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COMPARATIVE EVALUATION OF VESTIBULAR EXTENSION ALONE OR IN COMBINATION WITH PRF MEMBRANE OR AMNIOTIC MEMBRANE IN MANDIBULAR ANTERIOR REGION : A CLINICAL STUDY

Dental Science

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ABSTRACT

Introduction: An insufficient dimension of keratinised tissue is related to a shallow vestibular depth in many cases, which may prevent appropriate prosthetic rehabilitation and facilitate plaque retention. Thus, the aim of this study was to compare clinically vestibular extension alone or in combination with PRF membrane or amniotic membrane as graft materials for vestibuloplasty in mandibular anterior region.

Materials and Methods: 30 patients were selected based on the presence of shallow vestibular depth and divided into three groups: Group I (vestibular extension), Group II (vestibular extension with PRF membrane) and Group III (vestibular extension with amniotic membrane). Clinical parameters recorded were: Vestibular Depth, Thickness of Keratinised gingiva, Plaque index, Gingival Index, Healing Index & Visual Analog Scale.

Results: Amniotic group (p=0.005) showed the best reduction in PI score, whereas control group (p=0.02) showed a statistically significant difference in mean GI score. TKG depicted non-significant results. Mean VD and VAS scores depicted a statistically significant result. Better healing was observed in all the three groups but it was statistically non-significant, with Group III showing better results (p >0.05).

Conclusion: Thus, vestibular extension when performed with amniotic membrane showed better results in as compared to vestibular extension alone or when performed with PRF membrane.

KEYWORDS

Amniotic Membrane, PRF Membrane, Keratinised Tissue, Vestibular Extension

INTRODUCTION :

Mucogingival surgery, a relatively recent innovation in periodontal treatment is concerned with the problems of attached gingiva, alveolar mucosa and of the vestibular trough.[1] The concept of mucogingival surgery encompassed two intimately associated aims: the alteration of vestibular fornix depth and the production of a new and wider zone of attached ging iva.[2]

Following extraction, it has been well-demonstrated that hard tissue undergoes dimensional shrinkage.[3,4] However, the soft tissue also changes in a reductive way in terms of decrease in the keratinized tissue (KT) zone and vestibular depth to varying degrees, which may prevent appropriate prosthetic rehabilitation and facilitate plaque retention.[5]

Vestibuloplasty is a surgical procedure whereby the oral vestibule is deepened by changing the soft tissue attachments to increase the size of the denture bearing area and height of the residual alveolar ridge.[6] The most widely used techniques are Kazanjian's technique, Clark's apically positioned flap, the lip switch technique with different modifications and the Edlan-Mejchar technique.[7] The current vestibuloplasty techniques can be generally classified as secondary epithelialization, skin grafting, mucosal grafting, and local mucosal flaps.[8] In secondary epithelialization, wound contraction leads to the loss of newly attached mucosa and reattachment of muscle structures.[9]

Fetal membranes are biodegradable materials that have been used as graft material since their first introduction in 1910 by Davis.[10] Amnion is the innermost layer of the placenta and is derived from the epiblast. It has potential advantages of reducing loss of proteins, electrolytes and fluids, decreasing the risk of infection, minimizing pain, acceleration of wound healing and good handling properties; promotes secondary epithelialization and angiogenesis, antibacterial and causes no immune reactions; inexpensive and readily available in large amounts.[6,10] The use of amnion in vestibuloplasty has only been once reported by Guler et al., who concentrated on studying the blood flow to the graft.[11]

Platelet rich fibrin (PRF), a leukocyte and PRF biomaterial with a specific composition and three-dimensional architecture, was first developed in France by Choukroun et al in 2001. PRF has a dense fibrin network with leukocytes, cytokines, structural glycoproteins, and also growth factors such as transforming growth factor β1 (TGF-β), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and glycoproteins, that are gradually released as the fibrin matrix is resorbed, aiding the process of wound healing and regeneration.[12]

Thus, the aim of this study was to evaluate and compare clinically the vestibular depth and thickness of keratinised gingiva using vestibular extension alone or in combination with PRF membrane or amniotic membrane as graft materials for vestibuloplasty in mandibular anterior region.

