

Biocompatibility of Ti-8Ta-3Nb alloy with fetal rat calvarial cells

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I. INTRODUCTION

Titanium is the material of choice for oral implantology. The superiority of titanium comes from its mechanical, physicochemical, and biological properties, particularly immunotolerance and cytocompatibility^{1,2)}. However because of needs to improve its mechanical properties, titanium alloys such as Ti-6Al-4V, Ti-6Al-7Nb, have also been used for dental and orthopedic material³⁻⁵⁾.

Although titanium alloys are still widely used in medicine, some concern has been expressed over its use since it appears that small amounts of both vanadium and aluminum, released in the human body, induce possible cytotoxic effect and neurological disorders, respectively⁶⁻⁸⁾.

In terms of mechanical properties, Ti-6Al-4V alloy belongs to $\alpha + \beta$ type titanium alloy

family. The rigidity of $\alpha + \beta$ type titanium alloy is much higher (about 120 GPa) than that of the cortical bone (Max 30 GPa)⁹⁾. Such a strong rigidity (a large modulus of elasticity) causes insufficient loading of the bone adjacent to the implant. This is often referred to as the stress-shielding phenomenon and can lead to potential bone resorption and eventual failure of the implant¹⁰⁾. Consequently the recent trend in research and development of titanium alloys for biomedical application is to develop low rigidity titanium alloys composed of non-toxic and non-allergenic elements with excellent mechanical properties and workability^{9,10)}.

The most promising materials are the β -type titanium alloys because their elastic moduli are smaller than that of α - or $\alpha + \beta$ type titanium family. Therefore new β -type titanium alloys which are composed of non-toxic elements like Tantalum (Ta), Niobium (Nb), Zirconium (Zr),

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and Molybdenum (Mo), have been developed for next generation biomaterials⁹⁻¹².

One of new β -type titanium alloys, Ti-8Ta-3Nb has recently developed for biomedical application. This new Ti-8Ta-3Nb alloy is composed of non-toxic elements and presents low Young's modulus and excellent mechanical properties¹³. However, the biocompatibility of Ti-8Ta-3Nb alloy is poorly understood. Cui et al¹⁴ reported that Ti-Ta-Nb alloys, Ti-8Ta-3Nb and Ti-10Ta-10Nb alloys, exhibited better cytocompatibility as compared to the Ti-6Al-4V alloy and commercially pure titanium. They used indirect cytotoxicity method that was evaluated from extracts obtained by incubating test titanium alloys in Dulbecco's modified Eagle's medium without fetal bovine serum. However to better understand the interaction between the titanium surface and the biological environment, experiments should be done by direct method that cells were seeded directly onto the titanium materials.

The purpose of this study was assessed the biocompatibility of Ti-8Ta-3Nb alloy in comparison with pure titanium and Ti-6Al-4V alloy by scanning electron microscopy, cell proliferation, alkaline phosphatase activity analysis, and reverse transcription polymerase chain reaction method.

II. MATERIALS & METHODS

1. Alloy preparation and surface characterization of disks by atomic force microscopy

In this study, three different titanium alloys were prepared as follows; (1) titanium-8 tanta-

lum-3 neobium disks (weight percentage: 8% tantalum, 3% neobium, Ti-8Ta-3Nb), (2) titanium-6 aluminum-4 vanadium (weight percentage: 6% aluminum, 4% vanadium, Ti-6Al-4V), (3) commercially pure titanium (grade II, cp-Ti). The Ti-8Ta-3Nb, Ti-6Al-4V, and cp-Ti disks were formed into disks 15 mm diameter and 1 mm thickness and kindly provided by the Department of Materials Science and Engineering, Chonnam National University. Ti-8Ta-3Nb, Ti-6Al-4V, and 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. cp-Ti and two titanium alloys, Ti-8Ta-3Nb, and Ti-6Al-4V disks were examined with atomic force microscopy to provide qualitative and quantitative information on the 3 metallic surfaces.

2. Sample preparation

Ti-8Ta-3Nb, cp-Ti, and Ti-6Al-4V 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.

3. Cell culture of fetal rat calvarial cells (FRCC)

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 mg/ml penicillin, and 100 mg/ml streptomycin at 37°C in humidified atmosphere of 5% CO₂-95% air.

