Comparison of Biocompatibility of Various Membranes with Fibroblastsy

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Abstract

Objective: Different techniques have been suggested for the repair of bone defects at the injured sites. Use of biomembranes, or application of plasma rich in growth factor (PRGF) at the site of proliferation of osteoblasts are among the suggested techniques. The current study aimed to compare the biocompatibility of human periodontal ligament fibroblasts (hPLF) cultured on Hypro-Sorb F, Pericardium and Tutodent resorbable membranes coated with PRGF.

Methods: This experimental study was conducted on four resorbable membranes namely Hypro-Sorb F, Pericardium, Tutodent and Vicryl. Fibroblast cells isolated from the periodontal ligament of premolar tooth were passaged three times and 10^5 cells/ cm^2 were cultured on membranes coated with PRGF. After 72 hours, the cells were evaluated in terms of biocompatibility and alkaline phosphatase (ALP) activity. Statistical analysis was carried out using one-way ANOVA.

Results: PRGF increased cell adhesion and Tutodent membrane coated with PRGF showed the highest cell adhesion compared with Hypro-Sorb F and Pericardium membranes (p=0.005).

Conclusion: PRGF increases cell viability and ALP activity of cells on biomembranes. PRGF treatment increases the adhesion of fibroblast cells to these membranes.

Key words: Alkaline phosphatase, Fibroblast, MTT, PRGF

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Introduction:

The conventional periodontal treatments mainly focus on the elimination of microbial plaque and inflammation. These treatments almost always include scaling and root planning and in some cases periodontal surgery. These procedures along with proper oral hygiene practice by the patient often cease the disease process and preserve the remaining periodontium (1-3). However, the most important goal of periodontal treatment is to provide adequate conditions for regeneration of the injured or lost periodontium and restoring its function and primary characteristics (1).

Different techniques have been suggested for repair of bone defects at the injured sites. Use of biomembranes, or application of PRGF at the site of proliferation of osteoblasts are among the suggested techniques (4). Studies have demonstrated that following periodontal surgery, epithelial cells have the highest proliferation and accumulate on the denuded root surfaces sooner than other periodontal cells; this phenomenon is not accompanied by tissue regeneration or reconstruction and results in tissue repair with a long epithelial attachment to the root surface (1-5). Periodontal cells originated from the periodontal ligament (especially fibroblasts) can create the best type of attachment and the most suitable type of tissue regeneration (6). Different membranes have been suggested for use for this purpose. Considering the variable characteristics of membranes (7), they can be placed between

the denuded root surface and the connective tissue to prevent apical migration of the epithelial cells and the connective tissue during the early phase of healing after periodontal surgery. A placed membrane also maintains a space for the migration and accumulation of periodontal cells (with regenerative potential) originated from the periodontal ligament like the fibroblasts (8, 9). This issue was first confirmed by Gottlow in 1982 (10). Recent investigations have demonstrated that membrane parameters namely the orientation of fibers, their thickness and level of porosities can result in variable degrees of fibroblastic adhesion and proliferation on the surface of membranes (1, 2, 11, 12). Researchers have investigated the biocompatibility of membranes in fibroblast and osteoblast cell cultures and have reported their proliferation in presence of membranes (13).

To enhance guided tissue regeneration, use of polypeptide growth factors and enamel matrix proteins has been suggested in addition to the application of membranes (1). In 2007, the boosting effect of PRGF on fibroblast adhesion to several membranes was confirmed (1).

PRGF is a rich source of growth factors and has been introduced as an efficient material for tissue regeneration (14, 15). Thus, the current study sought to assess the effect of treatment with PRGF on the degree and type of adhesion of hPLF to resorbable membranes under *in-vitro* conditions.

