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Platelet-Derived Growth Factor-BB Promotes Proliferation and Osteogenic Differentiation of Rat Bone Marrow Mesenchymal Stem Cells

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Background: Previous research has indicated that platelet-rich plasma (PRP) promotes fracture healing and aids in the treatment of nonunion. A key component of PRP, platelet-derived growth factor BB (PDGF-BB), may play a crucial role in PRP, enhancing the biological functions of bone marrow mesenchymal stem cells (BMSCs). This study aims to investigate whether PDGF-BB is a key effector in PRP that promotes proliferation and osteogenic differentiation of BMSCs.
Methods: Rat BMSCs were isolated and cultured, then expanded to the third generation for morphological observation. Flow cytometry analysis was conducted to assess the expression of CD44, CD29, CD45, and CD11b. The BMSCs were cultured under different conditions: the control group received only basic culture medium, while experimental groups received 10 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, and 200 ng/mL PDGF-BB. Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) assay at 1, 3, 5, 7, and 10 days. The optimal PDGF-BB concentration was determined based on the CCK-8 results for subsequent experiments. Blood was collected from the rat's heart and used to prepare and activate platelet-rich plasma (PRP), which was then stored in liquid nitrogen for later use. According to the culture conditions for BMSCs, the experimental groups were as follows: a blank control group, a 10% PRP group, a 50 ng/mL PDGF-BB group, and a 10% PRP + 100 μM AG1295 [platelet-derived growth factor β receptor (PDGFR-β) inhibitor] group. Each experimental group was replicated three times. Cell proliferation was assessed using the CCK-8 assay, the cell cycle was analyzed using flow cytometry, and the expression of osteogenic differentiation markers was evaluated by Western blot.
Results: The cell viability of BMSCs treated with 50 ng/mL of PDGF-BB for 5 days was significantly higher than that of other concentration groups and time points. CCK-8 and flow cytometry results indicated that compared to the control group, both 10% PRP and 50 ng/mL PDGF-BB significantly promoted BMSCs proliferation and increased the proportion of BMSCs in the S phase of the cell cycle. Western blot results demonstrated that compared to the control group, both 10% PRP and 50 ng/mL PDGF-BB significantly upregulated the protein expression levels of osteogenic differentiation markers. The use of the PDGFR-β inhibitor AG1295 markedly attenuated the proliferative and osteogenic effects of 10% PRP on BMSCs.
Conclusions: A concentration of 50 ng/mL PDGF-BB significantly enhances the proliferation and osteogenic differentiation of rat BMSCs. PDGF-BB may play a key role in PRP, contributing to the enhancement of BMSCs' proliferation and osteogenic differentiation.

Keywords: platelet-derived growth factor (PDGF); bone marrow mesenchymal stem cells (BMSCs); platelet-rich plasma (PRP); proliferation; osteogenic differentiation

Introduction

The incidence of delayed fracture healing or nonunion globally ranges from 1.9% to 4.9% [1], causing severe physical and mental suffering as well as increased financial burden for patients [2]. Successful fracture healing requires not only adequate mechanical stability but also a favorable biological microenvironment [3]. Therefore, the use of the regenerative differentiation capabilities of stem cells and tissue engineering techniques involving cytokines holds considerable promise for treating nonunion. Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells with self-renewal and multidirectional differentiation potential. They can migrate and recruit to the fracture site, differentiate into osteoblasts, and secrete biologi-

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cally active molecules to regulate the microenvironment at the fracture site, thereby promoting fracture healing [4,5]. However, the number and activity of osteoblasts in the nonunion region are low. Consequently, transplanting BMSCs into the nonunion site and altering the local microenvironment to stimulate the proliferation and osteogenic differentiation of BMSCs present prospects for nonunion treatment [6,7].

Platelet-rich plasma (PRP) is a plasma preparation obtained by centrifuging autologous peripheral blood, containing platelets at concentrations 5 to 10 times higher and growth factors at concentrations 3 to 5 times higher than physiological baseline levels. PRP acts on the extracellular matrix, modulating the cellular microenvironment to enhance the biological functions of BMSCs [8–10]. Studies have reported that PRP can stimulate BMSC proliferation, and their combined transplantation shows certain efficacy in treating nonunion, although the specific mechanisms remain unclear [11,12]. Platelet-derived growth factor (PDGF) is a significant component of activated PRP, comprising five polymorphic subtypes: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD [13]. Among these subtypes, platelet-derived growth factor BB (PDGF-BB) is the most potent, capable of binding to all receptor molecules (PDGFR-$\alpha\alpha$, PDGFR-$\alpha\beta$, and PDGFR-$\beta\beta$), mediating cell chemotaxis and proliferation. It plays a crucial role in tissue repair and regeneration [14,15].

This study aims to investigate the proliferative effects of PDGF-BB on BMSCs, determine its optimal concentration, and compare the osteogenic differentiation capabilities of BMSCs under different culture conditions. The goal is to elucidate whether PDGF-BB is a key effector in PRP for promoting both the proliferation and osteogenic differentiation of BMSCs. These findings will provide experimental evidence for the potential application of PDGF-BB in tissue engineering techniques for the treatment of nonunion.

