Histological Comparison of New Bone Formation Using Amnion Membrane Graft Versus Resorbable Collagen Membrane: An Animal Study

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The purpose of this article was to evaluate the bone induction effects of an amnion membrane–protected graft compared with a collagen membrane–protected graft in the repair of tibial bony defects in dogs. This study was performed using the tibial bone of dogs. After the removal of periosteum, similar holes were made with a 16-mm trephine drill (38 holes in total). For the study group, 10 holes were covered by absorbable collagen and 16 holes by amniotic membrane. In the control group, 12 holes were made and covered by the overlying soft tissue. Tibial bones were exposed after 6 and 12 weeks, and the samples were harvested and histologically processed. New bone formation was evaluated by histomorphometric study. Four Iranian mixed dogs older than 1.5 years were included in this study. The new bone formation was less in the control group when compared with the collagen group ($P = .863$). The collagen group showed less bone formation than the amnion group ($P = .194$), but this difference was not significant. However, bone formation in the amnion group was significantly more than in the control group ($P = .050$). Using the amniotic membrane appears to accelerate bone formation in guided bone regeneration. However, further studies should investigate its clinical impact on bone healing.

Key Words: bone defect, osteogenesis, amnion membrane, graft, collagen membrane

INTRODUCTION

Guided bone regeneration is a surgical procedure for bone reconstruction that uses a membrane to control the growth of new bone. This membrane provides a barrier that limits fibroblast invasion to the bony defects and eventually leads to better osteogenesis.[1] Until now, choosing the best membrane for a predictable guided bone regeneration has been controversial, because different materials have different characteristics. Much research has been focused on finding the most effective material for guided bone regeneration and bone induction.

In 2012, Van Leeuwen et al[1] evaluated a new synthetic degradable barrier membrane based on poly-tri-methylene-carbonate for guided bone regeneration. Lei-Yen et al[2] used 2 new feasible media, periosteum progenitor cell sheet and acellular collagen sheet, in tendon-bone healing in anterior cruciate ligament reconstruction.

Amniotic membrane (AM) is one of the oldest biomaterials used as a biologic dressing. In 1910, Davis reported the first medical use of AM in skin burns, and he found faster repair and less scar formation with this graft.[3] In many studies, AM has been used as biodegradable graft or biologic dressing in skin burns, peritoneal surgery, orbital defects (cornea and conjunctiva), replacement of nasal mucosa in Rendu-Osler-Weber syndrome, spinal cord injuries, reconstruction of the vagina, and healing of leg ulcers.[4–10] It has several unique characteristics, including of bacteriostatic properties, an anti-adhesive nature, positive epithelialization effects, lack of antigenicity, angiogenesis induction, and reduction of inflammation and pain.[11] In addition, AM can be easily obtained, and it is inexpensive. Amniotic membrane is the innermost layer of the placenta lining of the amniotic cavity. AM has a firm matrix consisting of collagen and a layer of epithelial cells attaching through a basal lamina.[3] There are many investigations about the use of AM in the oral and maxillofacial region.[12–23] Tsuno et al[24] used hyperdry AM as a dressing for coverage of surgically exposed bone surfaces in the oral cavity. They reported 2 cases

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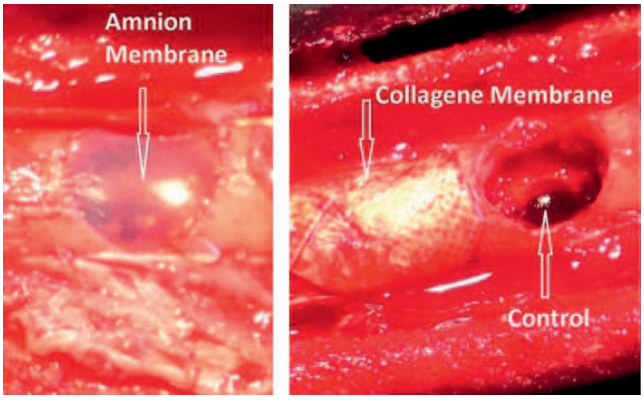


