The biological effects of fibronectin typeIII 7-10 to MC3T3-E1 osteoblast

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I. Introduction

Studies on the dental and orthopedic fields have revealed the biocompatibility of titanium(Ti) to achieve osseointegration. Titanium(Ti) is widely used for dental and orthopedic implants, due to its excellent biocompatibility. The initial success of implants may be dependents on rapid osteogenesis around the implant, in other words, on the early attachment of osteoblast to the implant surface[1-5].

Numerous studies have been tried to modify Ti surface mechanically to improve osseointegration. Most of the surface modifications have been aimed at achieving more bone-to-implant contact as determined histomorphometrically at the light microscopic level. Previous investigation in non-oral bone under short-term healing periods[6-10] indicated that sandblasted and acid-etched(SLA) Ti implant have a greater bone-to-implant contact than the machined surface Ti implant.[11-15] Dental fields have examined the ability of the macro and micro surface textures (e.g. fiber meshes,[16-17] sand blasted or acid etched surfaces,[18-19] plasma spray, coatings of hydroxyapatite,[20] and tricalcium phosphate[21-23]) to stimulate rapid bone formation *in vivo*[24]. To date, there are still long-term clinical prognosis in terms of degradation and delamination of ceramic coatings[25]. In addition, none of the coating technologies or surface macrotextures appears to act through biologically specific receptor-mediated mechanisms.

A more potent approach is the immobilization of biological factors onto an implant surface in order to induce a specific cellular response and promote osseointegration such as using Ti implant surface modified with growth factors and adhesion protein[26-27].

Despite the differences in the clinical success rates, it is not clear how the surfaces of implant materials promote or inhibit osteogenesis. The initial success of implants may be dependent on rapid osteogenesis around the implant, in other words, on the rapid attachment of osteoblasts to the implant surface. At present, however, there are insufficient basic data on the initial attachment of osteoblasts to implant materials. Recently other approaches

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involve treating Ti with biologically active substances such as adhesion proteins.

Fibronectin is an important matrix glycoprotein that mediates cellular activity and improves wound healing. Fibronectin is a large dimeric glycoprotein(M.W: 220,000-240,000Da). Fibronectin has certain proteolytic fragments that can promote chemotactic migration[33] and activate integrin signaling[34]. Each monomer is composed of type I, type II, and type III domains, identified as repeating amino acid motifs in the primary structure. An Arg-Gly-Asp (RGD) sequence located in the 10th type III repeating unit is a key cell-adhesive site for the cell-surface receptor integrin[35] and a Pro-His-Ser-Arg-Asn (PHSRN) sequence in the 9th type III repeating unit has been shown to act synergistically with RGD in cell adhesion mediated by integrin $\alpha 5\beta 1$ and $\alpha V\beta 3$[36].

One of the important properties of FN is its extremely wide distribution, which clearly suggests that they perform rather important functions. Intriguing feature is that fibronectin occur in two distinctly different situations; firstly, as a soluble glycoprotein in various body fluids or secreted by cells in culture and secondly, as an insoluble constituents of extracellular matrices and basement membranes[28].

It interacts with a variety of macromolecules including components of the cytoskeleton and the extracellular matrix, circulating components of the blood clotting, fibrinolytic, acute phase and complement systems, and with cell-surface receptors on a variety of cells including fibroblasts, neurons, phagocytes and bacteria[29]. To achieve optimal periodontal regeneration, many studies were performed on treating human tooth roots with plasma fibronectin during periodontal surgery. The application of fibronectin onto roots appears to enhance connective tissue attachment[30-32].

Fibronectin provides a crucial substrate for many forms of cell migration, such as in embryonic migratory pathways and in the provisional matrix of healing wounds. Among the many active sites in extracellular matrix proteins, the cell-adhesive domain of fibronectin has been well studied. We have chosen a fragment of human fibronectin encompassing the 7th through the RGD-containing 10th type III repeats.

The structure reveals an extended rod like molecule with a long axis of 140 Å and highly variable relationships between adjacent domains. An unusually small rotation between domains 9-10 creates a distinctive binding site, in which the RGD loop from domain 10 and synergy region from domain 9 are on the same face of FNIII 7-10 and thus easily accessible to a single integrin molecule. The cell binding RGD loop is well ordered in this structure and extends 10? away from the FNIII 7-10 core[37-38].

At the site of integrin-ligand interaction, integrin receptors cluster and several intracellular signaling molecules were recruited. Incremental extensions of the interdomain link between FNIII 9 and FNIII 10 reduced the initial cell attachment, but had a more pronounced effect on the downstream cell adhesion events of spreading and phosphorylation of focal adhesion kinase. Focal contacts cap the membrane-proximal ends of extensive actin filament networks(called stress fibers *in vitro*), enabling the cell to spread and eventually enter the cell mitotic cycle and proliferate. The activation of these two intracellular molecules is indicators of "outside-in" signal occurring between the extracellular matrix and the cell via integrin-mediated events. Widespread tyrosine phosphorylation is an important event following formation of focal contacts and is mediated by several kinases including focal adhesion kinase (FAK)[39-40]. The extent of disruption of cell adhesion depended upon the length of the interdomain linker.