4. 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/ml in the BGJb medium supplemented with 10% FBS. The control was cultured on cover glass (micro cover glass, VWR Science, U.S.A). After 24 hours, the medium was changed to the BGJb medium supplemented with 2% FBS and cells were cultured for an 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 absorbance at 490 nm by an enzyme-linked immunoabsorbent assay (ELISA) plate reader and analyzed. All experiments were carried out in triplicate.

5. Scanning Electron Microscopy (SEM)

Fetal rat calvarial cells were evaluated for cell attachment and growth using scanning electron microscopy. Cells were seeded at a density of 1×10^5 cells/ml in the BGJb medium with 10% FBS. After 3-day incubation period, dishes were washed three times with phosphate buffered saline (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.

6. Alkaline Phosphatase (ALP) Activity

The assay for ALP activity was carried out according to Bretauiere and Spillman¹⁵. For this purpose cells were seeded onto 12 well dishes at a density of 1×10^5 cell/ml in the BGJb medium containing 10% FBS, ascorbic acid 40 µg/ml and 20 µg/ml β-glycerol phosphate. Determination of ALP activity was performed as follows: At the day 7, cells were washed with PBS, lysed in Triton 0.1% (Triton X-100) in PBS, then frozen at -20°C and thawed. 100 µl of cell lysates was mixed with 200 µl of 10 mM p-nitrophenyl phosphate and 100 µl of 1.5 M 2-amino-2-methyl-1-propanol buffer. Samples were then incubated for 1 hour at 37 °C. ALP activity was measured for each sample by absorbance reading at 405 nm with a spectrophotometer (SmartSpec™, BioRAD, U.S.A) and corrected for cell number determined in parallel. All experiments were carried out in triplicate.

7. Reverse transcriptase polymerase chain reaction (RT-PCR) gene expression analysis

Gene expression of mineralized matrix markers was evaluated by RT-PCR. Ti-8Ta-3-Nb, cp-Ti, 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 $\mu\text{g}/\text{ml}$ and 20 $\mu\text{g}/\text{ml}$ 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 (Invitrogen, U.S.A). PCR was subsequently performed using amplification primer sets (Sigma-Genosys, U.S.A) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), collagen

PCR primers

Primer	Expected base pairs	Sequence (5'-3')
GAPDH-sense (+)	418	CACCATGGAGAAGGCCGGGG
GAPDH-antisense (-)		GACGGACACATTGGGGGTAG
COL I-sense (+)	250	TCTCCACTCTTCTAGGTTCT
COL I-antisense (-)		TTGGGTCATTTCCACATGC
BSP-sense (+)	1068	AACAATCCGTGCCACTCA
BSP-antisense (-)		GGAGGGGGCTTCACTGAT
OCN-sense (+)	198	TCTGACAAACCTTCATGTCC
OCN-antisense (-)		AAATAGTGATACCGTAGATGCG

PCR programs

GAPDH	94 °C 1 min	94 °C 1 min	60 °C 2 min	72 °C 1 min	72 °C 10 min
COL I	94 °C 1 min	94 °C 1 min	55 °C 2 min	72 °C 1 min	72 °C 10 min
BSP OCN	94 °C 1 min	94 °C 1 min	50 °C 2 min	72 °C 1 min	72 °C 10 min

Figure 1. Amplification primer sets and conditions used in polymerase chain reaction. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; COL I, type 1 collagen; BSP, bone sialoprotein; and OCN, osteocalcin.

type I (COL-I), bone sialoproteins (BSP) and osteocalcin (OCN) 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 procedures were performed twice.

7. Statistical analysis

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

III. RESULTS

1. Surface characterization and roughness test

Figure 2 showed SEM and AFM images of three different test surfaces, cp-Ti, Ti-8Ta-3Nb, and Ti-6Al-4V. Roughness measurements of the AFM information yielded root mean square (RMS) values of the surface topographies. Profile roughness measurements showed 3.3 nm on cp-Ti, 29.0 nm on Ti-8Ta-3Nb, and 7.7 nm on Ti-6Al-4V. For all three tested disks, there were little difference in the RMS roughness values (Table 1).

2. Scanning Electron Microscopy

For each specimen, cells were examined by SEM. The cells spreaded extensively and totally flattened on Ti-8Ta-3Nb alloy surface. 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 Ti-6Al-4V alloy surfaces, cells spreaded polygonally and cell projections connecting cells were visible (figure 3).

