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Evaluation and comparison number of gingival fibroblast and osteosarcoma cell (MG-63 cell line) adhesive to mocugraft, alloderm, and collagen membrane with or without advanced platelet-rich fibrin

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ABSTRACT

Background: The tissue engineering has recently shown a significant progress in the fields of membranes and biosynthetic materials. Advanced platelet-rich fibrin (A-PRF) contains functional molecules that have newly shown great interest in regenerative therapies. The purpose of this study was to evaluate the effect of A-PRF on the adhesion of gingival fibroblast cells and osteosarcoma cells to different membranes.

Materials and Methods: In this experimental *in vitro* study, three collagen, alloderm, and mucograft membranes were studied, which were cut into four 5 mm × 5 mm pieces and placed in the bottom of a 24-well culture medium. One milliliter of A-PRF was added to two wells from each group and the other two wells remained without A-PRF. The gingival fibroblasts and osteosarcoma cells were individually added to each well. The cell adhesion was studied using an electron microscope after 24 h. The data were analyzed by independent *t*-test, one-way analysis of variance, and least significant difference test.

Results: In the presence of A-PRF, there was a significant higher osteoblast adhesion to collagen membrane compared to alloderm and mucograft membranes ($P < 0.001$). In the absence of A-PRF, adhesion of osteoblasts to collagen membrane was significantly higher than alloderm and mucograft ($P = 0.019$). Moreover, in the presence of A-PRF, fibroblast adhesion to collagen membrane was significantly higher than alloderm and mucograft membranes ($P < 0.001$). Furthermore, in the absence of A-PRF, no significant difference was found among the study groups ($P = 0.830$).

Conclusion: A-PRF was effective on fibroblast adhesion to the collagen membrane, which is similar to its absence. A-PRF was also found to be very effective on the adhesion of fibroblast cells to the collagen membrane, and in its absence, even less adhesion was observed compared to the other membranes. The presence or absence of A-PRF showed no significant differences in both cells' adhesion for alloderm and mucograft membranes.

Key Words: Advanced platelet-rich fibrin, alloderm, cell adhesion, collagen membrane, guided tissue regeneration, mocugraft

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INTRODUCTION

The final objective of periodontal treatments is to control the periodontal tissue inflammation and predictable regeneration periodontium, which are lost as the results of periodontal diseases. In order to improve the periodontium regeneration, it is important to direct those tissues capable of regenerating.[1,2] Various clinical studies have shown a significant reduction in the pocket depth probe and clinical attachment level gain after performing regenerative treatments using different absorbable and nonabsorbable membranes.[3,4]

Fibroblasts and osteoblasts play an important role in wound healing. These cells are rapidly present and proliferate at the site of injury and can also accelerate the healing process. They are also responsible for the development of various growth factors, which finally lead to extracellular matrix deposition and epithelial cell differentiation.[5,6]

Periodontal wound healing requires a series of interactions among epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts. The presence of growth factors and cytokines in platelets also play a key role in the inflammation and in wound-healing process.[7] Platelets also act as a matrix for connective tissue by secreting fibrin and fibronectin, and by these adhesive molecules, they facilitate cell migration.[8] This has led to the theory of using platelets as a therapeutic tool to improve the tissue repairing, especially healing of periodontal wounds.[9]

The use of collagen membranes is recommended to avoid second surgery (it was indicated that the use of absorbable membranes eliminates the need for the second stage surgery to remove the membrane compared to nonabsorbable membranes.); however, it has been shown that, early membrane breakdown and downward epithelial growth can affect the treatment outcomes.[10]

The membrane used for regenerative treatments should have a number of specific features. Accordingly, an important feature of the membrane is its ability of improving the cell adhesion, proliferation, and differentiation. Several membranes are designed to meet these requirements, but some factors such as membrane composition, surface tissue morphology, size of the pores, and duration of membrane function that may affect the results of regenerative therapies, have not been still well understood.[11]

The cell adhesion to the surface substrate consists of a four-stage sequence including absorption of glycoproteins on the substrate surface, cell contact, adhesion, and diffusion, respectively.[12] The cell migration and proliferation occur after these four stages. Since the cell adhesion to all the tested membranes occurred, the cell migration and proliferation would also occur at the surface, which indicate the biocompatibility and nontoxicity of the studied membranes.

Hakki *et al*.[13] in their research studied the gingival fibroblast cell adhesion and proliferation on three collagen, alloderm, and glycolic lactic copolymer membranes and observed that adhesion and proliferation on two collagen and alloderm membranes were similar and also higher than glycolic lactic copolymer membrane group. Vahabi *et al*.[14] by evaluating the binding of fibroblast and osteoblast cells to three TXT-200, human collagen, and animal collagen membranes found that the binding of each cell in platelet-rich plasma (PRP)-activated group was >5% fetal bovine serum. Locci *et al*.[15] suggested that collagen membranes have the best ability in binding and inducing osteoblast cell adhesion.

