

# *In Vitro* Evaluation of Osteoblast Response to the Effect of Injectable Platelet-rich Fibrin Coating on Titanium Disks

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## ABSTRACT

**Aim:** Biomimetic implant surface coatings can improve osteoblast response and enhance osseointegration. This study aimed to assess the response of osteoblast-like cell line (MG-63) coating of injectable platelet-rich fibrin on titanium discs.

**Materials and methods:** Injectable-PRF (i-PRF) was prepared by centrifugation of blood at 700 rpm for 3 minutes without any anti-coagulant in i-PRF tubes. Ten commercially pure titanium discs were divided into control groups comprising plain discs, and test group, titanium discs were coated with i-PRF. These were then added to the cultured MG-63 cells. Cell proliferation, alkaline phosphatase production, and mineralization were assessed in both groups at day 1, 7, 14, and 21.

**Results:** The cell proliferation, alkaline phosphatase production, and mineralization increased significantly from day 1 to day 21 in both test and control groups and was significantly higher in the test group than in control group at day 1, 7, 14, and 21 ( $p < 0.001$ ).

**Conclusions:** Coating of titanium discs with i-PRF causes increased proliferation, alkaline phosphatase production, and increased mineralization at day 1, 7, 14, and 21 in MG-63 osteoblast-like cells.

**Clinical significance:** Improved osteoblast proliferation and mineralization demonstrate enhanced activity on the surface of an implant, which in turn may lead to increased bone to implant contact and faster/ and/or enhanced osseointegration.

**Keywords:** Bone, Dental implants, Osseointegration, Osteoblasts, Platelet-rich fibrin.

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## INTRODUCTION

Titanium dental implants have become the treatment of choice for rehabilitation of partial or total edentulism. Since their inception, several surface modifications have been proposed to alter the properties to achieve enhanced osteogenesis, optimum bone to implant contact, and eventually to achieve greater and faster osseointegration. These surface modifications include mechanical subtractive or additive surface treatments, treatment with lasers, and biomimetic coatings like hydroxyapatite.<sup>1</sup> Recently, the incorporation of certain drugs like zoledronate, simvastatin, or gentamicin has also been suggested. These modifications aim for implant surface decontamination, inhibition of collagenase activity, and increase in bone to implant contact.<sup>2</sup> The search for an ideal implant surface coating is still continued.

Platelet-rich fibrin is an autologous blood derivative that is obtained by centrifugation of blood without any external additives or anti-coagulant. It concentrates the blood platelets and has found application in several areas of dentistry including periodontal recession coverage, intrabony defects, furcation defects, soft tissue augmentation, periapical defects, and so on. Over the years, three major protocols of preparation of PRF have evolved.<sup>3,4</sup> These include L-PRF (leukocyte and platelet-rich fibrin), A-PRF (advanced platelet-rich fibrin), and i-PRF (injectable platelet-rich fibrin). Of these, L-PRF and A-PRF are obtained as gels whereas i-PRF is a liquid form of PRF which coagulates after its preparation. These platelet concentrates have demonstrated to release several growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF) that provide a jumpstart to the local healing response. This growth factor release occurs gradually for 10 days at the site of implantation.<sup>5</sup>

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In a recent study, the addition of PRF to sockets receiving titanium implants demonstrated a significant enhancement of implant stability in the early healing period.<sup>6</sup> Also, when titanium discs were immersed in liquid platelet concentrate for 10 minutes, they demonstrated a significantly thicker surface fibrin network formation on the surface as compared to non-treated ones.<sup>7</sup> It was hypothesized that this biologic coating may contribute to enhanced cell migration and differentiation on the surface of the implant. Also, it has been demonstrated that i-PRF induced significantly higher cell migration, differentiation, and release of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF) on Val fibroblasts.<sup>8</sup> These studies reinforce the positive biologic influence, i-PRF coating can play, on the decisive biologic events taking place in the immediate vicinity of implant after its placement.

Hence, this study aimed to assess the effect of i-PRF coating on titanium surface on the cell proliferation, alkaline phosphatase production, and mineral deposition of MG-63 osteoblastic-like cell line.

## MATERIALS AND METHODS

This *in vitro* study was approved by the institutional review board and ethical committee (Ref no. BDC/exam/747). Ten commercially pure titanium discs of diameter 10 mm and thickness 1.5 mm were used for the study (Bhagyashali metals, Mumbai, India). Discs were autoclaved prior to the analysis. All the tests were performed in triplicates. These discs were divided into two groups; group 1 control (untreated titanium discs,  $n = 5$ ) and group 2 test (titanium discs coated with i-PRF,  $n = 5$ ). MG-63 osteoblastic cell line was obtained from a commercially available source (National Centre of Cell Services, Pune, India).

