

PDGF-BB TGF- 1

I. , PDGF subunit . PDGF - AB PDGF - BB . 11), 가 PDGF A - chain 12), PDGF IGF - 1 13), TGF - 14,15), TGF - 16), TGF - 3,7 - 10), PDGF PDGF A - chain PDGF B - chain polypeptide PDGF AA, BB, AB 3 PDGF 17), TGF - 가 isoform PDGF subunit 가 Piche

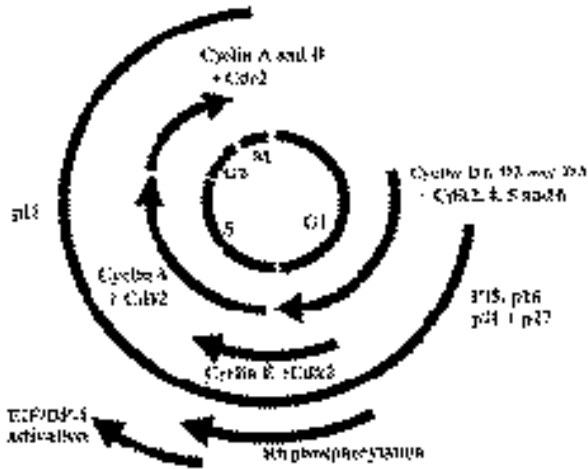


Fig 1. Cell cycle and cell cycle regulatory proteins

(1989)¹⁸⁾

TGF -

, PDGF가

가

Piche Graves (1989)¹⁹⁾

TGF - 가

. Oates (1993)²⁰⁾

PDGF - AA PDGF - BB

DNA 가

TGF -

TGF -

PDGF

Dennison

(1994)²¹⁾

TGF - 1 PDGF

TGF - 1

, PDGF

가 . DNA
S , M , S
M 가 G1
G2 4 (Fig. 1).

가 . cyclin cyclin - dependent
kinases(cdk)^{22, 23)}. cdk

serine/threonine protein kinase , cyclins

가

, cyclin cdk

. Cyclin threonine

residue cdk가

cyclin

. Cyclin cdk

²⁴⁾. cyclin - cdk

Cyclin ,
G1 cyclin G1

cdk S

. Dennison
M G2 cyclin
cdk cdk

²⁵⁾. G1 cyclin

cyclin, cdk, cdk p21, proliferating

cell nuclear antigen(PCNA) 4

^{26 - 33)}. G1 S

cyclin D 가 , G1

cyclin E ^{34 - 37)}. D -

cyclin(D1, D2, D3) cdk 4

cdk 6 , cyclin E cdk 2

. cyclin G1 S

, cdk cyclin
cyclin - activating kinase
38 - 40).
Cyclin dependent kinase inhibitor cdk
cdk
p21 G1 cyclin - cdk
41). p21 G1 cyclin
가 ,
가 cdk
42 - 44). p21 kinase
S
PCNA PCNA가 DNA
polymerase DNA
가 45, 46).
PDGF - BB TGF - 1

3
. .
PBS 2
PBS 0.25% Trypsin/EDTA(10
x, Gibco, USA) 2Mℓ 3 clean
bench
15Mℓ 1,200 rpm
10
PBS 가 Vortex mixer
1,200rpm 10
. DMEM 가
Vortex mixer
60mm
2 - 3
1:3 - 4
6
. 5,
2.

II.

1.
. .
60mm
5 - 6 30 37 ,
5% CO₂, 100%
, 10% fetal
bovine serum(FBS, Gibco, USA) 1%
(10,000units/Mℓ Penicillin G, 10,000μg/Mℓ
Streptomycin, 25μg/Mℓ Amphotericin B ,
Gibco, USA)가 DMEM(Dulbecco's
Modified Eagle Medium, Gibco, USA) 3Mℓ
가 2 -

trypsin
. .
6 well dish
2 × 10³ 가
TGF - 1(Sigma, USA) 10ng/Mℓ, PDGF -
BB(Sigma, USA) 20ng/Mℓ
3 trypan blue
exclusion
. .
2 × 10³ 6 well dish
3 (× 40)
. .
3. MTT assay
5, 6
0.25% Trypsin/EDTA trypan
blue

