



6).

## II.

가

1.

5' - cyclic AMP prostaglandin

E2

가

00

7 - 13).

3

osteonectin, bone - gla -

3

1/3

protein

가

Hank's Balanced Salt Solution(HBSS, Gibco BRL, Grand Island, NY )

14).

1 - 2mm<sup>3</sup>

60mm

(Falcon, Roskilde, Denmark)

Duarte <sup>15)</sup> S100 (S100

calcium - binding protein family)

S100A4 S100A2가 in -

situ hybridization

10% fetal bovine serum(FBS) Dulbecco's Modified

Eagles Medium(DMEM, Gibco BRL, Grand Island, NY)

5% CO<sub>2</sub>, 37 , 100%

humidity

가

3

, Horiuchi <sup>16)</sup>

2.

total RNA

MC3T3E1

poly(A)+RNA

OSF - 2

total RNA

SV

periostin

total RNA Isolation System(Promega, Madison, USA)

S100A4 S100A2

(S100 calcium - binding protein family)

periostin(OSF2) RT - PCR

Northern

, total RNA spectrophotometer(Amersham, Amersham, UK)

260nm 280nm

total RNA oligo(dT) -

latex(Invitrogen)

poly(A)+ RNA

Table 1, Oligonucleotide primers used in the PCR amplification of human S100A4, S100A2, periostin, and G3PDH.

Gene(expected size)	Primer sequence
Periostin(548bp)	5' primer 5' - AA ACTCTCTATCCAGCAGA - 3' primer 3' - AGCAGTCTTTTAAATTTCTTC -
S100A2 (278bp) GACAAGTTCAAGC - 3' CA - 3'	5' primer 5' - AAGAGGGC - 3' primer 5' - GAATGTTGCAGGAAACAGC -

S100A4 (295bp)  
Foster city, CA, USA) sequencing sequence tag blast search program(NIH) database  
S100A4, S100A2, periostin G3PDH

### 3. Reverse transcription(RT) - PCR sequence analysis

total RNA poly(A)<sup>+</sup> RNA 1µg 25 U  
AMV reverse transcriptase(Promega, Madison, USA) oligo - d(T) primer  
first - strand cDNA  
Calcium binding protein - S100A4, calcium binding protein - S100A2, periostin(OSF - 2, osteoblast - specific factor 2) G3PDH  
blast search program(NIH)  
sense antisense oligonucleotide primer (Table. 1).

Reverse transcription(RT)  
cDNA template primer  
94 : 4 min, 36 cycle(94 : 1 min, 55 : 30 sec, 72 : 2 min), 72 : 5 min  
PCR (PCR cycler, MJ research)  
1.5% agarose gel

T/A cloning vec - III.  
tor(Invitrogen) subcloning  
ABI automatic sequencer(Perkin - Elmer,

northern probe

### 4. northern

total RNA 15µg 0.8% agarose gel  
nylon membrane(Hybond N, Amersham) blotting  
S100A4, S100A2, periostin G3PDH  
probe [ - P<sup>32</sup>] - dCTP random priming kit(Amersham) labelling 30% formamide, 4X SSC, 1X Denhart's solution SDS7  
hybridization 42 , 16 - 20  
incubation 2X SSC, 0.1% SDS  
1 - 2 X - ray film(Kodak)

