

# Fibroblast Proliferation due to Exposure to a Platelet Concentrate: An *in vitro* Study

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## ABSTRACT

**Introduction:** The major cellular events in the tissue repair are mitogenesis, migration and metabolism. The proteins responsible for coordination of these events are called "growth factors". The activated platelets at the wound margins release several growth factors, such as PDGF, TGF- $\beta$  and EGF, etc., and plasma exudates also provide an important source of TGF- $\beta$  factors.

**Materials and methods:** Periodontal ligament fibroblast obtained from third molar impaction surgery, periodontal ligaments were cultured under standard conditions and spread on 96 well tissue culture plates. Platelet concentrate was obtained after centrifugation of 350-400 ml of blood at 1000 and 5000 rpm. 15  $\mu$ l of platelet concentrate was added to each well. The proliferation rate of test and control group was determined by Redox indicator (Alamar blue<sup>®</sup> assay). The number of cells were counted by neu bar counting chamber after 24, 48 and 72 hours.

**Results:** The proliferation activity of cells was considerably higher in the platelet concentrate group (test group) than the control group. The difference was highly significant upto 72 hours after addition of platelet concentrates (Mann-Whitney U test  $p < 0.001$ ).

**Conclusion:** A cellular effect of the platelet concentrate is clearly discernible. It was concluded that the use of platelet concentrate is an effective modality of regeneration.

**Keywords:** Fibroblast, Growth factors, Platelet concentrate, Proliferation, Regeneration.

## INTRODUCTION

A major goal of periodontal therapy is restoration of the damaged tissues to their original form and function. It requires regeneration of the destroyed periodontal connective tissue through formation of new cementum, new bone, and new attachment of new connective tissue fibers. Periodontal ligament cells have a key function in periodontal regeneration. Among them 'fibroblast' plays a major role. Proliferation of fibroblast could therefore be beneficial for the re-establishment of connective tissue attachment. In recent years, many studies have confirmed that growth factors are capable of aiding periodontal regeneration. The observation showed that growth factors are involved in the formation of new attachment and these polypeptides may have a beneficial effect in inducing periodontal regeneration.

Wound healing is a complex process involving cell migration, cell differentiation and proliferation. Many of these processes are controlled by cytokines and growth factors.<sup>1</sup> Platelet concentrate involves the sequestration and concentration of platelets in plasma.<sup>2</sup> PDGF and TGF- $\beta$  are known to be abundant in the  $\alpha$  granules of platelets (Assoian et al).<sup>3</sup> PDGF has been demonstrated to induce the adhesion of PDLF to the root surface (Gama et al)<sup>4</sup> and TGF- $\beta$  to promote extracellular matrix production (Wrana et al).<sup>5</sup>

Therefore this study, through the use of an *in vitro* model of wound healing, aimed to gain a better understanding about platelet concentrate enhancing wound repair, particularly soft tissue repair by investigating its effect on the proliferation of human periodontal ligament fibroblast cells (HPDLF).

## MATERIALS AND METHODS

The total sample size was 30. It was controlled trial and divided into two groups, the test group (with platelet) and control group (without platelet). Each group contained 15 samples.

### Inclusion Criteria

1. Age between 18 and 30 years.
2. Bony impacted third molar.

### Exclusion Criteria

1. Patients with systemic disease like diabetes mellitus.
2. Patients who have taken medications, such as cytotoxic drugs or are on steroid therapy.
3. Soft tissue impacted third molars.

## Method of Collection of Data

### Cell Culture\*

Periodontal ligament fibroblast (PDLF) cells were obtained during third molar impaction surgery. PDLF cells were maintained under standard conditions. The roots were cut into pieces and transferred to laboratory in phosphate buffer solution (PBS). It was placed on a petridish with 2 ml of Dulbecco's Eagle media supplemented with Penicillin G (100 U/ML), Streptomycin (100 mg/ml), gentamycin (100 mg/ml) and 10% fetal Bovine serum. Incubation was done at 37°C in atmosphere of 5% CO<sub>2</sub>/95% air, in 100% humidity. Medium was changed after every 5 to 6 days.<sup>6</sup>

Once emigrating fibroblast like cells had become confluent around the tissue fragments (4-6 weeks), then medium was removed. The cell layer was washed with phosphate buffer saline. After this, 0.25% trypsin in EDTA buffer was added and incubated for 2 to 4 minutes.

The cells were detached and spread on tissue culture plate. A solution contained  $1 \times 10^4$  cells/ml, the concentration of cells were counted under neu bar counting chamber with adjusting the dilution of Eagle's basal media, 10% FBS and antibiotics. 0.2 ml of the cell solution was added to each well of 96 well tissue culture plates with 15 ml of Alamar blue dye.

