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The Effect of Amniotic Chorion Membrane on Tissue Biotype, Wound Healing and Periodontal Regeneration

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Abstract: *Amniotic chorion membrane is considered to be a promising treatment modalities for furcation involvement .The aim of this study was to evaluate clinically and radiographically the effect of using Amniotic chorion membrane (ACM) in conjunction to alloplast bone graft in the management of grade II furcation involvement and to assess its effect on tissue biotype and healing at the furcation area..This study was conducted on fourteen patients with fourteen furcation defects grade II. Patients were divided into two equal groups: Test group: were treated by alloplast bone graft and amnion chorion membrane, and control group: were treated with alloplast bone graft and collagen membrane. Soft tissue examination and hard tissue measurements with cone-beam computed tomography were performed. .Test group has shown better healing, more gain in clinical attachment loss, improve tissue biotype and enhanced bone formation when compared to the control group. It was concluded that, ACM can promote periodontal regeneration, improve tissue biotype and enhance healing of periodontal wounds.*

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I. Introduction

Tissue biotype is a critical factor that determines the result of dental treatment. Different gingival biotypes respond differently to inflammation, restorative, trauma and parafunctional habits .However periodontal surgical techniques can enhance tissue quality resulting in a more favorable treatment outcome.[1] The furcation is an area of complex anatomic morphology, 2 that may be difficult or impossible to debride by routine periodontal instrumentation. [3, 4] Class II furcation defects have the strongest level of evidence for predictable outcomes after regenerative therapy, that being combination therapy with barrier membranes and bone replacement grafts. [5]At present multiple regenerative techniques used alone or in combination are widely available for periodontal regeneration including bone graft or substitute, guided bone regeneration (GTR), root surface modification and biological mediators. [6]The most widely investigated calcium phosphate grafts are either hydroxyappatite [HA] or tricalcium phosphate (TCP). A mixture of HA and β-TCP produces biphasic calcium phosphate (BCP) which possesses the reactivity of β-TCP and the stability of HA, providing more bioactivity, and involving more new bone growth. BCP has an intermediate resorbability which can be controlled by variation of the HA/β-TCP ratio [7].Moreover It was reported that BCP composed of 60% HA and 40% β-TCP is the most ideal osteo-conductive bone graft material .7Collagen is also hemostatic, it attracts and activates periodontal ligament and ginigival fibroblast cells, it promotes platelet aggregation, it stabilizes blood clots and during wound healing interactions between collagen and various cell types take place[8]. Amniotic chorion membrane (ACM) which is a placental based membrane, is composed of amniotic membrane (AM), and chorion membrane [9]. Unlike other barrier membranes, ACM is biologically active due to the presence of bioactive proteins and growth factors (GF) that hasten granulation tissue formation and act as a bioactive matrix that facilitates cell migration. The wound healing property is further enhanced by the physiological seal obtained with the gingival[9].Immunohistochemical (IHC) staining of amniotic chorion membrane shows intense concentrations of laminin and laminin-5 in the barrier. Laminin-5 has affinity for binding gingival epithelial cells [10,11]. It was reported that Amniotic membrane (AM) can facilitate migration of epithelial cells, reinforces basal cell adhesion, promotes epithelial differentiation, prevents epithelial apoptosis, and promotes epithelization in healing of wounds[9]. An important property of AM is its resistance to various proteolytic factors owing to the presence of interstitial collagens [10]. Elastin present in amnion is responsible for providing elasticity. It has multiple metabolic functions such as its role in water and soluble material transportation and production of bioactive peptides, growth factors, and cytokines, therefore it was used as GTR membrane in treatment of periodontal osseous defects, gingival recessions, and furcation defects[12]. In spite of the membrane safety, in the literature few studies were performed to evaluate the GTR procedure using ACM augmented with bone graft to manage furcation defects, or asses its effect on tissue biotype as it promote epithelization.Therefore the current study aimed at evaluating clinically

and radiografically the effect of using ACM in conjunction to biphasic calcium phosphate [BCP] bone graft in the management of type II furcation involvement and to assess its effect on tissue biotype and healing at the furcation area.

