Regenerative Therapy 30 (2025) 911–919

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: www.elsevier.com/locate/reth

Bone morphogenetic protein-2-derived osteogenic peptide promotes bone regeneration via osteoblastogenesis

Jong-Bin Lee [a,1], Ji-Youn Hong [b,1], Hyeeun Shim [c], Sehee Kim [c], Dong Won Lee [d], Bosun Kwon [c,*], Jeong-Ho Yun [e,f,**]

[a] Department of Periodontology and Research Institute of Oral Sciences, College of Dentistry, Gangneung-Wonju National University, Gangneung, Republic of Korea
[b] Department of Periodontology, College of Dentistry, Kyung Hee University Dental Hospital, Kyung Hee University, Seoul, Republic of Korea
[c] MedivelBio Co., Ltd., Seoul, Republic of Korea
[d] Department of Periodontology, Gangnam Severance Hospital, College of Dentistry, Yonsei University, Seoul, Republic of Korea
[e] Department of Periodontology, College of Dentistry and Institute of Oral Bioscience, Jeonbuk National University, Jeonju, Republic of Korea
[f] Research Institute of Clinical Medicine of Jeonbuk National University-Biomedical Research Institute of Jeonbuk National University Hospital, Jeonju, Republic of Korea

ARTICLE INFO

Keywords:
Bone morphogenetic protein
Mesenchymal stromal cells
Osteogenesis
Peptide
Regeneration

ABSTRACT

Introduction: Bone morphogenetic protein (BMP)-2 plays a critical role in stimulating human mesenchymal stromal cells (hMSCs) differentiation, a key process in bone regeneration. However, the clinical application of BMP-2 has been hindered by several adverse effects. This study evaluated the effectiveness of a newly synthesized BMP2-derived osteogenic peptide (OP), which may overcome the limitations of BMP-2 while preserving its osteogenic potential.

Methods: OP5, selected from the OP family based on its osteogenic potential, was tested *in vitro* to compare its effects on osteogenic signaling, osteoblast differentiation, and hMSC gene expression in with those of BMP-2. New bone formation stimulated by OP5 or BMP-2 was assessed *in vivo* using radiographic and histological analyses in a rat model of calvarial defects.

Results: The optimal OP5 concentration of 1 μM supported hMSC viability and exhibited potent osteogenic activity. OP5 significantly activated BMP receptor types IA and II binding and the osteogenic protein kinase A and phosphorylated cAMP response element-binding protein signaling pathway. OP5-induced gene expressions of alkaline phosphatase and osteocalcin peaked on day 4 (early osteogenesis) and were sustained until day 14 (late osteogenesis). *In vivo*, 100 μg OP5 demonstrated superior bone formation compared to other doses (50, 300, and 600 μg), but was less effective than BMP-2. The amount of bone regeneration varied with different doses of OP5.

Conclusions: OP5, a low-molecular-weight peptide with strong osteogenic potential, may be a viable alternative to BMP-2 for clinical bone regeneration, minimizing BMP2-associated adverse effects.

Abbreviations: BMP, bone morphogenetic protein; hMSCs, human mesenchymal stromal cells; OP, osteogenic peptide; TGF, transforming growth factor; BMPR, BMP receptor; P-SMAD, phosphorylated SMAD; PKA, protein-kinase A; P-CREB, phosphorylated cAMP response element-binding protein; Runx2, runt-related transcription factor 2; Cbfa1, core-binding factor subunit alpha-1; ALP, alkaline phosphatase; OCN, osteocalcin; rhBMP-2, recombinant human BMP-2; OPDs, osteopromotive domains; BOPDs, BMP-2 receptor-binding peptides, termed OPDs; OGP, osteogenic growth peptide; ARS, Alizarin red S; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; ODM, osteogenic differentiation medium; DMSO, dimethyl sulfoxide; O.D., Optical density; PBS, phosphate-buffered saline; CPC, cetylpyridinium chloride; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; ABTS, Azino-bis diammonium salt; SDS, sodium dodecyl sulfate; RT-PCR, reverse transcription-polymerase chain reaction; micro-CT, micro-computed tomography; H&E, hematoxylin and eosin; 3-D, three-dimensional.

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

* Corresponding author.
** Corresponding author. Department of Periodontology, Jeonbuk National University College of Dentistry, 20 Geonji-ro, Deokjin-gu, Jeonju-si, Jeollabuk-do, 54907, Republic of Korea.
E-mail addresses: bskwon9@medivelbio.co.kr (B. Kwon), grayheron@hanmail.net (J.-H. Yun).
[1] Jong-Bin Lee and Ji-Youn Hong contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.reth.2025.09.006
Received 7 August 2025; Received in revised form 15 September 2025; Accepted 20 September 2025
Available online 15 October 2025

1. Introduction

Growth factors, osteoinductive molecules, and proteins such as transforming growth factor (TGF)-β, bone morphogenetic protein (BMP)-2, and BMP-7 play crucial roles in stimulating the robust regeneration of damaged bone tissue [1]. These proteins are required to stimulate specific cellular responses, particularly the differentiation of mesenchymal stromal cells (MSCs) into osteoblasts, which is crucial for bone healing. The importance of these osteogenic factors lies in their ability to facilitate recovery from bone loss and bone-related diseases, making them indispensable in regenerative medicine. In recent decades, numerous studies have elucidated the mechanisms of bone healing and growth factor signaling, contributing to the development of cell-based therapies [2–4]. Under normal physiological conditions, bone metabolism is regulated by the balance between osteoblasts and osteoclasts, which are derived from MSCs and hematopoietic stem cells, respectively [5,6]. Furthermore, bone repair involves a complex series of cellular events in which MSCs are influenced by both local and systemic regulators such as transcription factors, hormones, and cytokines [7–9].

Among the osteogenic factors involved in bone regeneration, BMPs and members of the TGF-β family are recognized as critical inducers of osteogenesis [10–13]. BMPs initiate their signaling cascades by binding to heterodimeric complexes of two transmembrane serine-threonine kinase receptors, BMP receptor type-I (BMPR-I) and BMP receptor type-II (BMPR-II), leading to the phosphorylation of SMAD transcription factors [14,15]. Phosphorylated SMAD (P-SMAD) subsequently activates the expression of key osteogenic genes. Additionally, protein-kinase A (PKA) and phosphorylated cAMP response element-binding protein (P-CREB) signaling pathways are linked to SMAD-mediated regulation of osteogenic differentiation [16–18]. Recent studies have also identified the Wnt/β-catenin signaling pathway as a mediator of peptide-induced osteoblast differentiation through α5β1 integrin priming in MSCs [19]. During osteogenesis, the expression of transcription factors, such as runt-related transcription factor 2 (Runx2)/core-binding factor subunit alpha-1 (Cbfa1) increases [20–22], and the expression of osteogenesis-related genes, such as alkaline phosphatase (ALP), BMP-2, and osteocalcin (OCN) is upregulated [23,24].

The clinical application of BMPs, particularly recombinant human BMP-2 (rhBMP-2), have gained traction for enhancing bone formation in orthopedic, spinal, and dental surgeries. However, the use of rhBMP-2 has been limited by several challenges [25], including rapid degradation by proteases, a short half-life, and the need for supra-physiological doses to achieve therapeutic effects [26]. These high doses are associated with serious adverse effects such as tumor formation, bone resorption, and ectopic bone growth [27–30]. High-dose rhBMP-2 has also been reported to cause complications such as formation of cyst-like bone voids, vertebral osteolysis, ectopic bone formation, radiculitis, and soft tissue swelling [31,32].

To address these limitations, bioactive peptides derived from the active protein domain of BMP-2 have been explored as alternatives to the full-length growth factor [33–37]. These synthetic peptides mimic the bioactivity of BMPs, while minimizing their adverse effects. Lee et al. demonstrated that self-assembled nanostructures consisting of BMP-2 receptor-binding peptides, termed osteopromotive domains (BOPDs), can activate and maintain osteogenesis [38]. Similarly, the C-terminal pentapeptide sequence of osteogenic growth peptide (OGP) has been reported to promote osteogenic differentiation while inhibiting adipogenesis in MSCs [39].

This study aimed to develop a synthetic peptide, OP5, derived from the receptor-binding domain of BMP-2, hypothesizing that OP5 could serve as a more effective alternative to BMP-2. Previous studies indicated that low-molecular-weight BMP-2-derived osteogenic peptides can overcome the limitations of rhBMP-2 while maintaining osteogenic efficacy [40]. Based on these findings, several amino acid sequences were synthesized and selected using the ALP and Alizarin red S (ARS) assays. Among them, the synthetic osteogenic activating peptide OP5, comprising 13 amino acids, including DWIVA, showed better osteogenic effects on human MSCs (hMSCs) and other synthetic peptides *in vitro*. Here, the osteogenic potential of OP5 was evaluated in comparison to that of BMP-2, both *in vitro* and *in vivo*, to assess its clinical applicability for bone regeneration.

