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Application of Bone Morphogenetic Protein 7 Enhanced the Osteogenic Differentiation and Mineralization of Bone Marrow-Derived Stem Cells Cultured on Deproteinized Bovine Bone

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Abstract: The growth of bone morphogenetic protein 7 (BMP-7) has been applied for tissue regeneration due to its osteoinductive properties. The aim of this research is to analyze the enhancing effects of BMP-7 on the osteogenic differentiation and mineralization of human bone marrow-derived stem cells cultured on the bovine bone particle. After the stem cells were loaded onto the bone graft material, their morphology was observed on day 7. Viability assays based on the application of fluorescent stains were used for qualitative analyses. Alkaline phosphatase activity assays and Alizarin red staining were used for the assessment of osteogenic differentiation on days 7 and 14. Next-generation mRNA sequencing was applied to evaluate global gene expression. Gene ontology and pathway analysis was used to propose the underlying mechanism. Fibroblast-like morphology was attained with the stem cells. The cells were shown to be firmly attached to the bone particle. Most of the stem cells produced an intense green fluorescence. The relative cellular viability assay values for BMP-7 groups at 0, 10, and 100 ng/mL on day 7 were 0.295 ± 0.003, 0.250 ± 0.002, and 0.240 ± 0.003, respectively ($p < 0.05$). Alkaline phosphatase activity was significantly higher in BMP-7 groups at concentration of 100 ng/mL compared to the control on days 7 and 14 ($p < 0.05$). The results of the mineralization assay showed significantly higher values for BMP-7 groups at 100 ng/mL concentration when compared with the control ($p < 0.05$). The expression of RUNX2 was increased with application of BMP-7 and mitogen-activated protein kinase pathway was associated with the target genes. Overall, this study shows that in vitro application of BMP-7 increases alkaline phosphorylase activity and mineralization of stem cells culture on deproteinized bovine bone mineral. The study suggests that combining stem cells with osteoinductive growth factors with scaffolds can have synergy effects on osteogenic differentiation.

Keywords: bone morphogenetic protein 7; bone marrow; cell survival; cell differentiation; osteogenesis; stem cells

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1. Introduction

As expected, in the first half of the 21st century, the number of people over 65 years of age dramatically increased [1]. Moreover, there might be a demographic shift within the elderly population in the coming decades in which disease incidences and mortality increase [2]. Most adults suffer from periodontal disease, which is the second most-seen oral disease along with dental caries [3]. Periodontal disease is a major cause of tooth loss in the elderly population [4]. The economic burden and quality of life for the elderly tend

to drop significantly due to the need for immediate and continuous treatment of periodontitis [5]. Therefore, it is necessary to regenerate lost tissue and restore it to its functioning state [6].

In more recent years, the application of stem cells has been introduced to the tissue regeneration field [7]. Different medication treatments have been widely studied to enhance the tissue regeneration [8]. Moreover, research on the growth factors has been actively conducted and has contributed to the development of tissue regeneration concepts [9]. One of the most widely applied growth factors for tissue regeneration is bone morphogenetic proteins (BMPs) [10]. One of the widely noted BMPs, BMP-7, was applied in various studies due to its osteoinductive property [11–15]. The effects of the BMP-7 stimulation on osteoblasts cultured on various biomaterials was recently tested, and the loading osteoblasts on bovine bone mineral with rhBMP-7 led to a higher cell proliferation and collagen synthesis [11]. BMPs carried in a matrix was suggested to be able to serve as a substitute for the autologous bone graft in the critical size defects [12]. In a previous study, the reconstruction of the mandibular defect was done by local application of BMP-7 and bone grafting materials including xenogenic bone mineral [13]. The addition of BMP-7 resulted in the significantly stimulated bone formation in sinus augmentation procedures [14]. BMP-7 has been combined with the stem cells for tissue engineering purposes [15]. The purpose of this study is to evaluate the bone regeneration ability through the simultaneous application of BMP-7 and stem cells cultured on deproteinized bovine bone.

2. Materials and Methods

2.1. Culturing of Bone Marrow-Derived Mesenchymal Stem Cells on Bone

The Institutional Review Board of Seoul St. Mary's Hospital approved the present study (KC18SESI0199 and KC20SISE0582; approval date: 25 April 2018). Human bone marrow-derived mesenchymal stem cells (hBMSCs; Catholic MASTER cells) were obtained from the Catholic Institute of Cell Therapy (CIC, Seoul, Korea) [16]. CIC verified that cells showed >90% positive CD 73, >90% positive CD 90 expression, >90% negative CD 31, >90% negative CD 34, and >90% negative CD 45 expression.

The quantity of bone particle used per culture was 0.08 g/well. Cell-seeding density used for individual assays was 1.0×10^4. The hBMSCs were loaded onto the deproteinized bovine bone particle (granule size of 0.25–1 mm; Geistlich Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) and incubated for one day in growth media to make the hBMSCs grow on the bone particle. After that, the bone graft was moved to a new culture plate. The stem cell-loaded graft was cultured in osteogenic media. Along with this, the graft was also incubated with the BMP-7 at the final concentrations of 0 (= control), 10 and 100 ng/mL.