5 - 10

1. RT - PCR

S100A4, S100A2

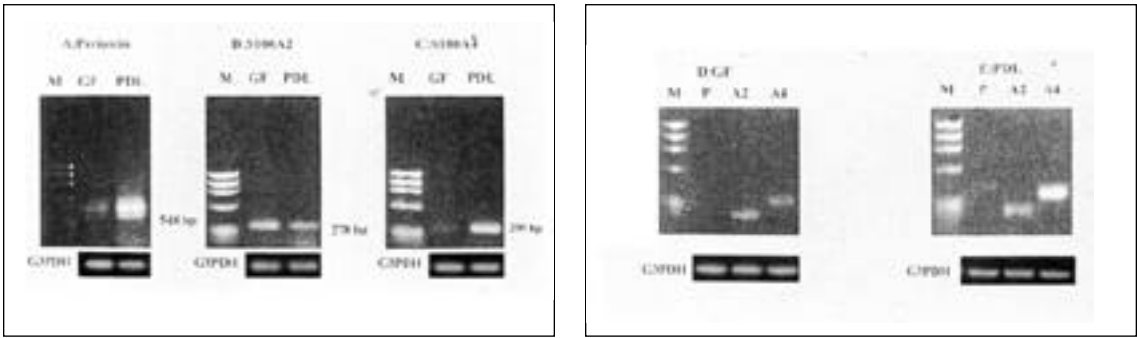


Figure 1. Expression of human periostin(A), S100A2(B), and S100A4(C) mRNAs in periodontal ligament fibroblasts and gingival fibroblasts analysed by RT - PCR(Left). Expression of periostin, S100A2, and S100A4 in periodontal ligament fibroblasts(D) and gingival fibroblasts(E) analysed by RT - PCR(Right). G3PDH: human glyceraldehyde - 3 - phosphate dehydroge - nase and was used as a positive control.

periostin mRNA

G3PDH  
(Figure 1A, 1B, 1C, 1D, 1E).

RT - PCR  
periostin mRNA  
periostin(OSF2)

S100A4, S100A2

2. RT - PCR  
periostin

RT - PCR  
sub - cloning  
sequencing  
sequence tag  
blast search program(NIH)  
database  
S100A4, S100A2,  
periostin G3PDH

S100A4

S100A4, S100A2, periostin  
G3PDH  
(Figure 2A, 2B, 2C, 2D).

(Figure 1C). RNA

3. Northern  
S100A4,  
S100A2 periostin mRNA

S100A4, S100A2  
periostin

Periostin(OSF2) 3,200bp

periostin, S100A2, S100A4 가  
(Figure 1D, 1E). (Figure 3A), S100A2



emb:Y07755.1|HSS100A2 Homo sapiens S100A2 gene, exon 1-3  
Length = 2670

Score = 551 bits (278), Expect = e-156  
Identities = 278/278 (100%)  
Strand = Plus / Plus

```
Query: 1   aagaggcgacacggttcaggctgagtaggaggaactgaaggacctctgcacaggagc 60
          |||.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Sbjct: 6385  aagaggcgacacggttcaggctgagtaggaggaactgaaggacctctgcacaggagc 6444

Query: 61   tggccagctttgtagggatgagtaggcacagacctgtagggagagctctggtagagtag 120
          |||.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Sbjct: 6445  tggccagctttgtagggatgagtaggcacagacctgtagggagagctctggtagagtag 6504

Query: 121  ggagtagcaggttaaatctctcccagctccaggtagctgtagatgcaggtgccaggatg 180
          |||.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Sbjct: 6505  ggagtagcaggttaaatctctcccagctccaggtagctgtagatgcaggtgccaggatg 6564

Query: 181  gggcccagccctcccactttagcttcatagctcacttaggagtagaatgagggccagag 240
          |||.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Sbjct: 6565  gggcccagccctcccactttagcttcatagctcacttaggagtagaatgagggccagag 6624

Query 241  tagagtagcttaattaatggaggttctcgaacattc 278
          |||.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
Sbjct 6625  tagagtagcttaattaatggaggttctcgaacattc 6682
```

Figure 2B. Identification of human S100A2. Results from a BLAST search indicating the homology between the nucleotide sequence of the cDNA obtained from RT-PCR to that of human S100A2(emb/Y07755.1/HSS100A2).

calcium - binding protein family)  
osteoclast specific  
, 1980 factor - 2(OSF - 2, periostin)  
17 - 23),  
, 1990  
epidermal growth  
factor(EGF) binding site,  
XII S100 (S100

>ref|NM\_002961.2| Homo sapiens S100 calcium-binding protein A4 (calium protein, calyasin, metastasin, murine placental homology) (S100A4), transcript variant 1, mRNA  
 Length = 512

