

## rhBMP-2

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:110-749

28

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1)

2)

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,

lamina propria

3-7)

PDGF-BB,

IGF-1, TGF-

bone morphogenetic protein(BMP)

BMP

8-13) BMP

,<sup>14)</sup> BMP가 *in vitro*

15-24) BMP가  
 ,  
 25-28)  
 bone sialoprotein osteopontin, osteocalcin  
 가 29-31)  
 Osteopontin osteocalcin, bone sialoprotein  
 32) Osteocalcin  
 , osteopontin bone sialoprotein  
 30,32)  
 가  
 가 BMP-2 가  
 recombinant human bone  
 morphogenetic protein-2 (rhBMP-2) alkaline phosphatase  
 alkaline phosphatase, osteopontin, osteocalcin, bone sialoprotein  
 mRNA

1. rhBMP-2

rhBMP-2(R&D Systems, USA) 0.1% bovine serum albumin(BSA)  
 phosphate buffered saline(PBS) 가 10 µg/ml  
 50ng/ml 100ng/ml .

2.

1 1/3  
 , 10cm , penicillin,  
 streptomycin amphotericin-B가 1% antibacterial-antifungal solution (Gibco,  
 USA) 10% FBS (Gibco, USA) 가 -MEM(Gibco, USA) , 3

95%, 37°C 5% CO<sup>2</sup>

5 Alkaline phosphatase 12 well culture plate well 1x10<sup>3</sup>  
Reverse transcriptase polymerase chain reaction(RT-PCR)  
10cm 5x10<sup>5</sup>, 24  
rh BMP-2 50ng/ml, rhBMP-2 100ng/ml

### 3. Alkaline phosphatase

12 well culture plate 1 2  
0.25% trypsin-EDTA 5 0.5ml  
가 1, 0.1ml 0.1ml  
0.1M glycine-NaOH buffer, 0.1ml 15mM para-nitrophenol phosphate(PNPP),  
0.1% Triton X-100 0.1ml 30 37°C  
2.5ml 0.1N NaOH 가  
microplate reader 405nm  
para-nitrophenol(PNP) Alkaline phosphatase  
one-way ANOVA test 0.05  
Tukey

### 4. Reverse transcriptase polymerase chain reaction(RT-PCR)

10cm 1 2  
High Pure RNA Isolation kit(Roche, Germany) total RNA  
RNA Reverse Transcriptase, RNase inhibitor *AccuPower*R RT  
PreMix (Bioneer, Korea) cDNA, RNA 1.0µg Oligo  
dT<sub>15</sub> primer 1.0µg 70°C 5  
50µl RT PreMix tube cDNA 42°C  
60, Reverse transcriptase 94°C 5

cDNA 4μl 20pmole primer DNA polymerase, dNTPs  
 reaction buffer *AccuPowerR* PCR PreMix(Bioneer, Korea)  
 50μl thermal cycler(ependorf, Germany) polymerase chain  
 reaction(PCR) . primer , PCR 1,2  
 PCR 2% agarose gel 0.5μg/ml ethidium  
 bromide GelDoc 2000 Gel Documentation System(BIO-RAD,USA)

### 1. Primer

β - actin (321bp)	5 : ATGAGGATCCTCACCGAGCGCGGCTACAGC 3 : ACACCACTGTGTTGGCGTACAGGTCTTTGC
type I Collagen (223bp)	5 : AGGGCTCCAACGAGATCGAGATCCG 3 : TACAGGAAGCAGACAGGGCCAACGTCCG
Alkaline phosphatase (475bp)	5 : ACGTGGCTAAGAATGTCATC 3 : CTGGTAGGCGATGTCCTTA
BMP - 2 (315bp)	5 : TTGCGGCTGCTCAGCATGTT 3 : CATCTTGCATCTGTTCTCGGAA
Osteopontin (430bp)	5 : TTGCTTTTGCCTCCTAGGCA 3 : GTGAAAACCTTCGGTTGCTGG
Osteocalcin (310bp)	5 : CATGAGAGCCCTCACA 3 : AGAGCGACACCCTAGAC
Bone sialoprotein (439bp)	5 : GCCTGTGCTTTCTCAATG 3 : TTCCTTCCTCTTCCTCCTC