Using gold-thiol reactions, thiol compounds that contain bioactive moieties (terminal cysteine) can be

chemisorbed to gold films to provide, in principle, correspondingly bioactive surfaces. For example, chemisorbed layers form spontaneously upon immersion of gold substrates in thiolate containing solutions. Depending on the structure of the thiolate, surfaces of well defined character and properties can be prepared.

The chemisorption[41] of organosulfur compounds onto metallic gold is powerful method for the preparation of well-defined surface. A cysteine on 7th segment in FNIII 7-10 added to custom made gold-coated Ti surfaces to form bioactive substrates. One advantage of this chemisorption method is that it can be readily applied to a range of biomaterial surfaces, including biometal and polymer[42].

The purpose of this study was to investigate on the biological effects of fibronectin typeIII 7-10 on MC3T3-E1 osteoblast on the modified Ti surface.

II. Materials and methods

1. Ti disks

The Ti disks(6mm in diameter and 2mm in thickness) used in this study were purchased from Avana(Seoul, Korea). Disks were cleaned according to American Standard Testing and Material (ASTM) specification F-86. Rods were degreased in acetone and ethanol and passivated in nitric acid.

2. Preparation of fibronectin

Native plasma Fibronectin was used (American Peptide Company, Sunnyvale, CA) and FNIII 7-10 was expressed by using recombinant DNA technology as following procedures.

Fibronectin cDNAs were amplified from adult human brain cDNAs library. Polymerase chain reaction(PCR) primers were designed to recognize FN type III repeats 7th to 10th as follows.

Forward: 5' -AGCCATATGGCTGTTCCTCCTCC-CACTGACCTG-3'

Reverse: 5' -TCGGATCCTAAGTTCGGTAAT-TAAT-3'

PCR was performed in a 50㎕ reaction containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl 100 u/ml gelatin, 0.2 mM dNTPs, 1.25 units of *Taq* polymerase (Perkin-Elmer, Boston, MA), and 50 pmol of each upstream and downstream primers. The thermocycling parameters used in PCR were as follows: annealing, 1 min at 55°C; extension, 2 min at 72°C; denaturation, 1 min at 94°C.

After 30 cycles, amplified cDNA products were digested with *Nde*I and *Bam*HI and separated using PCR purification kit (Qiagen, Chatsworth, CA). Nucleotide sequences were determined for inserts by using dideoxy terminator cycle sequencing (Applied Biosystems, Foster City, CA), and compared with those in GeneBank database by the BLAST program of NCBI (National Center for Biotechnology Information, Bethesda, MD).

The FNIII 7-10 fusion proteins containing poly histidine tag were expressed and purified using a Ni^{2+} affinity column under denaturing conditions according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). The cell lysates and purified fusion proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and visualized by Coomassie Blue staining.

3. Preparation of surface modified Titanium

In this experiment, Smooth surface (SS) is negative control, SLA is positive control. Experimental group is FN and FNIII7-10 coated Ti disks. Oxalic acid attack modification created numerous secondary micropores (2.0㎛)

on the basis of sandblasted surface macrotexture. This modified sandblasting surface treatment is feasible and reliable to apply to dental implants and does not decrease the biocompatibility on Ti.

Gold-coated Ti disks were custom fabricated. Cleaned Ti disks were first coated with a 20 Ålayer of pure Ti-oxide to increase gold adhesion and then with a 80 Å layer of gold using an electron beam evaporator (Model:NRC3177, Newton, MA).

Disks were immersed in a 3.5㎍/Ml solution (1:1 ethanol:water) of FN overnight at room temperature in closed containers with gentle mixing. All implants were sterilized in 70% ethanol and stored for 1 day in sterile PBS prior to cell seeding.

4. Cell cultures and in vitro experiments

1) Cell culture and seeding into Ti disks

MC3T3-E1 osteoblasts (Riken, Japan), derived from mouse calvaria, were maintained in Dulbecco's modified Eagle's media (DMEM, Life Technologies Inc., Rockville, MD) supplemented with 10% bovine serum (FBS, Life Technologies Inc.). 4,000cells/20㎕ to permit individual cells grown separately to avoid cell-to-cell contact. When confluent monolayer was reached, the cells were enzymatically lifted from the dishes using 0.25% trypsin in 4mM EDTA.

Aliquots of 20㎕ of cell suspension were seeded on the top of the Ti disks which are placed in the wells of 96-well plates. The matrices were left undisturbed in an incubator for 3 hours to allow the cells to attach to the matrices, after which, an additional 1 ㎖ of complete media was added to each well. Cultures were maintained in a humidified atmosphere consisting of 95% air and 5% CO_2 at 37°C. The media was changed every 2-3days.

2) Cell proliferation and cell morphology

Cell proliferation was measured at 4 hr, 1, 3, 7, and 14 days. At each time point, media was removed from the wells. The Ti disks were washed gently with Hank's balanced salt solution (HBSS) (Gibco, NY) to remove any unattached cells.

The adherent cells were removed from the sample by incubation in 0.5㎖ of 0.25% trypsin in 4mM EDTA for 10 minutes at 37°C and were counted by the hemocytometer. After counting, the cells in the media were centrifuged at 1260 rpm, 4.0°C, and for 10 minutes. The supernatant was suctioned away and the cell pellet was prepared for alkaline phosphatase activity test.

Cell morphology was examined at 4hr, 1,3, 7 and 14 days with scanning electron microscope.(JSM 840A, Jeol co. Japan).

