Journal of Dental Sciences 20 (2025) 2416–2426

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journal homepage: www.e-jds.com

Original Article

Propofol-induced osteogenic differentiation in dental pulp stem cells: Modulation of MAPK signaling under inflammatory conditions

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Received 17 March 2025; Final revision received 21 March 2025
Available online 4 April 2025

KEYWORDS
Bone regeneration;
Dental pulp stem cell;
Inflammation;
Mitogen-activated protein kinases;
Propofol

Abstract *Background/purpose:* Bone regeneration in an inflammatory environment remains a significant challenge in the field of regenerative dentistry. Previous studies have demonstrated that inflammation inhibits osteogenic differentiation, necessitating the development of novel therapeutic approaches to counteract these effects. We investigated the effects of propofol on the osteogenic differentiation of dental pulp stem cells (DPSCs) under inflammatory conditions and explored the underlying signaling mechanisms.
Materials and methods: DPSCs were cultured in the presence of lipopolysaccharide (LPS) and tumor necrosis factor-alpha (TNF-α) to mimic inflammatory conditions. Propofol (10, 50, and 100 μM) was administered, and its effects on cell viability, alkaline phosphatase (ALP) activity,

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https://doi.org/10.1016/j.jds.2025.03.025

and mineralization were assessed. In addition, the expression of osteogenic markers was analyzed and activation of the mitogen-activated protein kinase (MAPK) signaling pathway was examined by western blotting.
Results: Propofol significantly enhanced ALP activity and mineralization in DPSCs under inflammatory conditions. In addition, it upregulated the expression of osteogenic marker genes and proteins. Functionally, propofol treatment activated p38 phosphorylation and suppressed extracellular signal regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) phosphorylation.
Conclusion: Propofol promotes the osteogenic differentiation of DPSCs under inflammatory conditions by activating the p38/MAPK signaling while modulating the ERK and JNK pathways. This suggests that propofol has potential therapeutic applications in bone regeneration and regenerative dentistry, particularly in inflammatory environments.

Introduction

Periodontitis and pulpitis are inflammatory conditions that develop in the surrounding structures of teeth, including the root cementum, periodontal ligaments, and alveolar bone. These conditions primarily arise because of the accumulation of oral microbial biofilms and impaired immune–inflammatory responses, thereby contributing to the progressive destruction of dental tissues and ultimately causing tooth loss.[1] Regenerative medicine has emerged as a promising field, focusing on the reconstruction and restoration of damaged or lost tissues.[2] Although substantial advancements have been made in tissue regeneration across different medical fields, bone and tissue regeneration in dentistry present unique obstacles. These challenges stem from the high microbial load in the oral cavity and exposure to diverse bacterial species and toxins.[3] Among the factors exacerbating these inflammatory conditions, tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, can stimulate bone resorption while concurrently inhibiting bone formation, thus accelerating bone loss.[4] Additionally, lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, contribute to periodontitis pathogenesis and suppresses osteoblast differentiation, potentially leading to bone loss and osteoporosis.[5]

Mesenchymal stem cells (MSCs), originating from the mesodermal lineage, have multipotent properties that enable their differentiation into different specialized cell types, including osteoblasts, chondrocytes, and adipocytes. They are excellent candidates for tissue engineering and regenerative medicine because of their ease of isolation, self-renewal capabilities, and immunomodulatory properties.[6] MSCs can be harvested from multiple sources, such as bone marrow, adipose tissue, umbilical cord blood, and dental pulp.[7,8] Among MSCs, dental pulp stem cells (DPSCs) exhibit a proliferation rate superior to that of bone marrow-derived stem cells (BMSCs).[9] Moreover, DPSCs possess the potential to differentiate into both odontoblasts and osteoblast precursors, making them valuable for the development of novel biomaterials for dental applications.[10,11]

Propofol, an intravenous anesthetic widely used for general anesthesia and sedation, is recognized for its rapid onset, short duration of action, and smooth recovery. Therefore, it is commonly administered during dental procedures that require sedation. Studies have highlighted the anti-inflammatory effects of propofol, suggesting that its application in clinical dentistry may influence oral tissue health and treatment outcomes.[12,13] Although certain studies have investigated the role of propofol in osteoblastogenesis, the results are inconsistent. Propofol enhances the proliferation and differentiation of osteoblasts cultured under hypoxic conditions.[14] In addition, propofol stimulates bone nodular mineralization and upregulates osteogenic gene expression in human osteoblasts under oxidative stress.[15] However, a recent study reported conflicting results, wherein propofol attenuated the odontogenic/osteogenic differentiation of DPSCs *in vitro*.[16]

Given the discrepancies in previous findings, the effect of propofol on osteoblast differentiation remains unclear. To date, no studies have examined the effects of propofol on osteogenic differentiation under inflammatory conditions, highlighting the need for further investigation. Because propofol exhibits anti-inflammatory properties, and inflammation is known to inhibit osteoblast differentiation, we examined the effects of propofol on the osteogenic differentiation of DPSCs exposed to an inflammatory environment induced by LPS and TNF-α. In addition, we explored the signaling pathways involved in propofol-mediated osteogenesis under inflammatory conditions.

