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Behavioral Analysis Of Gingival Fibroblasts In Response To Antibiotic Fortified Injectable Platelet Rich Fibrin

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Abstract
Background: *Injectable platelet rich fibrin (i-PRF) is the liquid form of PRF which is obtained by low-speed centrifugation and has the capacity to stimulate tissue regeneration. With the addition of antibiotics to i-PRF, the resultant platelet concentrate would have an enhanced antibacterial property and furnish better results in periodontal regenerative surgeries.*
Methods and Methodology: *An in vitro experimental study was conducted to evaluate the regenerative potential of the antibiotic infused i-PRF when compared to i-PRF alone. Blood was collected from 5 participants following which i-PRF and its antibiotic infusion was formulated. Antibiotics infused were 5mg/ml Amoxicillin Group-1 and Group 2 was i-PRF alone. The groups were subjected to cell viability and migration assays with gingival fibroblast cell line.*
Results: *Group 2 showed highest cell viability and mobility of the gingival fibroblasts when compared to Group 1*
Conclusion: *Within the limitations of the present study, it can be concluded that the modified i-PRF preparation may be used to reduce the risk of post-operative infection in addition to the beneficial healing properties of i-PRF.*
Keywords: *i-PRF, antibiotic i-PRF, periodontal disease, amoxicillin*

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I. Introduction

Periodontitis is a polymicrobial, multifactorial, inflammatory condition that affects the tooth-supporting apparatus. It is associated with dysbiotic plaque biofilms and leads to progressive destruction of the periodontal tissues.

Platelet concentrates may be defined as an autologous biological product obtained following centrifugation of peripheral blood. Different centrifugal speeds result in various types of platelet concentrates. Platelet concentrates have three standard characteristics that is they act as scaffolds, serve as a source of growth factors and contain viable cells.

Platelet-Rich Fibrin (PRF) is a second-generation platelet concentrate that was introduced by *Choukroun et al.*, in 2001, it is an autogenous fibrin product that can be obtained from blood using low-speed centrifugation without the addition of anticoagulants [1]. PRF can improve tissue regeneration due to its effects on vascularisation, capturing the circulating stem cells, immune control and closure of the epithelium. Increased platelet counts in PRF acts as a biological connector between the different elements of healing and as a matrix which favours neo-angiogenesis, the capture of stem cells and migration of osteo progenitor cells. It also helps in postoperative protection of the surgical site and accelerates remodelling and integration of the bone.

Recent advances in PRF formulation have led to the development of an injectable form of PRF. Injectable PRF (i-PRF) is the liquid form of PRF, it is a bioactive agent obtained by low-speed centrifugation and it has the capacity to stimulate tissue regeneration. Low-speed centrifugation preserves more immune cells, growth factors and cytokines. At high concentrations, PRF may stimulate the secretion of several growth factors and trigger fibroblast migration and can be used in regenerative periodontal treatments. It was also found that i-

PRF decreases biofilm production at the minimal inhibitory concentration (MIC) and no biofilm production at the minimal bactericidal concentration (MBC).

Infections are one of the most common postoperative risks caused by pathogenic and opportunistic bacteria [2]**.** With the ability to produce biofilms, bacteria can evade the host immune system and can cause various local and systemic infections.

Along with biomaterials, the use of antibiotics in periodontal therapy is of primary importance. The success of antibiotic therapy depends on the potency of the drug used against the specific pathogen. The choice of antibiotic regimen is difficult in periodontal disease as it is a complex polymicrobial disease. A large array of antibiotics is used as adjuncts to nonsurgical and surgical therapy in the treatment of periodontal disease. The commonly used antimicrobials in periodontal therapy are tetracycline, metronidazole, penicillin, macrolides, ciprofloxacin and clindamycin. Metronidazole and amoxicillin are reported to be the most used combination antibiotic regimen. Hence, in the present study, amoxicillin and metronidazole were preferred.

Therefore, this study aims to evaluate the effect of antibiotic infusion in injectable platelet rich fibrin(i-PRF) as a carrier matrix on the gingival fibroblasts. To the best of our knowledge, there are very few studies evaluating the effect of antibiotic i-PRF on the viability and mobility of the gingival fibroblasts and its anti-inflammatory potential. Hence, our study focuses on the assessment of the cell viability and mobility of the gingival fibroblasts after exposure to i-PRF when used in an injectable form with and without antibiotics.

II. Materials And Methods

The present study was planned and conducted in the Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bangalore after the institutional ethical clearance.