Table 1. Cell number count and MTT assay in gingival fibroblasts supplemented with PDGF - BB and

	Cell number (× 10 ⁴)	MTT (absorbance)
Control	5.29 ± 0.82	2.89 ± 0.08
PDGF - BB	11.60 ± 0.63*	3.65 ± 0.05*
TGF - 1	7.42 ± 0.59*	3.06 ± 0.07*
PDGF - BB + TGF - 1	6.33 ± 0.66*	2.89 ± 0.03

* Significantly different from control(p<0.05)

24well plate well 1 × 10⁴ 가 (Molecular Research Center, Inc., USA)
 가 , Lowry
 1 37 , 5% CO₂, 100% 47)

50μg
 DMEM 1 12.5% SDS - polyacrylamide gel
 TGF - electrophoresis nitrocellulose
 1 10ng/ml, PDGF - BB 20ng/ml membrane(Bio - Rad, USA) transfer
 가 1 3

membrane blocking (Zymed, USA) 1
 MTT(3 - 1 90
 (4, 5 - dimethylthiazol - 2 - yl) - 2, 5 - diphenyl tetrazolium bromide ; Sigma, USA) 200
 μl well 가 3

200μl
 DMSO(Junsei, Japan) 가
 formazan 96 well plate
 . Plate ELISA
 analyser(Spectra MAX 250, Molecular Devices Co., USA) plate 570nm
 , 4

4. Western blot analysis

trypsin
 100mm 1 × 10⁵ 가
 .
 TGF - 1 PDGF - BB 가
 3 . 3 Tri - reagent
 , anti - mouse or anti - rabbit IgG - horse raddish peroxidase conjugated secondary antibody(Santa Cruz Biotechnology) 1
 PBS

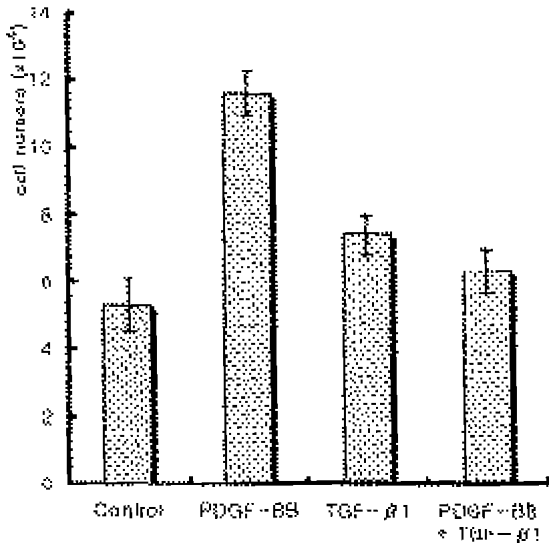


Fig 2. Effects of PDGF - BB and TGF - 1 on gingival fibroblasts growth. Cell numbers were counted by trypan blue exclusion test after 3 days incubation(Mean±S.D.).

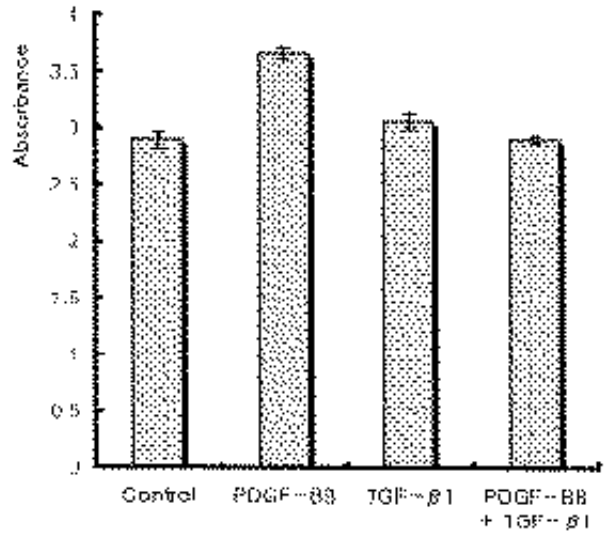


Fig 3. Effects of PDGF - BB and TGF - 1 on gingival fibroblasts growth. MTT assay was performed after 3 days incubation

membrane ECL western blotting detection reagent(Amersham, UK)
Hyperfilm - MP(Amersham)

membrane 1 x Ponceau S (Sigma, USA)

