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ORIGINAL RESEARCH

Human amniotic membrane, best healing accelerator, and the choice of bone induction for vestibuloplasty technique (an animal study)

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Objective: To investigate the effects of amniotic membrane (AM) in bone induction and wound healing after vestibuloplasty surgery on animal samples while receptacle proteins such as growth factors were considered as accelerators for wound healing and bone induction after these operations.

Material and methods: Ten adult dogs (5 females, 5 males; race, Iranian mixed; weight, 44 pounds) were included, which underwent surgery for transplantation on mandible and maxillary. AM was used for promoting bone induction and healing.

Results: The tissue samples were obtained after 2, 8, and 12 weeks for histology survey. No significant differences were observed between male and female or left and right jaws. AM decreased fibrinoleukocytic exudates and inflammation in the experimental group, had significant effects on bone formation, considerably improves wound healing, and gives rise to bone induction ($P < 0.0001$).

Conclusions: Our study findings indicate that the AM is a suitable cover for different injuries and acellular AM has the potential for rapid improvement and bone induction. The AM contains collagen, laminin, and fibronectin, which provide an appropriate substrate for bone induction. This substrate promoted bone induction and might contribute to induction of the progenitor cells and/or stem cells in the area where surgery had been undertaken and is also differentiated into bone. In comparison with the control group, the difference was significant and meaningful ($P < 0.0001$).

Keywords: inflammation, bone induction, fibrinoleukocytic exudates

Introduction

Amniotic membrane (AM), the innermost layer of fetal membranes, is applied as a biological membrane for the management of burns and skin ulcers.[1–3] Furthermore, it has been identified as a suitable membrane for vestibuloplasty surgery.[4] Alveoloplasty and ridge expansion are the surgical removal of a portion of the alveolar process and retract the mucogingival from top of ridge to vestibule.[5]

AM is suitable for supporting the growth of epithelial progenitor cells. It is composed of basement membrane and avascular stroma. The AM stroma contains growth factors, antiangiogenic factors, anti-inflammatory proteins, and natural protease inhibitors.[3] Basement membrane consists of collagen type IV and VII, laminin 1 and 5, fibronectin, and basic fibroblast growth factor (bFGF).[6,7] The side of amniotic tissue whose subchains are identified as having conjunction of collagen IV with laminin is especially effective in facilitating epithelial cell adhesion.[7] Application of AM is significant when considering the immunologic rejection caused by AM. In previous research, it has been reported that the acute inflammation declined when the wounds were covered

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with AM and this reduction developed and was defined by polymorphonuclear (PMN) neutrophils infiltration.[6] AM has been found to be a suitable wound dressing for reconstruction of oral cavity, bladder, and vagina.[8] The studies on corneal transplantation demonstrate that the AM may enhance epithelialization, reduces inflammation or scarring, regulates angiogenesis, and possesses antibacterial activity.[9]

Considering that AM has different growth factors, assists in improving physiologic wounds, accelerates wound healing, and stimulates bone induction, the aim of this study is to use AM as an appropriate cover for surgical sites and evaluation of bone inductive effect and loading of bone formation on the buccal or lingual side of the alveolar ridge after vestibuloplasty surgery in dogs.

Material and methods

Preparation of amniotic membrane

The placenta was prepared just after elective cesarean delivery. The woman's serum was negative for human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and syphilis. The placenta was washed free of blood clots with sterile saline under a lamellar flow hood. The inner AM was separated from the rest of chorion. Small clean sections (5×5 cm^2) of placenta were kept in 400 mL of saline, containing 1,000,000 IU specific antibiotic composition at 4°C for 24 hours. After a 24-hour period, the samples were cultured again to check whether they were microbial or not (all procedures were performed at the Iranian Tissue Bank, Imam Khomeini Hospital Complex, Tehran University Medical Sciences, Tehran, Iran).

Surgical procedures (wound preparation and amniotic membrane transplantation)

Ten adult dogs (5 females, 5 males; race, Iranian mixed; weight, 44 pounds) were kept in the same circumstances in Torabinejad Center (Isfahan, Iran). They did not have teeth between canines and their first molars (the canines and molars of the samples had been extracted 4 months ago). All animals were intramuscularly anesthetized with ketamin (20 mg/kg) and aspromize (0.2 mg/kg). After intubation, the procedure of anesthetization was maintained by 1.5% halotan. Following the removal of the buccal or lingual epithelium with full-thickness flap at the buccal or lingual vestibule by periosteal elevator from the top of alveolar ridge (crestal bone) to mouth floor or deep of buccal in mandible and to check or plate in maxilla jaw, a wound was created 7×5 cm^2 in size. Then, half of the ridge bone was subperiosteally exposed and the wound was completed. In control sides, after the procedure the wounds were dressed by Orabase® and acrylic cover, whereas in experimental sides, the wounds were covered by sterile AM (Iranian Tissue Bank), Orabase (dressing ointment; Bristol-Myers-Squibb, New York, NY), and acrylic cover (Bayer, Tehran, Iran). After 14 days, the acrylic cover was removed and we removed the tissue sample. The sampling times were in the 2nd, 8th, and 12th weeks. Full-thickness samples (1×3 cm^2), which had been obtained from the buccal or lingual epithelium and alveolar bone, were removed by chisel and the wound sites were dressed by surgery pack in both experiment and control groups. (There is a 1 cm space between the sample sites, because the new ulcer could bias a histopathology survey. Each dog had four sites: two sites were experimental and two sites were control, and they were located in the maxilla jaw and mandible jaw, respectively).

