

The Effect of Platelet-Rich Plasma on Osteoblast and Periodontal Ligament Cell Migration, Proliferation and Differentiation

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**Running Title:**

Effect of PRP on cell function

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## **ABSTRACT**

*Background and objective:* The use of platelet-rich plasma (PRP) aims to safely and conveniently deliver growth factors in order to enhance bone and periodontal regeneration. However, conflicting reports regarding its effectiveness suggest that further study of the relevant cellular mechanisms is required. The aim of this study was to investigate the *in vitro* effect of PRP on osteoblasts and periodontal ligament cell function.

*Methods:* Various concentrations of PRP (100%, 50%, 20% and 10%) and platelet-poor plasma (PPP) obtained from human donors was applied to primary cultures of human osteoblast and periodontal ligament cells. <sup>3</sup>H-Thymidine incorporation, crystal violet and MTT assays were utilized to assess DNA synthesis and proliferation. Migration was determined by assessing cell response to a concentration gradient, while differentiation was assessed using Alazarin Red staining.

*Results:* PRP and PPP had stimulatory effects on the migration of both cell types. At 24 hours, DNA synthesis was suppressed by the application of the various concentrations of PRP, but over a 5 day period, a beneficial effect on proliferation was observed, especially in response to the intermediate concentrations of 50% PRP. Platelet-poor plasma (PPP) resulted in the greatest enhancement of cellular proliferation for both cell types. 50% PRP and PPP facilitated differentiation of both cell types.

*Conclusion:* PRP can exert a positive effect on osteoblast and periodontal ligament cell function, but this effect is concentration specific with maximal concentrations not

necessarily resulting in optimal outcomes. Furthermore, PPP appears to also have the ability to promote wound healing associated cell function.

## **INTRODUCTION**

During the early stages of wound healing, platelet released growth factors, including platelet derived growth factor (PDGF), insulin like growth factor -1 (IGF) and transforming growth factor  $\beta$  (TGF $\beta$ ), initiate a cascade of cellular and molecular events which result in wound healing in a highly regulated and coordinated fashion (1-3). The understanding that platelet-derived growth factors play an important role in wound healing has led to the development of recombinant growth factors aimed at influencing and enhancing repair and regeneration. The application of these growth factors to bone and periodontal regeneration has been investigated using *in vitro* and *in vivo* models with promising results (4-9) that have provided the basis for subsequent human clinical studies (10, 11).

In addition to the use of recombinant growth factors, concentrated formulations of platelets, known as platelet rich plasma (PRP), have also been investigated as a potential source of autologous growth factors. PRP is a volume of autologous plasma that has a platelet concentration approximately three to four times above baseline levels (12). Hence, it can be considered a concentration of all the platelet derived growth factors and plasma components of the individual patient in their biologically determined ratios (13,14).

Whitman *et al* first described the use of PRP in the dental setting and showed that PRP application to the underlying tissues allowed more predictable flap adaptation and haemostasis and ensured a more definitive seal than primary closure alone (15). Subsequently, there has been considerable interest in examining this method of

promoting wound healing and regeneration. In particular, it has been proposed that PRP may be utilized alone or in conjunction with various graft materials to deliver growth factors to the wound site, especially in order to enhance bone and/or periodontal regeneration.

Many investigations have been conducted on the clinical effect of PRP on bone regeneration (for review see (3)) and periodontal regeneration (16-18), in addition to several *in vitro* studies (19-26) aimed at establishing the biological rationale for this treatment. However, there is a lack of consensus regarding the effectiveness of PRP with early promising clinical reports (16, 27-29) being tempered by subsequent negative findings (30-32). Hence, the aim of this study was to investigate the *in vitro* effect of platelet-rich plasma on the cells that are critical for periodontal and bone regeneration, namely periodontal ligament cells and osteoblasts, in terms of the key cellular functions associated with wound healing and regeneration, namely migration, proliferation and differentiation.

## **MATERIALS AND METHODS**

### **Cell culture**

During surgical extraction of third molars, teeth and bone chips were collected in explant media containing Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100units/ml Penicillin, 100µg/ml Streptomycin, Fungizone (2.5mg/ml) and non-essential amino acids. The study was approved by the human ethics committee of the University of Queensland and informed consent was obtained prior to the collection of the samples.

Periodontal ligament cells and osteoblasts were obtained as previously described (33, 34). Briefly, the bone chips and periodontal ligament fragments obtained from the middle third of the root surface were minced into smaller tissue portions, transferred to 25cm<sup>2</sup> tissue culture flasks (Corning Incorporated, Corning NY, USA) and incubated in explant media at 37°C and a humidified atmosphere containing 5% CO<sub>2</sub>. One week following establishment of the explants, the explant media was changed to standard media containing DMEM, 10% FCS, 50units/ml Penicillin, 50µg/ml Streptomycin and non-essential amino acids.

Following cell growth from the explants, the cells were detached from the plate using 0.2% trypsin and 0.02% EDTA (Sigma Chemical Co, St Louis, USA), and were subsequently propagated by passaging in a 1:3 split until sufficient numbers were obtained to carry out the required experiments. Cells from passages 3 to 5 were used in this study.

Each experiment described in this study was carried out using three individual primary cell lines from different donors and was repeated in triplicate for each cell line.

### **PRP Preparation**

PRP was prepared from healthy patients immediately prior to application on the cells. Whole venous blood was collected in lithium heparin coated collection tubes (Becton, Dickinson and Company, NJ, USA) and initially centrifuged at 1500RPM for 10 minutes to separate the red blood cell (RBC) portion from the platelet-rich and platelet-poor plasma. The upper layer of the RBC portion was included as the platelets containing the largest amount of growth factors, and hence having the greatest potential biological activity, are larger and mix with the upper 1mm of the red blood cells. The inclusion of this small RBC layers imparted a red tinge to the PRP (28, 35) . The PRP and PPP portions were then extracted and centrifuged again at 2500RPM for 10 minutes to separate the PRP from the PPP.

Dilutions of the PRP and PPP for the various experiments were obtained by mixing with standard serum-free media (DMEM, non-essential amino acids and penicillin/streptomycin). Both autogenous and allogenic PRP/PPP preparations were used with no differences on cell functions being observed (data not shown), and hence the results are combined.

### **Migration**

Using Transwell Permeable (TP) Supports (Corning Incorporated, Corning NY, USA) (26, 36) with 6.5mm diameter and 8µm pore size, migration from one side of a membrane to the other was examined after 6 hours in response to five treatments – 100% PRP, 50% PRP, and 10% PRP, as well as 50% PPP and serum free medium. 300µl of treatment media per well was placed into a 24-well plate and allowed to incubate for 30 minutes. Care was taken to ensure that the treatment media was settled at the bottom of the well, and then serum free media was gently applied on top until the layer just contacted the under side of the TP supports. Cells were seeded at a concentration of  $2 \times 10^3$  onto the outer surface of the TP supports and were incubated for six hours.

