



A CLINICAL STUDY TO EVALUATE THE EFFICACY OF HUMAN AMNION MEMBRANE IN MILLER'S CLASS I AND II RECESSION DEFECTS IN CONJUNCTION WITH CORONALLY ADVANCED FLAP

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ABSTRACT

Human Amniotic membrane is a pluripotent cellular element that is embedded in a semipermeable membranous structure. It has several properties like anti-inflammatory, anti-scarring, antimicrobial and antiviral properties, cell differentiation, lack of immunogenicity etc. Amniotic membrane is an excellent membrane because it is ethically acceptable, easy to use, cost effective, and easily stored. Successful applications of HAM in various clinical and surgical fields had been reported for over 100 years. The first documented use of amniotic membrane is as a skin substitute by Davis in 1910. Subsequently the foetal membrane was found to be useful in the management of burns, creation of surgical dressings, as well as reconstruction of the oral cavity, bladder, and vagina, tympanoplasty, arthroplasty and so forth. In Periodontics, these membranes have been used in furcation defects, intrabony defects, and gingival recession coverage.

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INTRODUCTION

Many patients come to the periodontal clinic with complaints of gingival recession. Etiologies of gingival recession can be varied. This may range from shrinkage of gingival tissue due to inflammation followed by degeneration to mechanical factors like improper methods of tooth brushing, tooth malpositioning, bone deficiencies, thin marginal soft tissues, high frenum attachment and sometimes orthodontic treatment may also leads to gingival recession.¹

Till date sub-epithelial connective tissue graft with or without coronally advanced flap has been advocated to be gold standard for treatment of gingival recession. However this is a technique sensitive procedure and often associated with increased post operative patient morbidity.² The time involved in the surgical procedure is also prolonged.

GTR membrane and other materials in place of sub epithelial connective tissue graft.

Recently some studies have been conducted using Amniotic membrane as an allograft along with coronally advanced flap for recession coverage. Amniotic membrane has been shown to stimulate re-epithelialization, decreased inflammatory response and modulates angiogenesis.

It is rich in growth factor like fibroblast growth factor (b FGF), epidermal growth factor (EGF), transforming growth factor- α (TGF- α) transforming growth factor- β (TGF- β), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF) which may be important in tissue engineering in the recession area.³ Besides Amniotic membrane has low immunogenicity, paving the path for its use in multiple medical and dental treatment areas.

Thus the present study was designed to evaluate the adjunctive effect of human amnion membrane and coronally advanced flap with coronally advanced flap alone in Miller's class I and class II recession defect

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MATERIALS AND METHODS

The present study was carried out on 30 selected sites having either Miller's Class I or II marginal tissue recession in patients recruited from the outpatient Department of Periodontology at Buddha Institute of Dental Sciences and Hospital, Post Graduate Institute and Research Centre, Patna, Bihar. This is a clinical study designed as split mouth randomised controlled S trial. Each site constitutes one sample of the study. A prior approval of institutional ethical committee was taken to conduct the study.

Inclusion criteria

Systemically healthy patients with Miller's class I and class II gingival recessions with no radiographic evidence of interdental bone loss were selected for the study.

Exclusion criteria

1. Patients with known systemic diseases or immune deficiency.
2. Patients under medication that are known to affect periodontal healing.
3. Patients with psychiatric disorder, pregnancy and lactating mothers.
4. Patients with a habit of tobacco use in any form.
5. Patients found to be incompetent in maintaining oral hygiene.
6. 6. Patients with inability or unwillingness to complete the trial and who were participating in another clinical trial.

30 Amniotic membranes (freezed dried irradiated) were procured from *Tata Memorial Hospital, Tissue Bank, Mumbai*.



1 Amnion membrane used for root coverage procedure

15 sites were randomly assigned for the test group (CAF+Amnion membrane) and another 15 sites were assigned for the control group (CAF alone). All subjects received clinical periodontal examination by a single examiner.

Recession depth (RD) was measured at mid-facial region of the tooth from CEJ to the free gingival margin with UNC-15 periodontal probe at baseline and compared at 1, 3 and 6 months post-operatively between test and control sites. Acrylic stent was made for standardization of the study



2 Pre operative view

Examiner calibration

Eight non-study patients with gingival recession were recruited for calibration. The single designated examiner recorded Recession Depth in each patient. The same examiner repeated the procedure after 24 hours. The intra examiner error was determined based on repeated RD measurement. The resultant correlation coefficient was 0.94 ± 1 mm and was accepted to proceed for the study

Surgical procedure

Informed consent was taken from all subjects recruited for the study.