2.2. Morphologic Evaluation of Stem Cells

The morphology of the hBMSCs grown on the bone graft material was perceived on day 7, using a confocal laser microscope (LSM800 w/Airyscan; Carl Zeiss, Zena, Oberkochen, Germany). The culture media was removed and the stem cell-bone graft was meticulously washed with phosphate-buffered saline. The hBMSC-bone graft was fixed with 4% paraformaldehyde (P2031, Biosesang, Seongnam-si, Korea) at room temperature for 20 min. Then hBMSC-bone particle was washed with Dulbecco's phosphate-buffered saline solution three times. Cells were then stained using a 1× Phalloidin conjugate working solution (ab235138, Abcam, Cambridge, UK) for 60 min at room temperature. The hBMSC-bone graft was washed and mounted with small drop volumes (approximately 25 μL) of VECTASHIELD Mounting Medium with DAPI (H-1200, VECTOR laboratories). The samples were stored at 4 °C, protected from all light.

2.3. Determination of Cellular Viability

Viability assays based on the application of fluorescent stains (Live/Dead Kit assay, Molecular Probes, Eugene, OR, USA) was used for qualitative analysis of the hBMSCs cultured on the bone particle on day 7. The hBMSC-bone particle was treated with working solution for one hour at room temperature following the manufacturer's recommendation. The hBMSC-bone particle was viewed using a confocal laser microscope (LSM800 w/Airyscan; Carl Zeiss, Jena, Germany). Cell Counting Kit-8 (Dojindo, Tokyo, Japan) was used for the evaluation of quantitative cellular viability at 37 °C for 1 hour. Three independent cultures were used for the evaluation.

2.4. Level of Alkaline Phosphatase Activity and Calcium Deposition

Alkaline phosphatase activity level was used to assess the osteogenic differentiation on days 7 and 14 (K412-500, BioVision, Inc., Milpitas, CA, USA) by obtaining hBMSCs grown on bone graft material with osteogenic media. The hBMSCs on the deproteinized bovine bone were re-suspended with an assay buffer, then decomposed with triton X-100. Insoluble material was removed, and the supernatant was mixed with p-nitrophenylphosphate substrate. The incubation of the mixture was continued for 60 min at 4 °C. We measured the spectrophotometric absorbance of the samples at 405 nm.

To assess the degree of mineralization, Alizarin red staining was used to evaluate calcium deposition. The hBMSCs on the bone particle was stained with 2% Alizarin-Red Solution (Cat. No. 0223; ScienCell Research Laboratories, Inc., Carlsbad, CA, USA) for 30 min at room temperature. The bound dye was solubilized by applying 10% cetylpyridinium chloride (Cat. No. C0732-100G; Sigma-Aldrich Co., St. Louis, MO, USA) solution for 15 min. The quantification of the bound dyes was performed on days 7 and 14 at 560 nm.

2.5. Extraction of RNA and Sequencing of mRNA

Isolation of total RNA was performed using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) and RNA quantification was performed using a spectrophotometer (ND-2000, Thermo Fisher Scientific Inc., Waltham, MA, USA). Library of control and test RNAs were built using SMARTer Stranded RNA-Seq Kit (Clontech Laboratories, Inc., Palo Alto, CA, USA) according to the manufacturer's instructions [17].

The alignment file was gained by mapping mRNA-Seq reads tool using TopHat software tool [18]. Differentially expressed genes were determined by counts from unique and multiple alignments [19]. The read count data were processed by quantile–quantile normalization method [20]. Transcripts were assembled by cufflinks and the expression level of the gene regions was evaluated using fragments per kilobase of exon per million fragments method. Data mining and graphic visualization were performed using ExDEGA (ebiogen Inc., Seoul, Korea))

2.6. Statistical Analysis

We presented the data as means ± standard deviations of the experiments. We conducted the tests of normality and equal of variances. We tested the differences among groups by applying one-way analysis of variance with Tukey's post hoc test (SPSS 12 for Windows, SPSS Inc., Chicago, IL, USA; $p < 0.05$).

3. Results

3.1. Cell Attachment of Stem Cells to the Deproteinized Bovine Bone Mineral

Fibroblast-like morphology was attained with hBMSC. The cells were shown to be firmly attached to the bone particle on day 7 (Figure 1). Addition of BMP-7 did not produce significant changes. However, the number of cells presented on deproteinized bovine bone mineral particles seemed to decrease with addition of BMP-7 (Figure 1).

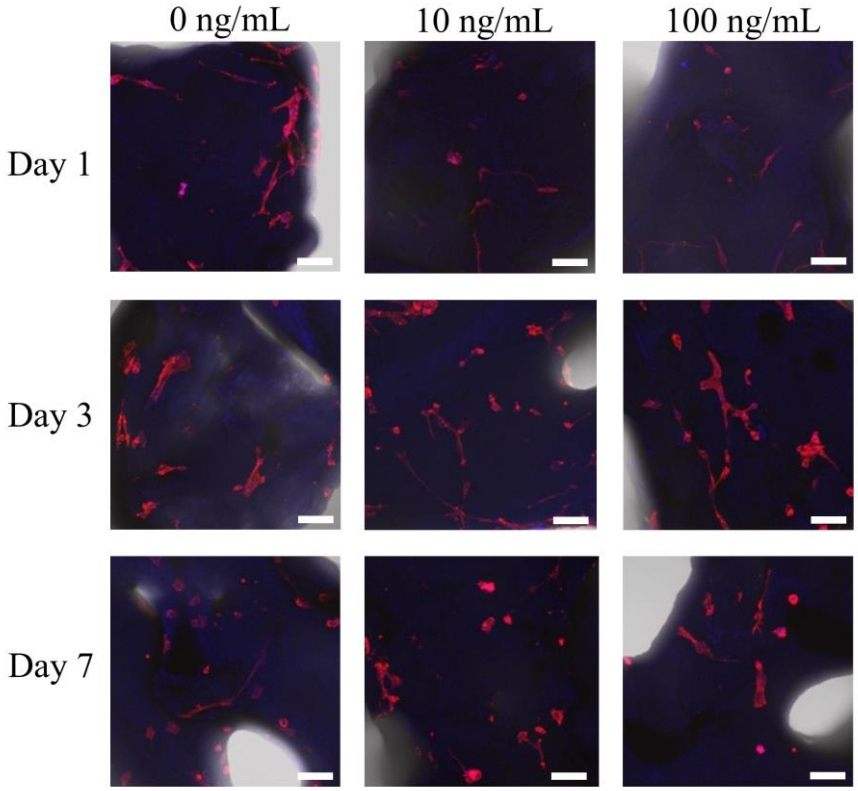


Figure 1. Stem cell attachment to the deproteinized bovine bone mineral. Scale bar indicates 100 μm (original magnification: x50).