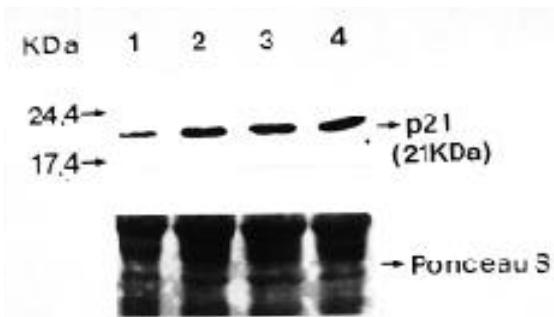


Fig 4. Western blot analysis for p21 in human gingival fibroblasts exposed to PDGF - BB and TGF - 1 for 3 days. Equal cellular proteins were loaded and transferred to a membrane as showed at the lower panel(KDa : kilodalton, 1 : control, 2 :

5.

1.

III.
가 PDGF - BB TGF - 1
Fig 2 Table 1
가 가 3
PDGF - BB TGF - 1
TGF - 1
PDGF - BB TGF - 1

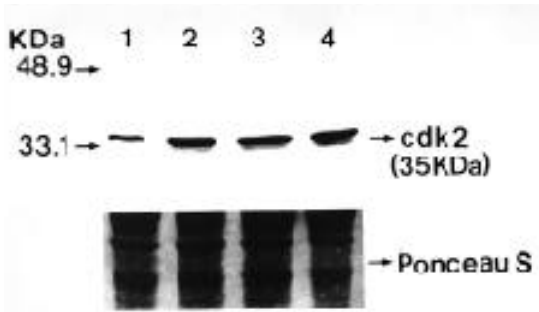


Fig 5. Western blot analysis for cdk 2 in human gingival fibroblasts exposed to PDGF - BB and TGF - 1 for 3 days. Equal cellular proteins were loaded and transferred to a membrane as showed at the lower panel(KDa : kilodalton, 1 : control, 2 :

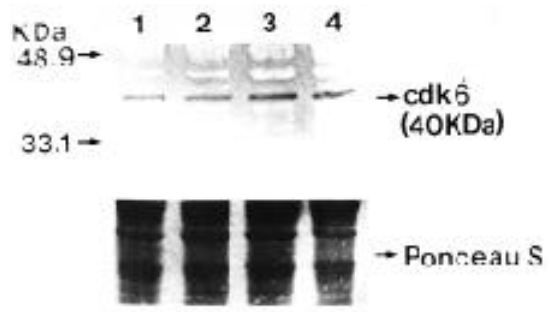


Fig 7. Western blot analysis for cdk 6 in human gingival fibroblasts exposed to PDGF - BB and TGF - 1 for 3 days. Equal cellular proteins were loaded and transferred to a membrane as showed at the lower panel(KDa : kilodalton, 1 : control, 2 :

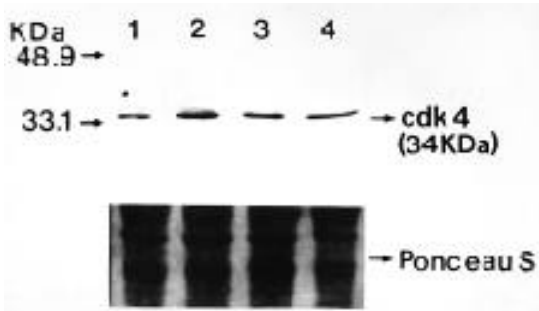


Fig 6. Western blot analysis for cdk 4 in human gingival fibroblasts exposed to PDGF - BB and TGF - 1 for 3 days. Equal cellular proteins were loaded and transferred to a membrane as showed at the lower panel(KDa : kilodalton, 1 : control, 2 :

TGF - 1 PDGF - BB
TGF - 1 PDGF - BB
(Photo. 1 - 4).