Considering that the use of different types of membranes is common for periodontal treatments, the higher the adhesion of fibroblasts and osteoblasts to the respective membranes, the lower the probability of membrane displacement and subsequent disruption of tissue regeneration. The number of gingival fibroblasts and osteosarcoma cells (MG-63 cell line) attached to the three mucogrel, alloderm, and collagen membranes was determined in the presence or in the absence of advanced platelet-rich fibrin (A-PRF).

MATERIALS AND METHODS

In this experimental *in vitro* study, gingival fibroblast cells (NCBI Codece C165) and human osteosarcoma cells (MG-63) were prepared from Pasteur Institute. The cells were incubated at 37°C under the moist conditions, exposed to 5% carbon dioxide, and then passaged five times in a 24-well culture medium to reach the sufficient numbers.

In this study, three collagen (Regen [Itb, Tehran, Iran]), alloderm (Regen [Itb, Tehran, Iran]) (Freeze derived, cell_ free human dermal matrix.), and mucograft (Botiss dental, Berlin, Germany) (Pure collagen type I and III matrix porcine origin without any future cross linkage) membranes were studied for

adhesion of osteoblast and gingival fibroblast cells. Furthermore, four pieces of 5 mm × 5 mm were prepared from each membrane.

In order to prepare A-PRF, the patient's blood sample was placed in a 2 ml tube without anticoagulant and then centrifuged immediately at 1500 rpm for 14 min. After centrifugation, the product was composed of three layers as follows: The top layer consisting of cell-free plasma containing low platelet counts, A-PRF clot in the middle, and the red blood cells at the bottom of the test tube. By removing the supernatant and the remainder of the product from the centrifuge tube and then isolating the sediment (red blood cells); fibrin clot (A-PRF) remained. After isolating the A-PRF layer, it was placed on a heater to be used at the same temperature of 36°C.

In order to prepare the membranes in groups containing A-PRF, 1 ml of prepared A-PRF was added to the membranes, then incubated for 2 h at 4°C, and finally washed with 5 ml of PBS. After that, 10^4 fibroblast cells and 10^4 MG-63 cells were added to all groups and incubated for 24 h at 37°C under the moist conditions, and exposed to 1% carbon dioxide. By passing 24 h, the cell binding in the control group (without any membrane and A-PRF) was evaluated using light microscopy and cell adhesion was assured.

Under the electron microscope, the surface of each membrane was divided into four parts. In each quadrant, a point image with a ×300 magnification was taken, in which, 16 images were obtained from each one of the membranes. Accordingly, eight images showed the number of fibroblasts attached to the membrane without the presence of A-PRF, and the other eight images showed cells attached to the surface of the membrane in the presence of A-PRF.

The obtained data were analyzed by independent *t*-test, one-way analysis of variance (ANOVA), least significant difference, and software SPSS 22 (IBM, Armonk, USA), and also the statistical level was considered as 0.05.

To evaluate the adhesion of osteoblast cells in each membrane alone, based on independent *t*-test.

RESULTS

According to two-way ANOVA, the membrane type was effective on the cell adhesion ($P < 0.01$).

To evaluate the adhesion of osteoblast cells in each membrane separately, using independent *t*-test, it was shown that the adhesion of osteoblast cells to alloderm ($P = 0.11$) and mucograft ($P = 0.643$) membranes was slightly higher in the absence of A-PRF, and this difference was not statistically significant. The cell adhesion to collagen membrane was higher in the presence of A-PRF, which was not statistically significant ($P = 0.736$) [Table 1].

The results of comparison between the studied membranes based on the one-way ANOVA showed that, in the presence of A-PRF, osteoblast adhesion to collagen membrane was higher than alloderm and mucograft membranes and this difference was statistically significant ($P < 0.001$). Furthermore, the adhesion of osteoblasts to collagen membrane was significantly higher than alloderm and mucograft ($P = 0.019$) when A-PRF was absent in the medium.

According to paired comparison of the groups, in the presence of A-PRF, the adhesion of the osteoblasts to collagen membrane was significantly higher than those of alloderm ($P < 0.001$) and mucograft ($P < 0.001$). The adhesion of osteoblasts to collagen membrane was significantly higher compared to alloderm ($P = 0.024$) and mucograft ($P = 0.009$), and in the presence of A-PRF, osteoblast adhesion to mucograft membrane was higher than alloderm; however, this difference was not statistically significant. The cell adhesion to alloderm was higher than mucograft in the absence of A-PRF, but again, this difference was not statistically significant ($P = 0.655$) [Figure 1].

