



**ORIGINAL RESEARCH**

**EVALUATION OF ROOT COVERAGE USING AMNIOTIC MEMBRANE IN PATIENTS WITH LINGUAL RECESSON USING IMAGE ANALYSIS SYSTEM: CASE**

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**Received: Jun 15, 2025; Accepted: Jun 1, 2025; Published: Jul.15,2025**

**ABSTRACT**

**Background:** Lingual gingival recession is a unique clinical concern because of its aesthetic and oral health implications. While many treatment approaches to root coverage have been explored, use of amniotic membrane (AM), known for its anti-inflammatory and regenerative capabilities, is comparatively less used in lingual recession defect treatment.

**Objective:** The case series was done to assess the efficacy of amniotic membrane to achieve root coverage of lingual recession defects, with an image analysis system for objective measurement.

**Methods:** Three patients with Cairo's RT I or II classification of lingual recession were included. After local anesthesia and meticulous root surface debridement, AM grafts were placed onto the recession areas and splinted with sutures. Postoperative assessments were performed over a period of six months. Clinical outcomes data were monitored using data such as recession depth (RD), recession width (RW), keratinized tissue width (KTW), mucosal thickness (MT), percentage of root coverage, and measurements that were further validated with an image analysis system for increased accuracy.

**Results:** All cases showed significant root coverage and KTW gain at the six-month follow-up. Image analysis revealed a mean root coverage of 92%, with significant soft tissue healing and esthetic improvement. Patients reported minimal postoperative pain and were highly satisfied with the result.

**Conclusion:** Treatment of lingual gingival recession using amniotic membrane produced satisfactory clinical and esthetic results, indicating its possibility as a good regenerative therapeutic modality in periodontal therapy.

**Keywords:** lingual recession, root coverage, amniotic membrane, image analysis, periodontal regeneration.

**1. INTRODUCTION**

The mandibular anterior lingual area is often deficient in a proper band of keratinized tissue (KT), which is less than 2 mm in thickness, with less than 1 mm of attached gingiva (AG). This zone is also often characterized by a thin gingival phenotype, with a gingival thickness (GT) of less than 1 mm and a narrow amount of attached

gingiva, often less than 1 mm. These traits may help in causing difficulties in periodontal stability and health, particularly under microbial or mechanical stress<sup>[1]</sup>. Voigt et al. reported that the lingual surface attached gingiva of canine and incisor had the minimum width (1.9 mm) in clinically healthy subjects, and as age goes on increasing, the lingual attached gingiva width decreases even more<sup>[2]</sup>. The significance of attached gingiva to protect the

gingival tissue includes threshold to withstand functional forces <sup>[3]</sup> and prevention of inflammatory spread by its dense collagenous band of connective tissue that retards or obstructs, as stated by Goldman and Cohen <sup>[4]</sup>.

An inadequate zone of attached gingiva is more susceptible to compromise periodontal health and results in gingival recession with bone loss. Recession results in sequence of events such as exposure of root surface, root sensitivity and poor oral hygiene that eventually leads to progression of root caries, gingivitis and periodontitis.<sup>[5]</sup>

The management of gingival recession is obtained by increasing keratinized gingiva width. In spite of many gingival augmentation procedures done for the management of lingual recessions of the mandible, the pouch and tunnel technique, a less invasive method assists in attaining coronal positioning of flap and stability of the graft or biomaterials with better healing. Periodontal regeneration involves the use of the guided tissue regeneration (GTR) principle, wherein a membrane or other biomaterial is placed as a barrier to facilitate selective cell repopulation in the site <sup>[6]</sup>. Literature reviews have discussed the characteristics of amniotic membrane and have suggested that it is a candidate regenerative membrane. It has collagen type 1, 3 and various growth factors. It therefore has anti-inflammatory, anti-scarring, antibacterial, and angiogenesis-inducing properties <sup>[7-8]</sup>.

Literature reviews suggested image analysis system as a promising method for evaluating root coverage following periodontal plastic surgery <sup>[9]</sup>. This innovative approach is found to help clinicians to accurately assess the success of root coverage procedures by analyzing pre- and post-operative images, which can capture subtle changes in the soft tissue. By using standardized imaging techniques, such as digital photography along with specialized software, it enables the clinicians to measure parameters like keratinized tissue changes, root exposure reduction, and the overall esthetic outcomes with a high degree of precision.