II.Materials and Methods

Study Design : The study design was a simple random sample technique clinical trial that was conducted on 14 patients having 14 grade II furcation defects(31) associated with Miller's class I gingival recession(67) attending the clinic of the Periodontology and Oral Medicine Department, Faculty of Dentistry, Alexandria University.

Study Location: This study was performed at the the clinic of the Periodontology and Oral Medicine Department, Faculty of Dentistry, Alexandria University, Egypt.

Study Duration: March 2017 to March 2018

Sample size: 14 patient**s.**

Sample size calculations: The sample size was 14 patients; 7 per group with critical size grade II furcation defects is required to estimate an average success [bone filling] for amnion chorion membrane = 66.7% using alpha error = 0.05 and precision = 10% will provide a study power of 80 %. The sample size was calculated using G.power software[15, 16].

Subjects and selection method: The subjects consisted of fourteen patients having lower posterior tooth with critical size grade II furcation defect with horizontal component of 4 mm and a vertical component of 4 to 6 mm detected using Naber's probe and William's periodontal probe, [13,14] who attend the clinic of the Periodontology and Oral Medicine Department, Faculty of Dentistry, Alexandria University. Both sexes are included in the study with age range between 25-50 years. Patients were divided randomly into two groups:

- Group I [Test group]: In which seven furcation defects were managed with alloplast bone graft covered by amniotic chorion membrane.
- Group II [Control group]: In which seven furcation defects were managed with alloplast bone graft covered by resorbable collagen membrane.

Inclusion Criteria

1.Presence of a lower posterior tooth with critical size grade II furcation defect (15) with horizontal component of 4 mm and a vertical component of 4 to 6 mm (18), detected using Naber's probe and William's periodontal probe.
2.Patient's age between 25 - 50 years.
3.Both sexes.
4.The patient should be psychologically accepting the procedures.
5.Patients should be systemically free.

Exclusion criteria

1.Uncooperative patients regarding oral hygiene measures performance.
2.Patients with para functional habits.
3. Smokers.
4.Pregnant or lactating women.
5.Patients that have been submitted to any periodontal surgeries in the previous six months.
6.Patients that have received medication in the previous six months.

Materials

1.Amnion Chorion Membrane (ACM) (10x25mm) allograft, (Bioxclude). †
2.Resorbable collagen membrane (15x20x0.3 mm) (T-Gen). ‡
3.Bone alloplast (biphasic calcium phosphate (BCP)): Beta tri calcium phosphate + Hydroxy apatite, (500-1000 µm-1g) (Genesis).§

† Snoasis Medical, Denver, USA
‡ Bioland, Songjeongri, South Korea
§ Dio Implant, Busan, South Korea

Procedure methodology:

The study design was a simple random sample technique (clinical trial**).** The research was approved by the Ethical Committee of the Alexandria University, (IRBNO:00010556-IORG0008839) an informed consent was obtained from each patient after providing detailed information and description of the study.

The following clinical and radiographic examination were done to all patients preoperatively for selection, and evaluation of all parameters. Clinical evaluation included , healing index of Landry (HIL) [17],

probing depth (PD) [18], clinical attachment loss (CAL) [18] ,depth of keratinized gingival (tissue biotype) [19]. They are all assessed preoperatively and after one, three and six months postoperatively except (HIL) was assessed one and two weeks after the surgery. While radiographic examination included orthopantomogram (OPG) at the first visit for selection of the patient. Cone beam computed tomography (C.B.C.T) using the high-resolution imaging system 3D J. Morita, to indicate level of alveolar bone at the furcation area.
The slice thickness will be set at 0.5 mm, and the pitch will be set at 0.125 mm.