Figure 1. Clinical views of amnion, collagen, and control groups.

in which intraoral alveolar wounds with bone exposure were successfully treated with the use of hyperdry AM. The results indicated that the hyperdry AM is a useful dressing material, not only for soft-tissue wounds but also for the exposed bone in the oral cavity in particular.[24]

The AM stroma contains growth factors, antiangiogenic factors, anti-inflammatory proteins, and natural protease inhibitors.[25] The basement membrane consists of collagen type IV and VII, laminae 1 and 5, fibronectin, and basic fibroblast growth factor.[26,27] Considering that AM has different growth factors, it contributes to improving physiologic wound healings, accelerates wound healing, and stimulates bone induction. In all reviewed studies, AM has been used for regeneration of soft tissue.

The purpose of this study was comparison of the effectiveness of AM with collagen membrane in bone formation in bony defects in dogs. The authors hypothesized that using AM is more effective in guided bone regeneration when compared with collagen membrane.

Materials and Methods

The authors conducted a prospective clinical trial. This study was approved by the ethics committee of Isfahan University of Medical Sciences. In this experimental study, we used 4 adult dogs. All were Iranian mixed dogs aged more than 1.5 years and 20 kg weight that were kept in similar conditions in an animal house under veterinarian control. After shaving and preparing the skin, the tibia was exposed and a 16-mm trephine drill was used to make similar holes on the lateral surface of tibia. The holes had a distance of 8 mm. Holes were made as follows: dog 1, 9 holes; dog 2, 9 holes; Dog 3, 10 holes; and dog 4, 10 holes.

In total, 38 holes were made in 4 tibias. Ten holes were covered by absorbable collagen membrane (T-Barrier Collagen Membrane, B&B Dental Implant Company, Casale, Italy), 16 holes were covered by AM (AM is produced under the standard protocol of the Iranian Tissue Bank, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran), and 12 other holes as a control group were covered only by overlying soft tissue (Figure 1). The number of specimens was different between groups because at the beginning of the study, we had 5 dogs and 1 of them died for a reason not related to the surgical procedure.

The soft tissues over the holes were closed primarily. Two tibias after 6 weeks and 2 other tibias after 12 weeks were exposed, and the defects were excised en bloc with the trephine drill. The samples were fixed in 10% formalin buffer and after demineralization with 10% formic acid were stained by hematoxylin-eosin and specifically with tricromason. New bone formation (woven or lamellar) was defined by histomorphometric study in each sample. The extent of inflammation found in samples was graded as non, mild, moderate, and severe (Figure 2a through c). Data were analyzed via *t* test and statistical variance analyses.

Table 1

Comparison of new bone formation (NBF) between collagen, amnion, and control groups

Group	Time		NBF	Woven	Lamellar
Collagen	6 wk	Mean	50.83	30.66	20.16
		n	6	6	6
		SD	6.794	4.457	4.262
	12 wk	Mean	48.00	32.00	16.00
		n	4	4	4
		SD	5.88784	6.48074	2.44949
Control	6 wk	Mean	48.66	25.33	23.33
		n	6	6	6
		SD	11.53	5.64	7.50
	12 wk	Mean	47.33	31.16	16.16
		n	6	6	6
		SD	8.06639	5.45588	3.43026
Amnion	6 wk	Mean	52.88	32.55	20.33
		n	9	9	9
		SD	7.99131	5.54777	4.15331
	12 wk	Mean	58.14	36.71	21.42
		n	7	7	7
		SD	2.79455	2.05866	2.76026

Results

The study group was composed of 38 bone defects in the tibias of 4 male dogs. All cases were more than 1.5 years old. Two-way variance analysis was used for new bone formation—woven and lamellar—for each group separately. We observed that new bone formation between the 3 groups was different and statistically significant ($P = .034$; Table 1). The effect of time was not statistically significant ($P = .888$). The reciprocal effect between the treatment technique and time was not statistically significant ($P = .364$). For more details, a complementary Tukey test was performed and showed that new bone formation between the collagen and control group ($P = .863$) and between the collagen and amnion group ($P = .194$) was not statistically significant. However, it was significant between the amnion and control group ($P = .050$; Figures 3 through 5).