3) Alkaline phosphatase activity

Production of alkaline phosphatase (ALPase) was measured spectroscopically at 3, 7, and 14 days. This test was done with the same cells used for the cell proliferation test. Removed cells from the matrices were homogenized with 0.5㎖ of double distilled water and sonicated for 1 minute in ice. 0.1㎖ of cell lysates were mixed with 0.1㎖ of 0.1M glycin-NaOH buffer, 0.1㎖ of 15mM paranitrophenol phosphate(PNPP), 0.1% triton X-100/saline and 0.1㎖ of DDW. Each aliquots was incubated at 37°C for 30 minutes. After incubation, each tube was added 2.5 ㎖ of 0.1N NaOH and placed on ice.

The production of para-nitrophenol (PNP) in the presence of ALPase was measured by monitoring light absorbance by the solution at 405㎚. The slope of absorbance versus time plot was used to calculate the ALPase activity.

4) Cell immunocytochemistry

Cytoskeletal organization in adherent, cultured osteoblast was observed as an indication of the relative degrees of cell spreading on various Ti disks.

Staining of filamentous actin with rhodamine phalloidin(Molecular Probes, Eugene, OR) was used to monitor stress fiber formation via fluorescence microscopy. In order to quantify the formation of focal adhesions, FAK, which are involved in the cytoplasmic domain of the adhesion plaque, were immunostained. Cells were plated onto the samples at 25,000cells/*ml* and incubated for 4 and 24 hours.

Then the cells were rinsed in PBS, fixed with 3.7% formaldehyde for 10min, and washed with PBS again. The fixed cells were permeabilized in 0.5% (V/V) Triton X-100 for 5min, washed three times in PBS.

After three additional washes in PBS, the cells were blocked with 1% bovine serum albumin (Nacalai Tesque, Inc.). Immunofluorescence staining for FAK was performed with rabbit anti-human FAK polyclonal antibody (p125fak, 1:40 dilution,

Upstate Biotechnology, NY) for 2 hours at 37°C, followed by incubation with FITC-conjugated sheep anti-mouse antibody (1:40 dilution, Sigma). For controls, the primary anitsera were replaced with nonimmune rabbit or mouse serum, respectively. Samples were examined with an fluorescence microscopy (Olympus, Japan).

5) Statistical analysis

Analysis of differences were performed with one-way and two-way ANOVA with Fisher LSD test and Turkey HSD (honestly significant difference) were performed, with values of $p < 0.05$ considered statistically significant using SAS program(SAS Institute Inc., Cary, NC)

III. Results

1. Cell proliferation and ALP activity

MC3T3-E1 cells were plated on smooth surface(SS), sandblasted and acid etched(SLA), native plasma fibronectin(FN) and fibronectin type III 7-10(FNIII 7-10). At 4 hr and 1 day, there is no difference in cell proliferation among groups, however, as time goes by(3, 7 and 14 days), there was significant difference between smooth surface(SS) and two FN treated groups($p<0.05$). There was also statistically significant difference between SLA and FNIII 7-10 groups($p<0.05$). In FNIII 7-10 group, cell proliferation was highest at each time point, but there was no statistically significant difference between FN and FNIII 7-10 groups(Table 1, Fig. 1).

2. Cell morphology study using SEM

At 4 hours, osteoblasts on smooth surface were not well spread on the substrate with short and thin process, while cells on SLA, FN and FNIII 7-10 were

Table 1. Cell Proliferation test by hemocytometry (X 10^3 cell count)

Time	SS(Control)	SLA	FN	FNIII 7-10
4Hrs	1.38±0.14	1.75±0.20	1.42±0.29	1.67±0.38
1 Day	3.13±0.43	4.81±0.63	4.25±0.50	4.33±0.63
3 Days	6.25±0.65	7.88±1.18	10.83±1.04*	15.00±0.50*†
7 Days	42.94±7.51	59.06±6.40	61.25±2.84*	83.75±12.05*†
14 Days	133.00±6.27	51.75±3.95	90.67±6.03*	218.33±7.64*†

Data represent mean±S.E. (N=6-7)

SS ; smooth surface, SLA ; sandblasted and acid etched, FN ; native plasma fibronectin, FNIII 7-10 ; fibronectin type III 7-10

* Significantly different from SS group at 3, 7, 14 days ($p<0.05$)

† Significantly different from SLA group at 3, 7, 14 days ($p<0.05$)