Measurements were done to the nearest 0.5 mm using the On Demand 3D software by Cybermed Inc. with a linear measurement tool and a digital magnification lens [20].Furcation involvement (FI) was calculated in the horizontal plane by measuring, to the nearest 0.5 millimeter, the distance between the outer root surface and the inter-radicular bone (bone loss in the horizontal direction – 'BL-H'). The degree of FI was graded according to Glickman's classification system[13] .Degree of bone loss in the vertical direction (BL-V) was calculated by measuring the distance from the furcation entrance to the base of the defect in the vertical direction [14]. The radiographic examination using cone beam computed tomography (C.B.C.T) was performed at the baseline, six and nine months. A correlation test was done then to assess the correlation between tissue biotype and underlying bone fill in the furcation area before and after treatment for both groups. All patients received first Phase 1 therapy in the form of oral hygiene instructions scaling and root planing. Coronoplasty will be done if needed.

Surgical procedure (Figure 1)

All patients were operated under local anaesthesia using nerve block technique in the surgical field then ingival sulcular incision was done using 15 c blade extending one tooth mesial and one tooth distal to the selected tooth.

A full thickness mucoperiosteal flap was reflected using a periosteal elevator and degranulation of the surgical field was done using CK6 surgical scaler and 4R-4L Colombia curette. The size of the defect was measured horizontally and vertically using Naber's probe and William's periodontal probe respectively.

Bone graft was mixed with the patient's own blood ‖and applied in the furcation defect using bone curette.

Concerning membrane application for ***Group I (Test group)*** ACM was applied over the alloplast bone graft in the defect according to the size of the defect extending and adapted over the defect using a damp gauze pressed on it for few minutes to ensure proper adherence.

While for ***Group II (Control group),*** resorbable collagen membrane was trimmed according to the size of the defect. Resorbable collagen membrane was applied over the alloplast bone graft and secured in place over the defect using 4-0 vicryl resorbable suture. The Flap was then repositioned using non-resorbable 4-0 silk interrupted sutures._Postoperative medications including Amoxicillin clavulanate ‖ 1 gm; 1 capsule every 12 hours for 5 days and non-steroidal anti-inflammatory drugs: Ibuprofen ¶ 1400 mg, 1 tablet 3 times daily after meals for 4 days._Sutures will be removed ten days post surgically.

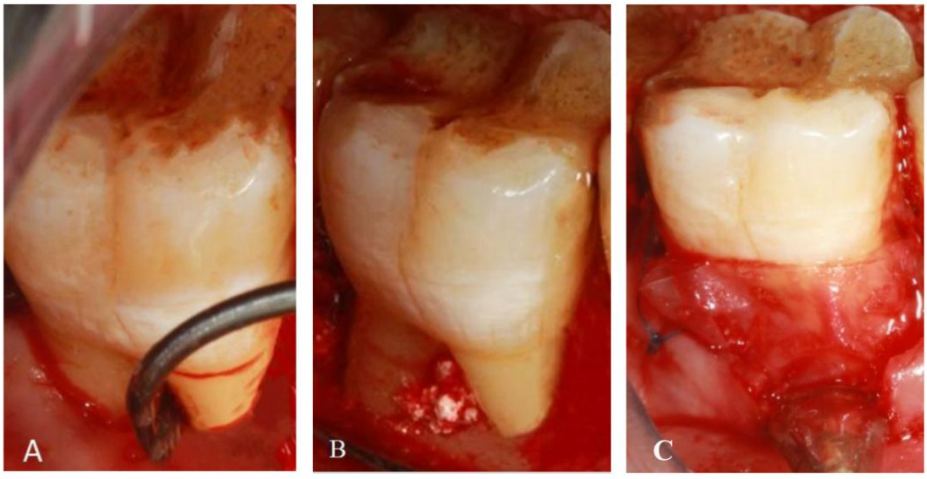


Fig . (1): Surgical procedure of the test group (A) Class II furcation defect, (B) Bone graft in the defect, (C) Application of the ACM.

