Assessment of the Impact of PRF- A Hemoderative on the Gingival Fibroblasts in Perspective of Their Migration and Proliferation

ABSTRACT: Periodontal diseases deteriorate the periodontium, leading to tooth loss. Therefore regeneration of periodontal tissue is a focus of various periodontal treatments. Despite high treatment demand and numerous procedures, healing is prolonged. In this context, platelet-rich fibrin (PRF), as a biomaterial, presents a promising natural alternative with favorable results as it has biological properties like wound healing and regenerative capacity. Our research focused on evaluating the efficacy of PRF in inducing gingival fibroblasts proliferation and migration.

KEYWORDS: platelet-rich fibrin, gingival fibroblasts, proliferation, migration, periodontitis

INTRODUCTION

Periodontal disease is one of the chronic oral inflammatory diseases affecting the supporting structure of tooth such as gingiva, periodontal ligament, cementum, and alveolar bone.[1,2] It is widespread worldwide, impacting approximately 20-50% of the global population.[3] The high incidence of periodontal disease among adolescents, adults, and the elderly highlights its significance as a public health issue. [3]

Periodontal disease is typically marked by the gradual destruction of the soft and hard tissues of the periodontal complex, driven by the interaction between dysbiotic microbial communities and abnormal immune responses within gingival and periodontal tissues. [4]

Periodontal wound healing involves interactions among epithelial cells, gingival fibroblasts, periodontal ligament cells, and osteoblasts.[5] Disrupted vasculature leads to fibrin formation, platelet aggregation, and the release of growth factors from platelets.[6] These factors, crucial for inflammation and wound healing, also include fibrin, fibronectin, and vitronectin, which provide a matrix for tissue and aid cell migration[6]. Thus, platelets are seen as potential therapeutic tools for improving periodontal tissue repair. [6]

Platelet-rich fibrin (PRF), first prepared by Choukroun et al, is a second-generation platelet concentrate made from the patient's blood without anticoagulants. It forms a strong fibrin matrix that concentrates platelets and growth factors, with unique architecture and mechanical properties distinguishing it from other platelet concentrates.[7,8]

Platelets within the fibrin matrix are vital not only for hemostasis but also for the wound healing process. They serve as significant reservoirs for growth factors, releasing high concentrations of biologically active proteins that aid in recruiting cells from surrounding host tissue, stimulating growth and cell morphogenesis, and thereby promoting the healing of bone and soft tissue.

Numerous studies have explored PRF applications in treating alveolar bone defects, implant restorations, periodontal treatment, and gingival recession. However, there is limited research on the mechanisms by which PRF stimulates wound healing, particularly in activating internal stem cell sources for periodontal regeneration. This study aims to assess the effectiveness of PRF in promoting the migration and proliferation of human gingival fibroblasts (hGFs).

MATERIALS AND METHODS

ETHICAL CLEARANCE

The ethical clearance was given by Institutional Ethical Committee of Malla Reddy Institute of Dental Sciences with the registration no: IEC/MRIDS/33/2022 dated on 14 May 2022.

Cell culture:

Revival of Gingival fibroblasts:

The cells used for the study were Human Gingival Fibroblasts (HGFs) from the repository of Central research Laboratory, Maratha Mandal’s Natahjirao Halgekar Institute of Dental Scinces and research center Belagavi. The cells were maintained in 96 wells micro titer plate containing alpha MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamicin (10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37ºC for 48-72 hours.

PREPARATION OF PRF

Blood sample was collected from a healthy, non-smoking volunteer aged 27 years. Volunteer was chosen based on the inclusion and exclusion criteria. Inclusion criteria included healthy, non-alcoholic, non-smoker, aged under 30years, and not on any medication. Exclusion criteria included suffering with systemic diseases, and had history of aspirin use or other medications affecting coagulation in the prior two weeks. In summary, 10 mL of blood was drawn from the antecubital vein into a test tube without anticoagulants and centrifuged immediately at 3000 rpm for 10 minutes. The PRF clots were then carefully extracted with sterilized forceps and separated from the RBC layer using scissors.