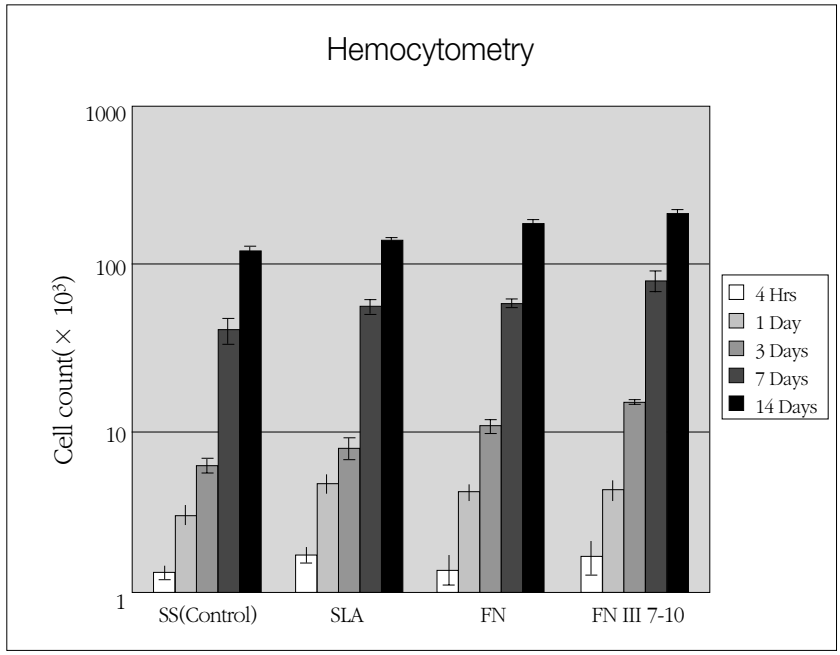

Figure 1. Cell Proliferation test by hemocytometry (X 10^3 cell count)

SS ; smooth surface, SLA ; sandblasted and acid etched, FN ; native plasma fibronectin, FNIII 7-10 ; fibronectin type III 7-10

Table 2. ALPase activity (ρM/ μg/30Min)

Days	SS(Control)	SLA	FN	FNIII 7-10
3	9.67±5.54	20.08±7.00	27.76±5.33*	33.72±7.73*†
7	19.80±7.29	32.59±8.26	33.9±6.03*	35.42±2.08*†
14	32.94±4.28	32.91±6.78	44.43±3.84*	63.8±10.7*†

Data represent mean±S.E. (N=6-7)

SS ; smooth surface, SLA ; sandblasted and acid etched, FN ; native plasma fibronectin, FNIII 7-10 ; fibronectin type III 7-10

* Significantly different from SS group at 3, 7, 14 days ($p<0.05$)

† Significantly different from SLA group at 3, 7, 14 days ($p<0.05$)

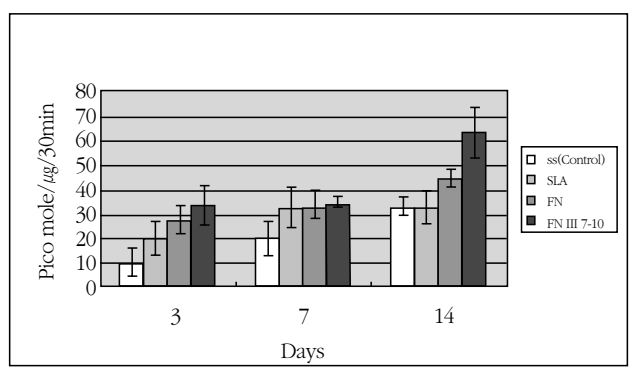

Figure 2. ALPase activity

SS ; smooth surface, SLA ; sandblasted and acid etched, FN ; native plasma fibronectin, FNIII 7-10 ; fibronectin type III 7-10

well attached and spread with long cytoplasmic process. Especially cells on SLA spreaded in spike-like fashion compare to other groups(Fig. 3-6).

At 1 day, attached cells on SS, FN and FNIII 7-10 disks were well spread similarly with developed process and cells on on FN and FN III 7-10 maintained their flattened and elongated pattern. Cells on SLA disks also maintained their spike-like fashion at 1 day (Fig. 7-10). At 3 days, the number of cells on disks was significantly increased in all groups. Proliferated cells on each disks were grown in a concentric circle pattern due to the turned line on the disk which was occurred during milling(Fig. 11-14). The confluent cells on SLA had many pieces of cracks and it was assumed that underlying rough surface affected attached cells during sample preparation for SEM(Fig. 12). At 7 and 14 days, all samples had a similar cell proliferation pattern(data are not shown).

3. Cell immunocytochemistry

1) Actin staining

Cytoskeletal organization in adherent osteoblast was observed as an indication of the relative degrees of cell spreading with rhodamine phalloidin. 4 hours after cell plating, cells were already spread on the disks, accordingly bundles of actin fibers forming stress fibers appeared in the cells on all groups (Fig. 15-22). On SLA, however, actin filaments were distributed in elongated spike-like fashion with clumps in proximal ends, which can be predicted as cytoplasmic process (Fig. 16). At 24 hours of culture, well-defined stress fibers showing regular arrangement with particular polarities were found in cells grown on SS, FN and FNIII 7-10 (Fig. 15-22).

2) Immunostaining for FAK

In the cells cultured for 4 hours on Ti disks, many immunoreactive plaques for FAK were found in the peripheral regions of the cells (Fig. 23, 25, 27, 29). Cells grown on SS and SLA showed similar immunoreactive in the periphery of the cells, although the immunoreactions for FAK were relatively stronger in the cells on FN and FNIII 7-10 disks than in those on SS and SLA.

In the cells cultured for 24 hours, increasing numbers of plaques with triangular and ovoid shapes were expressed along the periphery of spreaded cells in all samples with, however, comparing the plaque numbers between groups was somewhat difficult due to the limitation of the resolution(Fig. 24, 26, 28, 30).

IV. Discussion

Attachment, spreading, and growth of osteoblast are shown to be modulated by changing surface modification. While previous work came to similar conclusions regarding substrate-dependent cell behavior[43], substrate chemistries have rarely been consistent or controlled. These results demonstrate that molecular details in surface chemistry translate to molecular changes in adsorbed ECM, influencing subsequent cell signaling responses. These results indicate that outside-in signaling can be modulated by surface chemistry in anchorage-dependent cells.