Materials and methods

Reagents

Dental pulp stem cells (DPSCs) were obtained from Lonza (PT-5025, Basel, Switzerland). The culture medium, consisting of α-modified Eagle medium (α-MEM), fetal bovine serum (FBS), and penicillin/streptomycin, was procured from Gibco BRL Co. (Grand Island, NY, USA). Dexamethasone, β-glycerophosphate, ascorbic acid, and alizarin red S (ARS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). LPS were supplied by Sigma–Aldrich, and recombinant human TNF-α was obtained from PeproTech (Cranbury, NJ, USA). The StemTAG™ alkaline phosphatase (ALP) staining kit (CBA-300) was acquired from Cell Biolabs, Inc.

(San Diego, CA, USA). Antibodies against extracellular signal-regulated kinase (ERK), p-ERK, c-Jun N-terminal kinase (JNK), p-JNK, p38, and p-p38 were obtained from Cell Signaling Technology (Danvers, MA, USA). Additional antibodies, including anti-ALP, anti-osteopontin (OPN), anti-dentin matrix protein 1 (DMP1), anti-runt-related transcription factor 2 (Runx2), and anti-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and MBL (Nagoya, Japan). Antibodies targeting osteocalcin and bone morphogenetic protein 2 (BMP2) were procured from Abcam (Cambridge, UK).

Culture of DPSCs

DPSCs were cultured in α-MEM supplemented with 10 % FBS and 1 % penicillin—streptomycin under a controlled atmosphere of 5 % CO_2 at 37 °C. After reaching confluence, cells were passaged using 0.05 % trypsin—EDTA. Propofol (Fresenius Kabi Austria GmbH, Hafnerstrabe, Austria) was dissolved in dimethyl sulfoxide (DMSO) (Sigma—Aldrich, St. Louis, MO, USA) and applied to the culture at concentrations of 10, 50, and 100 μM.

Osteogenic differentiation

To promote osteogenic differentiation, DPSCs were cultured in an osteogenic differentiation medium (ODM) containing 10 mM β-glycerophosphate, 0.1 mM ascorbic acid, and 100 nM dexamethasone for 4, 7, 14, and 21 days. Control cells were maintained in the standard growth medium. The ODM was replaced every 2 days.

MTT assay

Cell viability and proliferation in response to propofol treatment were assessed using an MTT assay. DPSCs were seeded in 24-well plates and treated with varying concentrations of propofol (0, 10, 50, and 100 μM) along with LPS (100 ng/mL) and TNF-α (10 ng/mL) for 1, 2, and 3 days. After treatment, the cells were incubated in a fresh medium containing 0.5 mg/mL of MTT solution for 4 h. The formazan product was dissolved in 200 μL of DMSO, and absorbance was measured at 570 nm using a microplate reader.

Alkaline phosphatase (ALP) staining

The DPSCs were plated in 48-well culture plates at a density of 5×10^4 cells/well and incubated for 24 h before treatment. Cells were exposed to propofol (0, 10, 50, and 100 μM) with or without LPS (100 ng/mL) and TNF-α (10 ng/mL) in ODM for 4 and 7 days. The culture medium was replaced every 2 days. ALP staining was performed using a CBA-300 AP staining kit according to the manufacturer's protocol. After staining, the cells were washed with PBS, and images were captured under a microscope.

Alizarin red S (ARS) staining

For mineralization assessment, DPSCs were cultured in ODM for 7 or 14 days in the presence or absence of propofol. The cells were fixed with 4 % paraformaldehyde for 15 min, washed with PBS, and stained with 2 % ARS solution (pH 4.2) for 10 min at room temperature. After staining, the cells were rinsed with distilled water, and images were recorded using a digital camera. Staining intensity was quantified using the ImageJ software (NIH, Bethesda, MD, USA).

Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from DPSCs on days 4, 7, and 14 after osteogenic induction using the RiboEx reagent (GeneAll, Seoul, Korea) according to the manufacturer's instructions. One microgram of RNA was reverse transcribed into complementary DNA (cDNA) using a HiSenScript RH [-] RT PreMix Kit (INtRON Biotechnology, Seongnam, Korea). qPCR was performed using SYBR Green Q-PCR Master Mix with a Low Rox kit (Applied Biosystems, Foster City, CA, USA) on a QuantStudio 1 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Amplification involved 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The relative expression of target genes was determined using the ΔΔCt method, with GAPDH serving as the reference gene. Primer sequences used for real-time PCR are listed in Table 1.

Western blotting

Whole-cell lysates were extracted using a passive lysis buffer (Promega, Madison, WI, USA) and sonicated on ice. The lysates were centrifuged at 13,000 rpm at 4 °C for 10 min, and protein concentration was determined using the Bradford Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were denatured, separated using 8 % and 10 % SDS-PAGE, and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5 % non-fat milk in PBST for 30 min, followed by incubation with primary antibodies overnight at 4 °C. The blots were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Protein signals were visualized using enhanced

Table 1 Primer sequences used for the real-time PCR.

