

Comparing the release of growth factors when partially demineralized tooth matrix is mixed with platelet rich fibrin and injectable platelet rich fibrin: An *in-vitro* observational study

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Abstract

Context: Platelet concentrates are rich in growth factors that assist in regenerative medicine to promote healing and tissue regeneration. Similarly, partially demineralized tooth is a storehouse of many growth factors, assisting in bone regeneration. Hence, the present study aimed to quantify the release of growth factors from different platelet concentrates individually and when mixed with a partially demineralized tooth matrix.

Method: Human growth factors such as bFGF, EGF, PDGF-AB, TGF-beta-1, and VEGF-A present in platelet-rich fibrin and injectable platelet-rich fibrin from young and old male and female donors were quantified separately. Then these platelet concentrates were then mixed with a partially demineralized tooth matrix, which was powdered using a Smart Dentin Grinder. An enzyme-linked immunosorbent assay was used for the quantification of growth factors.

Results: The release of growth factors, such as platelet-rich fibrin and injectable platelet-rich fibrin, was not statistically significant; however, it was significantly greater when i-PRF was mixed with a partially demineralized tooth matrix.

Conclusion: The study revealed that the expression of growth factors was significantly greater when a partially demineralized tooth matrix was mixed with injectable platelet-rich fibrin than when combined with platelet-rich fibrin.

Keywords: Growth factors, I-PRF, partially demineralized tooth matrix, platelet concentrates, platelet rich fibrin

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INTRODUCTION

Angiogenesis, the process of new capillary formation from the existing vasculature and “neovascularization,” plays very important role in wound healing and tissue regeneration along with polypeptide growth factors.^[1] These growth factors are biological mediators that regulate the proliferation, differentiation, and formation of the extracellular matrix,^[2] thereby acting as scaffolds along with autologous cells. In recent decades, blood-based biomaterials such as platelet-rich plasma (PRP), platelet-rich fibrin (PRF), fibrin sealant, platelet fibrin glue, and platelet gel have been extensively researched in regenerative medicine.^[3] PRP was the first-generation platelet concentrate studied, mainly in periodontology for wound healing and bone formation; currently, second-generation PRF is used extensively, as it enhances healing and regeneration. The newer generation of platelet concentrates releases sustainable growth factors for longer durations, which may be attributed to variations in centrifugation techniques, including rotations per minute (rpm), time of exposure, and pressure applied.^[4]

Platelet concentrates contain many growth factors, such as platelet-derived growth factor (PDGF), transforming growth factors beta 1 and 2 (TGF- β 1 and TGF- β 2), insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and human vascular endothelial growth factor (VEGF).^[5] The use of PRP is limited due to presence of anticoagulants, which may affect the release of growth factors and healing. Therefore, Choukroun’s platelet-rich fibrin membrane (PRF), which has no anticoagulants,^[6] was more promising for its osteoblastic potential because of the increased release of growth factors; thus, used to treat gingival recession, socket preservation, and wound closure.^[7-9]

These growth factors have different functions: PDGF regulates the migration, proliferation, and survival of mesenchymal cells; TGF- β assists in tissue repair and activates the synthesis of macrophages and endothelial cells; and VEGF assists in angiogenesis. The process of wound healing is multifactorial, and these platelet concentrates enhance healing by acting as a scaffold rich in leukocytes.^[10] Although PRP contains 6–8-fold more blood-derived growth factors, Choukroun’s PRF membrane has shown sustained and prolonged release of growth factors due to its complex three-dimensional fibrin network. Injectable platelet-rich fibrin (i-PRF) obtained by low centrifugation speed and relative centrifugal force (RCF) is currently used in treating periodontal defects; however, more research

needs to be done to understand its benefits and risk in comparison with other platelet concentrates.^[11]

Studies have shown that by decreasing the relative centrifugal force (RCF), centrifugation speed, and time, the resulting PRF increases the release of leukocytes, platelets and growth factors, but its clinical relevance needs to be further researched, especially in the extracellular matrix.^[12-15] In the past decade, the use of demineralized teeth as a graft material has been studied extensively, as many growth factors are present in demineralized teeth, and, their composition is similar to that of alveolar bone.^[16]

Hence, the present study was undertaken to estimate and compare human growth factors bFGF, EGF, PDGF-AB, TGF-beta1, and VEGF-A expressed in PRF and i-PRF individually and, when combined with a partially demineralized tooth matrix (PDTM), on the basis of the age and sex of the donor. The null hypothesis states that there is no difference in the expression of growth factors from PRF or i-PRF individually or when PDTM is combined with both PRF and i-PRF, irrespective of age or sex.

