

Comparative Evaluation of Coronally Advanced Flap with and without Amniotic Membrane in the treatment of Localised Miller's Class I and Class II Gingival Recession Defects: A Randomised Controlled Trial

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ABSTRACT

Introduction: Gingival recession is the exposure of the root surface of the tooth, characterised by the displacement of the gingival margin apically from the Cementoenamel Junction (CEJ). Various techniques have been suggested for treating recession, such as Free Gingival Graft (FGG), Connective tissue grafts, laterally or Coronally Advanced Flaps (CAF), and Guided Tissue Regeneration (GTR)-based procedures. Different membranes have been used for GTR, one of which is the amnion membrane.

Aim: To evaluate root coverage using Coronally Advanced Flap (CAF) with and without amniotic membrane in the treatment of localised gingival recession.

Materials and Methods: A randomised controlled trial was conducted at Pandit Deendayal Upadhyay Dental College and Hospital, Solapur, Maharashtra, India from March 2017 to February 2018. In this study, 30 teeth were treated, with 15 teeth undergoing CAF with amniotic membrane (Group II-Test Group) and 15 teeth treated with CAF without amniotic membrane (Group I-Control Group). Probing Depth (PD), Clinical Attachment Level (CAL), Relative Attachment Level (RAL), Recession Width (RW), Recession Depth (RD), Width of Keratinised Gingiva (WKG), Width of Attached Gingiva (WAG), and gingival biotype were assessed between baseline and after three months. Statistical analysis was performed using paired

t-test, independent t-test, and Mann-Whitney U-Test. Statistical Package for Social Sciences (SPSS) software version 20.0 was used for analysis. The α error of 5% (p -value=0.05) and β error of 20% were taken into consideration.

Results: The mean age of the study population was 30.36 ± 4.55 years, with 18 males and 12 females. There was a decrease in RD, RW, PD, and a gain in CAL and RAL in both groups after three months compared to the baseline values. Additionally, there was an increase in WKG, WAG, and a significant change in the thickness of the gingival biotype from thin to thick in both groups at the end of three months. On inter group comparison, the changes in PD (p -value=1.00), RD (p -value=1.00), RW (0.176), CAL (0.664), RAL (1.00), WKG (p -value=0.313) were not statistically significant at the end of three months. WAG was higher in Group II compared to Group I (p -value=0.014*), and the mean root coverage was greater in Group II (15.62%) than in the control group (11.642%) (p -value=0.005) at the end of three months. There was a statistically significant difference (p -value=0.005*) in Group II (15.62%) compared to the control group (11.642%).

Conclusion: Amniotic membrane in combination with CAF proves to be a successful option for root coverage in localised recession defects. However, histological evidence and a greater number of future longitudinal studies are necessary to study the efficacy of this approach.

Keywords: Guided tissue regeneration, Laminin, Wound healing

INTRODUCTION

Overall appearance of an individual has become significant as we step into the future. Dental appearance is no exception. The attractiveness of a smile is characterised by various factors involving both the teeth and the surrounding soft tissues. Evaluation of various structures is required for an ideal smile [1].

Gingival recession is the display of the root surface of the tooth characterised by the displacement of the gingival margin apically from the Cemento-enamel Junction (CEJ) [2]. This causes hypersensitivity to teeth, root caries, and poor aesthetics. The treatment of gingival recession is to restore the gingival margin to the CEJ and create a normal sulcus with functional attachment [3]. There are two types of gingival recession: one is due to periodontal disease and the other is primarily related to mechanical factors, especially tooth brushing [4].

Various techniques have been suggested for treating recession, such as FGG, Subepithelial Connective Tissue Graft (SCTG),

laterally or CAF, GTR-based root coverage procedures. The CAF procedure has been successfully tried where there is the presence of adequate keratinised gingiva apical to the recession defect [5]. The SCTG with CAF is considered the gold standard technique [6]. An increase in the WAG and better aesthetics are the added advantages, but its main disadvantage is that it requires a second surgical site. This causes aversion in many patients towards mucogingival surgeries, so different membranes have been used for GTR. GTR offers advantages such as the possibility of achieving periodontal regeneration rather than connective tissue repair of the exposed root surfaces with no additional donor site. In 1990, Tinti C first performed GTR for root coverage [7].