After the incubation period, the TP supports was removed and washed with phosphate-buffered saline solution (PBS) and the outer surface was carefully wiped dry with a flattened cotton bud and a microbrush to remove non-migrated cells. The TP supports were then placed into a fresh 24-well plate containing 300µl crystal violet for 15 minutes, and subsequently removed, washed by flooding with tap water until free dye was no longer visible and allowed to air dry. This stain was then solubilised and extracted with 33% glacial acetic acid and absorbance was read in a spectrophotometer at 570nm. The absorbance reading is directly proportional to the number of cells that migrated from the outer to the inner surface of the TP supports, in response to the various treatments placed in the 24 well plates.

### **Proliferation**

Proliferation of the periodontal ligament cells and osteoblasts was assessed using



three methods, which were based on different outcome measures. The treatments utilized were 0% FCS (negative control), 10% FCS (positive control), 50% PRP, 20% PRP, 10% PRP and 50% PPP. All treatments were diluted with serum-free media. 100% PRP was not utilized as it resulted in total loss of cell viability (data not shown).

### ***<sup>3</sup>H Thymidine Incorporation (DNA synthesis) Assay***

The <sup>3</sup>H-Thymidine incorporation assay is based on the incorporation of radio-labelled <sup>3</sup>H-thymidine into the replicating DNA strands during mitosis and therefore measures DNA synthesis. As previously described (37), the cells were seeded at a concentration of  $2 \times 10^4$  cells per well in 24 well (Nunclon, Denmark) plates in standard cell culture media. These cells were allowed to attach overnight. Subsequently, the cells were exposed to media containing 0% FCS for 48 hours in order to synchronize the cells in the G<sub>0</sub> phase of the cell cycle. After this time, the treatment media was added and the cells were allowed to incubate for 24 hours. For the last four hours, the cells were pulse-labelled with 10 $\mu$ Ci <sup>3</sup>H-Thymidine per well. Cells were then lysed with 1.5% SDS for 15 minutes, combined with scintillation fluid and radioactivity was measured using a liquid scintillation counter (Beckman Instruments, Fullerton, California, USA).

### ***Crystal Violet (Colorimetric) Assay***

The crystal violet colorimetric assay directly measures cell numbers that are present. As previously described (37), cells were seeded into 96 well plates at a concentration of  $2 \times 10^3$  cells per well and allowed to attach overnight in standard media. This media

was then removed and the treatment media were added followed by incubation for 5 days. After this time, the media was removed; the wells were washed with PBS and stained with crystal violet for 30 minutes. Following the removal of excess stain, solubilization of the bound crystal violet was carried out with 33% glacial acetic acid solution and absorbance was measured by a spectrophotometer at 570nm. The relative spectrophotometer readings are directly proportional to cell numbers.

### ***MTT Proliferation (Cell viability) Assay***

The MTT™ (Roche Diagnostics GmbH, Mannheim, Germany) proliferation assay was also used to assess the proliferative potential of PRP (26). This is a colorimetric assay where the amount of colour produced is directly proportional to the number of viable cells. Cells were seeded into 96 well plates at a concentration of  $2 \times 10^3$  cells per well and allowed to attach overnight in standard media containing 10% FCS. Treatment media was added and the cells were allowed to incubate for 5 days. The MTT labelling reagent was then added to each well and allowed to incubate for a further 4 hours, after which the cells were washed with PBS and solubilization solution was added. The plates were then incubated overnight and subsequently read using a spectrophotometer at 570nm.

### **Differentiation**

Differentiation of the periodontal ligament cells and osteoblasts was assessed by quantification of Alazarin red staining (38). Cells were plated in 6-well plates at a concentration of  $1 \times 10^5$  cells per well and incubated with DMEM containing 10% FCS (standard media) and allowed to attach overnight. Subsequently, treatment media

of 50% PRP, 20% PRP, 50% PPP, mineralization media (consisting of standard media with the addition of 50 µg/ml ascorbic acid, 10 mM glycerophosphate and  $10^{-8}$  M dexamethasone), standard media and media containing 0% FCS were added and the cells incubated for 5 days. After this time, the media were 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. Calcium forms an alizarin red S-calcium complex in a chelation process, and red staining is evident in the well. This stain was then solubilized with 300µl of 33% glacial acetic acid solution and absorbance was measured by a spectrophotometer at 415nm.

### **Statistical Analysis**

One way ANOVA was used to assess whether there was a statistically significant effect of the different treatments on cell function. In order to identify statistically significant differences between the various treatments, post-hoc analysis was carried out using SPSS software (SPSS Inc. Chicago, Illinois, USA) and the LSD correction. Statistical differences between groups were accepted for p-values lower than 0.05.

## **RESULTS**

### **Migration**

The results of the migration assay indicated that PRP had a stimulatory effect on cellular migration of periodontal ligament cells (Figure 1). Concentrations of 100% (p<0.01), 50% (p<0.001) and 10% PRP (p<0.001) significantly enhanced migration compared to the negative control of 0%FCS. 50% PPP also significantly enhanced migration of the periodontal ligament cells (p<0.02), but no difference was found compared to the various PRP concentrations. There were no significant differences between the various concentrations of PRP.

The migration of osteoblasts was also significantly enhanced by the various concentrations of PRP with 100% PRP (p<0.001), 50% PRP (p<0.01) and 10% PRP (p<0.05) all showing significant stimulation compared to 0% FCS (Figure 2). No statistical differences were seen between the various concentrations of PRP. 50% PPP also had a significant migratory stimulus compared to 0% FCS (p<0.01), but was not significantly different compared to the various PRP concentrations.

### **Proliferation**

#### ***<sup>3</sup>H Thymidine Incorporation (DNA synthesis) Assay***

The <sup>3</sup>H-thymidine proliferation assays showed similar effects of PRP on the DNA synthesis of both periodontal ligament cells and osteoblasts (Figures 3 and 4 respectively). PRP exerted a statistically significant decrease in cellular DNA synthesis of periodontal ligament cells at all concentrations (p<0.001) compared to the

positive control. Furthermore, 50% PPP significantly increased DNA synthesis compared to all PRP concentrations ( $p < 0.001$  for all concentrations), as well as the positive control ( $p < 0.01$ ) of 10% FCS, indicating its mitogenic effect on these cells.

Similar results were obtained using the osteoblasts, whereby all PRP concentrations significantly inhibited DNA synthesis ( $p < 0.001$ ) compared to the positive control (10% FCS). PPP had a significant enhancing effect when compared to all concentrations of PRP ( $p < 0.001$ ), but showed no difference compared to the positive control of DMEM containing 10%FCS.

#### ***Crystal Violet (Colorimetric) Assay***

Continuing the trend from the DNA synthesis assay, in the five day colorimetric proliferation assay, the periodontal ligament cells (figure 5) showed the greatest proliferative activity in the presence of 50% PPP, which was significantly higher than the positive control of 10% FCS ( $p < 0.05$ ), 20% PRP ( $p < 0.001$ ), 10% PRP ( $p < 0.001$ ) and the negative control of 0% FCS ( $p < 0.001$ ). However, in this assay, 50% PRP induced a significant increase in cell numbers compared to 0% FCS ( $p < 0.01$ ) and the lower concentrations of PRP, namely 20% and 10% ( $p < 0.001$  for both). There was no significant statistical difference between 50% PRP, 50% PPP and 10% FCS. In addition, both 20% and 10% PRP showed no significant difference when compared to the negative control of 0% FCS.