Two-way variance analysis for woven bone formation showed that the technique effect ($P = .008$) and the time effect ($P = .031$) were statistically significant, but the reciprocal effect between treatment technique and time was not statistically significant ($P = .587$). Complementary Tukey tests

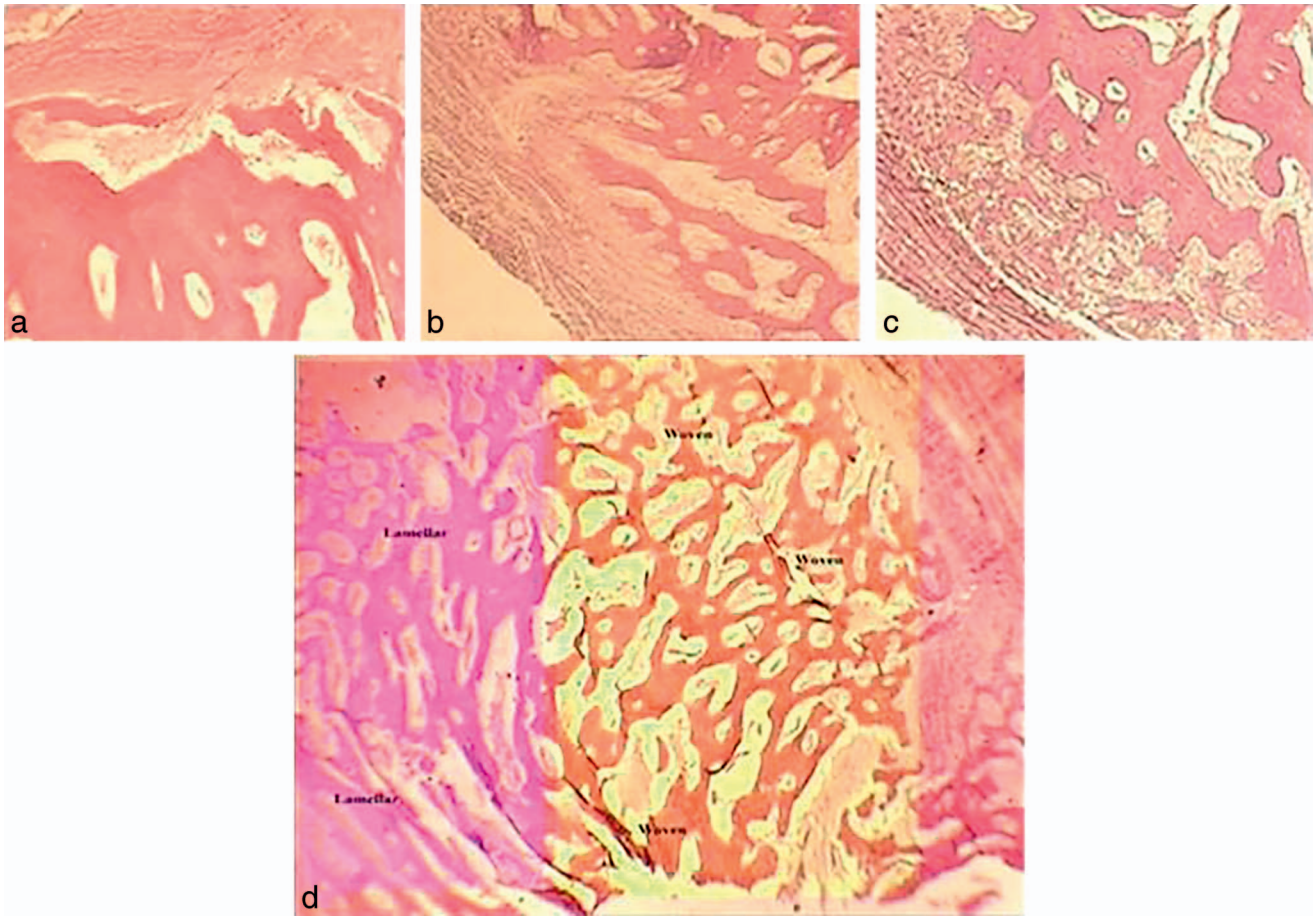


Figure 2. (a) New bone formation after 12 weeks in amnion membrane with mild inflammation. (b) New bone formation after 12 weeks in control group with no inflammation. (c) New bone formation after 12 weeks in collagen membrane with severe inflammation. (d) New bone formation after 12 weeks. Histomorphometric view of woven and lamellar bone.