METHODOLOGY

The *in vitro* observational study was conducted in Sterlix Laboratory, Peenya, Bangalore for a period of 1 month, and a total of 12 extracted teeth (3 young males and 3 young females and 3 each old males and females) were used in the study. The blood samples for preparation of the platelet concentrates were procured from 12 volunteers, of which 6 were aged between 40 and 50 years (3 males, 3 females) and 6 were aged 18 to 25 years (3 males, 3 females). The inclusion criterion for young patients was teeth extracted for orthodontic purposes only, and for older patients, extraction indicated for periodontal issues only. The exclusion criterion was tooth extracted due to trauma, root canal treatment, or caries. Blood drawn from healthy individuals with no underlying medical conditions was included in the study, and volunteers who were alcoholic, smokers, pregnant, or lactating were excluded from the study. Institutional ethical approval was obtained for the study (Ref: UECHT/2016-18/PhD_16DSDP732002), the results were tabulated, and statistical analysis was performed via an independent sample “*t*” test.

a. Preparation of PRF and i-PRF: PRF and i-PRF were prepared via a standard protocol of drawing 10 ml of blood for each preparation into sterile vials without anticoagulant from 12 volunteers (6 males, 6 females). The volunteers were further divided into 4 groups: 3

young male volunteers and 3 young female volunteers and old male and female volunteers aged 40–55 years. A total of 24 vials of blood were collected, 12 each for the PRF and i-PRF formulations. The blood was centrifuged (REMI; R-4C) at 3300 rpm for 10 minutes for PRF and at 700 rpm for 3 minutes for i-PRF. The middle fibrin layer from the PRF vials and the liquid from the i-PRF vials were carefully collected for the quantification of growth factors.

- b. Preparation of Partially Demineralized Tooth Matrix Powder:** The PDTM was prepared by using teeth extracted from each group. The teeth were cleaned, washed under running water, dried, and powdered using a Smart Dentin Grinder. This was followed by partial demineralization for 10 minutes with 0.6 M hydrochloric acid, and washed with phosphate-buffered saline (PBS, 1 mg/ml) and dried.
- c. ELISA:** The levels of growth factors expressed in PRF, i-PRF, and when both platelet concentrates were combined with PDTM were assessed via a 96-well plate ELISA Kit (Ray Biotech, Suite 100 Norcross, GA). The standards and samples were prepared according to the manufacturer's instructions and pipetted into the wells containing the target and, the growth factors in the samples bounded in the wells by the immobilized antibody. The wells were then washed, and biotinylated anti-human antibody was added. The unbound biotinylated antibody was washed away, and horseradish peroxidase (HRP)-conjugated streptavidin was pipetted into the wells. The wells were again washed, and TMB (3,3',5,5', tetramethylbenzidine) substrate solution was added, which developed color in proportion to the amount of growth factor. A stop solution of 0.16 M sulfuric acid was added to the wells, which changed the color of the bonded substrate from blue to yellow, and the intensity of the color obtained was measured. The unknown concentrations of all growth factors present in the samples were evaluated. The ratio of the mixture of platelet concentrates with PDTM was 1 mg/ml (1:1 ratio). The data were analyzed for growth factors present PRF and i-PRF samples and when they were combined separately with PDTM. The enzyme activity was measured via a spectrophotometer at a specific wavelength of 450 nm. The study was performed and triplicate for accuracy.

Statistical analysis

Independent *t* test was done to compare the difference between the means of two groups to determine the difference from each other.

RESULTS

The independent samples *t* test revealed statistical significance in VEGF ($P < 0.033$) and TGF beta ($P < 0.001$) in the i-PRF group YM and for VEGF ($P < 0.001$) and bFGF ($P < 0.002$) in the PRF group of the OM samples. In YF, TGF beta ($P < 0.003$) and PDGF-AB ($P < 0.001$) was significantly higher in the PRF groups, and in OF, VEGF ($P < 0.001$), TGF beta ($P < 0.001$), and PDGF-AB ($P < 0.001$) were significantly higher in the i-PRF groups [Table 1].

