The Effect of Alkali- and Heat-Treated Titanium Surfaces on Differentiation of Osteoblast

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In this study, the biological response of fetal rat calvarial cells on alkali- and heat-treated titanium was assessed. The results were as follows; Cell proliferation on alkali- and heat-treated surfaces showed significantly higher level than on the titanium-6aluminum-4vanadium (weight percentage: 6 % aluminum, 4 % vanadium, Ti-6Al-4V) surface ($p<0.01$). In ELISA analysis, concentration of IL-1β and IL-6 were raised when the cells were grown to day 7. Pre-treatment with herbimycin, a known tyrosine kinase inhibitor, suppressed the production of IL-6 ($p<0.01$). In comparison to commercially pure titanium (grade II, cp-Ti) and Ti-6Al-4V alloy, alkali- and heat-treated titanium enhanced alkaline phosphatase activity ($p<0.001$). In RT-PCR analysis, alkaline phosphatase, bone sialoprotein, receptor activated nuclear factor ligand mRNA expression was increased alkali- and heat-treated titanium. Herbimycin and SB203580, p38 MAPK inhibitor, were repressed of IL-1β-induced IL-6 mRNA expression. These results suggest that alkali- and heat-treated titanium stimulate osteoblasts differentiation and facilitate bone remodeling.

Key words: alkali-treated, differentiation, heat-treated, osteoblast, titanium

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INTRODUCTION

Titanium and titanium alloys are among the most popular materials for dental and orthopedic implants due to their biocompatibility, excellent corrosion resistance, good mechanical properties[1].There have been efforts to improve the osseointegration capability of the titanium implants by enhancing osteoconduction on their surfaces using surface morphology and chemistry[2-4].

Various techniques have been used to produce microrough titanium surfaces for promoting bone ingrowth and fixation between implants and bone. Besides surface topography, surface chemistry is another key variable for peri-implant bone apposition. Kokubo et al[3] introduced an alkaline and heat treatment on titanium surfaces, which provided strong bone-bonding ability and high bone

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affinity. After alkaline and heat treatments, titanium-based metals form bone-like apatite in simulated body fluid (SBF), which has ion concentrations nearly equal to human body fluid[4]. This phenomenon also occurs on the surfaces of bioactive glass and glass ceramics.

Osteoblasts are established as cells who respond to their substrate and rely heavily on signals induced to continue with an osteoblastic phenotype. If insufficient signals are provided by the substrate then a fibroblast phenotype is induced[5]. Other inflammatory mediators also regulate cellular activity, as do other molecules such as osteoprotegerin (OPG) or RANKL[6].

There had been reported that titanium prepared by alkali treatment could form bone-like apatite when it was soaked in SBF *in vitro* and had strong bone-bonding ability and high bone affinity *in vivo*[3, 7-10]. Considering these features of alkali- and heat-treated titanium, alkali- and heat-treated titanium implant is a useful candidate for the preparation of medical device.

Cytokines are a group of communicating molecules, originally identified in the leukocyte population, that are produced by both macrophages and osteoblasts. These protein molecules regulate many aspects of bone biology, including the cellular activity of both osteoblasts and osteoclasts[11]. The way in which osteoblasts or osteogenic cells react with alkali- and heat-treated titanium implants has not yet been elucidated. Osteoblasts may attach to the apatite formed on alkali- and heat-treated surfaces, and this may enhance growth and differentiation. As osteoblasts are pivotal in their control of bone remodeling, a biological response would need to be demonstrated to clarify the action of alkali- and heat-treated titanium *in vitro*. Thus, the purpose of this study was to elucidate cellular events that follow cell adhesion to alkali- and heat-treated titanium surface by cell proliferation, enzyme-linked immunosorbent assay (ELISA), alkaline phosphatase activity analysis and reverse transcription polymerase chain reaction (RT-PCR) analysis.