Genes	Primers	Sequences (5′-3′)
ALP	Forward	GGACGCTGGGAAATCTGTG
	Reverse	CCATGATCACGTCAATGTCC
Runx2	Forward	TCCCAGTATGAGAGTAGGTGTCC
	Reverse	GGCTCAGATAAGAGGGGTAAGAC
BMP2	Forward	TTCCACCATGAAGAATCTTTGG
	Reverse	AAACCTGAAGCTCTGCTGAG
DMP1	Forward	TAGGAAGTCTCGCATCTCAG
	Reverse	CCAGTGTCTCTGGAGTTGC
OPN	Forward	GCAACCGAAGTTTTCACTCC
	Reverse	ATCAGGGTACTGGATGTCAG
GAPDH	Forward	TATGACTCTACCCACGGCAAGT
	Reverse	ATACTCAGCACCAGCATCACC

ALP, alkaline phosphatase; Runx2, runt-related transcription factor 2; BMP2, bone morphogenetic protein 2; DMP1, dentin matrix protein 1; OPN, osteopontin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

chemiluminescence detection reagents (Millipore) and quantified using the ImageJ software (NIH).

Statistical analysis

All values are presented as means ± standard errors. Data were obtained from at least three independent experiments conducted in triplicates. Statistical analysis was performed using Student's *t*-test, and differences were considered statistically significant at $P < 0.05$.

Results

Effect of propofol on DPSC viability and proliferation under inflammatory conditions

As illustrated in Fig. 1A, MTT assay results demonstrated that LPS and TNF-α treatment, whether alone or combined with varying concentrations of propofol, did not exhibit cytotoxic effects on DPSCs. Furthermore, no significant differences in cell proliferation were observed among the

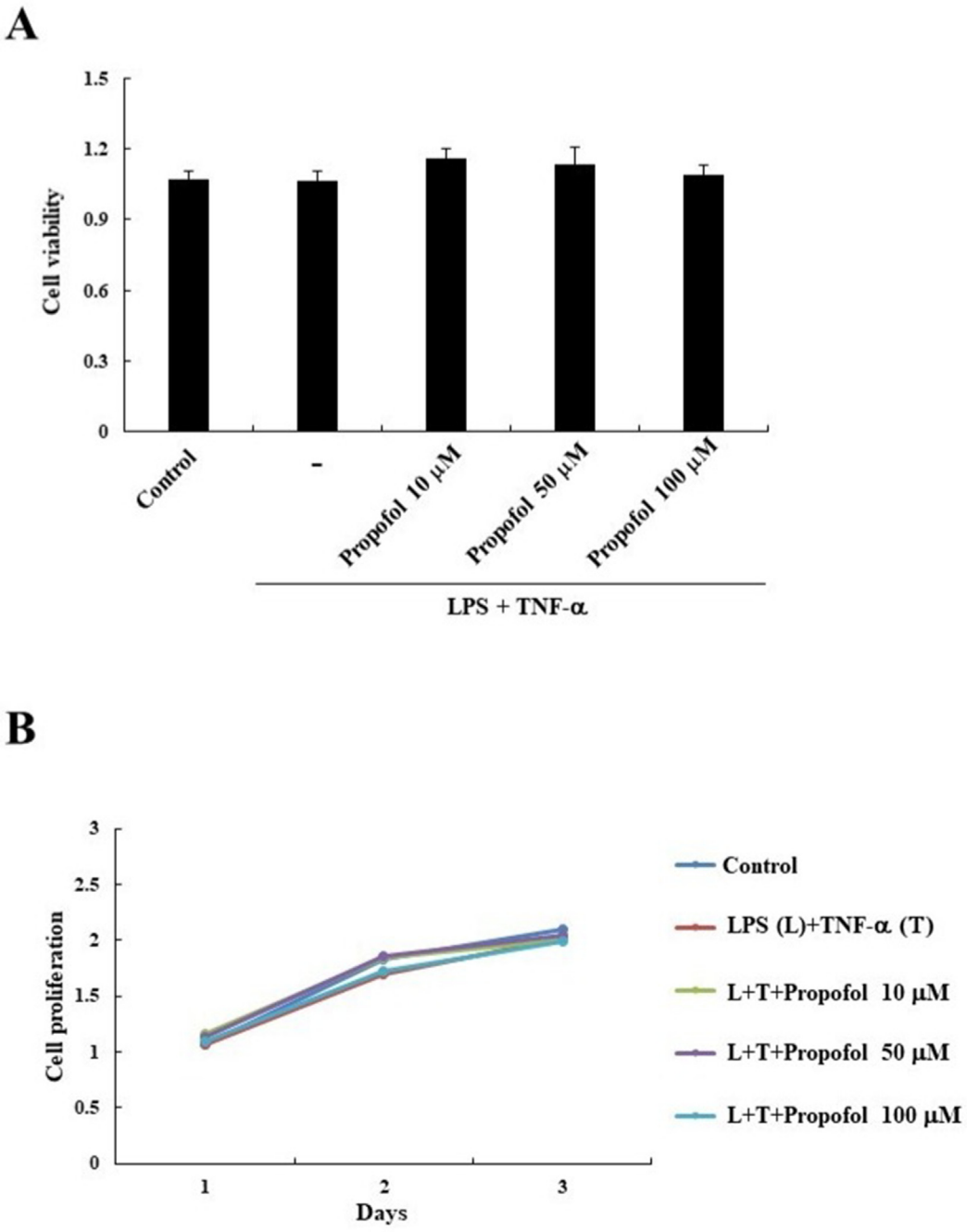

Figure 1 Effect of propofol (0, 10, 50, and 100 μM) and/or LPS (100 ng/mL) and TNF-α (10 ng/mL) on (A) cell viability and (B) proliferation of DPSCs, as evaluated by MTT assay on days 1, 2, and 3. LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; DPSCs, dental pulp stem cells.

different propofol concentrations (10, 50, and 100 μM) within 3 days of exposure to LPS and TNF-α (Fig. 1B).