The expression of growth factors associated with the combination of PDTM and i-PRF samples revealed that bFGF among YF ($P < 0.009$) and OF ($P < 0.028$) was significant, and VEGF expression was highest in all groups, YM, OM YF, and OF ($P < 0.014$), $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively. EGF and TGF beta expression was also similar to VEGF expression when PDTM was combined with i-PRF. EGF and TGF beta were significantly greater ($P < 0.001$), ($P < 0.015$), and ($P < 0.041$) ($P < 0.001$) in the YM, OM, and OF groups i-PRF mixed with PDTM group. PDGF-AB also had similar effects on the PDTM samples of YM, OM, YF, and OF mixed with i-PRF ($P < 0.007$), ($P < 0.002$), ($P < 0.002$), and ($P < 0.007$), respectively [Table 2].

Independent sample *t* tests revealed significantly higher levels of all growth factors when PDTM was combined with i-PRF than when it was combined with PRF.

The observed concentrations of bFGF in PRF alone were 0.4 pg/ml, 0.31 pg/ml, 0.29 pg/ml, and 0.34 pg/ml in young and old male and female (YM, OM, YF, and OF) samples and 0.28 pg/ml, 0.3 pg/ml, 0.28 pg/ml, and 0.3 pg/ml when combined with PDTM, respectively. There was little variation in the bFGF content between PRF and PRF mixed with PDTM [Table 3; Figures 1 and 1.1].

EGF in the PRF group alone was 1.22 pg/ml, 0.59 pg/ml, 0.89 pg/ml, and 0.71 pg/ml, and in combination with PDTM, it was 1.07 pg/ml, 0.93 pg/ml, 0.84 pg/ml, and 0.95 pg/ml in the YM, OM, YF, and OF samples, respectively. EGF expression was high when PDTM was combined with PRF in the OM and OF samples [Table 3; Figures 1 and 1.1].

The PDGF-AB concentrations in the PRF as well as the PRF mixed with PDTM were 4.73 pg/ml, 1.67 pg/ml, 3.62 pg/ml, and 1.67 pg/ml and 6.39 pg/ml, 3.17 pg/ml, 2.26 pg/ml, and 2.52 pg/ml in the YM, OM, YF, and OF samples, respectively, with a slight increase in the

Table 1: Comparison of growth factor expression between the i-PRF and PRF groups among young males (YMs), old males (OMs), young females (YFs), and old females (OFs)