MATERIALS AND METHODS

1. Alloy preparation

In this study, three different titanium alloys were prepared as follows; (1) commercially pure titanium (grade II, cp-Ti) (2) titanium-6aluminum-4vanadium (weight percentage: 6 % aluminum, 4 % vanadium, Ti-6Al-4V), (3) alkali- and heat-treated titanium. All Ti and Ti-based alloy disks were formed into disks 12 or 25 mm diameter and 1 mm thickness and kindly provided by the Department of Materials Science and Engineering, Chonnam National University. Cp-Ti disks were wet ground with 240, 400, and 600 grit silicon carbide papers. These surfaces were ultrasonically degreased in acetone and ethanol for 10 minutes each, with deionized water rinsing between applications of each solvent. Alkali treatment was performed by soaking the samples in a solution of 10 M NaOH at a temperature of 60 ℃ for 8 hours. They were then gently washed with deionized water and dried at 40 ℃ for 24 hours. The samples were heated to 500, 600, and 700 ℃ at a rate of 5 $℃^{-1}$, kept at the given temperature for 1 hour and then allowed to cool to room temperature in the furnace.

2. Surface characteristics of disks by atomic force microscopy (AFM).

Cp-Ti, Ti-6Al-4V, and alkali- and heat-treated Ti disks were examined with atomic force microscopy to provide qualitative and quantitative information on the 3 metallic surfaces.

3. Sample preparation

cp-Ti and alkali- and heat-treated titanium disks were placed under aseptic conditions in the bottom of 12- or 6-well culture dishes, then rinsed 3 times in 70 % ethanol, exposed to UV light for 1 hour and air dried in the cell culture hood.

4. Cell culture of fetal rat calvarial cells

Osteoblast-enriched cell preparations were obtained from Sprague-Dawley 21 day fetal calvaria by sequential collagenase digestion (Type II; Invitrogen, U.S.A). The resultant cells from the third to fifth 15-minute digestions were pooled and cultured in BGJb media (Life Technologies, U.S.A) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 100 ㎎/㎖ penicillin, and 100 ㎎/㎖ streptomycin at 37 ℃ in humidified atmosphere of 5 % CO_2-95 % air.

5. Measurement of cell proliferation

Cells were cultured on cp-Ti, Ti-6Al-4V, and Ti-8Ta-3Nb disks in 12-well plates at a density of 1×10^5 cells/㎖ in the BGJb medium. The control was cultured on tissue culture plate (TCP). After 24 hours, the medium was changed and the cells were cultured for additional 3 days. Following incubation, cell proliferation was assessed following the manufacturer's guidelines. In these experiments, the amount of reduced fomazan product is directly proportional to the number of viable cells. Fomazan accumulation was quantitated by absorbency at 490 nm by an enzyme-linked immunosorbent assay (ELISA) plate reader and analyzed. All experiments were carried out in triplicate.

6. Scanning electron microscopy (SEM)

Fetal rat calvarial cells were evaluated for cell attachment and growth using scanning electron microscopy (SEM). Cells were seeded at a density of 1×10^5 cells/㎖ in the BGJb medium. After 3-day incubation period, dishes were washed three times with PBS, fixed with 2.5 % glutaraldehyde in 100 mM cacodylate buffer. Samples were dehydrated in increasing concentrations of ethanol (30 %, 60 %, 95 %, and 100 %) at each concentration, immersed in hexamethyldisilazane (Sigma, U.S.A) for 15 mins, air-dried, and immediately mounted on aluminum stubs and coated with carbon. SEM was performed.

7. Alkaline phosphatase (ALP) activity

The assay for ALP activity was carried out according to Bretaudiere and Spillman[13]. For this purpose cells were seeded onto 12 well dishes at a density of 1×10^5 cell/㎖ in the BGJb medium containing 10 % FBS, ascorbic acid 40 ㎍/㎖ and 20 ㎍/㎖ β-glycerol phosphate. Determination of ALP activity was performed as follows; At the day 7, Cells were washed with phosphated buffered saline (PBS), lysed in Triton 0.1 % (Triton X-100) in PBS, then frozen at -20 ℃ and thawed. 100 ㎕ of cell lysates was mixed with 200 ㎕ of 10 mM p-nitrophenyl phosphate and 100 ㎕ of 1.5 M 2-amino-2-methyl-1-propanol buffer. Samples were then incubated for 1 hour at 37 ℃. ALP activity was measured for each sample by absorbance reading at 490 nm with a spectrophotometer (SmartSpec™, BioRAD, U.S.A) and corrected for cell number determined in parallel. All experiments were carried out in triplicate.

8. Measurement of cell products

It was examined standardized cell population and determined the changes in cytokine production, specifically the cytokines responsible for osteoclasts activation, interleukin 1β (IL-1β) and IL-6.