### Preparation and Coating of I-PRF

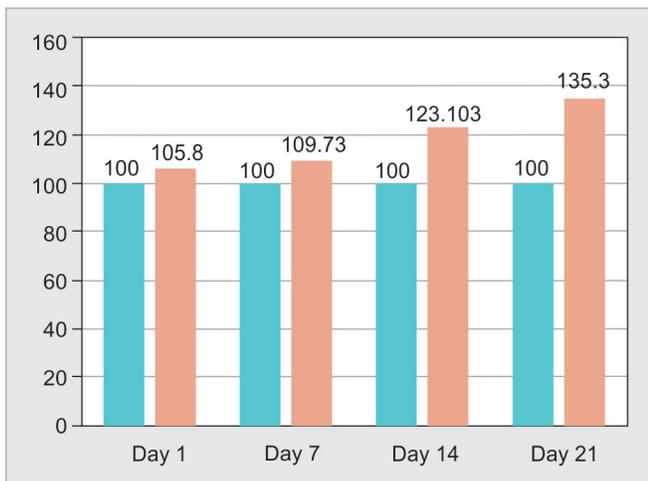
From systemically healthy male volunteers in the age range of 18–25 years, 9 mL of blood was collected in i-PRF tubes (IntraSpin, Intra-Lock International, Boca Raton, FL, USA). This was followed by immediate centrifugation at 700 rpm for 3 minutes (IntraSpin, Intra-Lock International, Boca Raton, FL, USA).<sup>8</sup> This led to separation of blood into orange-colored i-PRF concentrate at the top and RBCs at the bottom. The orange i-PRF concentrate was immediately aspirated in a syringe. In group I titanium discs were not subjected to any treatment but in group II they were immersed in i-PRF.

### Cell Culture

Human osteoblast-like cell line MG-63 (human osteosarcoma cell line) was procured from NCCS, Pune. The cells were seeded at a density of approximately  $1 \times 10^5$  cells/well in a 24-well flat-bottom microplate consisting of Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum and maintained at 37 °C in 95% humidity and 5% CO<sub>2</sub> for overnight. Group I (titanium discs only) and group II (titanium discs coated with i-PRF) were added into wells containing cells. The cells were incubated for a further 3 weeks and assessed at day 1, 7, 14, and 21 (endpoints) and the medium was replaced every 2–3 days coinciding with the endpoints.<sup>9</sup>

### Cell Proliferation

Cell proliferation was assessed using a commercially available CCK-8 kit (cell counting kit). At each endpoint, the cells in culture well were washed twice with phosphate buffer solution, and 25  $\mu$ L of the CCK-8 (cell counting kit) solution was added to each well,



**Fig. 1:** Comparison of cell proliferation between test and control group at day 1, 7, 14, and 21.

and the plate was incubated at 37 °C. After 4 hours of incubation, absorbance was recorded at 450 nm using a microplate reader.<sup>1,2</sup>

### Alkaline Phosphatase Activity

The alkaline phosphatase activity was assessed by collecting the supernatant using an alkaline phosphatase microplate test kit. A total of 20  $\mu$ L of collected supernatant from each test group and 960  $\mu$ L of assay buffer and 20  $\mu$ L substrate solutions were added. Following this, absorbance was recorded with 405 nm using a microplate reader as per manufacturer instructions. The following formula was used to calculate the values:

Alkaline phosphatase activity (units/mL) = mean OD of test compound/mean OD of negative control  $\times$  DF  $\times$  TV/18.5  $\times$  EV,

where, DF is the dilution factor, TV is the total volume of the assay (mL), EV is the volume of sample used, and 18.5 is the millimolar extinction coefficient of pNPP at 405 nm.

