# Effect of Liquid Platelet-rich Fibrin and Platelet-rich Plasma on the Regenerative Potential of Dental Pulp Cells Cultured under Inflammatory Conditions: A Comparative Analysis

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## SIGNIFICANCE

These findings show that liquid PRF (anticoagulant removal) promoted significantly greater regeneration potential of human dental pulp cells when compared with traditional PRP and offers novel regenerative solutions to clinicians working in the endodontic field.

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## ABSTRACT

**Introduction:** Platelet-rich plasma (PRP) has been widely used in regenerative dentistry for over 2 decades. Nevertheless, previous studies have shown that its growth factor content is released over a short time period, and the application of anticoagulants limits its regenerative potential. Therefore, a second-generation platelet concentrate (liquid platelet-rich fibrin [PRF]) was developed without the use of anticoagulants and with shorter centrifugation times. The purpose of the present study was to compare the cellular regenerative activity of human dental pulp cells (hDPCs) when cultured with either liquid PRF or traditional PRP. **Methods:** The regenerative potential of hDPCs isolated from healthy human third molars (18–22 years, $n = 5$) was investigated in both normal and inflammatorylike conditions (lipopolysaccharide [LPS]) and assessed for their potential for dentin repair. The effects of liquid PRF and PRP were assessed for cellular migration, proliferation, and odontoblastic differentiation using a transwell assay, scratch assay, proliferation assay, alkaline phosphatase assay, alizarin red staining, and real-time polymerase chain reaction for genes encoding collagen type 1 alpha 1, dentin sialophosphoprotein, and dentin matrix protein 1, respectively. The effects of both platelet concentrates were also assessed for their ability to influence nuclear translocation of nuclear factor kappa B (p65) by immunofluorescence, and reverse-transcription polymerase chain reaction for genes encoding interleukin-1β, tumor necrosis factor alpha, and nuclear factor kappa B (p65) during an inflammatory condition. **Results:** Both PRP and liquid PRF increased the migration and proliferation of hDPCs when compared with the control group, whereas liquid PRF showed a notable significant increase in migration when compared with PRP. Furthermore, liquid PRF induced significantly greater alkaline phosphatase activity, alizarin red staining, and a messenger RNA expression of genes encoding collagen type 1 alpha 1, dentin sialophosphoprotein, and dentin matrix protein 1 when compared with PRP. When hDPCs were cultured with LPS to stimulate an inflammatory environment, a marked decrease in dentin-related repair was observed. When liquid PRF was cultured within this inflammatory environment, the reduced regenerative potential in this LPS-produced environment was significantly and markedly improved, facilitating hDPC regeneration. The messenger RNA expression of inflammatory markers including tumor necrosis factor alpha, interleukin-1β, and p65 were all significantly decreased in the presence of liquid PRF, and, furthermore, liquid PRF also inhibited the transport of p65 to the nucleus in hDPCs (suggesting a reduced inflammatory condition). **Conclusions:** The findings from the present study suggest that liquid PRF promoted greater regeneration potential of hDPCs when compared with traditional PRP. Furthermore, liquid PRF also attenuated the inflammatory condition created by LPS and maintained a supportive regenerative ability for the stimulation of odontoblastic differentiation and reparative dentin in hDPCs. *(J Endod 2019;45:1000–1008.)*

## KEY WORDS

Human dental pulp cells; leukocyte and platelet-rich fibrin; odontoblastic differentiation; platelet-rich fibrin; platelet-rich plasma

More than two thirds of the world's population suffers from dental caries and/or pulpitis[1,2]. The treatments of dental caries and pulpitis range from coronary filling to root canal treatment in which the tooth is then permanently devitalized[3]. Ideally, maintaining the viability of the dental pulp is critical to dental homeostasis as well as durability, and there remains a critical need to develop novel clinical strategies and interventions to regenerate the dental pulp complex[4]. Although a number of regenerative strategies have been proposed, pulp capping remains 1 of the most recommended clinical options available for the repair of deep lesions. However, outcomes of currently available pulp capping materials are limited, with limited reported success because of irreversible pulp inflammation and a chance of reinfection[5]. Ideally, biomaterials used for pulp capping should be highly biocompatible with an ability to promote cell proliferation and differentiation, to promote or incorporate inductive growth factors, and to diminish the inflammatory environment.

Platelet concentrates are a concentration of autologous growth factors derived from peripheral blood offering a series of advantages including easily accessible, extremely biocompatible, economical, and reported regenerative potential[6,7]. They contain various growth factors such as transforming growth factor beta, platelet-derived growth factor, endothelial growth factor, and insulinlike growth factor, which induce cellular growth, migration, proliferation, and/or differentiation[8]. A first-generation platelet concentrate, platelet-rich plasma (PRP), has been shown to release the majority of its growth factor content within an early healing period (primarily within 8 hours), which has been shown to stimulate the healing of various tissues[9,10]. Therefore, PRP has been widely used as an adjunct to regenerative dentistry/medicine as well as esthetic medicine[11]. Despite this, some concerns have been raised within the past decade because of the use of anticoagulants during its preparation process[12]. As a result, platelet-rich fibrin (PRF) was developed as a second-generation platelet concentrate without the use of anticoagulants[13]. When compared with PRP, PRF contains a greater number of immune defense cells such as leukocytes, which play an important role in infection control/resistance[14]. In addition, the release of growth factors from the fibrin clots of PRF sustains a longer and more gradual release of growth factors over time, which is ideal for tissue repair[10]. It has previously been reported that the combination of PRP and PRF with human dental pulp cells (hDPCs) improved pulp regeneration in a canine tooth model[15–17]. Unfortunately, when PRP and PRF were used as a capping agent, there was no obvious reparative dentin formation in the PRF-alone group[18,19].