Materials and Methods

Optimal Intervention Time and Concentration of PDGF-BB on BMSCs

Isolation, Cultivation, and Identification of Rat BMSCs

Ten 4-week-old male Sprague-Dawley (SD) rats, weighing 200–220 g, were obtained from the Laboratory Animal Center of Kunming Medical University [Animal License Number: SYXK (Dian) K2020-0006]. The rats were kept under standard laboratory conditions with free access to food and water and were housed in a room maintained at 23 ± 2 °C, 60 ± 10% humidity, and a 12-hour light/dark cycle. Euthanasia was performed by cervical dislocation. The femurs were dissected, and external muscles were removed. The bone specimens were briefly immersed in 75% ethanol for 30 s, and the bone ends were excised. Bone marrow cells were flushed out into a culture dish using Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) (G4612, Servicebio, Wuhan, China) until the marrow cavity turned white. Subsequently, the wash fluid was pipetted to generate a single-cell suspension and centrifuged at 1000 rpm for 5 min using a centrifuge (TD-5Z, Shuke, Chengdu, China). The supernatant was discarded, and the cell pellet was resuspended in DMEM/F-12 medium. The suspended cells were then seeded into two new T25 culture flasks and statically cultured in a 37 °C, 5% CO_2 cell culture incubator (CL-191C, Crystal, Dallas, TX, USA). After 48 hours, the medium was replaced, and subsequent changes were made every two days until the cell confluence exceeded 90%. At this point, the cells were passaged. For the collection of BMSCs in the P3 generation, 0.25% trypsin (25200-056, Invitrogen Gibco, GrandIsland, NY, USA) was used to detach the cells. After removing the supernatant, the cells were washed twice with phosphate-buffered saline (PBS) (G4201, Servicebio, Wuhan, China). The BMSCs tested negative for mycoplasma. The cells were then divided into five tubes, with one tube serving as a blank control. The remaining four tubes were treated with CD11b (17-0112-82, eBioscience, San Diego, CA, USA), CD45 (11-0461-82, eBioscience, San Diego, CA, USA), CD44 (MA5-17522, eBioscience, San Diego, CA, USA), and CD29 (12-0291-82, eBioscience, San Diego, CA, USA) respectively, and incubated at 4 °C in the dark for 30 min. A flow cytometer (V0B4R2, Agilent, San Diego, CA, USA) was then used to assess the positivity rates.

Cell Counting Kit-8 (CCK-8) Assay

After being detached with 0.25% trypsin, cells were harvested, counted, and then seeded into a 96-well plate at a density of 5×10^3 cells per well, with 15 replicates per group. An additional 15 wells were used as a control group. Once the cell confluence reached approximately 70%, the culture medium was replaced with a basal culture medium. The control group was supplemented with a basic culture medium only, while the experimental groups were exposed to PDGF-BB (HY-P7278, MedChemExpress, Monmouth Junction, NJ, USA) at concentrations of 10 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, and 200 ng/mL, respectively. Each experimental group was replicated three times. Cell viability was assessed at 1, 3, 5, 7, and 10 days post-intervention using the Cell Counting Kit-8 (CCK-8) assay. Absorbance values were measured at a wavelength of 450 nm using an enzyme-linked immunosorbent assay reader (EL800, BIO-TEK, VT, USA). Optical density (OD) readings were converted to cell viability using the formula: Cell viability (%) = [(OD of experimental group – OD of blank group)/(OD of control group – OD of experimental group)] × 100%. The optimal intervention concentration and duration were selected for subsequent research conditions.

Assessment of PDGF-BB as the Key Effector in PRP for Promoting the Proliferation and Osteogenic Differentiation of BMSCs

Preparation and Activation of PRP

A 10 mL pre-loaded syringe containing 1 mL of sodium citrate (G0321, Servicebio, Wuhan, China) was used to collect 7 mL of blood from the hearts of genetically identical rats. The blood was then centrifuged at 2400 rpm for 10 min to remove the red blood cell and white blood cell layers. Subsequently, another centrifugation step was performed at 3600 rpm for 15 min, resulting in the preparation of 1.5 mL of PRP. A sterile 20% calcium chloride solution was prepared, and thrombin (G2411, Servicebio, Wuhan, China) was diluted to a concentration of 1000 U/mL to serve as the activator. The activator was then mixed with the prepared PRP at a ratio of 1:20, thoroughly pipetted, and stored at 4 °C for 12 h. After centrifugation at 4000 rpm for 30 min, the supernatant obtained was considered the activated PRP. The activated PRP was filtered through a 0.22 μm membrane and immediately used or stored in liquid nitrogen.

Cell Counting Kit-8 (CCK-8) Assay

Using 0.25% trypsin, cells were collected, counted, and seeded into 96-well plates at a density of 5×10^3 cells per well with three replicates per group. Additionally, cells were seeded into 6-well plates at a density of 1×10^5 cells per well with six replicates per group. When the cells reached about 70% confluence, the culture medium was replaced with basal culture medium, and the following treatment conditions were established: 10% fetal bovine serum (FBS) (10099-141, Gibco, NY, USA), 10% FBS + 10% activated PRP, 10% FBS + 50 ng/mL PDGF-BB, and 10% FBS + 10% activated PRP + 100 μM AG1295 (HY-101957, MedChemExpress, Monmouth Junction, NJ, USA). Each experimental group had three replicates. After 5 days of incubation, cells from the 96-well plates were subjected to CCK-8 assay, and absorbance values were measured at a wavelength of 450 nm using an enzyme-linked immunosorbent assay reader. The OD readings were then converted to cell viability.

Cell Cycle Assay

The experimental group was performed as described in the previous step, and each experimental group had three replicates. After 5 days of cultivation in the 6-well plates, the culture medium was aspirated, and the cells were washed twice with PBS. Each well was then treated with 150 μL of 0.25% trypsin for cell digestion. Digestion was terminated by adding 500 μL of fresh complete culture medium immediately upon observing cell contraction into spherical or sandy shapes. After centrifugation at 1000 rpm for 5 min, the supernatant was discarded, and the cell pellet was resuspended in 1 mL of pre-chilled PBS. Subsequent centrifugation at 1000 ×g for 5 min was performed to pellet the cells again. The cell pellet was gently mixed with 1 mL of pre-chilled 70% ethanol for fixation at 4 °C for at least 2 h or overnight. After another centrifugation at 1000 rpm for 5 min, the cells were resuspended in 1 mL of pre-chilled PBS. The cells were then centrifuged again at 1000 ×g for 5 min. Each cell sample was treated with 0.5 mL of propidium iodide staining solution, and after gentle mixing, the cells were resuspended. Following a 30-minute incubation at 37 °C in the dark, the samples were ready for analysis using a flow cytometer with excitation at 488 nm and detection of red fluorescence.