‖Augmentin: Galaxo Smith Kline, Brentford, United kingdom
¶ BRUFEN Abbotl, Chicago, USA

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** [20] Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range [minimum and maximum], mean, standard deviation and median. Significance of the obtained results was judged at the 5% level using Student t-test, Mann Whitney test, Wilcoxon signed ranks test, and Friedman test.

III. Results

Clinical results: All patients completed the study and attended all recall visits. The surgical procedures were tolerated well by the subjects. There were no postoperative complications as Pain, tenderness, infection or swelling so the membrane was totally biocompatible.

Healing index of Landry (HIL): Table no 1

In test group there was improvement in HIL after 1 and 2 weeks, but it was not statistically significant however in control group the improvement after 1 and 2 weeks was statistically significant .Concerning the difference between the two groups in HIL, there was statistically significant difference between them after both 1 and 2 weeks follow up periods.

Table no 1:Comparison between the two studied groups regarding healing index of Landry

Healing index of Landry	Control (n=7)	Test (n=7)	t_1	p_1
After 1 week				
Min. – Max.	2.0 – 4.0	4.0 – 5.0		
Mean ± SD.	2.71 ± 0.76	4.57 ± 0.53	5.307	<0.001*
Median	3.0	5.0		
After 2 weeks				
Min. – Max.	3.0 – 5.0	5.0 – 5.0		
Mean ± SD.	4.0 ± 0.82	5.0 ± 0.0	3.240	0.018*
Median	4.0	5.0		
$t_2(p_2)$	6.971 (<0.001*)	2.121(0.078)		
% of change	**↑51.19**	**↑10.71**		

t_1, p_1: t and p values for **Student t-test** for comparing between **test and control**

t_2, p_2: t and p values for **Paired t-test** for comparing between **1 week and 2 weeks**

*: Statistically significant at $p \leq 0.05$

Probing depth (P.D) : Table no 2

In the test group, there was reduction in the P.D when comparing the baseline to 30 days, yet the difference was not significant , Although a significant reduction was noted when comparing baseline to 90 and 180 days. However, no significant difference existed when comparing between the follow up periods. As for the control group, no significant difference was present neither when comparing the baseline to 30 ,90, and 180 days , nor when comparing between the follow up periods in the control group .On comparing both groups, there was a reduction of the probing depth of the test group throughout the follow up period more than the control group, yet the difference was not significant .

Clinical attachment loss (C.A.L) Table no 2

Concerning CAL , although there was reduction of the C.A.L throughout the follow up period in the test group, yet it was not statistically significant ,however when comparing baseline to 90, and 180 days , there was a statistically significant different .On comparing between both groups, no significant difference was noted at 30 or 90 days, while a significant difference was realized after 180 days for the test group compared to the control group .

Table no 2: Comparison between the two studied groups regarding probing depth and clinical attachment loss in millimeters.

		Baseline	Day			p
			30	90	180	
Probing depth	**Test (n=7)**					
	Median (Min. – Max.)	4.0(3.0 – 5.0)	3.0(3.0 – 4.0)	3.0 (2.0 – 4.0)	3.0 (2.0 – 3.0)	^{F}**p**= 0.005*
	Mean ± SD.	3.71 ± 0.76	3.43 ± 0.53	3.0♯± 0.58	2.71♯ ± 0.49)	
	% of change		**↓7.5**	**↓19.1**	**↓27.0**	
	Control (n=7)					
	Median (Min. – Max.)	3.0 (3.0 – 5.0)	4.0 (3.0 – 4.0)	3.0 (2.0 – 4.0)	3.0 (2.0 – 4.0)	^{F}**p**= 0.453
	Mean ± SD.	3.70 ± 0.95	3.57 ± 0.53	3.14 ± 0.90	3.14 ± 0.90	
	% of change		**↓3.8**	**↓15.4**	**↓15.4**	
Clinical attachment loss	**Test (n=7)**					
	Median (Min. – Max.)	4.0(3.0 – 4.0)	3.0 (2.0 – 4.0)	3.0 (2.0 – 3.0)	2.0 (1.0 – 3.0)	^{Fr}p= 0.03*
	Mean ± SD.	3.57 ± 0.53	3.0 ± 1.0	2.86♯ ± 0.38	2.0♯ ± 1.0	
	% of change		**↓16**	**↓19.9**	**↓44**	
	Control (n=7)					
	Median (Min. – Max.)	3.0 (3.0 – 5.0)	3.0 (3.0 – 4.0)	3.0 (3.0 – 4.0)	3.0 (3.0 – 4.0)	^{Fr}p= 0.950
	Mean ± SD.	3.56 ± 0.98	3.29 ± 0.49	3.29 ± 0.49	3.29†† ± 0.49	
	% of change		**↓7.8**	**↓7.8**	**↓7.8**	