2. p21

p21 cyclin - cdk

. PDGF - BB TGF -

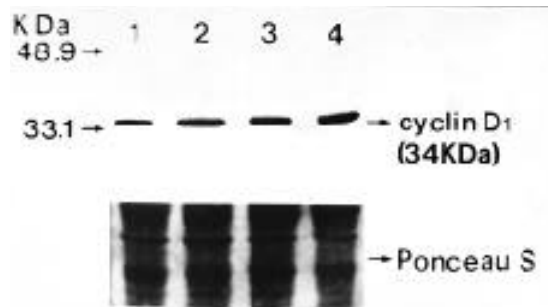


Fig 8. Western blot analysis for cyclin D1 in human gingival fibroblasts exposed to PDGF - BB and TGF - 1 for 3 days. Equal cellular proteins were loaded and transferred to a membrane as showed at the lower panel(KDa : kilodalton, 1 :

MTT
table 1 Fig 3 . MTT
가 PDGF - BB
가 가
TGF - 1 PDGF - BB TGF - 1
,
,
PDGF - BB
가 가

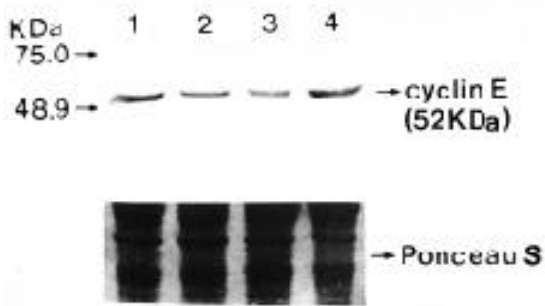


Fig 9. Western blot analysis for cyclin E in human gingival fibroblasts exposed to PDGF - BB and TGF - 1 for 3 days. Equal cellular proteins were loaded and transferred to a membrane as showed at the lower panel(KDa : kilodalton, 1 : control, 2 :

1 : PDGF - BB, 3 : TGF - 1, 4 : PDGF - BB + TGF - 1). p21 (Fig 4). p21 cyclin - cdk

TGF - 1 PDGF - BB p21 가

3. cdk

G1 cyclin D1 cdk 4 6 PDGF - BB TGF - 1 cyclin E cdk 2가

western blot . cdk 2 PDGF - BB 가 가 (Fig 5). PDGF - BB TGF - 1 가

BB TGF - 1 가 PDGF - BB TGF - 1 가 cdk 4 PDGF - BB

가 TGF - 1 PDGF - BB TGF - 1 가 (Fig 6).

6

. TGF - 1 . cdk

가 , PDGF - BB PDGF - BB TGF - 1 (Fig 7).

4. cyclin

cyclin D1 PDGF - BB TGF - 1

, PDGF - BB TGF - 1

(Fig 8). PDGF - BB TGF - 1 cyclin E

(Fig 9).

IV.

PDGF - BB TGF - 1 3 cdk 2, 4, 6, cyclin D1 가 .

PDGF - BB 가 PDGF - BB TGF - 1 , PDGF - BB TGF - 1 가 . ,

가 .

TGF - PDGF . Oates
 (1993)²⁰⁾
 PDGF TGF - 1
 TGF - 1 가
 cytokine
 TGF - 1 PDGF
 가 PDGF TGF - Piche
 Graves(1989)¹⁹⁾ PDGF TGF - 가
 가
 , Wrana (1986)⁴⁸⁾
 TGF -
 PDGF TGF - 1
 crystal violet
 , PDGF, TGF - 1
 PDGF가
 TGF - 1
 가 49).
 PDGF TGF - 가
 PDGF 가 . Oates
 TGF - (1993)²⁰⁾ TGF - 1
 . TGF - (1.0ng/Mℓ
 10.0ng/Mℓ) 가
 , PDGF TGF - PDGF
 , PDGF 가 50ng/Mℓ -
 가 . Pfeilschifter (1990)⁵⁰⁾
 21),
 PDGF - BB TGF - 1
 0.1nM 10nM(250ng/Mℓ)
 가 . 가

PDGF 1nM 10nM (320ng/Mℓ)
 Dennison (1994)²¹⁾ 10ng/Mℓ
 TGF-1 20ng/Mℓ PDGF
 , 3 PDGF
 가 , TGF-1
 TGF-1 PDGF
 가