### Platelet Concentration Preparation<sup>7</sup>

The platelet concentrate was obtained from 350 to 400 ml of blood that contained 49 ml of citrate phosphate dextrose as an anticoagulant. The glass tube containing blood was centrifuged at 1000 rpm for 10 minutes, which resulted in the separation of three basic fractions. Platelet poor plasma was on top, PRP in the middle and followed by RBC fraction at the bottom. The PRP was collected and again centrifuged at 5000 rpm for 9 minutes, which resulted in the separation of two basic fractions, platelet concentration and plasma. 15 ml of platelet concentrate was added to each well.

The percent yield was calculated as "No. of platelets in platelet Conc./ No. of platelets in the whole blood  $\times$  100."

The proliferation activity was determined by a simple, one step, nonradioactive assay with Alamar blue dye after 72 hours. The number of cells were counted with neu bar counting chamber to compare the proliferation rate of study and control group after 24, 48 and 72 hours.

### Proliferation Assay

Proliferation activity was determined by means of the Alamar blue assay. It is a simple non-radioactive assay used to monitor and determine the proliferation of various cell lines (Ahmed et al 1994).<sup>8</sup> After being taken up by the cells, the reduction-oxidation (Redox) indicator fluorescence and changes color in response to chemical reduction. Reduction related to cellular

growth causes the Redox indicator to change from oxidized (nonfluorescent) to reduced (fluorescent form).

### Reduction Oxidation Potential System

- Alamar blue oxide  $+2M^+ + 2e^- \leftrightarrow$  Alamar blue red + 380 mV (pH7, 25°C).
- $O_2 + 4H^+ + 40e^- \leftrightarrow 2H_2O + 820$  mV (Cytochromes oxide  $+1e^- \leftrightarrow$  Cytochromes red + 290 to + 80 mV).

The indicator is a substitute for molecular oxygen for any of the oxidoreductases reaction. It routinely utilizes molecular oxygen as an electron acceptor.

### Statistical Analysis

The value of each treatment group was presented as the arithmetic mean  $\pm$  standard deviation of the mean (mean  $\pm$  SD).

The comparison between the groups was made by the Mann Whitney (U test).  $p < 0.001$  was considered as highly significant results.

## RESULTS

### Platelet Concentrate

The mean number of platelet cells in samples of whole blood taken from different donors was  $252 \pm 10^6 m^{-1}$ . After centrifugation, the mean number of platelets in the platelet concentrate was  $520 \pm 18 \times 10^6 m^{-1}$  with a two-fold enrichment in platelets. In the experiments, the platelets were concentrated to  $4 \times 10^9 m^{-1}$ .

### Fibroblast Proliferation

In this experiment, proliferation activity in platelet concentration group (test group) was compared to the control group after 24, 48, 72 hours.

Table 1 illustrates that after 24 hours, the fibroblast number in platelet concentration group was  $2.4 \pm 1.4 \times 10^4$  and  $1.1 \pm 0.4 \times 10^4$  in control group, Z value (3.712) (Fig. 1).

After 48 hours, the fibroblast number in platelet concentrate group was  $3.53 \pm 1.5 \times 10^4$  and  $1.5 \pm 1.5 \times 10^4$  in control group, Z value (3.9612) (Fig. 2).

After 72 hours, the fibroblast number in platelet concentrate group was  $4.8 \pm 1.2 \times 10^4$  and  $1.9 \pm 0.9 \times 10^4$  in control group, Z value (4.663) (Fig. 3).

The difference between the samples incubated with platelet concentration (test group) and control group was highly significant ( $p < 0.001$ ) (Graph 1).

In this study, Alamar blue dye was used as Redox indicator for cell proliferation as the color change of the dye indicates

**Table 1:** Comparison between platelet concentration (test group) and control group by Mann Whitney (U test)

| Group   | Base line       | 24 hours      | 48 hours       | 72 hours      |
|---------|-----------------|---------------|----------------|---------------|
| Control | $1 \times 10^4$ | $1.1 \pm 0.4$ | $1.5 \pm 1.5$  | $1.9 \pm 0.9$ |
| Test    | $1 \times 10^4$ | $2.4 \pm 1.4$ | $3.53 \pm 1.5$ | $4.8 \pm 1.2$ |

\*Genetix Asia Pvt. Ltd., New Delhi, India.