3.2. Determination of Cellular Viability

Most of the stem cells produced an intense green fluorescence on day 7 (Figure 2). Minimal red fluorescence was noted, indicating there were a very low number of dead cells. The absorbance values at 450 nm on days 1, 3, and 7 are shown in Figure 3A. The relative cellular viability assay values for the BMP-7 groups at 0, 10, and 100 ng/mL on day 7 were 0.295 ± 0.003, 0.250 ± 0.002, and 0.240 ± 0.003, respectively ($p < 0.05$). The application of BMP-7 showed a statistical decrease in the cellular viability on day 7.

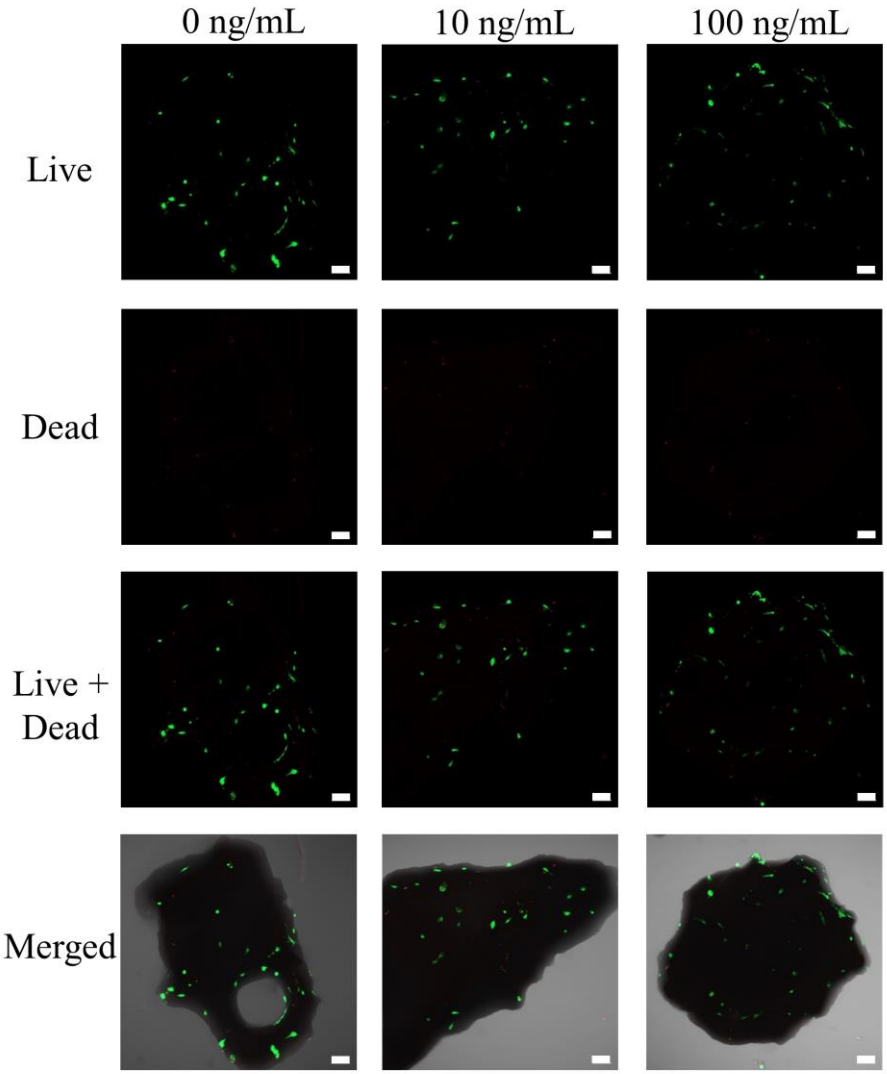
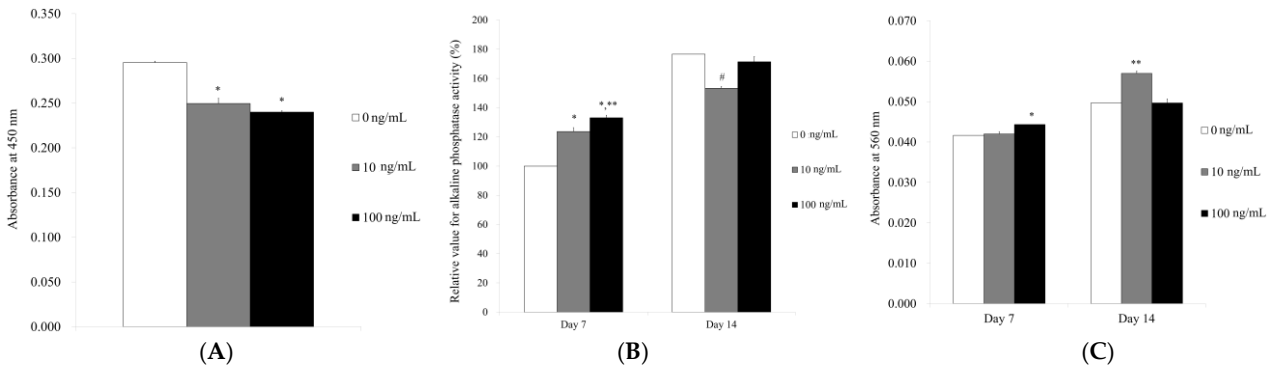


Figure 2. Live, dead, and merged images of stem cells cultured on the deproteinized bovine bone mineral. Scale bar indicates 100 μm (original magnification: x50).



