Original Article - In Vitro Study

Comparison of Attachment and Proliferation of Human Gingival Fibroblasts on Different Collagen Membranes

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Abstract

Background and Aim: Human gingival fibroblasts cultured on collagen membrane as an alternative treatment method used in tissue regeneration can lead to improved results in root coverage. The aim of this study was to evaluate the human gingival fibroblast proliferation and adhesion cultured on three types of collagen membranes. **Materials and Methods:** In this *in vitro* study, first-line human gingival fibroblast cells (HGF1-RT1) prepared and cultured on three membranes, including porcine pericardium (PP) (Jason, Botiss dental), human pericardium (HP) (Regen, Faravardeh Baft Iranian), and glutaraldehyde cross-linked (GC) (BioMend Extend, Zimmer Dental). Cell survival was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) after 24, 48, and 72 h and 7 days. Furthermore, morphology and adhesion of cells on the membrane were evaluated after 1 and 7 days by electron microscopy (scanning electron microscopy [SEM]). Statistical analysis was performed using two-way ANOVA with a significance level of 0.05. **Results:** Based on the results of MTT, cell survival on HP and PP membranes after 7 days significantly increased ($P < 0.001$), but for the GC membrane, it was reduced after 7 days ($P = 0.031$). Cell survival on HP and PP membranes did not differ ($P = 1$) and was more than GC ($P < 0.001$). SEM images showed that the adhesion of cells was better on HP and PP membranes than GC. **Conclusion:** The results of this study showed that natural collagen membranes (HP and PP) similarly support proliferation and adhesion of gingival fibroblasts. Survival and adhesion of gingival fibroblasts on cross-linked collagen membrane was less than two other membranes.

Keywords: Collagen membrane, fibroblasts, tissue engineering

Introduction

Gingival recession is a common clinical problem that could cause tooth hypersensitivity, pain, root caries, and esthetic issues.[1] The routine treatment modality is soft-tissue autograft which is associated with donor-site morbidity and patient discomfort. Application of tissue engineering by culturing fibroblasts on membranes, however, does not require donor site while providing unlimited amount of graft in a relatively shorter treatment time.[2] In addition, following guided tissue regeneration (GTR) in periodontal surgery, in which membranes are necessary,[3] it has been demonstrated that regeneration of fibers and cementum as well as limited new bone formation could be expected.[4,5] Furthermore, for root coverage, GTR has been associated with less failure compared to coronally advanced flap surgery.[6]

Pitaru *et al*.[7-9] and Blumenthal *et al*.[10-13] showed that collagen membrane could be used for GTR. It has been demonstrated that the results of application of collagen membranes in GTR are comparable to those of expanded polytetrafluoroethylene membranes.[3,14-16]

Fibroblasts are the most common gingival cells and have a vital role in soft-tissue regeneration. Using a proper scaffold, which supports adhesion and proliferation of fibroblasts, they could accelerate tissue repair. In this regard, some of the current tissue engineering approaches involve *ex vivo* fibroblast expansion on membranes followed by their implantation at the defect.[17-20] Collagen membrane with autologous gingival fibroblast has been introduced as a contemporary approach in GTR.[20]

Collagen membranes could be used either native or processed. Although there are several covalent bonds in natural collagen

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fibers, they could be processed to increase their strength. One of the most common chemical procedures on collagen membranes is cross-linking which could be done physically or chemically and would increase covalent bonds and strength of collagen fibers.[8,21,22] However, the effect of cross-linking on bioactivity and biocompatibility of collagen membranes has been less issued. Hence, the aim of the current *in vitro* study was to compare the human fibroblast adhesion and proliferation on two native collagen membranes compared to a cross-linked one.