Propofol enhances ALP activity and mineralization in DPSCs

ALP and ARS staining were performed on days 4, 7, 14, and 21 to investigate early and late osteogenic differentiation. LPS and TNF-α significantly reduced ALP staining relative to the ODM group, whereas propofol treatment enhanced ALP activity in a dose-dependent manner, as observed on days 4 and 7 (Fig. 2A and B). A notable increase in ALP-positive staining was observed on day 7 in the 50 and 100 μM propofol groups.

Bone matrix mineralization was assessed via ARS staining. Results indicated a significant reduction in mineralized calcium nodules in LPS and TNF-α treated DPSCs compared to the ODM group on days 14 and 21. However, propofol treatment, especially at higher concentrations, significantly enhanced mineral deposition at these time points (Fig. 2A and C). The most pronounced effect was observed at 100 μM propofol, which was subsequently used for further experiments.

Propofol upregulates osteogenic marker genes in DPSCs under inflammatory conditions

To understand the molecular mechanisms through which propofol influences osteogenic differentiation, key osteogenic markers were examined at the transcriptional and protein levels. LPS and TNF-α significantly reduced the mRNA expression of ALP, Runx2, OPN, BMP2, and DMP1 in comparison to the expression levels of the ODM group on days 7 and 14. However, treatment with 100 μM propofol markedly reversed this downregulation at both time points (Fig. 3).

Western blotting corroborated these findings, showing increased protein expression of ALP, Runx2, BMP2, OPN, and DMP1 in the propofol-treated groups compared to the LPS- and TNF-α-treated groups at days 7 and 14. Densitometric quantification confirmed a significant restoration of osteogenic markers in propofol-treated DPSCs, further supporting their osteoinductive properties (Fig. 4).

Propofol modulates MAPK signaling by activating p38 and suppressing ERK and JNK phosphorylation in DPSCs under inflammatory conditions

To explore the molecular mechanisms underlying the effect of propofol on osteogenic differentiation, the activation of MAPK signaling pathways, including p38, ERK, and JNK, was assessed via western blotting. Results demonstrated that propofol significantly enhanced p38 phosphorylation at both days 7 and 14 relative to the LPS and TNF-α groups. Conversely, the phosphorylation levels of ERK and JNK were significantly reduced in propofol-treated cells at day 14 compared to the LPS and TNF-α group, indicating that propofol modulates osteogenic differentiation by promoting p38 activation while suppressing ERK and JNK signaling (Fig. 5A and B).

Discussion

This study demonstrates that propofol enhances the osteogenic differentiation of DPSCs under inflammatory conditions. The administration of propofol significantly improved ALP activity, promoted mineralization, upregulated osteogenic markers, and activated p38/MAPK signaling. Increased p38 phosphorylation following propofol treatment suggests that it stimulates osteoblast differentiation. In addition, propofol reduced ERK and JNK phosphorylation at later stages, indicating its involvement in late-stage osteogenesis. Thus, propofol has a potential therapeutic value for bone regeneration under inflammatory conditions.

Our findings are in agreement with those of previous studies demonstrating that propofol enhances osteogenic differentiation. Kim et al.[15] revealed that propofol enhances bone mineralization and increases the expression of bone-related proteins such as collagen type I, BMP2, osterix, and TGF-β1 in human osteoblasts under oxidative stress. Similarly, Lee et al.[17] found that propofol positively affects bone remodeling by suppressing osteoclastogenesis. In contrast, our previous study[16] indicated that propofol inhibits the odontogenic and osteogenic differentiation of DPSCs *in vitro* under normal conditions. The current study was conducted under inflammatory conditions, whereas previous studies examined the effects of propofol under normal conditions. This suggests that the role of propofol in osteogenic differentiation may be context-dependent, particularly in inflammatory microenvironments. Our study further confirms that propofol promotes osteogenesis under inflammatory conditions, reinforcing its potential for use in regenerative dentistry.

In addition to its anesthetic properties, propofol has been recognized for its effects on cellular differentiation and inflammatory pathways.[18,19] Our findings indicate that the ability of propofol to enhance osteogenic differentiation under inflammatory conditions can be attributed to its immunomodulatory properties. Because inflammation has been implicated in different dental and orthopedic disorders, such as pulpitis, periodontitis, and bone fractures, the dual action of propofol in mitigating inflammation and promoting osteogenesis could be therapeutically relevant.[17,20] Previous studies have demonstrated that propofol inhibits nuclear factor-κB signaling and reduces pro-inflammatory cytokine levels.[19–22] The interplay between the anti-inflammatory and osteogenic effects of propofol warrants further investigation to determine whether these pathways act independently or synergistically in promoting bone regeneration under pathological conditions.