Similarly, with regards to the osteoblasts (figure 6), 50% PPP was the most beneficial in terms of proliferation compared to all PRP concentrations, as well as media containing 10% and 0% FCS ( $p < 0.001$ ). 50% PRP was significantly more mitogenic

compared to 0% FCS ( $p < 0.001$ ), 20% PRP ( $p < 0.001$ ) and 10% PRP ( $p < 0.001$ ), whereas 10% PRP significantly stimulated proliferation compared to 20% PRP ( $p < 0.05$ ) and 0% FCS ( $p < 0.05$ ). There were no statistical differences in proliferation between 20%PRP and 0% FCS, and 50% PRP and 10% FCS.

### ***MTT Proliferation (Cell Viability) Assay***

The MTT proliferation box-plots for the periodontal ligament cells and osteoblasts are shown in figures 7 and 8 respectively. Treatment with 50% PRP significantly stimulated cell proliferation of periodontal ligament cells compared to media containing 0% FCS ( $p < 0.001$ ), 10%PRP and 20% PRP (all  $p < 0.001$ ), but there was no statistical difference compared to 10% FCS and 50%PPP. Similarly, 50% PPP was shown to significantly increase proliferation compared to 0% FCS, 10% PRP and 20% PRP (all  $p < 0.001$ ), as well as 10% FCS ( $p < 0.05$ ).

With regards to the osteoblasts, 50% PRP ( $p < 0.001$ ) and 20% PRP ( $p < 0.01$ ) significantly increased proliferation compared to 0% FCS. Furthermore, 50% PPP significantly increased proliferation compared to 0% FCS ( $p < 0.001$ ) and 10% PRP ( $p < 0.01$ ). No significant statistical differences were seen between the various PRP concentrations and 10% FCS, although there appeared to be trend towards reduced proliferative activity with reduced PRP concentrations.

### **Differentiation**

The results show that PRP and PPP are capable of inducing differentiation of the periodontal ligament cells and osteoblasts as shown by the photographs of the wells

with the Alizarin Red S staining of  $\text{Ca}^{2+}$  deposits (Figure 9). This was reflected in the results obtained by solubilization of the staining and reading in the spectrophotometer at 415nm (Figures 10 and 11).

In the periodontal cells (Figure 10), 50% PRP ( $p < 0.001$ ) and 50% PPP ( $p < 0.05$ ) induced significant increases in differentiation compared to 0% FCS. In addition, 50% PRP significantly increased  $\text{Ca}^{2+}$  deposition compared to 10% FCS, mineralization media ( $p < 0.01$ ), 20% PRP ( $p < 0.01$ ) and 10% FCS ( $p < 0.01$ ). The lower concentration of PRP showed no difference when compared to 50% PPP or mineralization media.

The results obtained with the osteoblast cells indicated that mineralization media ( $p < 0.05$ ), 50% PRP ( $p < 0.01$ ) and 50% PPP ( $p < 0.05$ ) had a significant increase in differentiation when compared to 0% FCS and only 50% PRP induced significantly greater  $\text{Ca}^{2+}$  deposition compared to 10% FCS ( $p < 0.05$ ) (Figure 11). No other significant differences were noted.

## **DISCUSSION**

PRP is easily produced with minimal additional equipment using a two spin technique to ensure complete separation of the blood fragments (12). In the present study, the PRP was drawn from healthy volunteers immediately prior to application to the cell lines as platelets begin to release their growth factors immediately, with close to 70% of stored growth factors released within 10 minutes (12).

Several reports have previously assessed the effect of PRP on cell function *in vitro*. However, these studies utilized a wide range of methods for preparing the PRP formulation that was applied to the cells, and a wide variety of cell types were used. In this study, PRP was prepared and utilized in the same manner as it is used in the clinic, with the various dilutions of PRP being directly applied to the cells. Furthermore, primary cell lines of periodontal ligament cells and alveolar bone derived osteoblasts were used in order to replicate the clinical scenario.

This study report on the *in vitro* effects of PRP on the cell functions of periodontal cells and osteoblasts both in the short term (migration after 6hrs, DNA synthesis after 24 hrs), as well as long term (proliferation and differentiation after 5 days). The results were similar with regards to the two cell types. However, PRP concentration had a significant effect on cell function.

In the short term, the various concentrations of PRP significantly enhanced migration for both osteoblasts and periodontal ligament cells. This finding was consistent with a previous report of PRP promoting migration of the SaOS osteoblastic cell line (26). The migration data showed that the various components of PRP possess biological



activity, even at the lowest utilized concentration of 10%. Interestingly, PPP was also shown to possess biological activity.

The <sup>3</sup>H-thymidine incorporation assay showed that PRP does not enhance DNA synthesis within 24 hours. However, PPP significantly enhanced DNA synthesis in both cell types. This finding was in contrast to the findings of Celotti *et al.* (26), who showed that PRP increases DNA synthesis in osteoblast-like cells. However, the PRP formulation used by Celotti *et al.* was the ‘supernatant’ derived from PRP, rather than the whole PRP preparation that is utilized clinically and was also used in the current study. Conversely, the 5 day proliferation assays (crystal violet and MTT), in keeping with other reports (19, 21-23, 26), showed that PRP enhanced cell proliferation, with a concentration of 50% being the most effective. As was the case with the DNA synthesis data, these assays showed that PPP significantly enhanced proliferation, with the formulation of 50% PPP being equally effective as the most effective PRP concentration of 50%.

The differentiation results indicated that PRP and PPP are both capable of inducing differentiation of both cell types, as shown by the Alizarin Red S staining which identifies calcium deposits. This finding was in agreement with reports that PRP enhanced the expression of mineralized tissue associated proteins by osteoblasts and periodontal ligament cells (39, 40).

A major consideration in the interpretation of the results of this study, especially with regards to the DNA synthesis and proliferation assays, is that the PRP and PPP preparations were directly applied to the cells to mimic the clinical scenario. Indeed,

following the application of the 100% PRP gel-like preparation, the cells acquired an altered morphology with some cells detaching, suggesting that cell viability was being compromised and necessitating the exclusion of this formulation (100% PRP) from the proliferation and differentiation assays. Although the other preparations of PRP, 50%, 20% and 10%, did not appear to affect cell vitality, they also did not demonstrate a mitogenic effect at 24 hours. Since PPP also formed a gel-like complex in the plates, yet it demonstrated a markedly greater mitogenic effect compared to the various concentrations of PRP, it appears that the concentrated cocktail of active components present in PRP are inferior in terms of promoting wound healing associated functions, especially in the short term and on the cells that come into direct contact with the formulation.