The study aimed to evaluate the mobility and viability of gingival fibroblasts in i-PRF and to compare it with the antibiotic infused i-PRF. Study participants were selected based on the given inclusion and exclusion criteria and the blood samples were then congregated into four groups. The inclusion criteria included patients in the age group of 30 to 45 years, systemically healthy donors having a complete blood profile in the normal range. The exclusion criteria were patients diagnosed with systemic disease or condition**,** patients on any anti platelet or anti-coagulant medications**,** alcoholics or smokers or who use tobacco in any form**,** Pregnant or lactating women**.** Patients who have received any form of antibiotic therapy or periodontal intervention within the last 3 months were also excluded from the study.

Patient were recruited from Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bangalore. Informed consent was obtained from all the study participants willing to participate in the study.

The samples in the present comparative in vitro study were grouped into two groups based on the formulations. Group 1 was Amoxicillin infused i-PRF and Group 2 was i-PRF alone.

10ml of whole blood without anticoagulant was collected from 5 patients in i-PRF tubes. It was then centrifuged at 60 g RCF for 3 min at room temperature; 60 g RCF is equivalent to 700 rpm [3,4.] Antibiotics used: Amoxicillin 500 mg .According to the resultant extracted i-PRF, equivalent volume of the antibiotic was taken and infused into the i-PRF and centrifuged for equal distribution for 1 minute at 700 rpm. For group 1, Amoxicillin 500 mg powder formulation was employed where 2 ml of saline was mixed according to manufacturer's instructions following which amoxicillin was extracted in equivalent volume of the resultant i-PRF. It was then mixed and centrifuged again for two minutes at 700 rpm.

Human Gingival fibroblast cell line was procured from ATCC, stock cells was cultured in fibroblast growth medium supplemented with growth factors, penicillin (100 IU/ml), in a humidified atmosphere of 5% CO_2 at 37^{o}C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS)

After 24 and 48 h of culture, the cell cultures were rinsed with sterile phosphate-buffered saline (PBS) twice, and the proliferation and viability of the cells were evaluated using the methyl thiazolyl tetrazolium (MTT) assay. In brief, the MTT solution with a final concentration of 0.5 mg/mL was added to each culture dish at 24 and 48 h. The dishes were incubated at 37°C. After 4 h of incubation, the medium containing MTT was extracted and dimethyl sulfoxide was added to each dish, which resulted in the formation of insoluble crystals. Next, 100 µL of the solution was added to 96-well plates of enzyme-linked immunosorbent assay reader. The optical density was read at 570–620 nm wavelength.

In vitro scratch wound assay was used for assessment of cell migration. The cells were seeded into 6-well plates with 50,000 cell density. Using a sterile sampler, 100 vertical scratches were created in each well (time zero). Each well was rinsed with complete culture medium twice to remove the cells scraped off from the bottom of the wells and were stuck in the scratches. Each well was then treated with PRF using cell culture inserts. The control group did not receive any treatment. The insert along with the PRF membrane was removed from the wells after 24 and 48 h of exposure, and the wells were rinsed with cold PBS, fixed with ice-cold 100% methanol, and stained with 20% ethanol containing 0.5% crystal violet. The stained cells were inspected under an inverted

microscope at ×4 magnification, and digital images were obtained. The rate of cell migration was determined using Image J software (National Institutes of Health, Bethesda, MD, USA).

III. Results

Assessment of cell viability of the gingival fibroblast cell line in injectable PRF and antibiotic infused injectable using MTT Assay

The highest cell proliferation can be noted at 50% concentration of the test formulation. The results are calculated at triplicates; hence the mean and standard deviation is taken for the cell proliferation at 50 % concentration. At 50% concentration, Group 1 showed cell proliferation of 55.09%, 56.74% and 61.74% with a mean cell proliferation of 58.06±3.23%. Group 2 showed cell proliferation of 50.31%, 55.88% and 52.74% with a mean cell proliferation of 52.98±2.79%. Group 2 showed cell proliferation of 55.95%, 62.66% and 62.96% with a mean cell proliferation of 60.52±3.96% (Table 1). Highest cell proliferation was seen in group 2 and comparable proliferation in group 1.

The mean cell proliferation at 50% concentration for Group 1 was 58.057±3.233, for Group 2 it was 60.523±3.963. These differences in the mean cell proliferation of the gingival fibroblasts between 2 groups was statistically significant at $p<0.001$ (Table 2, Graph 3).

Assessment of cell mobility of the gingival fibroblast cell line in injectable PRF and antibiotic infused injectable using Scratch wound assay

Cell migration towards wound closure was evaluated at three-time intervals 50% concentration of the test formulations. Scratch wound approximately measured 2mm. The migration distance was measured as the distance from one side of the wound to the other using a scale bar in ImageJ software.

