbability. Little comparative information is available on the absorption rates of collagen membranes in the oral cavity. The three membranes used in this experiment were Gen Derm® (Baumer SA, Brazil) (20 x 20 mm), composed
of Type I native bone collagen obtained in a standardized process for removal of the mineral portion and lamination of cortical bovine bone; Kytinon® (Asmopul, Argentina) (4 x 2.5 mm), composed of lyophilized porcine dermis, Type I collagen (both membranes have an absorption time of 45 days, as described by the manufacturer at http://ar.unidentaldirect.com/kytinon-reabsorbable-membrane.html); and Collagen® (Dentium Co., Korea) (10 x 20 mm), pure Type I collagen derived from bovine tendon with an absorption period of 4–6 months, providing sufficient time for clot stabilization, together with the graft materials and supporting bone growth. The basement membrane complex (BMC) is retained in order to facilitate epithelial migration and attachment (4, 5).

The purpose of this study was to determine the rate of absorption of the three above mentioned specific collagen membranes in the oral cavity. Additional aims were to evaluate blood vessel penetration and fiber collagen penetration, and to examine whether membrane presence persists long enough to allow desirable healing events to occur.

MATERIALS AND METHODS

The study design followed the ARRIVE guidelines for reporting animal research. The protocol was approved by the University’s Ethics Committee (UCSUR-Lima, Peru) (Ethical Committee Number: PP004).

Four young adult mongrels (weighing approximately 10 kg to 15 kg each) were involved in our research project. The dogs were managed and cared for through the application of uniform acceptable standards in facilities designed for holding animals, and in caging which provided for their comfort and safety. Special emphasis was placed on the avoidance of unnecessary pain, suffering or injury to the animals during holding, experimentation and the post-experimentation period, through monitoring of their housing, environment, feeding and veterinary care. During our research the animals participated concomitantly in additional research projects within our institution which required animal sacrifice as part of the investigatory process. The scheduled animal sacrifice dates for these projects were incorporated preliminarily into our investigation in order to minimize and essentially eliminate the use of additional animal experimentation. These ongoing research projects did not influence in any way our own research process.

Experimental design

All procedures were performed under sterile conditions in an operating room. The animals were anesthetized intravenously via administration of 5 to 7 mg/kg of zolazepam tiletamine (Zoletil 50, Virbac, Carros, France), accompanied by anesthetic induction with fentanyl and local anesthesia in the form of Xylocaine 2% with Epinephrine 1:100,000 prior to all surgical procedures. A full-thickness flap was elevated and the selected collagen membrane was placed to ensure proper irrigation; small defects in edentulous areas between the first premolar and the canine were selected mesially and distally.

Each dog received one of each type of the three membranes being evaluated on opposite sites of the maxilla in a randomized manner: bovine collagen (GenDerm Baumer, Brazil), collagen (Genoss Dentium) and porcine dermis membrane (Kytinon M.O.R Asmopul, Argentina). The flap was sutured to facilitate primary intention healing.

For pain control, all dogs received Tramadol postoperatively for 7 days. Daily postoperative evaluations were performed to monitor healing and discomfort until the palate was sufficiently healed to enable dogs to return to a regular diet. A daily inspection of the wounds was performed to check for clinical signs of complications.

The dogs were randomly assigned a procedure number, indicating the material used and healing times. The treatment code was designed to record the side of the jaw treated and healing times.

The dogs were sacrificed at 15, 30, 60 and 90 days, depending on which group each dog was assigned to, based on preliminary knowledge of sacrifice dates. The animals were first anesthetized by intravenous injection of Zoletil® (5 to 7 mg/kg, Virbac, Carros, France). The animals were euthanized by lethal injection consisting of an overdose of barbiturate (Dolethal®, Vetoquinol Paris, France) and perfused through the carotid arteries with a fixative containing a mixture of 5% glutaraldehyde and 4% formaldehyde.

A biopsy of each tissue-containing sample was performed using a 4 mm diameter soft tissue punch to obtain a full-thickness cylindrical sample from the surface tissue to the bone.

Histological preparation

A total of 16 samples (four from each group) were included in this study (3 biopsies of the membranes in the test group and a sample of gingival tissue as a control). The soft tissue samples were placed in individual vials containing fresh 10% neutral buffered formalin. A total of 16 biopsies were included in the analysis; 4 biopsies for each dog (4). The specimens were embedded in a glycol methacrylate resin (Technovit 7200VLC, Heraeus Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned using a high-precision diamond blade (Exakt, Apparatebau, Norderstedt, Germany), delivering approximately 20 µm cleaving accuracy. The sections were prepared for histological analysis and subsequent histomorphometry after being stained with hematoxilin and eosin for standard optical microscopy analysis.