2. Methods

2.1. Cell culture

Bone marrow-derived hMSCs were obtained from Lonza Walkersville, Inc. (catalog no. PT-2501; Walkersville, MD, USA). Cells were cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM, catalog no. 11885084) supplemented with 10 % fetal bovine serum (FBS, catalog no. 16000044) and 1 % antibiotic/antimycotic (catalog no. 15240062) (10F medium; Gibco™; Life Technologies, Grand Island, NY, USA). For osteogenic differentiation, cells were maintained in osteogenic differentiation medium (ODM) containing 10 mM β-glycerophosphate, 0.2 mM ascorbic acid, and 10^{-8} M dexamethasone (i.e., positive control). Cultures were maintained in a humidified atmosphere of 5 % CO_2 at 37 °C, with media replaced every 2 days.

2.2. Cell viability assay

Osteogenic peptides derived from the BMP-2 sequence were synthesized as listed in Table 1 [40]. To assess the cytotoxicity and optimal concentration of the synthetic peptides, hMSCs were seeded in 96-well plates at a density of 3×10^3 cells/well and incubated overnight. On day 2, the culture medium was replaced with a serum-free medium containing antibiotics to prevent growth inhibition by the serum, followed by the addition of 1F medium (DMEM with 1 % FBS and 1 % antibiotic/antimycotic) containing OP5 at various concentrations (1 nM–1 mM). After 48 h of incubation, cell viability was measured using methyl thiazolyl tetrazolium (MTT, catalog no. M2128; 5 mg/ml; Sigma-Aldrich, St. Louis, MO, USA). After a further 3-h incubation, the supernatant was discarded and dimethyl sulfoxide (DMSO, catalog no. D2650; 100μl/well; Sigma-Aldrich) was added to dissolve the formazan crystals. Optical density (O.D.) was measured at 570 nm using a microplate reader.

2.3. ALP assay

To evaluate the osteogenic potential of the peptides, ALP activity was measured as an early marker of osteoblast differentiation. hMSCs were seeded at a density of 5×10^3 cells/well in 96-well plates and cultured overnight. On day 2, the medium was replaced with ODM containing OP5 or other peptides (BOPD, OGP, and OP1–6) at concentrations of 0.01 and 1 μM, based on the MTT assay results with the medium replaced every 3 days. After 4 and 7 days of culture, the cells were washed with phosphate-buffered saline (PBS) and lysed with a lysis

Table 1
Amino acid sequences of the osteogenic peptides derived from BMP-2.

Peptide	Amino acid sequences
BOPD	DWIVA-NH_2
OGP	YGFGG-NH_2
OP-1	DWIVAYGFG-NH_2
OP-2	DVEALYNK-NH_2
OP-3	YFGAGYGKSGDWIVA-NH_2
OP-4	DGEAGPGDWIVA-NH_2
OP-5	DWIVAGSGDWIVA-NH_2
OP-6	YGFGGSDWEA-NH_2

BMP, bone morphogenetic protein; BOPD, BMP receptor-binding peptide [termed osteopromotive domain (OPD)]; OGP, osteogenic growth peptide; OP, osteogenic peptide.

buffer (0.5 % Triton X-100 in PBS). ALP activity was measured using an ALP assay kit (catalog no. MAK530; Sigma-Aldrich) in quadruplicate in 96-well plates, and protein levels were determined using Bio-Rad protein assay reagent (catalog no. 5000002EDU; Bio-Rad, Hercules, CA, USA). ALP activity was normalized to total protein concentration.

2.4. Mineralized bone matrix formation assay

To assess the ability of the peptides to induce the formation of a mineralized bone matrix, hMSCs were seeded at a density of 5×10^3 cells/well in 96-well plates and cultured overnight. On day 2, cells were cultured in ODM with peptides (BOPD, OGP, and OP1–6) at concentrations of 0.01 and 1 μM, based on the MTT assay results. The medium was changed every 3–4 days. On days 11 and 14, the cultures were stained with 2 % Alizarin Red S (ARS, catalog no. 2003999; Sigma-Aldrich) to assess mineralization [41]. Cells were fixed with 4 % paraformaldehyde and washed with deionized water before ARS staining. Staining was quantified after destaining with 5 % cetylpyridinium chloride (CPC), and calcium content was measured by reading the absorbance at 562 nm using a microplate reader (SpectraMax Plus 384, catalog no. 735-0346; Molecular Devices, San Jose, CA, USA).

2.5. Receptor binding assay

Binding activity of the peptide to BMP receptors was examined using an enzyme-linked immunosorbent assay (ELISA) modified from the method described by Saito et al. [33]. Briefly, 96-well Maxisorp microtiter plates (catalog no. 439454; Nalgene Nunc International, Rochester, NY, USA) were coated with OP5 (20 μg/ml), DWIVA (20 μg/ml), or BMP-2 (2 μg/ml) in PBS at 4 °C overnight. Next, BMPR-IA and BMPR-II (catalog no. MAB24061 and MAB811; R&D Systems, Minneapolis, MN, USA) were added and incubated for 2 h at room temperature.

The plates were then incubated overnight at 4 °C with 1:1,000 mouse anti-BMPR-IA and anti-BMPR-II antibody (R&D systems), followed by reaction with 1:2000 horseradish peroxidase (HRP)-conjugate rabbit anti-mouse IgG (catalog no. 7076; Cell Signaling Technology Inc., Danvers, MA, USA) for 30 min at room temperature. The bound antibodies were detected using ABTS (2,20-Azino-bis diammonium salt, catalog no. 30931-67-0; Sigma-Aldrich). The reaction was stopped after 5 min by adding 1 % sodium dodecyl sulfate (SDS), and the absorbance was measured at 450 nm. Wells without any peptide or BMP-2 coating were used as background controls.

2.6. Western blotting

Western blotting was performed to examine OP5 activation of osteogenic signaling pathways. hMSCs were treated with OP5, BMP-2, or the control medium for various time intervals. Protein extracts were prepared by lysing the cells with tissue lysis buffer (catalog no. 9803; Cell Signaling Technology Inc.) and were subjected to SDS-PAGE (catalog no. 70607; Sigma-Aldrich). The proteins were transferred to nitrocellulose membranes, blocked, and incubated with primary antibodies against β-catenin (1:1000, catalog no. 9562), P-CREB (1:1000, catalog no. 9198), and P-SMAD (1:1000, catalog no. 3101) purchased from Cell Signaling Technologies inc. (Danvers, MA. USA). After washing, membranes were incubated with HRP-conjugated secondary antibodies, and bands were visualized using enhanced chemiluminescence reagents (catalog no. W1015; Promega Corporation, Madison, WI, USA). Chemiluminescent signals were quantified using a FluorChemE system (catalog no. 92-14860-00; ProteinSimple, Santa Barbara, CA, USA). The bar graphs represent the band density values obtained from Western blot analysis, with the results expressed as density percentages (%), normalized to β-actin as a control.

2.7. RNA analysis

Total RNA was extracted from hMSCs treated with ODM and peptides (OP5, BMP-2, and control) using TRIzol reagent (catalog no. T9424; Sigma-Aldrich) on day 7 of differentiation. After treatment with RNase-free DNase I (catalog no. EN0521; Invitrogen; Thermo Fisher Scientific Solutions LLC, Waltham, MA, USA) to remove contaminating DNA, reverse transcription was performed to generate cDNA using the RT Premix (catalog no. RT600; Enzynomics, Daejeon, South Korea). Reverse transcription-polymerase chain reaction (RT-PCR) was conducted to measure the expression of osteogenic markers such as *ALP*, *Runx2/Cbfa1*, *BMP*, and *OCN*. Gene expression was normalized to that of *GAPDH*. The primers used for the RT-PCR are listed in Table 2. The bar graphs represent the band density values obtained from RT-PCR analysis, with the results expressed as density percentages (%), normalized to GAPDH as a control.