MATERIALS AND METHODS :

This randomised controlled clinical study of a duration of 3 months was conducted in the Department of Periodontology and Oral Implantology, I.T.S. Centre for Dental Studies and Research, Ghaziabad on 30 patients, requiring both extension of vestibular depth and root coverage. This study followed the Declaration of Helsinki on medical protocol and ethics and the regional Ethical Review Board of I.T.S - Centre for Dental Studies and Research, Ghaziabad approved the study.Random allocation of participants t o individual treatment groups was achieved by a flip of coin:

- Group I – 10 patients underwent vestibular extension
- Group II– 10 patients underwent vestibular extension followed by placement of PRF membrane
- Group III – 10 patients underwent vestibular extension followed by placement of Amniotic membrane.

Inclusion criteria for the study was : patients with shallow vestibular depth, requiring root coverage procedures (Class 1 & 2 Miller's recession), radiographic evidence showing 15 – 20 mm of basal bone present in the anterior region of the mandible, no acute systemic disorders (ASA – I , ASA – II), no local pathology of either bone or soft tissue and motivated patients, and exclusion criteria was : patients with age ≥ 65 years, systemic (ASA –III, ASA – IV) and autoimmune diseases, pregnant or lactating women, patients undergoing or who have undergone radiotherapy or chemotherapy in the last 12 months, patients with para-functional habits and alcoholics, drug abusers and heavy smokers.

Detailed case history of the patients was recorded. Written and informed consent was taken by all the subjects. Data was recorded as per proforma, which included a clinical, radiological and haematological examination. Bimanual palpation and panoramic radiograph were used to determine the amount of possible extension of the vestibular sulcus.

Acrylic stents made of cold cure acrylic for positioning the probe and measuring the vestibular depth from a fixed reference point was fabricated for standardization of clinical measurements. UNC-15 probe was used to measure the clinical parameters, which were evaluated on Day 0, 7, 14 and 30 : Plaque Index[13] (PI), Gingival Index[13] (GI), Vestibular Depth (VD) (Fig 1a), Thickness of Keratinized Gingiva[14] (TKG) (Fig 1b), Healing Index[15] (HI) & Visual Analog Scale[16] (VAS).

Fig1:A)Pre-operative measurement of the vestibule with a periodontal probe and an acrylic stent B)Pre-operative measurement of the thickness of keratinised gingiva with a 6mm endodontic file

Prior to the surgery, all patients received thorough supragingival and subgingival scaling and root planing and oral hygiene instructions were given to the patient. Patients were reviewed after a period of 1 week and evaluated for optimal oral hygiene. Only those patients maintaining optimum oral hygiene were included in the study.

Platelet Rich Fibrin Membrane Preparation for Group II - Before the surgical procedure, 10 ml of blood was drawn from the subject's antecubital site. The blood sample was collected in glass coated plastic tubes without the addition of an anticoagulant. The blood containing tubes were immediately centrifuged for 10 min at 3000 revolutions per minute. The centrifuged blood mass presented with a structured fibrin clot in the middle of the tube between the red corpuscle layer at the bottom and the acellular plasma on the top. Fibrin clot could easily be retrieved from the tube and shaped freely and was used immediately after its collection. PRF was compressed between two glass slides to take the form of a consistent membrane.

Amniotic Membrane for Group III - The amniotic membrane was procured from the Tissue Bank, Tata Memorial Hospital, Mumbai, India.

Surgical Procedure (Fig 2,3,4) : Prior to the starting of procedure, extra oral preparation was done with 5% betadine. Oral scrubbing in 0.2% chlorohexidine gluconate was performed. Then, using 2% lignocaine hydrochloride with adrenaline (1: 80,000), bilateral mental nerve block was given. The lower lip was retracted outward and everted during the procedure. A transverse mucosal incision using a no. 15 B.P blade was placed in the mid vestibular region and extended deep into the labial sulcus from distal of lower right canine to the distal of lower left canine to create a suitable depth and width of vestibule. Supra-periosteal dissection was carried out and the muscle attachments were moved inferiorly to achieve the extended sulcus depth. After bleeding was controlled, PRF membrane or amniotic membrane was placed over the prepared recipient site and stabilized with absorbable sutures (4–0) tied in a criss-cross manner. Periodontal dressing was placed to provide comfort and protection over the surgical site in the groups I and II, whereas in the test group III, the surgical site was covered with a pre fabricated surgical stent.