Histopathology survey

The histopathological samples were obtained in the 2nd, 8th, and 12th weeks after surgery. The samples were reseated, fixed in 10% formalin buffer, and then kept in 5% phosphoric acid to dissolve the bone. After 48 hours, they were embedded in paraffin and 4-µm slices were prepared (10 slides provided for each paraffin block), and then stained with hematoxylin and eosin and viewed under an Olympus microscope with 10× and 40× magnifications (Olympus, Tokyo, Japan). Ten slides were provided for each sample block in the 2nd, 8th, and 12th weeks, giving 1,200 slides for histopathology survey (600 slides were experimental and 600 slides were control).

Results

Ten adult dogs (5 males, 5 females) were investigated after the 2nd, 8th, and 12th weeks. A thin strip (1×3 cm^2) was separated from the transplanted sites. To provide a histopathological assay, 10 slides were collected from each sample. In all, 1,200 samples from 10 dogs were obtained. The female and male samples were divided according to maxillary and mandibular jaws and then maxillary and mandibular jaws were divided into left and rightsides. Each group was labeled a, b,

Table 1 Summary of total group

Sex	Location	Type	Groups
Male	Upper	Control	a1
Male	Lower	Control	a2
Male	Upper	Experimental	b1
Male	Lower	Experimental	b2
Female	Upper	Control	c1
Female	Lower	Control	c2
Female	Upper	Experimental	d1
Female	Lower	Experimental	d2

Table 2 Independent samples *t*-test analysis

	Group	N	Mean	SD	*P* value
Healing 2	Case	120	1.052	0.0914	<0.0001
	Control	120	0.050	0.0603	
Healing 8	Case	120	2.002	0.0375	<0.0001
	Control	120	1.024	0.0793	
Healing 12	Case	120	3.017	0.0408	<0.0001
	Control	120	2.023	0.0513	
Fibrinoleukocytic 2	Case	120	0.0302	0.04326	<0.0001
	Control	120	3.0019	0.03275	
Fibrinoleukocytic 8	Case	120	0.0302	0.04326	<0.0001
	Control	120	2.0150	0.05048	
Fibrinoleukocytic 12	Case	120	0.0302	0.04326	0.026
	Control	120	0.0190	0.03364	

Abbreviation: SD, standard deviation.

c, and d, which were randomly changed in every animal. For example, if the left maxillary was control in the first animal, it was experimental in the second one (Table 1). Finally, one site was experimental and the opposite site was control (in the same jaw). The important variables were inflammation, fibrinoleukocytic exudates, healing, and bone induction. For data analysis and the histomorphometric survey, SPSS (version 16; SPSS Inc., Chicago, IL) and image tool software were used. Finally, parametric data for investigating the samples such as considerable changes in bone formation, healing, fibrinoleukocytic exudates, and inflammation rate analyses with independent samples *t*-test were used for comparative parametric variables in the experimental and control groups ($P < 0.0001$; Tables 2 and 3).

Table 2 shows the two groups (experimental and control) in which the healing and bone induction were found to be significant at the 2nd, 8th, and 12th weeks ($P < 0.0001$). It should be mentioned that there was no significant difference between male and female and left and right jaws, considering studied parameters (Figure 1). Based on the analyses, the most remarkable inflammation was observed among control groups in the 2nd and 8th weeks, whereas the least inflammation was in maxillaries and mandibles of dogs in experimental groups ($P < 0.0001$; Tables 2 and 3, Figures 2–13). In all experimental groups, the rate of healing was more than in control groups and the fibrinoleukocytic exudates were significantly less than in control groups (Figures 1–9, 11, 12). The rate of healing among females was more than males in experimental groups (not significant). Bone formation had occurred in mandible and maxilla jaws of the male and female dogs in experimental groups, but it did not occur in maxilla and mandible jaws of the male and female dogs in control groups ($P < 0.0001$; Figures 4–8, 10). Although very little inflammation was seen in control groups in the 12th week, it completely improved in experimental groups. Bone regeneration and general healing were meaningful and statistically significant in experimental groups, but fibrinoleukocytic exudates were still seen in control groups in the 12th week ($P < 0.0001$).

