

Platelet - derived growth factor가

. . .

I. 9) , , , , 6, 10),
 polypep - PDGF , 11 - 12)
 tide (autocrine factor),
 (paracrine factor) 1).
 polypeptide fibronectin,
 (Platelet - derived growth factor, PDGF),
 (Fibroblast growth fac - 13 - 14). Cochran 15) PDGF -
 tor), (Transforming growth BB가
 factor), - (Insulin - like , Canalis 16)
 growth factor), (Epidermal PDGF - BB가 interstitial collagenase 가
 growth factor) interleukin - 1 가
 , Centrella McCarthy¹⁾
 2). PDGF - BB
 PDGF가 가 DNA,
 . PDGF 가 . Lynch 17)
 28 - 35 KDa 3 - 4) Ross⁵⁾ PDGF - BB
 Kohler Lipton⁶⁾ 가 가 .
 Antoniades⁷⁾ Lynch 18)
 (PDGF - AA, BB) PDGF - BB
 (PDGF - AB)가 8). PDGF 가 가
 granule .

Rutherford¹⁹⁾ PDGF가 , 30-31).
 가 .
 , Matsuda²⁰⁾ 1 32),
 PDGF , PTH - adenylate
 , , 가 cyclase , prostaglandin E₂ ,
 , Oates²¹⁾ PDGF가 osteonectin, osteopontin³³⁾, bone phospho-
 . Piche Graves²²⁾ proteins³⁴⁾, proteoglycan collagenase
 PDGF 가 , 1,25(OH)₂D₃ - osteocalcin
 35) .

Rutherford¹⁹⁾ Giannobile²³⁾ PDGF -
 BB IGF - 1
 DNA .
 , Lynch²⁴⁾ PDGF - BB IGF
 가 7, 36).
 가
 가
 PDGF 가
 25) 가
 . PDGF 가
 가 1, 16).
 PDGF
 가 cyclo - oxygenase PDGF
 prostaglandin 1, 7). PDGF
 26). PDGF가 37).
 .
 PDGF가
 가
 PDGF
 , 가 ,
 가 27), PDGF
 . 28) PDGF
 5 - 7nm 29)가 , 가 ,

가 PDGF trypsin - EDTA (D - PBS, pH 7, Gibco, USA) (Nikon, Japan) hemocytometer(Sigma, USA) %

osteocalcin ROS 17/2.8 HOS PDGF - BB PDGF가 3.

II. 24 - well plate 10 ng/ml PDGF - BB (1, 2, 3)

1. ROS 17/2.8 HOS 5% fetal bovine serum(FBS, Gibco, USA) 가 F12 0.1% Triton X - 10% FBS가 Dulbecco's modified 100/saline 20 mM p - nitrophenyl phosphate(PNPP, Sigma, USA) glycine - NaOH buffer(pH 10.4) 37 30 가 1 : 10 PNPP PNP (spectrophotometer, Shimadzu, Japan) 405 nm 5% CO₂ 95% 24 well plate 6 well plate 가 4.

2. 24 - well plate well 2 × 10⁴ 가 PDGF - BB 가 (0.1, 0.4, 2, 10, 50 ng/ml) PDGF - BB 19 3.7% for -

malin 3% silver
nitrate(Sigma, USA)

15 - 30

1% toluidine blue(Sigma, USA)

(Nikon, Japan)

5. Osteocalcin

Osteocalcin ROS 17/2.8 HOS
6 - well plate 가
72 PDGF - BB
0.5% Triton X - 100/PBS

enzyme immunoassay(EIA)

kit (Takara, Biomedicals, JAPAN)

osteocalcin

osteocalcin

well 가

2

3

Antibody - POD

1

가

PBS

6.

Table 1. Effect of PDGF - BB on cell proliferation in ROS 17/2.8 and HOS cell culture

PDGF conc. (ng/ml)	Cell proliferation(% of control)	
	Ros 17/2.8	HOS
0	100 ± 6.11	100 ± 7.00
0.1	123.96 ± 7.93*	122.76 ± 5.97**
0.4	89.41 ± 8.41	135.07 ± 10.17**
2	85.25 ± 7.03	174.25 ± 10.26**
10	115.21 ± 10.37	182.84 ± 9.89**
4		가 ,
15	. 1N	well