MAPKs are activated under inflammatory conditions and play a complex and important regulatory role in multiple physiological processes, including cell cycle control, apoptosis, and cell fate specification.[23–25] In this study, by day 14, the phosphorylation levels of ERK and JNK significantly decreased in the propofol-treated groups compared to the LPS- and TNF-α-treated groups. ERK and JNK signaling pathways are involved in cell proliferation and differentiation.[26] JNK, in particular, regulates early osteogenic differentiation and is downregulated in the later stages.[27,28] Therefore, the observed reduction in p-ERK and p-JNK at day 14 suggests that propofol may promote late-

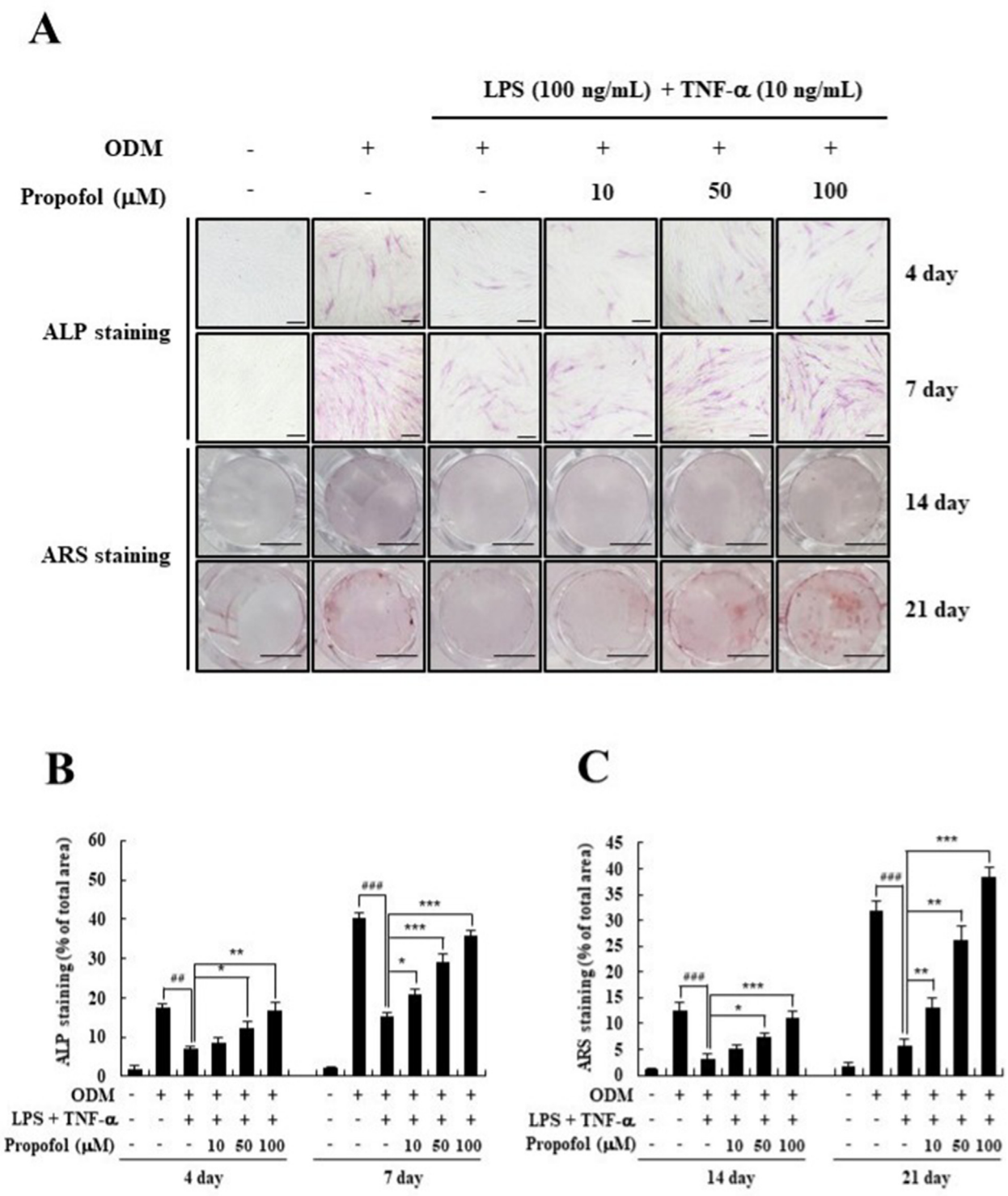

Figure 2 Effect of propofol on osteoblast differentiation in dental pulp stem cells (DPSCs) stimulated by LPS and TNF-α. (A) DPSCs were treated with LPS (100 ng/mL) and TNF-α (10 ng/mL) in osteogenic differentiation media (ODM) followed by treatment with different concentrations of propofol (0, 10, 50, and 100 μM) for 4, 7 days or 14, 21 days. The cells were fixed and stained for ALP (4, 7 days) or ARS (14, 21 days). Representative ALP (upper, scale bar = 200 μm) and ARS (lower, scale bar = 5 mm) stained images are shown. (B) The intensity of ALP and ARS staining was quantified. Results are presented as a percentage of the total area. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus ODM group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus LPS and TNF-α group. LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; DPSCs, dental pulp stem cells; ALP, alkaline phosphatase; ARS, alizarin red S.