Conversely, inhibitory peptides could also be used to block unwanted processes such as tumor cell invasion and metastasis. Conventional thought in this field has focused on the use of fibronectin or its mimetic as adhesive and migration substrates in wounds.

Such small peptide ligand: the sequence, arginine-glycine-aspartic acid or 'RGD' which has high affinity RGD peptides increase the overall adhesiveness of the surface for osteoblast[44] and enhance the rate of cell spreading compared to YIGSR containing

peptides from laminin[45]. Integrin adhesion and signaling events may contribute to the progressive differentiation of the osteoblast and to the initiation of a mineralized matrix.

Differential availability and exposure of the several conserved ECM cell binding domains, containing the RGD sequence recognized by cell surface integrin receptors, could lead to distinct differences in a material's ability to support cell attachment, spreading, and proliferation.

Exposure of this sequence was previously demonstrated to be increased upon adsorption to TCPS surfaces[46]. The data presented represent the percentage of the total 10th type III sequences found in surface-adsorbed FN exposed and recognized by the anti-FN antibody. Although no direct correlation can be made between FN binding domain availability, these results are consistent with reference for the integrin-FN surface and the observed cellular responses on these SAMs. An investigation of these outside-in signaling relationships between cell culture surfaces and RhoA activity in attached cells recently demonstrated important correlations between the cell morphological markers described in the study and the molecular signaling pathways responsible for gene regulation[47]. The clinical success of dental implants is closely associated with the properties of implant surface, which affect cell attachment as well as subsequent cell differentiation, matrix production, and mineralization.

However, cells in suspension do not possess FAK[48]. Thus, FAK is a molecule that requires the interaction of integrins with the extracellular matrix for its expression. This suggests that integrins aggregate on the cells attached on SLA or pure Ti, bind to the extracellular matrix secreted by these cells, and adsorbed onto the surface of SLA and Ti.

In addition, vitronection or fibronectin derived from serum also play an important role in the attachment between cells and materials. Actin staining by TRITIC-labeled palloidine is normally used to assess cell motility, cell spreading, and cell shape[49-52]. It is likely that the difference in migration on Ti, SLA, FN and FNIII7-10 disks reflects the difference in organization of actin. Therefore, these observations indicate that both surface chemistry and surface topography are contributing factors in migration of osteoblast. The immobilization of RGD on flat surfaces and in gels results in increased potency over its parent ligand (e.g. fibronectin) in encouraging cell adhesion [53-57] and supporting cell attachment and growth.[58-60]

V. Conclusion

In order to investigate the biologic effect of surface modified titanium with fibronectin type III 7-10 to osteoblast MC3T3-E1 cells, cell proliferation, ALPase activity, cell attachment and immunocytochemical analysis were done on cells cultured on Ti disks. The results were as follows:

1. Cell proliferation on FN and FNIII 7-10 were significantly higher than that of SS at 3, 7 and 14 days($p<0.05$). There were also significant differences between FNIII 7-10 and SLA at 3, 7 and 14days($p<0.05$).
2. ALPase activity of cultured cells on FN and FNIII 7-10 were significantly higher than that of SS at 3, 7 and 14 days($p<0.05$). There were also significant differences in ALPase activity of cultured cells between FNIII 7-10 and SLA($p<0.05$).
3. Cells were attached in flat and rhomboidal fashion in SS, FN and FNIII 7-10 at early time period and more spreading pattern with elongated cytoplasmic process was seen as time passed.
4. In immunocytochemical analysis for actin fila-

ment and FAK, cells cultured on FN and FNIII 7-10 revealed relatively well-arranged bundles and more plaques, respectively, than those of SS and SLA.

It was concluded that modified titanium surface with FN type III 7-10 fragment had favorable biologic effect on osteoblast cells as comparable as native plasma fibronectin.

VII. References

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사진부도 설명

사진부도 (I)

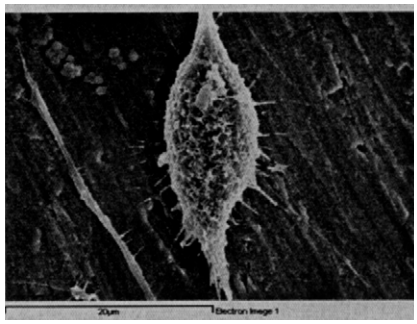
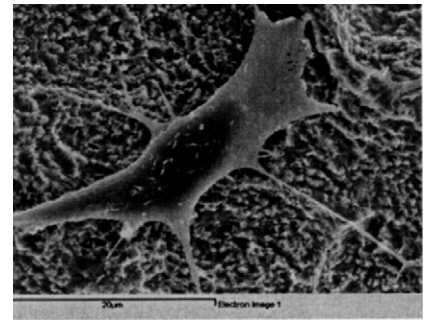


Figure 3



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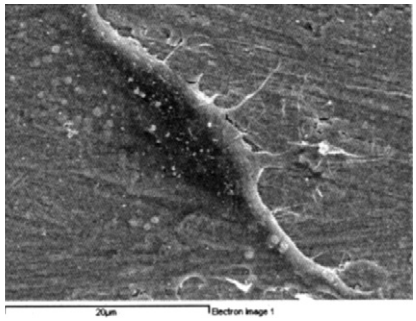