492nm

1

가 osteocal -

osteocalcin

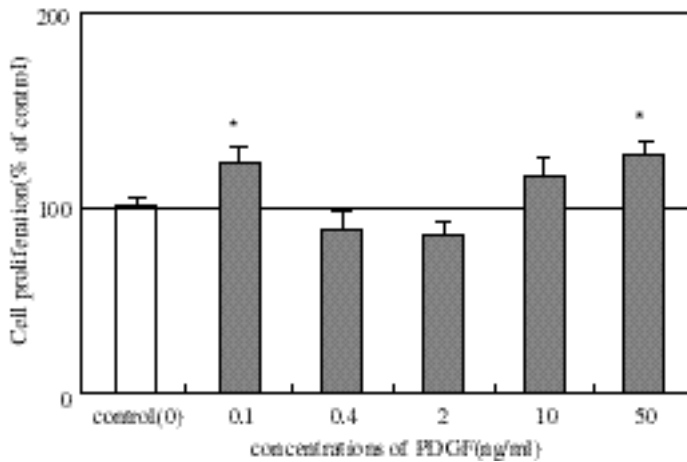


Figure 1. Effect of PDGF - BB on cell proliferation as determined by cell counting. Ros 17/2.8 cells were cultured for 48 hours in the DMEM/10% FBS with increasing concentrations of PDGF - BB. Cell proliferation was determined by cell counting.

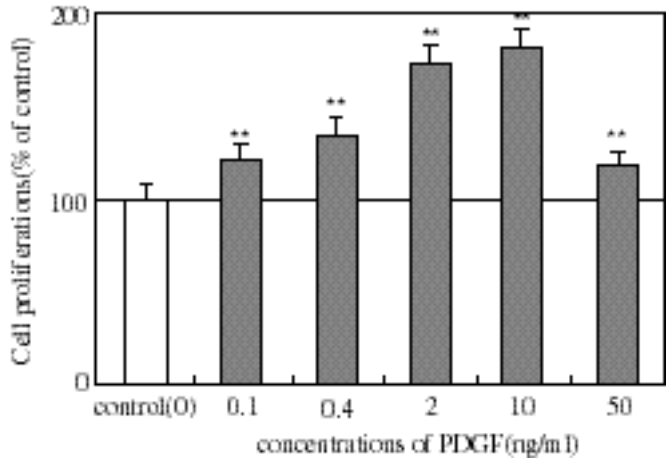


Figure 2. Effect of PDGF - BB on cell proliferation as determined by cell counting. HOS cells were cultured for 48 hours in the DMEM/10% FBS with increasing concentrations of PDGF.

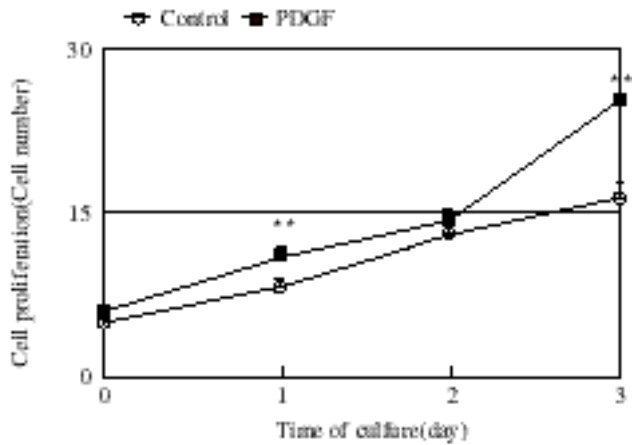


Figure 3. Time course effect of PDGF - BB on cell proliferation. ROS 17/28 cells were cultured with 10ng/ml PDGF.(**; $p < 0.01$ from Student's t test)

Student's t test	ROS 17/2.8 HOS 가 (0.1, 0.4, 2, 10, 50 ng/ml) PDGF - BB ROS 17/2.8 HOS (0.1, 0.4, 2, 10, 50ng/ml) 가
III.	
1.	