CELL PROLIFERATION ASSAY – XTT ASSAY

The kit for XTT assay was procured from HiMedia CellCulture (EZcountTM XTT Cell Assay Kit) with product code: CCK015.

The experimental protocol for the EZcount™ XTT Cell Assay Kit was optimized for cell density, incubation time, media composition, and agent concentration. Controls included media with cells but no experimental compound, and media with the compound cells. Phenol red-free media was used to avoid interference in color measurements. A uniform temperature was maintained for reproducibility, with absorbance measured at 450-500nm and a reference wavelength of 630-690nm. XTT reagent was prepared by mixing 0.1ml of electron coupling reagent with 5ml of XTT and equilibrated at room temperature before use. Freshly harvested cells were seeded at 1 x 10^6 cells/ml, serially diluted, and incubated at 37°C with 5% CO2 for 24 hours or until confluence. For proliferation assays, 100µl of cell suspension was seeded into 96-well plates and treated for 24 or 48 hours. After incubation, 50µl of activated XTT reagent was added, followed by a 2- to 4-hour incubation, and absorbance was measured at 450nm with a 630nm differential filter.

CELL MIGRATION- SCRATCH TEST

The experiment involved preparing a 12-well culture plate, with 2 mL of warmed media added to each well. Fibroblast cells were seeded at a density of 200,000 cells per well, aiming to reach 100% confluence within 24 hours. Once confluence was achieved, typically between 18-24 hours, a 1 mm pipette tip was used to scrape the cell layer, creating a straight line while keeping the tip perpendicular to the well bottom. After scratching, the cell monolayer was gently washed to remove any detached cell. The scratched areas were imaged using a phase contrast microscope at 4x magnification. The plates were then incubated, with images taken until the cells migrated to close the gap, which generally occurred within 48 hours.

RESULTS

CELL PROLIFERATION ASSAY

Human gingival fibroblasts demonstrated an 8% proliferation rate at 24 hours, which increased to 27% by 48 hours when exposed to PRF, compared to untreated cells. The PRF-treated cells exhibited a higher viability, with no cell death observed in either the treated or untreated groups.

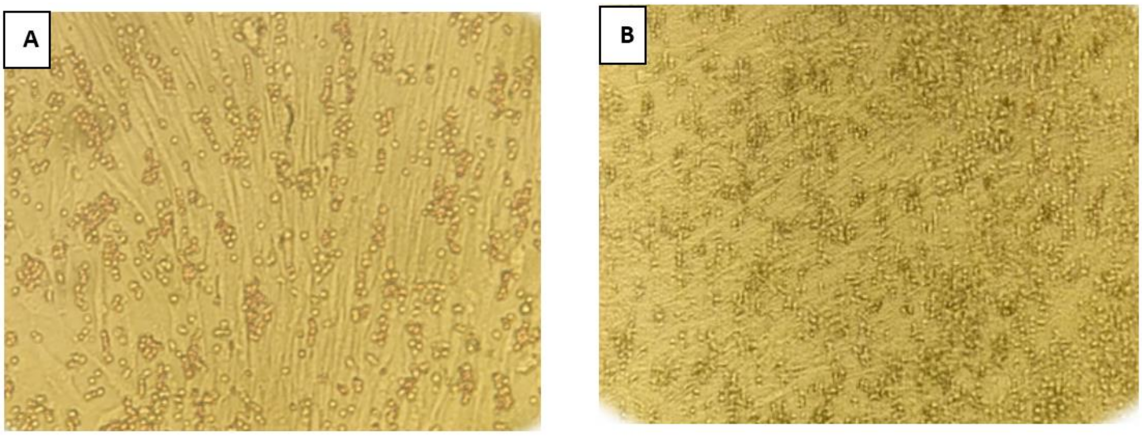

Fig 1: Demonstrates human gingiva fibroblast cells proliferation at (A) 24 hours and (B) 48 hours