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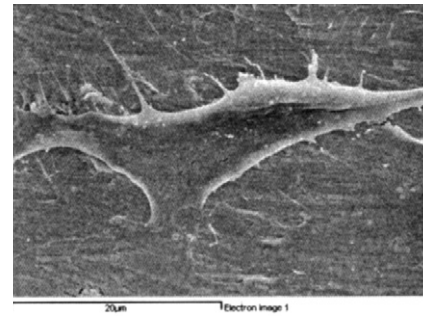

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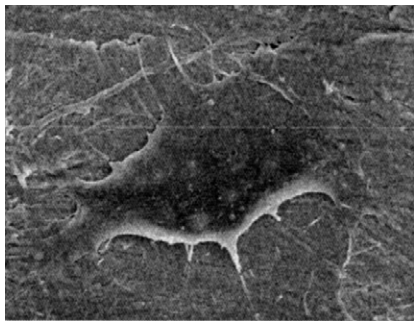

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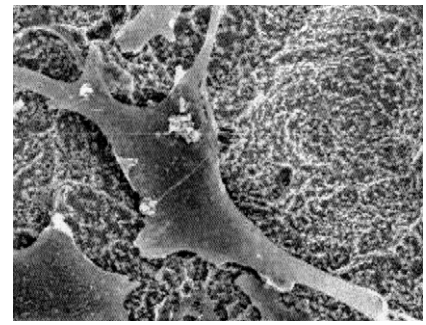

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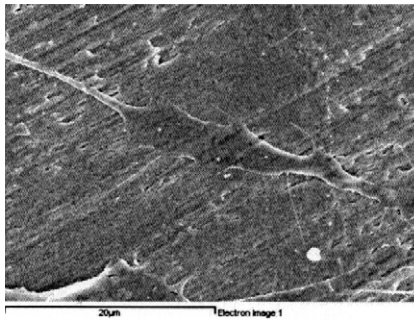

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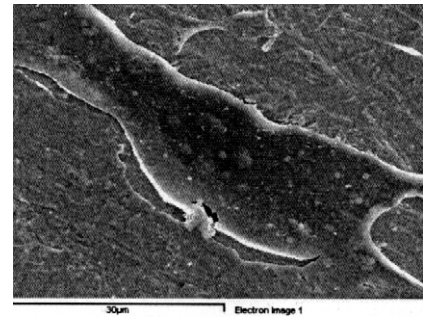
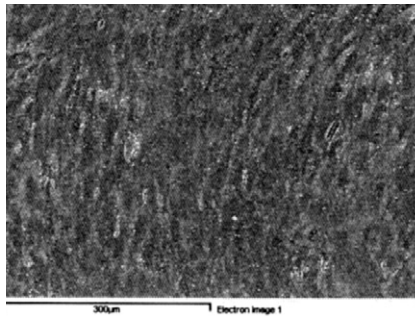


Figure 10

사진부도 (II)



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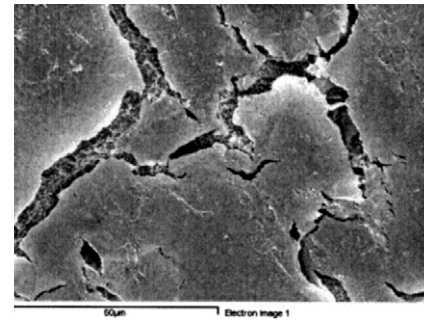

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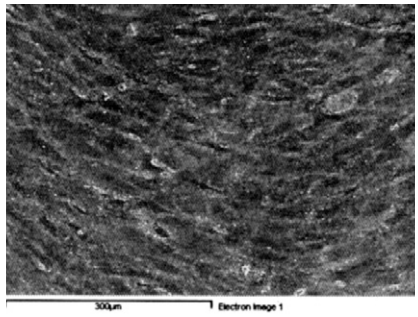

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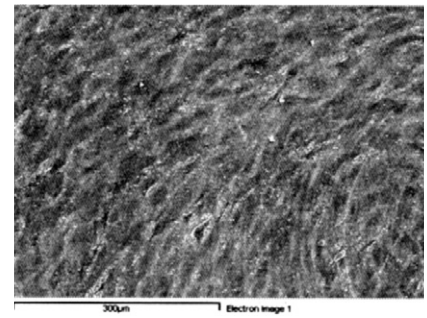

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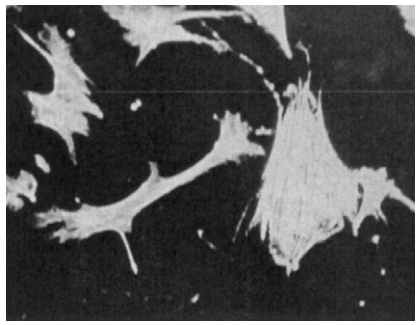

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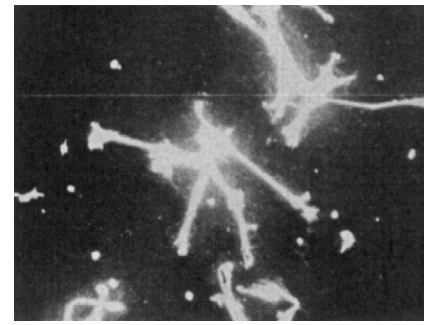