(**A**) (**B**) (**C**)

Figure 3. Cellular viability and osteogenic differentiation. (**A**) Cellular viability using a Cell Counting Kit-8 on day 7. * Statistically significant differences were shown when compared with 0 ng/mL ($p < 0.05$). (**B**) Alkaline phosphatase activity on days 7 and 14. * Significant increases were noted when compared with 0 ng/mL at day 7 ($p < 0.05$). ** Statistically significances were noted when compared with 10 ng/mL at day 7 ($p < 0.05$). # Significant differences were seen when compared with 0 ng/mL at day 14 ($p < 0.05$). (**C**) Results of Alizarin Red S staining on days 7 and 14. * Significant increases were noted were noted when compared with 0 ng/mL at day 7 ($p < 0.05$). ** Statistically significances were noted when compared with 0 ng/mL at day 14 ($p < 0.05$).

3.3. Level of Alkaline Phosphatase Activity and Calcium Deposition

The alkaline phosphatase activity assays on days 7 and 14 are shown in Figure 3B. The absorbance values at 405 nm on day 7 for BMP-7 at 0, 10, and 100 ng/mL were 0.074 ± 0.002, 0.077 ± 0.001, and 0.080 ± 0.001, respectively ($p < 0.05$). Significantly higher values were noted for BMP-7 groups at 100 ng/mL concentration when compared with the control group ($p < 0.05$). The results on day 14 were 0.076 ± 0.001, 0.074 ± 0.002, and 0.081 ± 0.001 for BMP-7 groups at 0, 10, and 100 ng/mL, respectively ($p < 0.05$). Statistically higher values were seen in BMP-7 groups at 100 ng/mL concentration when compared with the 0 ng/mL group ($p < 0.05$).

The results of the mineralization assay at days 7 and 14 are shown in Figure 3C. The absorbance values at 560 nm on day 7 were 0.042 ± 0.001, 0.042 ± 0.000, and 0.044 ± 0.001 for BMP-7 at 0, 10, and 100 ng/mL groups, respectively ($p < 0.05$). The quantification results showed significantly higher values for BMP-7 groups at 100 ng/mL concentration when compared with the 0 ng/mL group ($p < 0.05$). The results on day 14 were 0.050 ± 0.001, 0.057 ± 0.001, and 0.050 ± 0.002 for BMP-7 groups at 0, 10, and 100 ng/mL, respectively ($p < 0.05$). Statistically higher values were noted for BMP-7 groups at 10 ng/mL concentration when compared with the 0 ng/mL group ($p < 0.05$).

3.4. Evaluation of Gene Ontology and Pathway Analysis

Differentially expressed mRNA related to cell proliferation is shown in Table 1 (fold change of greater than 2, log2 normalized read counts >5 were selected). When comparing gene expression value between samples, the down-regulated gene was shown in green and up-regulated is in red. Comparison of the BMP-7 at 24 h versus control at 24 h revealed that 10 mRNAs were upregulated and 4 were downregulated. Comparison of the BMP-7 at 24 h versus BMP-7 at 3 h revealed that 10 mRNAs were upregulated and 5 were downregulated.

Clustering analysis of differentially expressed mRNA related to cell proliferation is shown in Figure 4.

Table 1. Differentially expressed mRNA related to cell proliferation (fold change of greater than 2, log2 normalized data >5 were selected). When comparing gene expression value between samples, the down-regulated gene was shown in green and up-regulated is in red.

Gene Symbol	BMP-7 24h/Control 24h	Gene Symbol	BMP-7 24h/BMP-7 3h
INSIG1	0.329	ITGA2	0.338
HMGA2	0.391	INSIG1	0.358
ITGA2	0.428	BMPER	0.401
BMPER	0.494	HMGA2	0.410
LTK	2.141	FGF8	0.497
PTCH1	2.172	BMP4	2.073
TCF7L2	2.304	OSR1	2.129
PTK2B	2.408	DDIT4	2.157
ADRA1B	2.441	ADRA1B	2.174
IRS2	2.604	IL15	2.510
BMP4	2.805	LTK	2.628
AXIN2	3.781	IRS2	2.696
KIT	15.821	TCF7L2	2.890
DDIT4	20.173	AXIN2	3.073
-	-	KIT	7.478