Media were removed from the wells and stored at -70 ℃ until required for ELISA measurements. For analysis, the media were thawed and centrifuged at 5000 rpm for 5 mins. The Quantikine™ immunoassay kits (R & D Systems, Oxford) were used for the analysis of cytokines. The assays employ a quantitative "sandwich" emzyme immunoassay technique which was used per the manufacturer's instructions. A color develops, the intensity of which is in proportion to the amount of cytokine present, and it then is measured and compared with known standards prepared from doubling dilutions. The plates were read at 450 nm with a correction at 570 nm using a spectrophotometer (SmartSpec™, BioRAD, U.S.A).

9. Reverse transcription polymerase chain reaction (RT-PCR) gene expression analysis

Gene expression of mineralized matrix markers was evaluated by RT-PCR. Cp-Ti, Ti-8Ta-3-Nb, and Ti-6Al-4V disks were placed under aseptic conditions in the 6-well tissue culture dishes. Then 2.0×10^5 cells in BGJb medium containing 10 % FBS were seeded into each well and incubated for 1 day. After an initial attachment period, the media was switched to mineralized media containing 10 % FBS, ascorbic acid 40 ㎍/㎖ and 20 ㎍/㎖ β -glycerol phosphate for the duration of experiment and was changed every 3 days.

Total RNA was isolated at day 7 using the methodology described by the manufacturer. First-strand cDNA synthesis was carried out in an Amplitron II thermocycler using SuperScript II (Invitrigen, U.S.A). PCR was subsequently performed using amplication primer sets (Sigma-Genosys, U.S.A) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), collagenase type I (COL-I), bone sialoproteins (BSP), interleukin (IL)-6, osteoporotegerin (OPG) and receptor activation of nuclear factor κB ligand (RANKL) as listed in Figure 1 for 30 cycles.

RT-PCR products were separated and analyzed by gel electrophoresis. Resulting images were captured using a Gel-Doc (BioRad, U.S.A) imaging system equipped with UV light and a gel scanner. These studies were performed twice.

10. Statistical analysis

An analysis of variance (ANOVA) for replicate measurements and DUNCAN multiple range test was done using SAS program.

RESULTS

1. Surface characteristics and roughness test

Fig. 2 showed SEM and AFM images of three different test surfaces, cp-Ti, Ti-6Al-4V, and alkali- and heat-treated Ti. Roughness measurements of the AFM information yielded root mean square (RMS) values of the surface topographies. For all three tested disks, there was little difference in overall surface roughness (Table I).

2. Scanning electron microscopy

For each specimen, cells were examined by SEM. The cells spreaded extensively and totally flattened

PCR programs

GAPDH	94 ℃ 1 min	94 ℃ 1 min	60 ℃ 2 min	72 ℃ 1 min	72 ℃ 10 min
		25 Cycles			
ALP, BSP, COL I	94 ℃ 1 min	94 ℃ 1 min	55 ℃ 2 min	72 ℃ 1 min	72 ℃ 10 min
		30 Cycles			
IL-6, OPG, RANKL	94 ℃ 1 min	94 ℃ 1 min	50 ℃ 2 min	72 ℃ 1 min	72 ℃ 10 min
		30 Cycles			

PCR primers

Fig. 1. Amplification primer sets and conditions used in polymerase chain reaction. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ALP, alkaline phosphatase; BSP, bone sialoprotein; COL I, type 1 collagen; IL-6, interleukin-6; OPG, osteoproteogerin; and RANKL

Primer	Expected base pairs	Sequence (5'-3')
GAPDH-sense (+)	418	CACCATGGAGAAGGCCGGGG
GAPDH-antisense (-)		GACGGACACATTGGGGGTAG
COL I-sense (+)	250	TCTCCACTCTTCTAGGTTCCT
COL I-antisense (-)		TTGGGTCATTTCCACATGC
BSP-sense (+)	1068	AACAATCCGTGCCACTCA
BSP-antisense (-)		GGAGGGGGCTTCACTGAT
IL-6-sense (+)	638	ATGAAGTTCCTCTCTGCAAGAGACT
IL-6-antisense (-)		CACTAGGTTTGCCGAGTAGATCTC
RANKL-sense (+)	499	CAGCACTCACTGCTTTTATAGAATCC
RANKL-antisense (-)		AGCTGAAGATAGTCTGTAGGTACGC
OPG-sense (+)	492	TGTAGAGAGGATAAAACGG
OPG-antisense (-)		CTAGTTATAAGCAGCTTTAT
ALP-sense (+)	372	CCATGATCACGTCGATATCC
ALP-antisense (-)		TCTGACAAACCTTCATGTCC