Considering the adhesion of fibroblast cells individually in each membrane, independent *t*-test showed that, adhesion of fibroblast cells to alloderm ($P = 0.745$) and mucograft ($P = 0.500$) membranes was slightly higher in the absence of A-PRF, and this difference was not statistically

Table 1: Mean of osteoblast cell adhesion in each membrane

Membrane	A-PRF	Osteoblast cell adhesion in each membrane, mean±SD	*P*
Alloderm	Yes	8.2500±1.2817	0.11
	No	11.7500±5.6505	
Mucograft	Yes	8.8750±2.4165	0.64
	No	9.6250±3.7773	
Collagen	Yes	52.1250±7.3180	0.73
	No	23.1250±14.7400	

SD: Standard deviation; A-PRF: Advanced platelet rich fibrin

significant. The cell adhesion to collagen membrane was higher in the presence of A-PRF, which was statistically significant ($P < 0.001$) [Table 2].

Comparison of the studied membranes by the one-way ANOVA showed that, in the presence of A-PRF, fibroblast adhesion to collagen membrane was higher than the other two membranes of alloderm and mucograft, and this difference was statistically significant ($P < 0.001$). When A-PRF was absent in the medium, adhesion of fibroblasts to mucograft membrane was higher than alloderm and collagen, which was not statistically significant ($P = 0.830$).

Furthermore, according to paired comparison of the groups, fibroblast adhesion to collagen membrane was significantly higher than alloderm ($P < 0.001$) and mucograft ($P < 0.001$). Moreover, in the presence of A-PRF, fibroblast adhesion to mucograft membrane was more than alloderm; however, this difference was not statistically significant ($P = 0.607$) [Figure 2].

DISCUSSION

Separately studying each membrane showed that, adhesion of osteoblast cells to alloderm and mucograft membranes was slightly higher when A-PRF was absent; however, this difference was not statistically significant. For collagen membrane, adhesion of osteoblast cells was higher in the presence of A-PRF, but the difference was not significant.

However, the results of comparison between the studied membranes showed that, the adhesion of osteoblast cells to collagen membrane was significantly higher than that of mucograft and alloderm in the absence of A-PRF, which is consistent with the results of the studies by Wang *et al*.[16] and Nagahara *et al*.[17]

Locci *et al*. in their study suggested that, collagen membrane had the best abilities in binding and inducing osteoblast cell adhesion.[15]

Table 2: Mean of adhesion of fibroblast cells in each membrane

Membrane	A-PRF	Adhesion of fibroblast cells in each membrane, mean±SD	*P*
Alloderm	Yes	6.2500±3.6154	0.74
	No	7.1250±6.5124	
Mucograft	Yes	7.5000±2.7775	0.5
	No	8.6250±3.6621	
Collagen	Yes	21.1250±6.9372	0.001
	No	7.8750±3.9799	

SD: Standard deviation; A-PRF: Advanced platelet rich fibrin

According to the results of the present study, in the presence of A-PRF, the adhesion of osteoblast cells to collagen membrane was significantly higher than mucograft and alloderm membranes, which is consistent with the results of studies by Vahabi *et al*.[14] and Simon *et al*.[18]

In a comparative study performed on PRF and collagen membrane, Gassling *et al*.[19] showed that the levels of proliferation and metabolic activities of osteoblast cells at PRF level was higher than that of collagen membrane. Furthermore, in a study by Wu *et al*.,[20] human-derived PRF was studied. Accordingly, it was found that binding and proliferation of osteoblast cells increased in the presence of PRF, and by evaluating the effective mechanisms using western blot test, it was found that a significant increase in collagen-dependent proteins (Heat shock protein 47), HSP47 (Lysyl oxidase), and LOX leads to the effect of PRF on bone regeneration. The study of the effect of TGF B-1, as a collagen gene inducer, that is normally secreted by PRF, on HSP47 that was produced from osteoblast cells showed that TGF B-1 could produce 3-fold mRNA HSP47 compared to its absence. This dose-dependent increase was observed in both HSP47 mRNA and collagen alpha 1 mRNA.[21]

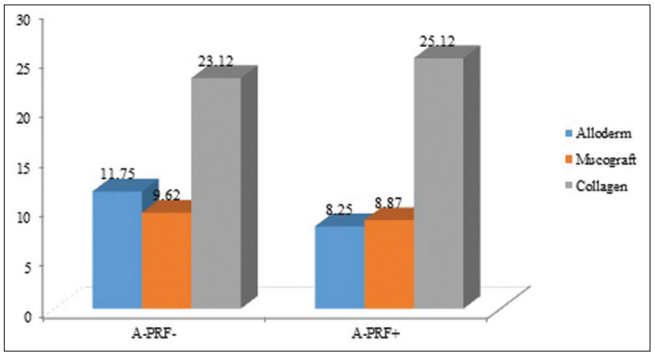

Figure 1: Mean of osteoblast cell adhesion in each membrane.