XTT ASSAY after 24hrs

S.No	Test Material	Cells used	OD values (450nm)	Mean OD values	Cell Viability	Cell proliferation	Cell Death
1	Test Group	Human Gingival Fibroblasts	0.519	0.513	108%	08%	NA
			0.511				
			0.511				
			0.514				
			0.512				
			0.510				
			0.510				
			0.518				
2	Negative Control – Untreated Cells	Human Gingival Fibroblasts	0.474	0.473	100%	NA	NA

Table 1: Demonstrates cell viability and proliferation after 24 hrs

XTT ASSAY after 48hrs

S.No	Test Material	Cells used	OD values (450nm)	Mean OD values	Cell Viability	Cell proliferation	Cell Death
1	Test Group	Human Gingival Firoblasts	1.800	1.805	127%	27%	NA
			1.802				
			1.800				
			1.809				
			1.805				
			1.808				
			1.808				
			1.805				
2	Negative Control	Human Gingival Fibroblasts	1.415	1.415	100%	NA	NA
			1.416				
			1.417				
			1.414				

Table 2: Demonstrates cell viability and proliferation after 48 hrs

CELL MIGRATION ASSAY

In this assay, the wound created by the pipette initially lacked cells. After introducing PRF, cell migration into the wound area was observed within 48 hours. Prior to the migration of gingival fibroblasts, a few red blood cells (RBCs) were present at the site of the scrape.

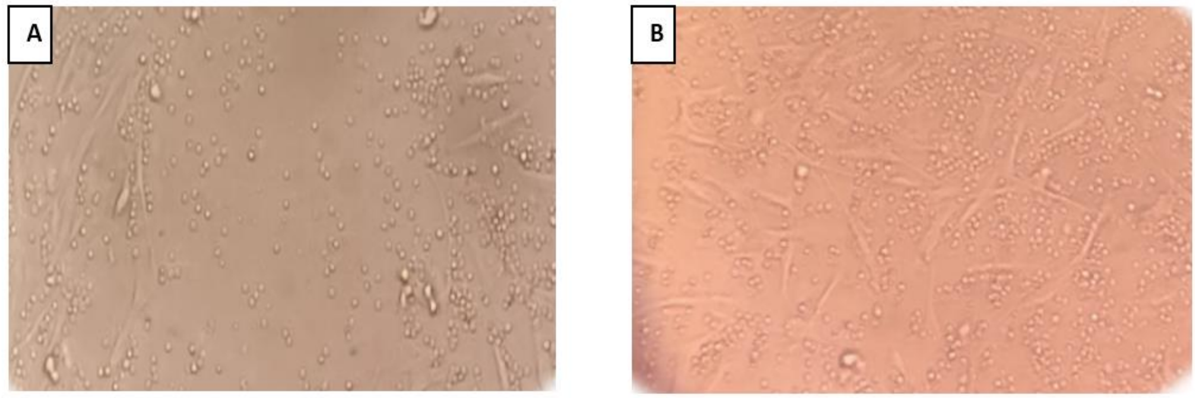

Fig 2: Demonstrates human gingival fibroblast cells migration (A) at 0 hours with scraped site showing few RBC cells and (B) migration of fibroblasts into scraped site in 48 hours

DISCUSSION

"Periodontitis" denotes a chronic inflammation that impacts the gingiva, periodontal ligament, alveolar bone, and dental cementum. This condition is recognized by the World Health Organization (WHO) as a prevalent chronic disease on a global scale. Its onset is associated with the accumulation of plaque around the teeth, subsequently forming bacterial biofilms and leading to localized gingival inflammation.[9]

The formation of bacterial biofilms initiates gingival inflammation. However, the progression of periodontitis is primarily influenced by dysbiotic alterations in the oral microbiome. These alterations are stimulated by nutrients from gingival inflammation, tissue degradation, and antibacterial mechanisms aimed at controlling microbial presence in the gingival sulcus. Consequently, these events trigger crucial molecular pathways leading to the activation of host-derived proteinases. Subsequently, these proteinases play a significant role in the loss of marginal periodontal ligament fibers, apical migration of the junctional epithelium, and the propagation of bacterial biofilms along the root surface.[10]