Materials and Methods

Membranes

In this *in vitro* study, two native collagen membranes including human pericardium (HP) membrane (Regen, Faravardeh Baft Iranian, Tehran, Iran) and porcine pericardium (PP) membrane (Jason membrane, botiss dental GmbH, Berlin, Germany) and one glutaraldehyde cross-linked (GC) collagen membrane (BioMend Extend, Zimmer Dental, Carlsbad, CA, USA) were used. HP is a 0.6–1.2-mm thick allograft membrane which includes basement membrane on one side. PP is a relatively thin (0.1–0.2 mm) membrane with high amount of collagen type III to increase its strength and resorption time which is designed for oral surgeries.[23] GC, on the other hand, is a chemically cross-linked bovine collagen membrane with a mean 0.4-mm thickness.[24]

Human fibroblast cell culture

Human gingival fibroblast cells (HGF1-RT1, Pasteur, Tehran, Iran) were cultured in DMEM (Gibco Laboratories, Grand Island, NY, USA) culture medium supplemented with 10,000 IU/ml penicillin (Gibco), 100 mg/ml streptomycin (Gibco), and 10% FBS (Gibco) in 37°C, 98% humidity, and 5% CO_2. Culture media were changed three times a week until cells reached subconfluent stage. Then, they were removed using 0.25% trypsin/EDTA and passaged. The fourth passage cells were used in the following experiments.

Cell seeding

Collagen membranes were cut into 5 mm × 5 mm pieces under sterile condition and rinsed three times with sterile saline for 10 min. They were placed in 96-well plates and each one was seeded with 6×10^4 human gingival fibroblasts. According to previous *in vitro* studies,[25,26] six samples for each group at each time point were used. No membrane was used in the control group and cells were seeded in empty wells. Following cell adhesion at 37°C for 1 h, the plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

To assess the amount of vital cells in each group, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test was performed at 24, 48, and 72 h and on 7 days after cell seeding using MTT (Sigma, USA) at described elsewhere.[27] The absorbance was determined by a microplate reader (Anthos, Austria) at 590 nm wavelength. Proliferation was estimated by comparing the MTT values at different time points.

Scanning electron microscopy

To visually observe cell attachment, scanning electron microscopic (SEM) images were taken 1 and 7 days after cell seeding. First, cells were fixed by 2.5% glutaraldehyde for 2 h and 1% osmium for 1 h followed by dehydration using sequential concentrations of ethanol. Then, they were sputtered with gold and observed under an SEM (VEGA, TESCAN, Czech Republic) at 10 kV.

Statistical analysis

A two-way ANOVA test followed by Tukey *post hoc* test was applied for the comparison of MTT values among different groups at different time points. Statistical analysis was performed using computer software (SPSS, Version 18, SPSS Inc., Chicago, IL, USA), with a significance level of 0.05.

Results

The trend of cell adhesion (MTT values) on different membranes is demonstrated in Figure 1. As it is apparent, the number of vital cells on all groups except GC was increased over time. Two-way ANOVA showed that the difference between groups and time points was statistically significant ($P < 0.001$) [Table 1].

A pairwise comparison of study groups revealed that control group had the most and GC membrane had the least amount of cells ($P < 0.001$) while the difference between HP and PP membranes was not statistically significant ($P = 1$) [Table 2].

Furthermore, comparison of different time points showed that only 7-day cultures had significantly more viable cells ($P < 0.001$) and the difference between shorter culture times was not significant ($P > 0.05$) [Table 3].

SEM images of membraned cultured with fibroblasts for 24 h show that fibroblasts were attached on the surface of all the study membranes [Figure 2a-f]. However, after 7-day culture, no cell was observed on GC membrane while fibroblasts were still attached on HP and PP native collagen membranes [Figure 3a-f].

Discussion

Membranes could prevent tissue ingrowth toward periodontal defects and clot stabilization as well as preserve new formed bone.

Table 1: Two-way ANOVA test for comparison of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide values between different groups at different time points

Source	Type III sum of squares	df	Mean square	*F*	*P*
Group	2.097	3	0.699	140.629	<0.001
Time	0.610	3	0.203	40.880	<0.001
Group × time	0.454	9	0.050	10.157	<0.001

Table 2: Pairwise comparison of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide values between collagen membranes and control group

Group (I)	Group (J)	Mean difference (I−J)	SE	*P*
PP	HP	−0.013	0.023	1.000
	GC	0.154*	0.020	<0.001
	Control	−0.264*	0.021	<0.001
HP	PP	0.013	0.023	1.000
	GC	0.167*	0.023	<0.001
	Control	−0.252*	0.024	<0.001
GC	PP	−0.154*	0.020	<0.001
	HP	−0.167*	0.023	<0.001
	Control	−0.419*	0.021	<0.001
Control	PP	0.264*	0.021	<0.001
	HP	0.252*	0.024	<0.001
	GC	0.419*	0.021	<0.001