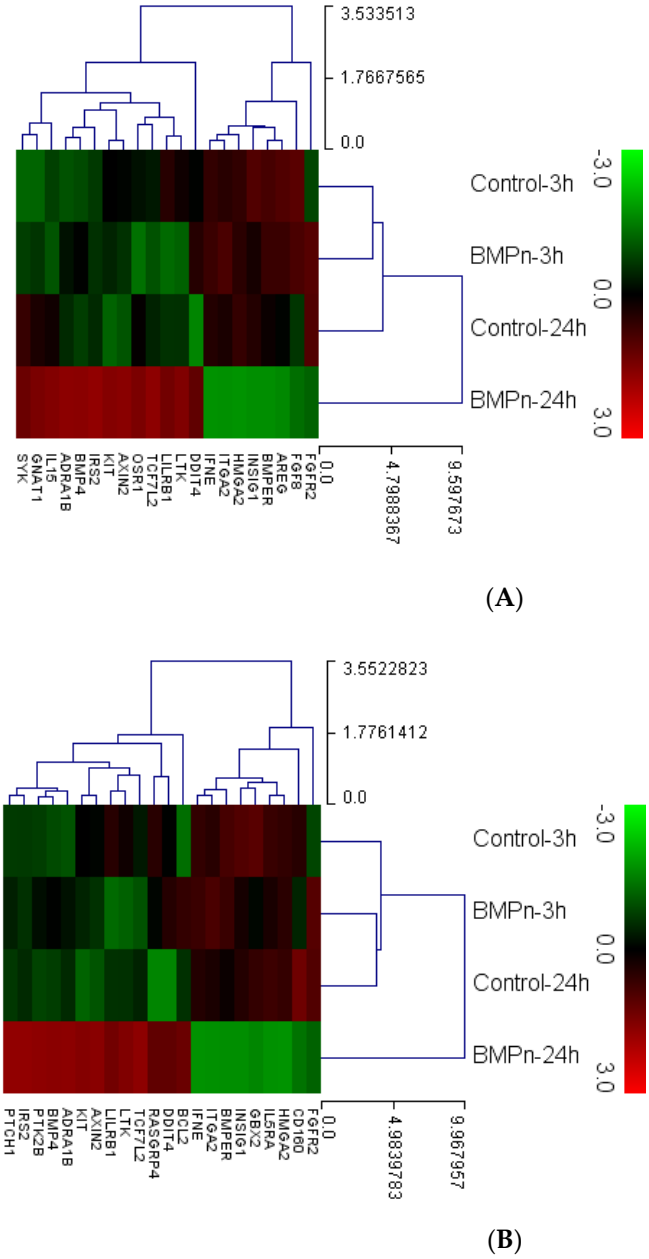


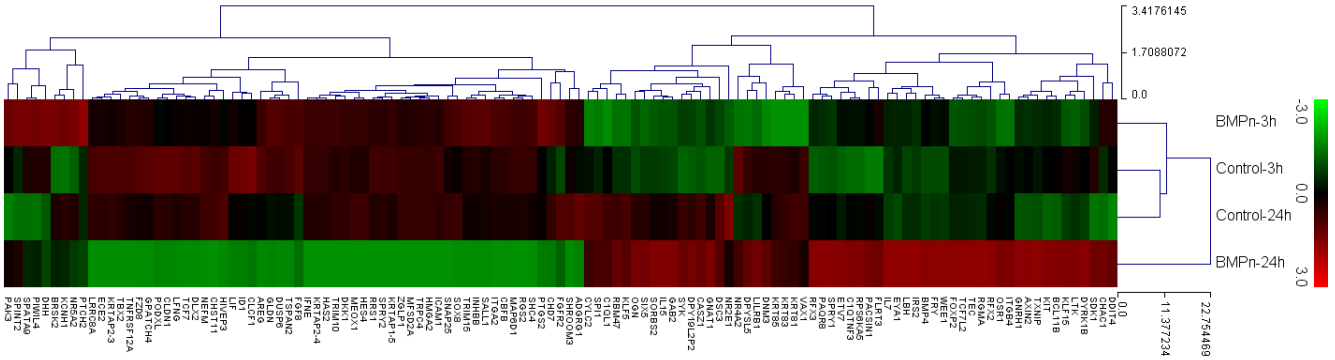
Figure 4. Clustering analysis of differentially expressed mRNA related to cell proliferation (**A**) BMP-7 at 24 h/control at 24 h and (**B**) BMP-7 at 24 h/BMP-7 at 3 h (fold change fold change of greater than 2, log2 normalized read counts >4 were selected).

Differentially expressed mRNA related to cell differentiation is shown in Table 2 (fold change of greater than 2, log2 normalized read counts >5 were selected). Comparison of the BMP-7 at 24 h versus control at 24 h revealed that 41 mRNAs were upregulated and 48 were downregulated. Comparison of the BMP-7 at 24 h versus BMP-7 at 3 h revealed that 38 mRNAs were upregulated and 50 were downregulated. Clustering analysis of differentially expressed mRNA related to cell differentiation is shown in Figure 5.

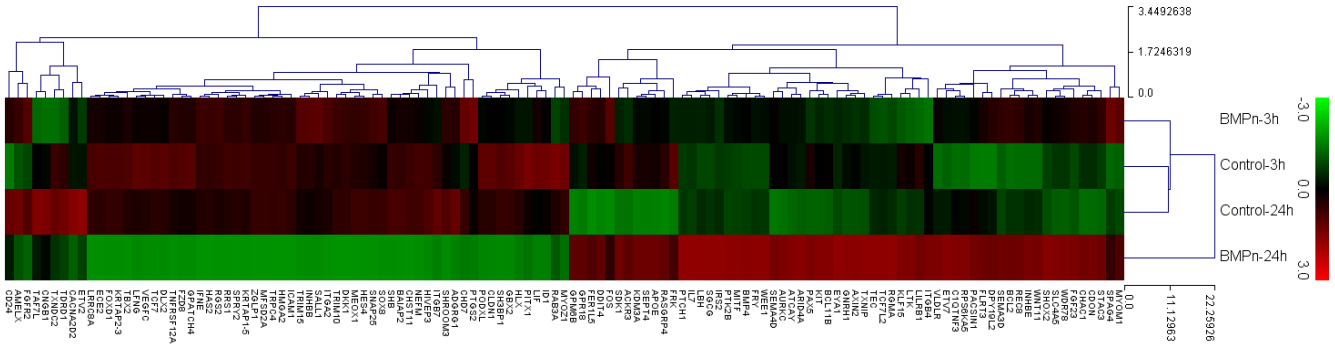
Table 2. Differentially expressed mRNA related to cell differentiation (fold change of greater than 2, log2 normalized data >5 were selected). When comparing gene expression value between samples, the down-regulated gene was shown in green and up-regulated is in red.