Membranes can be classified as the first generation of membranes (non absorbable), the second generation (absorbable), and the third generation (membranes as a product of tissue engineering) [8]. Various types of absorbable and non absorbable membranes have been used. Resorbable membranes are preferred over non-resorbable ones as their use eliminates the need for a second intervention for

removal, is cost-effective, and reduces the risks of complications. The major disadvantage of second-generation membranes is the low predictability of regeneration [5]. Predictable tissue regeneration depends on the crucial messenger molecules that stimulate progenitor cells. Third-generation membranes have been developed to function as barriers as well as delivery systems to release particular chemicals at the wound site, including growth factors, adhesion factors, and antibiotics, promoting natural wound healing [5]. The third generation of membranes includes the amniotic membrane. The amniotic membrane is a composite membrane consisting of pluripotent stem cells embedded in a semipermeable membrane that provides growth factors [9]. It contains specialised proteins and growth factors, reduces inflammation, minimises scar formation, and acts as a natural biological barrier [10]. Poor long-term stability has been associated with sites treated with CAF alone [11,12].

A comparison of the amnion allograft with the traditional connective tissue graft has previously demonstrated that the amnion allograft might be a suitable alternative to the connective tissue graft in covering denuded root surfaces [13]. A previous case study reported that the amnion allograft is well tolerated by the gingival tissues and results in excellent healing [14]. Another case series concluded that amniotic membrane is a useful substitute for autograft tissue when treating Miller's Class I and II recession defects that range from mild to moderate. Furthermore, as the amniotic membrane is self-adherent, the procedure takes much less time and is simpler. The study's findings could be interpreted as circumstantial evidence of the establishment of new attachment [15]. The amnion overcomes the drawbacks of other materials, such as ease of procurement, ease of use, and easy adaptability since it resembles the composition of gingival tissue. With all the added advantages mentioned above, when combined with CAF, it serves as an effective option in treating gingival recession [16].

Therefore, this study was conducted to evaluate root coverage using CAF with and without amniotic membrane in the treatment of localised gingival recession at baseline and after three months.

MATERIALS AND METHODS

This randomised controlled trial was conducted at the Department of Periodontics, Pandit Deendayal Upadhyay Dental College, Solapur, Maharashtra, India from March 2017 to February 2018. Approval from the Institutional Ethics Committee (IEC Approval No. 13) was obtained before the study commenced. Procedures followed were in accordance with the Helsinki Declaration of 1975. Informed consent was obtained from the patients. There were no ethical concerns regarding the study.

Inclusion criteria: The patients with no systemic history, patients with Miller's Class I or Class II Localised Gingival Recession [17] in relation to anterior and premolar teeth, patients with adequate width of attached gingiva (WAG)-at least 1 mm [18], and patients with good oral hygiene were included in the study.

Exclusion criteria: Patients with occlusal disharmony, smokers, alcoholics, immuno-compromised patients, pregnant or lactating females, and those with radiographic evidence of bone loss were excluded.

Sample size calculation: G*Power software version 3.1 was used for sample size estimation. Keeping the effect size=0.6 [19], α error of 5% (p -value=0.05), and β error of 20%, power ($1 - \beta$ error prob)=0.95, the sample size was estimated to be 31, rounded off to 30.

Procedure

Random allocation of patients was done using the lottery method. Patients with odd serial numbers (e.g., 1st, 3rd, 5th, etc.) were added to group I, and patients with even serial numbers (e.g., 2nd, 4th, 6th, etc.) were added to group II. It was a single-blinded trial. The patients did not know the group to which they were assigned. Thus, a total of 30 teeth were treated:

Group II- Test group: 15 patients: The patients who underwent CAF procedure using amniotic membrane.

Group I: Control group: 15 patients who underwent the CAF procedure without using amniotic membrane.

The amniotic membrane was procured from the Tissue Bank at Tata Memorial Hospital, Mumbai.