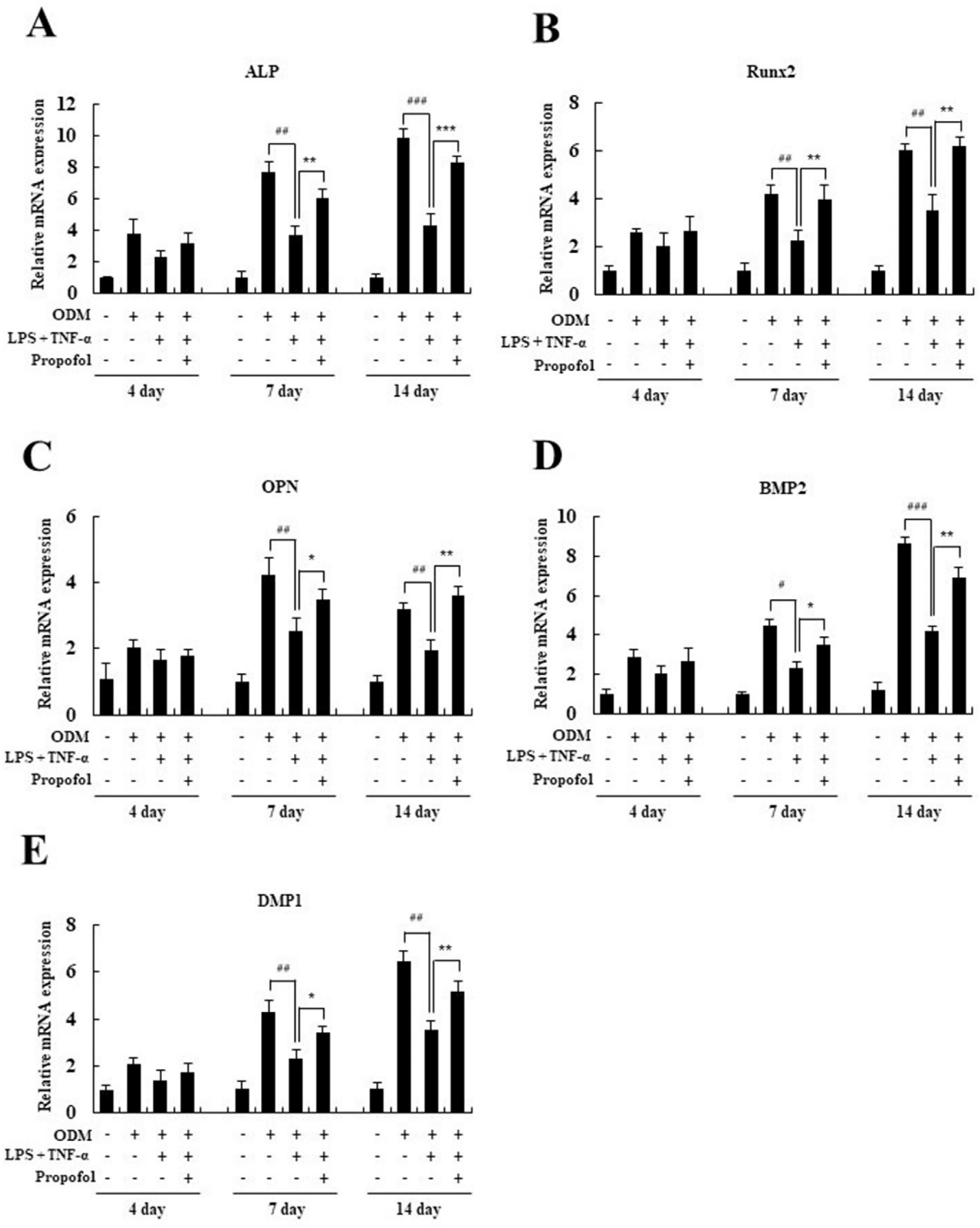

Figure 3 Effects of propofol on osteogenic differentiation-related gene expression in LPS and TNF-α treated DPSCs. DPSCs were treated with LPS and TNF-α in osteogenic differentiation media (ODM) followed by propofol for 4, 7, and 14 days. Total RNA was isolated during osteogenic differentiation and analyzed by real-time quantitative PCR (RT-qPCR). The expression of human (A) ALP, (B) Runx2, (C) OPN, (D) BMP2, and (E) DMP1 genes was determined by RT-qPCR. The expression in the basic media was considered to be 1.0, and the values were normalized to GAPDH mRNA levels. Data are expressed as means $\pm$ SDs of three independent experiments performed in triplicates. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus ODM group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus LPS and TNF-α group. LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; DPSCs, dental pulp stem cells; ALP, alkaline phosphatase; Runx2, runt-related transcription factor 2; OPN, osteopontin; BMP2, bone morphogenetic protein 2; DMP1, dentin matrix protein 1.