showed that the woven bone formation between the collagen and control group ($P = .366$) and between the collagen and amnion group ($P = .273$) was not statistically significant, but it was significant between the amnion and control group ($P = .009$). Two-way variance analysis showed that the treatment technique effect on the lamellar bone ($P = .320$) and reciprocal effect between the treatment technique and time ($P = .060$) were not statistically significant; however, the effect of time was statistically significant ($P = .028$).

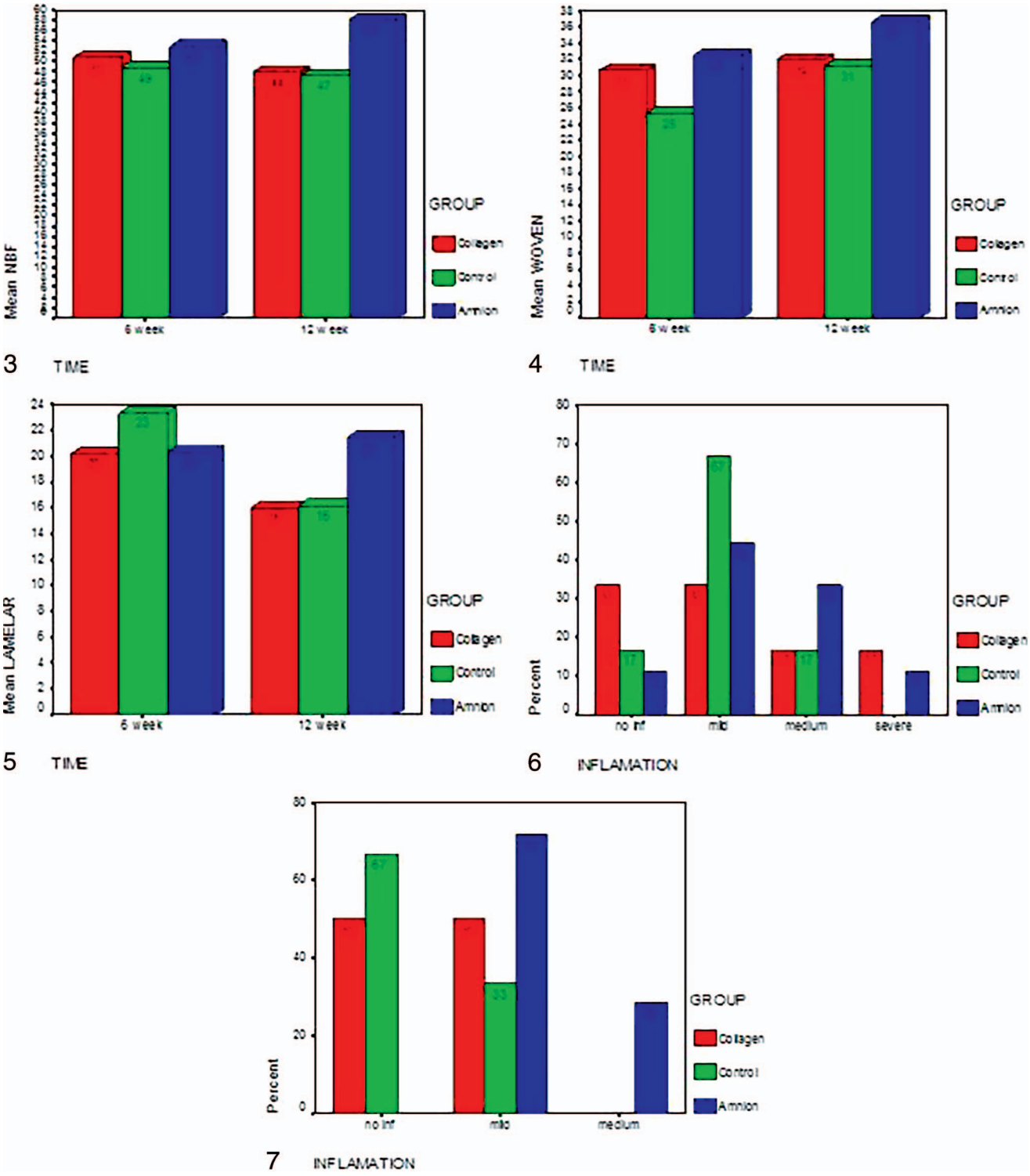
Mann-Whitney *U* test showed that the difference in inflammation in all groups was not statistically significant. *P* values for the collagen, control, and amnion groups were .476, .132, and .758, respectively. The Kruskal-Wallis test showed that the inflammation difference between the groups was statistically significant ($P = .045$). Complementary Mann-Whitney test showed that the inflammation difference between the collagen and control groups ($P = .722$) and between the collagen and amnion groups ($P = .150$) was not statistically significant, but it was significant between the amnion and control groups. Finally, the Mann-Whitney test showed that the inflammation between weeks 6 and 12 was not significantly different; thus, the time variable seems to be ineffective ($P = .280$; Figures 6 and 7; Table 2).

