

rhBMP - 2

가

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1

2

I.

15 - 19)

(BMP)

BMP

.20 -

26) Urist가²⁷⁾ BMP가

1-5)

. BMP TGF - superfamily

가 ,

가

, 6-10)

. rhBMP - 2가

11-14) 2,3

, rhBMP - 2가

가

20). Sigurdsson 28)

rhBMP - 2

PDGF - BB, IGF -

1, TGF -

, Ripamonti²⁹⁾ BMP - 7

가

39).

BMP가

pH 1
가

BMP가

2 - 3

,
,
30 - 32).

40,41).

BMP

rhBMP - 2

rhBMP - 2

42,43),

가

44 - 46).

33),

47).

34).

가

BMP

48).

BMP

가

가

가

49).

50)

가

35).

51).

가

가

36),

가

52)

37).

53)

38)

rhBMP - 2가
, rhBMP - 2

BMP units/ml, streptomycin sulfate 10,000 μ g/ml, amphotericin B 25 μ g/ml) 가 - MEM , 2 - 3

BMP 가 EDTA 1:4 ,

rhBMP - 2 가 3 - 4

⁵⁴⁾, BMP MC3T3 - E1(Riken, Japan)

가 ⁵⁵⁾ 5 - 6 BMP

rhBMP - 2 2.

가 1/3 6

80 , Exakt cutting system(Exakt Appa - rateb, Hamburg, Germany)

II.

1.

Somerman⁵⁶⁾ 4 x 2.5 x 1mm (288) 6

10 20 48

1% (penicillin G sodium 10,000 units/ml, streptomycin sulfate 10,000 μ g/ml, amphotericin B 25 μ g/ml, Gibco Co., U.S.A.) Hank's balanced salt solution(HBSS, Gibco Co., U.S.A.) 가 - minimum essential medium(- MEM; Gibco Co., U.S.A.) , 1/3

37 5% CO₂ 10% 3.

fetal bovine serum(FBS, Gibco., U.S.A.) 1% (penicillin G sodium 10,000

96

(Sigma, U.S.A.) 1
 , 96 10%
 (Tetracycline HCl, Chongge -
 undang, Korea.) 3
 pH=1.0
 10%
 pH meter(Benchtop pH/ISE
 Meters, Orion, U.S.A.)
 pH=2.04
 1000U/ml penicillin
 1000 µg/ml streptomycin -
 MEM 24 , 100U/ml penicillin
 100µg/ml streptomycin 가 -
 MEM

4 . rhBMP - 2

rhBMP - 2(R&D system , U.S.A.)
 10% FBS 1%
 (penicillin G sodium 10,000 units/ml, strep-
 tomycin sulfate 10,000µg/ml, amphotericin
 B 25µg/ml, Gibco Co., U.S.A.) -
 MEM 30µg/ml
 BMP ,
 CA+BMP TC+BMP
 30 µg/ml rhBMP - 2 10 ,
 10

5.

(24)
 0.25% trypsin
 4mM EDTA 0.5ml
 37 10 ,
 (1200rpm, 4 , 10)
 10% FBS 1% , 10mM

Na -glycerolphosphate(Sigma, U.S.A.), 50
 µg/ml L - ascorbic acid(Sigma, USA),
 , 10⁻⁷M Dexamethasone(Sigma. U.S.A.)
 - MEM ,
 가 5 × 10⁴cell/ml 가
 . 24 - well culture plate(Nunc,
 Rochester, NY, USA) well
 1 , 가
 well 1ml . -
 95% air, 5% CO₂, 37
 . 7
 3 1ml 가

6.

2 7 well
 1.5Mℓ 100µℓ
 (0.25% trypsin in 4mM EDTA)

7.