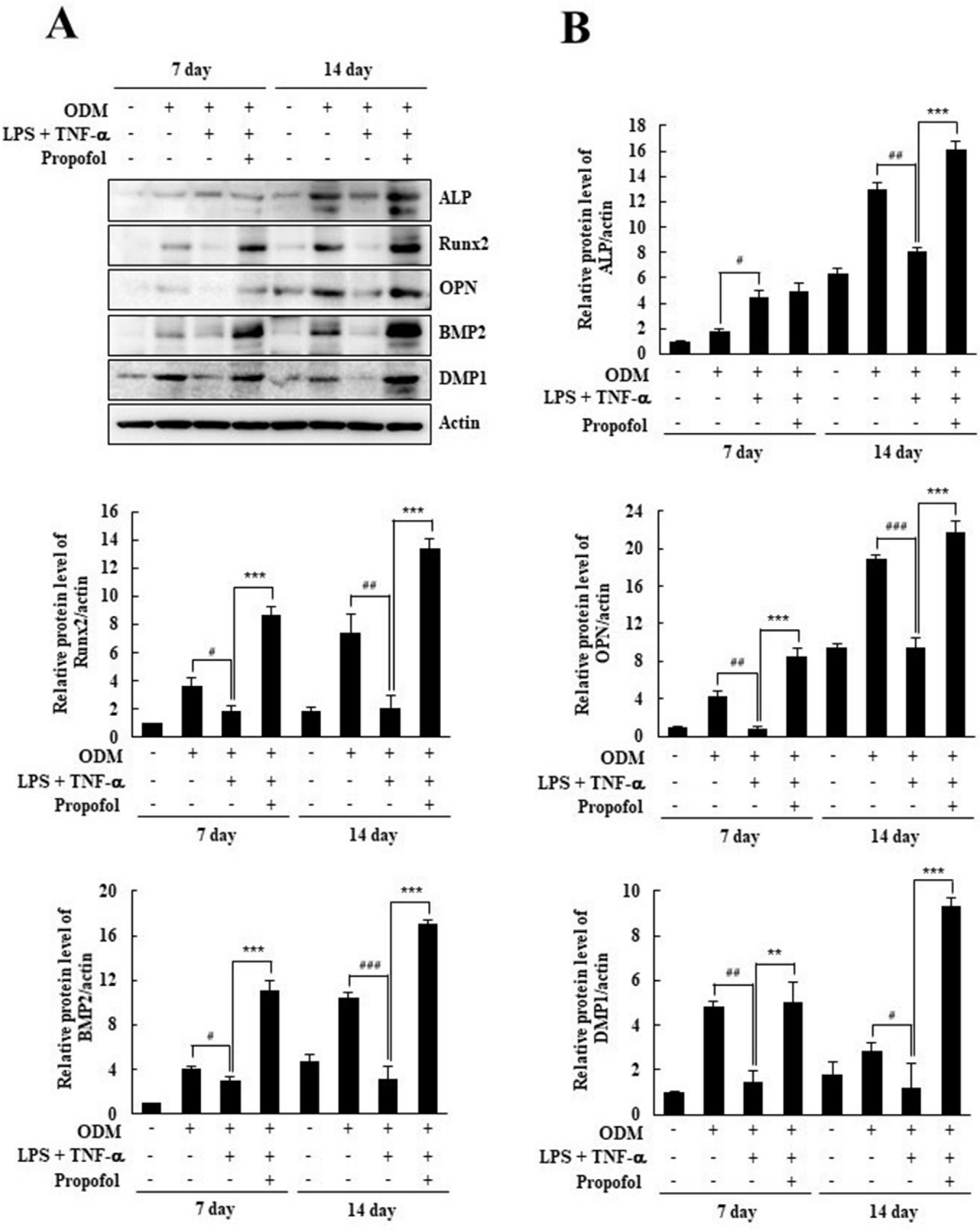

Figure 4 Effects of propofol on the protein expression of osteogenic differentiation marker in LPS and TNF-α treated DPSCs. (A) DPSCs were treated with LPS and TNF-α in osteogenic differentiation media (ODM) followed by propofol for 7 and 14 days. Human differentiation marker protein levels were determined by western blotting. Actin served as the loading control. (B) Band densitometry quantified by ImageJ and normalized to actin. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus ODM group, $^{***}P < 0.001$ versus LPS and TNF-α group. LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; DPSCs, dental pulp stem cells; ALP, alkaline phosphatase; Runx2, runt-related transcription factor 2; OPN, osteopontin; BMP2, bone morphogenetic protein 2; DMP1, dentin matrix protein 1.