| | Group | N | Mean | SD | T | P |
|----------|----------|---|------|------|---------|--------|
| b-FGF | YM-i-PRF | 3 | 0.22 | 0.01 | -0.472 | 0.661 |
| | YM-PRF | 3 | 0.23 | 0.02 | | |
| VEGF | YM-i-PRF | 3 | 0.65 | 0.02 | 3.195 | 0.033* |
| | YM-PRF | 3 | 0.58 | 0.03 | | |
| EGF | YM-i-PRF | 3 | 0.41 | 0.01 | -1.909 | 0.129 |
| | YM-PRF | 3 | 0.42 | 0.01 | | |
| TGF Beta | YM-i-PRF | 3 | 0.38 | 0.01 | 23.992 | 0.001* |
| | YM-PRF | 3 | 0.24 | 0.01 | | |
| PDGF-AB | YM-i-PRF | 3 | 0.99 | 0.02 | 2.345 | 0.079 |
| | YM-PRF | 3 | 0.86 | 0.09 | | |
| b-FGF | OM-i-PRF | 3 | 0.14 | 0.01 | -7.238 | 0.002* |
| | OM-PRF | 3 | 0.2 | 0.01 | | |
| VEGF | OM-i-PRF | 3 | 0.34 | 0.01 | -8.615 | 0.001* |
| | OM-PRF | 3 | 0.42 | 0.01 | | |
| EGF | OM-i-PRF | 3 | 0.28 | 0.05 | 0.479 | 0.657 |
| | OM-PRF | 3 | 0.27 | 0.01 | | |
| TGF Beta | OM-i-PRF | 3 | 0.27 | 0.01 | -1.851 | 0.138 |
| | OM-PRF | 3 | 0.3 | 0.02 | | |
| PDGF-AB | OM-i-PRF | 3 | 0.59 | 0.15 | 0.189 | 0.859 |
| | OM-PRF | 3 | 0.58 | 0.04 | | |
| b-FGF | YF-i-PRF | 3 | 0.17 | 0.01 | -1.502 | 0.207 |
| | YF-PRF | 3 | 0.19 | 0.03 | | |
| VEGF | YF-i-PRF | 3 | 0.55 | 0.01 | -1.488 | 0.211 |
| | YF-PRF | 3 | 0.57 | 0.03 | | |
| EGF | YF-i-PRF | 3 | 0.32 | 0.02 | -1.917 | 0.128 |
| | YF-PRF | 3 | 0.34 | 0.01 | | |
| TGF Beta | YF-i-PRF | 3 | 0.23 | 0.02 | -6.404 | 0.003* |
| | YF-PRF | 3 | 0.32 | 0.01 | | |
| PDGF-AB | YF-i-PRF | 3 | 0.43 | 0.03 | -20.744 | 0.001* |
| | YF-PRF | 3 | 0.8 | 0.01 | | |
| b-FGF | OF-i-PRF | 3 | 0.22 | 0.02 | 0.804 | 0.467 |
| | OF-PRF | 3 | 0.21 | 0 | | |
| VEGF | OF-i-PRF | 3 | 0.64 | 0.01 | 8.326 | 0.001* |
| | OF-PRF | 3 | 0.45 | 0.04 | | |
| EGF | OF-i-PRF | 3 | 0.35 | 0.02 | 2.252 | 0.087 |
| | OF-PRF | 3 | 0.3 | 0.04 | | |
| TGF Beta | OF-i-PRF | 3 | 0.41 | 0.01 | 13.535 | 0.001* |
| | OF-PRF | 3 | 0.31 | 0.01 | | |
| PDGF-AB | OF-i-PRF | 3 | 0.94 | 0.03 | 7.886 | 0.001* |
| | OF-PRF | 3 | 0.58 | 0.08 | | |

*Statistical significance set at 0.05; N: Number of samples; and SD: Standard deviation

concentration of the PRF combined with PDTM [Table 3; Figures 1 and 1.1].

The levels of TGF-beta recorded in the PRF and PRF combined with PDTM were 1.27 pg/ml, 1.55 pg/ml, 1.66 pg/ml, and 1.6 pg/ml and 1.79 pg/ml, 1.45 pg/ml, 1.19 pg/ml, and 1.77 pg/ml, respectively, in the YM, OM, YF, and OF samples. Increased levels of TGF-beta were observed in the combination samples from the YM and OF groups [Table 3; Figures 1 and 1.1].

The VEGF concentrations in the PRF and PDTM groups were 1.45 pg/ml, 0.77 pg/ml, and 1.37 pg/ml; 0.86 pg/ml; 2.07 pg/ml; 1.42 pg/ml; 1.18 pg/ml; and 1.41 pg/ml; and the YM, OM, YF, and OF samples, respectively. Increased levels of VEGF were observed in the combination samples,

Table 2: Comparison of growth factor i-PRF and PRF groups among young males (YM), old males (OM), young females (YF), and old females (OF) when combined with a partially demineralized tooth matrix (PDTM)