In 2016, a liquid version of PRF (liquid PRF) was made available by using slower and shorter centrifugation speeds and time (known as gentle centrifugation), which offered new advantages for regenerative medicine and dentistry[6]. Liquid PRF provides physicians a more operable and practical version of PRF because of its liquid formulation, which may then be used as an injectable biomaterial for a variety of applications including mixing with bone grafts/barrier membranes, injections into articular joints/temporomandibular joints, covering of serious foot ulcers, or cosmetic applications[20,21]. Furthermore, liquid PRF has been shown to promote the biological activity of various cell types[21]. Within liquid PRF, a larger concentration of platelets and leukocytes is obtained, further offering the potential advantages of enhancing the immune defense in inflammatory tissues with more growth factors available for release[22].

To date, no study has yet to investigate the regenerative potential of liquid PRF on pulp regeneration. Therefore, the aim of the present *in vitro* study was to compare the cellular regenerative activity of hPDCs when cultured with either liquid PRF or traditional PRP. Liquid PRF was also investigated on the expression of inflammation-related genes and the formation of reparative dentin under lipopolysaccharide (LPS)-induced inflammatory conditions and therefore aimed to provide a theoretical basis for the application of liquid PRF in regenerative endodontics.

## MATERIALS AND METHODS

### Preparation of PRP and Liquid PRF

The collection of whole blood samples was performed with the informed and written consent of each subject. All subjects were healthy young patients between the ages of 20 and 40 years old. Institutional review board approval was not required for the present study because patient data were not collected. PRP was prepared according to the protocol of Curasan as previously described[23]. In short, 10 mL peripheral whole blood was collected in a tube containing anticoagulant (EDTA). Centrifugation was performed at 900*g* for 5 minutes at room temperature to separate red blood cells from plasma. The supernatant was taken for a secondary centrifugation at 2000*g* for 15 minutes at room temperature to separate PRP from platelet-poor plasma. Approximately 1 mL PRP was obtained from 10 mL whole blood. The preparation of liquid PRF was obtained as follows: 10 mL whole blood was collected in polyethylene terephthalate plastic tubes (Chixin Biotech, Wuhan, China) without anticoagulant and immediately centrifuged at room temperature at 700*g* for 3 minutes. The upper plasma layer was collected as liquid PRF. The collected PRP and liquid PRF were then immediately transferred to 6-well plates and incubated with 5 mL medium (Dulbecco-modified Eagle medium [DMEM]; HyClone/Thermo Fisher Scientific, Waltham, MA) in a 37°C incubator with 5% $CO_2$ for 3 days. Afterward, the medium was collected for future experiments.

### Isolation of hDPCs

Fresh pulp tissue was obtained from healthy human third molars (18–22 years, $n = 5$). Briefly, the pulp tissue was cut into small pieces of 1 × 1 × 1 mm, and the tissue pieces were placed in T25 tissue culture flasks. DMEM (HyClone/Thermo Fisher Scientific) containing 20% fetal bovine serum (FBS; Gibco, Gaithersburg, MD) and 1% penicillin-streptomycin (HyClone/Thermo Fisher Scientific) were added. The pulpal tissue pieces were cultured in a 37°C incubator containing 5% $CO_2$. After growing the cells to confluency, they were passaged. hDPCs between passages 3–6 were used for this study.

### Cell Migration Assay

#### *Transwell Migration Assay*

The migration of hDPCs was performed using a transwell chamber with a pore size of 8 μm (Corning Costar, Corning, NY); 20% conditioned media from both the PRP and liquid PRF groups were added to DMEM supplemented with 10% FBS (500 μL/well) in the lower wells as previously described[24]. After starving the cells for 12 hours in DMEM containing 0.5% FBS, hDPCs were trypsinized, seeded in the upper transwell chamber ($10^5$ cells/100 μL/well), and cultured for 24 hours to allow for cell migration. Therefore, 4% formaldehyde was used to fix the cells for 5 minutes. Cells were then stained with 0.5% crystal violet (Amresco, Solon, OH) for 20 minutes followed by washing with phosphate-buffered saline (PBS). Nonmigrated cells were gently wiped from the upper side of the chamber using a cotton swab as per manufacturer's instructions. Migrated cells on the lower side of the chamber were then captured and counted

under a microscope (Olympus, Tokyo, Japan; 5 fields/well, ×200 magnification). The experiments were executed in triplicate with 3 independent experiments performed.

### *Scratch Wound Healing Assay*

hDPCs were seeded into 6-well plates at a density of $5 \times 10^5$ cells/well. After the cells reached confluency, a 10-μL pipette tip was used to scratch the cell layer according to the manufacturer's protocol. Then, cellular debris was washed away with PBS, and the culture medium containing PRP or liquid PRF was added. Images of the cells were immediately captured using a microscope (Olympus) and captured again after 24 hours. Reference points at the outer bottom of the plates were drew to find the location of the scratch to achieve the same field of view. The area of scratches after cell migration was measured using Photoshop (Adobe, San Jose, CA). The cell migration rate was calculated using the following formula: (D0h − D24h)/D0h × 100. D0h is the area at the start of the scratch, and D24h is the area after 24 hours.