Western Blotting

The experimental group was performed as described in the previous step, and three replicates were set for each group. The cells were collected and washed twice with PBS. Next, 300 μL of Radio-Immunoprecipitation Assay (RIPA) lysis buffer (P0013B, Beyotime, Beijing, China) was added to each well, and the cells were lysed on ice for 10 min. The samples were then centrifuged at 4 °C and 12,000 rpm for 10 min, and the supernatant was collected. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protein loading buffer (P0015L, Beyotime, Beijing, China) was added to the supernatant. The protein concentration of each sample was determined using a spectrophotometer (EzDrop1000, Blue-Ray Biotech, Taiwan, China), and the protein loading amount for each sample was adjusted to 60 μg. Subsequently, gel casting and sample loading were performed on a glass plate. Electrophoresis was conducted at a constant voltage of 60 V until the samples reached the separation gel, then switched to 90 V until the bromophenol blue traveled 0.7 cm from the bottom of the separating gel. The wet transfer method was used for membrane transfer. The polyvinylidene difluoride (PVDF) membrane (ISEQ00010, Millipore, St. Louis, MO, USA) was immersed in 5% skimmed milk at room temperature (20 °C–30 °C) for 1 h for blocking. Subsequently, it was placed on a shaker (WD-9405F, Liuyi, Beijing, China), and washed three times with buffer solution for 5 min each time. After blocking with skim milk, it was incubated with primary antibodies against transforming growth factor-β (TGF-β, 1:2000, bs-0086R, Bioss, Beijing, China), bone glaprotein (BGP, 1:2000, bs-6909R, Bioss, Beijing, China), and bone morphogenetic protein-9 (BMP-9,1:2000, bs-4896R, Bioss, Beijing, China) at 4 °C overnight, and then incubated with the secondary antibody IgG-HRP (1:4000, M21001L, Abmart, Shanghai, China) at 37 °C for 1 h. ImageJ (1.8.0, NIH, Bethesda, MD, USA) was used to measure and analyze the grayscale values of protein bands, detecting the relative protein expression levels.

Statistical Analyses

SPSS 23.0 (IBM, Armonk, NY, USA) statistical software was utilized for data processing. Additionally, data vi-

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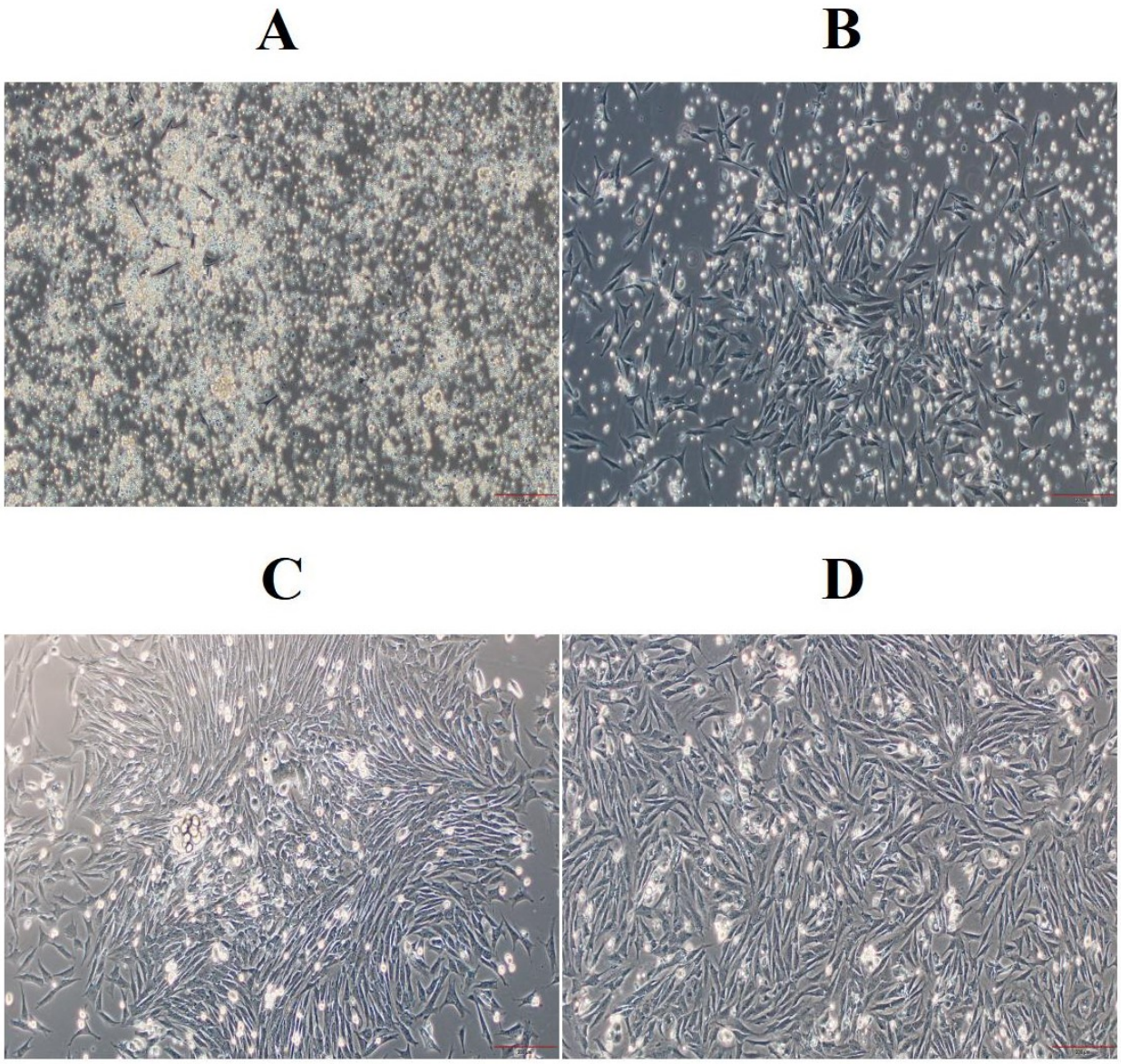

Fig. 1. Morphology of rat BMSCs. (A–C) Cell morphology of primary culture of rat BMSCs after 1 day, 3 days, and 7 days. The morphology of primary cells was irregular, and the growth was adherent to the wall. After 3 days, the growth of cells was not uniform, and colonies were formed, and the cells were overgrown after 7 days. The shape of primary cells was gradually uniform after multiple subcultures. (D) Cell morphology of the P3 generation. The cell morphology of the P3 generation began to be relatively uniform and fibrous, and it was arranged regularly and neatly. (scale bar: 200 μm). BMSCs, bone marrow mesenchymal stem cells.

sualization was performed using GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA) software. The means of multiple groups were compared by one-way analysis of variance (ANOVA), with a significance level of $p < 0.05$.