The management of periodontal disease treatment has progressively shifted from non-surgical and resective methods to regenerative therapies, owing to advancements in comprehending periodontal wound healing. Advancements in biomaterials, tools, and techniques have facilitated this transition, highlighting the genuine regeneration of periodontal tissues, such as the development of functional periodontal ligament (PDL).[11]

Considerable research has been undertaken on the use of biomaterial-based approaches for regenerating periodontal tissues. Progress in the fields of tissue engineering and nanotechnology has resulted in the creation of innovative biomimetic materials and scaffolding methods that faithfully replicate the natural microenvironment of platelet-rich fibrin (PRF) for the regeneration of periodontal tissues[12]

Platelet-rich fibrin (PRF) is an autologous matrix rich in leukocytes and platelets, characterized by a tetra-molecular structure containing cytokines, platelets, and stem cells. This matrix serves as a biodegradable scaffold that facilitates microvascularization and promotes the migration of epithelial cells to its surface. Furthermore, PRF is capable of transporting essential cells for tissue regeneration and demonstrates a sustained release of growth factors over a span of 1 to 4 weeks, establishing an optimal environment for efficient wound healing. This second-generation platelet-rich plasma (PRP) variant, produced according to Choukroun's protocol, does not necessitate the use of anticoagulants or external agents such as bovine thrombin or calcium chloride, thus positioning PRF as a natural, autologous biomaterial..[7]

Platelet-rich fibrin, a second-generation PRP, is produced following Choukroun's protocol without the use of anticoagulants or external agents like bovine thrombin or calcium chloride. As a result, PRF is regarded as a natural, autologous biomaterial.[13]

The Platelet-Rich Fibrin (PRF) functions as a mitogen, stimulating the proliferation of osteoblasts, gingival fibroblasts, and periodontal ligament cells. The cell proliferation induced by PRF is attributed to the release of multiple growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β. Recent research conducted by Dohan et al.21 demonstrated that the PRF membrane steadily releases crucial growth factors over at least one week, providing prolonged stimulation to its environment during the remodeling process..[14]

A recent study by Chang et al. the levels of specific growth factors (EGF, IGF-1, PDGF-BB, TGF-β1, and VEGF) were measured from PRF membranes and their exudates. They concluded that the concentration of growth factors in the PRF membranes was significantly higher compared to the PRF exudates.[15]

This study explored the potential role of PRF in wound healing by evaluating its effects on the proliferation and migration of human gingival fibroblasts (hGFs), aiming to provide evidence for its use in treating periodontitis. Our findings demonstrated that PRF enhanced both the proliferation and migration of hGFs. Cell viability improved with extended incubation periods in the PRF extract.

Consequently, it was effectively shown that PRF releases growth factors during incubation, which promotes cell proliferation. This outcome is consistent with prior research on PRF-derived growth factors, such as PDGF-AB, transforming growth factor β, vascular endothelial growth factor, and insulin-like growth factor.[16]

Future research should focus on correlating the clinical outcomes of PRF with its biological mechanisms, potentially uncovering new applications for this autologous platelet concentrate. Currently, there are limited studies on PRF's effects on cell proliferation and other biological functions. Hence, further investigations are necessary to explore innovative strategies for utilizing this platelet concentrate.

CONCLUSION

PRF, produced using Choukroun's technique, offers a straightforward and cost-effective method for successful periodontal tissue regeneration. A key advantage is that PRF preparation uses the patient's own blood, thereby reducing or eliminating the risk of disease transmission through blood. Using a protocol adapted from the Choukroun method, we successfully fabricated PRF and demonstrated its effects on enhancing the proliferation and migration of hGFs. These findings suggest that PRF can be effectively applied in clinical periodontitis treatments, particularly for inflamed gingiva and periodontal tissues. Evidence of PRF's effects on hGFs provides a foundation for further research and investigation into PRF's impact on wound healing cells, thereby encouraging its clinical application in dental care.

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