GC=Glutaraldehyde cross-linked; PP=Porcine pericardium; HP=Human pericardium; SE=Standard error. *The mean difference is significant at the 0.001 level

Table 3: Pairwise comparison of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide values between study time points

Group (I)	Group (J)	Mean difference (I−J)	SE	*P*
24 h	48 h	−0.001	0.022	1.000
	72 h	−0.052	0.022	0.144
	7 days	−0.207*	0.022	<0.001
48 h	24 h	0.001	0.022	1.000
	72 h	−0.050	0.022	0.166
	7 days	−0.206*	0.022	0.000
72 h	24 h	0.052	0.022	0.144
	48 h	0.050	0.022	0.166
	7 days	−0.155*	0.022	<0.001
7 days	24 h	0.207*	0.022	<0.001
	48 h	0.206*	0.022	<0.001
	72 h	0.155*	0.022	<0.001

SE=Standard error. *The mean difference is significant at the 0.001 level.

They also could increase the concentration of growth factors and osteogenic cells.[28] In this study, biocompatibility of three collagen membranes and the response of human gingival fibroblasts toward them were investigated. The results showed better response of native collagen membranes compared to cross-linked one.

The results showed that the number of attached fibroblasts on HP and PP native noncross-linked collagen membranes was increased from 1 to 7 days' culture. However, cell population was decreased on GC collagen membrane during 7-day culture. Attachment of surface-dependent cells is essential for cell proliferation.[29] The results of SEM which was used for the observation of cell attachment are also in agreement with MTT test. SEM images showed that fibroblasts were attached to all three membranes, while after 7 days, no cell was found on GC membrane.

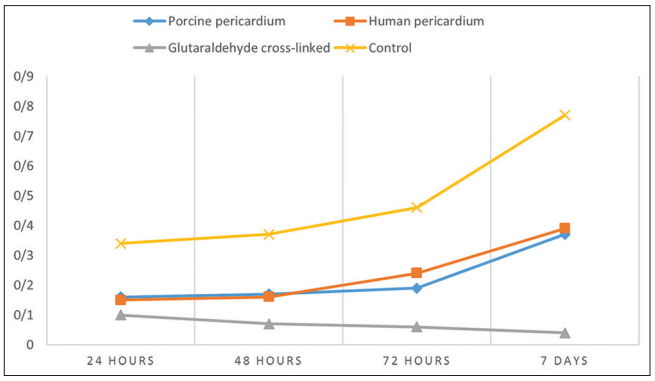

Figure 1: Comparison of the mean 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide values in different collagen membrane compared to control group at different time points

The chemical method which is used for cross-linking in the BioMend extend membrane is through application of glutaraldehyde. Although this method is a common approach, there are some reports on cytotoxic effect of the remnant aldehydes.[30,31] Some have suggested that use of glutaraldehyde does not have a significant influence on regenerative treatments using GC membranes.[32] However, the results of the current study showed that cell adhesion on GC membrane decreased during 7 days which might be due to gradual release of aldehydes in the culture media. Toxicity of glutaraldehyde is dose dependent[33] and its aggregation during 7 days might be the reason for reduction of cell population on GC membrane. The mean MTT values for GC after 7 days were about 5% of the control group. This might show that GC membrane is not compatible with human gingival fibroblasts. Similarly, it has been demonstrated that GC membrane could decrease its biocompatibility to a toxic level.[30,31,34] Speer *et al*.[30] reported that products released from GC membranes have a cytotoxic effect. However, van Luyn *et al*.[35] showed that constant change of cell substrate would prohibit toxic effect of these membranes on fibroblast cells. Changing cell media was done to mimic wash-out effect of body fluids. Nonetheless, implantation of GC membranes *in vivo* resulted in foreign body and inflammatory reactions in 10 days.[36] It seems that expected removal of toxic products needs more time and primary inflammation is inevitable.