There was no significant movement noticed between the groups. Migration distance in Group 1 was 1.98mm and Group 2 was 1.98 mm respectively. Phosphate buffered saline was taken as control with no apparent cell migration .Cell migration was prominent at 24 hours with a migration distance in Group 1 of 0.82mm and Group 2 of 0.65mm respectively. Control showed cell migration of 0.96mm .There was significant movement towards wound closure among the groups. Cell migration distance in Group 1 was 0.11mm and Group 2 was 0.01mm. Control showed cell migration of 0.43mm .Wound closure was measured by the percentage of cell migration through the scratch wounds at 24 hours and 48 hours.

Cell migration at 24 hours in Group 1 was 58.59% and Group 2 was 67.17% respectively (Table 3, Graph 4).
Cell migration at 48 hours in Group 1 was 94.44%, and Group 2 was 99.49% respectively (Table 3, Graph 4).

The multiple comparison of the mean differences of the migration distance between the groups at 24 hours obtained were highly statistically significant for Group 1 and Group 4 (p value<0.001*) The multiple comparison of the mean comparison of the migration distance between the groups at 48 hours obtained were highly statistically significant for both the Groups (p value<0.001*) (Table 5, Graph 6).

IV. Discussion

The study aimed to evaluate and compare injectable PRF and antibiotic infused injectable PRF for the effect on the behaviour of the gingival fibroblasts. The use of PRF in regenerative medicine has become increasingly popular because of its low-cost and enhanced curative effect [(6-9)]. After centrifugation at 700 rpm for 3 min, the faint yellow upper layer is i-PRF, and substratum is red corpuscles base. This liquid version of PRF, termed injectable-PRF, or i-PRF is intended to advance biomaterial blending with platelet concentrates and to form a fibrin network following coating [(7, 8).]

In the present study i-PRF (Group 2) was taken as a positive control and showed unsurpassed results in all the assays. Group 2 has shown to have highest cell proliferation and fastest wound closure.

Antibiotics are an effective treatment for various types of infections caused by bacteria (gram-positive and gram-negative). In turn, their misuse can lead to antibiotic resistance**.** Wound healing is a normal biological process in the human body, but in the postoperative period there is a high risk of infection. It is important to ensure a proper healing process and reduce the risk of infections [9]. For wound healing, PRF can also be used as a drug carrier in another system[10]. Therefore, our study aimed to formulate an autologous material, Injectable PRF infused with antibiotics (i-PRF + Antibiotics).

The addition of antibiotics to the injectable PRF(i-PRF) as a drug matrix showed comparable results to i-PRF alone (Group 2). These results exhibit that the conjugation with antibiotics did not harm the natural behaviour of the gingival fibroblasts. The use of PRF was found to promote micro vascularization, migration of epithelial cell and accelerated healing [11]. Its use has been reported for many surgical applications: treatment of bony defects, dental implant surgery, post-extraction healing and reducing rate of post-surgery complications [12].

Wound healing following surgery always bares a risk of infection ***(Yang et al., 2015)***. Even when disinfection has been stringently enforced, microbes can infiltrate and colonize the underlying wound tissues, resulting in loss of tissue integrity in the surgical site and impaired healing. Therefore, infection control is a prerequisite for successful surgical procedure. Weak evidence exists for the beneficial use of peri- and post-

operative systemic antibiotics following dental surgery and their adverse effects with the possibility for the development of resistant bacteria makes their use controversial [13]**.**

The present study evaluated the fibroblast migration for wound closure in the different formulations, where expeditious and complete wound closure was seen in Group 2 at 48 hours. Group 1 showed comparable wound closure at 48 hours. The combination i-PRF showed lesser proliferation and wound closure perhaps because of the different formulations of the available drug hence stating that the combination may influence the behaviour of the fibroblasts.

V. Conclusion

The study aimed to evaluate and compare i-PRF and antibiotic infused i-PRF for the effect on the behaviour of the gingival fibroblasts.In the i-PRF group, greatest cell proliferation of the gingival fibroblasts and complete wound closure, proving that the autogenous biomaterial has excellent regenerative and reparative potential.

Amoxicillin i-PRF group showed comparable cell proliferation of the gingival fibroblasts and wound closure. Amoxicillin i-PRF showed that the drug and formulation had no adverse effect on the natural behaviour of the gingival fibroblasts.

In conclusion, the present study provides the clinician with a novel tool to control post-operative infection using the modified i-PRF with antibacterial properties. This biomaterial can be highly important as a local surgical tool that prompts tissue healing and prevents infection.

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