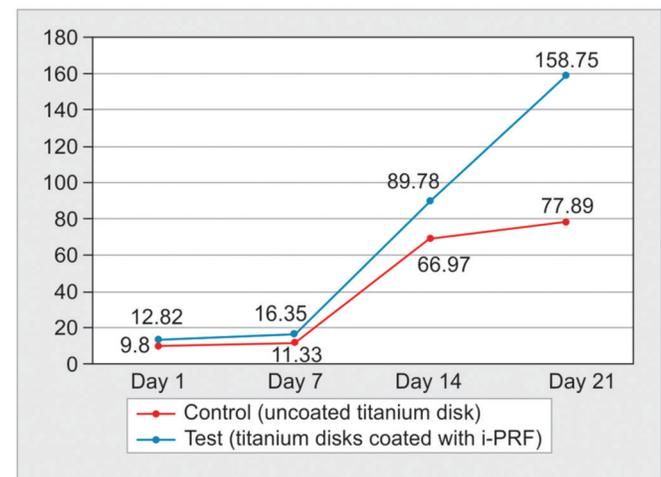
### Alizarin Red S Staining

At the end of 21 days, the culture was treated using DMEM media (concentration 400  $\mu$ g/mL) and incubated for 48 hours. After the incubation period, the media was removed and cells were washed and fixed with 95% ethanol for 15 minutes at 4°C. The cells were then stained with 2% alizarin red S (pH 4.1–4.3) for 15 minutes. The formation of red staining was observed and it was solubilized with 300  $\mu$ L of 33% glacial acetic acid solution. Following this, the absorbance was measured by a spectrophotometer at 415 nm.<sup>10</sup>

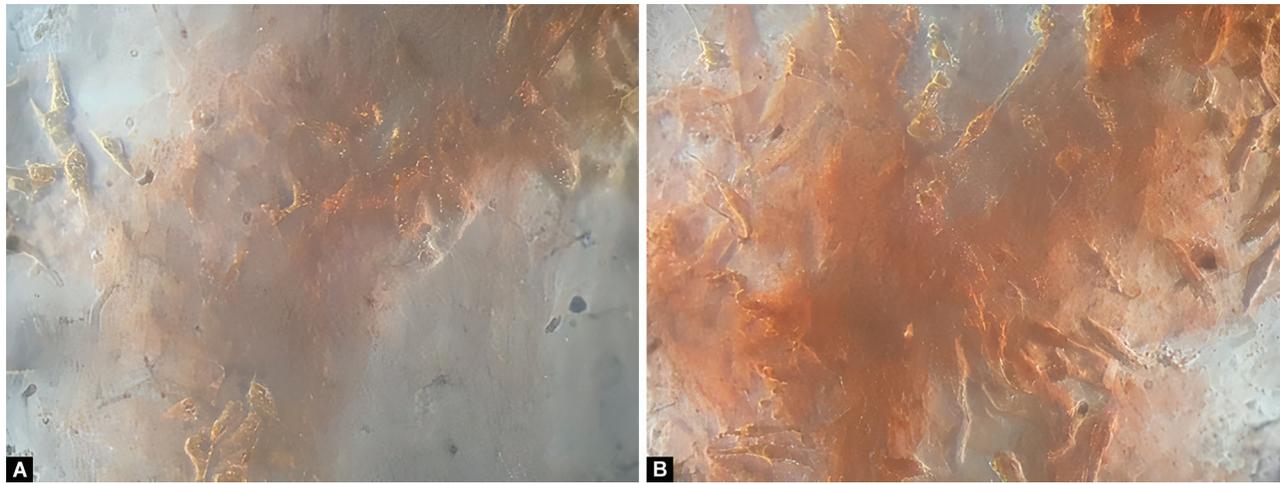
## RESULTS

All the values were entered in a pro forma and subject to statistical analysis using ANOVA. The cell proliferation as assessed by the CCK-8 assay increased significantly from day 1 to day 21 in both test and control groups. The proliferation of MG-63 cells in the test group was significantly higher than in the control group at day 1, 7, 14, and 21 ( $p < 0.001$ , Fig. 1).

The levels of alkaline phosphatase increased significantly from day 1 to day 21 in both test and control groups. However, the levels of alkaline phosphatase were significantly higher in the test group as compared to control group at the end of day 1, 7, 14, and 21 ( $p < 0.001$ , Fig. 2). Also, at the end of 21 days, the test group demonstrated significantly higher mineral deposition as assessed with alizarin red assay as compared to the control group ( $p < 0.001$ , Figs. 3A and B).