Fig 2 : a to d – Vestibular extension in control group : (a) Pre-operative view after scaling and root planning, (b) Vestibular incision extending from lower right canine to lower left canine, (c) Periodontal pack (Coe-Pak) was placed over the surgical site, (d) Post-operative photograph showing uneventful healing at 1 month and an increase in the vestibular depth

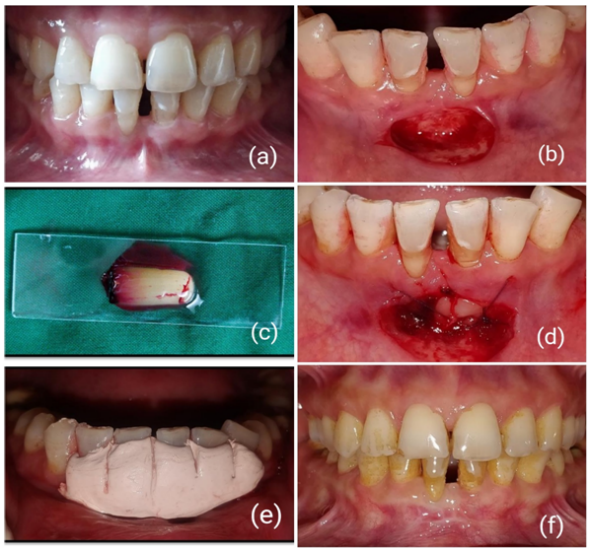

Fig 3 : a to f – Vestibular extension in Group II with PRF membrane (a) Pre-operative view after scaling and root planning, (b) Vestibular incision extending from lower right central incisor to lower left central incisor, (c) PRF membrane was prepared, (d) PRF membrane was placed and absorbable 4-0 sutures were given, (e) Periodontal pack (Coe-Pak) was placed over the surgical site, (f) Postoperative photograph showing uneventful healing at 1 month and an increase in the vestibular depth

Fig 4 : a to f – Vestibular extension in Group III with amniotic membrane : (a) Pre-operative view after scaling and root planing, (b) Vestibular incision extending from lower right lateral incisor to lower left central incisor, (c) Amniotic membrane was placed in the prepared surgical site, (d) Absorbable 4-0 sutures were placed, (e) Acrylic stent was placed over the surgical site, (f) Postoperative photograph showing uneventful healing at 1 month and an increase in the vestibular depth

Post-operative care - After surgery, a periodontal pack dressing (Coe Pak, De Trey/Denstply, Konstanz, Germany) was gently placed over the surgical site to cover the area for 72 hours in groups I and II. The surgical stent was placed for a period of 1 week in group III. All patients were put on a postoperative regime consisting of oral antibiotics (Amoxicillin 500mg 8th hourly for 5 days) and non-steroidal anti-inflammatory drugs (Ibuprofen 400mg 8th hourly for 5 days). Patients were instructed to maintain soft diet and to use warm saline rinses after meals and at bedtime. 0.2% chlorhexidine digluconate mouth rinses were advised twice daily for one month. The patients were made aware of the possibility of considerable pain, discomfort and swelling.

Post-Operative Evaluation - The patients were clinically evaluated at 7th, 14th and 30th day post therapy. Clinical evaluation was done on all the visits and the following were examined:

I. Healing of the mucosal flap and the effect of re-epithelization and its appearance (perforation, tearing, dehiscence etc).
ii. Postoperative course like pain or discomfort.
iii. Sensation of the lip and chin.
iv. Maintenance of vestibular depth
v. Clinical parameters were recorded

Data was analysed by Shapiro–Wilk test, one-way ANOVA, paired Sample t-test, Kruskal-Wallis test and Wilcoxon Signed Ranks test via SPSS version 16.0 (SPSS Inc., IL, USA) at 0.05 level of significance. The significance of association between 2 qualitative variables was tested by Pierson Chi Square Test. The level of significance and confidence interval were 5% and 95% respectively.

RESULTS:

In the present study, 30 patients fulfilling the inclusion and exclusion criteria were recruited; 10 each in group I, II and III, with age ranging from 27-65 years (mean age = 39.08±11.13 years). (Table 1)

Table 1 : Demographic status of the included patients

	Group I (n=10)	Group II (n=10)	Group III (n=10)
Age	33.75±6.80	44.75±13.96	38.75±11.52
Gender (Male/Female)	4/6	5/5	6/4
Smoking Status	NIL	NIL	NIL
Systemic Condition	Healthy	Healthy	Healthy

PLAQUE INDEX (PI) - (Table 2) Mean difference between the groups was statistically non-significant (p>0.05) at baseline and 30 days. *Post hoc* comparison of the scores showed that the mean PI score reduction was not statistically significant (p>0.05) from baseline to 30 days between groups I&II, II&III and I&III. On intra-group comparison for the groups I, II and III, the mean PI reduced significantly (*p<0.05*) at 30 days as compared to the baseline.