2.8. Animal model and surgical procedure

Animal studies were performed after receiving approval of the Institutional Animal Care and Use Committee (IACUC) of INHA University (IACUC No. INHA 140519-296) and were conducted in compliance with the ARRIVE guidelines and the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publication No. 85-23, 1985, revised 1996). Sprague-Dawley rats (8-week-old male, 250–300 g, n = 6/group) were anesthetized by intraperitoneal injection of combined zolazepam (10 mg/kg; Zoletil, NDC code: 51311-718-05; Virbac AH Inc., Westlake, TX, USA) and xylazine hydrochloride (2 mg/kg; Rumpun, catalog no. sc-551248Rx; Bayer, Leverkusen, Germany). A linear sagittal incision was made along the midline of the calvaria and full-thickness skin and periosteal flaps were elevated to expose the calvarial bone. Symmetrical 8-mm diameter critical-size bone defects were created in the calvarial bone using a trephine bur. The defects were treated with either no material (defect only) or a local hemostatic agent (GP, Greenplast Q®, catalog no. 214-82-06424; GC Biopharma, Yongin, Korea) as negative controls. For experimental groups, rhBMP-2 (1.5 μg; catalog no. GMP120-05ET-50UG; PeproTech Korea, Seoul, Korea) [42,43] or OP5 in various doses (50, 100, 300, 500, or 600 μg) combined with the scaffolds were implanted into the defects. The rhBMP-2 group served as a positive control. After 4 weeks, the animals were euthanized via CO_2 inhalation and calvarial bones were collected for micro-computed tomography (micro-CT) and histological analyses to evaluate new bone formation.

2.9. Micro-CT and histological analyses

The harvested calvarial specimens were fixed in 4 % paraformaldehyde and micro-CT scans were performed using a SKYSCAN1172 (Skyscan N.V., Antwerp, Belgium) with a resolution of 18.86 μm (50 kV, 200 μA). Regions of interest corresponding to the defect areas were reconstructed and analyzed using CTAn software (Skyscan N.V.) to determine the surface area (mm^2) and volume (mm^3) of the newly formed bone within the defects and compared to the control groups.

After micro-CT scanning, the specimens were decalcified, embedded in paraffin, and sectioned at a thickness of 5 μm. Sections were stained with hematoxylin and eosin (H&E), and histological analysis was performed to evaluate new bone formation using a light microscope (CKX41; Olympus, Tokyo, Japan) and digital image analysis software. New bone formation was measured at least three-times by the same examiner.

2.10. Statistics analysis

Results are presented as mean ± standard deviation (S.D.). Statistical comparisons between groups were performed using the student's t-test or one-way analysis of variance (ANOVA), followed by Tukey's post-hoc

Table 2
RT-PCR primer sequences.

Gene (accession no.)	Forward primer	Reverse primer
GAPDH (NM_002046.6)	5′-GTCAGTGGTGGACCTGACCT-3′	5′-AGGGGAGATTCAGTGTGGTG-3′
ALP (P00634)	5′-TTGCGGCTGCTCAGCATGTT-3′	5′-CATCTTGCATCTGTTCTCGGAA-3′
Runx2 (Q13950)	5′-AGATGGACCTCGGGAACCCA-3′	5′-AGAGTTCAGGGAGGGCCGTG-3′
Cbfa1 (Q13950)	5′-GATGACACTGCCACCTCTGA-3′	5′-GACTGGCGGGGTGTAAGTAA-3′
BMP (P12643)	5′-TTGCGGCTGCTCAGCATGTT-3′	5′-CATCTTGCATCTGTTCTCGGAA-3′
OCN (P02818)	5′-AGCGGTGCAGAGTCCAGCAA-3′	5′-AGGCCTCCTGAAAGCCGATG-3′

RT-PCR, reverse transcription-polymerase chain reaction; *ALP*, alkaline phosphatase; *Runx2*, runt-related transcription factor 2; *Cbfa1*, core-binding factor subunit alpha-1; *BMP*, bone morphogenetic protein; *OCN*, osteocalcin.

test when applicable. Statistical significance was set at a *P*-value of less than 0.05, with a higher significance level set at $P < 0.01$.

3. Results

3.1. Cytotoxicity and determination of optimal peptide concentration

The cytotoxicity of synthetic peptides OP5 was evaluated using the MTT assay to determine the optimal concentration for osteogenic differentiation assays. The hMSCs were treated with varying concentrations of OP5 (1 nM–1 mM) for 48 h. Cell viability decreased in a dose-dependent manner, with the peptides reducing hMSC viability at higher concentrations. OP5-treated hMSCs exhibited higher cell viability across about 1 nM–1 μM concentrations, with the IC50 of OP5 calculated to be ~20 μM (Fig. 1). Therefore, at concentrations below 1 μM, OP5 did not induce significant cytotoxic effects. Based on these results, 1 μM was selected as the optimal concentration for subsequent osteogenic differentiation experiments due to its non-cytotoxic nature and potential to maintain high viability in hMSCs.

3.2. ALP activity as an early osteogenic marker

ALP activity was measured on day 7 of differentiation to evaluate the early osteogenic effects of OP5. ALP activity, a key indicator of osteogenic differentiation, was significantly higher in hMSCs treated with OP5 than in the control groups, including the 10F medium and ODM groups. As shown in Fig. 2-A, OP5 demonstrated a dose-dependent increase in ALP activity, with 1 μM producing the highest levels of ALP among the peptides tested, including the other OP peptides. These results suggest that OP5 promotes early osteogenic differentiation, likely through enhanced activation of osteoblast-related genes.

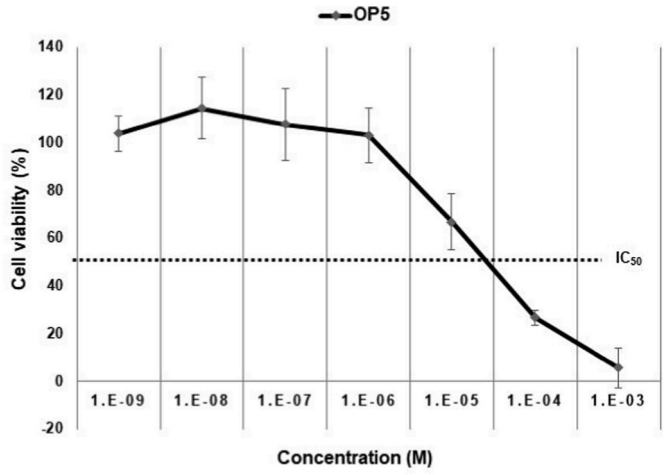

Fig. 1. Cell viability and cytotoxicity assessment of synthetic peptides derived from sequences of bone morphogenetic protein (BMP)-2. Each bar shows the mean ± standard deviation (S.D). OP, osteogenic peptide; IC_{50}, the half maximal inhibitory concentration.

3.3. Mineralized bone matrix formation

To confirm the osteogenic effects of OP5 on later-stage differentiation, the formation of mineralized bone matrix was assessed using ARS staining after 14 days of differentiation. ARS staining revealed significantly enhanced calcium deposition in OP5-treated hMSCs compared to treatment with the other synthetic peptides (OP1–6) and control groups (Fig. 2-B). These results suggest that 1 μM of OP5 promotes early osteogenic differentiation and facilitates late-stage mineralization, which is critical for bone tissue regeneration.

3.4. Receptor binding and activation of osteogenic signaling pathways

Binding affinity of OP5 to the BMP receptors BMPR-IA and BMPR-II was evaluated using ELISA. OP5 demonstrated strong binding to both BMPR-IA and BMPR-II, with a binding activity comparable to that of BMP-2, a well-known osteoinductive growth factor. Notably, OP5 exhibited a higher binding affinity for BMPR-II than DWIVA, a previously reported osteogenic peptide [38,40,44]. These findings suggest that OP5 interacts directly with BMP receptors to initiate osteogenic signaling pathways (Fig. 3-A).

Western blotting was performed to evaluate the activation of key osteogenic signaling molecules in OP5-treated hMSCs. P-SMAD, a downstream effector of BMP receptor signaling, was slightly increased in OP5-treated cells compared to control cells but not significantly. Additionally, OP5 induced the upregulation of P-CREB and β-catenin, which are both involved in osteoblast differentiation and bone formation. Notably, the expression of P-CREB was higher in OP5-treated cells than in BMP-2-treated cells after 6 h of treatment, suggesting that OP5 may exert stronger effects on CREB signaling than BMP-2 (Fig. 3-B).

Kim et al. demonstrated that the PKA/P-CREB pathway is involved in the osteogenic differentiation of hMSCs [45]. To validate the involvement of this pathway in OP5-mediated osteogenesis, we examined the effects of H89, a PKA inhibitor, on OP5-induced osteogenic differentiation. In the presence of H89 (10 μM) [16,18,46,47], P-CREB levels were reduced across all treatment groups, with OP5-treated cells showing the greatest downregulation of P-CREB, as observed through western blot analysis (Fig. 4-A).