Bessey⁵⁷⁾
 . 1mM MgCl₂ , 25mM sucrose, 5mM
 tris - HCl buffer (pH=9.8) 2mM p -
 nitrophenylphosphate (Wako Pure Chemical
 Co., Osaka, Japan)
 , 2 7 5 well
 25mM sucrose
 , 24 well
 well 300µℓ
 .
 24 well 37
 , 30
 24 well

0.6N 1.5ml NaOH 가 595nm
 well
 Bovine serum
 200 μ l 96 well , spec - albumin
 trophotometry 405nm
 9.
 p - Nitrophenol 가
 8. 24 well well 1.0ml
 2.5% glutaraldehyde . 4 20
 , 가 , PBS , 1% aqueous
 25mM sucrose OsO₄(Electron Microscopy Sciences, Fort
 Washington, PA, USA) 20
 2 10% SDS(sodium dodecylsul - fate, Sigma. U.S.A.) 100 μ l 가 70, 80, 90,
 95, 100%
 Bradford⁵⁸) Bio - Rad (Jeol, U.S.A.)
 protein assay kit(Bio - Rad, U.S.A.)

Table 1. Cell number of PDL cells cultured on dentin slices(Number/mm²).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	236 ± 52.7*	312 ± 89.2	362 ± 62.6	338 ± 73.6	310 ± 54.3	304 ± 68.8
Day 7	308 ± 55.4*	548 ± 144.3	836 ± 176.2	794 ± 213.6	582 ± 196.5	558 ± 164.5

*; Means ± S.D.

(: There were significant differences between Group 1(control) and each group(p<0.01).

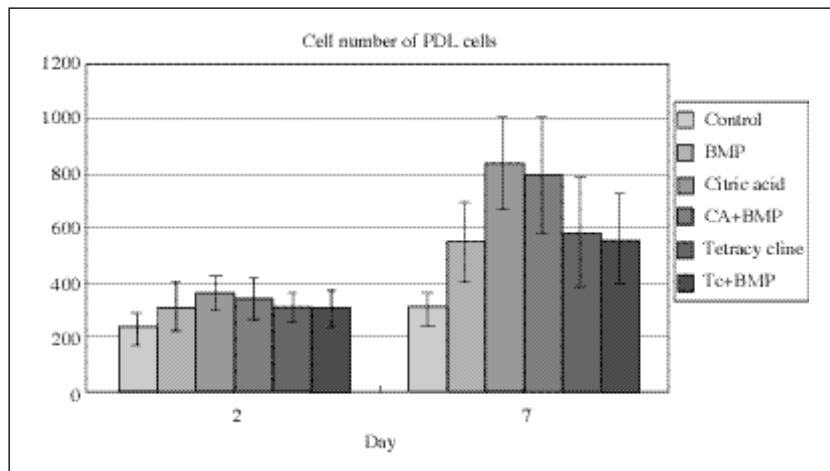


Figure 1. Cell number of PDL cells cultured on dentin slices(Number/mm²).

Table 2. Protein Assay of PDL cells cultured on dentin slices($\mu\text{g protein}/\text{mm}^2$)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	4.22 \pm 0.55*	4.63 \pm 1.11	8.21 \pm 1.08	8.27 \pm 1.17	7.20 \pm 0.91	7.15 \pm 0.58
Day 7	6.32 \pm 1.70	7.91 \pm 1.93	12.41 \pm 2.07	11.20 \pm 1.51	9.62 \pm 1.64	9.20 \pm 1.84

*; Means \pm S.D.

(: There were significant differences between Group 1 (control) and each group($p < 0.01$).

(: There were significant differences between Group 2 (BMP) and each group($p < 0.01$).