When platelets are activated, they release their growth factors almost immediately, with nearly 70% released in the first ten minutes and almost 100% within the first hour (12). Platelets can then be expected to continue to synthesize additional growth factors several days, after which they lose vitality (12). Therefore, it can be postulated that the highly concentrated PRP cocktail of molecules may be initially detrimental to the cells that it directly contacts, but at lower concentrations and over time, as its activity diminishes, it begins to promote cell function, as seen with the stimulation of cell proliferation by 50% PRP at 5 days. The suggestion that maximal concentrations of PRP may not be ideal and that better outcomes may be obtained by ‘intermediate’ concentrations of PRP is supported by both *in vitro* (19, 41) and *in vivo* (42) data. Indeed, in one of the few studies to investigate the clinical efficacy of different

concentrations of PRP, Weibrich et al (42) showed that intermediate concentrations were the most effective in terms of bone regeneration.

PPP significantly enhanced wound healing associated cell function which is consistent with previous reports which have shown a clinical benefit of using PPP (15). Furthermore, it has been suggested that the clinical action of PRP itself is due to the fibrin content (20), which is essentially PPP. It has also been demonstrated that PPP preparations contain significant amounts of certain growth factors, namely IGF-1 and BMP-2, at levels comparable to those found in PRP (43).

The results from the current study show that, in the short term, PRP appears to adversely affect DNA synthesis, with high concentrations adversely affecting cell viability in the immediate area of placement. This is accompanied by a strong migratory stimulus towards distant cell at all PRP concentrations (10%-100%). Over the longer term, and in intermediate concentrations (50%), PRP appears to enhance proliferation of periodontal ligament and osteoblast cells and these cells are then induced to differentiate. The short term detrimental effects of high concentrations of PRP may be a problem in periodontal regeneration or bone grafting where a limited number of progenitor cells are present in the ligament and graft particles respectively. This may account for the results of several recently published reports of a lack of clinical benefit following the use of PRP (30-32). Furthermore, the possibility that high concentrations may adversely affect cell viability is important in the context of PRP being proposed as a delivery vehicle for stem and progenitor cells in tissue engineering-based clinical therapies.

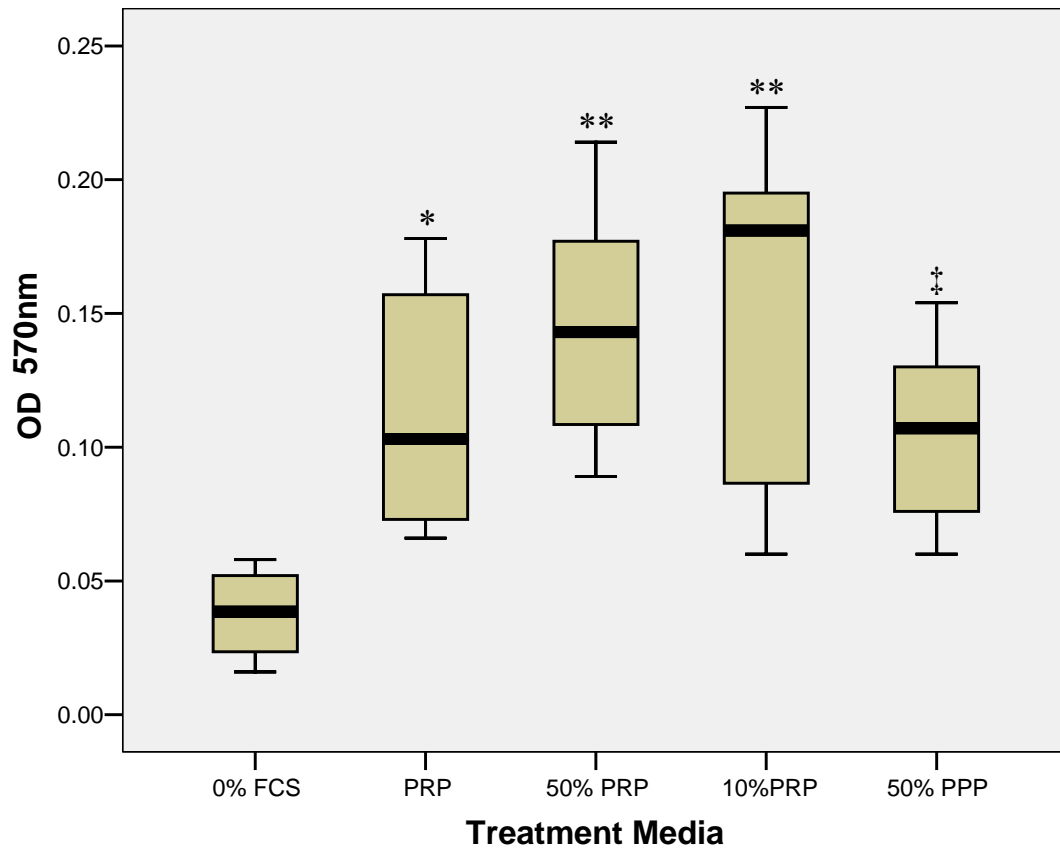
Interestingly, PPP demonstrated a better overall effect on cell function *in vitro*. PPP encouraged cellular proliferation in the short and long term, promoted cell migration and stimulated differentiation. Appropriately designed clinical studies comparing different concentrations of PRP, as well as PPP, are indicated to determine the effect of various PRP concentrations on clinical outcomes.

## **ACKNOWLEDGEMENTS**

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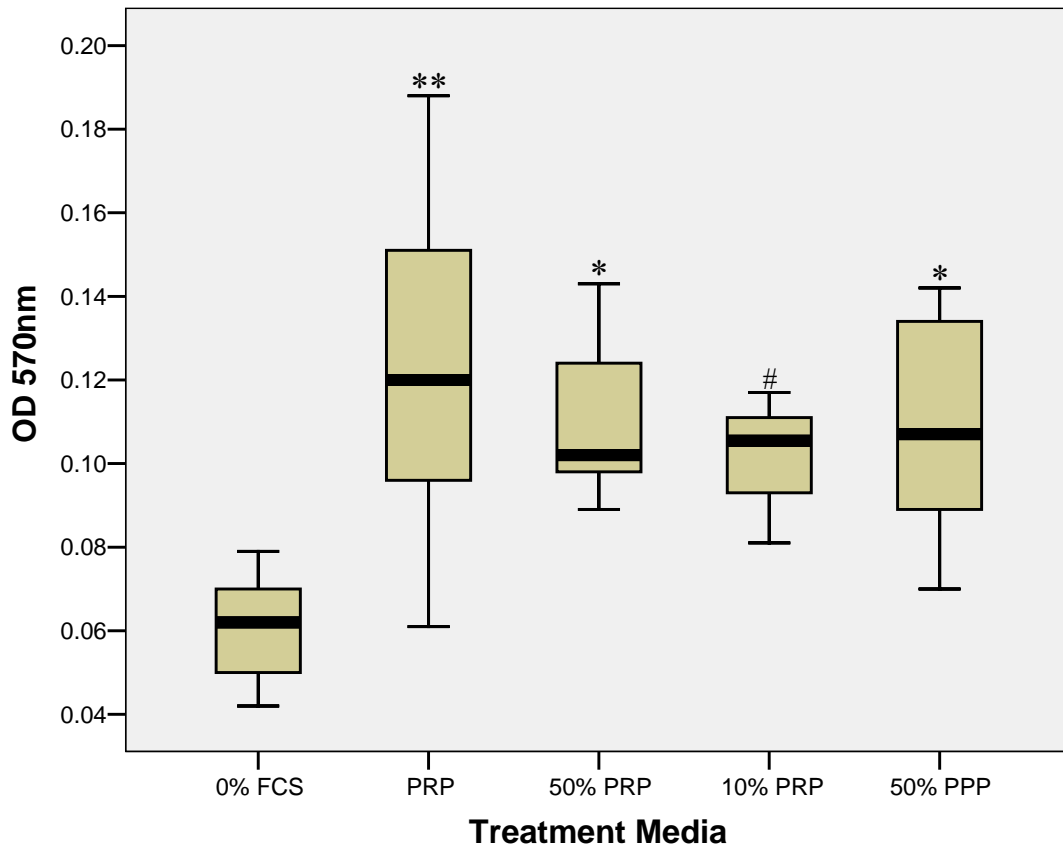
## **FIGURES**