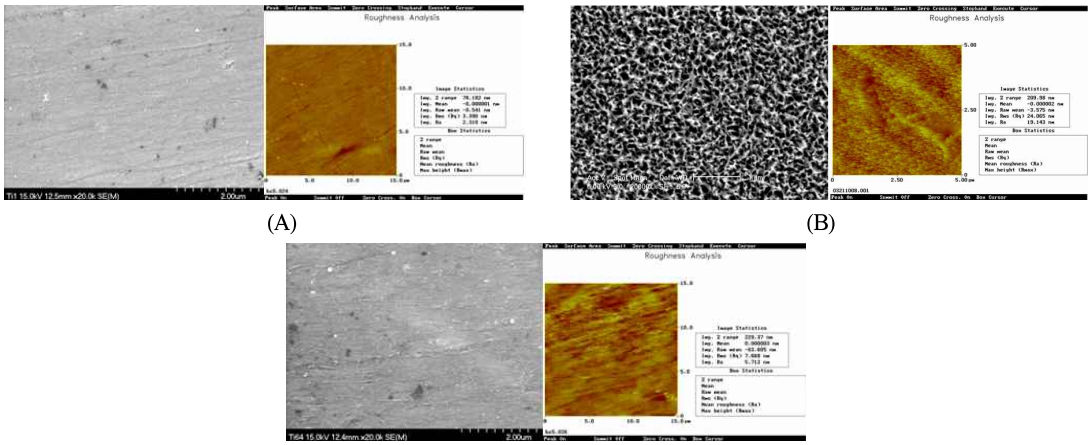


Fig. 2. Surface characteristics of cp- Ti (A), alkali- and heat-treated surfaces (B), and Ti-6Al-4V alloy (C)

Table I. Summary of AFM surface roughness of Ti-based alloys

Type of Ti-based alloys	RMS Roughness (nm)
cp-Ti	3.3
AH-Ti*	19.1
Ti-6Al-4V	7.7

AH-Ti*: alkali- and heat-treated titanium

on alkali- and heat-treated surfaces. They were polygonal shapes with filopodial extensions indicative of cell spreading. They did not have regular orientation and looked scattered in all directions.

On cp-Ti and alkali- and heat-treated surfaces, cells spreaded polygonally and cell projections connecting cells were visible (Fig. 4).

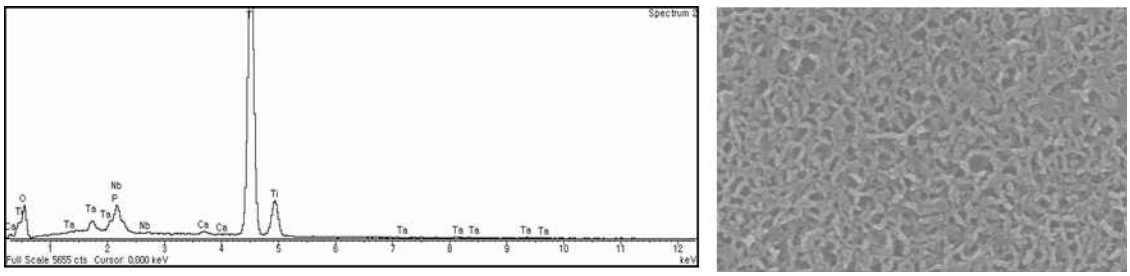
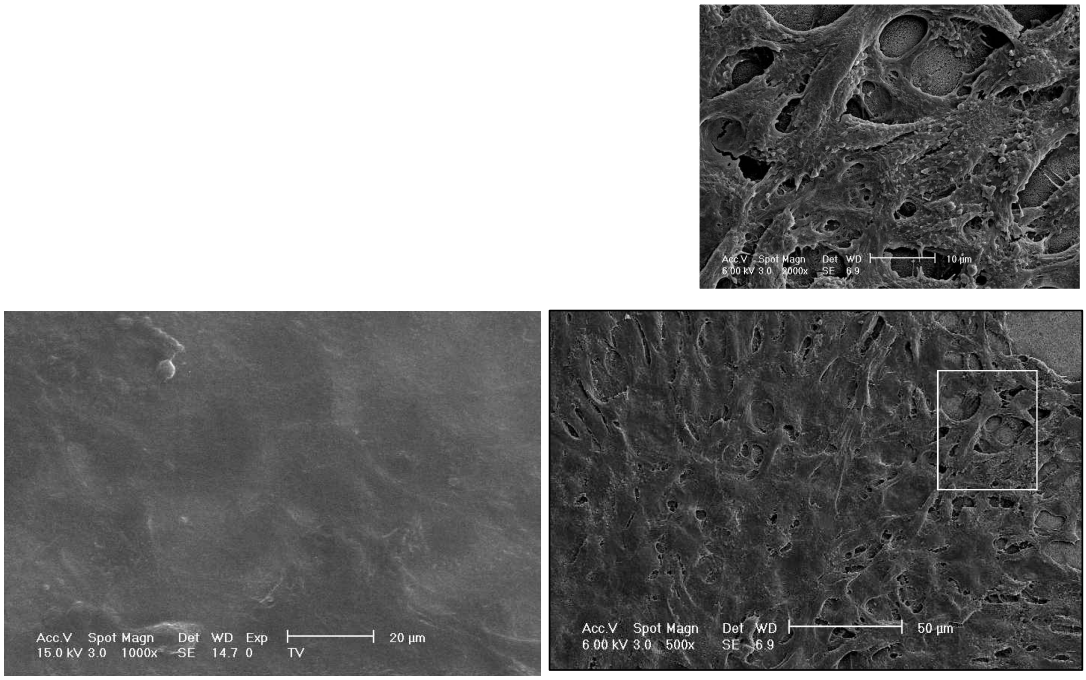