The inhibition of PKA by H89 also results in a reduction in OP5-induced osteogenesis. ALP activity, a key marker of early osteoblast differentiation, decreased as the concentration of H89 increased to 10 μM. Additionally, the mineralization activity, as assessed using the ARS assay, was significantly diminished after H89 treatment ($P < 0.01$) (Fig. 4-B). These results indicate that the PKA/P-CREB signaling pathway plays a critical role in OP5-induced osteoblast differentiation. The suppression of OP5's osteogenic effects by H89 further supports the conclusion that OP5-mediated osteogenesis is, at least in part, dependent on PKA/CREB pathway activation. These results indicated that OP5 activates multiple osteogenic signaling pathways, including the P-SMAD and P-CREB pathways, to promote osteogenesis.

3.5. Osteogenic gene expression

To confirm the osteogenic effects of OP5, RT-PCR was performed on

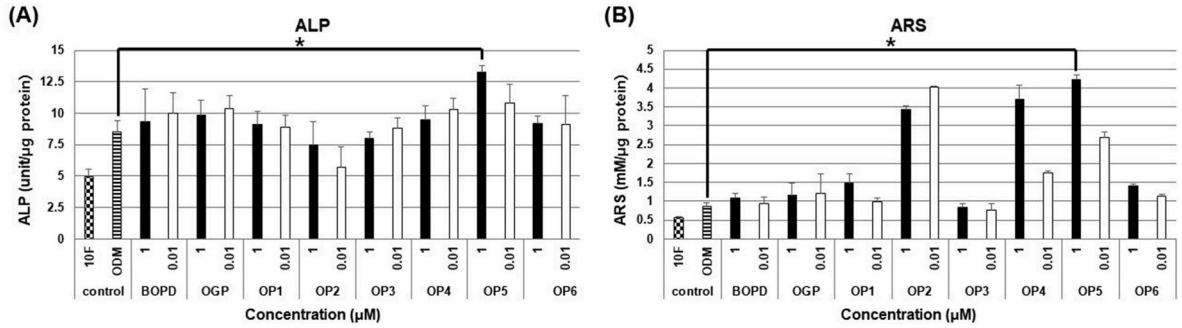

Fig. 2. Osteogenic peptide (OP) effects on osteogenesis in human mesenchymal stromal cells (hMSCs). **(A)** Alkaline phosphatase (ALP) activity of hMSCs cultured for 7 days in the control group [i.e., 10F medium {Low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % antibiotic/antimycotic}], positive control group [i.e., osteogenic differentiation medium (ODM; 10 mM β-glycerophosphate, 0.2 mM ascorbic acid, and 10^{-8}M dexamethasone)], and ODM containing the various peptidesas the test groups. The data are expressed as protein amount (nmoles of p-nitrophenol per μg) and are shown as mean ± S.D. $^{*}P < 0.05$. **(B)** Alizarin Red S (ARS) staining test of hMSCs cultured in the control and test groups. After continuous differentiation culture of hMSCs with several peptides for 14 days, mineralized matrix was evaluated by ARS staining. Each bar shows the mean ± S.D. BOPD, BMP receptor-binding peptide [termed osteopromotive domain (OPD)]; OGP, osteogenic growth peptide. $^{*}P < 0.05$.

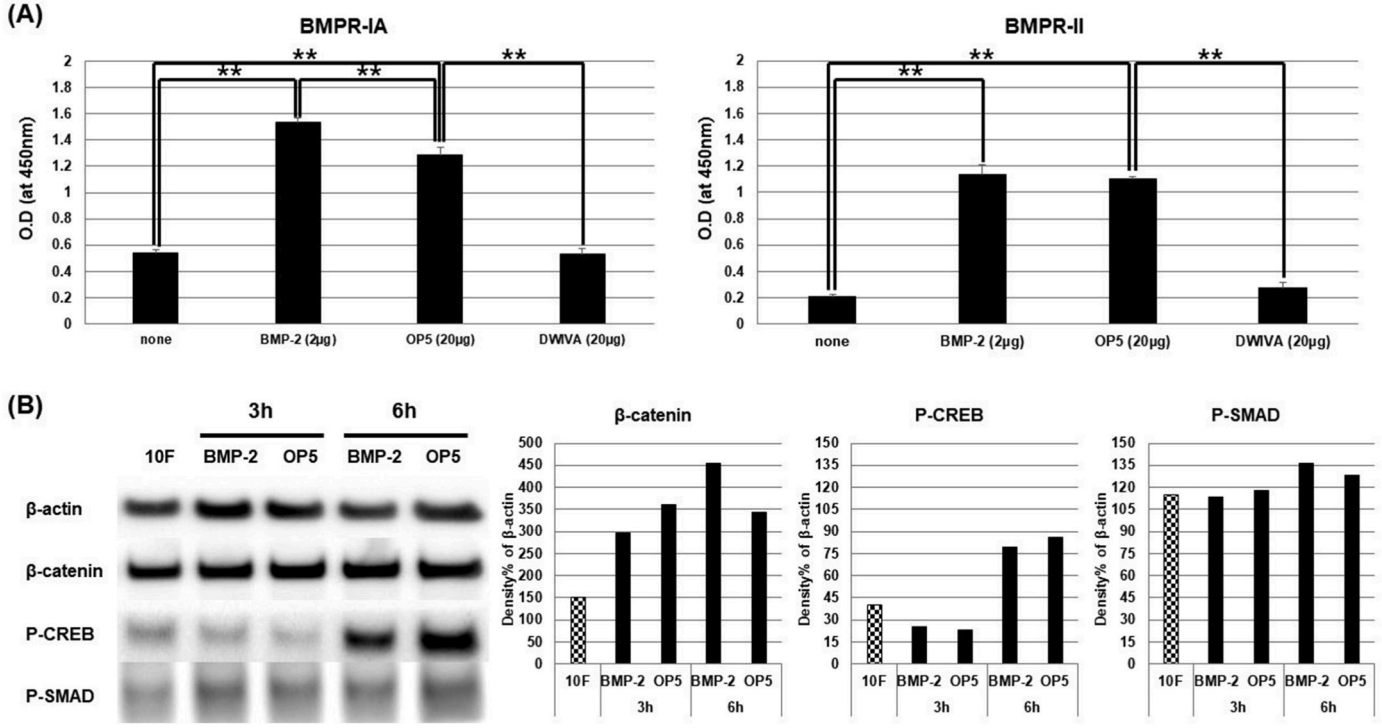

Fig. 3. The osteogenic signal transduction of OP5. **(A)** The binding affinity of the peptide to BMP receptor types IA and II (BMPR-IA and II). BMPR-IA or BMPR-II in lysate of human mesenchymal stromal cells (hMSCs) was immobilized on a microplate using a specific BMP receptor antibody in an ELISA. Data represents mean ± S.D. Binding activity of BMP-2 (2 μg/ml) and OP5 (20 μg/ml) are detected using anti-BMP-2 monoclonal antibody and 1:2000 horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG as a secondary antibody binding to primary antibody. Each bar shows the mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$. **(B)** Western blot analysis of β-catenin, phosphorylated cAMP response element-binding protein (P-CREB), and phosphorylated SMAD (P-SMAD) expression in hMSCs treated with BMP-2 and OP5 for 3 and 6 h. Cell lysates were prepared and analyzed by western blot using antibodies to β-catenin, P-CREB, and P-SMAD (all of 1:1000). The expression level of each group was normalized with the density % of β-actin in the same group. O.D., optical density; None, without peptide or protein group; BMP, bone morphogenetic protein; OP, osteogenic peptide; DWIVA, amino acid sequence from osteogenic peptide; 10F, 10F medium [Low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % antibiotic/antimycotic] as the control group.

days 4 and 14 of differentiation to quantify the expression of key osteogenic genes, including *ALP*, *Runx2/Cbfa1*, *BMP*, and *OCN* [48,49]. On day 4, OP5-treated hMSCs demonstrated a significant upregulation in the expression of *ALP*, a marker of early osteogenic differentiation. Additionally, the expression of *OCN*, a late-stage marker of osteoblast activity, was significantly increased on days 4 and 14 in OP5-treated cells. These findings indicate that OP5 facilitates both early osteogenic differentiation and osteoblast maturation. The expression of the transcription factor *Runx2/Cbfa1*, was also significantly upregulated in OP5-treated cells compared to that in control cells. This transcription factor is a critical regulator of osteoblast differentiation and bone formation. Moreover, the expression of BMP, an important osteogenic factor, was elevated in OP5-treated cells, indicating that OP5 may enhance the autocrine production of osteogenic factors (Fig. 5). Collectively, these findings demonstrate that OP5 induces a robust osteogenic gene expression profile in hMSCs, supporting its potential use