Results

Optimal Intervention Time and Concentration of PDGF-BB on BMSCs

Morphological Characteristics and Flow Cytometry Results of Rat BMSCs

The cells of P0 generation exhibited diverse morphology, characterized by irregular shapes and significant heterogeneity. Colony formation was visible after 3 d, and the bottom of the bottle was covered with spindle cells around 7

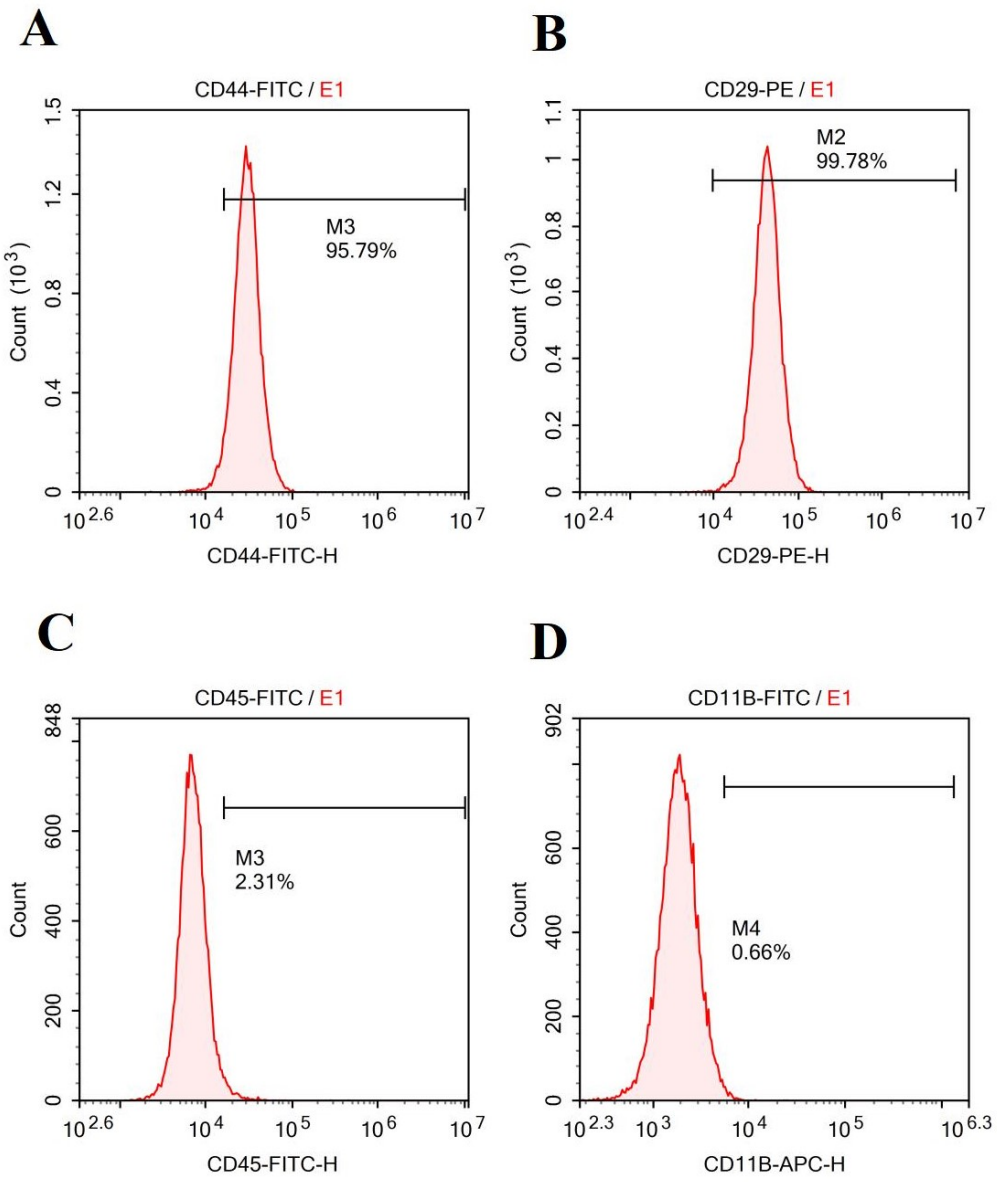

Fig. 2. Flow cytometry of rat bone marrow mesenchymal stem cells. (A) Positive expression of CD44 (95.79%). (B) Positive expression of CD29 (99.78%). (C) Negative expression of CD45 (2.31%). (D) Negative expression of CD11b (0.66%). Each experimental group was replicated three times.

d. Their morphology gradually became uniform after multiple subcultures. The cells of P3 generation showed a relatively uniform fibrous morphology (Fig. 1). Flow cytometry analysis of rat BMSCs' surface markers revealed positive expression of CD44 (95.79%) and CD29 (99.78%), while CD45 and CD11b showed negative expression at 2.31% and 0.66%, respectively (Fig. 2). These results were consistent with the characteristics of mesenchymal stem cells [16].

Concentration- and Time-Dependent Effects of PDGF-BB on BMSC Proliferation

The results of the CCK-8 assay revealed significant variations in the proliferative activity of BMSCs in response to different concentrations of PDGF-BB at various time points. This suggests a concentration- and time-dependent effect of PDGF-BB on BMSC proliferation. Specifically, when BMSCs were subjected to PDGF-BB at a concentration of 50 ng/mL for 5 days, their cellular viabilities were significantly higher compared to other concentrations and time points ($p < 0.05$), as shown in Fig. 3.