**Fig. 2:** Comparison of alkaline phosphatase levels between test and control group at day 1, 7, 14, and 21



Figs 3A and B: Alizarin red staining in control (A) vs test group (B) at day 21

## DISCUSSION

The long-term success of dental implants depends largely on successful osseointegration. One of the major prerequisites for osseointegration is early osteoblast differentiation and colonization on the surface of implants. An implant coating that can achieve this, would contribute to early osseointegration and in turn shorten the treatment time and reduce the risk of implant failure.<sup>11</sup>

Injectable platelet-rich fibrin provides a liquid platelet concentrate which releases a plethora of growth factors including BMP-2, VEGF, and TGF- $\beta$ .<sup>12</sup> Since i-PRF is obtained in a liquid form, it can be coated on to the surface of the implant before it coagulates. In a study by Lollobrigida et al., it has been demonstrated that contact with the liquid form of i-PRF results in the formation of a stable fibrin layer containing platelets and leukocytes on the surface of the implants.<sup>7</sup> As opposed to L-PRF and A-PRF, in which the fibrin polymerization is completed within the PRF tubes itself, this liquid form gives operator the control of the process where the fibrin formation occurs directly on the surface of implant. This allows the i-PRF to form a sort of biologic coating on the implant surface that bioactivates the otherwise biologically inert implant surface.<sup>13</sup> Injectable PRF is the most recent advance in PRF technology and the *in vitro* or *in vivo* efficacy have been studied by very few.<sup>12-16</sup>

In our study, we observed that i-PRF coating on titanium disc resulted in significantly higher cell proliferation and alkaline phosphatase production at day 1, 7, 14, and 21 in MG-63 osteoblast-like cell line. For cell culture analysis, MG-63 cells were used. This cell line exhibits typical features of undifferentiated osteoblast phenotype with relative stability, and is easily maintained over multiple passages in culture, provides several biological repeats and reproducibility of results, and is valuable in *in vitro* research.<sup>17</sup> Also, at the end of 21 days, discs coated with i-PRF demonstrated a significantly higher deposit of calcium as observed by alizarin red S staining than uncoated discs. These results demonstrate that i-PRF coating on titanium surface provides a biological advantage at a cellular level by promoting cell proliferation and activity. It can be hypothesized that this may clinically translate to enhanced osteoblast activity on the surface of an implant, which in turn

may lead to increased bone to implant contact and faster/ and/ or enhanced osseointegration.

A study by Wang et al. on the effect of i-PRF vs PRP on human osteoblasts observed that i-PRF induced a significantly higher cell proliferation at day 3 and day 5. Also, significantly higher alkaline phosphatase levels were observed at the end of 7 days and alizarin red staining at 14 days. The mRNA levels of alkaline phosphatase, Runx2, and osteocalcin all demonstrated higher levels with i-PRF.<sup>15</sup> The results obtained in the study by Wang et al. are in agreement with the results observed in our study that i-PRF exerts a positive cellular influence on osteoblast activity. Such an effect on osteoblasts may assume greater importance in cases of osteoporosis, those with diabetes, smokers, or other scenarios with impaired wound healing.

Also, in a recent study, it has been demonstrated that i-PRF has potent antibacterial activity against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.<sup>16</sup> *P. gingivalis* is one of the most common organisms isolated from peri-implantitis lesions.<sup>18</sup> Hence, it can also be hypothesized that this biologic coating with i-PRF not only has osteo-promotive activity but may also possess antibacterial activity. Such antibacterial coatings have long been researched in orthopedic implantology with potential implications for inhibition of early implant colonization by bacteria, inhibition of complex biofilm formation, and subsequent implant failure owing to periimplant infection.<sup>2</sup>

The results obtained from our study demonstrate a beneficial effect of i-PRF coating on titanium implant surface. One of the major advantages of this method of implant coating is that it is simple and rapid. Unlike other surface modifications, coating with i-PRF is a completely autogenous, biological chairside coating that provides an osteo-promotive and possible anti-bacterial effect. The fact that any conventional implant can be modified in this way chairside by the operator may result in greater clinical and patient acceptability without raising the cost of the procedure significantly. Also, in cases where the implant outcome is expected to be compromised, such as those with diabetes mellitus, osteoporosis, previously infected sites this approach may be beneficial. The limitation of our study is that it is an *in vitro* study and hence, further long-term clinical studies with a good sample size are required to validate the results obtained in this study. Also, negative control

without any discs was not performed. Studies should also focus to assess whether the cellular advantage of i-PRF can provide clinically superior outcomes for implant placement including improvement in primary and secondary implant stability and the possibility for early loading.

## CONCLUSION

Hence, within the limitations of this study, it can be concluded that coating of titanium discs with i-PRF causes increased proliferation, alkaline phosphatase production, and increased mineralization at day 1, 7, 14, and 21 in MG-63 osteoblast-like cells. Coating of the implant surface with i-PRF may provide early and improved osseointegration for implants.