Methods:

In this experimental study, 10^5 fibroblast cells/cm^2 isolated from the periodontal ligament of a premolar tooth were cultured in a solution containing Double Modified Eagle's Medium (DMEM), 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The resorbable membranes used in this study had 3x3 mm dimensions and were as follows:

Tutodent ® (Tutogen, Neunkirchen am Brand, Germany)

Hypro-Sorb F: Bilayer atelo-collagen membrane for GTR/GBR indications, Bioimplon GmbH (CAT No. = 0.23)

Pericardium membrane: 15x20 mm, collagen CopiOs® Pericardium Membrane (CAT No.= 0.23)

Vicryl Membrane (Lactic acid, German)

The membranes were coated with PRGF and placed at the bottom of the plates. All wells were washed with phosphate buffered saline and stored at 37°C under humid conditions for one hour. The cells were incubated for 24 hours in an incubator with 5% CO2.

ALP Activity Test:

ALP activity test is used for the assessment of cell activity especially the osteoblasts. The ALP activity assessment kit (Product No. 85, Sigma, Aldrich, Germany) was used for this purpose. The cells were exposed to the diazonium salt solution, stained with hematoxylin solution and evaluated under a light microscope.

MTT Assay:

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was used to assess cell viability. This assay measures the amount or ratio of cell proliferation and is a colorimetric assaydependent on the reduction of the tetrazolium salt, MTT, to form blue formazan crystals. After completion of incubation, the overlying culture medium was removed. After the addition of MTT, cells were incubated for 4 hours in an incubator containing CO2 at 37°C. Isopropanol acid was added and the optical density (OD) of the obtained solution was read at 630nm as the reference wavelength and at 570nm as the measurement wavelength using the ELISA reader. One-way ANOVA was used for data analysis.

Results:

In the resorbable membrane group without the PRGF, the highest number of flat cells was

observed in the Vicryl L membrane group with a mean number of 23.4 (4.66) cells and the lowest number of flat cells were noted in the Pericardium membrane group with a mean number of 4.6 (1.81) cells. In membranes without the PRGF, the highest amount of MTT belonged to Tutodent membrane with a mean value of 0.33 (0.305) and the lowest was seen in Pericardium membrane group with a mean value of 0.242 (0.231). The difference between groups was statistically significant (p=0.005). In terms of ALP activity in membranes without PRGF, the highest rate belonged to the Tutodent membrane (38 (5.38)) and the lowest rate belonged to the Pericardium membrane group (4 (2.23)). Based on one-way ANOVA, the difference in this respect among groups was statistically significant (p=0.005). Among resorbable membranes coated with PRGF, the highest number of flat cells was seen in Vicryl L membrane group (32.67 (7.37)) and the lowest cell count was seen in the Pericardium membrane group (1.52 (4.60)).