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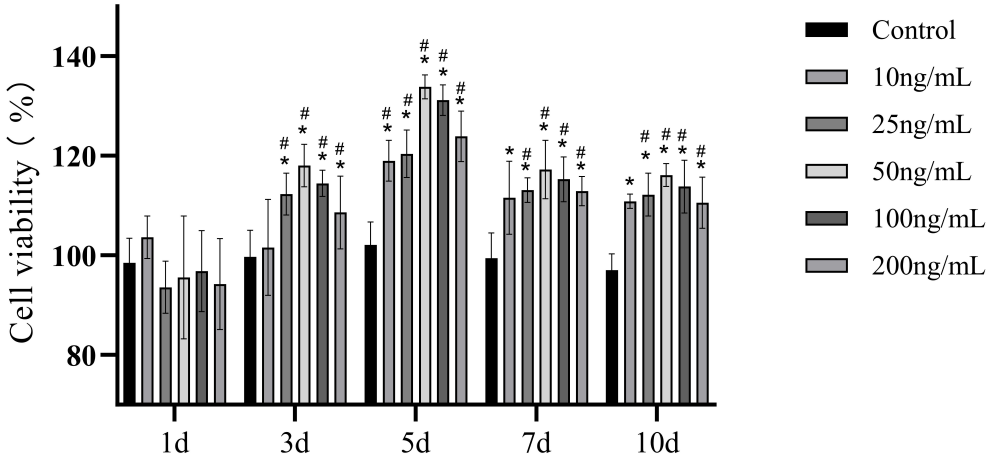

Fig. 3. Cell viability of BMSCs. CCK-8 assay was used to assess the cellular viability of BMSCs under various concentrations of PDGF-BB at different time points. Compared with the control group or Day 1 group, $^{*/\#}p < 0.05$ (n = 3). CCK-8, Cell Counting Kit-8; PDGF-BB, platelet-derived growth factor BB.

Assessment of PDGF-BB as the Key Effector in PRP for Promoting the Proliferation and Osteogenic Differentiation of BMSCs

Proliferative Effects of PDGF-BB on BMSCs

The CCK-8 results showed that compared to the control group, both 10% PRP and 50 ng/mL PDGF-BB significantly increased the viability of BMSCs and promoted their proliferation. Moreover, following treatment with 50 ng/mL PDGF-BB, the viability of BMSCs was significantly higher than that of the 10% PRP group ($p < 0.05$). Pre-treatment with the platelet-derived growth factor β receptor (PDGFR-β) inhibitor AG1295 markedly inhibited the proliferative effects of PRP on BMSCs, as shown in Fig. 4.

Effects of PRP and PDGF-BB on Cell Cycle Regulation in BMSCs

Cell cycle analysis showed that compared to the control group, both 10% PRP and 50 ng/mL PDGF-BB significantly decreased the proportion of cells in the G1 phase and elevated the proportion of cells in the S phase ($p < 0.05$). However, there was no significant difference between the two groups ($p > 0.05$). Pre-treatment with the PDGFR-β inhibitor significantly inhibited the promotive effect of PRP on the S phase of BMSCs ($p < 0.05$), as depicted in Fig. 5.

Effect of PRP and PDGF-BB on Osteogenic Protein Expression Levels in BMSCs

The Western blot results showed that 10% PRP and 50 ng/mL PDGF-BB significantly increased the protein expression levels of TGF-β, BGP, and BMP-9 compared to the control group. The differences in the expression levels of TGF-β and BGP between the two groups were statistically significant ($p < 0.01$), while no significant difference was observed for BMP-9 ($p > 0.05$). Additionally, the relative protein expression levels of TGF-β, BGP, and BMP-9 in the 10% PRP + AG1295 group were significantly lower than those in the 10% PRP group ($p < 0.01$), indicating that the PDGFR-β inhibitor AG1295 could markedly suppress the osteogenic differentiation-promoting effect of PRP on BMSCs. The result is shown in Fig. 6.

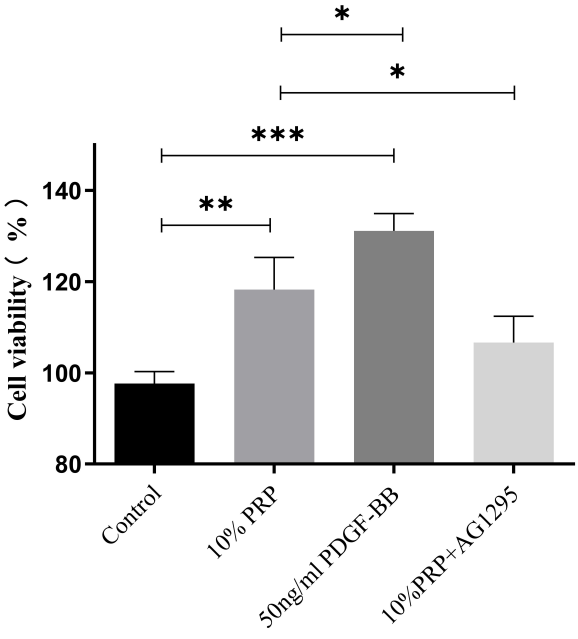

Fig. 4. Cell viability of BMSCs. The CCK-8 assay was used to evaluate the cellular viability of BMSCs under various treatments. Compared with the control group or 10% PRP group; $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$ (n = 3). PRP, platelet-rich plasma.