Table 3 Independent samples *t*-test analysis

Group 1		Group	N	Mean	SD	*P* value
Upper jaw	Inflammation 2	Case	60	0.0133	0.02549	<0.0001
		Control	60	2.9830	0.04295	
	Inflammation 8	Case	60	0.0290	0.12873	<0.0001
		Control	60	1.9852	0.07338	
	Inflammation 12	Case	60	0.0143	0.02520	0.748
		Control	60	0.0158	0.02580	
	Bone remodeling 2	Case	60	1.0433	0.08511	<0.0001
		Control	60	0.0095	0.02574	
	Bone remodeling 8	Case	60	1.5243	0.48967	<0.0001
		Control	60	0.0095	0.02574	
	Bone remodeling 12	Case	60	3.0140	0.03928	<0.0001
		Control	60	0.0095	0.02574	
Lower jaw	Inflammation 2	Case	60	0.0167	0.03256	<0.0001
		Control	60	3.0137	0.03936	
	Inflammation 8	Case	60	0.0157	0.03285	<0.0001
		Control	60	1.9938	0.05918	
	Inflammation 12	Case	60	0.0157	0.03285	0.803
		Control	60	0.0143	0.02520	
	Bone remodeling 2	Case	60	1.0500	0.08537	<0.0001
		Control	60	0.0427	0.05722	
	Bone remodeling 8	Case	60	2.0075	0.03487	<0.0001
		Control	60	0.0257	0.04612	
	Bone remodeling 12	Case	60	3.0140	0.03928	<0.0001
		Control	60	0.0443	0.05715	

Abbreviation: SD, standard deviation.

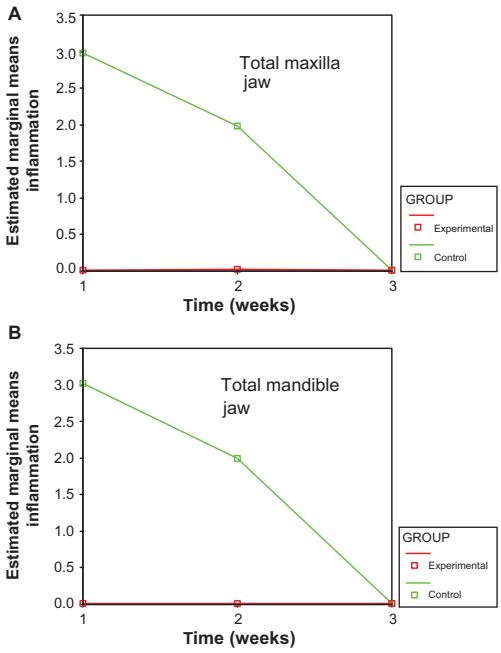

Figure 1 Inflammation condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (total maxilla [**A**] and mandible [**B**] male and female jaws).

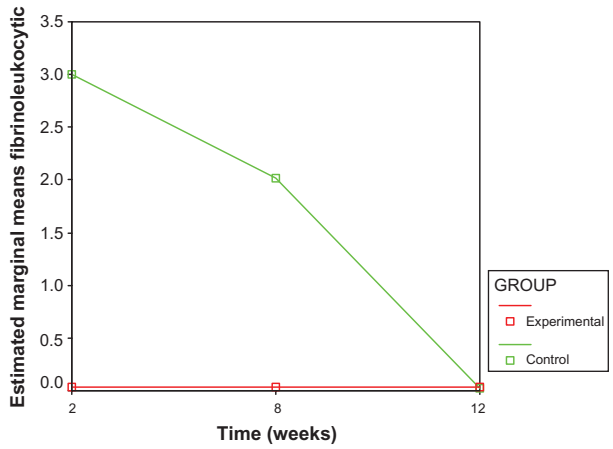

Figure 2 Fibrinoleukocytic exudates condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (male and female were identical).

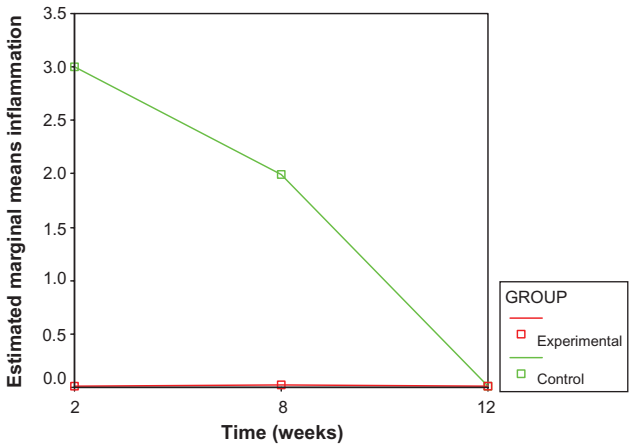

Figure 3 Inflammation condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (male and female were identical).