Gene Symbol	BMP-7 24h/Control 24h	Gene Symbol	BMP-7 24h/BMP-7 3h
PTPN22	0.039	DHH	0.034
RAB3A	0.065	MEOX1	0.045
NR4A2	0.125	TRIM10	0.053
PITX1	0.159	TRIM15	0.110
VAX1	0.184	DKK1	0.117
PODXL	0.197	FZD8	0.166
SNAI1	0.209	KRTAP2-3	0.222
HCLS1	0.220	PTGS2	0.238
CLDN1	0.236	HES4	0.248
LFNG	0.237	LFNG	0.261
ANKRD1	0.238	MFSD2A	0.273
MYOZ1	0.250	INHBB	0.281
LIF	0.256	ADGRG1	0.294
CLCF1	0.274	LIF	0.298
ETV4	0.279	TRPC4	0.298
DNAH1	0.314	PTCH2	0.320
MECOM	0.323	CHD7	0.324
HSF4	0.340	KRTAP1-5	0.330
SEMA3D	0.365	ICAM1	0.334
CEP290	0.365	ITGA2	0.338
EGR1	0.370	RRS1	0.343
TET1	0.371	KCNH1	0.344
MAK	0.376	TNFRSF12A	0.346
REC8	0.380	PODXL	0.350
IGSF10	0.391	CLDN1	0.353
SYNE2	0.404	RGS2	0.354
CHAC1	0.408	SALL1	0.360
JUP	0.411	NEFM	0.380
PTCH2	0.412	HAS2	0.382
BATF2	0.414	CHST11	0.400
HRNR	0.418	HMGA2	0.410
PRDM16	0.419	DLX2	0.410
DHH	0.420	ECE2	0.421
KCNH1	0.428	TBX2	0.423
-	0.429	CBFB	0.427
-	0.435	SHC4	0.427
-	0.443	TCF7	0.429
-	0.444	SNAP25	0.430
-	0.447	HIVEP3	0.432
-	0.451	SPRY2	0.448
-	0.457	MAP6D1	0.448
-	0.457	ID1	0.449
-	0.464	PIWIL4	0.453
-	0.479	CLCF1	0.463
-	0.488	GPATCH4	0.464
-	0.489	GLDN	0.476

-	0.491	DUSP6	0.476
-	0.494	SHROOM3	0.478
-	2.063	FGF8	0.497
-	2.141	LRRC8A	0.497
-	2.165	RBM47	2.004
-	2.169	ETV7	2.023
-	2.172	DYRK1B	2.047
-	2.174	DPY19L2P2	2.060
-	2.184	BMP4	2.073
-	2.206	CYLC2	2.095
-	2.304	WEE1	2.122
-	2.319	KLF5	2.123
-	2.346	OSR1	2.129
-	2.350	PAQR8	2.135
-	2.368	KRT81	2.138
-	2.408	DDIT4	2.157
-	2.417	SIX5	2.190
-	2.449	DNM3	2.193
-	2.554	CHAC1	2.294
-	2.604	RFX3	2.303
-	2.609	FOXP2	2.421
-	2.625	EYA1	2.464
-	2.661	IL15	2.510
-	2.682	LBH	2.558
-	2.684	GAB2	2.560
-	2.744	SPRY1	2.594
-	2.788	LTK	2.628
-	2.805	TXNIP	2.662
-	2.830	RFX2	2.675
-	2.893	NR4A2	2.683
-	2.973	IRS2	2.696
-	3.227	RPS6KA5	2.745
-	3.297	GNRH1	2.798
-	3.428	TCF7L2	2.890
-	3.455	DPYSL5	2.985
-	3.547	FRY	3.013
-	3.781	AXIN2	3.073
-	3.846	TEC	3.268
-	4.084	SORBS2	3.564
-	5.012	OGN	3.994
-	11.467	KLF15	6.356
-	15.821	KIT	7.478
-	20.173	-	-



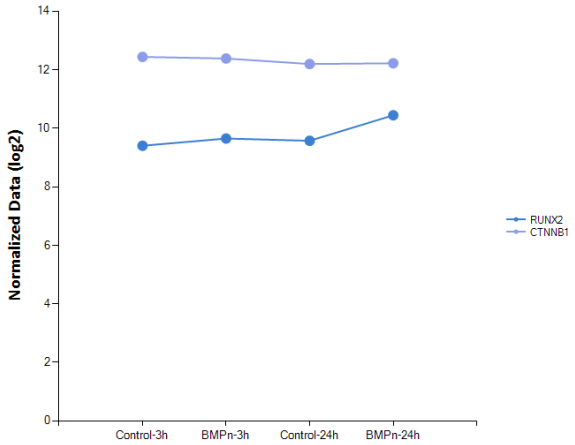
(**A**)



(**B**)

Figure 5. Clustering analysis of differentially expressed mRNA related to cell differentiation (**A**) BMP-7 at 24 h/control at 24 h and (**B**) BMP-7 at 24 h/BMP-7 at 3 h (fold change fold change of greater than 2, log2 normalized read counts >4 were selected).