Fig. 3. EDX profile of alkali- and heat-treated titanium-8tantalum-3neobium alloy surface soaked in simulated body fluid for 21 days



(A) (B)

Fig. 4. Photomicrographs of SEM findings at day 3. cp-Ti (A) and alkali- and heat-treated surfaces (B) show that cells grown on both Ti and alkali- and heat-treated surfaces , presented a flat, elongated spindle-like morphology (x 500)

3. Cell proliferation

Cell proliferation was measured by the MTT assay. After 3 days, there was a significant difference among glass, cp-Ti, Ti-6Al-4V, and alkali- and heat-treated titanium ($p<0.01$). Cell proliferation on alkali- and heat-treated surfaces showed significantly higher level than on the Ti-6Al-4V surface ($p<0.01$, Fig. 5).

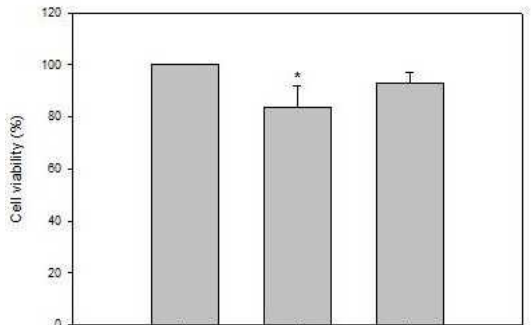


Fig. 5. Cell proliferation assay after 3 days on commercially pure Titanium (cp-Ti), Ti-6Al-4V alloy and alkali- and heat-treated cp-Ti (AH-Ti)
*: Significantly different compared to Ti-6Al-4V alloy ($p<0.01$)

4. Alkaline phosphatase (ALP) activity

Alkaline phosphatase activity on alkali- and heat-treated surfaces was the highest of all 3 test materials. Alkaline phosphatase activity on Ti-6Al-4V

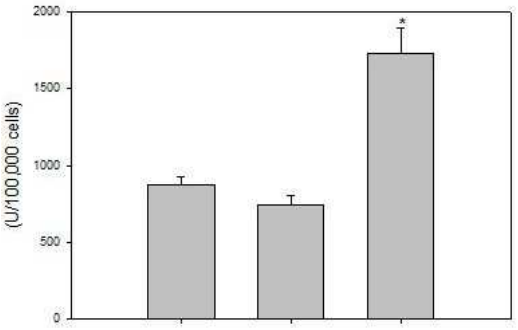


Fig. 6. Alkaline phosphatase activity of primary rat calvarial cells on commercially pure titanium (cp-Ti), Ti-6Al-4V alloy and alkali- and heat-treated cp-Ti (AH-Ti) at day 7 (U/ 100,000 cells)
*: A significantly higher value of alkali- and heat-treated Ti was observed. (p<0.001)

alloy was lower than cp-Ti and on tissue culture plate (TCP) controls (p<0.001, Fig. 6)

5. ELISA results

Cells without metallic disks served as control. The concentration of IL-1β were raised from day 3 to day 7 in all samples. However, there was statistical difference between control and Ti-based metal samples at day 7 (p<0.001, Table II). The concentration of IL-6 was negligible at day 3 (16~31 pg/㎖) however, was raised at day 7 in alkali- and heat-treated Ti. There was statistical difference between control and alkali- and heat-treated Ti. Culture on alkali- and heat-treated Ti with herbimycin produced media containing negligible quantities of IL-6 as compared to TCP controls (p<0.001, Table III).