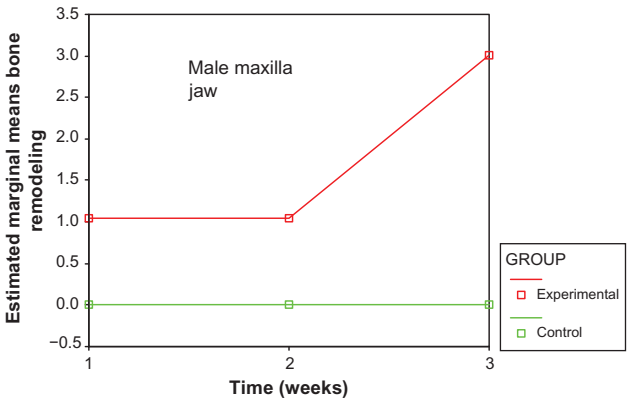

Figure 4 Bone remodeling condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (maxilla male jaw).

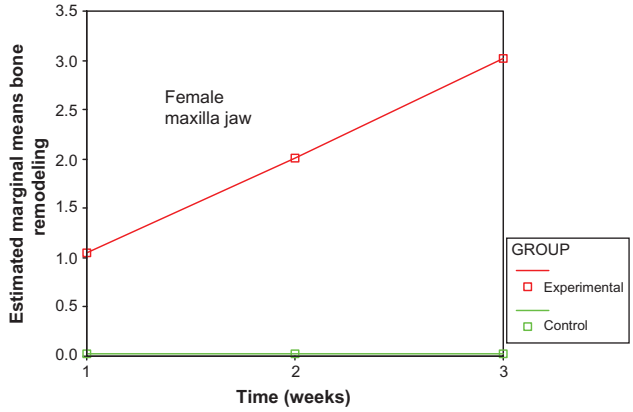

Figure 5 Bone remodeling condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (maxilla female jaw).

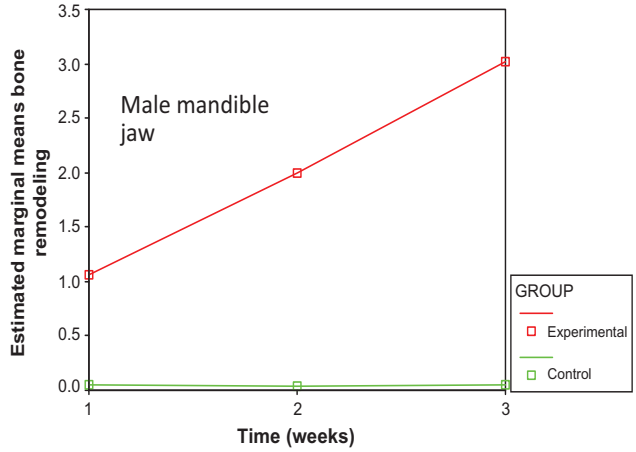

Figure 6 Bone remodeling condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (mandible male jaw).

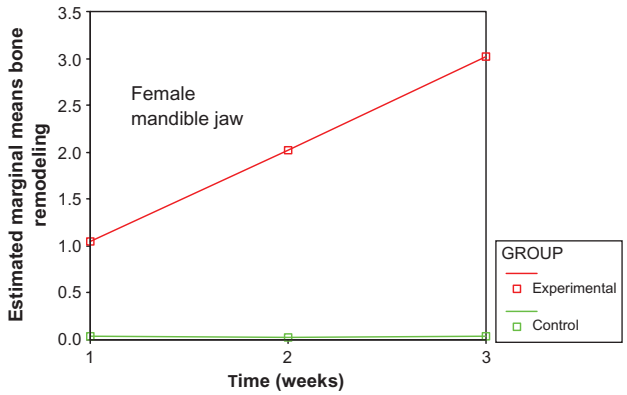

Figure 7 Bone remodeling condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (mandible female jaw).

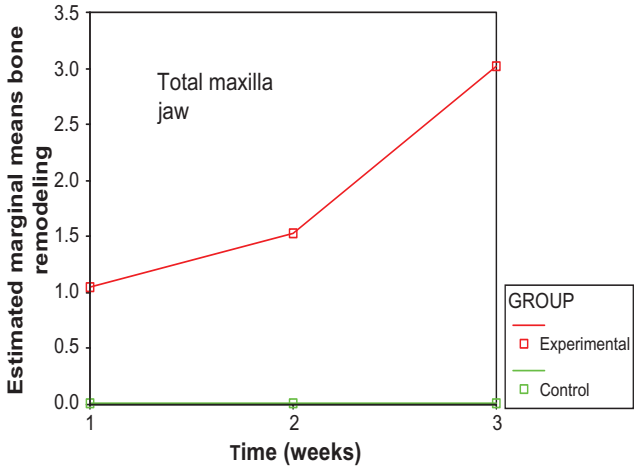

Figure 8 Bone remodeling condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (maxilla male and female jaws).

