A comparison of the in vitro resorption rate of human amnion membrane and commercially available bovine collagen membrane for potential periodontal applications

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Abstract

Introduction: There is a dire need for a reliable substitute for the connective tissue autografts for root coverage procedures. Recently, human amnion membrane is increasingly being used in this regard.

This study investigates the in vitro resorption rate of human amnion membrane and compares it with the resorption rate of commercially available bovine collagen membrane.

Materials and Methods: Thickness of human amnion membrane samples and bovine collagen membranes were measured. The in vitro resorption tests were conducted by placing the (i) human amnion membrane and bovine collagen membrane of equal sizes (10 mm × 5 mm) in 5 ml of pH 7.4 phosphate buffer solution on a shaker set at 40 rpm for 4 weeks. The resorption rates were expressed as the accumulated weight losses of the membranes at the end of first, second, third and fourth week.

Results: The average thickness of the amnion membranes used in this study was .46mm.The thickness of the bovine collagen membrane was .37mm.Amnion membranes degraded 21% of its initial weight at the end of the first week, 24.1%at the end of second week, 31.35% at the end of the third week and 70% at the end of four weeks.

Bovine collagen membranes degraded 6%of its initial weight at the end of the first week, 13% at the end of second week, 49% at the end of the third week and 80%at the end of four weeks

Conclusion: Human amnion membranes could assist root coverage procedures since they resist complete degradation even at 4 weeks.

Keyword: Human amnion membrane.

Introduction

Gingival recession poses both esthetic and functional challenges to the patient. The sub epithelial connective tissue graft is the gold standard technique in isolated gingival recessions. Recent literature indicates the bilaminar techniques as the most predictable root coverage surgical procedures.[1,2] Here a bilaminar vascular environment is created to nourish the connective tissue autograft. Donor site morbidity and lack of sufficient tissue for the coverage of multiple roots is a major limitation of the connective tissue autograft.[3] There is an intense need for a reliable substitute for autogenous connective tissue graft for gingival augmentation procedures. Acellular dermal matrix graft is an allograft which is being used in this regard.[4] Human amnion membranes are now being increasingly used in periodontal reconstructive procedures especially in root coverage. Amnion membranes possess distinctive properties that can be harnessed to promote periodontal healing. These placental allografts reduce inflammation and provide a matrix highly rich in protein and facilitate migration of cells at the area of defect.[5] Human amnion membranes have anti scarring, antimicrobial properties, they have low immunogenicity and is a reservoir of stem cells and growth factors. They also enhances wound healing and angiogenesis.[6] Though there is ample evidence in literature regarding their biologic properties, lacunae exists regarding how long the membrane survives at the wound healing site. This study provides evidence on the *in vitro* survival period of the amnion membrane in comparison to the commercially available bovine collagen.

Materials and Methods

Freeze dried irradiated amnion and membranes were purchased from the Tissue bank of Tata Memorial hospital.

Preparation of amnion: In the production of the amnion allograft used in this study, prescreened, consenting mothers donate the amnion and associated tissues during elective cesarean section surgery. All donated tissue follows strict guidelines for procurement, processing, and distribution, as dictated by the Tissue Bank, (Tata Memorial Hospital, Mumbai). These safety measures include testing for serological infectious diseases such as human immunodeficiency virus (HIV) type 1 and 2 antibodies, human T-lymphotropic virus type 1 and 2 antibodies, hepatitis C antibody, hepatitis B surface antigen, hepatitis B core total antibody, serological test for syphilis, HIV type 1 nucleic acid test, and hepatitis C virus nucleic acid test. Upon collection of the maternal tissue, the amnion and chorion tissues are carefully separated, and they are cleansed prior to processing.

The allografts are dehydrated, perforated, and terminally sterilized. (Fig. 1)

Commercially available bovine collagen (

Healiguide, EnColl, Freemont, CA, US) was purchased. The thickness of both the membranes were measured at six different points using a digital calliper (S. C. Decy Co) to calculate the average thickness.

In vitro resorption rates of the membranes was carried out at the Pushpagiri Research Centre. The in vitro resorption tests of the prepared membranes were conducted by placing five strips of each membrane of size 10 × 5 mm in 5 ml of pH 7.4 PBS on a shaker set (Orbital Kahn Shaker) (Fig. 2) at 40 rpm. All the membranes were pre weighed on an electronic micro weighing scale at baseline. They were weighed after hydration and at the end of 1st, 2nd, 3rd and 4th week. The degradation profiles were expressed as the accumulated weight losses of the membranes.