| Growth factors | Groups | N | Mean | SD | T | P |
|----------------|---------------|---|------|------|--------|--------|
| b-FGF | YM-iPRF+PDTM | 3 | 0.26 | 0.04 | 2.417 | 0.073 |
| | YM-PRF+PDTM | 3 | 0.19 | 0.03 | | |
| | OM-i-PRF+PDTM | 3 | 0.21 | 0.01 | 1.076 | 0.342 |
| | OM-PRF+PDTM | 3 | 0.2 | 0.02 | | |
| VEGF | YF-i-PRF+PDTM | 3 | 0.23 | 0.01 | 4.711 | 0.009* |
| | YF-PRF+PDTM | 3 | 0.19 | 0.01 | | |
| | OF-i-PRF+PDTM | 3 | 0.27 | 0.01 | 3.375 | 0.028* |
| | OF-PRF+PDTM | 3 | 0.2 | 0.04 | | |
| | YM-iPRF+PDTM | 3 | 0.78 | 0.02 | 4.193 | 0.014* |
| | YM-PRF+PDTM | 3 | 0.68 | 0.03 | | |
| | OM-iPRF+PDTM | 3 | 0.77 | 0.01 | 11.538 | <.001* |
| | OM-PRF+PDTM | 3 | 0.58 | 0.02 | | |
| EGF | YF-iPRF+PDTM | 3 | 0.67 | 0.02 | 8.054 | 0.001* |
| | YF-PRF+PDTM | 3 | 0.53 | 0.02 | | |
| | OF-iPRF+PDTM | 3 | 0.78 | 0.02 | 8.004 | 0.001* |
| | OF-PRF+PDTM | 3 | 0.58 | 0.03 | | |
| | YM-iPRF+PDTM | 3 | 0.46 | 0.01 | 5.954 | 0.004* |
| | YM-PRF+PDTM | 3 | 0.39 | 0.02 | | |
| | OM-iPRF+PDTM | 3 | 0.43 | 0.02 | 5.789 | 0.004* |
| | OM-PRF+PDTM | 3 | 0.36 | 0.01 | | |
| TGF-β | YF-iPRF+PDTM | 3 | 0.41 | 0.02 | 5.747 | 0.005* |
| | YF-PRF+PDTM | 3 | 0.33 | 0.02 | | |
| | OF-iPRF+PDTM | 3 | 0.44 | 0.01 | 4.466 | 0.011* |
| | OF-PRF+PDTM | 3 | 0.36 | 0.03 | | |
| | YM-iPRF+PDTM | 3 | 0.45 | 0.01 | 10.215 | <.001* |
| | YM-PRF+PDTM | 3 | 0.34 | 0.01 | | |
| | OM-iPRF+PDTM | 3 | 0.35 | 0.03 | 4.125 | 0.015* |
| | OM-PRF+PDTM | 3 | 0.28 | 0.01 | | |
| PDGF-AB | YF-iPRF+PDTM | 3 | 0.28 | 0.01 | 2.962 | 0.041* |
| | YF-PRF+PDTM | 3 | 0.23 | 0.02 | | |
| | OF-iPRF+PDTM | 3 | 0.47 | 0.01 | 7.811 | 0.001* |
| | OF-PRF+PDTM | 3 | 0.34 | 0.03 | | |
| | YM-iPRF+PDTM | 3 | 1.05 | 0.03 | 5.179 | 0.007* |
| | YM-PRF+PDTM | 3 | 0.91 | 0.03 | | |
| | OM-iPRF+PDTM | 3 | 0.95 | 0.04 | 7.67 | 0.002* |
| | OM-PRF+PDTM | 3 | 0.76 | 0.02 | | |
| | YF-iPRF+PDTM | 3 | 0.85 | 0.03 | 7.217 | 0.002* |
| | YF-PRF+PDTM | 3 | 0.67 | 0.03 | | |
| | OF-iPRF+PDTM | 3 | 0.96 | 0.04 | 5.192 | 0.007* |
| | OF-PRF+PDTM | 3 | 0.7 | 0.08 | | |

*Statistical significance set at 0.05; N: Number of samples; and SD: Standard deviation

with the exception of the YF samples [Table 3; Figures 1 and 1.1]. In the i-PRF group and the i-PRF with PDTM group, the growth factor levels were relatively greater in the i-PRF mixed with PDTM group.

The observed concentrations of bFGF in i-PRF were 0.39 pg/ml, 0.12 pg/ml, 0.2 pg/ml, and 0.38 pg/ml, and those in combination with PDTM were 0.52 pg/ml, 0.36 pg/ml, 0.41 pg/ml, and 0.56 pg/ml for the YM, OM, YF, and OF samples, respectively [Table 3; Figures 2 and 2.1].

The EGF levels in i-PRF were 1.16 pg/ml, 0.64 pg/ml, 0.78 pg/ml, and 0.92 pg/ml, and those in PDTM were 1.41 pg/ml, 1.29 pg/ml, 1.18 pg/ml, and 1.3 pg/ml for the YM, OM, YF, and OF samples, respectively.