**Figure 1:** Periodontal ligament cell migration (OD = optical density).



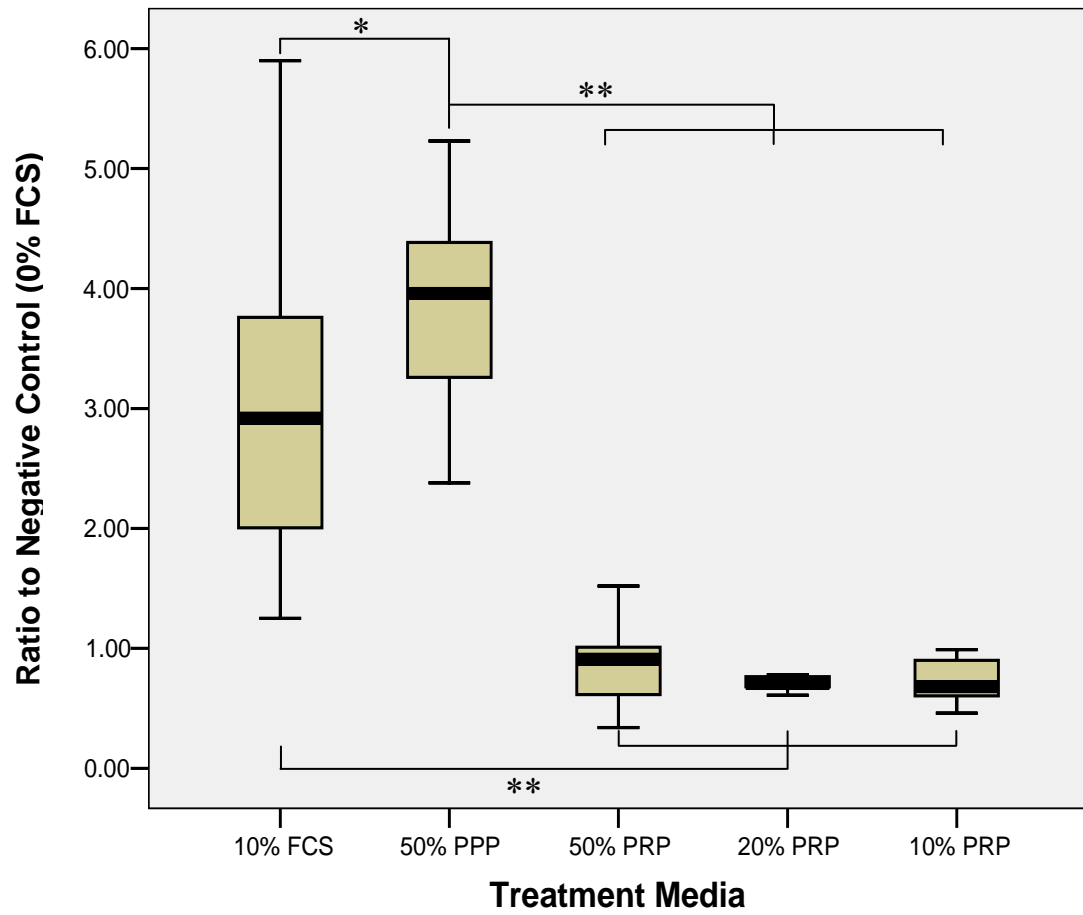
‡p<0.02, \*p<0.01, \*\*p<0.001

**Figure 2:** Osteoblast migration (OD = optical density).



#p<0.05, \*p<0.01, \*\*p<0.001

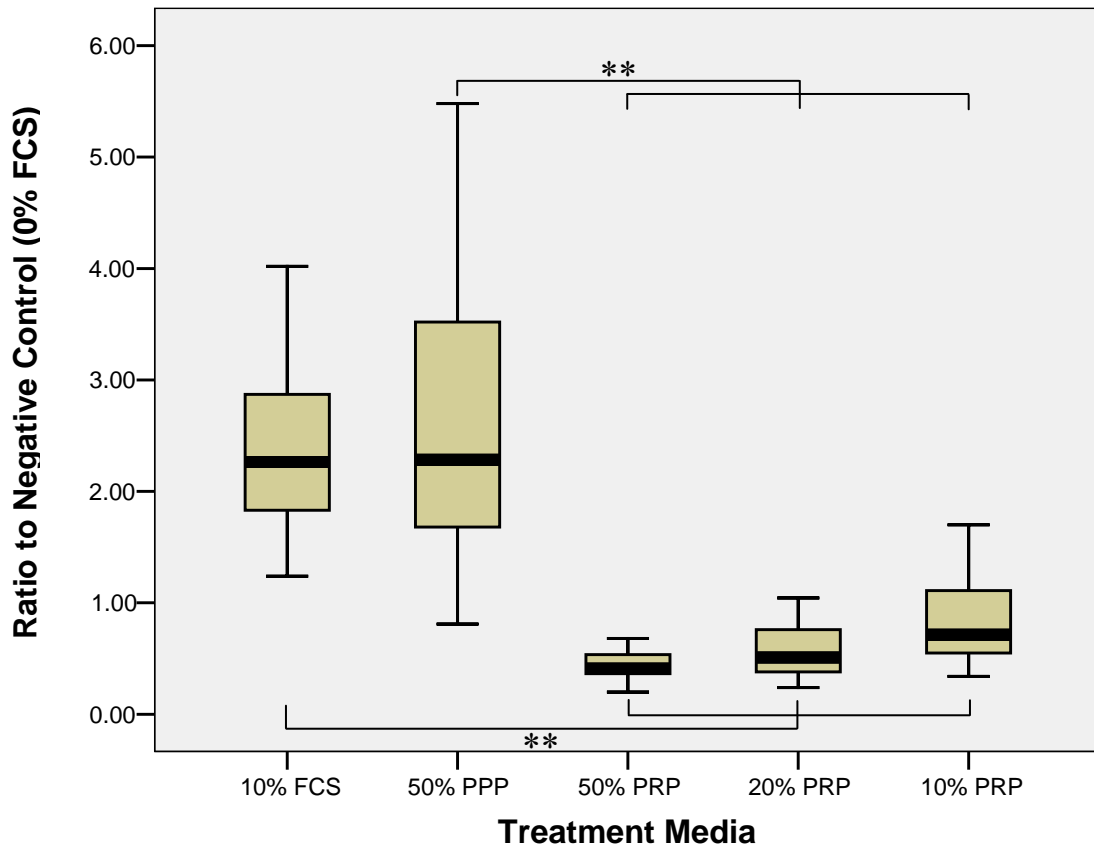
**Figure 3:** Periodontal ligament cell DNA synthesis (<sup>3</sup>H-Thymidine incorporation assay)