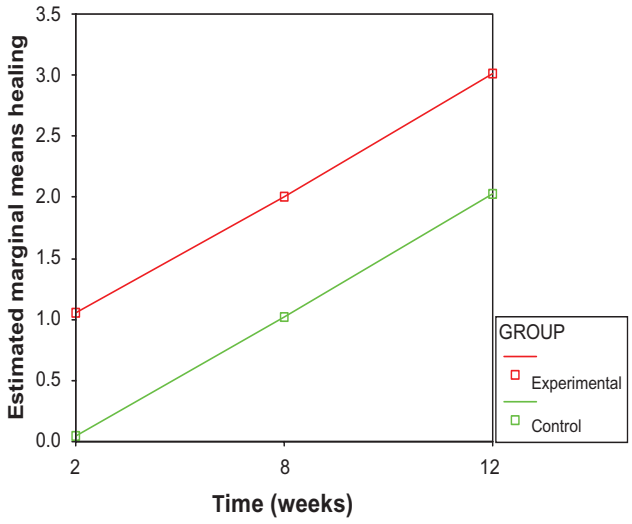

Figure 9 Healing condition in experimental and control groups at the 2nd, 8th, and 12th weeks after surgery (maxilla and mandible male and female jaws).

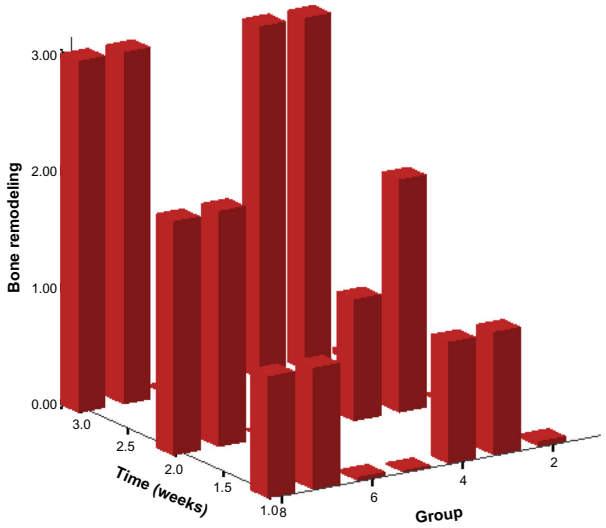

Figure 10 The rate of bone remodeling during 12 weeks. The analysis demonstrated that the rate of bone remodeling in experience groups was significantly greater than in controls.

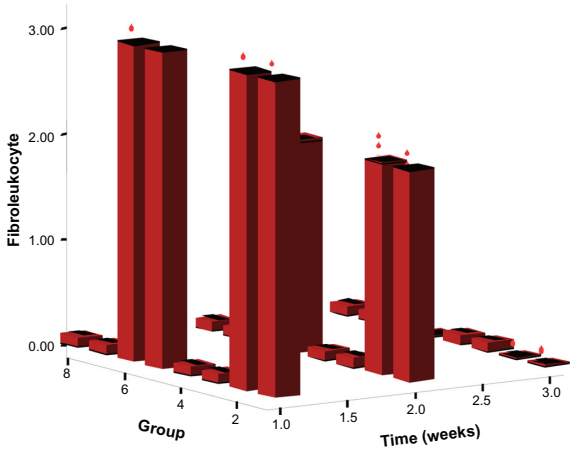

Figure 11 The changes of fibrinoleukocyte layer over 12 weeks. The fibrinoleukocyte layer was statistically significant in control groups at the 2nd and 8th weeks, but it was gradually recovered at the 12th week.

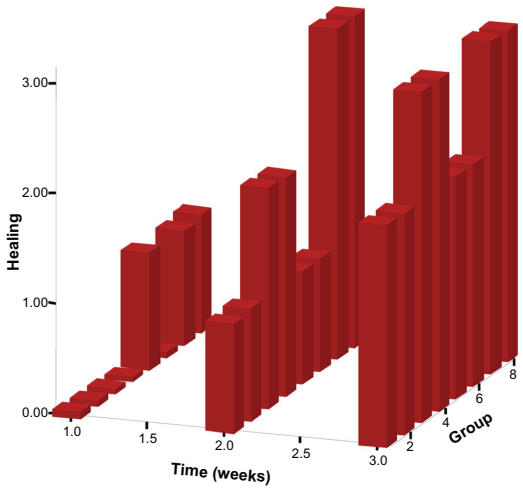

Figure 12 The rate of general healing during 12 weeks. In all groups the general healing was gradual but was more evident in female groups than in male groups.