Table 3: Summarized concentrations of growth factors observed in PRF and i-PRF alone and when partially demineralized tooth matrix (PDTM) is combined with PRF and i-PRF

| Growth factor | bFGF | EGF | PDGF | TGF-beta | VEGF |
|---------------|------------|------------|------------|------------|-----------|
| YM-PRF | 0.41±0.01 | 1.22±0.07 | 4.73±0.09 | 1.27±0.007 | 1.43±0.03 |
| OM-PRF | 0.31±0.06 | 0.59±0.01 | 1.67±0.03 | 1.55±0.19 | 0.77±0.08 |
| YF-PRF | 0.29±0.03 | 0.89±0.08 | 3.62±0.01 | 1.66±0.09 | 1.37±0.02 |
| OF-PRF | 0.34±0.01 | 0.71±0.03 | 1.67±0.07 | 1.6±0.09 | 0.86±0.03 |
| YM-PRF+PDTM | 0.28±0.02 | 1.07±0.01 | 6.39±0.03 | 1.79±0.01 | 2.07±0.03 |
| OM-PRF+PDTM | 0.3±0.02 | 0.93±0.01 | 3.17±0.01 | 1.45±0.01 | 1.42±0.02 |
| YF-PRF+PDTM | 0.28±0.01 | 0.84±0.01 | 2.26±0.03 | 1.19±0.02 | 1.18±0.02 |
| YM-iPRF | 0.39±0.01 | 1.16±0.009 | 11.39±0.02 | 1.99±0.07 | 1.83±0.01 |
| OM-iPRF | 0.12±0.01 | 0.64±0.047 | 1.76±0.15 | 1.42±0.01 | 0.56±0.01 |
| YF-iPRF | 0.2±0.01 | 0.78±0.024 | 1.02±0.02 | 1.18±0.02 | 1.25±0.01 |
| OF-iPRF | 0.38±0.02 | 0.92±0.015 | 7.62±0.02 | 2.11±0.01 | 1.73±0.01 |
| YM-iPRF-PDTM | 0.52±0.03 | 1.41±0.09 | 42.4±0.03 | 2.31±0.008 | 3.05±0.02 |
| OM-iPRF- PDTM | 0.36±0.01 | 1.29±0.01 | 7.92±0.03 | 1.81±0.02 | 2.92±0.01 |
| YF-iPRF- PDTM | 0.41±0.005 | 1.18±0.01 | 4.57±0.02 | 1.44±0.01 | 2.01±0.02 |
| OF-iPRF-PDTM | 0.56±0.007 | 1.3±0.01 | 9.05±0.03 | 2.46±0.01 | 3.07±0.02 |

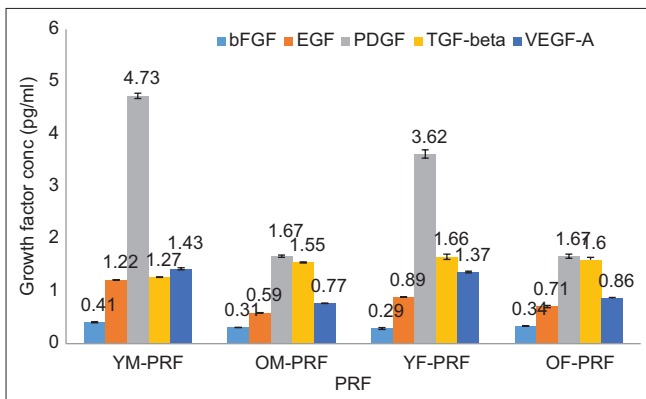


Figure 1: Overlaid bar graph depicting the concentration of growth factors expressed in the PRF samples