\*p<0.01, \*\*p<0.001



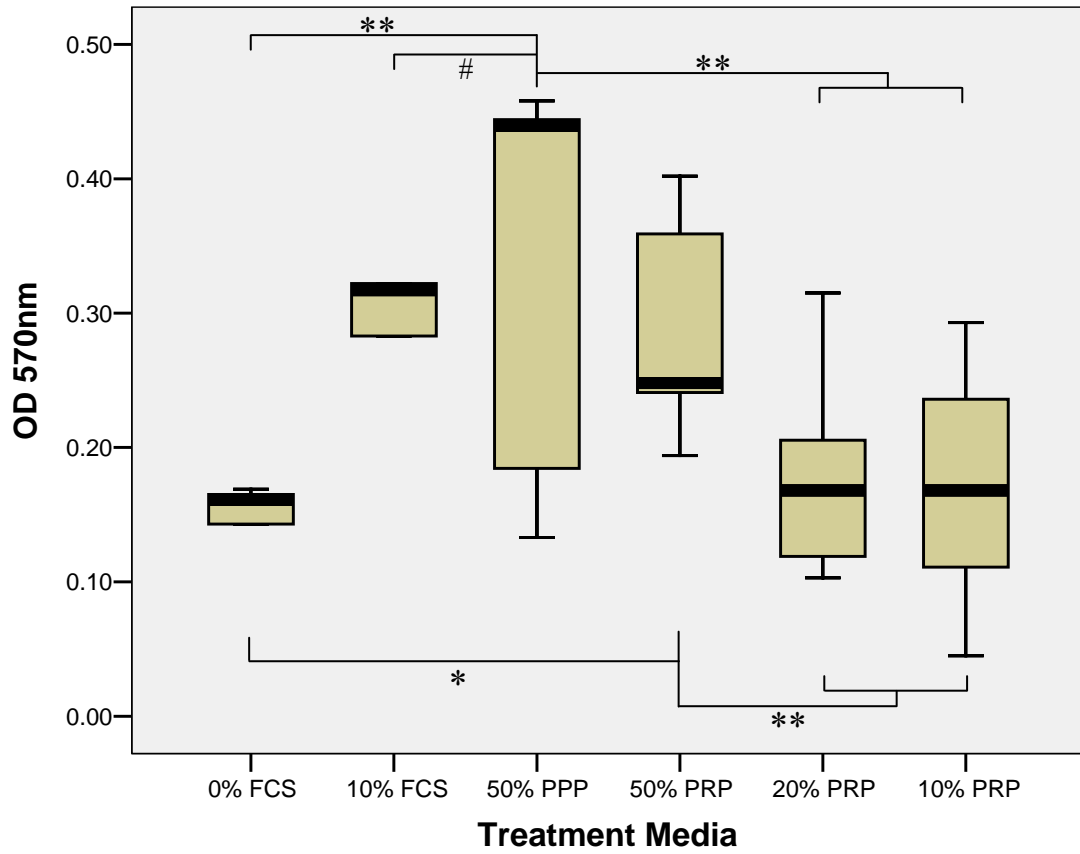
**Figure 4:** Osteoblast DNA synthesis (<sup>3</sup>H-Thymidine incorporation assay)



\*\*p<0.001

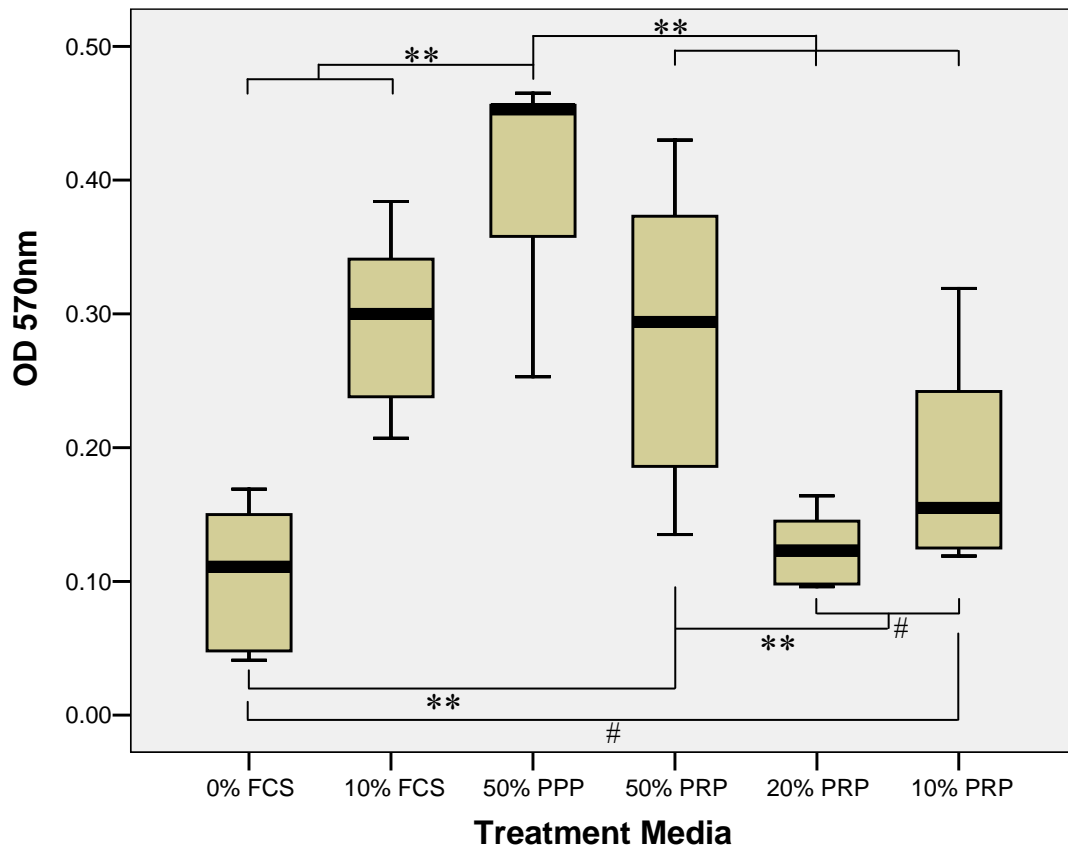
**Figure 5:** Periodontal ligament cell proliferation (crystal violet/colorimetric) assay.

OD=optical density.



