AN *IN VITRO* INVESTIGATION AND COMPARISON OF THE MECHANICAL PROPERTIES OF HUMAN AMNION AND BOVINE COLLAGEN MEMBRANE

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ABSTRACT

Introduction: The human amnion membrane is now increasingly used in Guided Tissue Regeneration(GTR). These placental allografts have excellent biologic properties. There is limited information regarding their mechanical properties as compared to other collagen membranes used in GTR. The aim of this study was to test and compare the mechanical properties of human amnion membrane with commercially available bovine collagen.

Materials and methods: After measurement of average thickness, three strips of 5×1cm were cut from both amnion and bovine collagen membranes. Mechanical testing was done in a universal testing machine. Test speed was 1mm/min with a load cell of 100N. Tensile strength, Young's modulus and elongation at break were calculated. A suture retention test was also carried out using 3x1 cm size strips. Groups were compared through the Mann-Whitney U test.

Results: Average thickness of amnion membrane was 0.46mm and thickness of bovine collagen membrane was 0.37mm.Tensile strength, Young's modulus and elongation at break of amnion membrane were 0.156MPa, 0.645MPa and 17mm; and that of bovine collagen membrane were 2.94MPa, 7.42MPa and 11.00mm, respectively ($p = 0.05$). Maximum load which can be applied during suturing for amnion membrane was 0.104N and for bovine collagen membrane was 2.07N ($p = 0.037$).

Conclusion: There is no statistically significant difference in terms of tensile strength, Young's modulus and elongation at break between human amnion and bovine collagen membranes.($p=0.05$)Membranes can only be considered different on the maximum load which can be applied during suturing.($p=0.037$)

Key words: Collagen, Mechanical testing. R Periodontia 2018; 28: 07-12.

INTRODUCTION

Guided tissue regeneration encompasses procedures attempting to regenerate specific anatomical structures through differential tissue responses (Quiñones & Caffesse, 1995). Guided tissue regeneration involves the exclusion of epithelial cells and gingival fibroblasts during the healing phase of periodontal defect by devices called barrier membranes (Tatakis *et al.*, 1999). GTR facilitates the colonisation of the wound space by undifferentiated mesenchymal cells from the periodontal ligament.

The use of a cellulose acetate laboratory filter provided the first human histological evidence of periodontal regeneration in response to GTR (Nyman *et al.*, 1982). Since then, several barriers made out of a variety of materials have been introduced.Design criteria necessary for guided tissue regeneration devices are biocompatibility, Cell exclusion, Space maintenance, Tissue integration, Ease of use (Scantlebury, 1993; Gottlow, 1993; Hardwick *et al.*, 1995) and Biological activity (Tatakis *et al.*, 1999). The non-resorbable barrier membranes were first developed. Though they have structural integrity,the need for a surgicalre-entry was a major limitation. Most non-absorbable devices are made from polytetrafluoroethylene or expanded polytetrafluoroethylene. Absorbable barriers do not require additional surgery for removal and hence has more patient acceptability, less chair-side time and cost and also eliminates potential surgery-related morbidity (Tatakis *et al.*, 1999). Absorbable barrier membranes can be either natural products or synthetic materials. Natural products tested as guided tissue regeneration devices include collagen,dura mater, cargile membrane, oxidized cellulose and laminar bone. Bovine, porcine and fishcollagen membranes have been tried extensively in GTR.

The human amnion membrane is now being increasingly used both in intra bony defects (Holtzclaw & Toscano, 2013; Kiany & Moloudi, 2015) and also in the coverage of denuded roots (Esteves *et al.*, 2015).The amnion as its Greek name suggests is a membranous sac that contains the foetus and the amniotic fluid. Amniotic membrane or amnion is the innermost layer of the placenta and consists of a thick basement membrane and an avascular stromal matrix. Human amniotic membrane (HAM) has been successfully used over the years for a wide range of surgical applications (Davis, 1909; Bennett *et al.*, 1980; Morton & Dewhurst, 1986; Güler *et al.*, 1997; Lee & Tseng, 1997; Luanratanakorn *et al.*, 2006; Memarzadeh *et al.*, 2006).The amnion serves as a natural barrier to bacterial infection and trauma (Shubert *et al.*, 1992).The membrane can be a scaffold for proliferation and differentiation due to the presence of fibronectin, elastin, nidogen, collagen types I, III, IV, V, and VI, elastin, and hyaluronic acid (Fukuda *et al.*, 1999).This placental membranelacks immunogenicity and also has anti-inflammatory properties, antifibrotic properties, antibacterial properties, and antiangiogenic properties (Niknejad *et al.*, 2008).