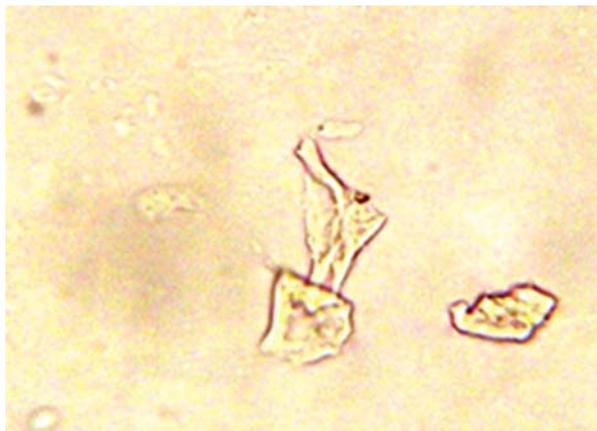
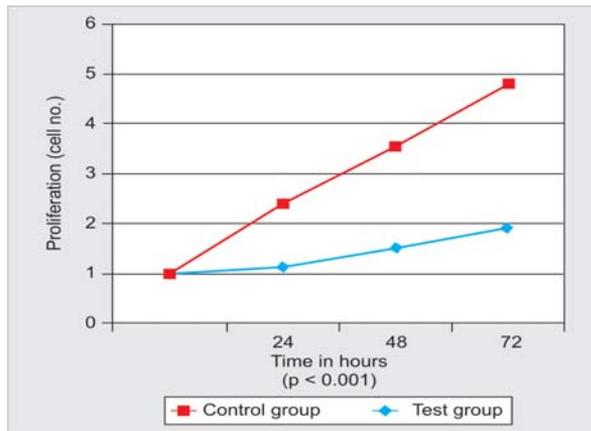


Fig. 1: Cell proliferation after 24 hours



Graph 1: Proliferation rate in test and control group after 24, 48 and 72 hours



Fig. 2: Cell proliferation after 48 hours

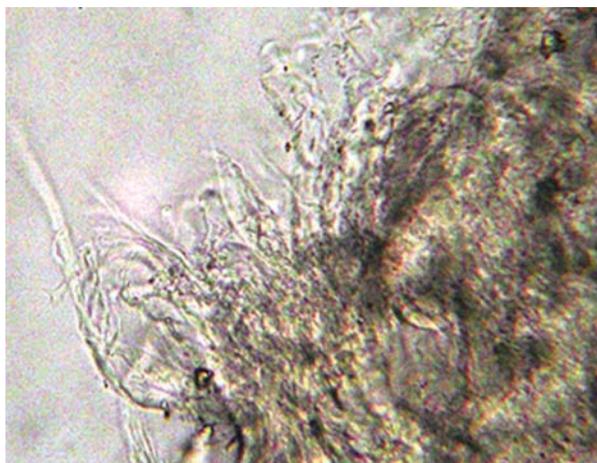


Fig. 3: Cell proliferation after 72 hours

the cell proliferation activity. After 72 hours, the color changed in the test group from red → black, indicating the proliferation activity of the cell.

### DISCUSSION

Recent advances in our understanding of functions and the use of paracrine growth factors to regulate specific components of healing process have provided incidence that these proteins may serve as therapeutic agents in the healing.

Fibroblast cells are key cells involved in the regeneration. These growth factors may interfere with cell surface interaction, which is essential for early cell repopulation and fiber development.<sup>9</sup>

In this study, the number of cells of test group were  $(4.8 \pm 1.2) \times 10^4$  and the control group were  $(1.9 \pm 0.9) \times 10^4$  after 72 hours. In both the groups, number of cells at baseline were  $1 \times 10^4$  per well. Results of the current study essentially suggested that platelet concentrate group has showed higher proliferation of periodontal fibroblast. Platelet concentrate is a sequestrum of growth factors. They are potent mitogen (stimulate of cell proliferation) and chemotactic (cause directed cell migration) protein for PDL fibroblasts.<sup>10</sup> The presence of some deflecting reports of Oates,<sup>11</sup> and Lynch<sup>12-14</sup> also suggested that PDGF plays a major role in human fibroblast repair.

Growth is a fundamental process and a unique characteristic of all living organisms. The most fundamental processes of tissue growth development begin with cell proliferation. Cell growth and division is a prerequisite for regeneration and repair.

PDGF and TGF- $\beta$  are produced by platelets and act on target cells, such as fibroblast and osteoblast.<sup>15,16</sup> They provoke a number of intracellular events that activate cells to repair the connective tissue matrix. Importantly, the rationale for using platelet concentrates is an attempt to regenerate periodontal tissue.<sup>17, 18</sup>

In periodontal wound healing growth factors regulate cell proliferation and differentiation.<sup>19</sup> These growth factors are present in the local wound healing environment.<sup>20,21</sup> PDGF has been demonstrated to induce the adhesion of periodontal ligament fibroblast to the root surface.<sup>4</sup> They promote wound healing and regeneration.

In conclusion, the results of this study indicate that the potential therapeutic use of platelet and its recombinant growth factors showed periodontal regeneration. But still there is a need to obtain more information about effect of its recombinant growth factors on fibroblast proliferation about the carrier and optimal concentration to use these factors for periodontal regeneration.

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