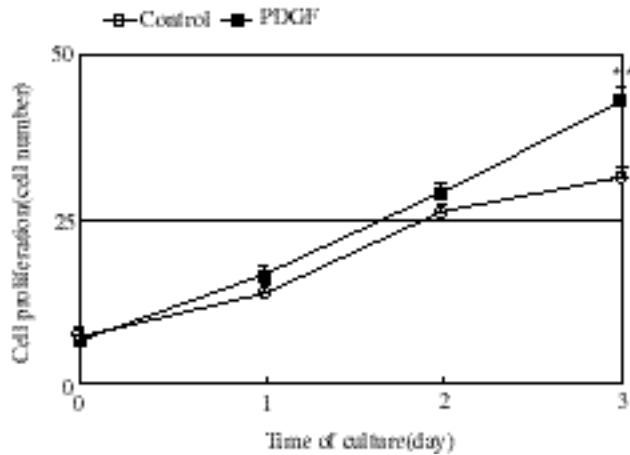


Figure 4. Time course effect of PDGF - BB on cell proliferation. HOS cells were cultured with 10ng/ml PDGF. (**: $p < 0.01$ from Student's t test)

Table 2. Effect of PDGF - BB on ALP activity in ROS 17/2.8 and HOS cell culture

PDGF concn. (ng/ml)	ALP activity per cell number	
	ROS 17/2.8	HOS
0	8.78	6.72
0.1	9.00	4.18
0.4	10.46	4.14
2	9.42	3.97
10	8.04	3.16
50	7.79	4.40

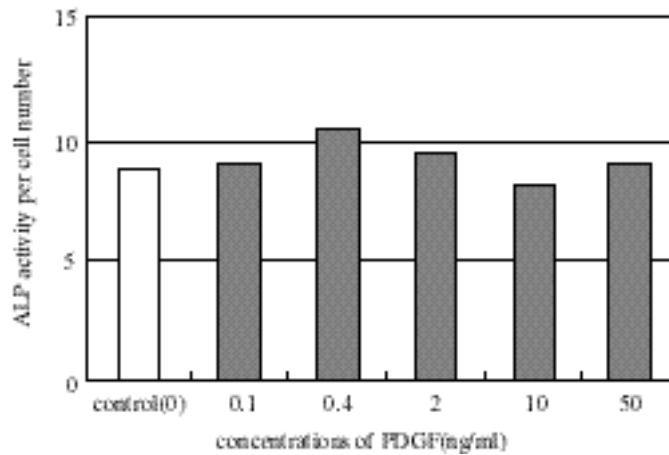


Figure 5. Effect of PDGF - BB on ALP activity. ROS 17/2.8 cells were cultured for 48 hours with increasing concentrations of PDGF.

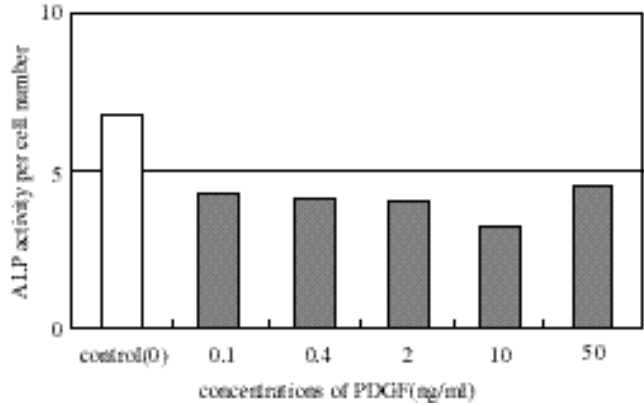


Figure 6. Effect of PDGF - BB on ALP activity. HOS cell were cultured for 48 hours with increasing concentrations of PDGF.

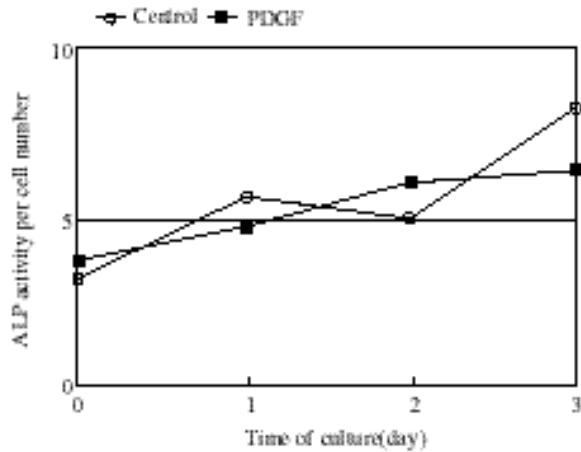


Figure 7. Time course effect of PDGF - BB on ALP activity. ROS 17/2.8 cells were cultured with 10ng/ml