In this study, the Image analysis system using a python software (OPEN CV) with calibrated measurements that convert pixel distances to real-world dimensions was used. This approach enhanced the reliability of periodontal assessments by minimizing human error, standardizing measurements, and improving reproducibility across cases. Additionally, it also offered the flexibility for further customization, like automating tissue margin detection and tracking changes over time, which could be beneficial in follow-up studies.

Therefore, the objective of the present study was to assess the gain in width of keratinized tissue as a function of root coverage in lingual anterior region,

employing amniotic membrane along with image analysis system.

## MATERIALS AND METHODS

The current research was an observational case series of 6 months duration. Out of 10 patients with chief complaint of lower anterior region sensitivity, an age group of 30–60 years from both genders were chosen from the outpatient department of the Department of Periodontology and Oral Implantology.

The patients were assessed for the following clinical parameters: Recession depth (RD), recession width (RW), width of keratinized gingiva (KG), mucosal thickness, % of root coverage at baseline and 6 months postoperatively.

Images of pre-op and post-op were captured with camera in high resolution to avoid the break in the pixel of the image for precise measurements. Follow-up imaging sessions at intervals such as 1 month, 3 months, and 6 months to track tissue changes were done. Each imaging sessions were performed at the same camera angle, lighting, and patient positioning. The materials used in this study includes tunneling knife 2- (TKN2 #6), prescreened amniotic membrane (Tata Memorial Hospital, Mumbai) AM size: 3 cm × 3cm, Ethicon vicryl 5-0 plus suture, Image analysis software (OPEN CV) and digital camera.

The inclusion criteria include Cairo's RT1 recession in lower anterior lingual region, 30 to 60 years, both genders, adequate oral hygiene (plaque index and gingival index < 2) and systemically healthy individuals with no contraindications for minor oral surgery. The Exclusion criteria include Grade II & III mobility, adjacent anatomical structures interfering with surgery (e.g., large lingual frenum), history of smoking or tobacco use within the last 6 months, presence of active caries, periapical infections, or severe periodontal disease (probing depths > 3 mm), pregnant or breastfeeding individuals due to potential systemic implications and patients with history of previous recession management on the same site.

## CASE REPORTS

### CASE 1

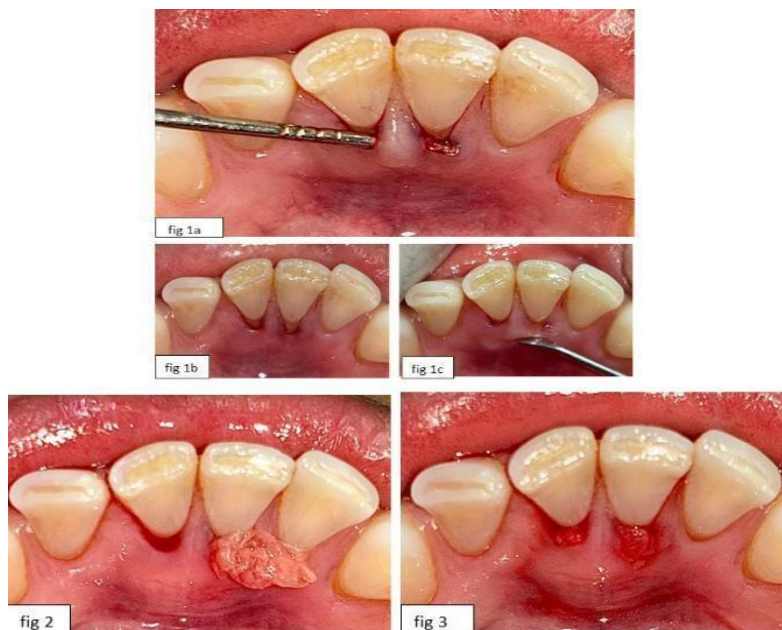
A case of Cairo's RT 1 gingival recession in the mandibular anterior lingual region involving 31,41 was reported to the Department of Periodontics [Figure 1]. Phase 1 therapy was performed. Patient was asked to maintain oral hygiene and report back for review after 1-week. Pre-operative images were captured at high resolution before surgical procedure.

### Surgical procedure:

A local anesthesia was administered using 2% lignocaine hydrochloride with epinephrine (1:200,000). Using tunneling knife 2 (TKN2 #6), gingival tissue was separated from the underlying bone or tooth structure to create a tunnel and pouch [Fig 1]. Amniotic membrane was inserted [Fig 2] and placed

inside the created tunnel over the denuded root surface [Fig 3], thereby preserving the blood supply and maintaining tissue integrity. Followed by the placement of membrane, it was then stabilized with double criss-cross suture

(Zuhr et al) and approximated coronally [Fig 4]. After a week of healing, the patient was recalled and sutures were removed. Postoperative image was captured at high resolution.