Discussion

The purpose of this article was evaluation of the bone induction effects of amnion membrane graft compared with collagen membrane graft in tibial bony defects in Iranian mixed dogs. We found that osteogenesis in the amnion membrane group was better than in the collagen group but was not statistically significant.

In the bone-healing process, formation of woven bone in the amnion group was the same as in the two other groups after 6 weeks; nevertheless, after some passage of time, there was greater improvement in the formation of lamellar bone in the amnion group compared with the 2 other groups, and this difference was statistically significant ($P = .050$; Figure 5). In other words, after 6 weeks, the difference in lamellar bone formation between the amnion group and the other 2 groups was not significant, and even in the collagen group was a little greater, but gradually, the percentage of lamellar bone formation in the amnion group increased and surpassed that of the other groups. The presence of some factors, such as basic fibroblast growth factor and laminae 1 and 5, were effective in changing the woven bone to lamellar form.[22]

Previous studies compared the properties of other membranes with the collagen membrane or defined the effects of



Figures 3–7. Figure 3. Mean new bone formation between collagen, control, and amnion groups. **Figure 4.** Mean woven bone formation between collagen, control, and amnion groups. **Figure 5.** Mean new lamellar formation between collagen, control, and amnion groups. **Figure 6.** Mean Inflammation after 6 weeks between collagen, control, and amnion groups. **Figure 7.** Mean Inflammation after 12 weeks between collagen, control, and amnion groups.

amnion membrane on soft-tissue healing; however, the present research evaluated the ability of osteoinduction in reconstructing bony defects directly. Fundamentally, in addition to faster repair and reestablishment of function, one of the most important advantages is in reconstruction of pathologic or traumatic defects in the maxillofacial region with better quality of bone. Also, mild inflammation avoids infection and additional problems. In this study, new bone formation in the

Table 2

Comparison of inflammation*

Time	Inflammation		Group			Total
			Collagen	Control	Amnion	
6 wk	No inf	Count	2	1	1	4
		%within GROUP	33.3%	16.7%	11.1%	19.0%
	Mild	Count	2	4	4	10
		%within GROUP	33.3%	66.7%	44.4%	47.6%
	Medium	Count	1	1	3	5
		%within GROUP	16.7%	16.7%	33.3%	23.8%
	Severe	Count	1	0	1	2
		%within GROUP	16.7%	0%	11.1%	9.5%
	Total	Count	6	6	9	21
		%within GROUP	100.0%	100.0%	100.0%	100.0%
12 wk	No inf	Count	2	2	5	9
		%within GROUP	50.0%	66.7%	0%	35.3%
	Mild	Count	2	2	5	9
		%within GROUP	50.0%	33.3%	71.4%	52.9%
	Medium	Count	0	0	2	2
		%within GROUP	0%	0%	28.6%	11.8%
	Total	Count	4	6	7	17
		%within GROUP	100%	100%	100%	100%