Table II. Mean IL-1β levels in TCP, cp-Ti, and alkali- and heat- treated Ti

Group	day	
	3 days	7 days
TCP	42 ± 19 *	132 ± 48
cp-Ti	75 ± 13	237 ± 60
AH-Ti	68 ± 22	308 ± 35^{+}

TCP:tissue culture plate, AH-Ti: alkali- and heat-treated titanium
* : Mean ± standard deviation (pg/ml)
$^{+}$: Significantly different compared to tissue culture plate (p<0.01)

Table III. Mean IL-6 levels in TCP, cp-Ti, and alkali- and heat-treated Ti(AH-Ti)

Group	day	
	3 days	7 days
TCP	16 ± 10*	68 ± 30
cp-Ti	20 ± 17	89 ± 21
AH-Ti	31 ± 10	156 ± 78^{+}
AH-Ti+HB	31 ± 8	60 ± 20

AH-Ti: alkali- and heat-treated titanium, HB;herbimycin
*: Mean ± standard deviation (pg/ml)
$^{+}$: A significantly higher value of alkali- and heat-treated Ti was observed (p<0.01)

6. RT-PCR gene expression analysis

After 7 days culture, the expression of COL-I, BSP, ALP, OPG, and RANKL mRNA from cells was evaluated by RT-PCR. Total RNA isolated from cells on Ti-6Al-4V alloy did not yield enough products for use with RT-PCR analysis. In contrast, enough total RNA was obtained on alkali- and

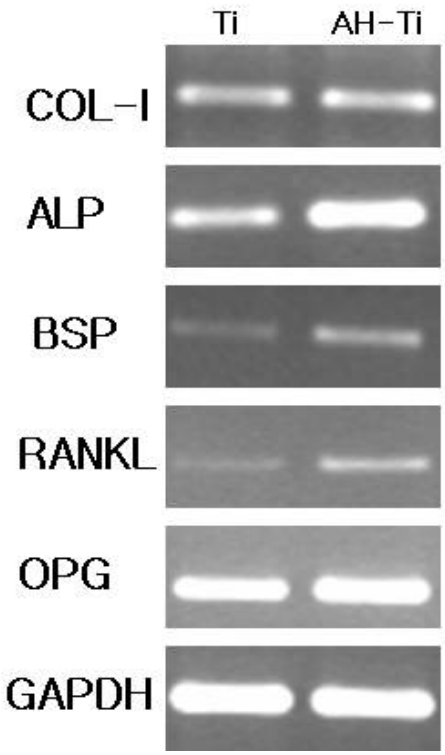


Fig. 7. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of collagen type I (COL-1), alkaline phosphatase (ALP), bone sialoprotein (BSP), receptor activated nuclear factor ligand (RANKL), osteoproteogerin (OPG), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) m RNA expression in rat calvarial cells (lane 1: cp-Ti, lane 2: alkali- and heat-treated titanium)

heat-treated Ti and cp-Ti. For the type I collagen gene(COL-I), the expression level of COL-I mRNA was not different on cp-Ti, alkali- and heat-treated surfaces. Alkali- and heat-treated Ti was shown that increased of alkaline phosphatase mRNA expression (2.5 folds), bone sialoprotein mRNA expression (2.3

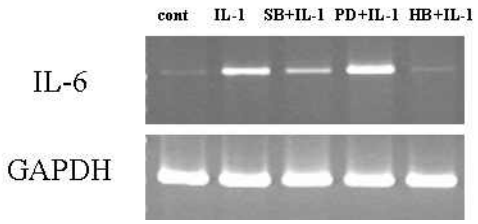


Fig. 8. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of interleukin -6 (IL-6) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) m RNA expression in rat calvarial cells (lane 1: control, lane 2: IL-1b (1 ng/ml), lane 3:SB203580+IL-1b, lane 4:PD + IL-1b lane 5: Herbimycin + IL-1b)

folds), and RANKL mRNA expression (2.8 folds) than those of cp-Ti-induced genes(Fig. 7). Herbimycin, a known tyrosine kinase inhibitor was repressed of IL-1β-induced IL-6 mRNA expression(Fig. 8).