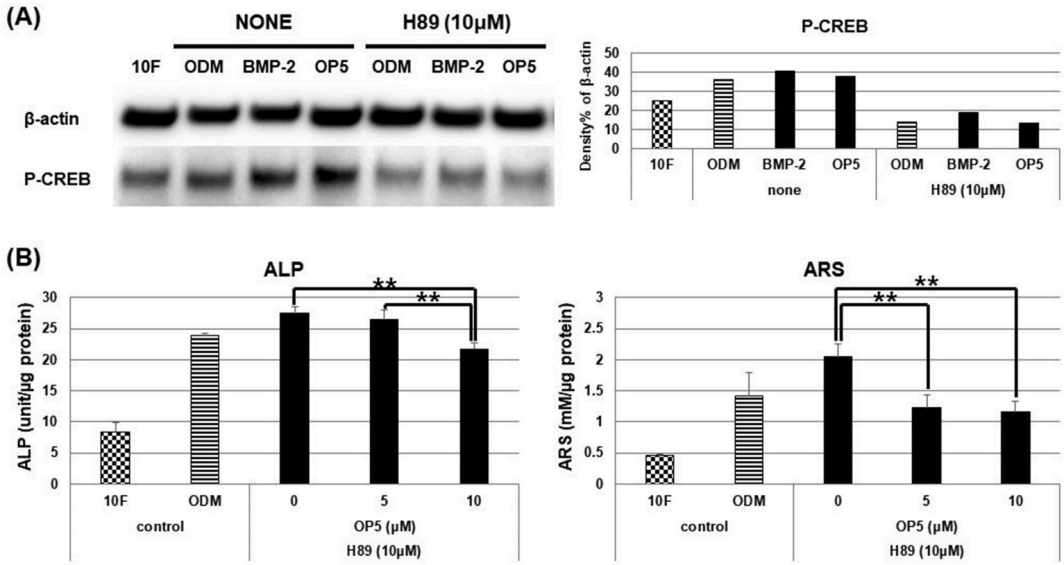

Fig. 4. Downregulation of OP5 in osteogenesis by inhibition of the protein-kinase A (PKA)/P-CREB pathway. **(A)** human mesenchymal stromal cells (hMSCs) were treated with OP5 (1 μM) or BMP-2 (400 ng/ml) in the absence or presence of H89 (PKA inhibitor; 10 μM) for 6 h and P-CREB was examined by western blot analysis. The expression level of each group was normalized with the density % of β-actin in the same group. **(B)** Inhibition of OP5-induced osteogenesis by H89 treatment was confirmed by ALP activity and ARS assay at 7 and 14 days, respectively, after osteogenesis induction. Each bar shows the mean ± S.D. 10F, 10F medium [Low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % antibiotic/antimycotic] as the control group; ODM, osteogenic differentiation medium (10 mM β-glycerophosphate, 0.2 mM ascorbic acid, and 10^{-8} M dexamethasone) as the positive control group; BMP, bone morphogenetic protein; OP, osteogenic peptide; P-CREB, phosphorylated cAMP response element-binding protein; ALP, alkaline phosphatase; ARS, Alizarin Red S. $^{*}P < 0.05$, $^{**}P < 0.01$.

in bone regeneration.

3.6. *In vivo* bone formation in a rat calvarial defect model

The *in vivo* bone-regeneration capacity of OP5 was evaluated using a rat calvarial defect model. Micro-CT and histological analyses were conducted to assess new bone formation within the defect sites. In micro-CT analysis, the surface area and volume of newly formed bone was higher in the OP5-treated groups compared to the control group, with the highest bone volume observed in the group treated with 100 μg of OP5. Notably, bone formation in the 100 μg OP5 group was comparable to that observed in the BMP-2 group, suggesting that OP5 is as effective as BMP-2 in promoting new bone formation. Additionally, the 100 μg OP5 group exhibited superior bone density and quality compared to the 300 μg and 500 μg OP5 groups, suggesting that 100 μg is the optimal concentration of OP5 for *in vivo* bone regeneration (Fig. 6). Histological analysis confirmed the micro-CT findings. H&E staining revealed that the newly formed bone in the OP5-treated defects was well organized, with dense bone trabeculae and fewer cyst-like voids than in the control group. The quality of newly formed bone in OP5-treated defects was also comparable to that observed in BMP-2-treated defects. Although OP5 did not achieve the full osteoinductive potential of BMP-2, it exhibited the capacity to promote both bone formation and high-quality bone regeneration (Fig. 6).

4. Discussion

BMP-2 plays an important role in bone regeneration by inducing the osteogenic differentiation of hMSCs [1]. Based on these characteristics, BMP-2, functioning as an osteoinductive factor in bone formation, has played an essential role in bone healing and regeneration during recovery from bone-related diseases in cell-based therapy [11–13]. However, because clinical application requires high concentrations of commercial BMP-2, side effects, such as tumor formation and unintended deformation of bone and soft tissue, may occur [28,32], thus, careful use and effective alternatives for BMP-2 are essential.

This study aimed to develop replacements for BMP-2, mitigating associated side effects, by screening synthetic peptides. A newly synthesized OP5—a peptide with a 13 amino acid sequence (DWIVAGSGDWIVA)—contains two DWIVA peptide sequences, known to have osteogenic activity [38]. DWIVA induces osteoblast differentiation by increasing ALP activity, and DWIVA peptide sequence from BMP-2 has high receptor affinity and specificity for BMPR-I and BMPR-II [38,44]. OP5 exhibited significantly higher receptor-binding activity than the control and DWIVA peptides in this study, confirming that OP5's effect on osteogenic induction capacity is superior to that of DWIVA and ultimately comparable to that of BMP-2. The high affinity of OP5 for BMPR-II provides clear evidence for this possibility.

BMP-2 induced BMP receptor signaling triggers osteogenic signals [50–53]. This interaction activates P-SMAD to mediate osteoblast differentiation [33]. Several pathways, including Wnt/β-catenin, PTH/CREB [45,54] and PKA/P-CREB signaling [16–18], which were closely related to SMAD phosphorylation and stimulation, have been reported to promote osteoblast differentiation and enhance osteogenic activity. In this study, OP5 sustained the expression of these signals over time, although it did not reach the level of BMP-2. Moreover, OP5 exhibited a superior effect to BMP-2 in inducing P-CREB protein expression in hMSCs. This was confirmed by the decrease in osteogenic and mineralization tendencies, which were sensitive to the application of H89, an inhibitor of CREB phosphorylation. It was verified that OP5, a bone growth factor, was involved in the regulation of CREB signaling during osteoblast differentiation. This confirmed that OP5 shares the same osteogenic signaling pathway as BMP-2, increasing the possibility of substitution between them.

The osteogenesis of hMSCs induces the upregulation of ALP at an early stage [55], and high levels of ALP are followed by the induction of OCN synthesis, which reflects calcium deposition [56]. In the OP5 group, the remarkable increase in *ALP* mRNA levels in the early stage and maintenance of the highest *OCN* mRNA levels in the early and late stages demonstrated osteoblastic gene expression. In addition, OP5 has the potential to maintain high *ALP* levels during the late stages. Runx2/Cbfα1 is a transcriptional activator for osteoblast differentiation [57,