**Fig 1 a) Tunneling performed irt to 31,41 b) Denuded root surface c) Maximum coronal advancement tunneling.**

**Fig 2- Amniotic membrane insertion into the tunnel.**

**Fig 3- After membrane placement over denuded root surface.**

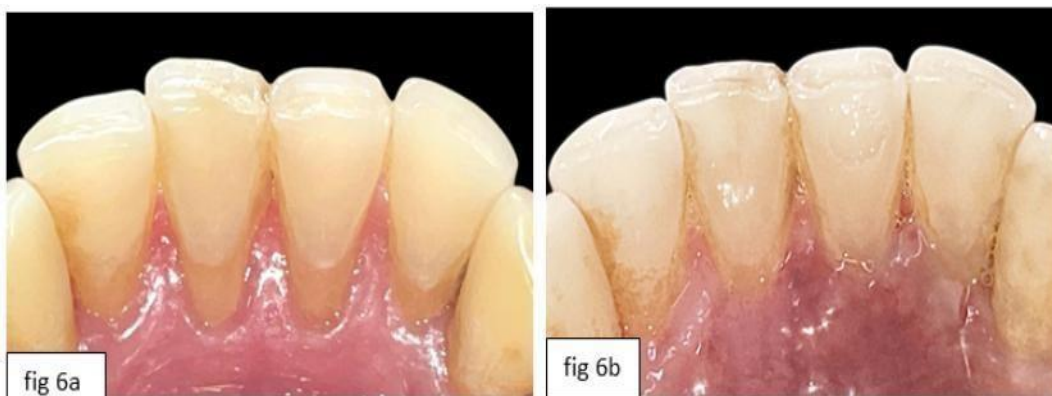
## CASE 2

A case of Cairo's RT 1 gingival recession in the mandibular anterior lingual region extending from 32 to 42 was reported to the Department of Periodontics. Scaling and root planning was performed, patient was asked to maintain oral hygiene and reviewed after a week. Pre-operative images were captured [Fig 6a] at high resolution before surgical procedure. Pouch and tunnel technique with amniotic membrane placement was performed and post operative image was captured [Fig 6b]. Finally, the images were analyzed using the image analysis system to compare the parameters in order to evaluate the preoperative and post operative images.



**Fig 4- Stabilization using double criss-cross suture.**

**Fig 5 a) Pre-op image with 3mm of recession in relation to 31,41. b) Post-op image with complete root coverage of 31,41**



### CASE 3

A case of Cairo's RT 1 gingival recession in the mandibular anterior lingual region extending from 31 to 41 was reported to the Department of Periodontics. Scaling and root planning was performed. Pre-operative images were captured [Fig 7a] at high resolution before surgical procedure. Pouch and tunnel technique with amniotic membrane placement was performed and post-op image captured [Fig 7b]. Finally, the images were analyzed using the image analysis system to compare the parameters in order to evaluate the pre operative and post operative images.



Fig 7a) Pre-op image with 3 mm of recession in relation to 31,41 b) Post-op shows complete root coverage in relation 31,41.

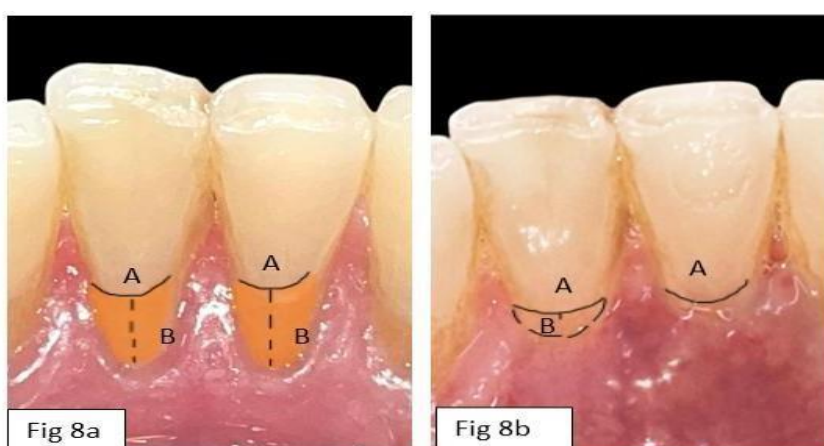


Fig 8 - A- anatomical CEJ from extending the arrow, reveals the recession depth. B - orange coloured surface reveals the recession surface area

**Image acquisition:**

Pre-op images and post-op images of 3 cases were uploaded in the software (OPEN CV) and further measurements were marked and analyzed. The recession surface area and recession depth with respective coding was created for the parameters and then they were analyzed and compared between the pre-operative and post-operative values, which resulted in precise measurements of the parameters.