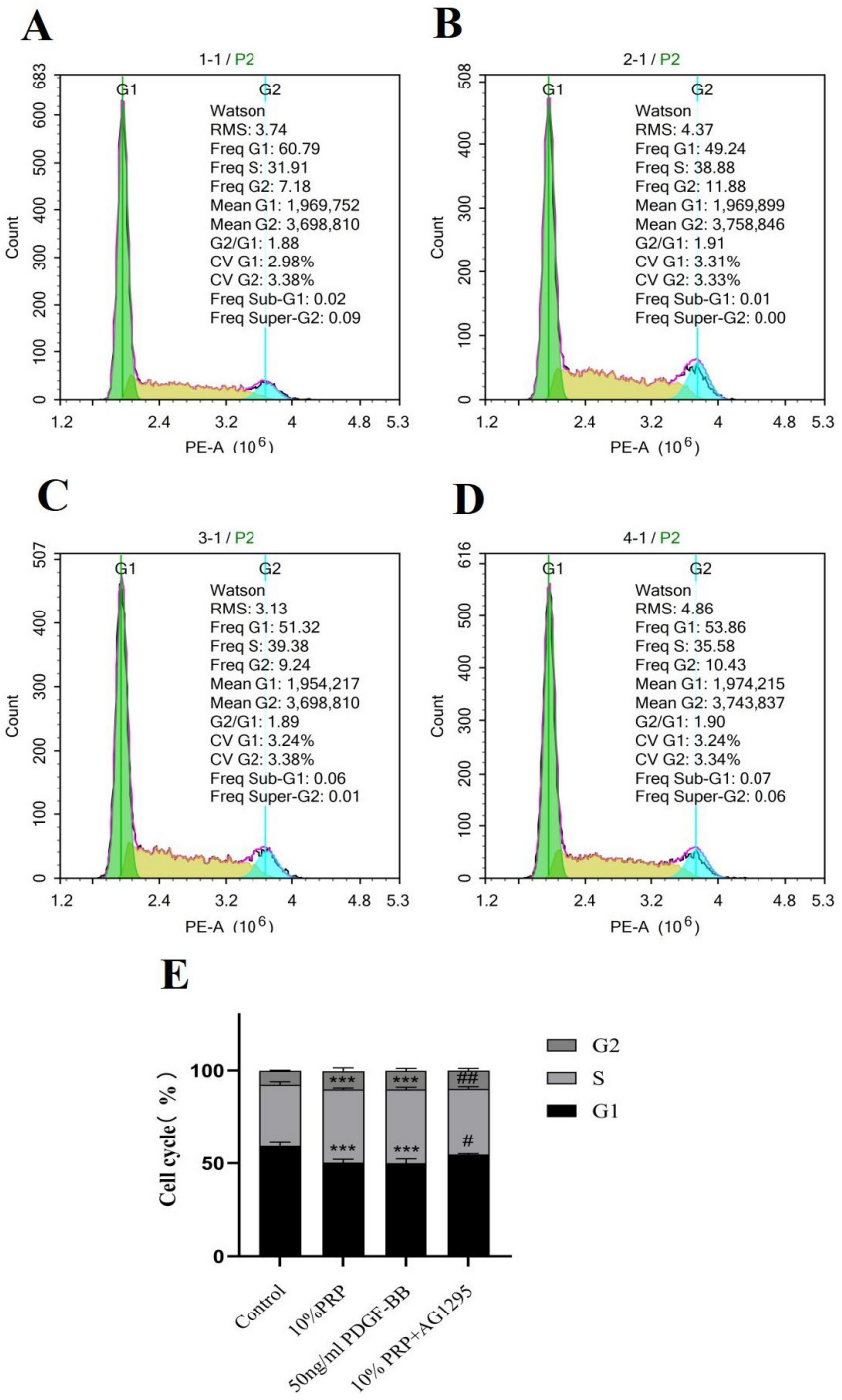

Fig. 5. Flow cytometry analysis of cell cycle in BMSCs. (A) Cell cycle of the control group. (B) Cell cycle of the 10% PRP group. (C) Cell cycle of the 50 ng/mL PDGF-BB group. (D) Cell cycle of the 10% PRP + AG1295 group. (E) Statistical comparison of the cell cycle. Compared with the control group, $^{***}p < 0.001$; compared with the 10% PRP group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (n = 3).

Discussion

Among the treatment modalities for nonunion fractures, bone tissue engineering technology holds considerable promise. BMSCs, due to their robust self-renewal capacity and wide differentiation potential, are considered ideal seed cells [17]. However, the optimal conditions for their proliferation and directed differentiation remain unclear. The results of this study revealed a positive dose- and time-dependent proliferative effect of PDGF-BB on BMSCs *in vitro*, with an optimal condition identified at a concentration of 50 ng/mL for a 5-day treatment period. Additionally, our results found that the proliferative and os-

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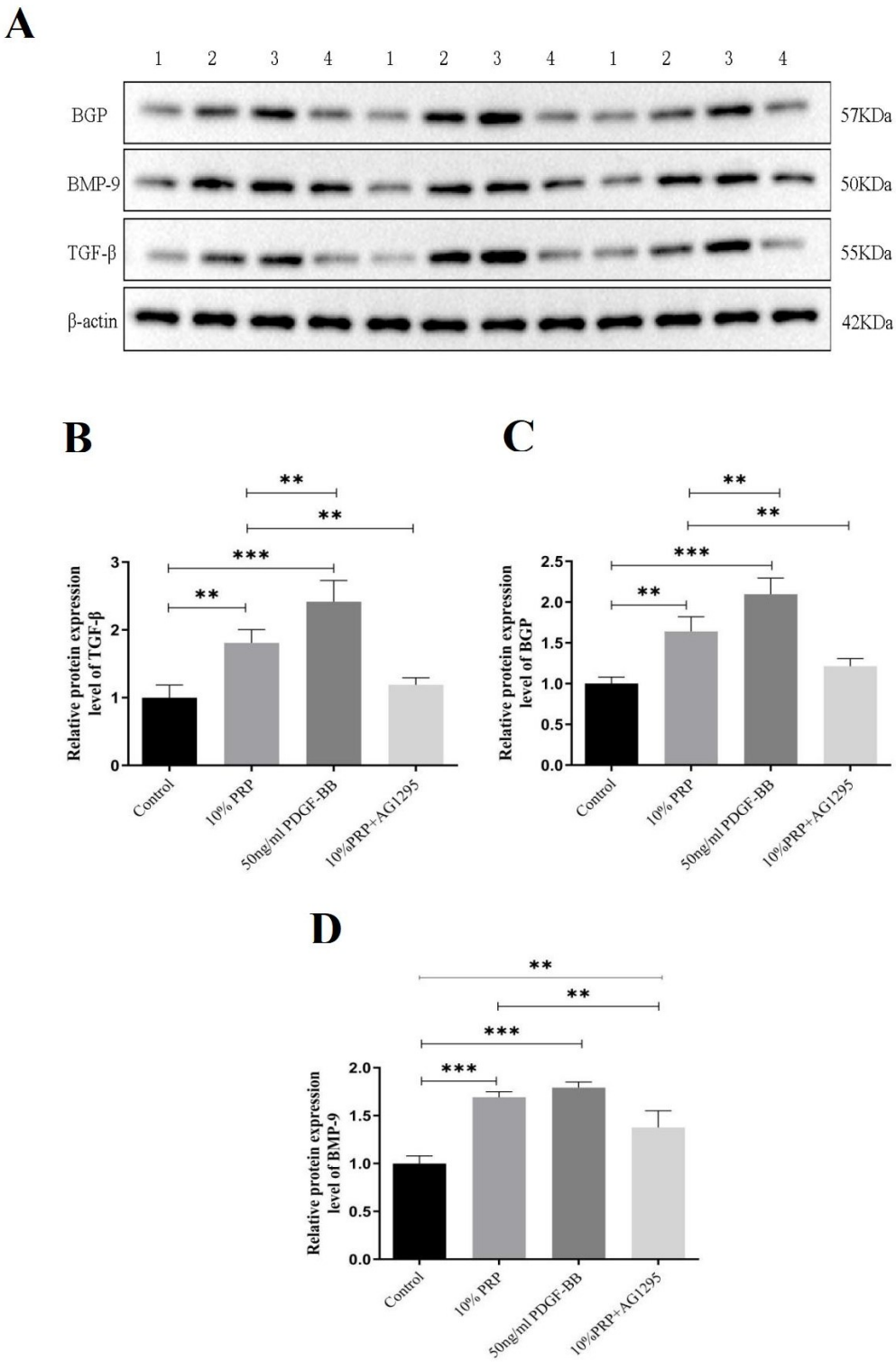