Table 2: Mean plaque index and mean difference in plaque index (PI) at various time intervals between the groups a nd within the groups.

		Group 1		Group 2		Group 3		
		Mean	SD[ρ]	Mean	SD	Mean	SD	p-value[α]
PI	Baseline	1.45	0.34	1.50	0.29	1.15	0.34	0.30
	30 days	1.12	0.17	1.02	0.15	0.85	0.28	0.23
MD[ε]		0.32		0.47		0.30		
p-value[β]		0.05*		0.02*		0.005*		

*Significant at p<0.05 ; [α]By one way ANOVA (Inter group comparison) ; [β]By paired t- test (Intra group comparison) ; MD[ε]– Mean Difference ; SD[ρ]– Standard Deviation

GINGIVAL INDEX (GI) - (Table 3) Mean difference between the groups was statistically significant (*p<0.05*) at baseline and 30 days. *Post hoc* comparison of the scores showed that the mean GI score reduction was statistically significant (*p<0.05*) from baseline to 30 days between group I&II and at baseline between group I&III, but was statistically non-significant (p>0.05) from baseline to 30 days between group II&III and at 30 days between group I&III. On intra-group comparison for group I, the mean GI reduced significantly (p<0.05) at 30 days as compared to the baseline but was statistically non-significant in group II and III at day 30 as compared to baseline.

Table 3: Mean ging ival index (GI) and mean difference in gingival index at various time interval s between the groups and within the group.

		Group 1		Group 2		Group 3		
		Mean	SD[ρ]	Mean	SD	Mean	SD	p-value[α]
GI	Baseline	0.70	0.18	0.22	0.22	0.26	0.18	0.01*
	30 days	0.38	0.10	0.09	0.10	0.09	0.10	0.04*
MD[ε]		0.31		0.12		0.16		
p-value[β]		0.02*		0.18		0.22		

[α] By one way ANOVA (Inter group comparison) ; [β] By paired t- test (Intra group comparison) ; Significant at p<0.05 ; MD[ε] – Mean Difference ; SD[ρ]– Standard Deviation

THICKNESS OF KERATINISED GINGIVA (TKG) - (Table 4) Mean difference between the groups was statistically non-significant (p>0.05) at baseline and 30 days. Post hoc comparison of the scores showed that the mean TKG score reduction was not statistically significant (p>0.05) from baseline to 30 days between group I&II, II&III and I&III. On intra-group comparison for the groups I, II and III, the mean TKG reduced significantly (p<0.05) at 30 days as compared to the baseline.

Table 4: Mean thickness of keratinized gingiva (TKG) and mean difference in thickness of keratinized gingiva at various time intervals between the groups between the group and within the group

		Group 1		Group 2		Group 3		
		Mean	SD[ρ]	Mean	SD	Mean	SD	p-value[α]
TKG	Baseline	2.25	1.32	2.25	0.64	1.50	0.40	0.41
	30 days	2.87	1.18	3.00	1.08	3.00	1.08	0.98
Md[ε]		-0.62		-0.75		-1.50		
p value[β]		0.01*		0.05*		0.03*		

[α] By one way ANOVA (Inter group comparison) ; [β] By paired t- test (Intra group comparison) ; Significant at p<0.05 ; MD[ε] – Mean Difference ; SD[ρ]– Standard Deviation

VESTIBULAR DEPTH (VD) - (Table 5) Mean difference between the groups was found to be statistically non-significant (p>0.05) at baseline, but was statistically significant (p<0.05) at 30 days. Post hoc comparison of the scores showed that the mean VD score reduction was not statistically significant (p>0.05) from baseline to 30 days between groups I&II and II&III, but was statistically significant (p<0.05) between I&III. On intra-group comparison for the groups I, II and III, the mean VD reduced significantly (p<0.05) at 30 days as compared to the baseline.

Table 5: Mean vestibular depth (VD) and mean difference in vestibular depth a t various time intervals between the groups and within the group.