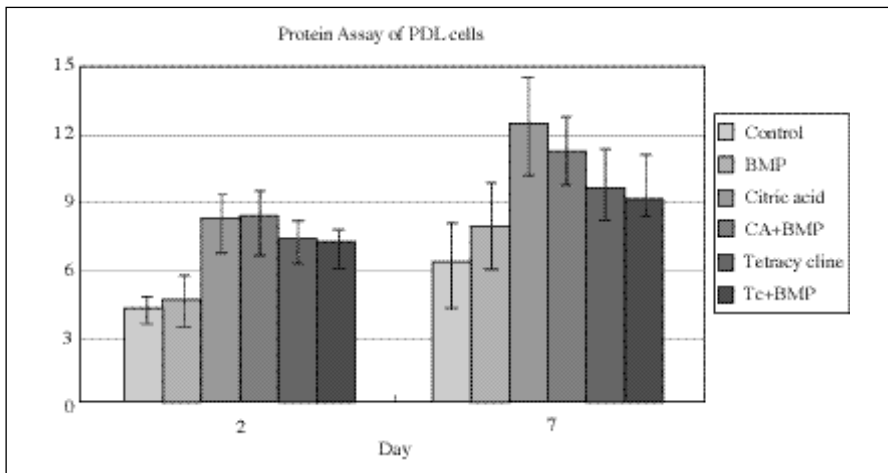


Figure 2. Protein Assay of PDL cells cultured on dentin slices($\mu\text{g protein}/\text{mm}^2$).

10. 1. 3) 2) 7) CA

(M \pm S.D) . SPSS

8.0(SPSS, U.S.A.)

t - test

ANOVA

Tukey

CA+BMP

, BMP 가

. CA 가

, CA+BMP CA

, TC TC+BMP

2 7

, CA CA+BMP 7

(Table 1, Figure 1).

Table 3. ALP activity of PDL cells cultured on dentin slices(nmolPNP/min/ μ g protein).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	34.4 \pm 11.7*	66.0 \pm 17.6	48.2 \pm 13.4	52.8 \pm 11.7	44.4 \pm 10.5	48.6 \pm 8.2
Day 7	59.8 \pm 14.5	143.2 \pm 23.8	74.6 \pm 16.6	124.2 \pm 25.6	76.4 \pm 20.3	83.8 \pm 28.3

*; Means \pm S.D.

(: There were significant differences between Group 1(control) and each group($p < 0.01$).

(: There were significant differences between Group 2(BMP) and each group($p < 0.01$).

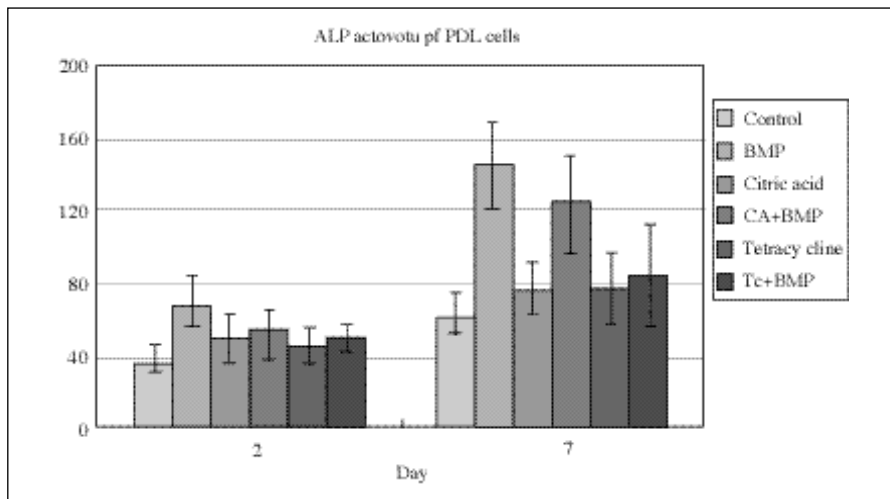


Figure 3. ALP activity of PDL cells cultured on dentin slices(nmolPNP/min/ μ g protein).

2) , BMP CA
 CA+BMP 가
 2 7 CA
 CA+BMP
 , BMP BMP
 CA , CA+BMP , TC TC+BMP
 CA CA+BMP TC TC+BMP CA CA+BMP 7
 가 (Table 2,
 Figure 2).
 7 CA
 , CA+BMP
 3)

Table 4. Cell number of Osteoblasts cultured on dentin slices(Number/mm²).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	258 ± 40.8*	364 ± 52.7	446 ± 55.5	436 ± 47.7	398 ± 58.9	396 ± 60.7
Day 7	374 ± 70.6	742 ± 128.5	1062 ± 164.2	984 ± 157.7	782 ± 114.0	772 ± 131.7

*; Means ± S.D.

(: There were significant differences between Group 1 (control) and each group(p<0.01).

(: There were significant differences between Group 2(BMP) and each group(p<0.01).