### 2. The PCR condition used in this study

Pre-denaturation	94°C	5
Denaturation	94°C	1
Annealing	*	2
Polymerization	72°C	1

\* Annealing temperature

β-actin : 65°C, type I collagen : 55°C, alkaline phosphatase : 44°C, BMP-2 : 60°C,  
 osteopontin : 44°C, osteocalcin : 60°C, bone sialoprotein : 55°C

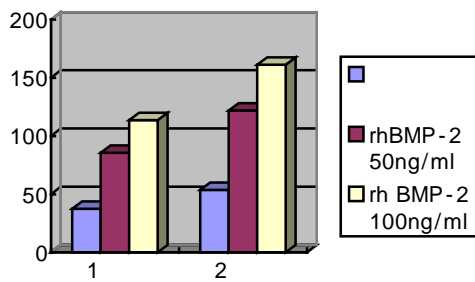


rhBMP-2  
가

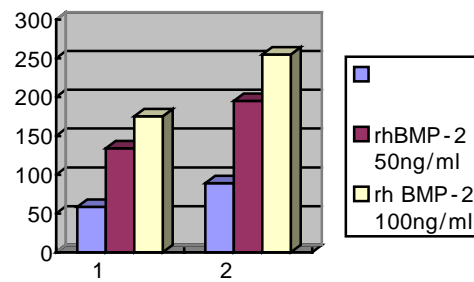
가

5.	alkaline phosphatase (nmolPNP/30min/ µgprot.)		
		rhBMP-2 50ng/ml	rhBMP-2 100ng/ml
1	37.4±10.3	85.7±19.5	113.5±20.4
2	53.8±12.1	121.8±26.7	161.2±23.9

3. alkaline phosphatase (nmolPNP/30min/ µgprot.)



4. alkaline phosphatase (nmolPNP/30min/ µgprot.)



6.	alkaline phosphatase (nmolPNP/30min/ µgprot.)		
		rhBMP-2 50ng/ml	rhBMP-2 100ng/ml
1	58.9±13.3	134.2±21.6	175.3±22.7
2	89.3±16.8	195.3±28.3	254.7±29.2

3. RT-PCR

RT-PCR agarose gel

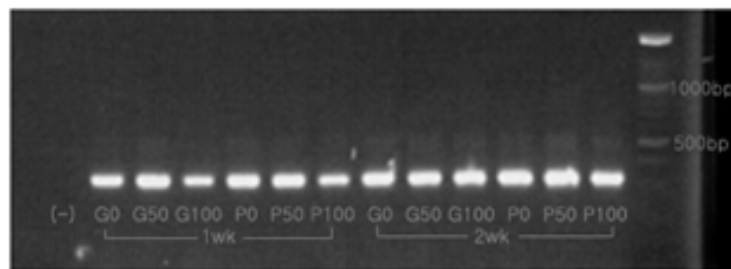
5-11 . β-

actin, type I collagen, alkaline phosphatase, BMP-2 mRNA  
osteopontin, osteocalcin

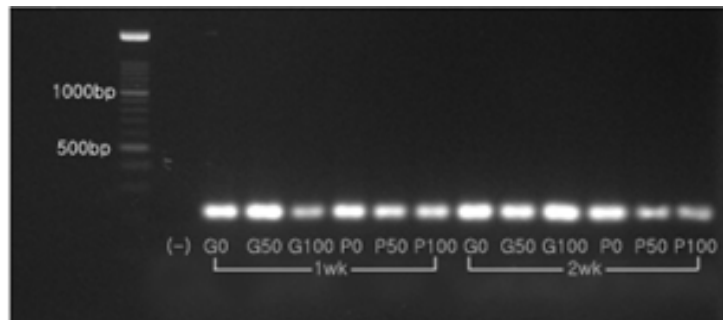
2

rhBMP-2 mRNA . Bone sialoprotein 2  
rhBMP-2 mRNA .