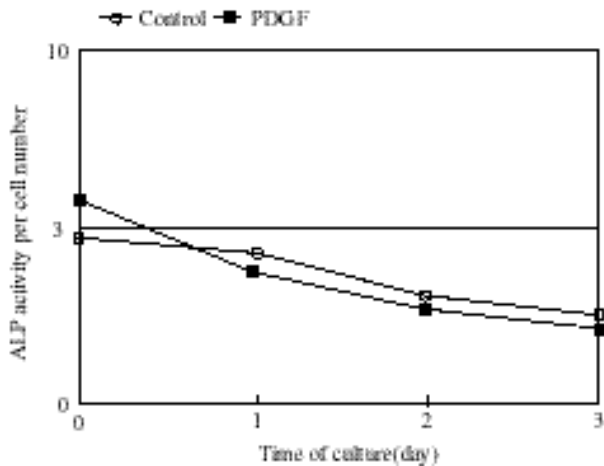


Figure 8. Time course effect of PDGF - BB on ALP activity. HOS cells were cultured with 10ng/ml PDGF

10ng/ml 가
(Table 1 Figure 1 2).

10 ng/ml PDGF - BB
1, 2, 3
PDGF - BB 가
3 가
(Figure 3 4).

Table 3. Effect of PDGF - BB on the calcified nodule formation in HOS cells in culture

PDGF conc.(ng/ml)	No. of calcified nodule
0	71.00 ± 27.78
0.1	69.50 ± 8.45
0.4	71.50 ± 12.92
2	58.25 ± 10.38
10	51.50 ± 9.49
50	38.50 ± 8.43

Data represent Mean ± S.E of 4 replicates.

2.

ROS 17/2.8 HOS

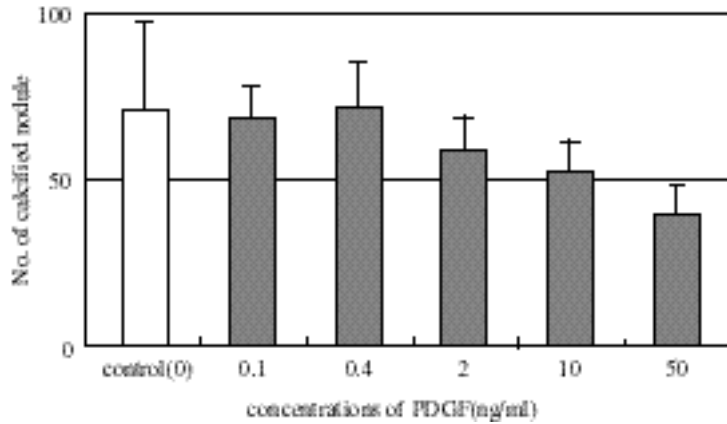


Figure 9. Effect of PDGF - BB on the calcified nodule formation in HOS cells in cultures

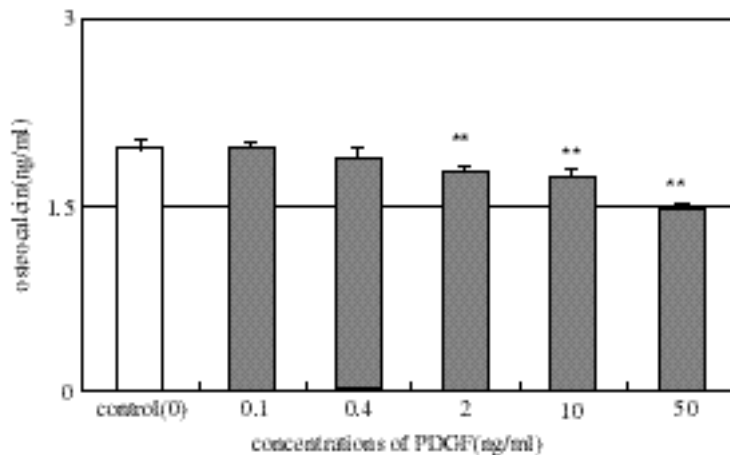


Figure 10. Effect of PDGF - BB on the osteocalcin production in ROS 17/28 cells in culture (**:P<0.01 form Student's t test)