10ng/Mℓ TGF-1 20ng/Mℓ PDGF-BB
 .
 cyclin D cdk 4, 6
 G1
 , cyclin E
 cdk 2 G1

PDGF-BB
 가 , 가
 가 cdk
 PDGF-BB
 가
 PDGF-BB

cyclin D cyclin E
 ,
 cyclin
 가 cyclin cdk

PDGF-BB TGF-1
 . PDGF-BB
 TGF-1 가
 51, 52).
 cdk p21
 cyclin-cdk G1-S

53, 54).
 cyclin-cdk kinase
 가 . p21
 cyclin-cdk
 Zhang (1994)³³⁾ Chen
 (1995)⁵⁵⁾ .
 p21

cyclin-cdk
 . Hiyama (1997)⁵⁶⁾
 p21 가
 cdk kinase cyclin D1
 ,

PDGF-BB TGF-1 p21
 western blot , p21 가
 가 cyclin-cdk
 가
 p21 cyclin-cdk 가
 p21

55). cyclin 가 p21
 가 가 cyclin p21
 cyclin-cdk-p21
 가 , p21
 cyclin-cdk 가

cyclin cdk 가
 p21
 . PDGF-BB TGF-1
 p21
 cdk

가
 , PDGF-BB 가
 가 .

- kinase 가
- V.
- PDGF - BB TGF - 1
- western blot
1. PDGF - BB가 가
TGF - 1 , PDGF - BB
TGF - 1
 2. cdk p21 가
 3. PDGF - BB cdk 2, 4 가
TGF - 1 PDGF - BB TGF - 1 가
 4. PDGF - BB TGF - 1 cyclin D1 가
cyclin E 가
- VI.
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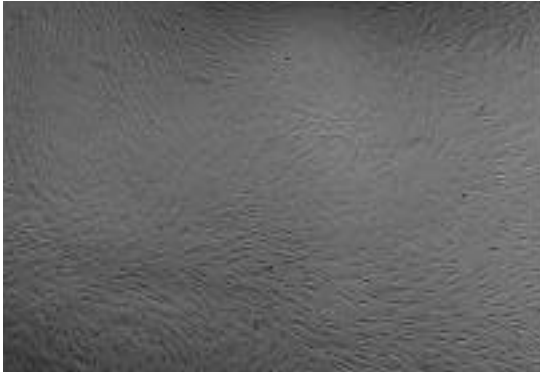


Photo 1. Cell density by light microscopic level after 3 days incubation supplemented with 10% FBS only($\times 40$).

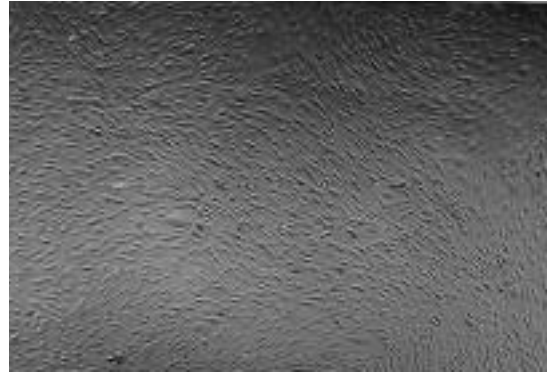


Photo 2. Cell density by light microscopic level after 3 days incubation supplemented with 10% TGF - 1($\times 40$).

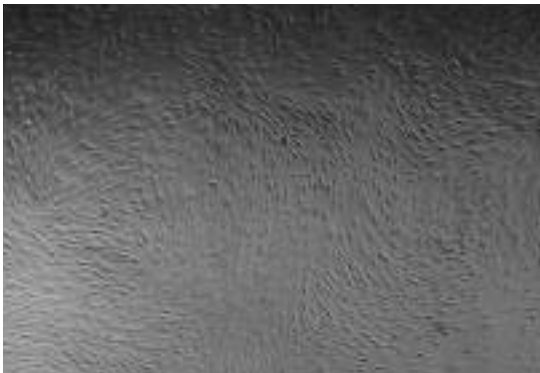


Photo 3. Cell density by light microscopic level after 3 days incubation supplemented with combination of PDGF - BB and 10% TGF - 1($\times 40$).



Photo 4. Cell density by light microscopic level after 3 days incubation supplemented with 10% FBS only($\times 40$).

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