

Treponema Denticola Treponema Lecithinolyticum

1 . 2 . 1 . 1 . 1 . 1

1

2

I.

Porphyromonas gingivalis, Fusobacterium nucleatum, spirochetes가

Prevotella intermedia, Pleomorphic bacteroides, Eikenella corrodens, Actinomyces species, spirochetes가 10).

33,38,39), 1800

Spirochetes

53),

300가

가

22,25).

13 19

(early onset periodontitis)

30 100

Porphyromonas gingivalis

10).

Treponema denticola가

7,8,15,16,50 - 52),

1).

17),

Riviere

가

spirochetes

, Treponema denticola

spirochetes

20,27,28,29,58),

가

43).

42).

가

Spirochetes

가

, Actinobacillus actinomyces comitans Capnocytophaga species가 가 ,

가

15가

spirochetes

(rapidly progressive periodontitis)

10),

(small,

medium, large)

spirochetes

가

spirochetes

II.

가

19,31,56),

1.

spirochetes

(1)

spirochetes

1

Hank's balanced salt

가 DNA

solution (HBSS)

3

1/3

15 blade

(oligonucleotide probe)

25mm

20% fetal

Loesche

spirochetes

bovine serum (FBS), penicillin (100 unit/ml),

keratinolytic enzyme

streptomycin (100mg/ml), amphotericin -

B (0.5mg/ml) 가

- minimal essential

medium (- MEM)

37 , 100% ,

5% CO₂

가

6,30,44).

75mm

7 10

가

26),

5 7

spirochetes

Treponema denticola, Treponema pecti -
novorum, Treponema socranskii, Treponema
vincentii, Treponema maltophilum⁶²),
Treponema medium⁵⁹), Treponema
amylovorum⁴⁸) , spirochetes

(2) Treponema culture

Treponema denticola ATCC 33521

Treponema lecithinolyticum ATCC 700332

OMIZ - Pat

37 ,

가

Treponema denticola

3

5000 x g 10

phosphate buffer

solution (PBS)

¹(Branson model 250 sonifer)

Treponema denticola 가

13,000rpm

10

spirochetes

Protein Assay

가

Kit (Pierce, USA)

³⁶), 가

Treponema

lecithinolyticum

1. Fisher Scientific, USA

filter pore size가 0.22 μ m membrane

immunosorbent assay(ELISA) reader

85 1

2.

(1) MTT test(Microtiter assay which uses the tetrazolium test)

(2) (Phase - contrast microscopy)

MTT test

(1, 2) 400

tetrazolium salt가

succinate dehydrogenase tetrazolium salt가 formazan salts formazan

(3) LDH(Lactate dehydrogenase) test

가 lactate

96 - well microtiter plates 1 x 10⁴ , 10% FBS가 - MEM 37 24 . 2% FBS가 - MEM 가 1, 2, 3

dehydrogenase

2.5ml 0.1ml 30 340nm , 1, 2, 3 25 warming (Sigma) 0.4ml LDH A 10ml LDH B

MTT test

100 μ l MTT(3 - [4,5 - dimethylthiazol - 2 - yl -] - 2,5 - diphenyltetrazolium bromide) 4 . MTT

(4) Gelatin zymography

collagenase gelatinase

50 μ l dimethyl sulfoxide(DMSO) 가 formazan 570nm Light filter enzyme - linked

zymography (1 x 10⁴) - MEM(10% FBS) 96 - well microtiter plate , - MEM 37

LDH A : NADH 0.194mmol/l phosphate buffer, pH7.5 0.54mmol/l

serum 1, 2, 3

LDH B pyruvate 16.2mmol/l

matrix metalloproteinase - 2 (MMP - 2 gelatinase A) gelatin

pyruvate+NADH+H⁺ LDH L - Lactate+NAD⁺

sodium dodesyl sulfate - polyacrylamide gel(SDS - PAGE)

(2.5% SDS, 3% sucrose, 0.005% bromophenol blue) 0.2% gelatin SDS - PAGE (2.5% Triton X - 100 50mM Tris -

HCl, pH 7.5) 30 2 SDS . Gel 37 (0.15M NaCl, 10mM CaCl₂, 50mM Tris - HCl, pH 7.5) 18 Coomassie Blue R - 250(0.05%, isopropyl