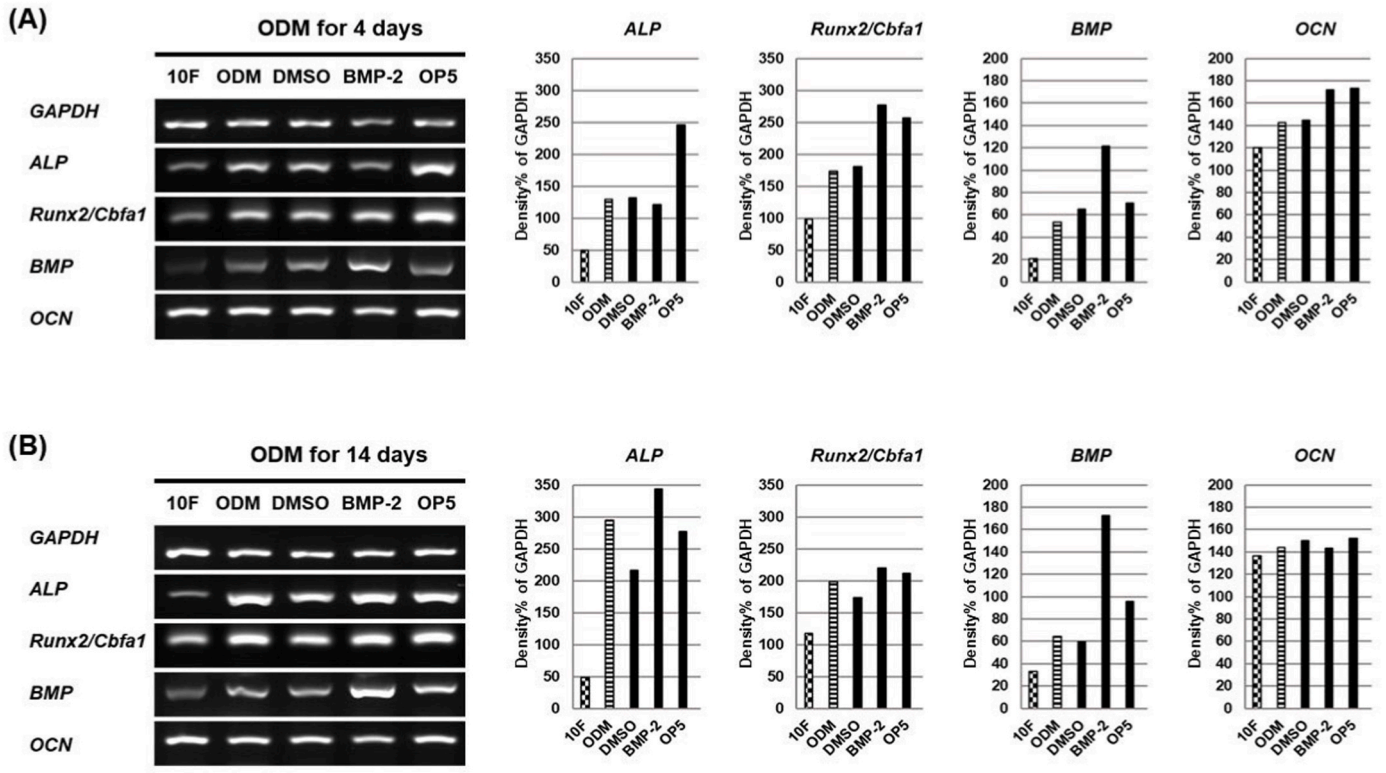

Fig. 5. Osteogenic gene expression in human mesenchymal stromal cells (hMSCs). The cells were cultured for 4 and 14 days, and total RNA was isolated. The RNA from each sample was reverse-transcribed into cDNA and the expression of each gene was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) analysis with primers. The band expression level of each gene (i.e., *ALP*, *Runx2/Cbfa1*, *BMP*, and *OCN*) was normalized with the density % of *GAPDH* in the same group. Noticeable difference appears when compared with control, BMP-2 (400 ng/ml) and OP5 (1 μM). **(A)** Gene expression levels in hMSCs on day 4. **(B)** Gene expression levels in hMSCs on day 14. 10F, 10F medium [Low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % antibiotic/antimycotic] as the control group; ODM, osteogenic differentiation medium (10 mM β-glycerophosphate, 0.2 mM ascorbic acid, and 10^{-8} M dexamethasone) as the positive control group; DMSO, dimethyl sulfoxide; BMP, bone morphogenetic protein; OP, osteogenic peptide; *ALP*, alkaline phosphatase; *Runx2/Cbfa1,* runt-related transcription factor 2/core-binding factor subunit alpha-1; *OCN*, osteocalcin.

58]. Our results showed that *Runx2/Cbfα1* was highly expressed in OP5 group. These experiments demonstrated that OP5 could be a successful substitute for BMP-2 because it can induce osteogenic conditions. However, since this is a qualitative endpoint evaluation through band density comparison, additional confirmation through quantitative PCR (i.e., real-time PCR) is required for a more quantitative and absolute evaluation.

Therefore, OP5 warrants strong focus. Because OP5 is a commercially synthesized peptide, it is less expensive to produce than biologically produced BMP-2 (e.g., rhBMP-2). This is important for cost reduction. Additionally, because OP5 is a low-molecular-weight peptide, the risk is lower than that of BMP-2, which has several side effects when applied at high concentrations. Low-molecular-weight synthetic peptides can be easily applied to scaffolds using various methods, therefore, they have the advantage of being incorporated into tissue-engineered treatments. Therefore, the fact that OP5 has effects similar to those of BMP-2 underscores its clinical utility.

In this study, the concentration 0.01–1 μM of OP5 did not affect hMSC viability. Furthermore, the optimal concentration 1 μM of OP5 showed higher osteogenic potency than other peptides by ALP activity and ARS assay. Comparative tests between OP5 (1 μM) and BMP-2 (300 ng/ml) showed that OP5 is not comparable to BMP-2 in 10F medium, but OP5 shows a similar level (about 90 %) of osteogenic inductive potency on hMSCs as BMP-2 in osteogenic medium (i.e., ODM) (Supplement Fig. 1). Thus, under appropriate conditions, OP5 can replace BMP-2.

Numerous animal studies have demonstrated the beneficial effects of BMP-2 on new bone formation using various methods, including bone defect and fracture models [59,60]. New bone formation in the rat calvarial defect was higher in the OP5 group compared to that in the control groups, following the BMP-2 group. We confirmed that 1 μM of OP5 was the optimal concentration for the viability and osteogenesis of hMSCs. Because concentration is directly affected by sample volume, the amount of peptide in a sample is a more appropriate indicator for evaluating sample activity than peptide concentration [61]. Therefore, osteogenic efficacy depends on the applied amount of OP5, and 100 μg of OP5 in this study was the most effective quantity. Additionally, regarding new bone quality, the new bone in the 100 μg of OP5 group was denser and had fewer voids, whereas the BMP-2 group was rather more porous. Thus, 1 μM of OP5 which contains 100 μg of OP5 can more stably induce high-quality bone formation in the *in vivo* model than BMP-2.

Therefore, OP5 is an ideal substitute to overcome the disadvantages of BMP-2 while maintaining its advantages. A previous study confirmed the formation of new vasculogenic bone through the immobilization of BMP-2-derived peptides onto nanoparticles [40]. These peptides can reduce side effects that occur in response to the full protein and can maintain their three-dimensional (3-D) structures under physiological conditions owing to their simple structural conformations. In addition, higher amounts of peptides could be available to be delivered *in vivo* compared to that of BMP-2. It suggests that OP5 is a promising alternative to BMP-2 for *in vivo* bone regeneration, with the potential to replace BMP-2 in clinical applications due to its comparable efficacy and a lower risk of side effects associated with high-dose BMP-2.

This study had several limitations. First, results would have been more conclusive with additional correlation experiments between