## REFERENCES

- Jemat A, Ghazali MJ, Razali M, et al. Surface modifications and their effects on titanium dental implants. *Biomed Res Int*. 2015;2015:791725. DOI: 10.1155/2015/791725.
- Romanò CL, Scarponi S, Gallazzi E, et al. Antibacterial coating of implants in orthopaedics and trauma: a classification proposal in an evolving panorama. *J Orthop Surg Res* 2015;10:157. DOI: 10.1186/s13018-015-0294-5.
- Borie E, Oliví DG, Orsi IA, et al. Platelet-rich fibrin application in dentistry: a literature review. *Int J Clin Exp Med* 2015;8(5):7922–7929.
- Shah R, Triveni MG, Thomas R, et al. An update on the protocols and biologic actions of platelet rich fibrin in dentistry. *Eur J Prosthodont Restor Dent* 2017;25(2):64–72. DOI: 10.1922/EJPRD\_01690Shah09.
- Bansal S, Garg A, Khurana R, et al. Platelet-rich fibrin or platelet-rich plasma—which one is better? An opinion. *Indian J Dent Sci* 2017;9(5):49–52. DOI: 10.4103/IJDS.IJDS\_55\_17.
- Öncü E, Alaaddinoğlu EE. The effect of platelet-rich fibrin on implant stability. *Int J Oral Maxillofac Implants* 2015;30(3):578–582. DOI: 10.11607/jomi.3897.
- Lollobrigida M, Maritato M, Bozzuto G, et al. Biomimetic implant surface functionalization with liquid L-PRF products: in vitro study. *Biomed Res Int* 2018;2018:9031435. DOI: 10.1155/2018/9031435.
- Wang X, Zhang Y, Choukroun J, et al. Behaviour of gingival fibroblasts on titanium implant surfaces in combination with either injectable-PRF or PRP. *Int J Mol Sci* 2017;18(2):331. DOI: 10.3390/ijms18020331.
- Jeon C, Oh KC, Park KH, et al. Effects of ultraviolet treatment and alendronate immersion on osteoblast-like cells and human gingival fibroblasts cultured on titanium surfaces. *Sci Rep* 2019;9(1):2581. DOI: 10.1038/s41598-019-39355-3.
- Park SJ, Bae SB, Kim SK, et al. Effect of implant surface microtopography by hydroxyapatite grit-blasting on adhesion, proliferation, and differentiation of osteoblast-like cell line, MG-63. *J Korean Assoc Oral Maxillofac Surg* 2011;37(3):214. DOI: 10.5125/jkaoms.2011.37.3.214.
- Ma T, Ge X, Zhang Y, et al. Effect of titanium surface modifications of dental implants on rapid osseointegration. In: Sasaki K, Suzuki O, Takahashi N, eds. *Interface Oral Health Science* 2016. Singapore: Springer; 2017. DOI: 10.1007/978-981-10-1560-1\_20.
- Varela HA, Souza JCM, Nascimento RM, et al. Injectable platelet rich fibrin: cell content, morphological, and protein characterization. *Clin Oral Investig* 2019;23(3):1309–1318. DOI: 10.1007/s00784-018-2555-2.
- Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? *Clin Oral Investig* 2017;21(8):2619–2627. DOI: 10.1007/s00784-017-2063-9.
- Abd El Raouf M, Wang X, Miusi S, et al. Injectable-platelet rich fibrin using the low speed centrifugation concept improves cartilage regeneration when compared to platelet-rich plasma. *Platelets* 2019;30(2):213–221. DOI: 10.1080/09537104.2017.1401058.
- Wang X, Zhang Y, Choukroun J, et al. Effects of an injectable platelet-rich fibrin on osteoblast behavior and bone tissue formation in comparison to platelet-rich plasma. *Platelets* 2018;29(1):48–55. DOI: 10.1080/09537104.2017.1293807.
- Kour P, Pudukalkatti PS, Vas AM, et al. Comparative evaluation of antimicrobial efficacy of platelet-rich plasma, platelet-rich fibrin, and injectable platelet-rich fibrin on the standard strains of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. *Contemp Clin Dent* 2018;9(Suppl 2):S325–S330. DOI: 10.4103/ccd.ccd\_367\_18.
- Staehele S, Rebl H, Nebe B. Phenotypic stability of the human MG-63 osteoblastic cell line at different passages. *Cell Biol Int* 2019;43(1):22–32. DOI: 10.1002/cbin.11073.
- Pokrowiecki R, Mielczarek A, Zaręba T, et al. Oral microbiome and peri-implant diseases: where are we now? *Ther Clin Risk Manag* 2017;13:1529–1542. DOI: 10.2147/TCRM.S139795.