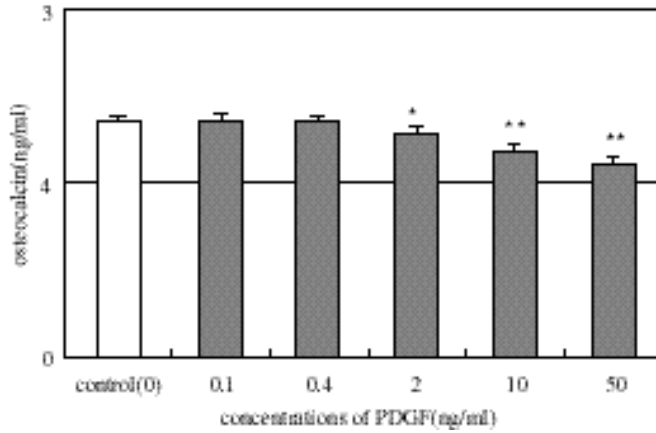


Figure 11. Effect of PDGF - BB on the osteocalcin production in HOS cells in cultures(*:p<0.05, **:p<0.01 from Student t test)

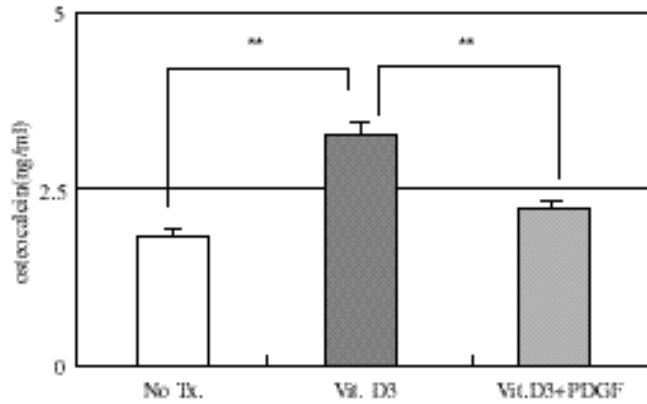


Figure 12. Effect of PDGF - BB on the 1,25(OH)₂D₃ - stimulated osteocalc in cultures of ROS 17/2.8 cells in cultures.(** :p<0.01 from Student t test)

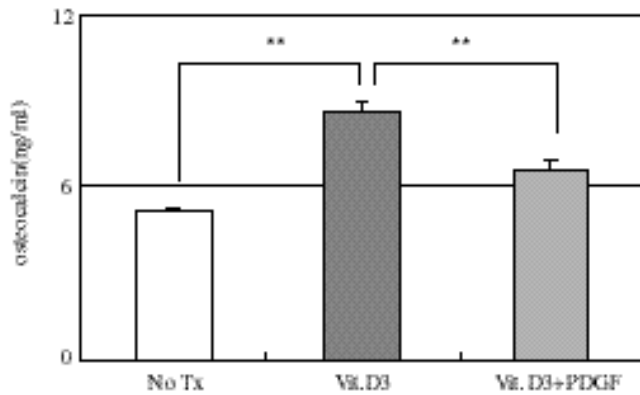


Figure 13. Effect of PDGF - BB on the 1,25(OH)₂D₃ - stimulated osteocalc in production in cultures of HOS cells in cultures.(** :p<0.01 from Student t test)

17/2.8 PDGF - BB 가 , ROS osteocalcin
 0.1ng/ml, 0.4ng/ml, 2ng/ml 가 BB , 1,25(OH)₂D₃ PDGF -
 가 , 10ng/ml, 50ng/ml 가 osteocalcin
 . HOS PDGF - BB 가 가 (Figure 12 13).
 . HOS
 10 ng/ml PDGF - BB 가 IV.
 (Table 2 Figure 5 6).

10 ng/ml , ,
 PDGF - BB 1,
 2, 3 ROS 17/2.8 가 ,
 1 3
 가 HOS 1,
 2, 3 가
 (Figure 7 8).

3. 가 , 10 - 20%가³⁸⁾.

HOS 19 von Kossa

, HOS
 0.1ng/ml, 0.4ng/ml PDGF - BB 가
 , 10ng/ml
 50ng/ml PDGF - BB osteocalcin (Bone gla -
 가 , protein), procollagen I carboxyl terminal
 (Table 3 Figure extension peptide
 9).