*Comparison of inflammation between Amnion group (mild inflammation [inf]), Control group (no inflammation), and Collagen group (severe inflammation) after 6 and 12 months.

amnion membrane group was greater than in the collagen and control groups after 6 and 12 weeks. Of course, the difference was statistically significant only between the amnion and control groups. Osteoinductive effects of AM probably can be because of the existence of fibronectin and laminine.[22]

AM is the innermost layer of the fetal membrane. It consists of a thick basement membrane and avascular stroma.[22] Amnion can be easily obtained and maintained for a long time without any infection and/or deterioration. There are many modalities used for preservation of amnion such as cryopreservation, freeze drying, and gamma radiation.[11] Amniotic membrane and its extracellular matrix contain components such as growth factor and antimicrobial peptides that are suggested as an excellent candidate for use as a scaffold.[13]

Several characteristics explain why AM can be useful for promoting epithelium healing. Oral epithelium produces various growth factors, and the basement membrane facilitates migration of epithelial cells and may promote epithelial differentiation. The probable mechanism of amnion's anti-adhesive characteristic is due to an arrest in tissue proliferation when it is in contact with healthy tissue.[17] In addition, AM transplantation may also function as an anatomical barrier against fibrous tissue migration.[28]

Another point to be taken into account is inflammation. Inflammation is a normal and useful stage of wound healing, but its aggravation and distribution may be noxious and even lead to treatment failure. In this study, inflammation in the control group was lower, but the difference between the groups was not significant, and the passage of time did not contribute to its significance. In fact, inflammation in the amnion group was a little higher than in the control group, but this does not interrupt bone formation, and some mild inflammation is useful for bone regeneration.

A previous study by the author on the vestibuloplasty technique in 2010[28] is the single study that reported the osteoinduction effect of AM. In that study, it was suggested that AM could provide suitable substances for bone induction and promote the bone formation process. According to these studies, it is recommended that AM alone as a biological dressing has unique properties in bone induction that can be useful in reconstruction of bone defects in the maxillofacial region.

Abbreviation

AM: Amniotic membrane

Note

The authors do not declare any conflict of interest.

References

1. Van Leeuwen AC, Huddleston SJJ, Gielkens PF, de Jong JR, Grijpma DW, Bos RR. Guided bone regeneration in rat mandibular defects using resorbable poly (trimethylene carbonate) barrier membranes. *Acta Biomater*. 2012;8:1422–1429.

2. Lei-Yen C, Shu W, Chih-Hwa C, et al. Single-layer periosteum progenitor cell sheet significantly promotes tendon–bone healing in comparison with acellular collagen sheet in anterior. *Formosan J Musculoskelet Disord*. 2012;3:1–8.

3. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. *Am J Obstet Gynecol*. 1979;134:833–845.

4. Prabhasawat P, Tseng SC. Impression cytology study of epithelial phenotype of ocular surface reconstructed by preserved human amniotic membrane. *Arch Ophthalmol*. 1997;115:1360–1367.

5. Lu R, Liu J, Zhang J, Zheng H, Yuan Z, Lin J. The histological changes after preserved human amniotic membrane transplantation for conjunctival reconstruction of rabbits eyes. *Yan Ke Xue Bao*. 2000;16:224–227.

6. Stoiber J, Muss WH, Pohla-Gubo G, Ruckhofer J, Grabner G.

Histopathology of human corneas after amniotic membrane and limbal stem cell transplantation for severe chemical burn. *Cornea*. 2002;21:482–489.

7. Sankar V, Muthusamy R. Role of human amniotic epithelial cell transplantation in spinal cord injury repair research. *Neuroscience*. 2003;118: 11–17.

8. Gris O, Guell JL, Lopez-Navidad A, Caballero F, Del Campo Z. Application of the amniotic membrane in ocular surface pathology. *Ann Transplant*. 1999;4:82–84.