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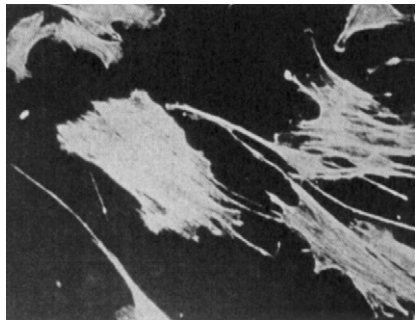

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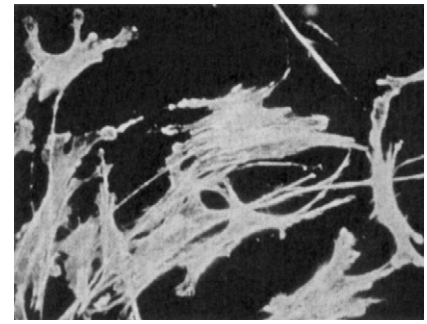

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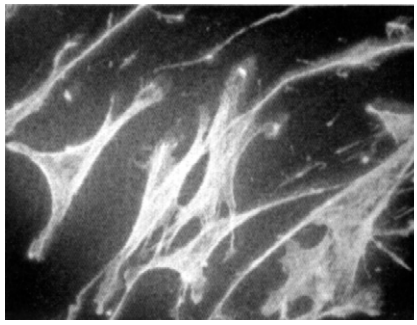

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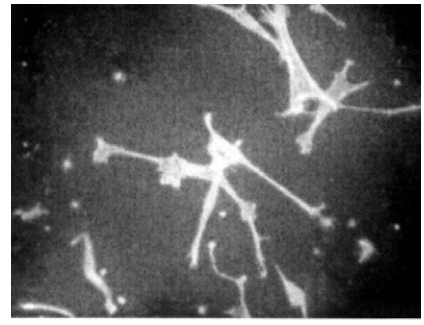

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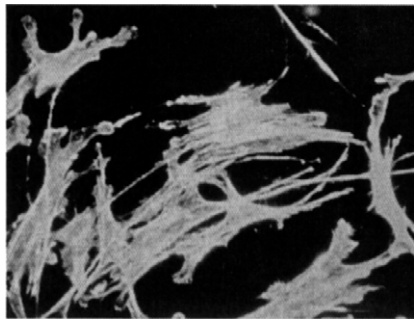

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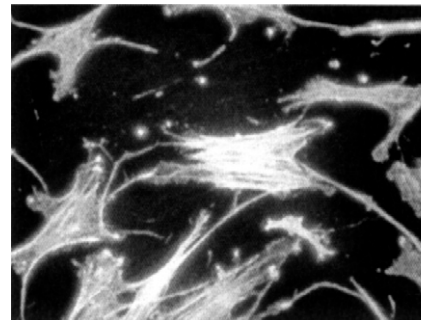

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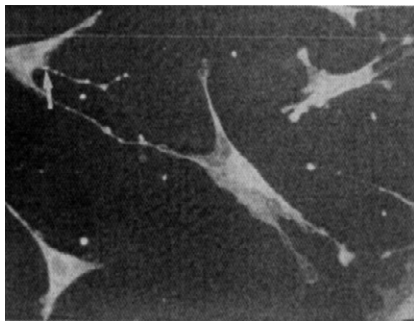

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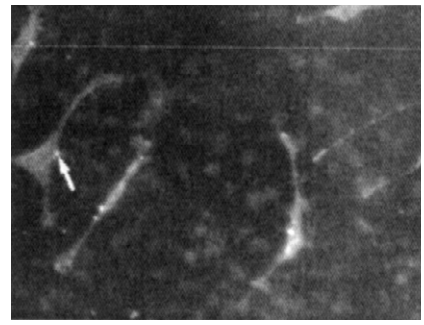

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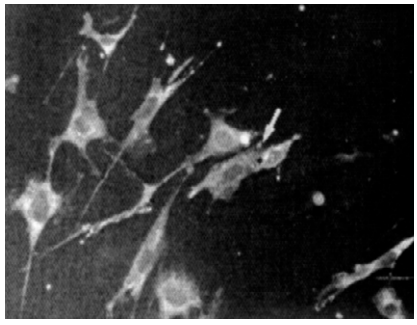


Figure 25

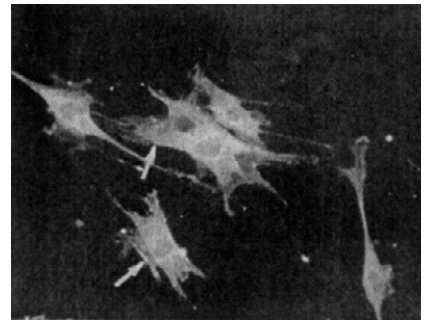
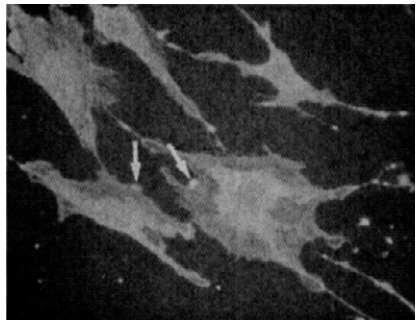


Figure 26

사진부도 (IV)