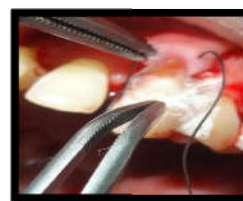
In control site, the surgical site was anesthetized by using 2% xylocaine with adrenaline (1:800000). CAF was performed by making two horizontal incisions with respect to the distal and mesial interdental papillae of the surgical sites, followed by a crevicular incision. Then two vertical releasing incisions at the mesial & distal aspects of the surgical site were given (*Photo3*). Full thickness flap followed by partial thickness flap was reflected.



3 Sulcular and vertical incision

The exposed root surface was scaled and root planed. The cervical step if present at the CEJ was eliminated by using an air rotor and diamond abrasive point.

The flap was coronally advanced so that the gingival margin was 1 mm coronal to the cemento-enamel junction and sutured. For the test group sites, the surgical preparation was similar to the control sites and the amnion membrane was placed over the denuded root surface just below the CEJ (*Photo 4*) and the flap was sutured (*Photo5*). Recession depth was recorded at 1, 3, and 6 months post-operatively and were compared with the baseline data.



4 Amnion membrane placed



5 Suture placed

RESULTS

Results obtained in both the groups were compared at baseline, and one month, three months and six months after the procedure.

The findings were tabulated and statistically analysed. Data was entered in Microsoft excel and Tukey's Multiple Comparison Test was applied for the inter group comparison and student t-Test was applied for comparison between two groups using statistical analysis software Graphpad Prism (Version 5). 'p' value of less than 0.05 was accepted as indicating significance.

Table 1& graph 1: depict the recession depths (RD) of the sites in groups A and B. No significant difference was found in the recession depths between the two groups at baseline (p=0.83); one month (p=0.13) and six months (p=0.64) after the procedure. However, statistically significant difference was noted at third month follow up between the two groups (p value=0.05).

Table 2: depicts the intra group comparison of RD for group A at baseline, first month, third month and sixth month after the procedure. However no significant difference was noted.

Table 3: depicts the intra group comparison for group B. Statistically significant difference was noted between values of baseline vs first month (p= <0.001), baseline vs third month (p=<0.001), and baseline vs sixth month (p=<0.01), no significant difference were found between the values of RD for the first month vs third month or 6 month follow up and third month vs sixth month follow up.

Table 1 Comparison of RD at different time intervals between Group A (Test group) and Group B (control group).

RD	Group A	Group B	P Value*
Baseline	2.53 ± 0.83	2.60 ± 0.83	0.83
First month	2.10 ± 1.51	1.23 ± 1.55	0.13
Third month	2.43 ± 1.61	1.33 ± 1.23	0.05 (s)
Sixth month	1.90 ± 1.54	1.67 ± 1.18	0.64

Student t Test

Table 2 Intra group comparison (Group A -Test group) of RD at different time intervals

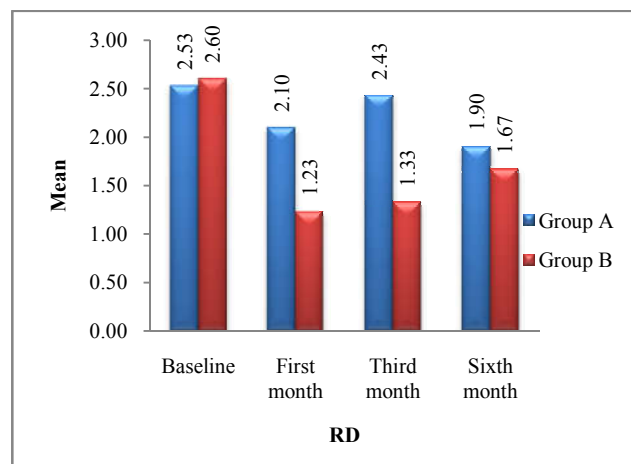
Group A	
Baseline vs First month	Ns
Baseline vs Third month	Ns
Baseline vs Sixth month	Ns

Tukey's Multiple Comparison Test

Table 3 Intra group comparison (Group B - Control group) of RD at different time intervals

Group B	
Baseline vs First month	<0.001 (s)
Baseline vs Third month	<0.001(s)
Baseline vs Sixth month	<0.01 (s)

Tukey's Multiple Comparison Test



Graph 1 Comparison of RD at different time intervals between Group A (Test group) and Group B (control group).