### Proliferation Assay

hDPCs were seeded in 96-well plates at a density of $10^3$ cells/well with culture medium alone or supplemented with 20% conditioned media containing either PRP or liquid PRF. On days 1, 3, and 5 after seeding, cells were quantified using the Cell Counting Kit-8 assay (Dojindo, Kumamoto, Japan), and absorbance was measured at 450nm using a microplate reader (PowerWave XS2; BioTek, Winooski, VT). Samples were executed in triplicate with 3 independent experiments performed.

### Mineralization Induction of hDPCs

HDPCs were seeded in 12-well plates at a density of $10^4$ cells/well with normal growth media. After the cells reached 70%−80% confluence, the medium was replaced with mineralization-induction medium (DMEM plus 5 mmol/L beta-glycerol phosphate plus 10 nmol/L dexamethasone) containing 20% conditioned media from PRP or liquid PRF. The medium was replaced every 2 days.

### Alkaline Phosphatase Activity Assay

After 7 days of mineralization induction as described in the previous section, hDPCs were fixed with 4% formaldehyde for 15 minutes. After washing with PBS, hDPCs were then stained using alkaline phosphatase (ALP) staining (Alp; Beyotime, Shanghai, China). The ALP activity of HDPCs was determined using an ALP activity assay (BioQuest, San Francisco, CA). Briefly, hDPCs were treated with 0.1% Triton X-100 (Merck, Darmstadt, Germany) for 15 minutes at 4°C. After sonication, ALP activity of the cellular lysate was determined (p-nitrophenol mmol/L · $min^{-1}$ · $mg^{-1}$ protein) by measuring the optical density at 405 nm/optical density at 562 nm following the manufacturer's protocol. Total protein was quantified using the BCA Protein Assay Kit (Thermo Fisher Scientific). Samples were performed in triplicate with 3 independent experiments.

### Alizarin Red Staining

After 14 days of mineralization induction with either 20% conditioned media from PRP or liquid PRF, hDPCs were fixed with 4% formaldehyde for 15 minutes. After washing with distilled water, hDPCs were then incubated with 0.1% alizarin red (Sigma-Aldrich, St Louis, MO) at 37°C for 1 hour. Images were then captured using a laboratory microscope (Olympus). For quantity analysis, 10% hexadecylpyridinium chloride was used to dissolve the mineralization nodules for 1 hour and thereafter quantified using an absorbance measurement at 562 nm.

### Real-time Polymerase Chain Reaction for Differentiation and Inflammatory Markers

Total messenger RNA from cells was isolated with the AxyPrep Multisource Total RNA Miniprep Kit (Axygen Inc, Union City, CA) following the manufacturer's instructions. The concentration of RNA was determined using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific). A total of 1 μg RNA was then reverse transcribed to complementary DNA by a cDNA Synthesis Kit (GeneCopoeia, Rockville, MD). The following primer sequences were used: collagen type I alpha 1 *(Col1a1)*: forward, 5′-GATTCCCTGGACCTAAAGGTGC-3′; reverse, 5′-AGCCTCTCCATCTTTGCCAGCA-3′; dentin sialophosphoprotein *(DSPP)*: forward, 5′-TGCTGGAGCCACAAAC-3′; reverse, 5′-AAACCCTATGCAACCTTC-3′; the primer of dentin matrix protein 1 *(DMP-1)*: forward: 5′-F ACAGGCAAATGAAGACCC-3′; reverse: 5′-TTCACTGGCTTGTATGG-3′; tumor necrosis factor alpha *(TNF-α)*: forward, 5′-TGGGATCATTGCCCTGTGAG-3′, reverse, 5′-GGTGTCTGAAGGAGGGGGTA-3′; interleukin 1 beta *(IL-1β)*: forward, 5′-CTCAGCCTCCTACTTCTGCTTT-3′; reverse, 5′-GTGCCTCGAAGAGGTTTGGT-3′; *p65*: forward, 5′-CAATCACGATCGTCACCG GA-3′; reverse, 5′-CGTAAAGGGATAG GGCTGGG-3′; and glyceraldehyde 3-phosphate dehydrogenase: forward, 5′-GCACCGTCAAGGCTGAGAAC-3′; reverse, 5′-TGGTGAAGACGCCAGTGGA-3′. A 20-microliter final reaction volume was used for quantification using the All-in-One qPCR Mix Kit (GeneCopoeia) on the CFX Connect Real-Time PCR Detection System (BIO-RAD, Hercules, CA). Glyceraldehyde 3-phosphate dehydrogenase was used to normalize the amount of messenger RNA of interest.

### Experimental Inflammation Induced by LPS

In order to create a consistent inflammatory condition *in vitro*, cells were pretreated with 10 μg/mL LPS (*Escherichia coli*, Sigma-Aldrich) for 7 days. To examine the anti-inflammatory effect of liquid PRF, cells were cultured with the concomitant treatment of LPS and liquid PRF for 7 days. To determine odontoblastic differentiation within these inflammatory conditions, cells were cultivated in odontogenic medium with/without LPS and with/without liquid PRF for up to 14 days. After culture, alizarin red staining and quantitative polymerase chain reaction for gene expression of Col1a1, DSPP, and DMP-1 was investigated as previously described in this study.