DISCUSSION

For surface chemistry modification, various calcium phosphate (Ca-P) coatings on titanium implants have been proposed to improve osseointegration. Whether primary cell or immortalized cell lines would be used when testing biomaterial is controversial. Primary osteoblasts have a diploid chromosome pattern, are characterized by growing slowly and have a finite lifespan. Established cell lines have been used in previous studies investing cell morphology and cytotoxicity in the presence of various titanium alloys[12,13]. Primary cell strains derived from living tissues are necessary and have been recommended by the ISO for specific testing to simulate the in vivo situation[14].

In this study, primary osteoblasts were obtained from fetal rat calvaria. This is an excellent source of osteoblasts because cells from young animals proliferate rapidly. Cells from the third, fourth and fifth digests were collected because these later digests provide a more pure culture, containing most cells that express an osteoblast-like phenotype[15].

Surface roughness can greatly affect the proliferation and protein synthesis of osteoblast cells that are cultured on a metal substrate characteristics in the healing of bone[16,17]. Many studies have demonstrated that roughness has a great influence on cell responses. According to this respects, the test samples used in this study was controlled that they had a similar roughness after they were polished.

Alkali- and heat-treated Ti implants have a thin reactive layer (Ca-P layer) which can form apatite in simulated body fluid. In this study, as the same as other reports[3,9,10,18,19], a layer of Ca-P was formed and confirmed by EDX profile however, the elements of calcium and phosphorus were not found on cp-Ti and Ti-6Al-4V alloy when soaked in culture media for 21 days(Fig. 3).

The osteoblastic cells undergo a temporal sequence of phase during the development of their completely differentiated phenotype: proliferation, differentiation, and mineralization[20]. Briefly, cells initially increase their number and produce extracellular matrix. The phase of differentiation follows, which is characterized by the production of high levels of alkaline phosphatase and modifications of the matrix that lead to the deposition of hydroxyapatite crystals. This phase of mineralization is also characterized by the synthesis of collagen, bone sialoprotein, and osteocalcin (OCN) that play a fundamental role in bone remodeling and represents the specific markers of final differentiation of osteoblasts.

In general, cell synthesis activity is sensitive to the type of material[16]. Cells grown on alkali- and heat-treated Ti showed alkaline phosphatase levels showed significantly higher than on cp-Ti and Ti-6Al-4V alloy ($p<0.001$). Alkaline phosphatase activity of cp-Ti and TCP exhibited the similar activity, but showed significantly higher than on Ti-6Al-4V alloy. This might be in case of Ti-6Al-4V alloy, due to the release of aluminum and/or vanadium in culture medium. In particular, vanadium is known to cytotoxic[21], this might affect cells to protein synthesis. This result means that Ti chemical composition interfered with alkaline phosphatase activity.

IL-1β and IL-6 are cytokines that involved indirectly or directly in osteoclastogenesis[22]. In RT-PCR analysis, mRNA expression was analyzed at 7 days. Type I collagen expression is an essential component of the extracellular matrix that is required before mineralized matrix formation. In this study, mRNA level of Type I collagen on cp-Ti and alkali- and heat-treated Ti was consistent. This means that cp-Ti and alkali- and heat-treated Ti did not inhibit mineralized matrix expression of fetal rat calvarial cells.

Alkaline phosphatase (ALP) is an enzyme belonging to a group of membrane-bound glycoproteins. Although its physiological function still remains unclear, ALP may play a key role in the formation and calcification of hard tissues[23], and its expression and enzyme activity are frequently used as markers of osteoblastic cells. Bone sialoprotein (BSP) is a 34-kDa protein that is highly sulfated, phosphorylated and glycosylated, and is expressed almost exclusively in mineralizing connective tissues[24]. Studies on the developmental expression of BSP have shown that BSP mRNA is expressed at high levels by osteoblasts at the onset

of bone formation[25], and under steady-state conditions in vitro BSP nucleates hydroxyapaptite crystal formation[26] indicating a role for this protein in the initial mineralization of bone. In this study, alkali- and heat-treated surface increased of alkaline phosphatase (ALP) mRNA and bone sialoprotein mRNA expression. This result is in accordance with Chosa et al[27]and could be explained that alkali- and heat-treated surface increased in osteoblastogenesis and bone formation.