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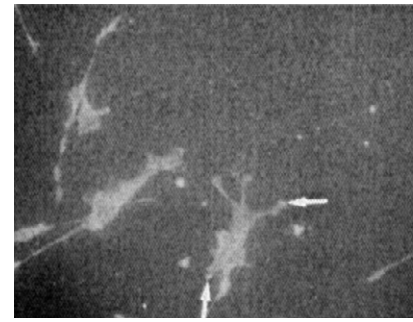

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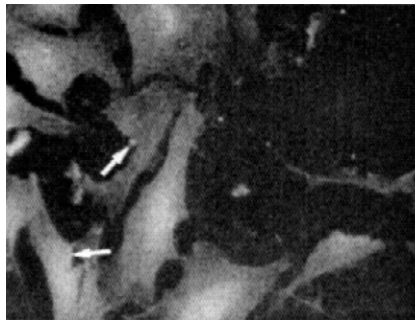

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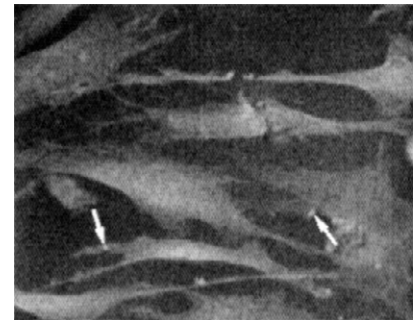

Figure 30

-국문초록-

Fibronectin type III 7-10 이 조골세포에 미치는 영향

홍정욱 · 최상묵 · 한수부 · 정종평 · 류인철, · 이용무 · 구 영

서울대학교 치과대학 치주과학교실

타이태늄은 뛰어난 생체적합성과 적절한 물리적 성질을 바탕으로 치과 및 정형외과 영역의 매식재로 널리 사용되어져 왔으며, 골과 매식재 사이의 골 융합 정도를 증가시킬 목적으로 물리, 화학적인 방법을 이용한 타이태늄의 표면처리에 관한 많은 연구들이 진행되어 왔다. 최근에는 부착단백질 또는 성장인자를 이용한 생체재료의 표면개질을 통하여 조직적합성 및 치유 능의 개선을 위한 시도들이 있어왔다. Fibronectin(FN)은 주요 세포외기질중의 하나로 생체 내 널리 분포하여 세포의 부착, 이동 및 증식에 관여하는 거대 당단백으로, RGD및 PHSRN 펩타이드 서열이 세포의 인테그린과 결합하여 세포의 활성을 조절하는 것으로 알려져 있다. 이 연구에서는 FN으로 처리된 타이태늄이 조골세포의 부착, 증식 및 분화에 미치는 영향과 이에 따른 석회화 정도에 미치는 영향을 관찰하여 부착분자를 이용한 타이태늄 표면개질의 효과를 규명하고자 하였다.

상업용 순수 타이태늄을 gold thiol법을 이용하여 표면처리 후, 혈장 FN(plasma FN, pFN)과 유전자재조합법을 이용하여 얻은 FN조각(FN type III 7-10, FNIII 7-10)을 피복한 시편을 실험군으로, 아무런 처리를 하지 않은 것(smooth surface, SS)과 산 부식(Sandblasted and acid etched, SLA)처리된것을 대조군으로 이용하였다.

배양된 조골세포주(MC3T3-E1)를 사용하여 타이태늄 표면 처리에 따른 세포의 증식, 형태변화, 알칼리성 인산분해효소(ALPase) 생산 및 세포면역형광법을 이용한 분화정도를 시간 경과에 따라 관찰하였다.

조골세포증식의 경우 FNIII 7-10 처리군에서 pFN 처리군 및 대조군에 비해 시간경과에 따라 유의성있는 세포수의 증식이 관찰되었으며($p<0.05$), ALPase 생성의 경우에도 FNIII 7-10 처리 군에서 아무 처리도 하지 않은 군에 비해 유의성 있게 높은 효소의 생성이 관찰되었다($p<0.05$). 주사전자현미경을 이용한 세포의 형태관찰결과 아무 처리도 하지 않은 군에서는 마름모형태를 나타내었으며, 산 부식 처리된 군에서는 세포가 가시모양의 형태를 보인 반면 FN으로 처리된 두 군에서는 세포의 부착 및 펴짐이 매우 발달되어 있는 모습이 관찰되었다. 세포의 분화정도를 관찰하기 위하여 국소부착키나제(focal adhesion kinase, FAK), 및 actin stress fiber의 분포양상을 세포면역형광법을 이용하여 관찰한 결과 FN으로 표면처리된 두 군에서 아무런 처리도 하지않은 군 및 산 부식처리 한 군에 비해 프라크의 발현이 높게 나타났으며 잘 발달된 actin stress fiber의 소견을 나타내었다.

이 실험의 결과들은 gold thiol 법을 이용한 표면처리 후 FN부착을 통한 타이태늄의 표면개질이 조골세포의 부착, 증식 및 분화에 중요한 역할을 담당하여 석회화 정도를 촉진시키는 것을 보여주었으며, 이런 결과들은 더 짧은 FN조각을 이용한 다른 생체재료의 표면개질에 폭 넓게 응용할 수 있으리라 생각된다.

주요어 : titanium, fibronectin, osteoblast, cell proliferation, ALP activity, cell differentiation