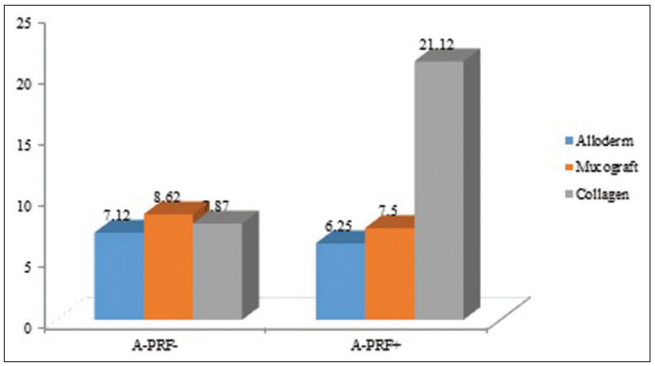

Figure 2: Mean of adhesion of fibroblast cells in each membrane.

However, the results of a study by Döri *et al*.[22] showed that, clinical parameters for the treatment of intracerebral guided tissue regeneration (GTR) with PRP, collagen membrane and autogenous mineralized bone were not different from those that were treated with similar materials, but without the use of PRP. The reason for this difference may be the use of PRP rather than PRF, and the study was an *in vivo* study, not an *in vitro* study.

Separately studying of each membrane revealed that, the adhesion of fibroblast cells to alloderm and mucograft membranes was slightly higher when A-PRF was absent; however, this difference was not statistically significant. For collagen membrane, the adhesion of fibroblast cells was higher in the presence of A-PRF, and the difference was statistically significant.

The adhesion of fibroblast cells was not significantly different among the three membranes studied in the absence of A-PRF; however it can be said that, the most important effect of A-PRF on fibroblast cell absorption is on collagen membrane, as in its absence fibroblast binding is lower than other membranes, which is consistent with the results of studies by Hakki *et al*.,[13] Alpar *et al*.,[23] and Wang *et al*.[24]

Rodrigues *et al*.[5] examined the binding and aggregation of fibroblasts on alloderm membrane and finally concluded that, on the 7th day, the cells were distributed as a discontinuous layer on the membrane, which continued until the day 14, but from day 14 to day 21, it reduced a little. In a study by Ojeh *et al*.,[25] the structure of dans alloderm reduced the cell migration into it. But Hillmann *et al*.[26] stated that, denser the matrix fibers carrying alloderm cell, the lower the cell migration, and low porosity and small pores in alloderm reduced the nutrient distribution and consequently caused disruption of migration and cell binding, which is inconsistent with the result of the present study, because it requires a longer study period that was less in the present study. Also, perhaps fibroblasts were more likely to be adsorbed to other membranes than alloderm if the study duration was longer.

In a study by Chang *et al*.,[27] the level of fibroblast cell absorption on the three types of membranes of Gore-Tex, Gore-Resolut XT (nonabsorbable membranes), and INION GTR (absorbable membranes) was higher in the presence of PRP compared to the absence of PRP. The highest cell adhesion was for INION membrane.

According to the study results, the adhesion to the absorbable membrane is better than the nonabsorbable membrane. Kasaj *et al*.[1] in their study showed that, the materials in the used membranes can improve cellular uptake and proliferation in the periodontal or bone tissue regeneration process, and also the absorbable membranes can stimulate the cell proliferation better than the nonabsorbable membranes.

Therefore, it seems that collagen receptor substrate could promote the growth and differentiation of many cells in the culture medium to a greater extent compared to those with glass and plastic substrates.[28,29]

According to the results, although the effect of A-PRF was solely on the adhesion of fibroblasts to collagen membrane and it was not very effective on the cell adhesion to membranes generally, due to its important role in the cell differentiation, its usage in adjacent membrane should not be undamaged.

CONCLUSION

The adhesion of both osteoblast and fibroblast cells to alloderm and mucograft membranes were slightly higher when A-PRF was absent; however, these differences were not statistically significant in both of these cell types.

For collagen membrane, osteoblast cell adhesion was higher in the presence of A-PRF, but again this difference was not statistically significant. However, the most significant effect of A-PRF was on the adhesion of fibroblast cells to the collagen membrane, and the cell adhesion to the membrane was higher in the presence of A-PRF, and this difference was statistically significant.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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