Results

The average thickness of the amnion membranes used in this study was .46mm.The thickness of the bovine collagen membrane was .37mm.Amnion membranes degraded 21% of its initial weight at the end of the first week, 24.1%at the end of second week, 31.35% at the end of the third week and 70% at the end of four weeks. (Fig. 3)

Bovine collagen membranes degraded 6%of its initial weight at the end of the first week, 13% at the end of second week, 49%at the end of the third week and 80%at the end of four weeks.

Discussion

Harvesting connective tissue from the palate for gingival augmentation procedures causes donor site morbidity, discomfort to the patient and thereby decreased patient acceptance. Many times adequate tissue necessary for coverage of multiple roots cannot be obtained. Hence the search for a reliable substitute allograft is ongoing. The amnion membrane represents the innermost layer of the placenta and is composed of a single epithelial layer, a thick basement membrane and an avascular stroma. The special structure and biological viability of the membrane allows it to be an ideal candidate for creating scaffolds used in tissue engineering. Human amnion membrane has excellent biologic properties and is now increasingly used in root coverage procedures sandwiched beneath a coronally repositioned flap.[7-9] As a potential allograft for root coverage procedures, it is important to understand how long the amnion membrane could survive at the defect site. In our study we observe the resorption rate of human amnion membrane in comparison to commercially available bovine collagen membrane. Both membranes resorb completely in vitro. Human amnion membranes retain their physical form up to end of three weeks and is not completely resorbed at the end of 4 weeks. Commercially available bovine collagen also resists total resorption upto 4 weeks.

Periodontal wound healing/regeneration largely appears complete within 2–3 weeks of wound closure, to be followed remodelling/tissue maturation to meet functional demands.[10] In this context, biomaterials like foetal allografts targeting regeneration serve their purpose if they can retain their physical form for a period of 2-3 weeks. amnion membrane can also be used as a reliable mucosal substitute or drug delivery surgical patch in the oral cavity. In this study, amnion membranes resist complete resorption upto a period of 4 weeks. Pollard S. M., Aye N. N., Symonds E. M. (1976)[11] attribute the resistance of amnion membranes to proteolytic degradation to the presence of interstitial collagens. Their rate of disintegration is similar to commercially available bovine collagen.

Bunyaratavej P, Wang HL (2001)[12] report that collagen membranes have unpredictable resorption rates, which can vary from 4 to 24 weeks. They also state that non-cross-linked collagen membranes have a half life that varies between 7 and 28 days. Most resorbable membranes degrade during a period of four to eight weeks.[13] Platelet rich fibrin membrane degrades in a period of less than one week.[14]Proteolytic degradation could accelerate degradation invivo but when used in the bilaminar technique for root coverage, the sandwiched location of the membrane beneath the flap could prolong degradation in vivo. Results of in vitro degradation rates of amnion is in concordance with the report of Chopra A, Thomas BS (2013).[15]

Limitations of this study was the inherent biologic nature of the human amnion membrane. Thickness differs at different areas. Malak and Bell, 1994[16] report areas of unique morphological features, which were only found within a restricted area, termed as "zone of altered morphology" (ZAM). This feature includes structural weaknesses and a marked disruption of the connective tissue layers as well as a marked reduction of the thickness and cellularity of the membrane. These areas are to be discarded. All the membrane samples tested do not reveal identical resorption rates. The membranes lack rigidity and they could collapse into the defect if not supported by a graft if they are used as barrier membranes in GTR.

Fig. 1: Human amnion membrane and bovine collagen membrane

Fig. 2: Shaker set

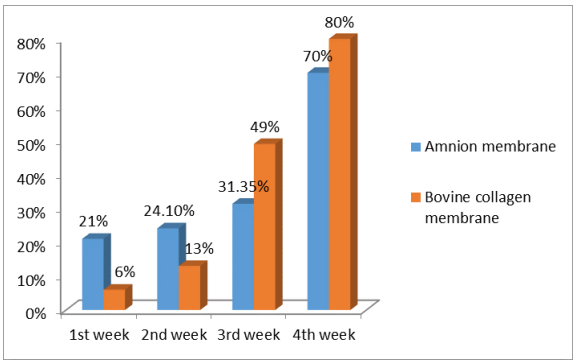

Fig. 3: Graph showing resorption rate of HAM and bovine collagen

Conclusion

Adequate stability to in vitro degradation do support the use of human amnion membrane as an allogenic alternative to the connective tissue autograft in the bilaminar technique of root coverage. Foetal membranes are cost effective and easily available. More clinical and in vivo histologic studies are needed to underline the effective use of foetal membranes in root coverage procedures.

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