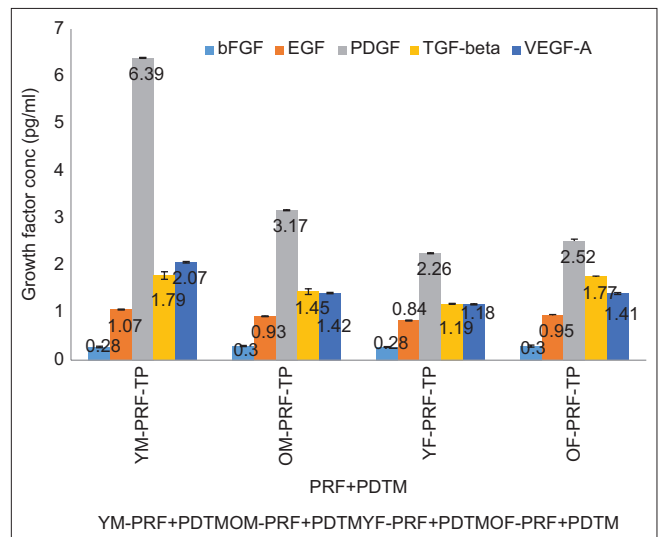


Figure 1.1: Overlaid bar graph depicting the concentration of growth factors expressed in different PRFs mixed with a partially demineralized tooth matrix

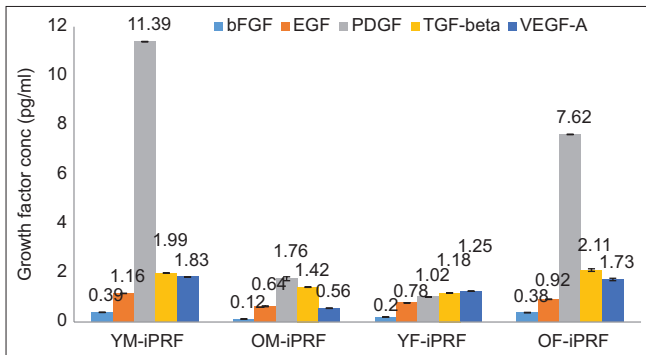


Figure 2: Overlaid bar graph depicting the concentrations of growth factors expressed in different i-PRF at 700 rpm

EGF expression was increased in the combination samples [Table 3; Figures 2 and 2.1].

The PDGF-AB concentrations in i-PRF and its combination were 1.39 pg/ml, 1.76 pg/ml, 1.02 pg/ml, and 7.62 pg/ml and 42.4 pg/ml, 7.92 pg/ml, 4.57 pg/ml, and 9.05 pg/ml in the YM, OM, YF, and OF samples, respectively, indicating relatively high PDGF contents when PDTM was combined with i-PRF [Table 3; Figures 2 and 2.1].

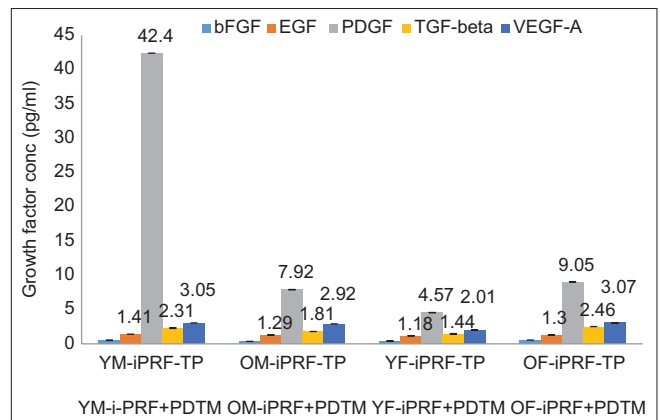


Figure 2.1: Overlaid bar graph depicting the concentration of growth factors expressed in different i-PRFs mixed with partially demineralized tooth matrix

The levels of TGF-beta recorded in i-PRF and its combination with PDTM were 1.99 pg/ml, 1.42 pg/ml,

1.18 pg/ml, and 2.11 pg/ml and 2.31 pg/ml, 1.81 pg/ml, 1.44 pg/ml, and 2.46 pg/ml, respectively, in the YM, OM, YF, and OF groups, with increases in the combination group [Table 3; Figures 2 and 2.1].

The VEGF concentrations in i-PRF and i-PRF mixed with PDTM were 1.83 pg/ml, 0.56 pg/ml, 1.25 pg/ml, and 1.73 pg/ml and 3.05 pg/ml, 2.92 pg/ml, 2.01 pg/ml, and 3.07 pg/ml in the YM, OM, YF, and OF samples, respectively [Table 3; Figures 2 and 2.1].