4. osteocalcin ,

Osteocalcin , ,
 PDGF - BB 가 가 ,
 osteocalcin , 가
 HOS ROS 17/2.8³⁹⁾.
 osteocalcin , ,
 (Figure 10 11). 1,25 (OH)₂D₃ 가

47), 48), 49) 50)
 PDGF
 AA, BB, AB 가
 가
 DNA
 가
 PDGF
 가
 PDGF
 가
 PDGF가
 40, 41), 42, 43), 44, ROS
 45) 17/2.8 HOS
 가
 가
 46). PDGF - ROS 17/2.8 HOS
 BB PDGF - BB 가 PDGF - BB
 Piche Graves²²⁾ ROS 17/2.8
 HOS
 PDGF - BB PDGF - BB (0.1, 0.4, 2,
 Rutherford¹⁹⁾ 10, 50 ng/ml)
 Lynch²⁴⁾ PDGF - BB 가 가 , 10 ng/ml PDGF - BB
 IGF - I 가
 PDGF - BB
 가
 PDGF Ross⁵⁾ Kohler Lipton³²⁾ Canalis⁵¹⁾
 0.1, 10, 100 ng/ml PDGF - BB
 가 가 DNA 가
 , Antoniades⁷⁾ , Oates²¹⁾
 PDGF 0.1, 0.5, 10, 20, 50 ng/ml PDGF - BB,
 AA 가 가

DNA 가
 52) PDGF - BB
 , 20 ng/ml 가
 . Blom

53) PDGF가 가 , 50 ng/ml DNA 54) pH
 274% 0.1 - 15 ng/ml , 56) 가⁵⁵⁾,
 PDGF 57), 59)
 58),
 50 ng/ml PDGF

PDGF -
 BB 가 10 ng/ml 가 50 ng/ml Robinson 60)
 가
 52) 20 ng/ml , Blom 53) 50 ng/ml Siffert⁶¹⁾
 가 가 , Stein 62)
 PDGF - BB
 0.4, 0.1 ng/ml 50, 10, 2,
 20 ng/ml

10 ng/ml 50 ng/ml
 가 가
 PDGF - BB ROS 17/2.8 HOS
 PDGF - BB , ROS 17/2.8
 (0.1, 0.4, 2 ng/ml) 가
 가 (10, 50 ng/ml)
 . HOS (0.1,
 0.4, 2, 10, 50 ng/ml) , 10
 ng/ml 가
 Centrella 13-15)
 PDGF - AB, BB
 가
 PDGF가

19-21). PDGF - BB
 30 가
 , 1, 2, 4 8 PDGF - BB가

BB 1, 2, 3 PDGF -
 가
 1, 2, 3 가 10 ng/ml PDGF - BB

가 PDGF - BB

3. ROS 17/2.8 HOS

가 (0.1, 0.4, 2, 10, 50 ng/ml) PDGF - BB

가 가 가

HOS

10 ng/ml PDGF - BB

가

4. , 10 ng/ml PDGF - BB , ROS 17/2.8 1 3 가 , HOS 1, 2, 3 가

5. HOS 0.1 ng/ml, 0.4 ng/ml PDGF - BB , 10 ng/ml 50 ng/ml PDGF 가

6. Osteocalcin , PDGF - BB osteocalcin , HOS ROS 17/2.5 osteocalcin 1,25(OH)₂D₃ osteocalcin 가 , 1,25(OH)₂D₃ PDGF - BB 1,25(OH)₂D₃ 가 osteocalcin

BB

PDGF -

VI.

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- Abstract -

Effects of Platelet - derived Growth Factor on the Activity of Osteoblastic Cells

Hyoung - Ho Choi, Jung - Keun Kim, Sung -
Bin Lim, Chin - Hyung Chung
Department of Periodontology College of
Dentistry Dan - Kook University

The cell activities of bone metabolism is affected by growth factor rather than by hormone. The affects of growth factors on the bone activity were observed using various culture methods. Platelet - derived growth factor (PDGF) is produced from the well differentiated bone cell. It stimulates cell mitosis, synthesizes collagen in bone tissue and plays a role in healing response. The purpose of this study is to evaluate the effects that PDGF has on the activity and the proliferation of osteoblast by measuring the activity of alkaline phosphatase, the growth formation of calcified nodules, and osteocalcin production.

In this study, HOS and ROS 17/2.8 osteoblastic cell line was used, along with variable concentrations of PDGF the were measured with osteoblastic proliferation. The cell proliferation of HOS and ROS 17/2.8 cells was stimulated dose - dependently. Alakline phosphatase activity was significantly decreased by PDGF in osteoblastic cells. A number of small calcified nodules were observed in HOS cell

treated with low concentrations (0.1, 0.4 ng/ml) of PDGF - BB and no significant difference from control group was found. High concentrations (10, 50 ng/ml) of PDGF suppressed calcified nodule formation. And osteocalcin production was inhibited with PDGF. These results suggest that PDGF stimulates the osteoblastic proliferation, whereas suppresses the individual cellular functions.