9. Dhall K. Amnion graft for treatment of congenital absence of the vagina. *Br J Obstet Gynaecol*. 1984;91:279–282.

10. Ward DJ, Bennett JP. The long-term results of the use of human amnion in the treatment of leg ulcers. *Br J Plast Surg*. 1984;37:191–193.

11. Nanda S, Chakraborty S, Ray A, Inamuddin. Healing of cervical necrotizing fasciitis using amniotic membrane as a dressing material. *Natl J Maxillofac Surg*. 2011;2:147–151.

12. Kothary PM. Total glossectomy and repair with amniotic membrane. *J Indian Med Assoc*. 1974;62:87–88.

13. Kesting MR, Loeffelbein DJ, Classen M, et al. Repair of oronasal fistulas with human amniotic membrane in minipigs. *Br J Oral Maxillofac Surg*. 2010;48:131–135.

14. Amemiya T, Nakamura T, Yamamoto T, Kinoshita S, Kanamura N. Immunohistochemical study of oral epithelial sheets cultured on amniotic membrane for oral mucosal reconstruction. *Biomed Mater Eng*. 2010;20:37–45.

15. Tuncel U, Ozgenel GY. Use of human amniotic membrane as an interpositional material in treatment of temporomandibular joint ankylosis. *J Oral Maxillofac Surg*. 2011;69:58–66.

16. Ozkaya O, Egemen O, Yesilada A, Sakiz D, Ugurlu K. The effect of nonpreserved human amniotic membrane on the survival of ischaemic skin flaps in rats. *J Plast Reconstr Aesthet Surg*. 2012;65:1700–1705.

17. Samandari MH, Yaghmaei M, Ejlali M, Moshref M, Saffar AS. Use of amnion as a graft material in vestibuloplasty: a preliminary report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;97:574–578.

18. Velez I, Parker WB, Siegel MA, Hernandez M. Cryopreserved amniotic membrane for modulation of periodontal soft tissue healing: a pilot study. *J Periodontol*. 2010;81:1797–1804.

19. Rai M, Ramaraj PN, Sharma A. Use of amniotic membrane as dressing in cervical necrotizing fasciitis. *J Oral Maxillofac Surg*. 2011;69:1125–1128.

20. Mucke T, Loeffelbein DJ, Holzle F, et al. Intraoral defect coverage with prelaminated epigastric fat flaps with human amniotic membrane in rats. *J Biomed Mater Res B Appl Biomater*. 2010;95:466–474.

21. Arai N, Tsuno H, Okabe M, et al. Clinical application of a hyperdry amniotic membrane on surgical defects of the oral mucosa. *J Oral Maxillofac Surg*. 2012;70:2221–2228.

22. Lawson VG. Oral cavity reconstruction using pectoralis major muscle and amnion. *Arch Otolaryngol*. 1985;111:230–233.

23. Guler R, Ercan MT, Ulutuncel N, Devrim H, Uran N. Measurement of blood flow by the 133Xe clearance technique to grafts of amnion used in vestibuloplasty. *Br J Oral Maxillofac Surg*. 1997;35:280–283.

24. Tsuno H, Arai N, Sakai C, et al. Intraoral application of hyperdry amniotic membrane to surgically exposed bone surface. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2014;117:e83–e87.

25. Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. *Exp Eye Res*. 2000;70:329–337.

26. Rinastiti, M, Harijadi, Santoso AL, Sosroseno W. Histological evaluation of rabbit gingival wound healing transplanted with human amniotic membrane. *Int J Oral Maxillofac Surg*. 2006;35:247–251.

27. Samandari MH, Adibi S, Khoshzaban A, et al. Human amniotic membrane, best healing accelerator, and the choice of bone induction for vestibuloplasty technique (an animal study). *Transplant Res Risk Manage*. 2010;2011:1–8.

28. Kumar KA, Chakravarthy M, Selvarajan S, et al. Use of an amniotic membrane as a novel barrier in a tooth with a questionable prognosis. *J Indian Soc Periodontol*. 2017;21:237–240.