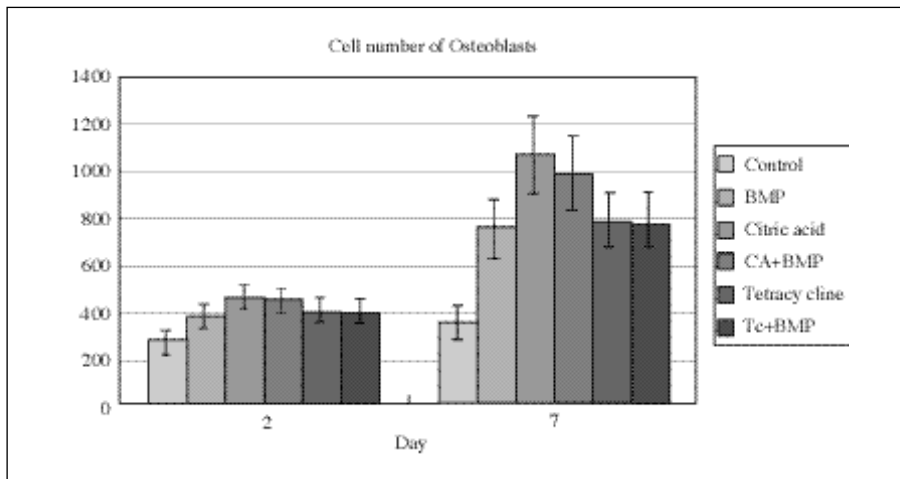


Figure 4. Cell number of Osteoblasts cultured on dentin slices(Number/mm²).

2 , CA+BMP 2 7
 , BMP CA+BMP
 . CA
 , CA+BMP
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 7 BMP CA+BMP
 , BMP CA+BMP
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 2 7
 BMP CA+BMP
 (Table 3, Figure
 3).
 BMP 가 , CA+BMP
 TC+BMP . CA 2.
 가 , BMP
 1)
 2 CA
 , CA+BMP CA

Table 5. Protein Assay of Osteoblasts cultured on dentin slices(μg protein/ mm^2).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	$5.67 \pm 0.92^*$	7.14 ± 1.26	9.24 ± 1.31	8.92 ± 1.16	8.20 ± 1.40	8.42 ± 1.21
Day 7	6.78 ± 1.30	10.40 ± 2.07	15.21 ± 2.29	13.70 ± 2.12	11.71 ± 2.28	11.40 ± 2.46

*; Means \pm S.D.

(: There were significant differences between Group 1(control) and each group($p < 0.01$).

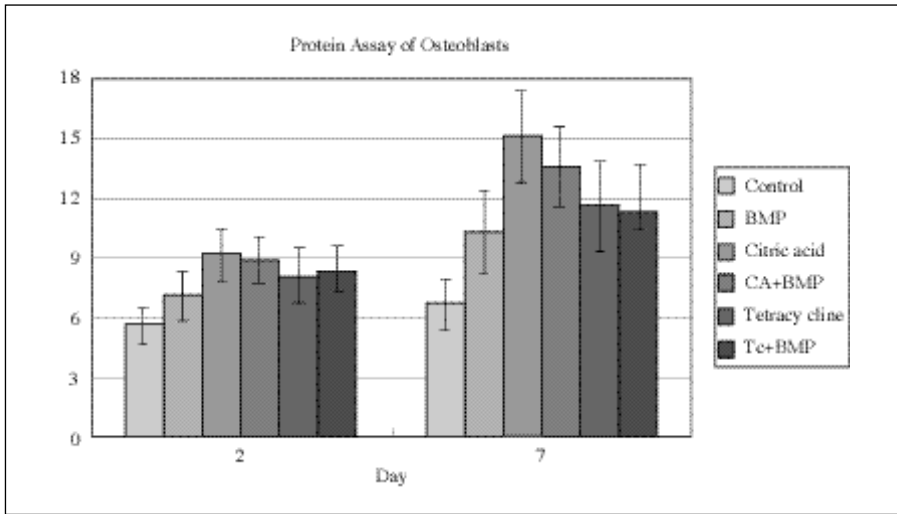


Figure 5. Protein Assay of Osteoblasts cultured on dentin slices(μg protein/ mm^2).