Informed written consent was obtained from a total of 30 selected patients. Detailed case histories were recorded, and scaling and root planing were performed (Phase I Therapy). Oral hygiene instructions were given, and patients were recalled after three weeks. Clinical parameters were recorded at baseline. The surgical procedure was performed under local anaesthesia. The procedure [14] began with horizontal incisions made at the base of the interdental papilla on both sides. A sulcular incision was made buccally, and two vertical releasing incisions were made on the mesial and distal surfaces of the involved defect, extending beyond the mucogingival junction. A trapezoidal flap was reflected. The full-thickness flap was reflected up to 3 mm apical to the bone dehiscence, and a split-thickness flap was reflected by sharp dissection apical to it. De-epithelialisation of the interdental papillae was performed, and the flap was mobilised coronally. Root planing was then carried out.

In Group I, without any membrane, the flap was advanced without using any membrane. In Group II, the amniotic membrane was placed up to the CEJ and 3 mm adjacent to the root on the sound bone. Since the membrane is self-adhesive, it did not require additional suturing. The flap was coronally advanced so that the gingival margin was positioned about 2 mm coronal to the CEJ. Sling sutures were used for better anchorage, and the vertical incisions were sutured using an interrupted suturing technique with 4-0 braided silk (Mersilk™).

A periodontal dressing was applied, and appropriate antibiotics, analgesics, and anti-inflammatory drugs were prescribed. Patients were given Capsule Amoxicillin 500 mg three times daily for 5 days, Tablet Aceclofenac (100 mg) + Paracetamol (325 mg) three times daily for five days, and Tablet Omeprazole 20 mg once daily for five days. Patients were advised to rinse with 0.2% chlorhexidine gluconate mouthwash twice daily for three weeks, and routine postoperative surgical instructions were followed. Periodontal dressing and sutures were removed two weeks after surgery, and a recall was scheduled three months later.

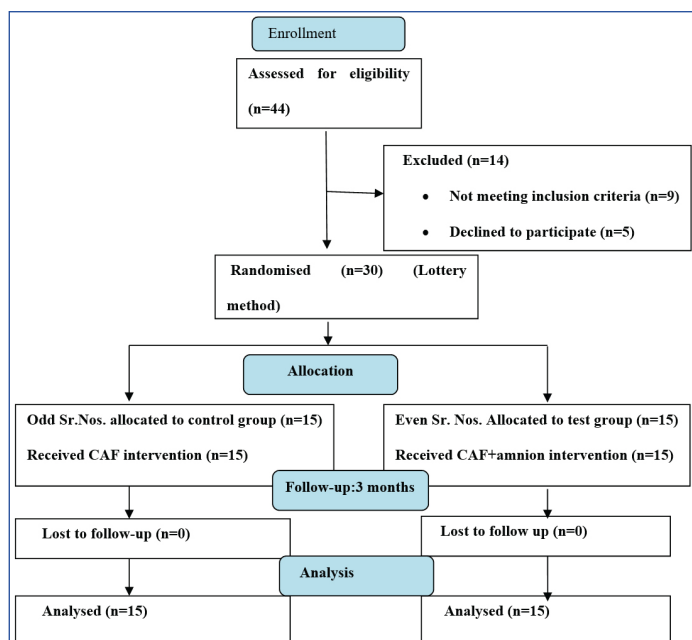
The following clinical parameters were evaluated: Recession Depth (RD) was measured from a fixed point (groove) on the stent to the gingival margin [20,21], and Recession Width (RW) was recorded as the distance between the mesial and distal papillae along the cemento-enamel junction [21].

Clinical Attachment Level (CAL) was measured from the CEJ to the base of the gingival sulcus [21], and Relative Attachment Level (RAL) was measured from a fixed reference point (groove on the stent) to the base of the gingival sulcus [20]. As RAL is a more reproducible and standardised parameter, it was also recorded.

WKG was measured from the gingival margin to the mucogingival junction [18], and Width of Attached Gingiva (WAG) was calculated as WKG minus Probing Depth (PD) [18]. Gingival Biotype (Periodontal Phenotype) was assessed using the probe transparency method. A periodontal probe was inserted into the gingival tissue, and if the probe was visible, the gingival biotype was considered thin (≤ 1 mm); if not visible, it was considered thick (> 1 mm) [22].

$$\text{Root coverage percentage (\%)} = \frac{(\text{preoperative RD} - \text{postoperative RD}) \times 100}{\text{Preoperative RD}}$$

All of these parameters were evaluated at baseline and three months postoperatively for localised recession. [Table/Fig-1] shows the CONSORT flow diagram.