To the extent of authors' knowledge, no previous study compared membranes used in the current experiment. The results of the current study is in agreement with some of previous studies which reported that noncross-linked membranes support cell adhesion and proliferation.[37-39] It has been reported that PP collagen membrane (Jason membrane) can support proliferation SaOS-2 osteoblasts during 7-day culture.[23] Further, SEM observation of this membrane revealed that it has interconnected pores which would permit cell infiltration[23] and its surface topography supports cell adhesion.[40]

Two studies reported that cross-linking does not necessarily cause a reduction in cell proliferation.[41,42] The methods for

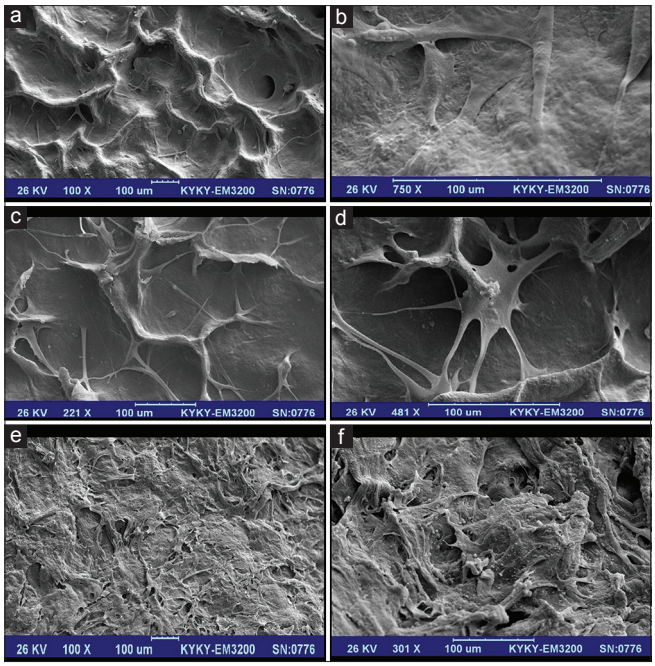

Figure 2: Attachment and distribution of human gingival fibroblasts after 24 h culture on (a and b) glutaraldehyde cross-linked, (c and d) porcine pericardium, and (e and f) human pericardium collagen membranes

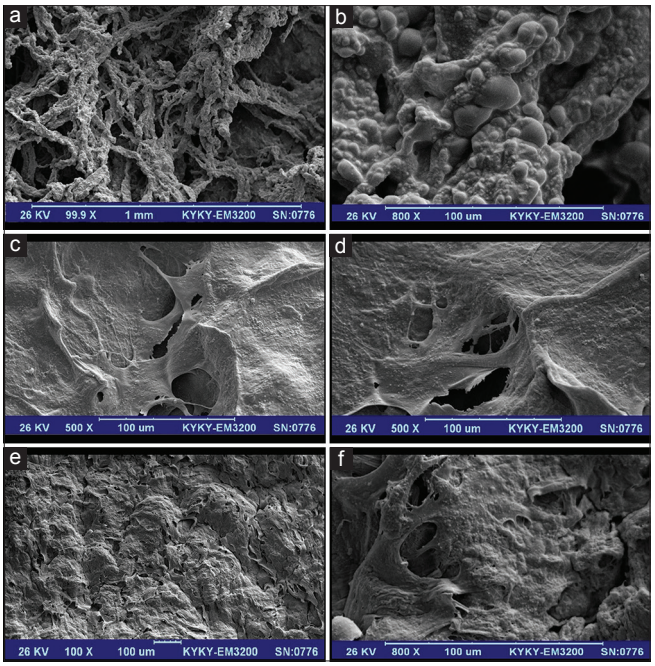

Figure 3: Attachment and distribution of human gingival fibroblasts after 7 days culture on (a and b) glutaraldehyde cross-linked, (c and d) porcine pericardium, and (e and f) human pericardium collagen membranes

cross-linking in these studies were by nordihydroguaiaretic acid and chitosan. However, the results of the current study revealed that cross-linking by glutaraldehyde would cause a significant reduction in the proliferation of human gingival fibroblasts. This reduction might be due to the effect of production method rather than cross-linkage.