Interestingly, in this study, the expression of RANKL mRNA increased in alkali- and heat-treated Ti surface. In previous studies, a number of stimulators of bone resorption such as parathyroid hormone (PTH) and IL-6 were shown to increase RANKL expression in osteoblasts[28,29]. In contrast, inhibitors of bone resorption and stimulators of bone formation such as bone morphogenic proteins inhibited RANKL. Li et al[30] indicated the involvement of RANKL in bone resorption by showing osteoclastic bone resorption can be inhibited by inhibitors that interfere with RANKL production. Although the precise role of RANKL in bone resorption, it has been proposed that RANKL differentiate osteoclasts by binding RANKL receptors[31]. Thus, it is possible that alkali- and heat-treated Ti stimulate the secretion of RANKL that cause the differentiation of osteoclasts at bone remodeling sites. Taken together, these results indicated that alkali- and heat-treated Ti surface facilitated bone turn over rate and eventually stimulate bone remodeling.

In this study, alkali- and heat-treated Ti had better cell response than cp-Ti for bone remodeling. This property could make alkali- and heat-treated Ti a useful candidate for the preparation of medical and dental device. However, more animal and human studies will be needed before using this alloy in medical and dental use.

CONCLUSION

Osseointegration of dental implant depends on the cellular response around implant. Changes in osteoblastic proliferation, differentiation, and maturation are important events in bone remodeling. Alkali- and heat-treated titanium formed an apatite surface layer which was reported to facilitate osteogenic differentiation. As osteoblasts are pivotal in their control of bone remodeling, a biological response would need to be demonstrated to clarify the action of alkali- and heat-treated titanium *in vitro*.

In this study, the biological response of fetal rat calvarial cells on alkali- and heat-treated titanium was assessed by cell proliferation, ELISA, alkaline phosphatase activity, and RT-PCR method.

The results are as follows;

1. Cell proliferation on alkali- and heat-treated surfaces showed significantly higher level than on the Ti-6Al-4V surface ($p<0.01$).
2. In ELISA analysis, concentration of IL-1β and IL-6 was raised when the cells were grown to day 7. Pre-treatment with herbimycin, a known tyrosine kinase inhibitor, suppressed the production of IL-6.
3. In comparison to cp-Ti and Ti-6Al-4V alloy, alkali- and heat-treated titanium enhanced alkaline phosphatase activity ($p<0.001$).
4. In RT-PCR analysis, ALP, BSP, and RANKL mRNA expression increased alkali- and heat-treated titanium. Herbimycin and SB203580, p38 MAPK inhibitor, were repressed of IL-1β-induced IL-6 mRNA expression.

These results suggest that alkali- and heat-treated titanium stimulate osteoblasts differentiation and facilitate bone remodeling.

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티타니움 표면의 알칼리-열처리가 골모세포의 분화에 미치는 영향

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본 연구에서는 알카리 열처리 티타늄 임플란트상에서 백서 태자 두개관 세포의 생물학적 반응을 알아보고자 하여 다음과 같은 결과를 얻었다. 알카리 열처리 티타니움 표면에서 Ti-6Al-4V 합금 표면에서보다 세포증식도가 유의하게 높았다 ($p<0.01$). 면역효소 분석에서 interleukin (IL)-1β와 IL-6의 합성은 배양 시간이 길수록 증가하였다. Herbimycin으로 전처치한 경우에는 세포 배양 7일 째에 IL-6의 합성이 유의하게 억제되었다($p<0.01$). 알카리인산 분해 효소 활성은 알카리 열처리 임플란트에서 타이타늄, Ti-6Al-4V 합금 표면에서 보다 유의하게 더 높았다 ($p<0.001$). 유전자 분석에서 알카리 열처리 임플란트에서 배양된 세포들의 alkaline phosphatase, bone sialoprotein, receptor activated nuclear factor ligand 유전자의 발현이 증가하였다. Herbimycin과 SB203580으로 전처치시 IL-1β-induced IL-6 유전자의 발현이 감소하였다. 이상의 결과는 알카리 열처리된 티타늄 임플란트가 조골세포의 분화를 자극하고 골개조를 촉진시킴을 시사한다.

주요어: 분화, 알카리처리, 열처리, 조골세포, 티타늄

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