### Immunofluorescence Staining

At 14 days after seeding, hDPCs were fixed using 4% formaldehyde for 15 minutes, treated with 0.5% Triton X-100 for 10 minutes at 4°C, and then washed with PBS and incubated with 2% bovine serum albumin (Roche, Basel, Switzerland) at 37°C for 1 hour. Thereafter, cells were incubated with p65 antibodies (1:100; abcam, Cambridge, UK) at 4°C overnight. After washing with PBS, the samples were then incubated with a secondary antibody of FITC-conjugated goat antirabbit (1:400; Invitrogen, Carlsbad, CA) at 37°C for 1 hour in dark conditions. After rinsing with PBS again, cells were then incubated with 4′,6-diamidino-2-phenylindole for 3 minutes. Images were captured by a fluorescence microscope (DP71, Olympus).

### Statistical Analysis

Statistical analysis was performed using 1- or 2-way analysis of variance, and the Student-Newman-Keuls test using GraphPad Prism Software v.6 (GraphPad Software, La Jolla, CA). Statistical significance was considered at $P < .05$. All data were expressed at the mean ±standard deviation.

## RESULTS

### The Effect of Liquid PRF and PRP on Cell Migration

To examine the effects of liquid PRF and PRP on the migration of hDPCs, a scratch wound

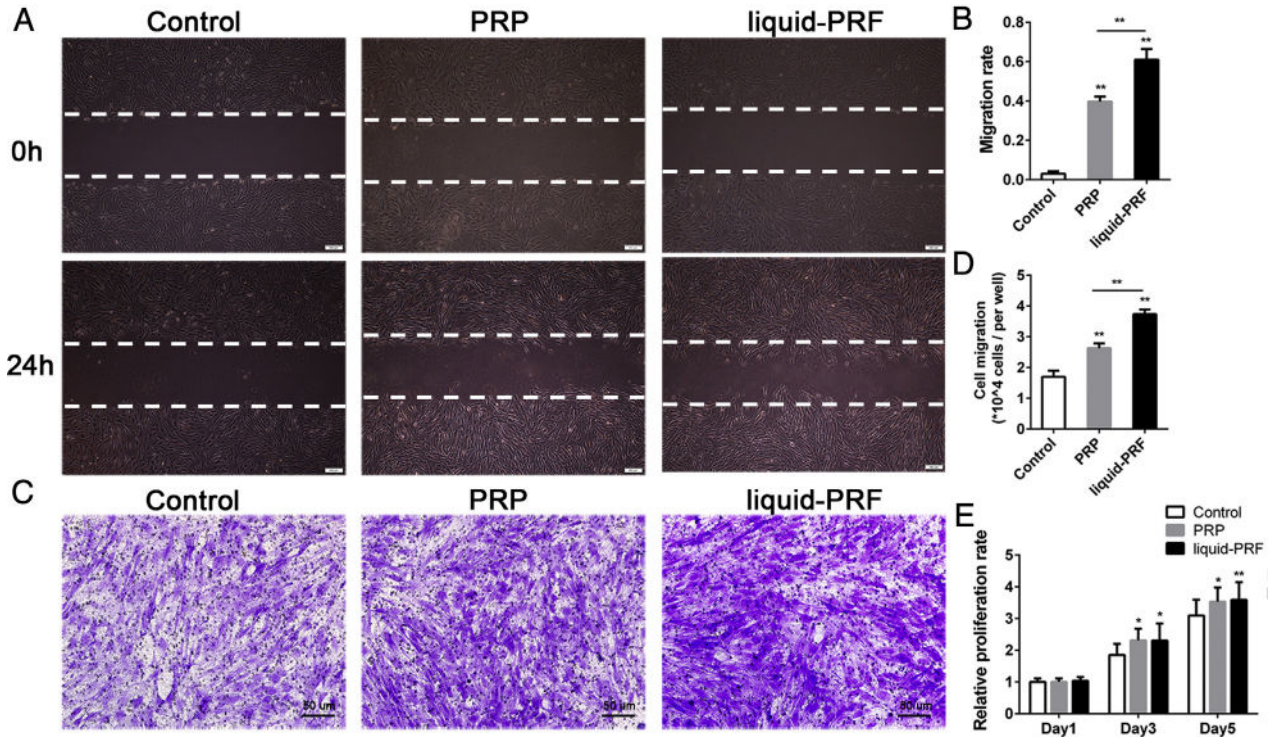


**FIGURE 1 –** The migration and proliferation of hDPCs cultured with PRP or liquid PRF. (*A*) Cell images of a scratch wound healing assay. (*B*) The migration rate of each group was calculated according to the area's coverage after 24 hours. (*C*) Cell images of a transwell migration assay. (*D*) The number of migrated cells ($10^4$ per well) from the transwell migration assay was analyzed after being cultured for 12 hours. PRP and liquid PRF significantly increased cell migration compared with the control. (*E*) The Cell Counting Kit-8 assay was used to investigate the proliferation ratio of the PRP- and liquid PRF–treated cells up to 5 days. A significant increase in cell numbers was found after cell culture with PRP and liquid PRF when compared with the control. The error bars correspond to the means ± standard deviation. Significant differences are indicated: $^*P < .05$, $^{**}P < .01$ when compared with the control. ns, not statistically significant versus the control group.

healing assay and a transwell migration assay were performed (Fig. 1). In the first experiment, the scratched areas in both the PRP and liquid PRF groups were significantly filled faster when compared with the control group ($P < .05$). Furthermore, a significant improvement was also observed in the liquid PRF group when compared with PRP (Fig. 1*A* and *B*). Similarly, quantitative analysis of migrated cells using the transwell assay confirmed the positive effect of either liquid PRF or PRP on cell migration (Fig. 1*C* and *D*).