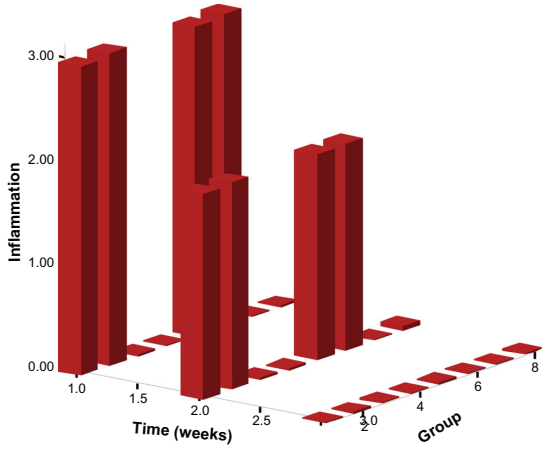

Figure 13 The rate of inflammation during 12 weeks. The analysis indicated that the rate of inflammation was noticeable in control groups at the 2nd and 8th weeks.

Summary of result with analysis

Analysis was by independent samples *t*-test. All variables in each group included control and experimental and all results in male and female were identical, therefore we could not separate the results according to sex. However, the results were different according to mandible and maxilla jaws general healing, and fibrinoleukocytic exudates.

First we analyzed bone formation and inflammation according to control and experimental mandible and maxilla jaws (Table 3).

In the 2nd and 8th weeks, inflammation in control groups was more evident than in experimental groups ($P < 0.0001$), but the difference was not significant in the 12th week ($P > 0.0001$; Figure 1, Table 3).

In the 2nd, 8th, and 12th weeks, bone formation in experimental groups was greater than in control groups ($P < 0.0001$; Table 3, Figures 4–8).

Healing at the 2nd, 8th, and 12th weeks in experimental groups was greater than in control groups ($P < 0.0001$; Table 2, Figure 9).

In the 2nd and 8th weeks, fibrinoleukocyte exudates in control groups were more evident than in experimental groups ($P < 0.0001$), but in the 12th week, it was not significant ($P > 0.0001$; Table 2, Diagram 1). Figures 14–16 are histopathological microscopic views at two sites, the experimental and control with inflammation, fibrinoleukocytic exudates, healing and new bone.

Discussion

The innermost layer of fetal membranes, AM consists of a thick basement membrane and avascular stroma. The avascular stroma includes bFGFs at high concentration and constructs a thick basement membrane, which is rich in various growth factors, basement membrane components, unknown trophic factors, and matrix proteins promoting the migration of epithelial cells, adhesion, and differentiation[1,7] In 1910, Davis applied the AM as a surgery material for the first time and in 1913, Stem and Sabella introduced the AM as a treatment of scorches and skin burns. This method decreased the infection and pain and also promoted the procedure of epithelialization. In 1964, Simon and Sorsby used the AM for chemical eye burns. The AM contains proteinase inhibitors, such as α_1-antichymotrypsin, α_1-macroglobulin, α_1-antitrypsin, α_2-antiplasmin, and inter-α_1-trypsin.[7] Different studies suggest that the AM has been effective in promoting epithelialization. This could be attributed to the production of growth factors while the AM facilitated the epithelial cell migration. This characteristic of the AM can support the adhesion of the basal epithelial cells and may accelerate the epithelial differentiation.[9–11] The AM provided a suitable substrate, such as laminins for rapid attachment of epithelial cells. In addition, it stimulates rapid cell proliferation, differentiation, inflammation inhibition, and fibrosis where the AM likely acts as a barrier to fibrous tissue proliferation.[9] The results demonstrated that human AM can be used for reconstruction of conjuctival defects in the surgery on ear and in vaginal epithelialization.[9] The new applications of this membrane are in tissue engineering by isolation of stem cells or creating apoptosis, and activating interferon γ-macrophage in the laboratory. Previous studies have shown that the AM likely suppressed the migration of the PMN cells. Similarly, some other studies have shown that human AM transplantation may reduce PMN infiltration in acute corneal alkali burn, thereby inhibiting inflammation. This can be attributed to cell apoptosis, inhibition of cell migration due to the suppression of the synthesis of both chemokine and anti-inflammatory cytokine interleukin-10 and finally to the prevention of microbial contamination due to the AM graft on the wound.[7,9] There are two cell types of different embryological origins in the AM: (1) human amnion epithelial cells derived from embryonic ectoderm, and (2) amnion mesenchymal cells from embryonic mesoderm.[12] Zhang et al[13] described that the mesenchymal stem cells in human placenta have the potential to differentiate into osteogenic, adipogenic, and chonrogenic lineages and are able to suppress T-cell proliferation. Yen et al[14] confirmed these results. They showed that placenta-derived stem cells have the same surface markers of embryonic stem cells such as SSEA-4 and also they have neurogenic differentiation capability.[14] In't Anker et al[15] demonstrated that the AM contains a high number of mesenchymal stem cells with bipotential osteogenic and adipogenic differentiation. Although these studies indicated that the inductive potential