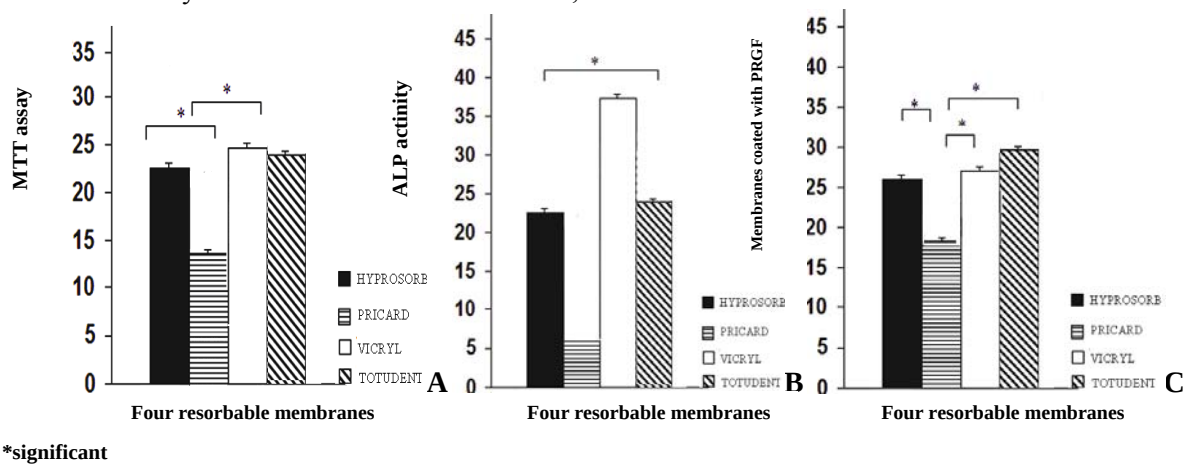

Diagram 1- The frequency distribution of the understudy specimens in terms of morphology (number of flat cells)(A), MTT assay (B) and ALP activity (C) for membranes coated with PRGF (p=0.005)

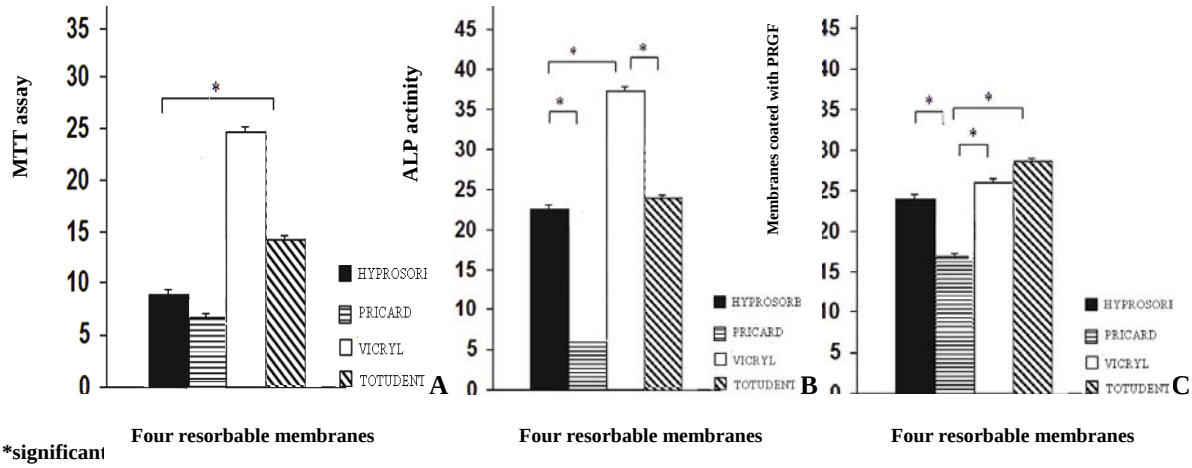

Diagram 2- The frequency distribution of the understudy specimens in terms of morphology (number of flat cells)(A), MTT assay (B) and ALP activity (C) for membranes without the PRGF (p=0.005)

The highest amount of MTT in membranes coated with PRGF was seen in Tutodent membrane group (0.331 (0.304)) and the lowest belonged to the Pericardium membrane group (0.33 (0.38)). According to the ANOVA, the difference among groups was statistically significant (p=0.005). The highest ALP activity in membranes coated with PRGF was noted in Tutodent membrane group (43 (2.64)) and the lowest was observed in the Pericardium membrane group (6.33 (3.78)). The difference among groups in this regard was statistically significant (p=0.005).

In terms of resorbability, Vicryl membrane coated with PRGF had the highest and Pericardium membrane without the PRGF had the lowest number of flat cells.

Discussion:

The main goal of periodontal treatment is to control inflammation and regenerate the lost tissue. Membranes as physical barriers prevent the unwanted proliferation of epithelial cells and the connective tissue and enhance the attachment of fibroblasts. Using membranes increases osteogenesis via two mechanisms. First, membranes prevent growth and migration of fibroblasts to the bone defect site. Second, membranes enhance the migration of osteoblasts from the adjacent bone margin into the injured site (4). On the other hand, use of polypeptide growth factors like PRGF enhances the process of cell adhesion.