## The Effect of Liquid PRF and PRP on Cell Proliferation

The Cell Counting Kit-8 (Dojindo, Japan) assay was then used to investigate cell proliferation (Fig. 1*E*). After cell culture with PRP or liquid PRF, a significant increase in cell number was observed at 3 or 5 days after seeding when compared with the control ($P < .05$). No significant difference was observed between liquid PRF and PRP.

## The Effect of Liquid PRF and PRP on Odontogenic Differentiation

The ALP activity of hDPCs was then investigated after culture with liquid PRF and PRP (Fig. 2). It was found that ALP activity was significantly increased in both platelet concentrates when compared with controls. However, no statistically significant difference was observed between the liquid PRF and PRP groups (Fig. 2*A* and *B*). Alizarin red S staining also showed that more mineralized nodules were formed in both platelet concentrate groups when compared with the control group, especially in the liquid PRF group (Fig. 2*C*). The results from the gene expression level analysis revealed that Col1a1, DMP-1, and DSPP all showed significantly elevated expression in the liquid PRF and PRP groups when compared with the control (Fig. 2*D*–*F*).

## The Anti-inflammation Effect of Liquid PRF

To further evaluate the ability for PRF to influence odontoblast differentiation during an inflammatory environment, LPS was cultured with hDPCs. According to the experimental time line, cells were pretreated with/without LPS for 7 days, and then liquid PRF was added into the media for 7 days (Fig. 3*A*). After culturing the cells with/without LPS as well as with/without liquid PRF, immunofluorescence staining of p65 was evaluated, showing that immunofluorescence intensity of *p65*-positive cells in the LPS-treated group was higher than those cultured without LPS (Fig. 3*B*). Additionally, the gene expression levels of proinflammatory mediators including *TNF-α*, *IL-1β*, and *p65* also significantly increased in the presence of LPS (Fig. 3*C*). When liquid PRF was added to LPS-cultured cells, a significant reduction in the expression levels of inflammatory markers as well as immunofluorescence staining of *p65* was observed, suggesting that liquid PRF could partially attenuate the inflammatory condition induced by LPS (Fig. 3*B* and *C*).

## The Effect of Liquid PRF on Odontoblast Differentiation during an LPS-induced Inflammatory Environment

To examine the odontoblastic differentiation of hDPCs during an inflammatory condition, the LPS-pretreated cells were cultured in odontogenic induction medium in the presence of LPS for an additional 14 days (Fig. 4). Alizarin red S staining revealed an intense reduction in mineralized deposition observed in cells treated with LPS (Fig. 4*A*). Furthermore, gene expression levels of