[Table/Fig-1]: Consort flow diagram.

Clinical parameters were assessed with the fabrication of acrylic stents on diagnostic study casts. Cold-cure acrylic was used to fabricate customised acrylic occlusal stents by taking alginate impressions and pouring the cast in dental stone. The stents covered the incisal/occlusal one-third of the selected site on the buccal and palatal aspects. Measurements were taken by creating a groove on the stent that guided the periodontal probe. This provided a definite reference point and a specific angulation for measurements at each site [20].

STATISTICAL ANALYSIS

The intra group comparisons in both groups were conducted using the paired t-test. The inter group comparisons in both groups were assessed using the independent T-test. The Mann-Whitney U-test, a non parametric test, was employed for comparing gingival biotype and root coverage. A p-value less than or equal to 0.05 (p≤0.05) was considered statistically significant. The software utilised was SPSS software version 20.0.

RESULTS

A total of 30 sites were randomly selected with Miller’s Class I and Class II Gingival recession. The mean age of the study population was 30.36±4.55 years, with 18 males and 12 females.

In Group I, a statistically significant reduction from baseline to three months was found in PD (p-value=0.041), RD (p<0.001), RW (<0.001), CAL (p-value=0.11), RAL (p<0.001). WAG showed an increase from baseline to three months, which was statistically significant (p-value=0.014). WKG over a period of three months showed a decrease from baseline to three months, which was statistically significant (p<0.001) [Table/Fig-2].

In Group II, there was a statistically significant reduction from baseline to three months in PD (p-value=0.19), RD (p<0.001), RW (p<0.001), CAL (p<0.001), RAL (p<0.001). WAG and WKG showed an increase from baseline to three months, which was statistically significant (p<0.001) [Table/Fig-3].

At baseline, none of the parameters showed a significant difference between the two groups [Table/Fig-4].

After three months, there was no statistically significant difference in PD, RD, and RW with p-values of 1.00, 1.00, and 0.176, respectively. The CAL gain was higher in Group I than in Group II, but the difference was not statistically significant (p-value=0.664). The RAL was the same in both Group I and Group II (p-value=1.00).

Variables	Time intervals	Mean	Standard deviation	Mean difference	T	p-value
Probing Depth (PD) (in mm)	Baseline	1.53	0.51	0.26	2.25	0.041*
	3 months	1.26	0.45			
Clinical Attachment Level (CAL) (in mm)	Baseline	4.53	2.55	1.93	2.90	0.011*
	3 months	2.60	.82			
Relative Attachment Level (RAL) (in mm)	Baseline	12.13	0.83	1.60	8.41	<0.001**
	3 months	10.533	0.83			
Recession Width (RW) (in mm)	Baseline	4.33	0.61	1.13	12.47	<0.001**
	3 months	3.20	0.67			
Recession Depth (RD) (in mm)	Baseline	10.60	0.63	1.33	8.36	<0.001**
	3 months	9.26	0.70			
Width of Keratinised Gingiva (WKG) (in mm)	Baseline	3.40	0.50	-0.80	-4.58	<0.001**
	3 months	4.20	0.77			
Width of Attached Gingiva (WAG) (in mm)	Baseline	1.86	0.63	-0.46	-2.82	0.014*
	3 months	2.33	0.89			

[Table/Fig-2]: Intragroup comparison of various clinical parameters between baseline and after 3 months in Group-I.