#p<0.05, \*p<0.01, \*\*p<0.001

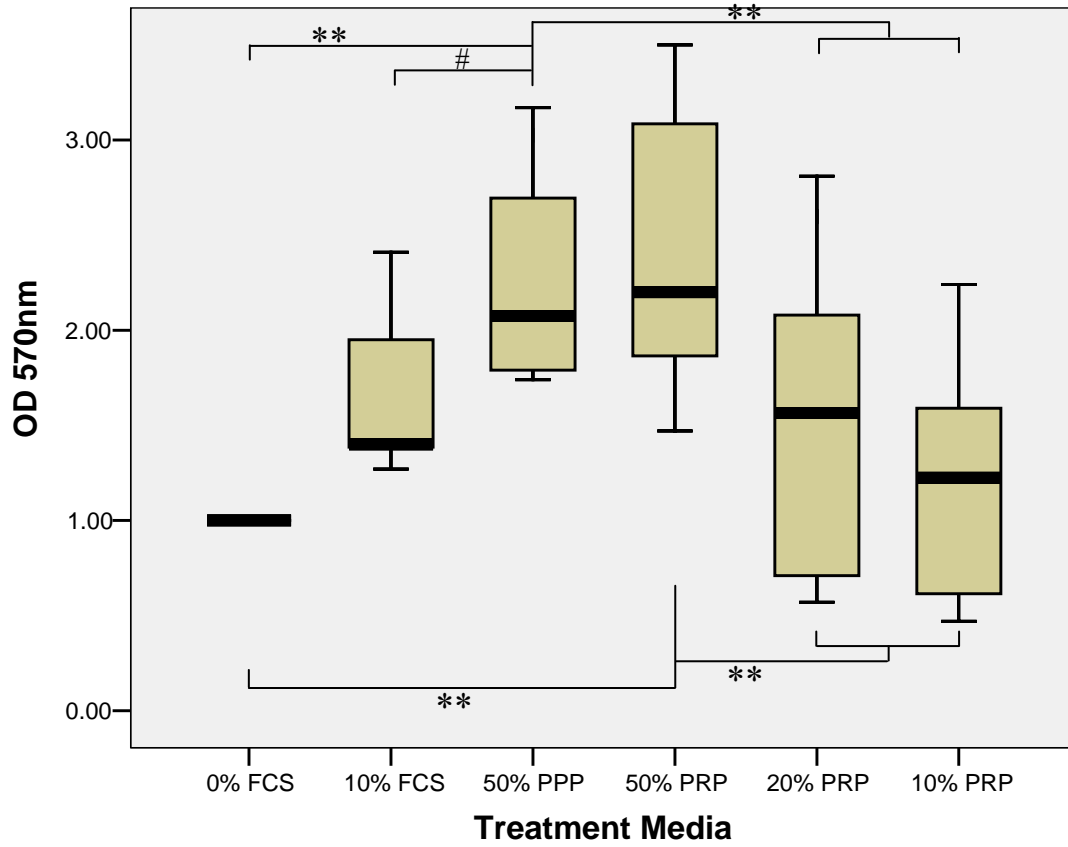
**Figure 6:** Osteoblast proliferation (crystal violet/colorimetric) assay. OD=optical density.



#p<0.05, \*\*p<0.001

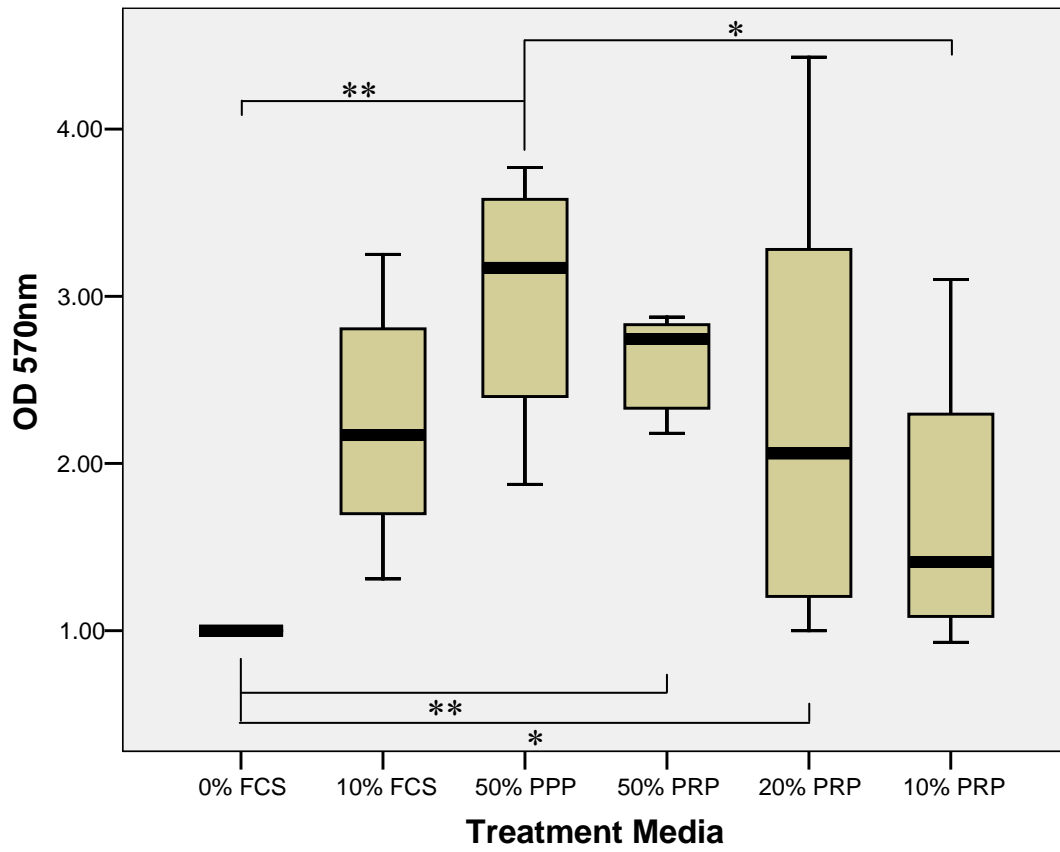
**Figure 7:** Periodontal ligament cell proliferation (MTT/cell vitality) assay.

OD=optical density.