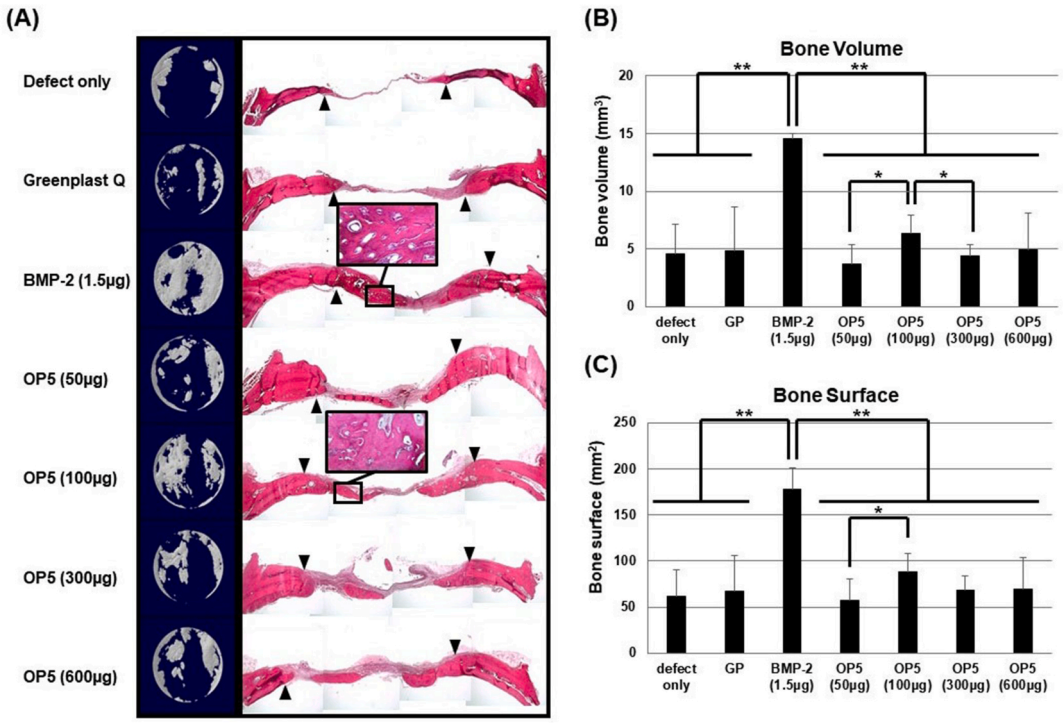

Fig. 6. New bone regeneration in an *in vivo* transplantation model after 4 weeks**. (A)** Representative micro-CT view and photomicrograph of calvarial defects with no material (defect only), scaffold only (negative control group; Greenplast Q®; GC Biopharma, Yongin, Korea) and scaffolds containing 1.5 μg of BMP-2 (positive control group) or OP5 in various doses (50, 100, 300, 500 and 600 μg) shown at 40x magnification. In OP5, immature and newly formed bone can be observed with embedded scaffold remnants irrespective of the loaded dose. The black box indicates the area with the highest new-bone density 3–5 mm away from defect margin at 200x magnification. **(B)** Micro-CT measurements of bone surface area (mm^2) of newly formed bone within rat calvarial defect. BMP-2 and OP5 (100 μg) groups show the highest values in that order. **(C)** Micro-CT measurements of bone volume (mm^3) of newly formed bone. Significant increases in BMP-2 followed by the 100 μg OP5 group indicates the critical concentration of OP5 that can be used as a substitute for BMP-2 in bone regeneration. GP, Greenplast Q®; BMP, bone morphogenetic protein; OP, osteogenic peptide. $^*P < 0.05$, $^{**}P < 0.01$.

osteoblasts and osteoclasts. Second, although Greenplast was used as scaffolds, further research using bone graft materials as scaffolds would be helpful for verifying the effects of OP5 and BMP-2. Third, Additional *in vivo* studies are required to observe the effects of OP5 on soft tissues regeneration and potential adverse effects. Fourth, while the 14-day results in this study were referred to as the late stage, this reflects a relative timeline, and late-stage osteogenesis could be more clearly confirmed through extended *in vitro* studies. Fifth, the *in vitro* experimental conditions—including analysis methods and sample size—need to be optimized to allow for more meaningful analysis. In the future, OP5 treatment validation in the induction of bone regeneration should be confirmed through human clinical studies on the jawbone and in large-scale and long-term follow-up animal studies including 3-D analysis.

5. Conclusions

The results of this study provide strong evidence that OP5, a synthetic osteogenic peptide derived from the receptor-binding domain of BMP-2, can effectively promote osteogenic differentiation *in vitro* and bone regeneration *in vivo*. These findings indicate that OP5 is a promising alternative to BMP-2, with several advantages, including lower-molecular-weight, easier synthesis, and reduced potential for adverse effects associated with high doses of BMP-2.

Declaration of competing interest

HS, SK, and BK are employed by the MedivelBio Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

This work was supported by Fund of Biomedical Research Institute, Jeonbuk National University Hospital and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, RS-2024-00338812).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2025.09.006.

References

[1] Karageorgiou V, Meinel L, Hofmann S, Malhotra A, Volloch V, et al. Bone morphogenetic protein-2 decorated silk fibroin films induce osteogenic differentiation of human bone marrow stromal cells. J Biomed Mater Res A 2004; 71(3):528–37.
[2] Lou J, Tu Y, Li S, Manske PR. Involvement of ERK in BMP-2 induced osteoblastic differentiation of mesenchymal progenitor cell line C3H10T1/2. Biochem Biophys Res Commun 2000;268(3):757–62.
[3] Palcy S, Goltzman D. Protein kinase signalling pathways involved in the up-regulation of the rat alpha1(I) collagen gene by transforming growth factor beta1 and bone morphogenetic protein 2 in osteoblastic cells. Biochem J 1999;343:21–7.
[4] Chen D, Zhao M, Harris SE, Mi Z. Signal transduction and biological functions of bone morphogenetic proteins. Front Biosci 2004;9:349–58.
[5] Alliston T, Derynck R. Medicine: interfering with bone remodelling. Nature 2002; 416(6882):686–7.
[6] Cohen Jr MM. The new bone biology: pathologic, molecular, and clinical correlates. Am J Med Genet A 2006;140(23):2646–706.
[7] Denburg JA, van Eeden SF. Bone marrow progenitors in inflammation and repair: new vistas in respiratory biology and pathophysiology. Eur Respir J 2006;27(3): 441–5.