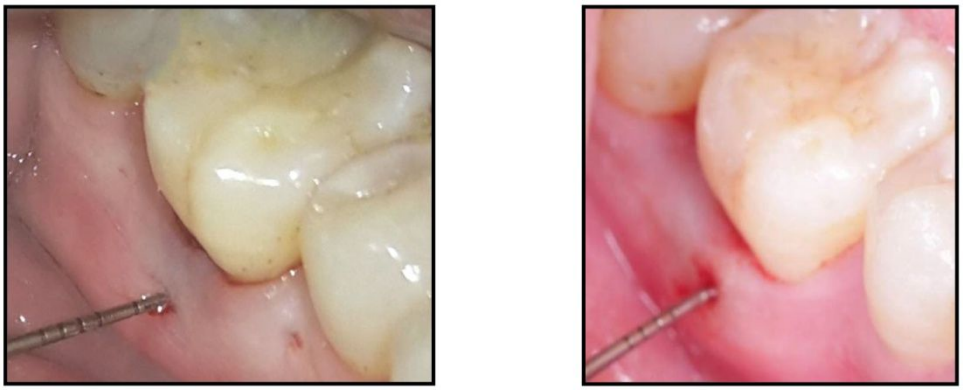
††: Statistically significant between **Test and Control**
♯: Statistically significant with **baseline**
^{F}p: p value for **ANOVA** with repeated measures, Sig bet grps was done using Hoc Test (**Bonferroni**)
^{Fr}p: p value for **Friedman test** Sig .between periods was done using **Wilcoxon signed ranks test**
*: Statistically significant at $p \leq 0.05$

Depth of keratinized gingiva in millimeters: Table no 3
Although there was increase in the mean depth of keratinized gingiva in the test group throughout the follow up period, yet the difference was not statistically significant.. As for control group, no change was noted in control group neither when comparing baseline to 30, 90, and 180 days nor during all the follow up periods. When comparing both groups, no significant difference was noted at 30days, while a significant difference was noted when comparing both groups at 90 and 180 days. Concerning the percentage of increase in test group, a slight improvement was noted in test group, as depth of keratinized gingiva increased in test group, while no change was found in control group.(Figure 2)

Table no 3:Comparison between the two studied groups regarding depth of keratinized gingival in millimeters.

Depth of keratinized gingival	Baseline	Day			^{F}p
		30	90	180	
Test (n=7)					
Median (Min. – Max.)	4.0(3.0–5.0)	4.0(3.0–5.0)	4.0(4.0–5.0)	4.0(4.0–5.0)	0.054
Mean ± SD.	3.71±0.76	4.14±0.69	4.29±0.49	4.43±0.53	
% of change		**↑11.59**	**↑15.63**	**↑19.41**	
Control (n=7)					
Min. – Max.	4.0(3.0–4.0)	4.0(3.0–4.0)	4.0(3.0–4.0)	4.0(3.0–4.0)	-
Mean ± SD.	3.57±0.53	3.57±0.53	3.57††±0.53	3.57††±0.53	
% of change		**0%**	**0%**	**0%**	

††: Statistically significant between **Test and Control**
^{F}p: p value for **ANOVA** with repeated measures, Sig bet groups was done using Hoc Test (**Bonferroni**)
*: Statistically significant at $p \leq 0.05$



A) Before B) After

Fig.(2): (A) Depth of keratinized gingival before treatment and (B) after 6 months.