Clinical significance

On the one hand, membranes used in GTR procedures should be obviously compatible to the cells including gingival fibroblasts. On the other hand, when primary wound closure is not achievable during periodontal surgery, cell migration and attachment are necessary for closure and preventing infection.[43] Fibroblasts are among the most important cells that should attach properly to the exposed membraned.[44]

Furthermore, as the fibroblast can produce collagen and growth factors, their application in regenerative medicine in the field of periodontal surgeries has increased.[20,45] The common approach is *ex vivo* proliferation of fibroblasts followed by their transplantation to the defect site using proper scaffold.[19,46] Various materials including collagen membranes have been used as scaffold.[19,45] The results of this study showed the ability of two native collagen membranes for fibroblast support.

However, it should be considered that the current study was an *in vitro* experiment and the effect of several confounding factors such as body fluids, recipient site, and cell interactions was not assessed. Therefore, extrapolation of the results to the clinical situations should be done with precaution. *In vitro* studies, nonetheless, allow controlling all the factors and evaluation of the aimed variable which is not easily achievable in *in vivo* studies. Another limitation of this study was that we did not measure release of glutaraldehyde or other products from studied membranes. Further studies evaluating the amount of toxic products released from membranes could reveal the causal relationship between toxicity and glutaraldehyde released from GC membrane.

Conclusion

Considering the limitations of the current *in vitro* study, the results could be summarized as follows:

1. Viable human gingival fibroblasts were increased on PP and HP natural collagen membranes from 24 h to 7 days culture while it was decreased on the cross-linked collagen membrane
2. These was no significant difference between amount of living cells on HP and PP membranes and both were significantly more than GC membrane
3. Morphology and attachment of cells on three membranes were relatively similar after 24-h culture while no cell was observed on GC membrane after 7-day culture.

These results show that natural collagen membranes support attachment and proliferation of the human gingival fibroblasts while GC collagen membrane does not. In addition, the results showed the toxic effect of GC membrane on the human gingival fibroblasts.

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Conflicts of interest

There are no conflicts of interest.