An essential design criteria for a GTR device is its ability to provide adequate space for the regenerating alveolar bone, periodontal ligament, and cementum. Space provision requires mechanical properties and/or structural features that enable the barrier to withstand forces exerted by tissue tensionfrom the overlying flaps and forces of mastication or physiologic forces which may be transmitted through the flaps.Lack of adequate mechanical properties may prevent collapse of the soft tissue and elimination/ reduction of the wound space (Wikesjo *et al.*, 1992; Haney *et al.*, 1993; Sigurdsson *et al.*, 1994). Even though the use of human amnion in guided tissue regeneration is adequately backed by its excellent biologic properties, there is limited knowledge regarding their mechanical properties. This study compares the mechanical properties of human amnion membrane with commercially available bovine collagen membrane.

MATERIALS AND METHODS

Study design: In this study an in vitro qualification of the mechanical properties of human amnion membrane and bovine collagen membranes were done in a comparative manner. Freeze dried irradiated human amnion membranes were purchased from the Tissue bank of Tata Memorial hospital. Bovine collage membranes were also procured. (Healiguide, EnColl, Freemont, CA, US).Purposive sampling plan was followed.

Preparation of human amnion membrane: For the placental allograft used in this study, prescreened, consenting mothers donated the amnion and associated tissues during elective caesarean section surgery. All donated tissue followed strict guidelines for procurement, processing, and distribution, as dictated by the Tissue Bank, (Tata Memorial Hospital, Mumbai). These safety measures included 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. After collection of the maternal tissue, the amnion tissues were carefully separated, and cleaned prior to processing. The amnion membranes werethen dehydrated, perforated, and sterilized and packed for distribution.

Mechanical testing of the membranes was conducted at the Biomedical wing of the Sree Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram. The thickness measurements of both the membranes was done by a digital calliper (Baker.002-.5mm, Type J11) prior to mechanical testing. The thickness of both the membranes was recorded at six different points to calculate the average thickness.

Three samples (5×1cm) each from both membrane types were drawn for mechanical testing. Another three samples 10mm×3 cm) each from both membrane types were drawn for evaluating the maximum load which could be applied during suturing

Three strips of 5×1cm were cut from both amnion and bovine collagen membranes. The membrane strips were immersed in phosphate buffered saline solution for fifteen minutes before mechanical testing. The mechanical testing device used in the study is Instron, model 3345 (Made in the United Kingdom) Gage length (distance between the clamps) used was 3 cm. During mechanical testing, a longitudinal strain is applied at a constant rate along the main size of the rectangular piece of membrane. Test speed was 1mm/mt with a load cell of 100N. Tensile strength, Young's modulus and elongation at break were calculated.(figures 1and 2) A suture retention test was also carried out. In the suture retention test,

FIGURE 1: MECHANICAL TESTING FOR HUMAN AMNION MEMBRANES

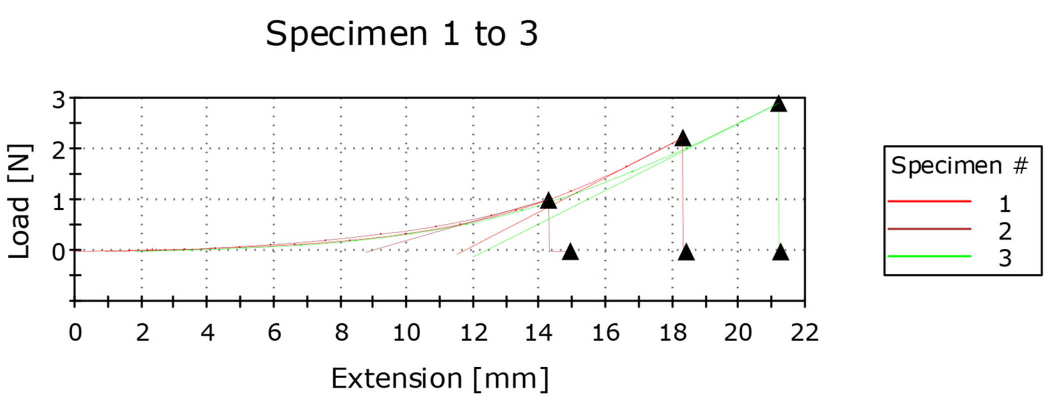
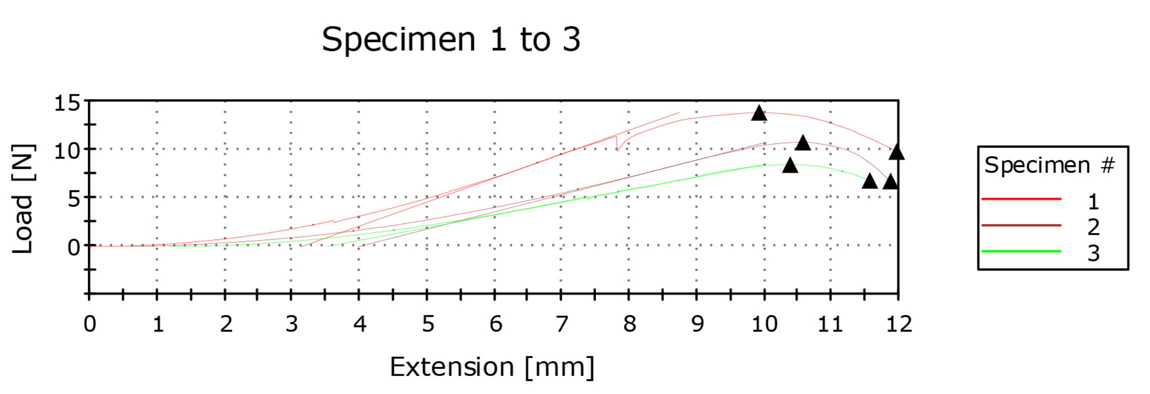