The pre-op (fig 8a) and post-op (fig 8b) images of a case were analyzed using the respective measurements (the recession surface area[B] and recession depth[A]) of 31 and 41 in python software (OPEN CV). It resulted in increased keratinized tissue and decreased recession surface area and recession depth. The post-op image showed complete root coverage in 41 and partial root coverage in 31.

**Statistical analysis:**

Statistical analysis was conducted and compared by paired t test. Statistical measurement was obtained with mean and standard deviation for all the 6 parameters in sample size of 3, which exhibited variability. Wilcoxon Signed Ranks Test, a non-parametric test was applied to compare pre-op and post-op measurements, for variability and small sample size.

**RESULT**

This study demonstrated the effectiveness of using amniotic membrane for root coverage in periodontal plastic surgery in all the three cases. Using an image analysis software (OPEN CV) shows the values of pre-operative and post operative (Table 1 & 2) using the respective coding which helped us to analyze the required parameters.

**Table 1 Paired Sample Statistics**

|        |                                    | Mean    | N | Std. Deviation | Std. Error Mean |
|--------|------------------------------------|---------|---|----------------|-----------------|
| Pair 1 | root coverage - pre-op             | 96.100  | 3 | 2.0075         | 1.1590          |
|        | root coverage - post-op            | 98.900  | 3 | 1.2490         | .7211           |
| Pair 2 | recession depth - pre-op           | 4.333   | 3 | 2.2591         | 1.3043          |
|        | recession depth - post-op          | 2.590   | 3 | 1.8567         | 1.0720          |
| Pair 3 | recession width - pre-op           | 4.533   | 3 | 2.2030         | 1.2719          |
|        | recession width - post-op          | 2.767   | 3 | 1.5695         | .9062           |
| Pair 4 | keratinized tissue width - pre-op  | 1.1067  | 3 | .10066         | .05812          |
|        | keratinized tissue width - post-op | 1.9833  | 3 | .64485         | .37231          |
| Pair 5 | mucosal thickness - pre-op         | 1.5200  | 3 | .35000         | .20207          |
|        | mucosal thickness - post-op        | 1.9400  | 3 | .25239         | .14572          |
| Pair 6 | recession surface area - pre-op    | 23.0167 | 3 | .95553         | .55167          |
|        | recession surface area - post-op   | 20.9933 | 3 | 1.48372        | .85663          |

**Table 2 Test Statistics**

| Test statistics      |                                              |                                                  |                                                 |                                                                        |                                                     |                                                                  |
|----------------------|----------------------------------------------|--------------------------------------------------|-------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------|------------------------------------------------------------------|
|                      | Root coverage post op – root coverage pre op | Recession depth post op - recession depth pre op | Recession width post op- recession width pre op | Keratinized tissue width – post Op - keratinized tissue width – pre op | Mucosal thickness– post op Mucosal thickness pre op | Recession surface area – post op Recession surface area – pre op |
| Z                    | -1.604 <sup>a</sup>                          | -1.604 <sup>b</sup>                              | -1.604 <sup>b</sup>                             | -1.604 <sup>a</sup>                                                    | -1.604 <sup>a</sup>                                 | -1.604 <sup>b</sup>                                              |
| Asymp.Sig (2 tailed) | .109                                         | .109                                             | .109                                            | .109                                                                   | .109                                                | .109                                                             |

a. Based on negative ranks.

b. Based on positive ranks.

c. Wilcoxon Signed Ranks Test

The average root coverage increased is found to be 1.89% that is from 98.0% pre-operatively to 99.89% post-operatively, indicating substantially complete coverage across treated sites. The average recession depth showed a 50% reduction, with a mean decrease of 2.0 mm, from 4 mm pre-operatively to 2 mm post-operatively. A significant reduction of exposed root surfaces was apparent by the recession's average width, which reduced from 5.62 mm to 3.32 mm, with a mean reduction of 2.3 mm. The keratinized tissue width increased by over two times, from an average of 1.20 mm prior surgery to 2.70 mm after, with a mean increase of 1.5 mm. This suggested enhanced tissue stability and periodontal health post-operatively. An average increase in mucosal thickness of 0.4 mm, ranging from 1.77 mm to 2.17 mm was also seen. The recession surface area is also reduced from 22.46 mm<sup>2</sup> to 20.46mm<sup>2</sup>, which resulted in a mean decrease of 2.0 mm<sup>2</sup> (Figure 9). Paired t test (Table 1) and Wilcoxon Signed Ranks test (Table 2) suggested variability among the parameters with in no statistically significant difference in any of the parameters between pre operative and post operative ( $p > 0.05$ ), due to limited sample size.