DISCUSSION

Growth factors play a significant role in tissue healing and regeneration. The most common autogenous graft material used for bone regeneration is the iliac crest, which contains TGF- β , FGF, VEGF, PDGF, IGF, and BMPs.^[17] Bone morphogenic proteins (BMPs) and growth factors present in demineralized bone matrix in large quantities, especially BMP-4 and TGF- β 1; however, their release varies on the basis of donor conditions.^[18] The current study also revealed that the maximum number of growth factors released was PDGF-AB, followed by TGF- β and VEGF.

The demineralized dentin matrix (DDM) contains BMPs and growth factors expressed in picograms (pg); therefore, issues of toxicity or ectopic mineralization are rare. Studies on DDMs via ELISA have demonstrated the presence of TGF- β , VEGF, FGF-2, PDGF, BMPs, and inulin-like growth factor-1 (IGF-1). The results of the current study revealed that TGF- β was followed by FGF-2, VEGF, PDGF, and IGF-1.^[19] In the present study, the release of growth factors when the PDTM was mixed with i-PRF and PRF was as follows: PDGF-AB, TGF- β , h VEGF, EGF, and b FGF. IGF-1 was not assessed in the current study because a study by Richard D. Finkelman reported that IGF-I was the least expressed.^[20]

When the platelet-rich fibrin matrix (PRFM) and PRF were compared *in vitro*, the release of PDGF, VEGF, EGF, FGF, TGF- β , and IGF was found to be significantly greater in the PRFM initially, but the release of growth factors from the PRF was sustained up to 23 days.^[21] The results of the present study, however, revealed the release of growth factors in the order of PDGF-AB, TGF- β , VEGF, EGF, and bFGF in PRF.

Another *in vitro* study on the release of growth factors in PRF at different time intervals revealed the sustainable release of growth factors, highest being IGF-1, followed by TGF- β 1, PDGF-AB, VEGF, and EGF.^[22] In the present study, IGF-1 release was not assessed, but there was a

change in the order of release of growth factors which were PDGF-AB, TGF- β , VEGF, and EGF.

The release of growth factors from i-PRF resulted in increased concentrations of PDGF-AB and TGF- β , which was in accordance with our study, in which PDGF-AB and TGF- β were highly expressed in i-PRF. Many studies on i-PRF have concluded that, at different centrifugation forces, there is greater release of VEGF and TGF- β 1 growth factor^[6,12]; these results are similar to those of the current study, which revealed similar values of VEGF and TGF- β 1.

The correlation of growth factor release with donor age and sex in PRP revealed that the release of PDGF-AB and TGF- β was more similar to that reported in the present study, but there was no significant difference in age or sex.^[5] In the present study, however, there was a statistically significant difference in terms of sex and age, with young males and old females in the TGF- β group ($P = 0.001$), old females in the PDGF-AB group ($P = 0.001$) in the i-PRF and young females in the TGF- β ($P = 0.003$) and PDGF-AB ($P = 0.001$) groups in the PRF.

A study performed by Gummaluri SS compared the clinical efficacy of titanium-prepared platelet-rich fibrin (T-PRF) and leucocyte platelet-rich fibrin (L-PRF) for infrabony defects and concluded that T-PRF filled the defect better than L-PRF clinically and radiographically; however, this difference was not significant.^[23] In the present study, the addition of PDTM to platelet concentrates resulted in a statistically significant release of growth factors, especially with i-PRF, which can promote the treatment of bony defects.

SUMMARY AND CONCLUSION

Within the limitations of the study, the following conclusions were drawn:

1. The i-PRF samples from young males significantly released growth factors from h VEGF ($P = 0.033$) and TGF- β ($P = 0.001$), whereas in old males, bFGF ($P = 0.002$) and h VEGF ($P = 0.001$) were more abundant in the PRF samples.
2. In the PRF samples from older females, PDGF ($P = 0.001$), VEGF ($P = 0.001$), and TGF- β ($P = 0.001$) were significant, whereas in young females, were significant for TGF- β ($P = 0.003$) and PDGF-AB ($P = 0.001$).
3. When the partially demineralized tooth matrix of all the groups was mixed with i-PRF and PRF, the release of growth factors was significantly greater in the i-PRF groups than in the PRF groups.

Further studies need to be performed to evaluate BMP release from PDTM, PRF, and i-PRF, which should be evaluated clinically.

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Conflicts of interest

There are no conflicts of interest.

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