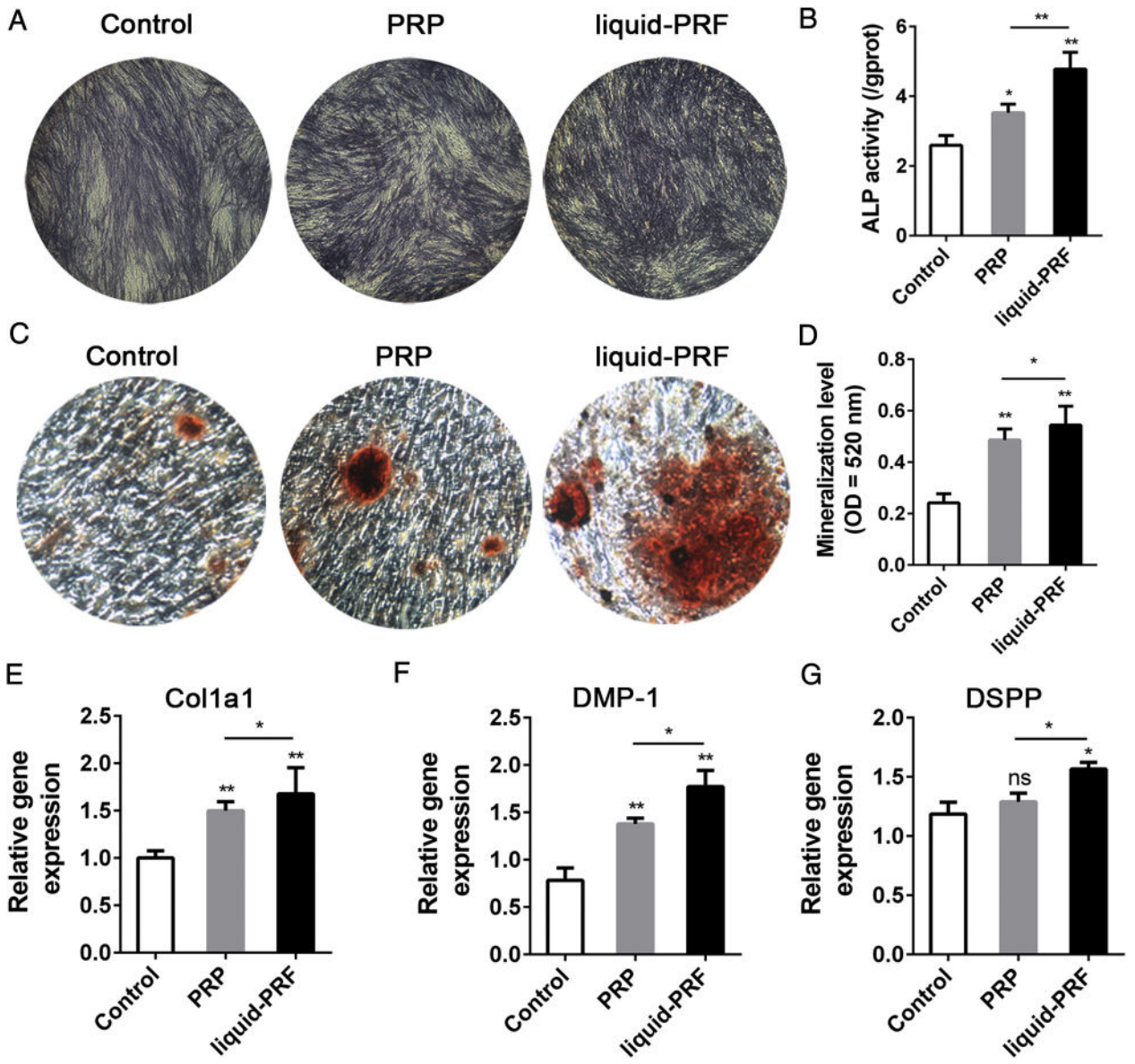


**FIGURE 2 –** The effect of PRP and liquid PRF on odontoblast differentiation of hDPCs. (*A* and *B*) The effects of PRP and liquid PRF on ALP activity were detected by (*A*) ALP staining and (*B*) ALP activity test. (*C* and *D*) Alizarin red S staining showed the mineralized nodules in each group after induction for 14 days; the right panel indicates the semiquantification of the mineralization level. (*E–G*) Relative gene expression levels of (*E*) *Col1a1*, (*F*) *DMP-1*, and (*G*) *DSPP* after culture with PRP or liquid PRF for 14 days. The error bars correspond to the means ± standard deviation. Significant differences are indicated: $^{*}P < .05$, $^{**}P < .01$; ns, not statistically significant versus the control group.

Col1a1, DMP-1, and DSPP were all significantly down-regulated after LPS treatment (Fig. 4*B*). After additional culture with liquid PRF, it was revealed that liquid PRF was able to attenuate some of the lost odontogenic potential induced by LPS (Fig. 4). However, complete recovery was not observed (Fig. 4).

## DISCUSSION

The purpose of the present study was to compare the cellular regenerative activity of hDPCs cultured with either PRP or liquid PRF. This is the first study to evaluate the potential effect of liquid PRF on hDPCs as a potential regenerative adjunct to pulp capping for the treatment of pulpitis. In the present study, it was observed that in general both PRP and liquid PRF showed significant regenerative potential of hDPCs. Of the 2 platelet formulations, liquid PRF did show a further significant increase in a number of experiments when compared with PRP.

Naturally, the role of the centrifuge is to separate blood cell layers based on cell density. The original centrifugation speed of PRF was described at high speeds (ranging from 2500–2700) for 10 to 12 minutes[25]. The theoretical advantage of PRF over PRP is that it forms fibrin after centrifugation because anticoagulants are not used. As a result, growth factors and cells (platelets and leukocytes primarily) promoted the slow and sustained release process of bioactive factors[14,23]. More recently, it was found that lower centrifugation speeds and time resulted in an optimized PRF clot with a higher concentration of entrapped platelets and leukocytes, resulting in a higher release of growth factors[26,27]. Because PRF is found in the upper portion of blood collection tubes, centrifugation at high speeds and prolonged periods of time has been known to push cells toward the bottom of centrifugation tubes, away from the upper layer where PRF clots are