FIGURE 2: MECHANICAL TESTING FOR BOVINE COLLAGEN MEMBRANES



10mm×3 cm size strips were used. In the universal testing machine the lower part of the membrane was inserted into the lower clamp and upper part of the membrane is left free. At 0.2mm from the upper part of the membrane a suture is inserted into the membrane and then into the upper clamp and pulled upwards at a speed of 1mm/min. The suture was pulled continuously till the membrane ruptured. Maximum load prior to rupture of each membrane was recorded and this gave the maximum load which could be applied during suturing.

Statistical Analysis: Mean, standard deviation,median and Coefficient of variation were calculated for Tensile strength, Young's modulus, Extension at break and Suture retention load for both the membranes. Mann-Whitney U test was used for testing the statistical significance in the difference between these membranes. A p value of less than 0.05 was considered to be significant ($p<0.05$) for the study.

RESULTS

Average thickness of amnion membrane was 0.46mm and thickness of bovine collagen membrane was 0.37mm. The meantensile strength of amnion membrane was 0.156MPa (median was 0.186MPa) and that of bovine collagen membrane was 2.938MPa(median was 2.87MPa.) ($p=0.05$)

Mean Young's modulus was 0.645MPa (median was 0.744MPa) for the human amnion membrane and 7.42 (median - 7.129MPa) for the commercially available bovine collagen. ($p=0.05$)

The mean value for the elongation at break was17.25mm (median-18.393mm) for the amnion membrane and was11.18mm (median 11.26mm) for the bovine collagen membrane. ($p=0.05$)

The average value for the maximum load that can be applied during suturing for the human amnion membrane

TABLE 1: COMPARISON OF MECHANICAL PROPERTIES OF HUMAN AMNION MEMBRANE AND COMMERCIALLY AVAILABLE BOVINE COLLAGEN								
Mechanical Property	Tensile strength		Young's Modulus		Extension at break		Max.load that can be applied during suturing	
Type of Membrane	Bovine collagen	Human Amnion	Bovine	Human Amnion	Bovine	Human Amniotic	Bovine	Human Amniotic
Mean (SD)	2.9384 (0.7202)	0.1560 (0.0826)	7.4205 (2.3414)	0.6452 (0.2605)	11.1863 (0.7960)	17.2515 (2.8748)	2.0782 (0)	0.1044 (0.0592)
Median	2.87	0.186	7.129	.744	11.26	18.393	2.0782	.137
Coefficient of Variance (%)	24.51	52.95	31.55	40.37	7.11	16.66	0	56.7
P Value	0.050		0.050		0.050		0.037	

was 0.14N (median was 0.137N) and that for the bovine collagen membrane was 2.078N (median was2.08) (p=0.037)

Coefficient of variation was higher for human amnion membrane. The descriptive statistics of the mechanical properties and the results of the tests of significance of both the membranes are given in Table 1.