The results of the current study demonstrated that PRGF increased the attachment of fibroblasts to the resorbable and non-resorbable membranes and subsequently increased ALP activity and cell viability (MTT). In line with our findings, another study showed that Pericardium membrane enhanced the adhesion and proliferation of fibroblasts and osteoblasts (14).

In terms of morphology, the highest attachment was observed to Vicryl membrane coated with PRGF. When it comes to adhesion, MTT and ALP are important parameters. Tutodent membrane with and without PRGF showed the highest MTT and ALP activity among the resorbable membranes.

Some researchers have discussed that adhesion of fibroblasts to different membranes is influenced by external factors such as the platelet-rich plasma (PRP) (15, 16). No significant difference has been noted in cell adhesion to membranes with and without PRP. Different adhesion of cells to membranes is due to several factors including the microscopic structure of membranes. Unsmooth and porous surfaces significantly affect the adhesion. Difference in adhesion of cells to membranes with and without PRP may be due to the presence of factors like platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) causing different levels of cell adhesion.

In the current study, PRGF enhanced the adhesion of fibroblasts. Tutodent membrane due to its porous structure and cross-linked fibers caused the highest attachment in terms of MTT parameter (0.305 (0.33)) and ALP activity (38 (5.38)). Vicryl membrane due to the presence of more flat cells with a mean value of 23.4 (4.66) showed the highest attachment (the distance between cells was smaller). But, in terms of MTT (0.46 (0.270)) and ALP activity (27.8 (6.30)), it showed lower adhesion than Tutodent. Based on the obtained results and the importance of these parameters in determination of cell attachment, Tutodent in both groups of with and without PRGF showed the highest adhesion potential. In fact, by using PRGF, the mean adhesion of Tutodent membrane based on morphology (compared to the use of PRGF) increased from a mean value of 12.40 (0.305) to a mean value of 16.67 (2.30). Its ALP activity increased from a mean value of 38 (3.8) to a

mean value of 43 (2.64). Thus, it appears that PRGF is the most important parameter for increasing cell adhesion. This finding has been very well demonstrated in studies reporting the results of PRGF application for bone grafting prior to implant placement. Takata, *et al*. (2011) (7) reported that the most important membrane property causing ideal adhesion is their biocompatibility. However, other factors such as the toxicity, composition, shape and structure of membranesas well as the size of membrane porosities also play a role in this regard. In fact, porosities act as a scaffold to entrap blood clot; affecting the migration of cells into the scaffold (7).

Kasaj, *et al*. (2008) believed that resorbable membranes significantly enhance cell adhesion in comparison with non-resorbable membranes. Among the tested membranes, Tutodent caused the highest adhesion. They explained that this finding is attributed to the enhanced cell proliferation and extracellular macromolecules by the collagen resorbable membranes (2). The reason for the positive efficacy of PRGF in the current study was its biological effects and having many of growth factors such as TGFB and PDGF. These factors induce osteogenesis. The TGFB family plays a role in differentiation of osteoblasts and cementoblasts and can strongly induce proliferation and differentiation of PDL cells and alveolar bone. Stimulation of fibronectin causes the attachment of fibroblasts to root surface and angiogenesis (17-19). PRGF has cell adhesion properties due to its fibrin content. It can be homeostatic and stabilize the blood clot in the area (20, 21). In general, positive effects of PRP can be explained by tissue engineering principles. The three main components of tissue engineering include a scaffold, messenger molecules and growth factors for regeneration. Combination of these factors under suitable biological conditions and adequate time for cell adhesion to membranes will determine the behavior of cells.

Conclusion:

PRGF increases cell viability and ALP activity of cells on biomembranes. Treatment with PRGF enhances the adhesion of fibroblasts to membranes.

Conflict of Interest: "None Declared"

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