The changes in expression of RUNX2 and CTNNB1 are shown in Figure 6A. Expression of RUNX2 was increased in the BMP-7 group at both 3 h and 24 h when compared with the control group of each time point. The expression of RUNX2 in BMP-7 groups was higher with longer incubation of 24 h (Figure 6B). However, the expression of CTNNB1 was decreased both at 3 and 24 h when compared with the control group of each time point. The expression of CTNNB1 in BMP-7 groups was lower at 24 h. The signaling pathway associated with the target genes related to cell proliferation and cell differentiation was mitogen-activated protein kinase pathway (Figure 7).



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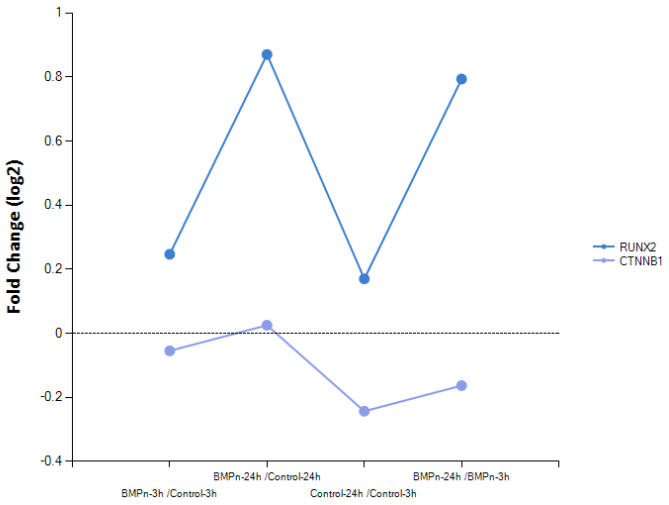

(**B**)

Figure 6. (**A**) Log2-normalized read counts regarding the expression of RUNX2 and CTNNB1 (for β-catenin expression) and (**B**) Log2 fold change regarding the expression of RUNX2 and CTNNB1.

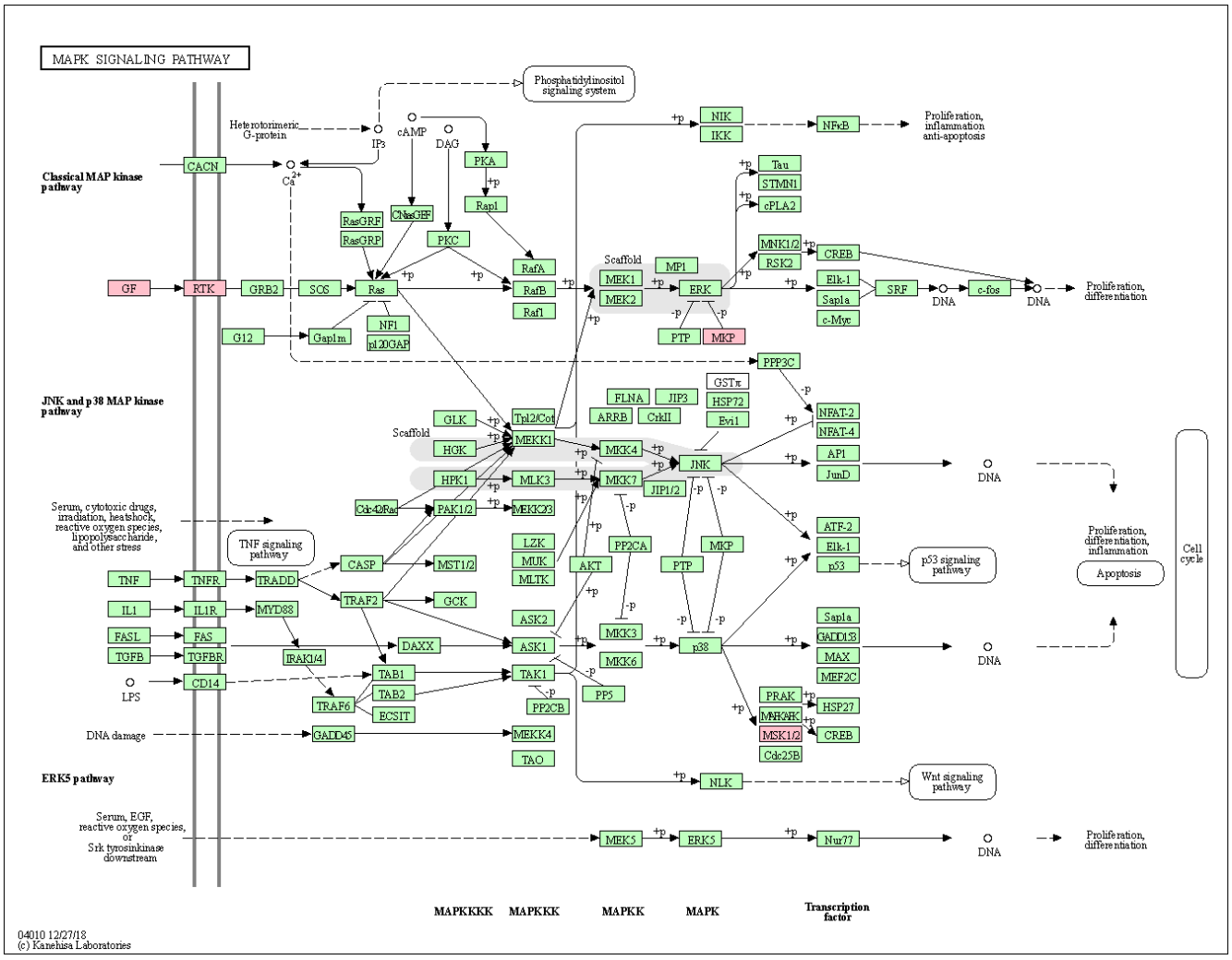

Figure 7. Signaling pathway.