		Group 1		Group 2		Group 3		
		Mean	SD[ρ]	Mean	SD	Mean	SD	p-value[α]
VD	Baseline	12.88	1.65	14.12	0.62	15.08	0.86	0.06
	30 days	13.75	1.55	15.38	1.10	16.70	1.26	0.03*
MD[ε]		-0.87		-1.25		-1.62		
p-value[β]		0.00*		0.03*		0.02*		

[α] By one way ANOVA (Inter group comparison) ; [β] By paired t- test (Intra group comparison) ; Significant at p <0.05 ; MD[ε] – Mean Difference ; SD[ρ]– Standard Deviation

HEALING INDEX (HI) - (Table 6) Mean difference between the groups was statistically non-significant (p>0.05) at 7, 14 and 30 days. Intra-group comparison was performed at three-point time intervals viz. 7th day, 14th day and 30th day. In Group I – difference between the HI scores at different time intervals was not significant ; Group II – mean HI scores reduced significantly (*p<0.05*) from 7 to 30 days and from 14 to 30 days ; In Group III - mean HI scores reduced significantly (*p<0.05*) from 7 to 14 days and from 7 to 30 days.

Table 6: Mean healing index (HI) and mean difference in healing index at various time intervals between the groups and within the group

		Group1		Group2		Group3		
		Mean	SD[ρ]	Mean	SD	Mean	SD	p-value[α]
HI	7days	1.75	0.50	1.75	0.50	1.75	0.50	1.00
	14days	3.25	0.50	3.25	0.50	3.50	0.57	0.70

	30 days	4.75	0.50	4.50	0.57	5.00	0.00	0.29
MD$^{\varepsilon}$		-0.87		-1.25		-1.62		
p-value$^{\mu}$	7-14 days	0.06		0.06		0.05*		
	14-30 days	0.06		0.05*		0.06		
	7-30 days	0.06		0.05*		0.05*		

$^{\pi}$ By Kruskal-Wallis test (Inter group comparison) ; $^{\mu}$ By Wilcoxon Signed Ranks test (Intra group comparison) ; Significant at p <0.05 ; MD$^{\varepsilon}$– Mean Difference ; SD$^{\rho}$– Standard Deviation

VAS SCORE (VAS) - (Table 7) Mean difference between the groups was found to be statistically significant (*p<0.05)* at 14 days. *Post hoc* comparison of the scores showed that the mean VAS score reduction was statistically significant (*p<0.05*) at 14 days between group I&II and II&III. On intra-group comparison for the groups I, II and III, the mean VAS reduced significantly (p<0.05) at all time intervals viz. 7th - 14th day, 14th - 30th day and 7th - 30th day.

Table 7: Mean visual analog score (VAS) and mean difference in visual analog score at various time intervals between the groups and within the group

		Group1		Group2		Group3		
		Mean	SD$^{\rho}$	Mean	SD	Mean	SD	p-value$^{\alpha}$
VAS	7days	6.25	0.50	7.00	0.81	6.75	0.95	0.42
	14days	2.50	1.29	4.25	0.50	1.75	0.95	0.01*
	30days	1.00	0.81	1.00	0.81	0.25	0.50	0.29
MD$^{\varepsilon}$	7-14days	3.75		2.75		-		
	14-30days	1.50		3.25		1.50		
	7-30days	5.25		6.00		6.50		
p-value$^{\beta}$	7-14days	0.00*		0.01*		-		
	14-30days	0.01*		0.00*		0.01*		
	7-30days	0.00*		0.00*		0.00*		

$^{\alpha}$ By one way ANOVA (Inter group comparison) ; $^{\beta}$ By paired t- test (Intra group comparison) ; Significant at p <0.05 ; MD$^{\varepsilon}$ – Mean Difference ; SD$^{\rho}$– Standard Deviation

DISCUSSION :

Pre-prosthetic surgery includes procedures for bone and soft tissue augmentation. Decreased residual alveolar ridge and keratinised mucosa is a prerequisite for compromised esthetics and plays an important role in prosthesis retention and stability.[7] It was believed that an inadequate zone of gingiva with a shallow vestibular fornix would facilitate subgingival plaque formation, favour attachment loss, soft-tissue recession and impede proper oral hygiene maintenance. Hence, vestibular deepening should be regarded where patients experience discomfort during brushing and chewing.[17]