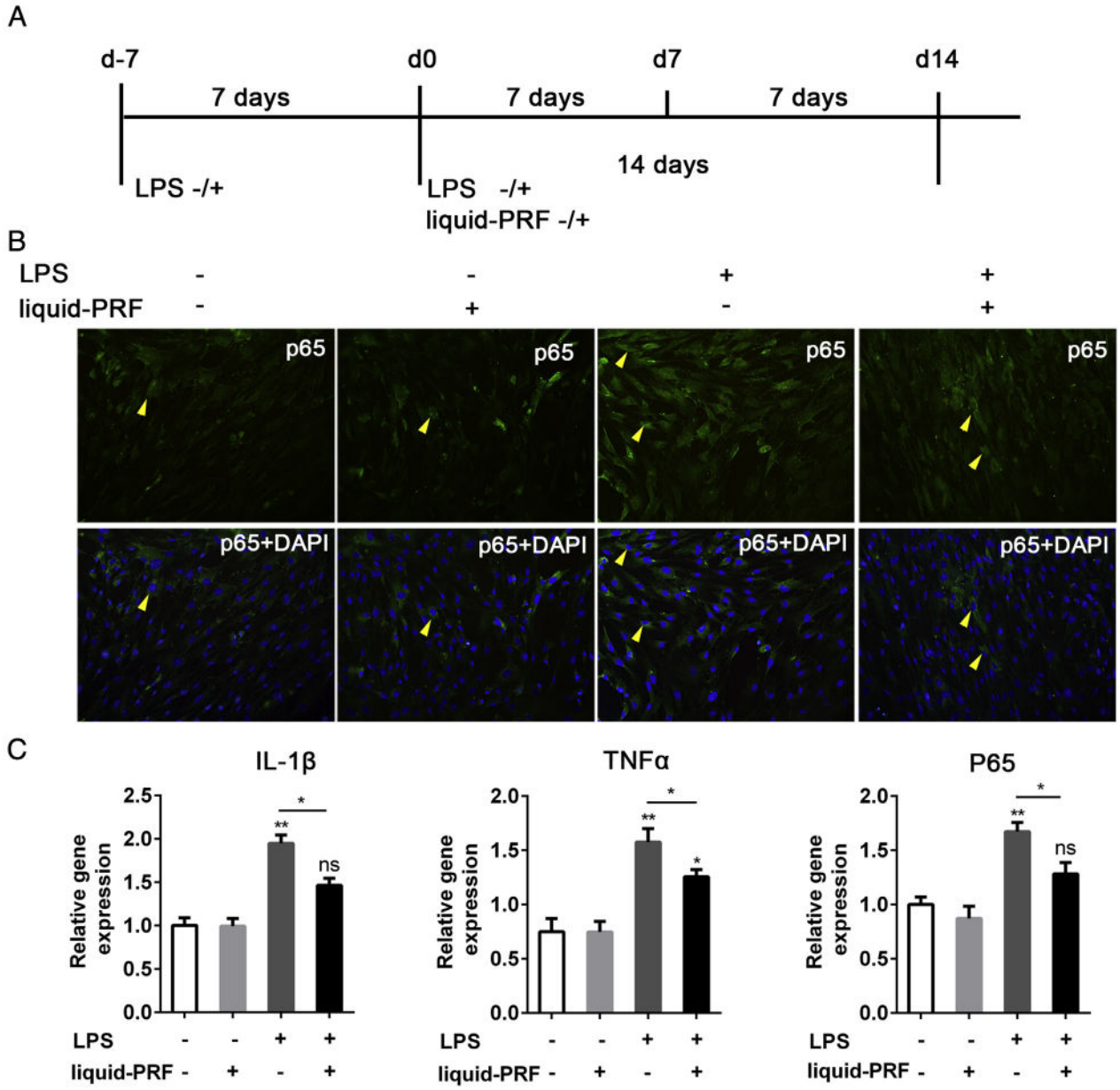


**FIGURE 3 –** The expression of inflammatory markers in LPS-treated hDPCs. (*A*) An overview time line of the experimental setup. (*B*) Immunofluorescence staining of *p65* in hDPCs after cultured with or without LPS and/or liquid PRF. The *arrows* correspond to areas of enhanced expression of *p65*. (*C*) The relative gene expression levels of inflammatory markers including *IL-1β, TNF-α,* and *p65*. The error bars correspond to the means ± standard deviation. Significant differences are indicated: $^{*}P < .05$, $^{**}P < .01$; ns, not statistically significant versus the control group.

collected[26,27]. Shortly after this development, it was further reported that by reducing the centrifugation speeds and time even further, a separation of layers could be achieved even before clot formation[6]. This new formulation of PRF remained liquid for ~20 to 25 minutes before clot formation and could be used as an injectable biologic similar to PRP. Within the present study, we investigated for the first time this new formulation of liquid PRF on pulp cell regeneration in comparison with PRP. Because this liquid PRF can be used as an injectable PRF, it offers the advantage of being used with a higher penetration ability (because it is liquid) and could also be used easily to flow into root canals (if needed) and only thereafter produce a 3-dimensional fibrin scaffold within the pulp chamber/roots. Therefore, it offers great regenerative potential as a flexible biomaterial, and, equally importantly, it is 100% autologous and therefore will not cause an inflammatory reaction. It further provides the benefit of containing supraphysiological concentrations of defense-fighting leukocytes.

Yeom et al[28] reported that PRP increased the expressions of odontoblastic markers, such as *DSPP* and *DMP-1*, in dental pulp cells derived from the dental papilla of rat incisors. Similarly, Otero et al[29] found that the potential of PRP to induce osteogenic differentiation of hDPCs was better than culture medium with ascorbic acid or melatonin. Because of this previous research on the topic, our research group was interested in investigating and comparing the activity of liquid PRF and PRP on hDPCs. In a first set of experiments, it was found that liquid PRF induced significantly greater cell migration when compared with PRP (Fig. 1). Although no