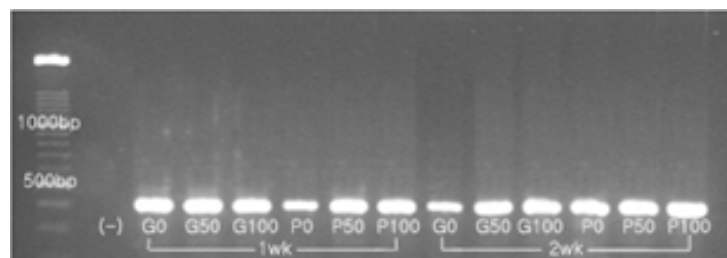
### 5. $\beta$ -actin



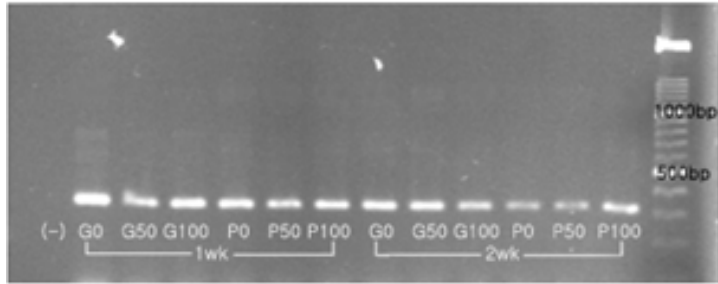
### 6. type I Collagen



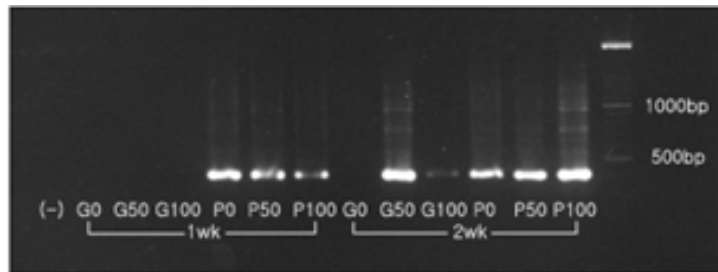
### 7. Alkaline phosphatase



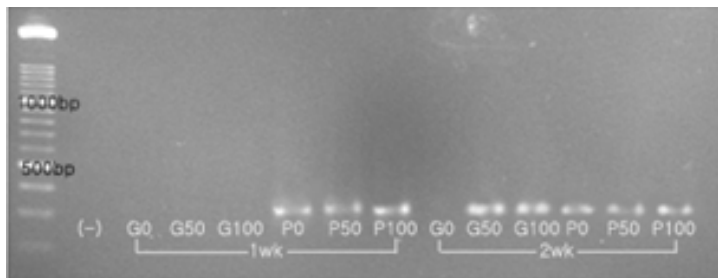
### 8. BMP-2



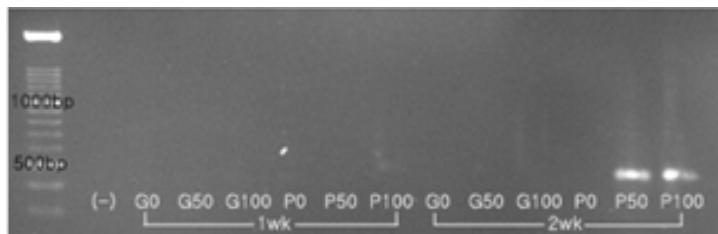
### 9. Osteopontin



### 10. Osteocalcin



### 11. Bone sialoprotein





가 BMP 가  
 . BMP *in vivo in vitro*  
 BMP BMP BMP  
 BMP가  
 가  
 rhBMP-2가  
 BMP  
 ,  
 18,20,23,33-35)  
 rhBMP-2  
 . Zheng rhBMP-2가  
 35), Kobayashi rhBMP-2가  
 15) Zaman rhBMP-2가  
 alkaline phosphatase 가  
 48)  
 alka- line phosphatase rhBMP-2  
 가 가 alkaline phosphatase 가 가  
 ,  
 15,17-22,24,36,37) rhBMP-2가  
 ,  
 38-41)  
 Collagen alkaline phosphatase가  
 가 osteopontin, osteocalcin, bone sialoprotein ,<sup>41)</sup> bone  
 sialoprotein mineralization  
 osteocalcin mineralization crystal growth  
 . Osteopontin bone sialoprotein osteocalcin  
 crystal growth ,  
 collagen  
 alkaline phosphatase mRNA가 Osteopontin,

osteocalcin  
, bone sialoprotein 2  
가

2 rhBMP-2  
rhBMP-2 mRNA  
가 . BMP

rhBMP-2가 alkaline  
phosphatase  
osteocalcin ,  
, 21,22) 17) , 24,36) , 20)  
. Lecanda  
rhBMP-2가 alkaline  
phosphatase osteocalcin 가  
가 , 42)  
rhBMP-2 가  
가 가  
Shibano rhBMP-2가 osteocalcin  
, 43) Kobayashi rhBMP-2가 osteocalcin  
mRNA , 15) BMP가 *in vitro*  
가 .

BMP가  
가 가 ,

Alkaline phosphatase mRNA alkaline  
phosphatase 가 ,  
alkaline phosphatase  
, 44-47)  
가 RT-PCR  
alkaline phosphatase

Osteopontin, osteocalcin, bone sialoprotein ,  
rhBMP-2 mRNA

rhBMP-2가

1. rhBMP-2

2. 가  
가 rhBMP-2 alkaline phosphatase  
가 alkaline phosphatase 가  
가

3. rhBMP-2

가 alkaline phosphatase

mRNA

가 , rhBMP-2 가

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- Abstract -