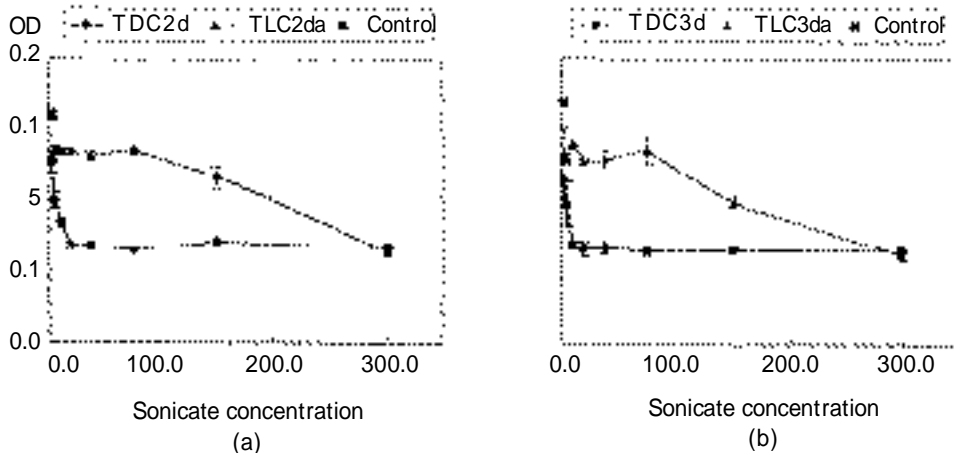


Figure 1. The effect of concentration on cell proliferation with time (a) 2 - day incubation (b) 3 - day incubation