Fig. 6. Relative protein levels of TGF-β, BGP, and BMP-9 in BMSCs of each group. (A) Western blot analysis was used to assess the expression of TGF-β, BGP, and BMP-9. Three replicates were set for each group (1. Control group; 2. 10% PRP; 3. 50 ng/mL PDGF-BB; 4. 10% PRP + AG1295). (B–D) Statistical analysis of the relative protein expression for TGF-β, BGP, and BMP-9, respectively. In comparison to the control group or the 10% PRP group, $^{***}p < 0.001$, $^{**}p < 0.01$ (n = 3). TGF-β, transforming growth factor-β; BGP, bone glaprotein; BMP-9, bone morphogenetic protein-9.

teogenic differentiation effects of 50 ng/mL PDGF-BB on BMSCs surpass those of 10% platelet-rich plasma (PRP), while the PDGFR-β inhibitor inhibited the biological functional enhancement of BMSCs induced by PRP.

The local microenvironment during bone tissue regeneration is influenced by multiple growth factors. Among these, PDGF-BB is the most potent mitogen and is one of the five biological subtypes of PDGF. Its role in promoting migration, proliferation, and osteogenic differentiation of mesenchymal stem cells has been confirmed. PDGF-BB is widely used to study the osteogenic effects of bone tissue engineering [18–20]. By binding to surface receptors on stem cells, PDGF-BB stimulates cell mitosis, exerts its proliferative effects, and demonstrates concentration- and time-dependent effects [21]. However, the optimal effective concentration of PDGF-BB for BMSCs remains to be elucidated. In a study by Yang *et al*. [22], it was observed that PDGF-BB enhances the proliferation and differentiation of vascular smooth muscle cells, with a dose-dependent effect that begins to decline after 24 h of treatment with 20 ng/mL PDGF-BB. Mihaylova *et al*. [23] conducted a study on human periodontal ligament mesenchymal stem cells to investigate the effects of different concentrations of PDGF-BB. They found that the most significant proliferative effect was achieved after 48 h of treatment with 50 ng/mL PDGF-BB. In line with previous research, our present study used gradient concentrations of PDGF-BB (0, 10, 25, 50, 100, 200 ng/mL) and co-cultured with BMSCs for 1, 3, 5, 7, and 10 days. The proliferation of BMSCs was assessed using the CCK-8 assay, which revealed a concentration- and time-dependent proliferative promotion effect of PDGF-BB on BMSCs. When the concentration of PDGF-BB exceeded 50 ng/mL at different time points, the cellular viability of BMSCs decreased with increasing concentration. Moreover, beyond an intervention time of 5 days, a declining trend in the time-dependent effect of PDGF-BB on BMSCs was observed. These findings are consistent with previous literature [22,23], suggesting that an appropriate concentration of PDGF-BB expression can effectively accelerate cell replication. Lower concentrations of PDGF-BB have been found to stimulate mesenchymal stem cells, while higher concentrations may have inhibitory effects. Chen *et al*. [24] reported that concentrations of 3 ng/mL and 30 ng/mL of PDGF-BB significantly promoted BMSC proliferation, but the osteogenic mineralization effect was relatively weaker at 30 ng/mL. Xu *et al*. [25] observed that a concentration of 1 µg/L PDGF-BB enhanced cell recruitment *in vitro* but was detrimental to cartilage repair in a mouse model. Furthermore, high doses of PDGF-BB may lead to bone softening and secondary hyperparathyroidism [24]. Persistent elevation of PDGFR-β expression in bone tissue may contribute to vascular aging and arterial sclerosis [26]. Therefore, cautious selection of an appropriate dosage of PDGF-BB is advisable.

PRP is abundant in growth factors and other protein particles with osteoinductive capabilities. It has been demonstrated to facilitate the healing of nonunion fractures when combined with other therapeutic approaches such as autogenous bone grafting, mesenchymal stem cells, and internal fixation [27–29]. However, a study has reported that when PRP is used alone, its therapeutic effect is not as effective as that of bone morphogenetic protein [30]. This difference may be attributed to the comparatively lower concentrations of extractable growth factors in PRP, as the concentration range of PDGF-BB in human serum is approximately 2.19 to 10.67 ng/mL [31]. Therefore, further investigation is needed to clarify the osteogenic differentiation-promoting abilities of PRP on BMSCs and its primary effector factors. Lai *et al*. [32] observed that the proliferative effect of PRP on adipose-derived mesenchymal stem cells is comparable to the individual application of PDGF-BB. Concurrently, the addition of PDGF antibodies to the culture medium inhibits the proliferative effect of PRP. Thus, it is postulated that PDGF-BB may be the primary effective component of PRP in enhancing the biological functions of mesenchymal stem cells. Based on the results of the first part of our experiments, we selected the condition of culturing BMSCs with 50 ng/mL PDGF-BB for 5 days. The experimental groups were compared with a blank control group, a 10% PRP group, and a 10% PRP + 100 µM AG1295 (PDGFR-β inhibitor) group. The results demonstrated that both 10% PRP and 50 ng/mL PDGF-BB significantly promoted the proliferation and osteogenic differentiation ability of BMSCs. The PDGFR-β inhibitor reduced the effects of PRP on the proliferation and osteogenic differentiation effects of BMSCs. These findings suggest that PDGF-BB may play a key role in the PRP-mediated promotion of BMSC proliferation and osteogenic differentiation. Cao *et al*. [33] found that local application of PRP in the treatment of steroid-induced osteonecrosis promotes bone formation and vascular regeneration. High levels of PDGF-BB in PRP are considered the primary biological basis for this therapeutic approach. The mechanism involves the activation of the PDGFR/Akt/GSK3β/CERB signaling pathway, mediating the self-renewal of mesenchymal stem cells and maintaining their osteogenic potential. Fang *et al*. [34] co-cultured mesenchymal stem cells with different growth factors, including PDGF-AA, PDGF-BB, EGF, and bFGF. They observed a significant decrease in PDGF-BB levels and an increase in the proportion of PDGFR-β^+ mesenchymal stem cells, indicating a preferential utilization of PDGF-BB by mesenchymal stem cells. The key to bone repair and regeneration depends on the activation and recruitment of skeletal stem and progenitor cells with osteogenic capacity, and bone homeostasis depends on the formation of bone by osteoblasts and the absorption of bone by osteoclasts, and the PDGF/PDGFR-β signaling pathway is involved in the regulation of the above processes [35]. Sanchez-Fernandez *et al*. [36] confirmed