CA+BMP , TC , CA , TC+BMP , BMP , 2 7 , CA , CA+BMP , 7 (Table 4, Figure 4). , BMP , CA , 2 , CA CA+BMP , 2 , CA , CA+BMP , 7 , CA , CA+BMP , TC , BMP , CA+BMP , BMP , BMP

Table 6. ALP activity of Osteoblasts cultured on dentin slices(nmolPNP/min/ μ gprotein)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	50.8 \pm 9.5*	70.6 \pm 9.9	57.2 \pm 12.7	65.6 \pm 9.6	52.8 \pm 8.3	54.0 \pm 8.8
Day 7	80.2 \pm 21.5	195.4 \pm 62.5	111.2 \pm 35.3	185 \pm 46.0	104 \pm 39.6	162 \pm 41.9

*; Means \pm S.D.

(: There were significant differences between Group 1 (control) and each group(p<0.01).

(: There were significant differences between Group 2 (BMP) and each group(p<0.01).

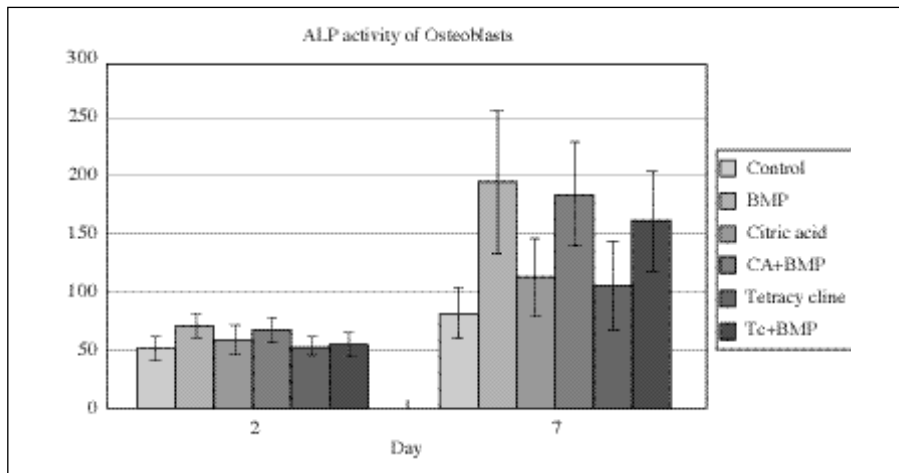


Figure 6. ALP activity of Osteoblasts cultured on dentin slices(nmolPNP/min/ μ g protein).

CA
 . 2 7
 , CA CA+BMP 2 7
 , BMP
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 (Table 5,

Figure 5).

3)

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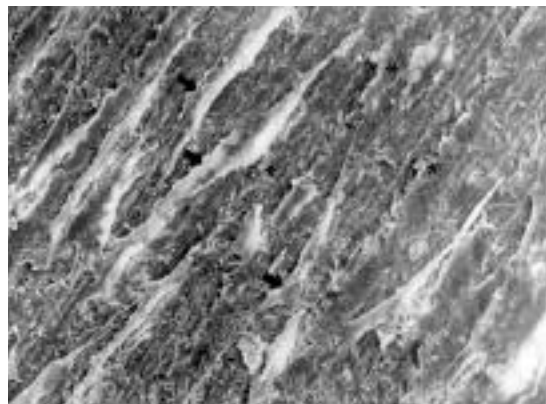


Figure 7. SEM view of group 2 with periodontal ligament cells two days after seed - ing(x400). Smear layer can be seen(*). Arrow marks indicates attached PDL cells. Cell attachment