Radiographic results

1-Horizontal component of the Furcation :Table no 4

A significant reduction of the horizontal component of the furcation was denoted in the test group when comparing baseline to 6 and 9 months, and when comparing 6 months to 9 months.

As for the control group, a significant decrease was noted when comparing baseline to 6 and 9 months , and when comparing 6 months to 9 months.

Comparing both groups, a significant reduction was realized in the test group after 6 and 9 months , denoting reduction in the horizontal component of the furcation in the test group by the end of the follow up period compared to the control group . (Figure 3)

2. Vertical component of the furcation : Table no 4

A significant decrease was denoted in the test group when comparing baseline to 6 and 9 months. However, this difference was insignificant when comparing 6 months to 9 months.

As for the control group, no significant difference was denoted neither when comparing baseline to 6 and 9 months ,nor when comparing between the different follow up periods.

Comparing both groups, a significant reduction of the vertical component of the furcation was realized after 6 and 9 months in the test group compared to the control group, denoting improvement in the vertical component of the furcation in the test group by the end of follow up period compared to the control group.(Figure 3)

Table no 4: Comparison between the two studied groups regarding horizontal and vertical component of the furcation in millimeters.

		Baseline	6 Months	9 Months	^{Fr}p
Furcation horizontal component	**Test (n=7)**				
	Median (Min. – Max.)	5.0(4.0 – 5.0)	3.0(0.0 – 3.50)	2.0(0.0 – 2.50)	<0.001*
	Mean ± SD.	4.64 ± 0.48	2.64♯ ± 1.28	1.93♯ ± 0.89	
	% of change		**↓43.1**	**↓58.4**	
	Control (n=7)				
	Median (Min. – Max.)	4.5(4.0 – 5.0)	4.0(3.0 – 4.50)	3.0(3.0 – 4.0)	<0.001*
	Mean ± SD.	4.57 ± 0.45	3.86♯†† ± 0.63	3.29♯†† ± 0.39	
	% of change		**↓15.5**	**↓28.0**	
Furcation vertical component	**Test (n=7)**				
	Median (Min. – Max.)	4.5(4.0 – 4.50)	2.0(0.0 – 3.0)	2.0(0.0 – 2.0)	0.002*
	Mean ± SD.	4.29 ± 0.27	1.86♯ ± 0.94	1.57♯ ± 0.79	
	% of change		**↓56.6**	**↓63.4**	
	Control (n=7)				
	Median (Min. – Max.)	4.5(4.0 – 4.50)	3.0(2.50 – 4.0)	3.0 (2.50 – 4.0)	0.058
	Mean ± SD.	4.30 ± 0.28	3.32†† ± 0.70	3.29†† ± 0.70	
	% of change		**↓23.3**	**↓23.3**	

††: Statistically significant between **Test and Control**

♯: Statistically significant with **baseline**

^{Fr}p: p value for **Friedman test** for comparing between the different periods in each group