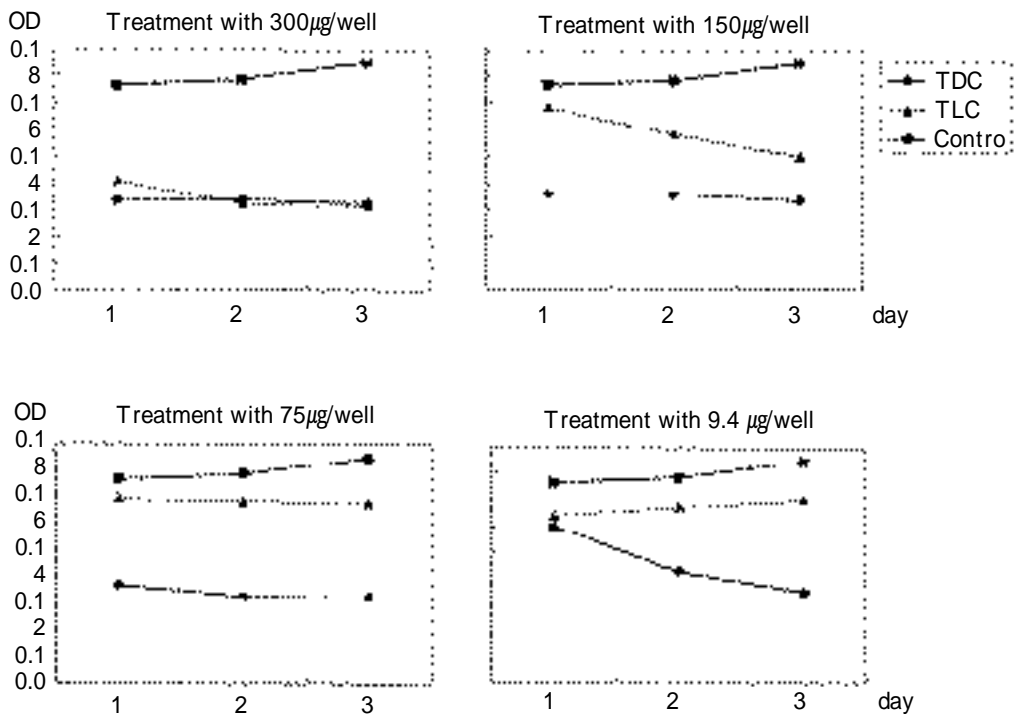


Figure 2. The effect of time on cell proliferation with concentration

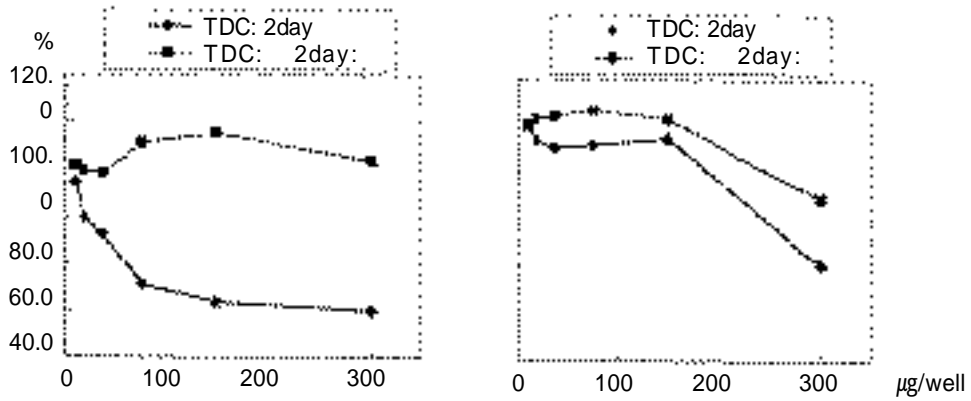


Figure 3. The effect of heat - treated TDC and TLC at 2 - day incubation

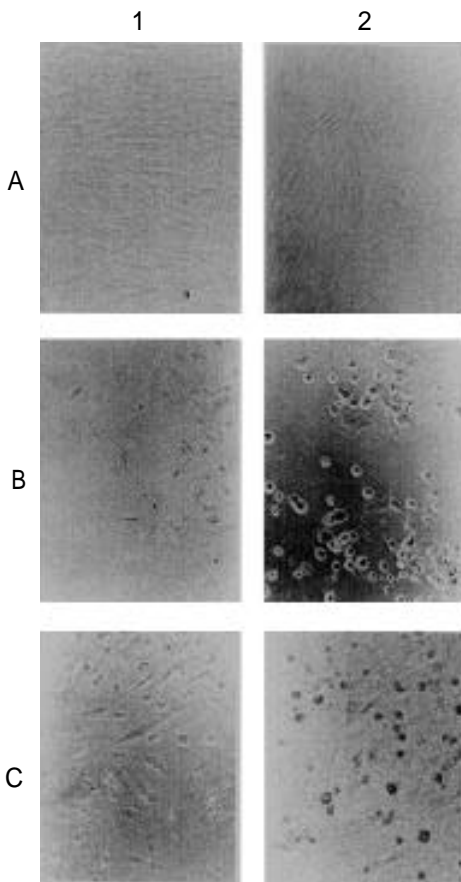


Figure 4 Cell morphology change which was observed by phase - contrast microscopy

1A: control(1day incubation), 2A: control(2day incubation), 1B: treatment with TDC(150µg/well) for 1day, 2B: treatment with TDC(150µg/well) for

alcohol: glacial acetic acid: dH₂O = 1:1:8)
 isoprophyl alcohol: glacial acetic acid: dH₂O (1:1:8) clear band

T. phagedenis T.
 denticola T. lecithinolyticum

III.

1. MTT test

(1)

2 Treponema lecithinolyticum cell sonicates(TLC) 75µg/well

가 150µg/well 가

, Treponema denticola cell sonicates(TDC) 9.4 µg/well

가 . 3

2

(Figure 1).

(2)

sonicated bacterial extracts(SBEs)
 Porphyromonas gingi -
 valis Fusobacterium
 , Porphyromonas gingivalis
 가

cytokine
 가 Gram(-)
 lipopolysaccharide
 (LPS)가

가
 , SBEs
 (non -
 dose dependent manner)
 TDC TLC

protease
 TDC
 .
 34,40,56,60) SBEs

Steenbergen Porphyromonas gingivalis
 SBEs

60), Stevens Hammond⁵⁶⁾
 Fusobacterium nucleatum SBEs

, Pissiotis Spanberg⁴⁰⁾
 Porphyromonas gingivalis SBEs

Porphyromonas intermedia SBEs

가
 TDC TLC SBEs
 가

가

Lactate dehydrogenase test TLC,
 TDC 가

가
 Zymography TDC TLC가

matrix metalloproteinase(MMP)

MMP matirixin collagenase

,
 . MMP
 interstitial
 collagenase(MMP - 1, - 8, - 13), gelati -
 nase(MMP - 2 - 9 type collagenase

), stromelysin(MMP - 3, - 10, -
 11) membrane - bound group(MMP -
 14, - 15, - 16, - 17)
 m a t r i l y s i n (M M P - 7)
 metalloelastase(MMP - 12)

¹³⁾. collagenase collagen ¼ ¼

endopeptidase interstitial
 collagen (remodeling)

⁹⁾.