## RESULT

The management of lingual recession in mandibular anterior teeth always remains as a challenge for the clinician due to its anatomical challenge of having thin lingual mucosa which eventually leads to the surgical complication of elevating a lingual flap that compromise the blood supply<sup>[10]</sup>. According to Tavelli et al, tunnelling approach is a very successful periodontal plastic surgery for treating both single and multiple gingival recessions<sup>[11]</sup>. The tunneling technique was modified from the envelope flap and originally introduced by Allen et al in 1994<sup>[12]</sup>. Azzi et al in 1998<sup>[13]</sup> released the interdental papilla to mobilize the tunnel flap and accomplish coronal advancement, while Zabalegui et al. 1999<sup>[14]</sup> claimed that the tunnel connects multiple envelopes to form the sub-periosteal tunnel. This study utilized the concept of tunneling including the interdental papilla which results in appropriate coverage of denuded root surfaces.

The membranes that have been used in the field of periodontal regeneration were natural and synthetic biomaterials. There are several studies where autografts were also used in the treatment of lingual recession. In order to avoid secondary surgical site due to few setbacks such as limited blood supply, necrosis of tissue and high chance of donor site morbidity, biomaterials were preferred in the treatment of recession.

Amniotic membranes contain collagen type 1 and 3 and also found to be rich in growth factors, cytokines, and extracellular matrix components, making them a biocompatible and regenerative material<sup>[15]</sup>. Gurinsky et al discovered that the dehydrated amnion allograft resulted in increase of new gingival tissue with increase in attached gingiva which resulted in 97% of the defect coverage in gingival recession<sup>[15]</sup>. Shetty et al in a study compared amniotic membrane with platelet-rich fibrin (PRF), and suggested that amniotic membrane resulted in significant root coverage of 97% over 7 months of follow-up.<sup>[16]</sup> The current study was in concurrence with these previous studies suggesting that there was significant increase in root coverage of about 98% postoperatively. Conversely, few studies comparing PRF to the amniotic membrane in the management of root coverage found that the PRF-treated sites achieved more than 50% complete root coverage, which made it superior to the amniotic membrane<sup>[17]</sup>.

The scientific evidences reported amniotic membrane to have various anti-inflammatory factors such as tissue inhibitors of metalloproteinase1-4, activin A, IL-1 and IL-2 receptor antagonists, IL 10, and endostatin, which promote angiogenesis and prevent the proliferation of endothelial cells. A study demonstrating the anti-inflammatory qualities of amniotic membrane shows that areas treated with amniotic membrane have much lower levels of interleukin-1 beta (IL-1 $\beta$ ) and higher levels of human beta defensins (hBD-2) in the gingival crevicular fluid (GCF), which are necessary for pathogen defense.<sup>[18]</sup> The other properties include anti-microbial (presence of secretory leukocyte proteinase inhibitor and elafin), low immunogenicity which are important to create a biocompatible scaffold for tissue engineering and anti-scarring (downregulation of TGF- $\beta$ )<sup>[19]</sup>. The limitations of the current study include sample sizes and longer follow-up intervals which would have elaborated more about the authenticity and its clinical significance in oral and periodontal surgery.

## CONCLUSION

This study emphasizes the potential of amniotic membrane as an alternative biomaterial for soft tissue augmentation and root coverage procedures. This approach may enhance esthetic outcomes and periodontal health by providing both functional and aesthetic advantages.

What is new about this study?

**Management of Lingual Recession:** Lingual gingival recession often receives less attention in clinical research compared to buccal recession, making this study novel in addressing this unique anatomical and clinical challenge.

## Use of Amniotic Membrane for Lingual

**Recession:** While amniotic membranes have been widely studied for root coverage in buccal recession, their application in managing lingual recession is less explored.

**Image Analysis System for Assessment:** Using an image analysis system introduces an objective and standardized approach to evaluating root coverage. This method offers precise measurement changes in gingival dimensions and root exposure.

## DECLARATIONS

### Conflicts of interest

The authors declare no conflict of interest.

### Funding

This research received no external funding.

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