*: Statistically significant at $p \leq 0.05$

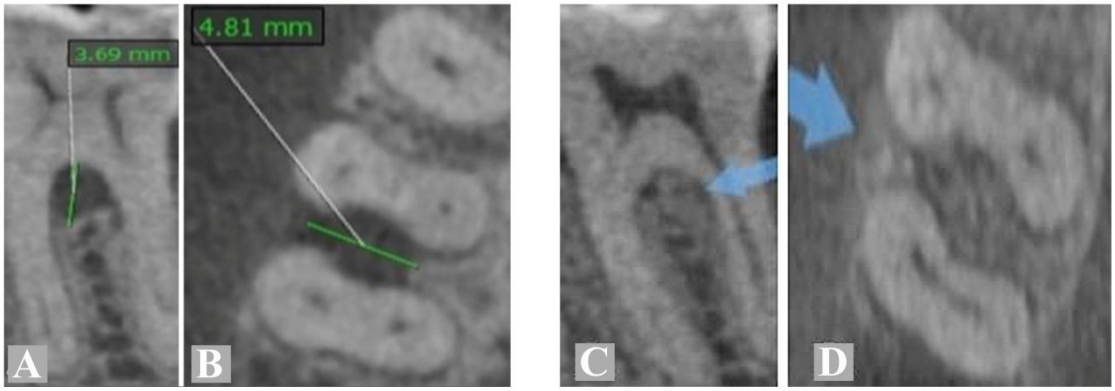


Fig. (3): CBCT image showing. **(A)** Vertical component **(B)** Horizontal component of the furcation defect at baseline,(C) Vertical component **(D)** Horizontal component of the furcation defect at 9 months all in test group.

IV. Discussion

The present study was carried out to evaluate the effect of using amnion chorion membrane augmented with (biphasic alloplast) in the management of grade II furcation defects clinically and radiographically. The study also assessed the two treatments effect on tissue biotype and healing at the furcation area.

The selection of ACM in management of critical size grade II furcation involvement was due to presence of biologic growth factors, which may enhance periodontal tissue regeneration. [21]Moreover it has anti-inflammatory, and anti-bacterial properties. It has the ability to promote epithelization and regeneration. In addition to anti fibrosis properties, cell adhesion and cell differentiation properties. ACM has a thickness of 300 micrometers, and a rate of resorption in a range of eight to 12 weeks according to the manufacture [21].

Amniotic chorion membrane (ACM) acts in a different manner from traditional GTR barriers in the fact that it encourages rapid epithelial cell growth rather than epithelial exclusion. As epithelial cells quickly migrate across the ACM barrier, they form a seal over the underlying bone graft and do not apically migrate into the defect [22].In the present study healing index of Landry, probing depth (PD), and clinical attachment loss (CAL) were assessed and evaluated throughout the follow up period. As those clinical parameters and their results indicate the progress of healing and regeneration . The depth of keratinized gingiva were assessed in the current study, as the depth of keratinized gingival considered as one of the methods of assessment of gingival biotype and is also an important parameter when defining the qualitative nature of the gingival. [19,23]ACM membranes are easier to apply, due to the self-adhering properties, so they do not require sutures for fixation. This property could be due to the presence of adherent molecules as laminin and fibronectin , in addition to the presence of elastin which made it elastic and self-adherent. All these properties allow ACM to ultimately mold to the defect anatomy and adapt to the contours around the roots' surfaces [24].Results of the current study show progress and improvement of healing in test group when compared to control group. As most of the patients in test group showed excellent healing according to healing index of Landry after 1 and 2 weeks follow up period and the difference between the two groups was statistically significant. The significant improvement in healing in test group may be due to the anti-inflammatory properties of ACM, which may decrease the influx of inflammatory cells and inflammatory mediators to the wound area and serve as a barrier [25].Also it was proved that ACM releases inhibitors of matrix metalloproteinases (MMPs), e.g: 1, 2, 3, and 4, which are released by infiltrating neutrophils and macrophages. In addition to the presence of interleukin-10 antagonists and endostatin which inhibit inflammation. [26] Moreover, the matrix of amniotic membrane stroma has the ability to suppress pro-inflammatory mediators e.g: interleukin-1α and interleukin-1β [27, 28].