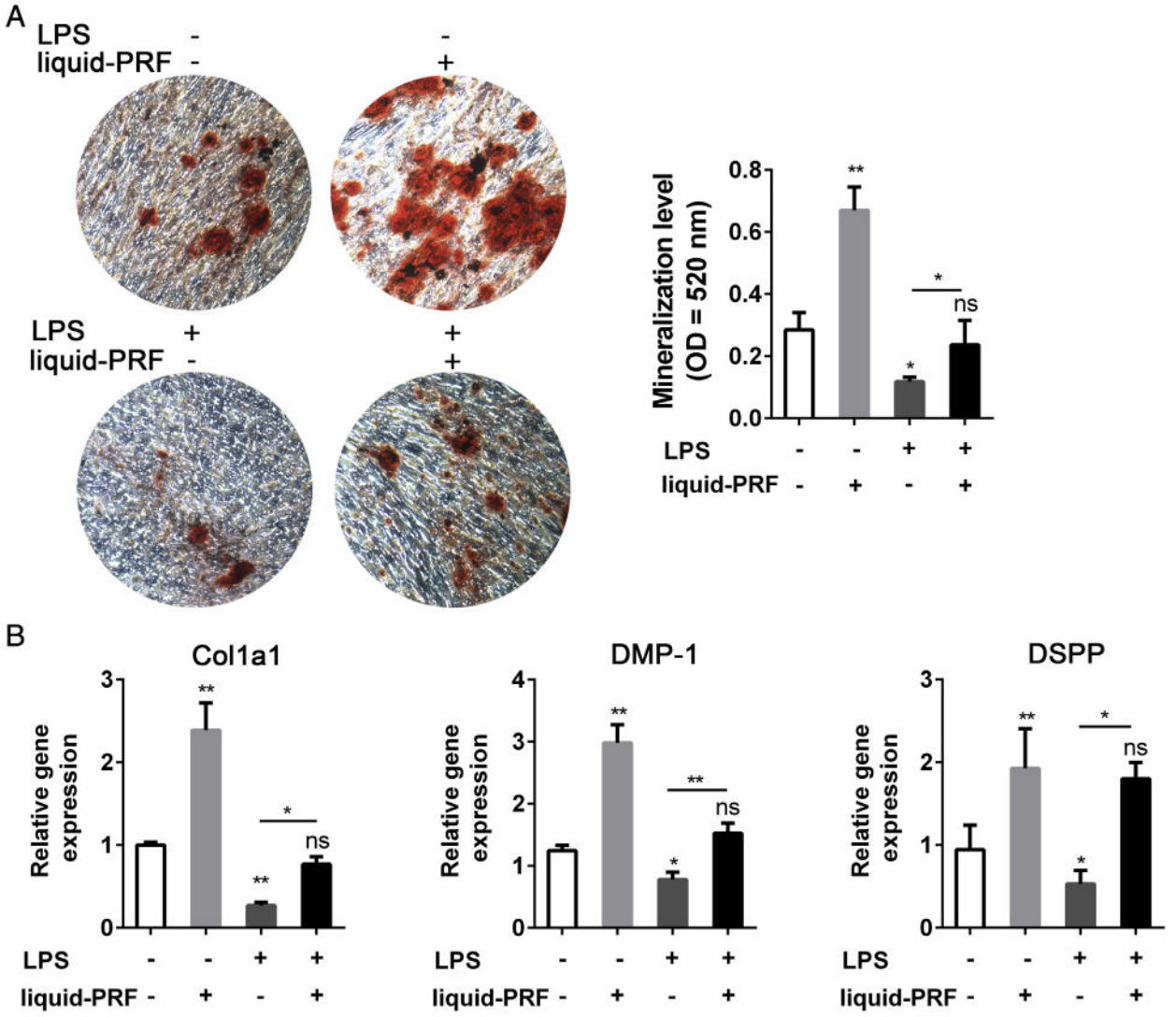


**FIGURE 4 –** Odontoblast differentiation of hDPC cultures in LPS inflammatory conditions with liquid PRF. (*A*) Alizarin red S staining of hDPCs cultured in the presence of LPS and/or liquid PRF. (*B*) Gene expression levels of Col1a1, DMP-1, and DSPP in inflammatory conditions. The error bars correspond to the mean ± standard deviation. Significant differences are indicated: $*P < .05$, $**P < .01$. ns, not statistically significant versus the control group.

differences in cell proliferation were observed, liquid PRF also significantly promoted the odontogenic differentiation of hDPCs when compared with PRP (Fig. 2).

Interestingly, PRP has also been used for pulp regeneration in combination with various other regenerative modalities including enamel matrix derivative and hydroxyapatite as a direct capping agent in various rat or monkey animal models[18,30]. In those studies, it was found that the added benefit of platelet concentrates further induced odontoblastlike cells and the formation of reparative dentin[18,30]. In a recent article, we described the potential for liquid PRF to be used as a carrier system because it clots upon injection/use[31]. Remarkably, standard PRF has already been used on patients with success for carious pulp exposures[19,32]. After a 22-month follow-up period, the tooth recovered positively, responded to pulp sensibility tests, and radiographically showed a normal periodontal ligament space[19]. Therefore, we aimed to introduce a potential type of autologous and liquid pulp capping agent with an ability to clot into a 3-dimensional matrix.

It is also known that PRF (often referred to as leukocyte) contains a supraphysiological concentration of leukocytes, which are 1 of the primary cells in the body responsible for fighting against bacterial invasions. Therefore, we hypothesized that their incorporation into LPS-induced environments may facilitate a pro–wound healing environment. LPS is the major component of the bacterial membrane of gram-negative bacteria that contributes to pulpitis[33]. We mimicked the environment of pulpitis by treating hDPCs with LPS. In our study, it was found that the liquid PRF attenuated the inflammatory level in LPS-treated hDPCs and facilitated potential repair of dentin under these inflammatory conditions. It was shown that the expression levels of inflammatory-related genes was significantly reduced after treatment with liquid PRF, including TNF-α and IL-1β (genes expressed during pulpitis)[34]. These inflammatory cytokines play important roles in a variety of signaling cascades, such as the nuclear factor kappa B signaling pathway[35]. It was further found that liquid PRF partially inhibited the transport of nuclear factor kappa B (p65) to the nucleus (Fig. 4*B*) and therefore may reduce the activation of the downstream signaling pathways responsible for creating an inflammatory response with hDPCs. Future research on this topic, potential even with various bacteria found during pulp infection, is needed to further characterize the ability of PRF not only to impact odontoblast cell activity but

also to act as a natural autologous defense-fighting biomaterial containing living cells.

In summary, the present study showed that this newly developed liquid PRF promotes hDPC migration, proliferation, and differentiation and was associated with a partial immune defense in response to an LPS-induced inflammatory condition. This study further provides the theoretical basis for further investigation of liquid PRF in further animal and human studies as a conservative, autologous, and low-cost treatment modality for pulpitis.

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*The authors deny any conflicts of interest related to this study.*

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