Type collagenase gelatinase
 가 , 72kDa gelatinase
 A(MMP - 2) mesenchymal cell
 95kDa gelatinase B(MMP - 9)

Type (basement
 membrane) . Plasmin

prometalloproteinase
 gelatinase A

progelatinase A incubation

¹³⁾.

collagenase, radiolabeled collagen oligopeptide substrates, ELISA (sensitivity), ELISA (specificity),

collagenase Birkedal - Hansen Taylor⁴⁾, Heussen Dowle Substrate - containing polyacrylamide gel 72kDa 95kDa Type collagenase¹²⁾,

collagenase interleukin 1 collagen zymography, interleukin 1 collagenase

collagenase 2.7 collagen zymography collagenase⁹⁾,

Treponema phagedenis TLC, TDC zymography TLC TDC collagenase 72kDa progelatinase A가

TLC TDC Type IV progelatinase A TLC TDC가 가 가 가

V.

Treponema denticola Treponema lecithinolyticum

MTT test

LDH test gelatin

zymography

1.

TLC (150µg/well) 가 , TDC (9.4µg/well) 가

2.

TLC 2 150 µg/well 가 , TDC 9.4µg/well 2 가

3.

TDC

4.

TLC

5.

LDH test

6. Zymography

TDC TLC 72kDa progelatinase A가

TLC TDC

Type IV

progelatinase A

VI.

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Treponema Denticola Treponema Lecithinolyticum

spirochetes
Treponema denticola(TDC) 가
Treponema
lecithinolyticum(TLC)
spirochetes
MTT test
LDH(lactate dehydrogenase) test
gelatinase

TLC 가
4.
LDH test 가
6. Zymography
TDC TLC
72kDa progelatinase A가
TLC TDC
Type IV
progelatinase A

1. TLC
(150µg/well) 가 , TDC
(9.4µg/well) 가
2. TLC 2 150
µg/well 가 , TDC 9.4µg/well
2 가
3. TDC

: Treponema denticola,
Treponema lecithinolyticum, MTT test,
, gelatinase A
- Abstract -

The Effect of Treponema Denticola and Treponema Lecithinolyticum on Peri - odontal Ligament Cells

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This study was investigated to observe the effect of *Treponema denticola*(TDC) and *Treponema lecithinolyticum*(TLC) on cultured human periodontal ligament cells. Several experiments were performed including MTT test for the inhibition effect of cell proliferation, LDH test for the cytotoxicity , gelatin zymography for the gelatinase activation and observation of cell morphology change using the phase - contrast microscopy. The results were as follows.

1. The effect of concentration on cell proliferation with time showed an inhibitory effect at high concentration(150 μ g/well) for TLC and at low concentration(9.4 μ g/well) for TDC.
2. The effect of time on cell proliferation with concentration showed an inhibitory effect at 150 μ g/well on 2 - day incubation for TLC and at 9.4 μ g /well on 2 - day incubation for TDC.
3. The effect of heat - treated TDC and TLC on the inhibition of cell proliferation showed the difference in the heat - treated group compared to the non - heat treated group for TDC, whereas no difference was found for

TLC.

4. The morphological changes which were observed from the phase - contrast microscopy showed the difference in the test group compared to the control group. The loss of spindle - like appearance, cell - to - cell detachment and inhibition of cell proliferation were observed.
5. There was no difference of the cytotoxicity effect between the test group and the control group in the LDH test.
6. The active form of progelatinase A with molecular weight 72kDa was activated in both TDC and TLC on the gelatin zymography.

Regarding to the above results, TDC and TLC have an effect on periodontal ligament cells by playing an inhibitory role in cell proliferation and appears to activate progelatinase A which degrades type IV collagen.

Key words : *Treponema denticola*(TDC), *Treponema lecithinolyticum* (TLC), MTT test, inhibition effect of cell proliferation, gelatinase A