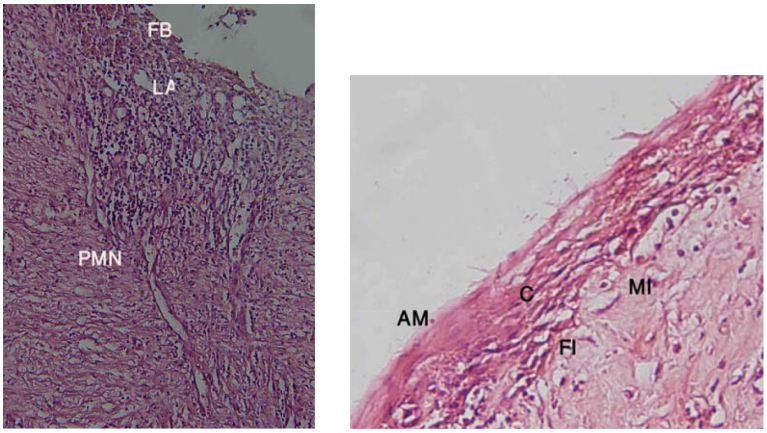

Figure 14 Fibrinoleukocyte layer and PMN infiltration in wounded site from the control group at the 2nd week is compared with that from the AM-transplanted group at the same time where the mild inflammation was observed (H&E 20×).
Abbreviations: AM**,** amniotic membrane; FB, fibrinoleukocyte layer; LA, lymphocyte; C, collagen; MI, mild inflammation; FI, fibroblast; H&E, hematoxylin and eosin; PMN, polymorphonuclear cells.

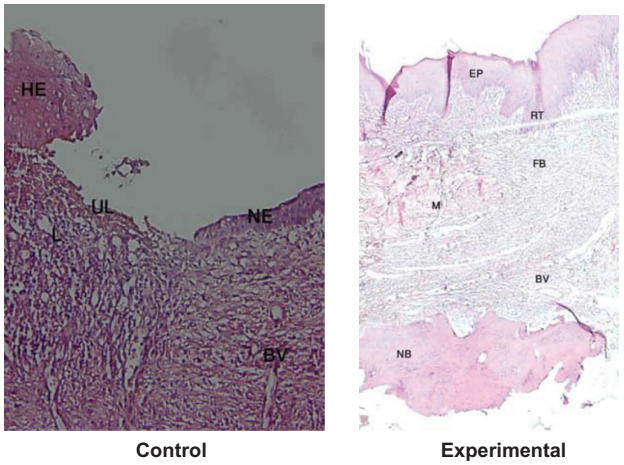

Figure 15 Hypertrophy and ulcer are obvious in the control group, whereas the new bone is generated at the 8th week in the experimental group (H&E 20×).
Abbreviations: HE, hypertrophy in epithelium; UL, ulcer; L, lymphocyte; NE, normal epithelium; BV, blood vessel; EP, epithelium; RT, Retepag; FB, fibroblast; M, muscle; NB, new bone; BV, blood vessel; H&E, hematoxylin and eosin.

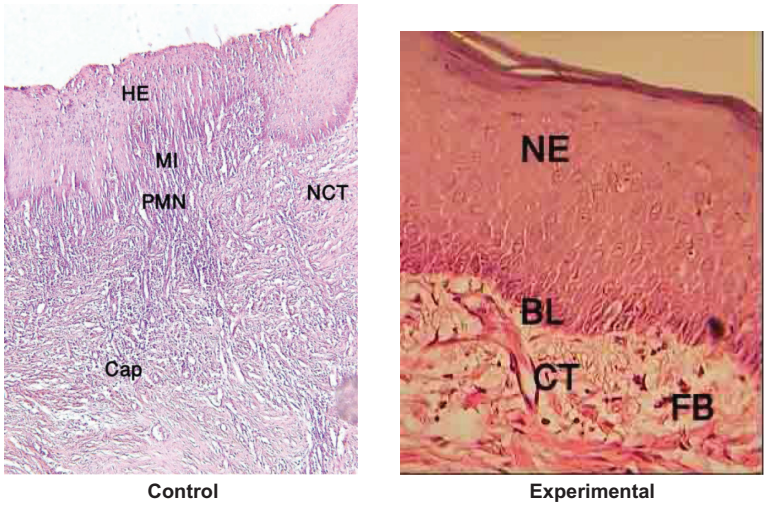

Figure 16 Mild inflammation and PMN were observed in the wound site in the control group at the 12th week, whereas the wound has completely recovered in the experimental group (H&E 20×).
Abbreviations: NE, new bone; BL, basal lamina; CT, connective tissue; FB, fibroblast; HE, healing epithelium; MI, mild inflammation; CAP, capillary; NCT, normal connective tissue; H&E, hematoxylin and eosin.