Two trained histologists (kappa intra-class correlation coefficient >0.82) independently evaluated each slide, using an Eclipse E200LED microscope (Nikon Corporation, Tokyo, Japan) equipped with a video camera (Digital Sight DS-Fi2, Nikon Corporation, Tokyo, Japan), and a camera controller (DS-L3, Nikon Corporation, Tokyo, Japan). Using ImageJ software, the amount of remaining membrane could...
be measured and the number of vessels and collagen fibers counted, to assess degradation of the membranes and the presence/absence of these indicators in the histological samples, and these scores were later compared. In the event of any discrepancy, the two investigators reviewed the slide together in order to arrive at a consensus on the score. A millimeter scale was used in the eyepiece of the 20x magnification microscope. Results were expressed as the number of positive cells per square millimeter. The biopsies were then compared to normal dog tissue samples. Each specimen was given a score from 0 to 4 for membrane condition: 0 = intact, easily identifiable; 1 = slight degradation (35%); 2 = moderate degradation (35%–65%); 3 = severe degradation (65%); and 4 = absent, not identifiable.

RESULTS

Postoperative healing after insertion of the membranes was successful in all cases.

Histological analysis

Membrane degradation rates were variable over different time periods and among individual dogs (Table 1, Fig. 1). Scores ranged from 1 to 2 for all membranes at 30 days. Scores ranged from 2 to 3 for all membranes at 60 days. Only the bovine collagen membrane demonstrated complete biodegradation (score of 4) after 3 months. Blood vessel penetration varied among the samples. Blood vessel penetration was found to be slight and moderate for most of the samples at 90 days (Table 2, Fig. 2). Only one sample showed any signs of blood vessel penetration at 90 days. Slight and moderate inflammation was found in the collagen membranes (Gen Derm®, Collagen®) samples after the first 30 days. No signs of inflammation were found at 60 days (Table 3). The bovine collagen membrane showed the highest penetration of collagen fibers at 90 days, compared with porcine dermis and bovine collagen II membrane (Table 4, Fig. 4). The absorption rate was <35% at 15 days for all the membranes, and between 35%-65% at 30 days. After 60 days, only the bovine collagen GD membrane displayed >65% absorption (Fig. 5). After 90 days the bovine collagen GD was completely absorbed (Fig. 6), the bovine collagen C demonstrated >65% absorption, while the porcine dermis membrane displayed a 35% to 65% absorption (Fig. 7).

DISCUSSION

The majority of research has shown that the wound clot
must be kept stable and protected during the first period of healing in order to promote regeneration (9, 15, 16). If the membrane dissolves or fragments before 4 weeks its goal will not be accomplished (17, 18). Bragger et al. (19) found that all alveolar bone adjacent to sites exposed to guided tissue regeneration demonstrated a slow consolidation of the tissues, represented by delayed mineralization, compared with changes in probing attachment levels, when used for periodontal regeneration. Using reinforced titanium ePTFE membranes, Sigurdsson et al. (20) demonstrated that dogs produce no regeneration at 3 weeks, whereas at 8 weeks 75% of the bone has regenerated, together with 40% of the cementum. If collagen barriers are completely resorbed before day 30, new cementum may be found in the healing area, but no new bone (21). Miller et al. (22) found that in rabbits certain cross-linked collagen membranes were resorbed within 2 weeks. Owens and Yukna (23) compared three different collagen membranes (BioGide, AlloDerm porcine-derived, and Allo-Derm human-derived membrane), and at one month all membranes demonstrated slight to moderate degradation. At two months, all membranes demonstrated moderate to severe degradation with the exception of one AlloDerm human-derived membrane, which remained intact. At three months, all the membranes were scored from severe degradation to not identifiable. At four months, all the membranes demonstrated severe degradation to complete absence. Oh et al. (24) compared the clinical and histological features of two collagen membranes (Bio Gide and Bio-Mend Extend) for their effect on implant dehiscence defects in dogs. After four weeks no significant differences were observed between the groups; however, at four months a higher linear percentage of bone fill in large areas was observed. It was concluded that treatment with collagen membranes can stimulate bone regeneration significantly during later stages (four months), and that maintaining coverage of the space and the membrane represented two major factors affecting collagen membrane outcomes in periodontal and other oral surgical procedures.

In our study, we obtained interesting results concerning membrane biodegradation; whereas in some cases biodegradation was gradual, in the case of the bovine collagen it was much faster, leading to the rapid penetration of soft tissue, which could disrupt the occlusivity supposedly offered by this type of membrane. The aim of our study was to test the membranes in order to establish their actual behavior in our patients, and to use these findings to enable the correct selection of the membranes used in regenerative treatment of our patients. One criterion could be the number of walls of the defects, as this could influence the stability of a membrane.

**CONCLUSIONS**

The rate of absorption of the three specific collagen membranes in the oral cavity was similar at 15 and 30
membranes. collagen penetration in the more rapidly absorbed different membranes, with faster vascularization and penetration and fiber collagen penetration in the three Significant differences were found in blood vessel displayed different absorption rates (>65% and 35-65% bovine collagen C and the porcine dermis membranes still and after 90 days it was completely absorbed, while the GD membrane displayed higher absorption rates (>65%) days (<35% at 15 days and between 35%-65% at 30 days), whereas after 60 days, only the bovine collagen GD membrane displayed higher absorption rates (>65%) and after 90 days it was completely absorbed, while the bovine collagen C and the porcine dermis membranes still displayed different absorption rates (>65% and 35-65% respectively).

Significant differences were found in blood vessel penetration and fiber collagen penetration in the three different membranes, with faster vascularization and collagen penetration in the more rapidly absorbed membranes.

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REFERENCES