Results of the current study are in agreement with another study that used amniotic membrane (AM) in management of intra-bony defects combined with demineralized freeze dried bone allograft (DFDBA) and open flap debridement (OFD). Healing promotion ability of ACM was proved through occurrence of significant reduction in the mean plaque index, gingival index, and bleeding index from baseline to 12 months in that study, and it was suggested that the membrane reduces inflammation through entrapment of inflammatory cells , and the presence of proteinase inhibitors [29].Regarding PD and CAL in the current study, there was reduction in PD and gain in clinical attachment level in test group compared to control group throughout the follow up period. These reduction and gain may be explained by the action of ACM which encourages rapid epithelial cell growth rather than epithelial exclusion which is a different manner of action from traditional GTR barriers, in other words the presence of the laminin proteins cause the rapid epithelial growth along the membrane, so the new junctional

epithelium forms faster which allows for epithelial exclusion from the bone defect [21].Results of the present study are in consistence with another one that showed a reduction in PD and gain in clinical attachment level after six months follow up period. When used a composite allograft containing mesenchymal cells with ACM to treat mandibular class III furcation defects [30] .Also in accordance with a retrospective study by Holtzclaw [31], revealed that localized moderate-to-severe chronic periodontitis patients treated by ACM combination-GTR therapy had average PD reduction and clinical attachment level improvement after 12 months follow up period. It was concluded that ACM may hold promise for regeneration and treating the most challenging clinical dilemmas. Regarding depth of keratnized gingiva the increase of it was noticed in test group only, this may be explained by the fact that, once the stability of the soft tissue margin has been obtained at the level of cemeno-enamel junction (CEJ), keratinized tissue (KT) is able to increase with time [32]. This stability may be due to the presence of laminin and fibronectin, with their role in promotion of cell attachment, growth, and differentiation of a number of cell types. As well as, their involvement in many cellular processes, including tissue repair, blood clotting, cell migration, and adhesion [23, 33] .Moreover it was found that ACM reinforces basal cell adhesion, due to the presence of significant amounts of laminin and laminin-5. [24, 34] Additionally, chorion matrix contains abundant growth factors, such as keratinocyte growth factor (KGF), basic fibroblast growth factor (b-FGF), and transforming growth factor beta (TGF- β), that promote periodontal regeneration, 35 and provide a natural environment for accelerated healing, all those factors can attribute to increase in keratinized gingival depth or soft tissue biotype which was statistically significant in test group in comparison to control group[31].The current results are also in accordance with another study used chorion membrane with coronally advanced flap for gingival biotype enhancement, and the results revealed a one millimeter increase in gingival thickness at three months follow up[23].

The radiographic results (CBCT results) from the current study show a significant improvement of vertical and horizontal furcation components (gain in bone fill) in test group after six, and nine months when compared to control group. These results are consistent with those of the study used composite allograft containing mesenchymal cells with ACM to treat a mandibular class III furcation, in which three-dimensional imaging at six months using CBCT suggested complete furcation closure [30]. Provided that this study used composite allograft containing mesenchymal cells, while in the current study alloplast (BCP) was used.The current results are also in agreement with Kumar et al [36] who used AM with hydroxyapatite (HA) bone graft to manage contained interdental defects, and found a significant gain in bone fill at test group from baseline to 24 weeks. They concluded that AM has the potential to function as a barrier for GTR and can act as a matrix for periodontal regeneration. Similarly Kothiwale et al 37 evaluated the efficacy of DFDBA and bovine derived xenogeneic bone graft with AM in treatment of Grade II furcation defects, and showed significant PD reductions, CAL gains, and significant improvement in bone fill.The improvement of horizontal and vertical furcation components may be attributed to the bone inductive potential of ACM, due to its ability to upregulate the recruitment of mesenchymal progenitor cells which demonstrate osteogenic and adipogenic differentiation. At the same time, ACM shows excellent acceptability with bone grafts by demonstrating excellent containment of the material and its resorption without the formation of voids [38] .

V. Conclusion

Amnion chorion membrane is an effective, easy to handle and safe membrane, that can promote periodontal regeneration, improve tissue biotype and enhance healing of periodontal wounds .Further studies are recommended to assess the effect of the ACM on tissue biotype in other areas specially thin biotype gingival tissue in anterior teeth.

Conflict of interest

Drs Taalab and Gamal report no conflict of interest related to this study.

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