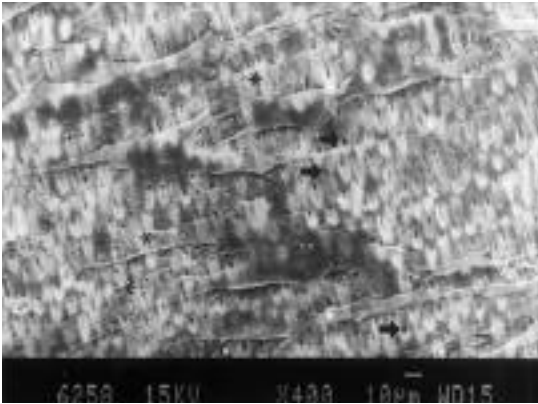


Figure 8. SEM view of group 3 with periodontal ligament cells two days after seed - ing(x400). PDL cells are well attached to the etched dentin surfaces and evenly distributed through all the dentin surfaces. Fully extended cell processes(*), exposed dentinal tubules(arrow marks) can be seen.

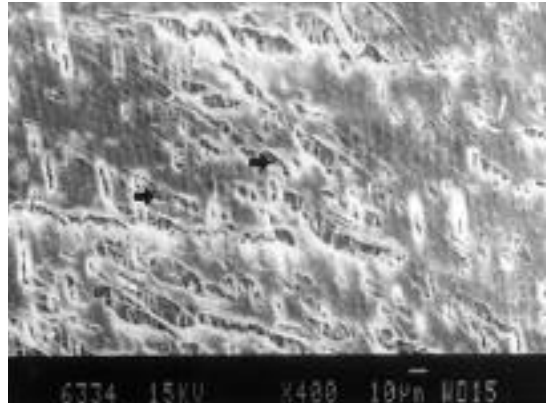


Figure 9. SEM view of group 4 with osteoblasts two days after seeding(x400). Almost all of the dentin surfaces are covered with seeded cells. The cells are in good shape and cell processes are well extended. Dentinal tubules(arrow marks) can be seen in the picture. Left arrow mark indicates obscure dentinal tubule which might be due

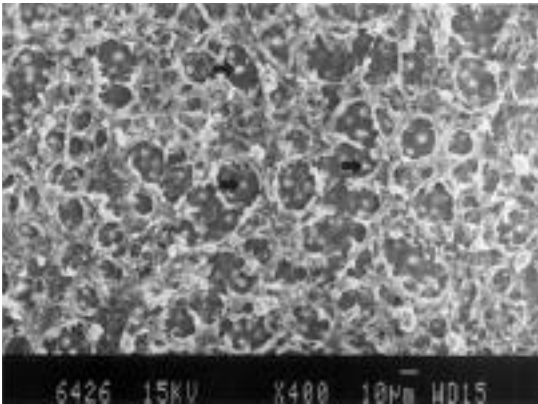


Figure 10. SEM view of group 5 with periodontal ligament cells two days after seed - ing (x400). Attached cells are narrow in shape, and cell processes exten - sions are limited. Exposed dentinal tubule (arrow mark) can be seen.

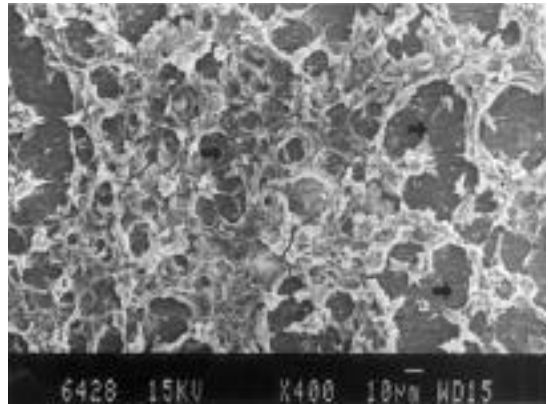


Figure 11. Sem view of group 6 with periodontal ligament cells two days after seed - ing (x400). Cell morphology is similar to Fig 10. Dentinal tubules(arrow marks) is obscure in the picture.

7	BMP	, CA	
CA+BMP			. BMP CA+BMP
	, BMP 가		가
, CA+BMP	TC+BMP	2 7	.

, BMP , CA+BMP TC+BMP

(Table 6, Figure 6).

3.

2 7

(Figure 7 - 11).

BMP CA , CA+BMP
TC , TC+BMP
가 .