4. Discussion

In this report, we evaluated the effects of BMP-7 on stem cells cultured on deproteinized bovine bone at the concentration of 10 and 100 ng/mL. Applying BMP-7 caused an increased alkaline phosphatase activity and mineralization of the hBMSCs.

BMP-7 is considered anabolic and anti-catabolic growth factor on cell homeostasis [21]. In a previous report, BMP-7 distinctly stimulated chondrocyte proliferation and inhibited chondrocyte hypertrophy [22]. It should be also noted that even though BMP-7 is potent bone anabolic substances, but it may also act as a booster for catabolism, and this may lead to resorption [23]. A previous report showed that BMP-7 upregulated the genes involved in osteogenic differentiation of gingiva-derived stem cells [24]. Moreover, BMP-7 was involved in the induction of cementogenic differentiation of stem cells derived from periodontal ligament [25]. In this report, BMP-7 enhanced the osteogenic differentiation of hBMSCs. However, 50 and 100 ng/mL of BMP-7 induced proliferation of stem cells derived from periodontal ligament, contrary to this study [25] and this may be due to the differences in culture condition, passage and origin of the stem cells [26].

The concentration of the BMP-7 may affect the results. Local injection of a single dose of 200 μg of rhBMP-7 enhanced bone density during distraction osteogenesis in the rabbit mandible model [27]. Six hundred micrograms of BMP-7 per 8 mL xenogenic bone mineral in the block was used for mandibular reconstruction in a miniature pig model [28]. The use of xenograft bone mineral granules with 1,000 μg BMP-7 per gram can be applied for bone regeneration in a minipig model [29]. For the adult chimpanzee model, 2.5 mg BMP-7 per gram collagen matrix was used [14]. Upregulation of osteocalcin and osteopontin expression was seen in periodontal ligament-derived stem cells with BMP-7 at 100 ng/mL [30]. Previous report showed that BMP-7 at 100 ng/mL increased the expression of target genes associated with osteoblast differentiation [24]. Similarly, this report showed a significantly higher osteogenic differentiation was noted for BMP-7 groups at 10 and 100 ng/mL concentration when compared with the control group.

There has been a great level of interest in stem cell application for many years [7]. More recently, stem cells are being actively applied to tissue regeneration approaches [31]. When the tissue is damaged, the stem cells are activated by the substances secreted in situ [6]. The activated stem cells receive additional signals from the tissue, and they proliferate or differentiate into mature cells [32]. Newly formed mature cells migrate to the damaged tissue and contribute to regenerating the tissue [33]. Based on the role of the stem cells listed above, stem cells are applied for tissue regeneration purposes [34]. The application of stem cells needs to be done very carefully because they can dislocate or penetrate into blood vessels and form ectopic or fibrotic tissue [35]. This study showed that the application of BMP-7 led to increased expression of RUNX2, which was similar to the previous reports [36,37]. However, in this study, the expression of CTNNB1 was not increased in BMP-7 applied groups. This study also mitogen-activated protein kinase pathway was associated with the target genes related to cell proliferation and cell differentiation.

Moreover, stem cells have been used as growth factor-producing devices [38]. Stem cells are known to secrete various growth factors, including platelet-derived growth factor, vascular endothelial growth factor, and hepatocyte growth factor, to name a few [39,40]. The use of scaffolds may allow and facilitate the application of stem cells exactly to the desired area and their maintenance at the area [41]. It is possible that the type of carrier/scaffold used may influence the morphology and the amount of newly formed bone [42]. In another report, the application of BMP on an allograft, xenograft or cell binding peptide-loaded xenograft enhanced osteogenic differentiation when compared with non-loaded group, but the type of scaffold did not produce significant differences [11]. In this report, we used one of the most widely used biomaterial for bone augmentation namely deproteinized bovine bone [43].

However, there are several things to consider when applying growth factors in stem cell therapy. One of the main strengths of this study is that next-generation mRNA se-

quencing was used to evaluate the global gene expression [44]. Pathway analysis performed in this study suggested the underlying signaling pathway [45]. A limitation of this study is that further analysis of in vivo models and of human participants may be needed for wider application [46]. When the growth factor is applied, an initial burst phenomenon occurs in the early stage [47]. The half-life of the growth factor is then reduced due to an enzymatic action [48]. The applied growth factor may be washed away by the blood flow [49]. For the desired effect of growth factors, it is necessary to consider the high cost and various side effects when applying a high dose [50]. Though doing this has led to a conflict between the effectiveness and side effects of the growth factors [50]. Modification of surface of titanium surface with BMP-7 led to increased adhesion and differentiation of stem cells [51]. It was also suggested that mesenchymal stem cells, which overexpress BMP-7, may be applied for the enhanced bone healing [52]. Attempts were made to apply a combination of growth factors for optimal results [53,54]. The co-application of BMP-7 and PDGF-BB has been applied for the differentiation of stem cells [53]. Heterodimeric BMP-2/7 was also used for the osteogenic differentiation of adipose-derived stem cells [54]. Moreover, other BMPs have been applied for bone tissue regeneration as these are emerging BMPs for orthotopic bone regeneration [55–57].

5. Conclusions

This study shows that the application of BMP-7 increases alkaline phosphatase activity and the mineralization of stem cells culture on deproteinized bovine bone mineral. This report suggests the combined use of stem cells and osteoinductive growth factors with a scaffold may have synergistic effects on osteogenesis.

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