of the AM is due to the existence of mesenchymal progenitor cells, but the acellular AM was used in our study. Therefore, it can be suggested that the acellular AM has some unknown capabilities to induce as it has been shown that osteogenesis occurred in this assay. In the present study, the inflammation reduction took place as described in the previous studies. There were no remarkable differences between the results observed in male and female samples, but females showed more improvement than males. This could be ascribed to the female hormones. Bone induction was observed to be significant which probably could be attributed to the permissive induction of AM. In a permissive interaction, the responding tissue (the injured jaw) has enough potential to express the determined genes. For this purpose, it requires only a suitable environment to make the expression of these traits possible. Many tissues need a solid substrate, containing fibronectin and laminin.[16] It is suggested that due to the induction process, the fibronectin and laminin components of AM could provide a suitable substrate on the jaw's bone surface and promote bone formation. It should be noted that there could be some progenitor cells, or even stem cells that can use the AM as a suitable substrate for bone induction. Finally, AM may have some unknown role conductive to bone formation. Moreover, the AM could be applied as a barrier to guided bone regeneration and guided tissue regeneration activities, and also be an appropriate cover for the exposed jaw in vestibuloplasty surgery.

Acknowledgment

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Kubo M, Sonoda Y, Muramatsu R, Usui M. Immunogenicity of human amniotic membrane in experimental xenotransplantation. *Invest Ophthalmol Vis Sci*. 2001;42(7):1539–1546.
2. Ghalambor AA, Pipelzadeh MH, Khodadadi A. The amniotic membrane: a suitable biological dressing to prevent infection in thermal burns. *Med J Islam Acad Sci*. 2000;13(3):115–118.
3. Tseng SCG. Literature summary on uses of amniotic membrane in ocular surface reconstruction. DJBLOCH 2003.
4. Samandari MH, Yaghmaei M, Ejlali M, Moshref M, Saffar AS. Use of amnion as a graft material in vestibuloplasty: a preliminary report. *Oral Surg Med Oral Pathol Oral Radiol Endod*. 2004;97(57):4–8.
5. Kruger GO. *Textbook of Oral and Maxillofacial Surgery*, 5th edn. Chapter 5. St Louis, MO: The CV Mosby Company; 1979.
6. Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits protease activity on wound healing following acute corneal alkali burns. *Exp Eye Res*. 1998;70:329–337.
7. Rinastiti M, Harijadi, Santoso ALS, Sosroseno W. Histological evaluation of rabbit gingival wound healing transplanted with human amniotic membrane. *Int J Oral Maxillofac Surg*. 2006;35:247–251.
8. Niknejad H, Peirovi H, Jorjani M, Ahmadian A, Ghanavi J, Seifalian AM. Properties of amniotic membrane for potential use in tissue engineering. *Eur Cells Mater*. 2008;15:88–99.
9. Azuara-Blanco A, Pillai CT, Dua HS. Amniotic membrane transplantation for ocular surface reconstruction. *Br J Opthalmol*. 1999;83:399–402.
10. Muraine M, Descargues G, Franck O, et al. La greffe de membrane amniotique dans les pathologies oculaires de surface. *J Fr Ophtalmol*. 2001;24(8):798–812.
11. Ho Y, Yamamoto Y, Teramachi M, et al. Mesothelial cell regeneration in purified human amniotic membrane grafts implanted in dog pericardium. *Tissue Eng*. 1998;4(2):131–137.
12. Sakuragawa N, Kakinuma K, Kikuchi A, et al. Human amnion mesenchymal cells express phenotypes of neuroglial progenitor cells. *J Neurosci Res*. 2004;78:208–214.
13. Zhang Y, Li C, Jiang X, et al. Human placenta-derived mesenchymal progenitor cells support culture expansion of long-term culture-initiating cells from cord blood CD34+ cells. *Exp Hematol*. 2004;32(7):657–664.
14. Yen BL, Huang HI, Chien CC, et al. Isolation of multipotent cells from human term placenta. *Stem cells*. 2005;23(1):3–9.
15. In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem cells*. 2004;22(7):1338–1345.
16. Scott GF. *Developmental Biology*. 6th edn. Chapter 6. Philadelphia, PA: Swarthmore College Sinaure Associates Inc; 2000:147.
17. Russo RG, Liotta LA, Thorgeirsson U. Polymorphonuclear leukocyte migration through human amniotic membrane. *J cell Biol*. 1981;91:459–467.
18. Orsini G, Scarano A, Degidi M, Caputi S, Iezzi G, Piattelli A. Histological and ultrastructural evaluation of bone around Bio-Oss particles in sinus augmentation. *Oral Dis*. 2007;13:586–593.
19. Bauer TW. An overview of skeletal substitute materials. *Arch Pathol Lab Med*. 2007:1–6.
20. Solomon A, Espana EM, Tseng SCG. Amniotic membrane transplantation for reconstruction of the conjunctival fornices. *Ophthalmology*. 2003;110:93–100.