References

1. Tugnait A, Clerehugh V. Gingival recession-its significance and management. J Dent 2001;29:381-94.
2. Wang HL, Modarressi M, Fu JH. Utilizing collagen membranes for guided tissue regeneration-based root coverage. Periodontol 2000 2012;59:140-57.
3. Khojasteh A, Kheiri L, Motamedian SR, Khoshkam V. Guided bone regeneration for the reconstruction of alveolar bone defects. Ann Maxillofac Surg 2017;7:263-77.
4. Parma-Benfenati S, Tinti C. Histologic evaluation of new attachment utilizing a titanium-reinforced barrier membrane in a mucogingival recession defect. A case report. J Periodontol 1998;69:834-9.
5. Leknes KN, Amarante ES, Price DE, Bøe OE, Skavland RJ, Lie T, *et al.* Coronally positioned flap procedures with or without a biodegradable membrane in the treatment of human gingival recession. A 6-year follow-up study. J Clin Periodontol 2005;32:518-29.
6. Lee EJ, Meraw SJ, Oh TJ, Giannobile WV, Wang HL. Comparative histologic analysis of coronally advanced flap with and without collagen membrane for root coverage. J Periodontol 2002;73:779-88.
7. Pitaru S, Tal H, Soldinger M, Azar-Avidan O, Noff M. Collagen membranes prevent the apical migration of epithelium during periodontal wound healing. J Periodontal Res 1987;22:331-3.
8. Pitaru S, Tal H, Soldinger M, Grosskopf A, Noff M. Partial regeneration of periodontal tissues using collagen barriers. Initial observations in the canine. J Periodontol 1988;59:380-6.
9. Pitaru S, Noff M, Grosskopf A, Moses O, Tal H, Savion N, *et al.* Heparan sulfate and fibronectin improve the capacity of collagen barriers to prevent apical migration of the junctional epithelium. J Periodontol 1991;62:598-601.
10. Blumenthal N. The use of collagen materials in bone grafted defects to enhance guided tissue regeneration. Ill Dent J 1987;56:176-9.
11. Blumenthal N, Sabe T, Barrington E. Healing responses to grafting of combined collagen-decalcified bone in periodontal defects in dogs. J Periodontol 1986;57:84-90.
12. Blumenthal NM. A clinical comparison of collagen membranes with e-PTFE membranes in the treatment of human mandibular buccal class II furcation defects. J Periodontol 1993;64:925-33.
13. Blumenthal NM, Alves ME, Al-Huwais S, Hofbauer AM, Koperski RD. Defect-determined regenerative options for treating periodontal intrabony defects in baboons. J Periodontol 2003;74:10-24.
14. Zitzmann NU, Naef R, Schärer P. Resorbable versus nonresorbable membranes in combination with bio-oss for guided bone regeneration. Int J Oral Maxillofac Implants 1997;12:844-52.
15. Carpio L, Loza J, Lynch S, Genco R. Guided bone regeneration around endosseous implants with anorganic bovine bone mineral. A randomized controlled trial comparing bioabsorbable versus non-resorbable barriers. J Periodontol 2000;71:1743-9.
16. Moses O, Pitaru S, Artzi Z, Nemcovsky CE. Healing of dehiscence-type defects in implants placed together with different barrier membranes: A comparative clinical study. Clin Oral Implants Res 2005;16:210-9.
17. Jhaveri HM, Chavan MS, Tomar GB, Deshmukh VL, Wani MR, Miller PD Jr., *et al.* Acellular dermal matrix seeded with autologous gingival fibroblasts for the treatment of gingival recession: A proof-of-concept study. J Periodontol 2010;81:616-25.
18. McGuire MK, Scheyer ET, Nevins ML, Neiva R, Cochran DL, Mellonig JT, *et al.* Living cellular construct for increasing the width of keratinized gingiva: Results from a randomized, within-patient, controlled trial. J Periodontol 2011;82:1414-23.
19. Morelli T, Neiva R, Nevins ML, McGuire MK, Scheyer ET, Oh TJ, *et al.* Angiogenic biomarkers and healing of living cellular constructs. J Dent Res 2011;90:456-62.
20. Lotfi G, Shokrgozar MA, Mofid R, Abbas FM, Ghanavati F, Bagheban AA, *et al.* A clinical and histologic evaluation of gingival fibroblasts seeding on a chitosan-based scaffold and its effect on the width of keratinized gingiva in dogs. J Periodontol 2011;82:1367-75.
21. Minabe M, Kodama T, Kogou T, Tamura T, Hori T, Watanabe Y, *et al.* Different cross-linked types of collagen implanted in rat palatal gingiva. J Periodontol 1989;60:35-43.
22. Houshmand B, Tabibzadeh Z, Motamedian SR, Kouhestani F. Effect of metformin on dental pulp stem cells attachment, proliferation and differentiation cultured on biphasic bone substitutes. Arch Oral Biol. 2018;95:44-50.
23. Rothamel D, Schwarz F, Fienitz T, Smeets R, Dreiseidler T, Ritter L, *et al.* Biocompatibility and biodegradation of a native porcine pericardium membrane: Results of *in vitro* and *in vivo* examinations. Int J Oral Maxillofac Implants 2012;27:146-54.
24. Norowski PA, Mishra S, Adatrow PC, Haggard WO, Bumgardner JD. Suture pullout strength and *in vitro* fibroblast and RAW 264.7 monocyte biocompatibility of genipin crosslinked nanofibrous chitosan mats for guided tissue regeneration. J Biomed Mater Res A 2012;100:2890-6.
25. Khojasteh A, Motamedian SR, Sharifzadeh N, Zadeh HH. The influence of initial alveolar ridge defect morphology on the outcome of implants in augmented atrophic posterior mandible: An exploratory retrospective study. Clin Oral Implants Res. 2017;28:e208-e217.
26. Motamedian SR, Tabatabaei FS, Akhlaghi F, Torshabi M, Gholamin P, Khojasteh A, *et al.* Response of dental pulp stem cells to synthetic, allograft, and xenograft bone scaffolds. Int J Periodontics Restorative Dent 2017;37:49-59.
27. Hosseinpour S1, Ghazizadeh Ahsaie M, Rezai Rad M, Baghani MT, Motamedian SR, Khojasteh A. Application of selected scaffolds for bone tissue engineering: A systematic review. Oral Maxillofac Surg. 2017;21:109-29.
28. Behfarnia P, Khoroushi M, Fakhari E, Foroughi MR. A review of biomaterials used to treat peri-dental and peri-implant lesions. J Isfahan Dent Sch 2014;10:179-93.
29. Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA. Toward guided tissue and bone regeneration: Morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008;96:1-1.
30. Speer DP, Chvapil M, Eskelson CD, Ulreich J. Biological effects of residual glutaraldehyde in glutaraldehyde-tanned collagen biomaterials. J Biomed Mater Res 1980;14:753-64.
31. Wiebe D, Megerman J, L'Italien GJ, Abbott WM. Glutaraldehyde release from vascular prostheses of biologic origin. Surgery 1988;104:26-33.
32. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J, *et al.* Biodegradation of differently cross-linked collagen membranes: An experimental study in the rat. Clin Oral Implants Res 2005;16:369-78.
33. Sun HW, Feigal RJ, Messer HH. Cytotoxicity of glutaraldehyde and formaldehyde in relation to time of exposure and concentration. Pediatr Dent 1990;12:303-7.
34. Rothamel D, Schwarz F, Sculean A, Herten M, Scherbaum W, Becker J, *et al.* Biocompatibility of various collagen membranes in cultures of human PDL fibroblasts and human osteoblast-like cells. Clin Oral Implants Res 2004;15:443-9.
35. van Luyn MJ, van Wachem PB, Olde Damink LH, Dijkstra PJ, Feijen J, Nieuwenhuis P, *et al.* Secondary cytotoxicity of cross-linked dermal sheep collagens during repeated exposure to human fibroblasts. Biomaterials 1992;13:1017-24.
36. van Wachem PB, van Luyn MJ, Olde Damink LH, Feijen J, Niewenhuis P. *In vivo* interactions with (tissue culture pretreated) dermal sheep collagen. In: Tissue-inducing biomaterials symposium. Mater Res Soc 1992;252:117-24.
37. Wang HL, Miyauchi M, Takata T. Initial attachment of osteoblasts to various guided bone regeneration membranes: An *in vitro* study. J Periodontal Res 2002;37:340-4.
38. Takata T, Wang HL, Miyauchi M. Attachment, proliferation and differentiation of periodontal ligament cells on various guided tissue regeneration membranes. J Periodontal Res 2001;36:322-7.
39. Takata T, Wang HL, Miyauchi M. Migration of osteoblastic cells on various guided bone regeneration membranes. Clin Oral Implants Res 2001;12:332-8.
40. Stähli A, Miron RJ, Bosshardt DD, Sculean A, Gruber R. Collagen membranes adsorb the transforming growth factor-β receptor I kinase-dependent activity of enamel matrix derivative. J Periodontol 2016;87:583-90.