PRF which is enriched in platelets, growth factors and cytokines, helps in increasing the healing potential of both hard and soft tissues. α-granules present in platelets contain growth factors (PDGF, TGF-β, VEGF and EPDF). PDGF functions as a chemoattractant for fibroblasts and osteoblasts in gingiva and periodontal ligament resulting in their activation.[12]

Guler et al.[18] concluded that grafts of amnion origin might be better than other grafts in mandibular vestibuloplasty because of early healing. It has been observed that when the amnion's mesenchymal side is applied to the host tissue, then vascularisation and rejection phenomenon are not seen leading to better graft survival. The process of using amniotic membrane is technique sensitive, manipulation of the membrane is not easy and requires experience. However, it has certain advantages such as : rapid healing, minimal scarring, relapse and post-operative pain, which makes it a viable and desirable option. Also, it is easily available, relatively cheap and appears to be a good alternative to other grafts, making it potentially an ideal material to cover exposed periosteal bone surface after vestibuloplasty.[6]

Thus, the present study compared three modalities for vestibuloplasty in the anterior mandibular region : vestibular extension only (Group I), vestibular extension with PRF (Group II) and vestibular extension with amniotic membrane (Group III) and to the best of our knowledge, these three modalities have yet to be compared in one study.

Adequate width of attached gingiva is required for the proper maintenance of oral hygiene. Any discrepancy in vestibular depth interferes in proper oral hygiene maintenance and may cause various mucogingival problems.[19] In our study, amniotic group (p=0.005) showed the best reduction in PI score, whereas control group (p=0.02) showed a statistically significant difference in mean GI score.

TKG depicted non-significant results in the present study. Gingival biotype has a significant impact on the aftereffect of restorative and regenerative therapy. Since tissue biotypes have unalike gingival and osseous architectures, they do exhibit different pathological responses, which could dictate different treatment modalities. Periodontal surgical technique can improve the tissue quality and treatment outcome.[20]

Mean VD scores depicted a statistically significant result, which was in concordance with similar studies by Al-Belasy[21] and Sikkerimath et al.[22] Thus, amnion graft is a viable and reliable option that stimulates early healing and maintains postoperative vestibular depth. In a case report by Singhal and Deepa[12], PRF membrane was used as a graft in vestibular extension and 3mm gain was found in vestibular depth post-operatively, concluding that adequate depth of vestibule and width of attached gingiva was obtained using PRF as an adjunct for vestibular extension.

Better healing was observed in all the three groups but it was statistically non-significant, with Group III showing better results (p >0.05). In another study by Keerthi et al[6], following the removal of the splint a week after the operation, a white necrotic soft tissue layer could be seen with underlying hyperemic tissue. By the end of the second week, the necrotic layer had faded, leaving slightly hyperemic mucosal tissue. Three weeks after the procedure the graft area was observed but the amnion had completely degenerated and disappeared. Recent studies[23,24] have shown that amniotic membrane is an abundant source of stem cells with the ability to differentiate into chondroblasts, osteoblasts, adipocytes, myocytes and neuronal cells. According to Alviano et al.[25], these cells have the ability to differentiate into endothelial cells and also properties to regulate the angiogenic processes.

In our study, the mean VAS showed significant results. Similar results were found in a study by Keerthi et al[6] in which the patient complained of very little discomfort and mild pain with no burning sensation. No complications such as infection or graft rejection was detected. There was negligible scarring or decrease in sulcular depth that was achieved after the vestibuloplasty procedure. Contrasting results were seen by Kalakonda et al[16], which compared VAS scores of pain and discomfort within the laser group and reported that patients in the laser group had lower VAS scores for pain and discomfort compared to the scalpel group.

The main strength of this study is that it is the first one to directly compare the clinical effects of control group v/s PRF membrane v/s amniotic membrane in vestibular extension in lower anterior region. However, there were certain limitations in the study design: the clinical data obtained was from 12 subjects only; a longer follow-up period would have shown better results; sites other than the mandibular anterior region could have been included; histologic sections at 1 month could have been taken to assess the healing. This is the first study in the best of our knowledge to clinically evaluate PI, GI and TKG in the three groups. In our opinion, further prospective studies evaluating all the clinical parameters with a larger sample size is recommended, to fully determine the success rate and predictability of this procedure.

CONCLUSION :

Thus, it was concluded that vestibular extension when performed with amniotic membrane showed better results in context of vestibular depth, thickness of keratinised gingiva, healing index, plaque index etc. as compared to vestibular extension alone or when performed with PRF membrane.

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