*Paired t-test

Variables	Time intervals	Mean	Standard deviation	Mean difference	T	p-value
Probing Depth (PD) (in mm)	Baseline	1.60	0.50	0.33	2.64	0.019*
	3 months	1.26	0.45			
Clinical attachment level (in mm)	Baseline	4.26	1.22	1.80	6.44	<0.001**
	3 months	2.46	0.83			
Relative Attachment Level (RAL) (in mm)	Baseline	12.60	0.73	2.06	8.32	<0.001**
	3 months	10.53	0.63			
Recession Width (RW) (in mm)	Baseline	4.46	0.51	.93	14.00	<0.001**
	3 months	3.53	0.63			
Recession Depth (RD) (in mm)	Baseline	11.00	0.75	1.73	8.40	<0.001**
	3 months	9.26	0.45			
Width of Keratinised (WKG) Gingiva (in mm)	Baseline	3.33	0.48	-1.20	-5.39	<0.001**
	3 months	4.53	0.99			
Width of Attached Gingiva (WAG) (in mm)	Baseline	1.80	.67	-1.53	-5.27	<0.001**
	3 months	3.33	1.17			

[Table/Fig-3]: Intragroup comparison of various clinical parameters between baseline and after three months in Group-II.

*Paired t-test

Variables	Time interval	Groups	Mean	Standard deviation	Mean difference	p-value
Probing Depth (PD) (in mm)	Baseline	Group-I	1.53	0.51	-0.06	0.724
		Group-II	1.60	0.50		
Recession Depth (RD) (in mm)	Baseline	Group-I	10.60	0.63	-0.40	0.127
		Group-II	11.00	0.75		
Recession Width (RW) (in mm)	Baseline	Group-I	4.33	0.61	-0.13	0.526
		Group-II	4.46	0.51		
Clinical Attachment Level (CAL) (in mm)	Baseline	Group-I	4.53	2.55	0.26	0.719
		Group-II	4.26	1.22		
Relative Attachment Level (RAL) (in mm)	Baseline	Group-I	12.13	0.83	-0.46	0.115
		Group-II	12.60	0.73		
Width of Attached Gingiva (WAG) (in mm)	Baseline	Group-I	1.86	0.63	0.066	0.784
		Group-II	1.80	0.67		
Width of Keratinised Gingiva (WKG) (in mm)	Baseline	Group-I	3.40	0.50	0.06	0.716
		Group-II	3.33	0.48		

[Table/Fig-4]: Inter group comparison of clinical parameters between Group-I and Group-II at baseline using independent t-test.

*Independent t-test

There was a change in WAG and WKG, which was greater in Group II than in Group I, with p-values of 0.014 and 0.313, respectively [Table/Fig-5].

Variables	Time interval	Groups	Mean	Standard deviation	Mean difference	p-value
Probing Depth (PD) (in mm)	3 months	Group-I	1.26	0.45	0.00	1.000
		Group-II	1.26	0.45		
Recession Depth (RD) (in mm)	3 months	Group-I	9.26	0.70	0.00	1.000
		Group-II	9.26	0.45		
Recession Width (RW) (in mm)	3 months	Group-I	3.20	0.67	-0.33	0.176
		Group-II	3.53	0.63		
Clinical Attachment Level (CAL) (in mm)	3 months	Group-I	2.60	0.82	0.13	0.664
		Group-II	2.46	0.83		
Relative Attachment Level (RAL) (in mm)	3 months	Group-I	10.53	0.83	0.00	1.00
		Group-II	10.53	0.63		
Width of Attached Gingiva (WAG) (in mm)	3 months	Group-I	2.33	0.89	-1.00	0.014*
		Group-II	3.33	1.17		
Width of Keratinised Gingiva (WKG) (in mm)	3 months	Group-I	4.20	0.77	-0.33	0.313
		Group-II	4.53	0.99		

[Table/Fig-5]: Inter group comparison of clinical parameters between Group-I and Group-II after 3 months. *Independent t-test

The gingival biotype changed from thin to thick in both Groups I (p-value=0.0466) and II (p-value=0.046) from baseline to three months, which was statistically significant [Table/Fig-6].

Time interval	Groups	Z	p-value
Group-I	Baseline	-2.00	0.046*
	3 months		
Group-II	Baseline	-2.00	0.046*
	3 months		

[Table/Fig-6]: Intragroup comparison of gingival biotype between baseline and at three months in Group-I and Group-II. *Wilcoxon signed Rank test

When both groups were compared for the change in thickness in the gingival biotype, there was no statistically significant difference at baseline and at three months [Table/Fig-7].