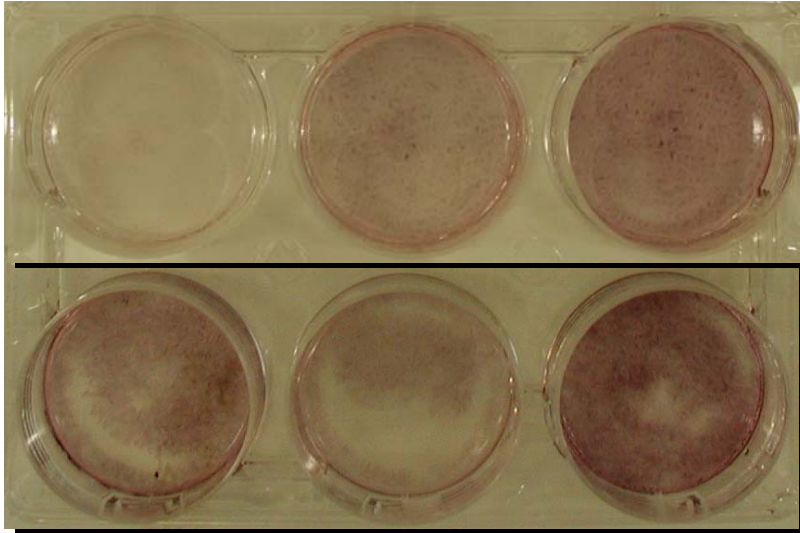
#p<0.05, \*\*p<0.001

**Figure 8:** Osteoblast proliferation (MTT/cell vitality) assay. OD=optical density.

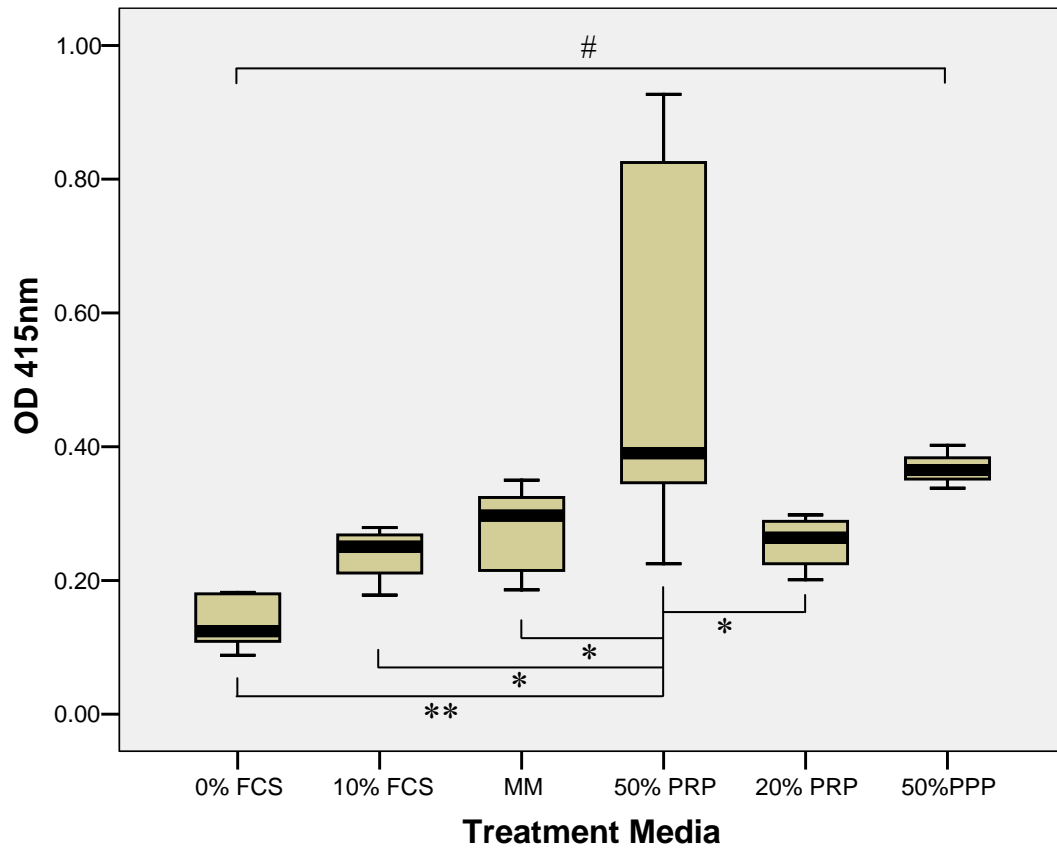


\*p<0.01, \*\*p<0.001

**Figure 9:** Visual representation of osteoblast differentiation assessed by Alazarin Red staining. Top row, left to right: 0% FCS, 10% FCS, Mineralization Media. Bottom row, left to right: 50% PRP, 20% PRP and 50% PPP

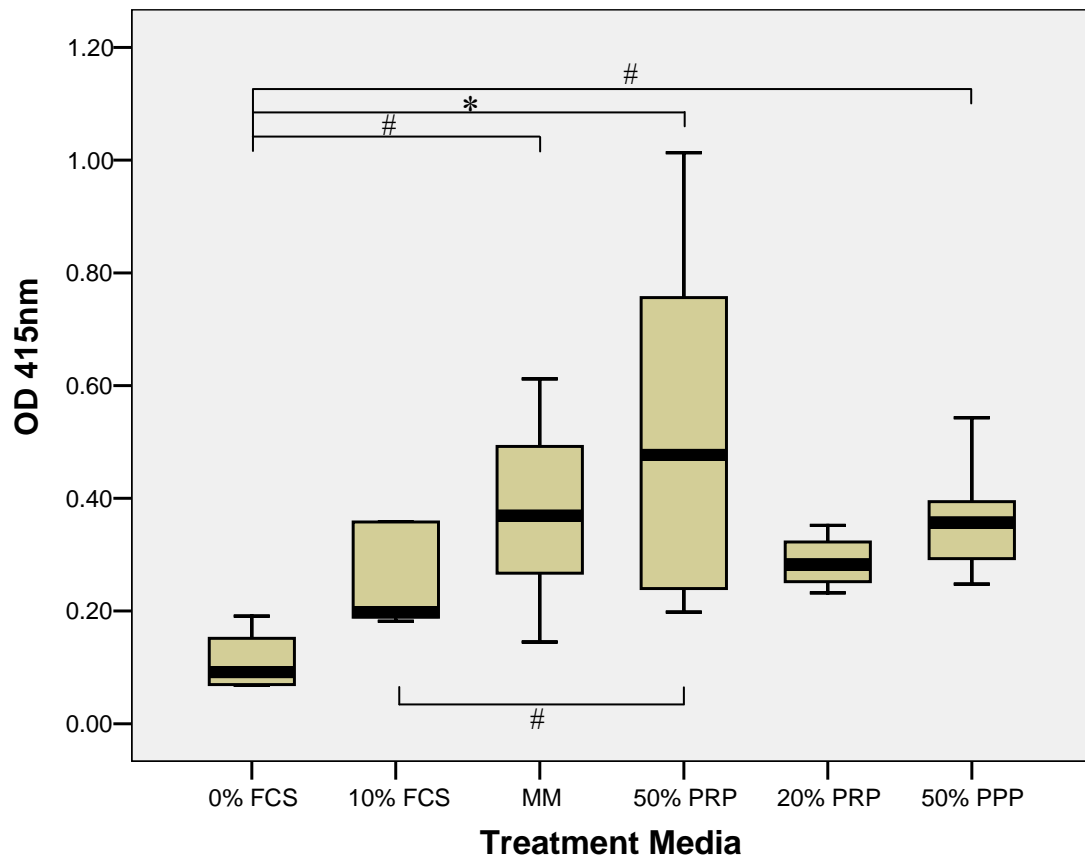


**Figure 10:** Quantification of periodontal ligament cell differentiation (OD = optical density).



#p<0.05, \*p<0.01, \*\*p<0.001

**Figure 11:** Quantification of osteoblast differentiation (OD = optical density).



#p<0.05, \*p<0.01



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