DISCUSSION

In guided periodontal tissue regeneration, a physical barrier is placed between the gingival flap and the debrided root surface prior to flap repositioning and suturing. Barrier membranes used in GTR are eitherresorbable or non-resorbable. The absorbable barriers may be of either natural or synthetic origin. A variety of resorbable barriers have been tested for safety and efficacy in promoting guided tissue regeneration such as collagen, polyglycolic acid, polylactic acid, or copolymers of these materials (Quiñones & Caffesse, 1995). The potential value of these barriers is that they resorb and consequently do not require a second surgical procedure for their removal. The "ideal" resorbable barrier material must effectively exclude gingival epithelium and connective tissue, permit the selective repopulation of the root surface and adjacent alveolar wound area by periodontal ligament and/or alveolar bone cells, resorb without exerting adverse effects on the healing process and must be replaced by the periodontal connective tissues (Scantlebury, 1993; Gottlow, 1993; Hardwick *et al.*, 1995). They must have enough stiffness to facilitate positioning and must be soft enough to be closely adapted to different defect morphologies (Greenstein &Caton, 1993). The search for the "ideal"GTR device is ongoing.

The human amnion membrane is now being widely used in periodontal regeneration (Gurinsky, 2009; Singh & Singh, 2013; Shetty *et al.*, 2014) and this placental allograft has good biologic properties to support their use. The structure of amniotic membrane has three parts which are epithelial monolayer, a thick basement membrane, and an avascular stroma. This epithelium is firmly fixed to a basement membrane which is in turn attached to a condensed acellular layer. This layer is composed of collagen types I, II, and V. The human amnion membrane is avascular (Niknejad *et al.*, 2008).

However, to be used as a GTR device, the membrane requires mechanical properties to ensure space maintenance and wound protection (Tatakis *et al.*, 1999).In our study we compare the mechanical properties of human amnion membrane to commercially available bovine collagen used for GTR. The commercially available bovine collagen (Healiguide) used in this study is made of high purity Type 1 collagen derived from selected animal tissues.

The average thickness of human amnion membrane used in the study was 0.46mm and that of commercially available bovine collagen membrane was 0.37mm.These results agree with previously reported data (Rao & Chandrasekharam, 1981).

Rao and Chandrasekharan(1981) report that the thickness of amnion ranges from .02 to 0.5mm and that, one of the major advantages of foetal membranes in comparison to other biodegradable membranes, is their thinness and good adaptability. The thickness of the human term amnion varies among individuals and also depends on the location of the sample (Rao & Chandrasekharam, 1981).

In our study, Mechanical testing reveals that both the membranes are elastic. There was no statistically significant difference in tensile strength between the two membranes. (p=0.05) Tensile strength values of amnion membranes previously reported by K Tanaka is 6.80 ± 0.22 MPa (Tanaka *et al.*, 2010) are higher than that obtained in our study.

The Young's modulus is a measure of elasticity which is normally applied in mechanical physics and is defined as the ratio of applied stress to strain. The membranes did not show statistically significant difference (p=0.05) in terms of their Young's modulus. The difference in elongation at break values between the two membranes was also not statistically significant. (p=0.05)

Elastin present in amnion is responsible for providing elasticity (Fukuda *et al.*, 1999). The thin human amnion is remarkably strong and elastic. Amnion withstands the progressive stretching of the growing embryo, internal and external traumas, and fast and slow pressure changes (Niknejad *et al.*, 2008).

Even though only three tests were made for each membrane, a good repeatability has been found for both amnion and bovine collagen membranes. The ability of amnion membrane to self-adhere eliminates the need for suturing (Holtzclaw & Toscano, 2013), thus making it easier to use in posterior defects. However, if need for suturing arises as in areas of complex anatomic features, sutures can help stabilize the membranes. In this study, The difference in the maximum load which can be applied during suturing was statistically significant (p=0.037)and the results indicate that higher suture retention loads can be tolerated by bovine collagen membrane.

Three commercially available collagen membranes used in GTR techniques, namely BioGide, Collprotect and Jason, were compared by Emanuela Ortolani *et al.* (2015). The tested membranes exhibited different behaviours, different deformability values and thickness, Jason being the thinnest and Bio-Gide the thickest.

Most collagen-based, resorbable GTR devices appear to have limited, if any, potential to support guided tissue regeneration, as evaluated in various animal models and clinically, primarily because of their limitations in providing/maintaining a wound space (Tatakis *et al.*, 1999).

Results of our study point out that, even though the human amnion membrane was slightly inferior to commercially available bovine collagen membrane in terms of their tensile strength, Young's modulus and elongation at break, the differences in these properties between the two membranes were not statistically significant. However, the bovine collage membrane can withstand significantly higher loads during suturing.

Limitations of this study was the small sample size and the inherent biologic nature of these membranes.

CONCLUSION

The human amnion membrane has no statistically significant difference between the commercially available bovine collagen membrane in terms of their tensile strength, Young's modulus and elongation at break. These membranes can only be considered different on the maximum load which can applied during suturing.

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