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that PDGF-BB is the most effective secreted growth factor that triggers osteoblast chemotaxis *in vitro* among several growth factors that are up-regulated during osteoclast formation. However, the knockdown of PDGFR-β gene in osteoblasts or the knockdown of PDGF gene in mature osteoclasts can cause the loss of osteoblast migration ability and inhibit bone formation and bone remodeling process [37]. It was found that PDGFR-β^+ MSC showed significantly stronger differentiation potential for osteogenesis, chondrogenesis and adipogenesis than PDGFR-β^- MSC [38]. Furthermore, a study has indicated a significant inhibitory effect of high doses of PDGF-BB or PDGFR-β inhibitors on the biological functions of mesenchymal stem cells. This aligns with the results of our study, suggesting that the actions of PDGF-BB may be mediated through the activation of the PDGF-BB/PDGFR-β signaling pathway and downstream pathways [39]. PDGF-BB, serving as a coupling regulatory factor for angiogenesis and osteogenesis, has been investigated for its potential to accelerate fracture healing and treat osteoporosis when administered via intraosseous injection [40]. However, its mechanism of action remains unclear. The proliferation ability of BMSCs modified with the PDGF-BB overexpression gene and osteogenic related markers, such as type I collagen, osteopontin, and osteocalcin, were found to be in the modified stromal stem cells, which showed stronger proliferation and differentiation ability and alkaline phosphatase activity [41]. PDGF-BB, after binding to its receptor, can increase osteogenic differentiation of BMSCs and inhibit fat formation through ERK1/2 and Src/JAK2 signaling pathways [42]. Li *et al*. [43] demonstrated that PDGF-BB can enhance the proliferation, migration, and osteogenic differentiation of BMSCs by upregulating phosphorylated levels of AKT and ERK. Other study has suggested that the PI3K/Akt pathway is involved in PDGF-BB-induced proliferation of mesenchymal stem cells, while the MEK/Erk pathway is primarily associated with their differentiation [44]. The above study showed that PDGFBB showed a key role in affecting bone formation related cells, and could promote the proliferation and osteogenic differentiation of BMSCs, which was also consistent with the conclusions of this experiment. In addition, although this study shows that PDGFR-β inhibitors can significantly reduce the proliferative and osteogenic differentiation effects of PRP on BMSCs, it indicates that PDGFBB may be the key effect factor for PRP to promote the biological function of BMSCs. However, other growth factors such as insulin-like growth factor (IGF) and transforming growth factor-β (TGF-β) that promote bone osteogenic activity of BMSCs also exist in PRP, and their synergistic mechanism with PDGF-BB is still unclear. IGF is involved in the proliferation and differentiation of mesenchymal cells, periosteum cells and osteoblasts during bone repair, and can synergistically promote the regeneration of epidermis and vascular endothelium with PDGF-BB [45]. TGF-β can induce the phosphorylation of Smad2 and Smad3, activate R-Smads to form complexes translocated Smads into the nucleus and companion proteins to regulate transcription of specific target genes to promote osteogenesis, and stimulate MSCs to induce expression of basic leucine zipline structure factor (C/EPBP-β) [46]. In addition, a study has shown that PDGF-BB coupling TGF-β can act as a central signal between cell components and osteogenic factors, and PDGF-BB has a stronger effect on promoting the expression of bone-related genes than TGF-β [47].

Despite the study's findings, the following limitation exist: (1) Although Western blot can reflect the final expression level of osteogenic differentiation markers, the expression level of osteogenic differentiation markers should be detected by alkaline phosphatase staining or alizarine red staining in subsequent studies, so as to further confirm the role and mechanism of PDGF-BB/PDGFR-β in osteogenic differentiation of BMSCs. (2) Studies on the synergistic effect and interaction between PDGF-BB and other growth factors of PRP are also lacking in this paper, which will be further improved in the future.

Conclusions

In conclusion, treatment with 50 ng/mL PDGFBB for 5 days is a suitable condition for promoting proliferation and osteogenic differentiation of BMSCs, and its accreting effect is close to 10% PRP, while its osteogenic effect is slightly better than 10% PRP. PDGFR-β inhibitors can reduce the proliferative and osteogenic differentiation effects of PRP on BMSCs, suggesting that PDGFBB may be a key effect factor for PRP to promote the biological function of BMSCs, and the tissue engineering technology of PDGFBB combined with BMSCs has considerable research prospects for the treatment of bone nonunion. However, further experiments are needed to explore its mechanisms, and *in vivo* animal experiments are warranted to investigate its therapeutic effects.

Availability of Data and Materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

ZGL, HDC and JY designed the research study. ZGL, YY and ZS performed the research. HL and CJS provided help and advice on the experiments. XHS analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal experiment ethics committee of the The First Affiliated Hospital of Dali University approved this study and supervised all animal experiments in this study (DFY20230401001).

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Conflict of Interest

The authors declare no conflict of interest.

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