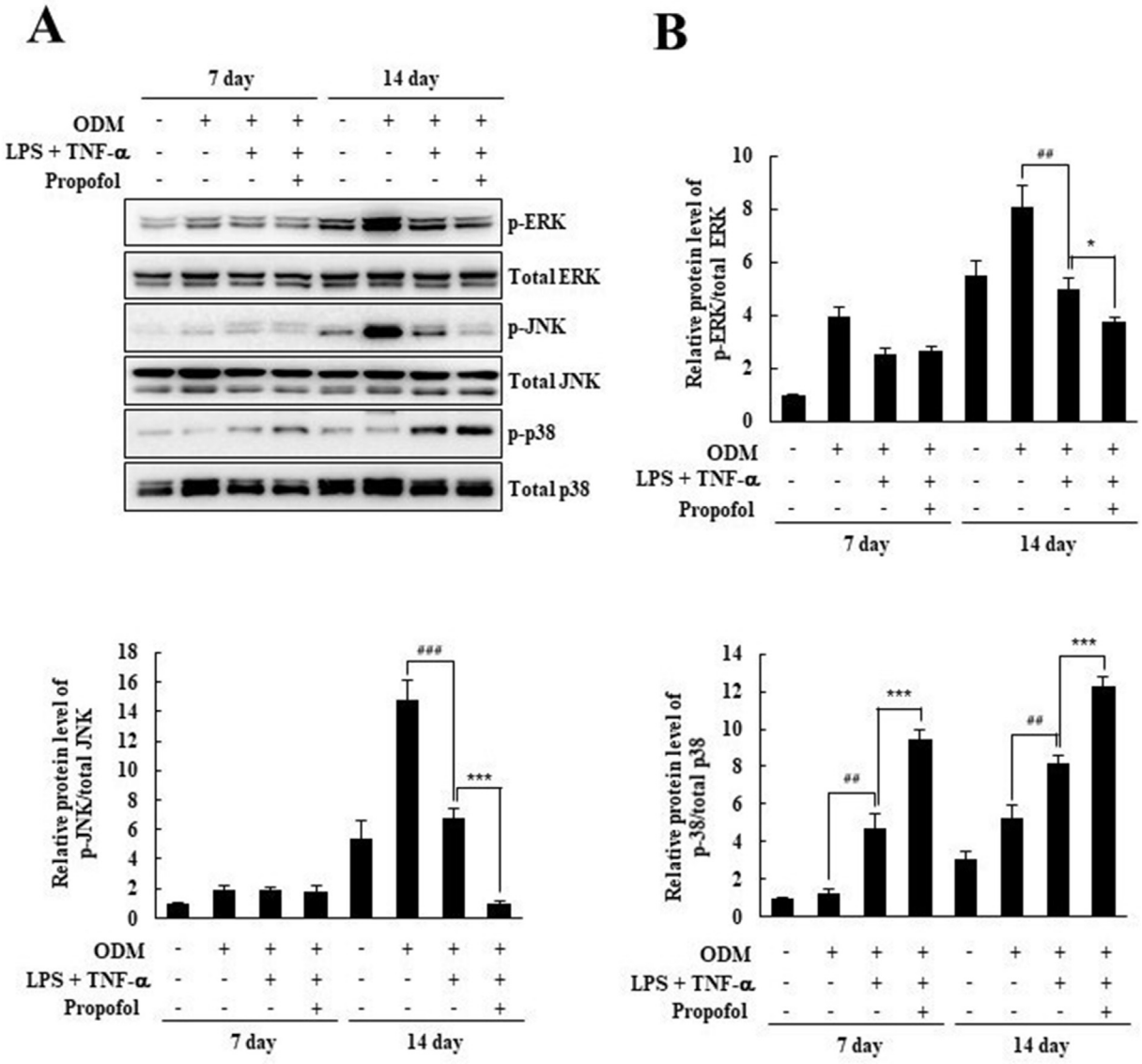

Figure 5 Propofol regulates osteoblast through the MAPK signaling pathway. (A) After culturing DPSCs in osteogenic differentiation media (ODM) for 7 and 14 days in the presence or absence of propofol, proteins were extracted and subjected to western blotting. Immunoblots were developed using antibodies directed against p-ERK, p-JNK, and p-p38. ERK, JNK, and p38 were used as controls. (B) Band densitometry quantified by ImageJ and normalized to ERK, JNK, and p38. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus ODM group, $^{*}P < 0.05$, $^{***}P < 0.001$ versus LPS and TNF-α groups. LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; DPSCs, dental pulp stem cells; MAPK, mitogen-activated protein kinases; ERK, extracellular signal regulated kinase; JNK, c-Jun N-terminal kinase.

stage osteogenic differentiation by modulating these signaling pathways. This is consistent with a previous study that reported that ERK and JNK inhibition enhanced osteoblast differentiation.[29,30]

Additionally, our findings showed that p38 phosphorylation was further increased in the presence of LPS and TNF-α, and this activation was intensified by propofol treatment. Given that p38 is a critical regulator of osteogenesis, particularly under stress conditions,[31,32] its precise role in propofol-induced osteogenic differentiation remains to be clarified. While some studies suggest that p38 activation enhances Runx2 expression and promotes osteoblast differentiation,[33,34] others indicate that excessive p38 activation may trigger apoptotic signaling and impair bone formation.[35,36] Thus, future studies employing pharmacological inhibition or gene silencing approaches targeting p38 will be necessary to determine whether propofol-mediated p38 activation contributes directly to osteogenesis or represents a compensatory response to inflammatory stress.

Although this study provided valuable insights into the function of propofol in promoting osteogenic differentiation under inflammatory conditions, it had several limitations. First, the experiments were conducted *in vitro*, and the effects of propofol on DPSCs may differ in *in vivo* environments, where complex cellular interactions and

systemic factors influence bone regeneration. Second, although we demonstrated the involvement of the p38/MAPK pathway in propofol-induced osteogenesis, additional mechanistic studies, such as pharmacological inhibition or gene knockdown experiments, are required to confirm the causality of this pathway. Third, we focused on the effects of propofol in an inflammatory microenvironment induced by LPS and TNF-α; however, other inflammatory conditions commonly found in the oral cavity, such as those caused by bacterial biofilms, may exhibit different responses. Finally, the clinical relevance of our findings requires further validation in preclinical animal models to assess the long-term effects of propofol on bone formation and dental tissue regeneration. Future studies addressing these limitations are essential for translating our findings into clinical applications.

In conclusion, our study demonstrated that propofol facilitates the osteogenic differentiation of DPSCs under inflammatory conditions by activating the p38/MAPK pathway while modulating ERK and JNK signaling. By enhancing ALP activity, extracellular matrix mineralization, and osteogenic marker expression, propofol effectively counteracted the inflammation-induced suppression of osteogenesis. These findings suggest that propofol has potential therapeutic applications in bone regeneration and regenerative dentistry, particularly for inflammation-associated conditions.

Declaration of competing interest

The authors declare no conflict of interest for this submission.

Acknowledgments

This work was supported by a 2-Year Research Grant of Pusan National University.

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