**The effect of rhBMP-2 on the osteoblastic differentiation of human periodontal ligament cells and gingival fibroblasts *in vitro***

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BMP can induce ectopic bone formation when implanted into sites such as rat muscle and can greatly enhance healing of bony defects when applied exogenously. In addition, BMP stimulated osteoblastic differentiation *in vitro* in various types of cells. The aim of this study was to investigate the effect of recombinant human bone morphogenetic protein(rhBMP-2) on the proliferation and osteoblastic differentiation of human periodontal ligament cells and gingival fibroblasts.

The cell number and alkaline phosphatase activity were measured in 3 experimental groups of human periodontal ligament cells and gingival fibroblasts (control group, rhBMP-2 50ng/ml group, and rhBMP-2 100ng/ml group) at 1 and 2 weeks after culture. At the same time, total RNA of cultured cells were extracted and reverse trascription polymerase chain reaction(RT-PCR) was performed to determine the expression of mRNA of bone matrix protein.

RhBMP-2 had no effect on the cell proliferation of human periodontal ligament cells and gingival fibroblasts. Alkaline phosphatase activity was elevated significantly by rhBMP-2 in both cells. And periodontal ligament cells showed significantly higher alkaline phosphatase activity than gingival fibroblasts.  $\beta$ -actin, type I collagen, alkaline phosphatase, BMP-2 mRNA were expressed in all of the samples. Osteopontin, osteocalcin mRNA were expressed in all periodontal ligament cell groups, and rhBMP-2 50ng/ml group, rhBMP-2 100ng/ml group of 2

week culture period of gingival fibroblasts. Bone sialoprotein mRNA was only expressed in rhBMP-2 50ng/ml group and rhBMP-2 100ng/ml group of 2-week culture period. These results suggest that rhBMP-2 stimulates osteoblastic differentiation in human periodontal ligament cells and gingival fibroblasts *in vitro*.

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Key Words: recombinant human bone morphogenetic protein-2 (rhBMP-2), periodontal ligament cell, osteoblast, differentiation