, BMP CA CA+BMP

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TC , TC+BMP

. Figure 7 BMP

가

Figure 8 CA

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rhBMP - 2

. Figure 9 CA+BMP

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가

가

가

가

가

. Figure 10 TC

가

. Figure 11 TC+BMP

가

Figure 10

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IV.

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BMP가

20,22 - 24),

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25). BMP

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54). rhBMP - 2 BMP 가

37% 20 62).

, rhBMP - 2가 가 ,

rhBMP - 2 rhBMP - 2 rhBMP - 2

가 . rhBMP - 2

, , 가 ,

가 , PDGF IGF 가 ,

Gamal 59) ,

. Rompen 63)

3% 2.5%

, 24 , ,

가 , 가

60), 가 . Erdinc⁶⁴⁾

61). 가 5 2 4

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50, 100, 200 μ g/ml
rhBMP - 2
44), rhBMP - 2가 50ng/mL

Madison 65)

Kobayashi 26) rhBMP - 2가 50ng/mL 66)

rhBMP - 2
marker
(PTH) 3',
rhBMP - 2가
5' - cyclic adenosine monophosphate
가 rhBMP - 2가

rhBMP - 2
22) 37%
rhBMP - 2 10
38 10
38 가
55) 35%
10
가
가
가
pH 가
가
rhBMP - 2가
가

rhBMP - 2 가
가
rhBMP - 2
가 , rhBMP - 2 1 μ g/ml 3 μ g
/ml 54) 2
rhBMP - 2 가 4
가 7
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rhBMP - 2 가 BMP . rhBMP - 2
가가

Takayama 67)
bFGF

rhBMP - 2

2.

rhBMP - 2

rhBMP - 2

rhBMP - 2

. rhBMP - 2

rhBMP - 2

2 7

가

rhBMP - 2

VI.

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V.

rhBMP - 2가

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rhBMP - 2

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- Abstract -

Effect of Citric Acid and Tetracycline HCl Root Conditioning on rhBMP - 2 on Human Periodontal Ligament Fibroblast and Osteoblast

Cell

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The goal of periodontal treatment is pre-
dictable periodontal regeneration. But until
now, many products including GTR materi-
als and growth factors are beyond of com-
plete regeneration.

BMP can induce ectopic bone formation
when implanted into sites such as rat mus-
cle and can greatly enhance healing of bony
defects when applied exogenously. BMP
can promote periodontal regeneration by
their ability to stimulate new bone and new
cementum formation. But little is known
about optimal conditions required for the
application. Root conditioning is used for
bioactive root change so altered root sur-
face provides a substrate that promotes
chemotaxis, migration and attachment of
periodontal cells encouraging connective
attachment to the denuded root surface.
The aim of this study is to investigate
whether the acid conditioning change effect
of rhBMP - 2 on human periodontal ligament
cell and osteoblast cell line. 288 periodon-
tally involved root dentin slices are divided
into 6 groups, each 48, 1)control, 2)treated

with BMP, 3)treated with citric acid
4)treated with citric acid+BMP 5)treated
with tetracycline 6)treated with TC+BMP.
Each group was divided half, so 12 root
dentin slices were seeded with periodontal
ligament cells and 12 were seeded with
osteoblasts. At day 2 and 7, cell number,
protein assay, ALP activity was measured.
To investigate morphology of cultured cells,
SEM was employed. Statistical analysis was
performed with SPSS 8.0 either t - test or
ANOVA test.

The results are ; Protein assay and cell
number was slightly decreased in CA+BMP
group compared to CA group but it was not
statistically significant and ALP activity was
much more increased in CA+BMP group
compared to CA group so there was no
statistical significance between BMP and
CA+BMP group and statistically significant
compared to control group.

Cell number and protein assay was
slightly increased in TC group and ALP
activity was much less than BMP group and
CA group. Cell number and protein and ALP
activity was not much increased in
TC+BMP group. TC group and TC+BMP
group showed cell morphology change in
SEM. This results suggested that applica-
tion of root surface with citric acid before
BMP treatment might give better result in
periodontal regeneration.

Key word : PDL cell, osteoblast, citric acid,
tetracycline, attachment, proliferation, dif-
ferentiation