Score = 577 bits (291). Expect = e-162  
 Identities = 254/268 (95%)  
 Strand = Plus / Plus

```

Query: 1   ctccagcgcctctctctcttcttgggtttgatctctgactgctgctccctggcgtgcccctctcagga 60
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Subject: 29  ctccagcgcctctctctcttcttgggtttgatctctgactgctgctccctggcgtgcccctctcagga 60

Query: 61   aggcctctggatctgatgctgctcccccctcccaagtaacagggaaggggggggggggggggggg 120
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Subject: 89   aggcctctggatctgatgctgctcccccctcccaagtaacagggaaggggggggggggggggggg 120

Query: 121  tcaagctcacccaagtcagacatcaagagagctgctgaccaggggggggggggggggggggggggg 180
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Subject: 149  tcaagctcacccaagtcagacatcaagagagctgctgaccaggggggggggggggggggggggggg 180

Query: 181  ggaaataggacagatgaaagctgcttccagaagcctgatgagcaacttggaccaggaacaggggg 240
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Subject: 209  ggaaataggacagatgaaagctgcttccagaagcctgatgagcaacttggaccaggaacaggggg 240

Query: 241  acaaacaggggggggggggggggggggggggggggggggggggggggggggggggggggggggggg 300
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Subject: 259  acaaacaggggggggggggggggggggggggggggggggggggggggggggggggggggggggggg 300
  
```

Figure 2C. Identification of human S100A4. Results from a BLAST search indicating the homology between the nucleotide sequence of the cDNA obtained from RT-PCR to that of human S100A4(ref/NM - 002961.2).

	MC3T3 - E1	NIH3T3	cDNA
			subtraction hybridization
			differential screening
	4	(transcripts)	가
	90 kDa		
		24,25),	Horiuchi 16)
		periostin	,
system	TGF -		(periosteal
	expansion)		
	(attachment of osteoblast precursors in		
Osteoblast - specific factor 2(OSF - 2)	the periosteum)		

```

sqh1322642.1:NR033950: Human glyceraldehyde 3-phosphate dehydrogenase mRNA
Length = 1234

Score = 872 bits (440), Expect = 0.0
Identities = 448/452 (99%)
Strand = Plus / Plus

Query: 1   accacagtcacatgcaatcaactgcaacacccagagaaactgtcgatggccctccagagaaactg 66
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 527 accacagtcacatgcaatcaactgcaacacccagagaaactgtcgatggccctccagagaaactg 66

Query: 51   tggcctggatccaccgagggactccagacatcatccctgctctcactggcgtggccag 132
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 587 tggcctggatccaccgagggactccagacatcatccctgctctcactggcgtggccag 132

Query: 131  gatgtggcacaagtctctccctgagctgaaccggaaggctcactggcctggcctccgtgctc 182
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 647 gatgtggcacaagtctctccctgagctgaaccggaaggctcactggcctggcctccgtgctc 182

Query: 161  ctcaactgcccactatggtcagttggtggaaccggacctggcgtctagaaaacctggcaactat 232
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 707 ctcaactgcccactatggtcagttggtggaaccggacctggcgtctagaaaacctggcaactat 232

Query: 231  gatgacatcaggagaggtggtgaaaccagaaatcggaaaggcccccgaagggaacccctggac 302
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 777 gatgacatcaggagaggtggtgaaaccagaaatcggaaaggcccccgaagggaacccctggac 302

Query: 331  tacactgagcaacaggtagttctctctgactcctaacggagccacacactcctccacctt 360
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 827 tacactgagcaacaggtagttctctctgactcctaacggagccacacactcctccacctt 360

Query: 361  gaggtcagggctggcaatggcccaaggaccacatctgtcaagctcatctctctgcatggac 422
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 857 gaggtcagggctggcaatggcccaaggaccacatctgtcaagctcatctctctgcatggac 422

Query: 421  aaggaatggatccagccacaggatggaggc 452
          ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct: 917 aaggaatggatccagccacaggatggaggc 452

```

Figure 2D. Identification of human G3PDH. Results from a BLAST search indicating the homology between the nucleotide sequence of the cDNA obtained from RT - PCR to that of human G3PDH(gb:HUMG3PDC).