Time interval	Groups	Z	p-value
Baseline	Group-I	-0.76	0.446
	Group-II		
3 months	Group-I	-1.4	0.150
	Group-II		

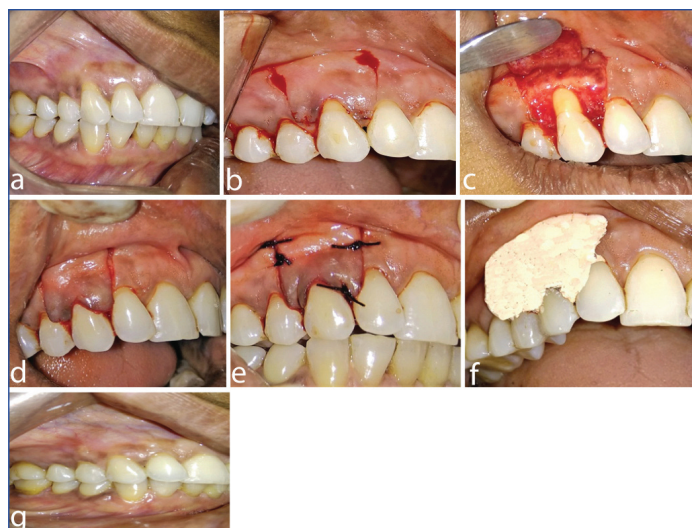
[Table/Fig-7]: Inter group comparison of gingival biotype between Group-I and Group-II at baseline and at three months. *Mann whitney U-test

The mean root coverage percentage was higher in Group II (15.62±6.049) than in Group I (11.642±5.031), which was statistically significant (p-value=0.005) [Table/Fig-8].

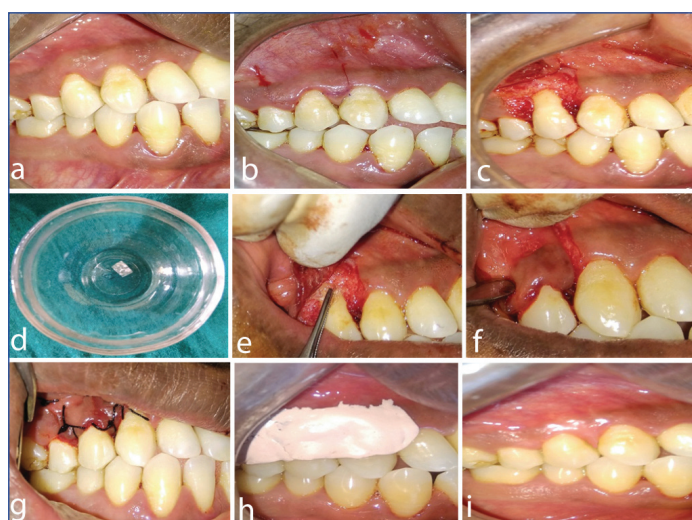
Groups	Mean (%)	Standard deviation	Z	p-value
Group-I	11.64	5.03	-2.81	0.005*
Group-II	15.62	6.04		

[Table/Fig-8]: Difference in RC (Root Coverage) percentage between Group-I and Group-II. *Mann whitney U-test

[Table/Fig-9a-g,10a-i] show clinical pictures of the procedures followed in Group I and Group II.



[Table/Fig-9]: CAF (Group I): a) Pre-operative image; b) Incision placed; c) Flap reflection; d) Coronal approximation; e) Sutures placed; f) Perio pack placed of flap; g) Postoperative at 3 months.



[Table/Fig-10]: CAF+Amnion (Group II): a) Preoperative image; b) Incision placed; c) Flap reflection; d) Amniotic membrane; e) Amniotic membrane adaptation; f) Coronal approximation; g) Sutures placed; h) Perio pack placed; i) Postoperative at 3 months.

DISCUSSION

One of the most prevalent issues associated with periodontal disease, both aesthetically and functionally, is gingival recession [23]. In the present study, the CAF procedure was performed with and without the use of amniotic membrane to treat recession defects, and the parameters were evaluated. There was a decrease in RD, RW, PD, and a gain in CAL and RAL from baseline to three months in both groups. There was an increase in WKG, WAG, and a significant change in the thickness of the gingival biotype from thin to thick at the end of 3 months in Groups I and II. The changes in PD and RD were similar in both groups after three months. The increase in WAG was greater in Group II than in Group I (p-value=0.014). The root coverage showed a statistically significant difference in the group using amniotic membrane (15.62%) compared to the control group (11.642%) (p-value=0.005).