DISCUSSION

CAF with Subepithelial connective tissue graft has been shown to be a predictable procedure to treat Miller's class I and class II mucogingival recession. Subepithelial connective tissue graft is often used in combination with coronally advanced flap for root coverage. This combination has shown high success and predictability rates.⁴

But sub epithelial grafts have several adverse effects such as discomfort with or without pain associated with a donor sites source of the connective tissue grafts and the second wound area. Hence there is a search for an alternative to connective tissue graft which would render better results than the coronally advanced flap procedure alone in case of treatment of Miller's class I and class II recession defects.⁵ HAM has been tried by some periodontists but the literature regarding HAM is mostly in the form of case reports.^{6,7} Therefore, this present RCT was conducted to see the additional benefits of using HAM, if any, along with the standard procedure of CAF. The present randomized controlled trial has shown that the result of recession coverage with CAF alone is significantly better than that with CAF + AM. Comparable results have also been achieved with the use of CAF + AM or amnion chorion membrane.^{8,9}

The reason for limited success of HAM in the treatment of soft tissue defect could not be found in the literature.

When HAM is used as an adjunct to CAF in the treatment of recession defects, the presence of additional material in the form of HAM makes it difficult to manipulate soft tissue, whereby primary flap closure requires stretching the tissue to a great extent, rendering it difficult to suture the flap in a tension free fashion. This might be one of the reasons for the less than optimal results achieved by HAM in soft tissue augmentation. Additionally stretching the tissue in the esthetic zone might change the tissue quality. In case the facial flap requires a lot of stretching the vestibular tissue is pulled coronally resulting in a thin biotype and insufficient volume of attached tissue. Equally if the tissue is pulled too taut in patients with thick tissue, it stretches thin and becomes prone to rupture.¹⁰ It may be because of the same CAF reasons that the amount of root coverage obtained with CAF is greater than that observed with

CAF+GTR, although the use of GTR results in significantly greater alveolar crest level gain.¹¹ Comparable results have also been shown with the adjunctive use of acellular dermal matrix with CAF, due mainly to the aforesaid reasons.¹¹

It has also been noted during and after the surgical procedures that HAM undergoes some shrinkage with time, which has the potential to create a dead space between the root surface and the tissues, which might invite microorganisms and jeopardise with the healing process. No mention of this observation has been noted in the literature.

The present study has some limitations. Firstly it was not blinded at any stage. A single operator performed all the surgical procedures and it was the same person who examined, monitored and maintained all the patients at all stages of the study which may have contributed to observer bias.

Secondly, the sample size of the present study was small and the duration of the study was only for 6 months. The AM used for the present study, procedure from Tata Memorial Hospital Tissue Bank, was freeze dried and irradiated which is not available commercially, so its accessibility is limited and these processing methods might have led to deterioration of some of the properties of the material. So HAM preserved by other techniques like cryopreservation may also be used in future studies for appraisal of the full spectrum of benefits of this material.

Moreover, atraumatic needles and suture materials, which are ideal for mucogingival procedures could not be used for this study 4'-0 silk sutures and 3/8 circle stainless steel needle have been used for all the cases.

It has also to be noted that recession coverage correlates inversely with initial recession depth, indicating that deeper defect would benefit more with root coverage procedures.¹² However, in the present study the mean baseline recession depth was small for both the groups (2.60 mm for CAF and 2.53 mm for CAF+HAM groups). Thus the results of the present study should not be extrapolated to patients with deeper recessions beyond 3mm.

The most reliable outcome variable for assessing periodontal regeneration is human histology.¹² Due to ethical considerations and patient's management limitations, no histological evaluation was performed in the present study; therefore the effect of HAM on overall regenerative capacity remains to be determined.

CONCLUSION

The present study has shown that CAF alone is superior to CAF + HAM in root coverage procedure in Miller's class I & class II recession defects at 6 months post-operatively. Within the limitations of the study it can be concluded that:

Human amniotic membrane along with coronally advanced flap has been found to be inferior to CAF alone in providing a consistent reduction of the baseline recession in Miller's class I & II defects.

Further studies with longer follow-up and involving a larger sample size are required to evaluate the full potential of amniotic membrane.

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