가 (domain) 가 -  
periostin 811 (cell - cell adhesion molecule)  
fascin



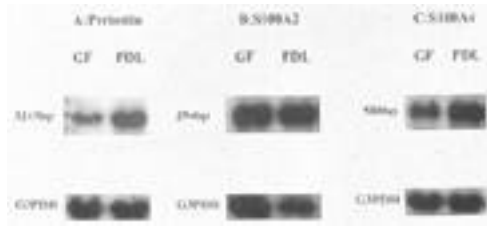


Figure 3. Northern analysis of human periostin, S100A2 and S100A4 cDNA. Total RNA(15 $\mu$ g) isolated from cultured periodontal ligament(PDL) fibroblasts and gingival fibroblasts (GF) were fractionated on a 1% formaldehyde/agarose gel and Northern blotting was carried out as described in material and methods.(A) Expression of periostin mRNA in cultured periodontal ligament fibroblasts and gingival fibroblasts. 548 bp periostin cDNA fragment from Figure 1(A) was used as a probe.(B) Expression of S100A2 mRNA in cultured periodontal ligament fibroblasts and gingival fibroblasts. 278 bp S100A2 cDNA fragment from Figure 1(B) was used as a probe.(C) Expression of S100A4 mRNA in cultured periodontal ligament fibroblasts and gingival fibroblasts. 295 bp S100A4 cDNA fragment from Figure 1(C) was used as a probe. GF, gingival fibroblast; PDL, periodontal ligament. G3PDH is human glyceralde -

TGB -

(spreading) (attachment)  
ig - h3

Periostin(OSF2) 가 RT - PCR  
Northern S100A2 S100

perioestin (osteoblast precursor cell) Duarte <sup>15)</sup> Northern S100A4

, S100A2

S100 (S100 calcium - RT - PCR Northern  
binding protein) S100A2  
EF - hand Ca<sup>2+</sup> - binding domain  
. S100 17  
A4 S100 가 , S100A4

S100A4

periostin

S100A2 S100A4가

. S100A4

가

S100

V.

26,27)

S100A4 S100A2  
periostin(OSF2)

RT - PCR Northern

가

(epithelial - mesenchymal relationship)

1. S100A2  
PCR Northern

RT -

가

2. S100A4  
PCR Northern

RT -

가

3. Periostin(OSF2)  
Northern

RT - PCR

가 가

two hybrid method

S100A4

periostin(OSF2)

## VI

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---

- Abstract -

# Expression of Periostin and S100A2 - S100A4 - Calcium Binding Proteins mRNA in Human Gingival Fibroblasts and Periodontal Ligament Fibroblasts

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Chosun University, Oral Biology Research Institute<sup>+</sup>  
College of Dentistry, Chosun University

Gingival fibroblasts (GF) and periodontal ligament fibroblasts (PDLF) are the major cellular components of periodontal soft connective tissues, but the precise molecular biological differences between these cells are not yet known. In the present study, we investigated the expression of S100A4, S100A2 calcium - binding protein and osteoblast - specific factor 2 (OSF - 2, Periostin) mRNA in GF and PDLF in vitro through the process of reverse transcription - polymerase chain reaction (RT - PCR) and Northern blot analysis in each. Human GF and PDLF were isolated from the gingival connective tissue and the middle third of freshly extracted healthy third molars. They were cultured in Dulbecco's Modified

Eagle Medium (DMEM) containing 10% fetal bovine serum and cells in the third passage were used in the experiments. After extracting total RNA from cultured cells, RT - PCR and Northern analysis were performed using S100A4 - , S100A2 - and Periostin - specific oligonucleotide primers and subcloned cDNA probes in each.

In RT - PCR and Northern analysis, the expression of S100A4 and Periostin mRNA in GF was slightly detectable. Interestingly, the expression of S100A4 and Periostin mRNA in PDLF was much higher than that in GF. On the other hand, S100A2 mRNA was highly expressed in both GF and PDLF. Since there was a marked difference of S100A4 and Periostin expression between GF and PDLF in vitro, these data suggest that S100A4 and Periostin could be used as a useful marker for distinguishing cultured gingival fibroblasts and periodontal ligament cells.

**Key words;** Periostin, S100A4, S100A2

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