41. Koob TJ, Willis TA, Hernandez DJ. Biocompatibility of NDGA-polymerized collagen fibers. I. Evaluation of cytotoxicity with tendon fibroblasts *in vitro*. J Biomed Mater Res 2001;56:31-9.
42. Kumar TR, Shanmugasundaram N, Babu M. Biocompatible collagen scaffolds from a human amniotic membrane: Physicochemical and *in vitro* culture characteristics. J Biomater Sci Polym Ed 2003;14:689-706.
43. Brunette DM. The effects of implant surface topography on the behavior of cells. Int J Oral Maxillofac Implants 1988;3:231-46.
44. Payne JM, Cobb CM, Rapley JW, Killoy WJ, Spencer P. Migration of human gingival fibroblasts over guided tissue regeneration barrier materials. J Periodontol 1996;67:236-44.
45. Köseoğlu S, Duran İ, Sağlam M, Bozkurt SB, Kırtıloğlu OS, Hakkı SS, *et al.* Efficacy of collagen membrane seeded with autologous gingival fibroblasts in gingival recession treatment: A randomized, controlled pilot study. J Periodontol 2013;84:1416-24.
46. Rodrigues AZ, Oliveira PT, Novaes AB Jr., Maia LP, Souza SL, Palioto DB, *et al.* Evaluation of *in vitro* human gingival fibroblast seeding on acellular dermal matrix. Braz Dent J 2010;21:179-89.