[8] Kloss FR, Gassner R. Bone and aging: effects on the maxillofacial skeleton. Exp Gerontol 2006;41(2):123–9.
[9] Fleet JC, Cashman K, Cox K, Rosen V. The effects of aging on the bone inductive activity of recombinant human bone morphogenetic protein-2. Endocrinology 1996;137(11):4605–10.
[10] Ducy P, Karsenty G. The family of bone morphogenetic proteins. Kidney Int 2000;57(6):2207–14.
[11] Wozney JM. The bone morphogenetic protein family and osteogenesis. Mol Reprod Dev 1992;32(2):160–7.
[12] Kopp JB. BMP receptors in kidney. Kidney Int 2000;58(5):2237–8.
[13] Yamaguchi A, Katagiri T, Ikeda T, Wozney JM, Rosen V, et al. Recombinant human bone morphogenetic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation in vitro. J Cell Biol 1991;113(3):681–7.
[14] Cheng H, Jiang W, Phillips FM, Haydon RC, Peng Y, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). J Bone Joint Surg Am 2003;85(8):1544–52.
[15] Kirsch T, Sebald W, Dreyer MK. Crystal structure of the BMP-2-BRIA ectodomain complex. Nat Struct Biol 2000;7(6):492–6.
[16] Lee YS, Chuong CM. Activation of protein kinase A is a pivotal step involved in both BMP-2- and cyclic AMP-induced chondrogenesis. J Cell Physiol 1997;170(2):153–65.
[17] Ionescu AM, Drissi H, Schwarz EM, Kato M, Puzas JE, et al. CREB Cooperates with BMP-stimulated Smad signaling to enhance transcription of the Smad6 promoter. J Cell Physiol 2004;198(3):428–40.
[18] Siddappa R, Martens A, Doorn J, Leusink A, Olivo C, et al. cAMP/PKA pathway activation in human mesenchymal stem cells in vitro results in robust bone formation in vivo. Proc Natl Acad Sci U S A 2008;105(20):7281–6.
[19] Saidak Z, Le Henaff C, Azzi S, Marty C, Da Nascimento S, et al. Wnt/beta-catenin signaling mediates osteoblast differentiation triggered by peptide-induced alpha5beta1 integrin priming in mesenchymal skeletal cells. J Biol Chem 2015;290(11):6903–12.
[20] Phimphilai M, Zhao Z, Boules H, Roca H, Franceschi RT. BMP signaling is required for RUNX2-dependent induction of the osteoblast phenotype. J Bone Miner Res 2006;21(4):637–46.
[21] Yang S, Wei D, Wang D, Phimphilai M, Krebsbach PH, et al. In vitro and in vivo synergistic interactions between the Runx2/Cbfa1 transcription factor and bone morphogenetic protein-2 in stimulating osteoblast differentiation. J Bone Miner Res 2003;18(4):705–15.
[22] Franceschi RT, Xiao G, Jiang D, Gopalakrishnan R, Yang S, et al. Multiple signaling pathways converge on the Cbfa1/Runx2 transcription factor to regulate osteoblast differentiation. Connect Tissue Res 2003;44(Suppl 1):109–16.
[23] Harris SE, Bonewald LF, Harris MA, Sabatini M, Dallas S, et al. Effects of transforming growth factor beta on bone nodule formation and expression of bone morphogenetic protein 2, osteocalcin, osteopontin, alkaline phosphatase, and type I collagen mRNA in long-term cultures of fetal rat calvarial osteoblasts. J Bone Miner Res 1994;9(6):855–63.
[24] Huang WC, Xie Z, Konaka H, Sodek J, Zhau HE, et al. Human osteocalcin and bone sialoprotein mediating osteomimicry of prostate cancer cells: role of cAMP-dependent protein kinase A signaling pathway. Cancer Res 2005;65(6):2303–13.
[25] Kim MS, Lee JS, Shin HK, Kim JS, Yun JH, et al. Prospective randomized, controlled trial of sinus grafting using Escherichia-coli-produced rhBMP-2 with a biphasic calcium phosphate carrier compared to deproteinized bovine bone. Clin Oral Implants Res 2015;26(12):1361–8.
[26] Shields LB, Raque GH, Glassman SD, Campbell M, Vitaz T, et al. Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion. Spine 2006;31(5):542–7.
[27] Kang HJ, Jun CM, Yun JH. Radiographic and Histologic Evaluation of a Bone Void that Formed After Recombinant Human Bone Morphogenetic Protein-2-Mediated Sinus Graft Augmentation: A Case Report. Int J Periodontics Restor Dent 2016;36:s151–8.
[28] Langenfeld EM, Calvano SE, Abou-Nukta F, Lowry SF, Amenta P, et al. The mature bone morphogenetic protein-2 is aberrantly expressed in non-small cell lung carcinomas and stimulates tumor growth of A549 cells. Carcinogenesis 2003;24(9):1445–54.
[29] Kaneko H, Arakawa T, Mano H, Kaneda T, Ogasawara A, et al. Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. Bone 2000;27(4):479–86.
[30] Kleeff J, Maruyama H, Ishiwata T, Sawhney H, Friess H, et al. Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterology 1999;116(5):1202–16.
[31] Zara JN, Siu RK, Zhang X, Shen J, Ngo R, et al. High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo. Tissue Eng Part A 2011;17(9–10):1389–99.
[32] Shimer AL, Oner FC, Vaccaro AR. Spinal reconstruction and bone morphogenetic proteins: open questions. Injury 2009;40(Suppl 3):S32–8.
[33] Saito A, Suzuki Y, Ogata S, Ohtsuki C, Tanihara M. Activation of osteo-progenitor cells by a novel synthetic peptide derived from the bone morphogenetic protein-2 knuckle epitope. Biochim Biophys Acta 2003;1651(1–2):60–7.
[34] Gabet Y, Muller R, Regev E, Sela J, Shteyer A, et al. Osteogenic growth peptide modulates fracture callus structural and mechanical properties. Bone 2004;35(1):65–73.
[35] Behnam K, Phillips ML, Silva JD, Brochmann EJ, Duarte ME, et al. BMP binding peptide: a BMP-2 enhancing factor deduced from the sequence of native bovine bone morphogenetic protein/non-collagenous protein. J Orthop Res 2005;23(1):175–80.
[36] Mercado AE, Yang X, He X, Jabbari E. Effect of grafting BMP2-derived peptide to nanoparticles on osteogenic and vasculogenic expression of stromal cells. J Tissue Eng Regen Med 2014;8(1):15–28.
[37] Chen J, Shi ZD, Ji X, Morales J, Zhang J, et al. Enhanced osteogenesis of human mesenchymal stem cells by periodic heat shock in self-assembling peptide hydrogel. Tissue Eng Part A 2013;19(5–6):716–28.
[38] Lee JY, Choo JE, Choi YS, Suh JS, Lee SJ, et al. Osteoblastic differentiation of human bone marrow stromal cells in self-assembled BMP-2 receptor-binding peptide-amphiphiles. Biomaterials 2009;30(21):3532–41.
[39] Chen ZX, Chang M, Peng YL, Zhao L, Zhan YR, et al. Osteogenic growth peptide C-terminal pentapeptide [OGP(10-14)] acts on rat bone marrow mesenchymal stem cells to promote differentiation to osteoblasts and to inhibit differentiation to adipocytes. Regul Pept 2007;142(1–2):16–23.
[40] Lee J, Perikamana SK, Ahmad T, Lee MS, Yang HS, et al. Controlled Retention of BMP-2-Derived Peptide on Nanofibers Based on Mussel-Inspired Adhesion for Bone Formation. Tissue Eng Part A 2017;23(7–8):323–34.
[41] Stanford CM, Jacobson PA, Eanes ED, Lembke LA, Midura RJ. Rapidly forming apatitic mineral in an osteoblastic cell line (UMR 106-01 BSP). J Biol Chem 1995;270(16):9420–8.
[42] Intini G, Andreana S, Buhite RJ, Bobek LA. A comparative analysis of bone formation induced by human demineralized freeze-dried bone and enamel matrix derivative in rat calvaria critical-size bone defects. J Periodontol 2008;79(7):1217–24.
[43] Jung JH, Yun JH, Um YJ, Jung UW, Kim CS, et al. Bone formation of Escherichia coli expressed rhBMP-2 on absorbable collagen block in rat calvarial defects. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111(3):298–305.
[44] Nickel J, Dreyer MK, Kirsch T, Sebald W. The crystal structure of the BMP-2:BMPR-IA complex and the generation of BMP-2 antagonists. J Bone Joint Surg Am 2001;83-A(Suppl 1):S7–14.
[45] Kim JM, Choi JS, Kim YH, Jin SH, Lim S, et al. An activator of the cAMP/PKA/CREB pathway promotes osteogenesis from human mesenchymal stem cells. J Cell Physiol 2013;228(3):617–26.
[46] Shi Q, Gao J, Jiang Y, Sun B, Lu W, et al. Differentiation of human umbilical cord Wharton's jelly-derived mesenchymal stem cells into endometrial cells. Stem Cell Res Ther 2017;8(1):246.
[47] Kondo M, Yamaoka K, Sonomoto K, Fukuyo S, Oshita K, et al. IL-17 inhibits chondrogenic differentiation of human mesenchymal stem cells. PLoS One 2013;8(11):e79463.
[48] Lee MH, Javed A, Kim HJ, Shin HI, Gutierrez S, et al. Transient upregulation of CBFA1 in response to bone morphogenetic protein-2 and transforming growth factor beta1 in C2C12 myogenic cells coincides with suppression of the myogenic phenotype but is not sufficient for osteoblast differentiation. J Cell Biochem 1999;73(1):114–25.
[49] Marie PJ. Transcription factors controlling osteoblastogenesis. Arch Biochem Biophys 2008;473(2):98–105.
[50] Chen G, Deng C, Li YP. TGF-beta and BMP signaling in osteoblast differentiation and bone formation. Int J Biol Sci 2012;8(2):272–88.
[51] Knaus P, Sebald W. Cooperativity of binding epitopes and receptor chains in the BMP/TGFbeta superfamily. Biol Chem 2001;382(8):1189–95.
[52] Kudo TA, Kanetaka H, Watanabe A, Okumoto A, Asano M, et al. Investigating bone morphogenetic protein (BMP) signaling in a newly established human cell line expressing BMP receptor type II. Tohoku J Exp Med 2010;222(2):121–9.
[53] Fukuda T, Scott G, Komatsu Y, Araya R, Kawano M, et al. Generation of a mouse with conditionally activated signaling through the BMP receptor, ALK2. Genesis 2006;44(4):159–67.
[54] Zhang R, Edwards JR, Ko SY, Dong S, Liu H, et al. Transcriptional regulation of BMP2 expression by the PTH-CREB signaling pathway in osteoblasts. PLoS One 2011;6(6):e20780.
[55] Whyte MP. Hypophosphatasia and the role of alkaline phosphatase in skeletal mineralization. Endocr Rev 1994;15(4):439–61.
[56] Lian JB, Stein GS, Stein JL, van Wijnen AJ. Regulated expression of the bone-specific osteocalcin gene by vitamins and hormones. Vitam Horm 1999;55:443–509.
[57] Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 1997;89(5):747–54.
[58] Sila-Asna M, Bunyaratvej A, Maeda S, Kitaguchi H, Bunyaratavej N. Osteoblast differentiation and bone formation gene expression in strontium-inducing bone marrow mesenchymal stem cell. Kobe J Med Sci 2007;53(1–2):25–35.
[59] Park SY, Kim KH, Koo KT, Lee KW, Lee YM, et al. The evaluation of the correlation between histomorphometric analysis and micro-computed tomography analysis in AdBMP-2 induced bone regeneration in rat calvarial defects. J Periodontal Implant Sci 2011;41(5):218–26.
[60] Hong YR, Kim TH, Park KH, Kang J, Lee K, et al. rhBMP-2-Conjugated Three-Dimensional-Printed Poly(L-lactide) Scaffold is an Effective Bone Substitute. Tissue Eng Regen Med 2023;20(1):69–81.
[61] Lamster IB, Oshrain RL, Gordon JM. Enzyme activity in human gingival crevicular fluid: considerations in data reporting based on analysis of individual crevicular sites. J Clin Periodontol 1986;13(8):799–804.