Norberg introduced the CAF procedure [24]. It provides satisfactory root coverage, good colour blending with adjacent soft tissue [25]. It also offers advantages such as being easy to perform, less technique-sensitive, no requirement for a second surgical site, optimum blood supply to the flap as it is a pedicle flap, and excellent aesthetics. This also results in minimal postoperative discomfort to the patient while enhancing periodontal regeneration. GTR-based root coverage shows new attachment formation histologically [26,27]. Therefore, this procedure was selected as the study procedure.

Amniotic membrane serves as a good alternative in GTR-based root coverage procedures. Firstly, the amniotic membrane enhances

wound healing by reducing scar formation and inflammation. Secondly, it helps to form a scaffold for cells to proliferate and differentiate. Thirdly, it acts as a scaffold for tissue engineering due to the presence of growth factors. Lastly, it is easy to obtain and transport [28]. Gurinsky B studied dehydrated Amnion allograft in gingival recession and obtained significant results [29]. Laminin-5, which is present in the basement membrane of amnion, plays a role in the cellular adhesion of gingival cells, invasive growth of fibroblasts, and angiogenesis, which are very helpful in the early phases of wound healing [30-32]. Shah R et al., in a case report, used amnion allograft and found that it results in predictable regeneration [14]. Sharma A and Yadav K evaluated in a case series that amnion can be successfully used in treating recession defects [15]. Joshi CP et al., reported a case of a 52-year-old male with bilateral gingival recession in the maxillary arch, which was treated with CAF along with the placement of Amnion-Chorion Membrane (ACM) and chorion membrane [33]. They achieved 100% root coverage with pronounced gingival biotype improvement [33].

Katkurwar AA and Mahale SA conducted a systematic review and compared the efficacy of Amnion Membrane in combination with CAF by considering seven relevant articles [34]. It was found that it can be successfully used in the treatment of gingival recession with respect to a decrease in RD, RW, and a gain in CAL, which supported the findings of this study. The gain in CAL suggests periodontal regeneration, new attachment, or reattachment, but there is no histological evidence in the present study. Therefore, the actual phenomenon behind the gain in CAL cannot be explained [35]. Abdel-Fatah R and Saleh W in a systematic review and meta-analysis in 2024 [36], concluded that amniotic membrane can be considered a viable treatment option for gingival recession with satisfactory treatment outcomes. Better root coverage, gain in CAL, and WAG were observed in the group using amniotic membrane than CAF alone, which were similar to this study. Ghahroudi AA et al., conducted a study in which CAF+CTG was compared with CAF+Amnion and found that the usage of amnion showed significant improvement in PD, RD, and root coverage [13]. Chakraborty S et al., performed CAF with Amnion and Chorion membranes and concluded that both amnion and chorion allografts seem to be promising novel tissue-engineered biomaterials, similar to the current study [37]. Navarasu M et al., performed CAF versus CAF + Amnion, in which he found out that the use of amniotic membrane as a barrier along with CAF did not influence the clinical outcome of the root coverage procedure, which was contrasting to the study [38]. Nath J et al., compared CAF with CAF using Amnion and concluded that the decrease in RD and RW was more in test sites than in the control sites, which supports the findings of this study [39].

In summary, in this study, amniotic membrane further enhanced the results with a better outcome in recession coverage, with a significant difference when comparing with the group without amniotic membrane. Thus, it can be used as an effective option to treat root coverage in recession defects without the need for a second surgery.

Limitation(s)

The surgical procedure is technique-sensitive and also depends on the patient's level of maintenance of oral hygiene. This may act as a limitation. Histological evidence and a greater number of future longitudinal studies are necessary to study the efficacy of this approach.

CONCLUSION(S)

This study was conducted to evaluate root coverage using CAF with and without Amniotic membrane in localised recession defects. The results of the study were encouraging. There was a significant improvement in all the clinical parameters in both groups from baseline to three months. The root coverage was observed to be

more significant statistically in the group using Amniotic membrane, which can be attributed to the efficient healing and regenerative capacity of the membrane.

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