3. Cell proliferation

Cell proliferation was measured by the MTT assay. After 3 days, there was a significant difference between on the glass and cp-Ti or titanium alloys ($p < 0.001$). However, cell proliferation cultured on the Ti-8Ta-3Nb surface was nearly the same as that of cp-Ti. Cells on Ti-8Ta-3Nb surface showed significantly higher level than on the Ti-6Al-4V surface (figure 4).

4. Alkaline phosphatase (ALP) activity

Alkaline phosphatase activity on glass was the highest of all test materials. Among three metallic test materials, both cp-Ti and Ti-8Ta-3Nb alloy exhibited the similar activity, but showed significantly higher than on Ti-6Al-4V alloy ($p < 0.01$, figure 5).

Table 1. Summary of AFM surface roughness of Ti-based alloys.

Type of Ti-based alloys	RMS Roughness (nm)
cp-Ti	3.3
Ti-8Ta-3Nb	29.0
Ti-6Al-4V	7.7

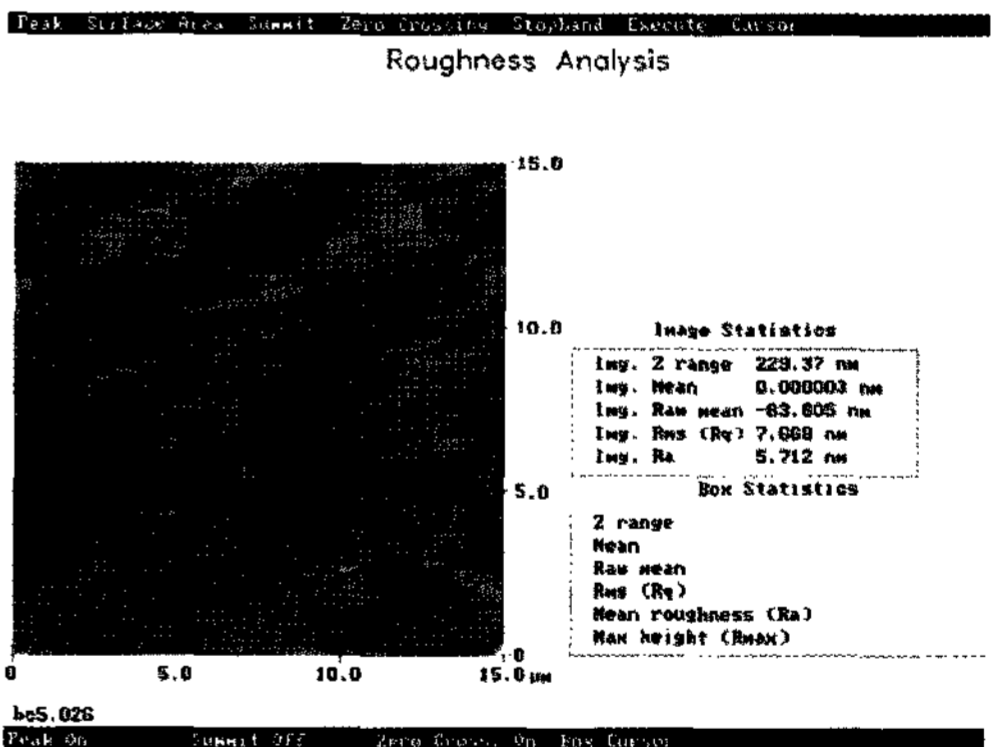
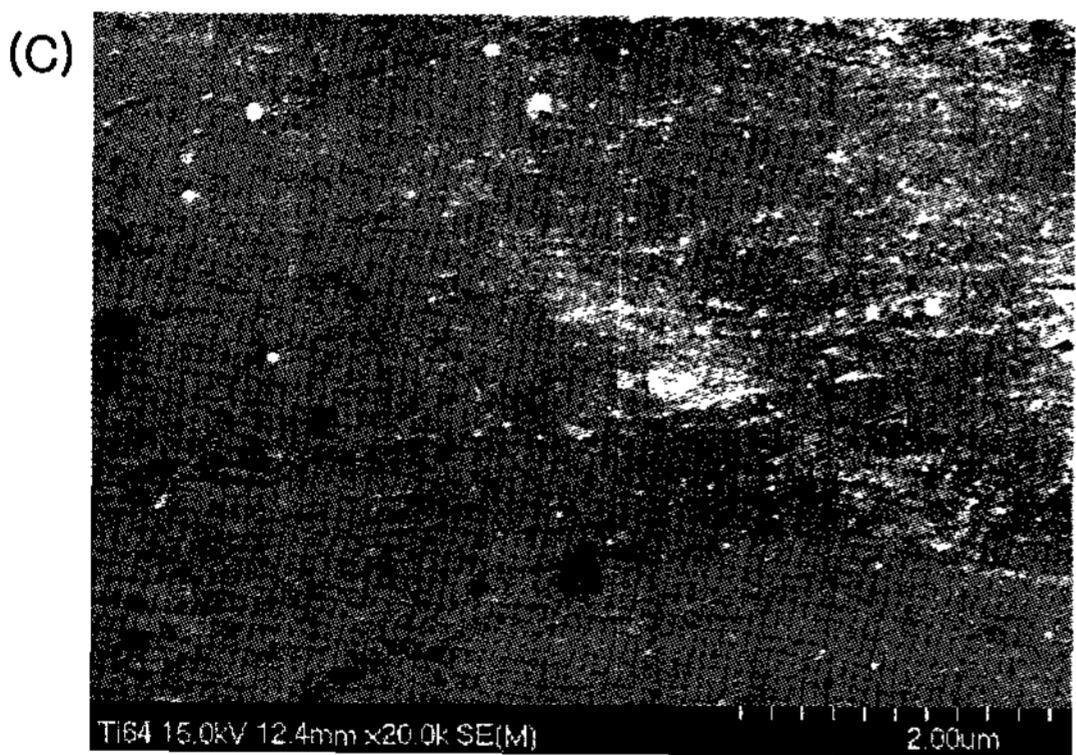
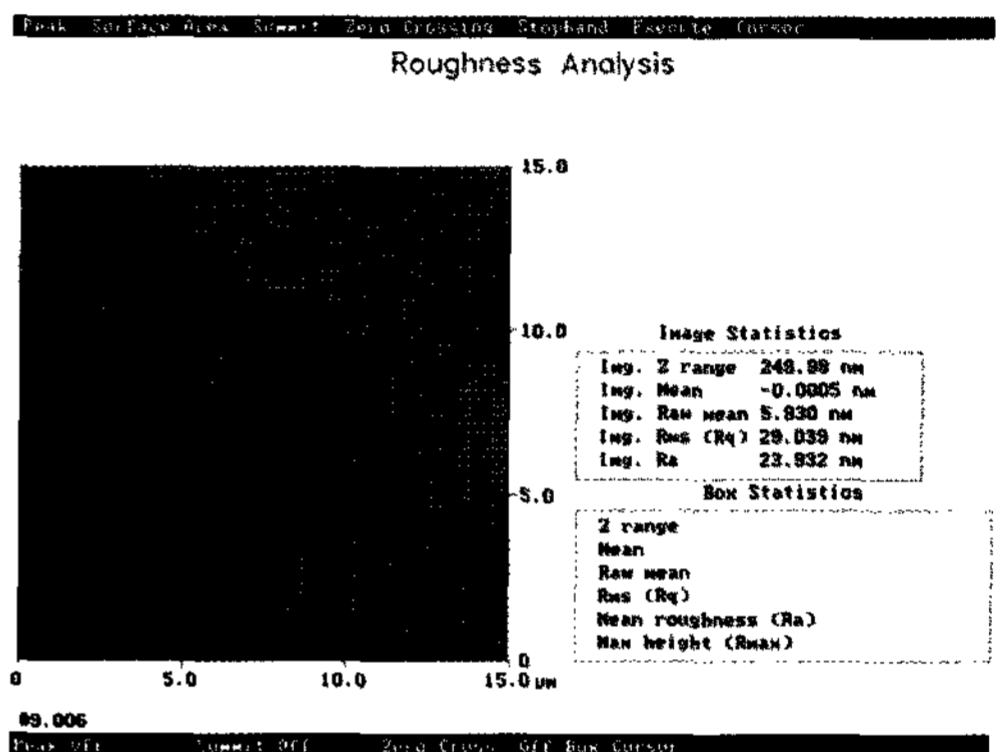
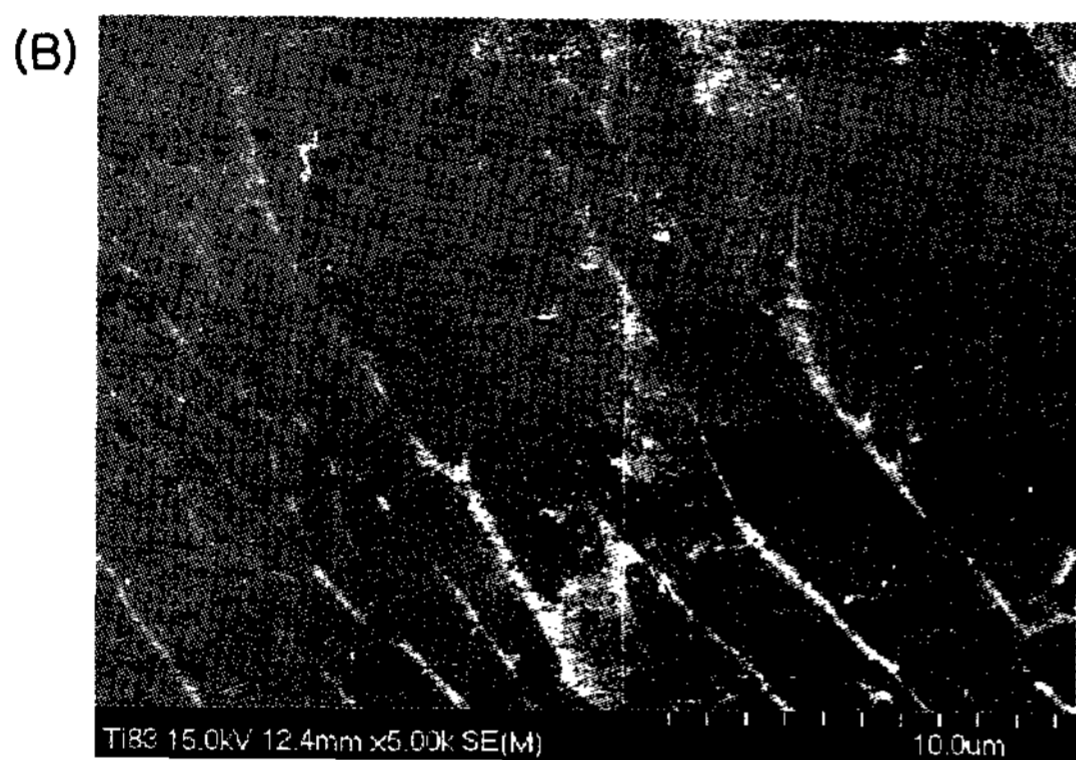
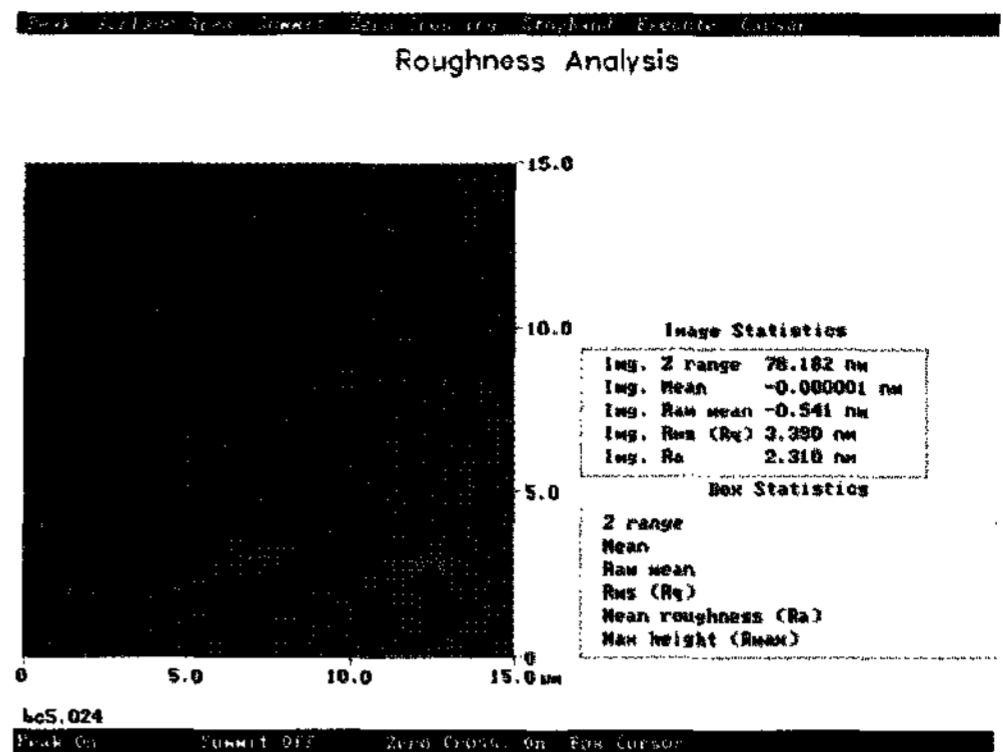
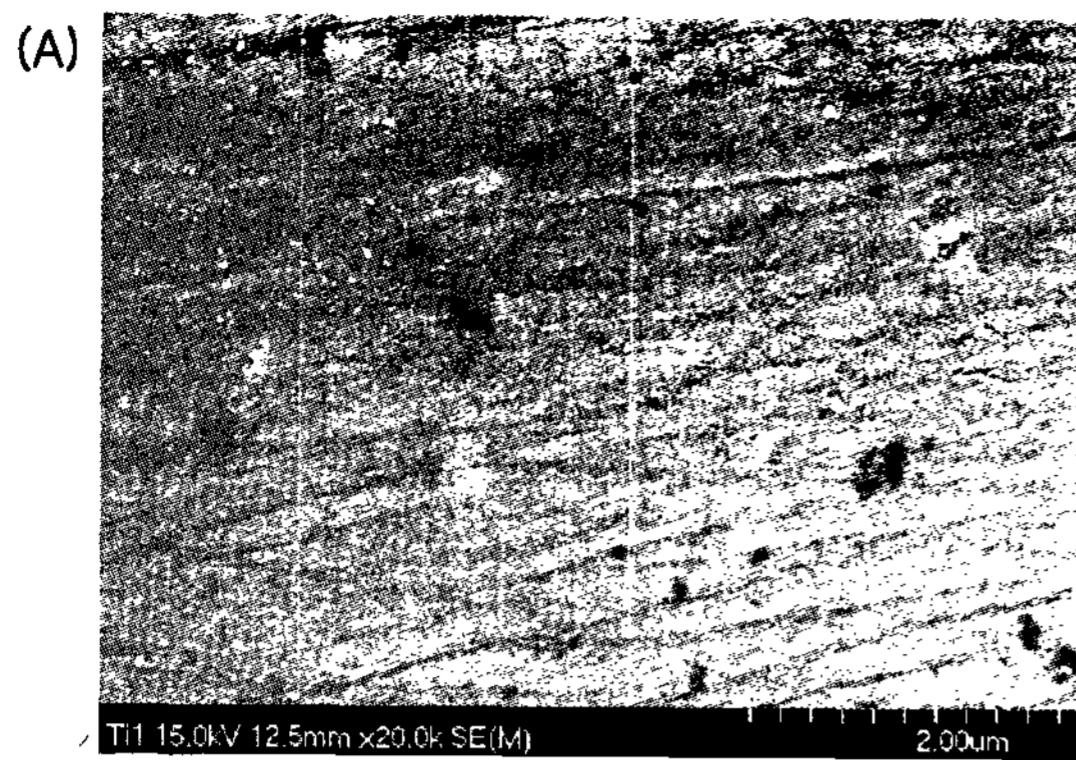


Figure 2. Surface characteristics of cp-Ti (A), Ti-8Ta-3Ta alloy (B), and Ti-6Al-4V alloy (C).



Figure 3. Photomicrographs of SEM findings at day 3. Pure Ti (A) and Ti-8Ta-3Nb (B) surfaces show that cells grown on both Ti and Ti-8Ta-3Nb surfaces, presented a flat, elongated spindle-like morphology, while cells on Ti-6Al-4V (C) surface were flat, but some cells were oval or round in shape (x 500).

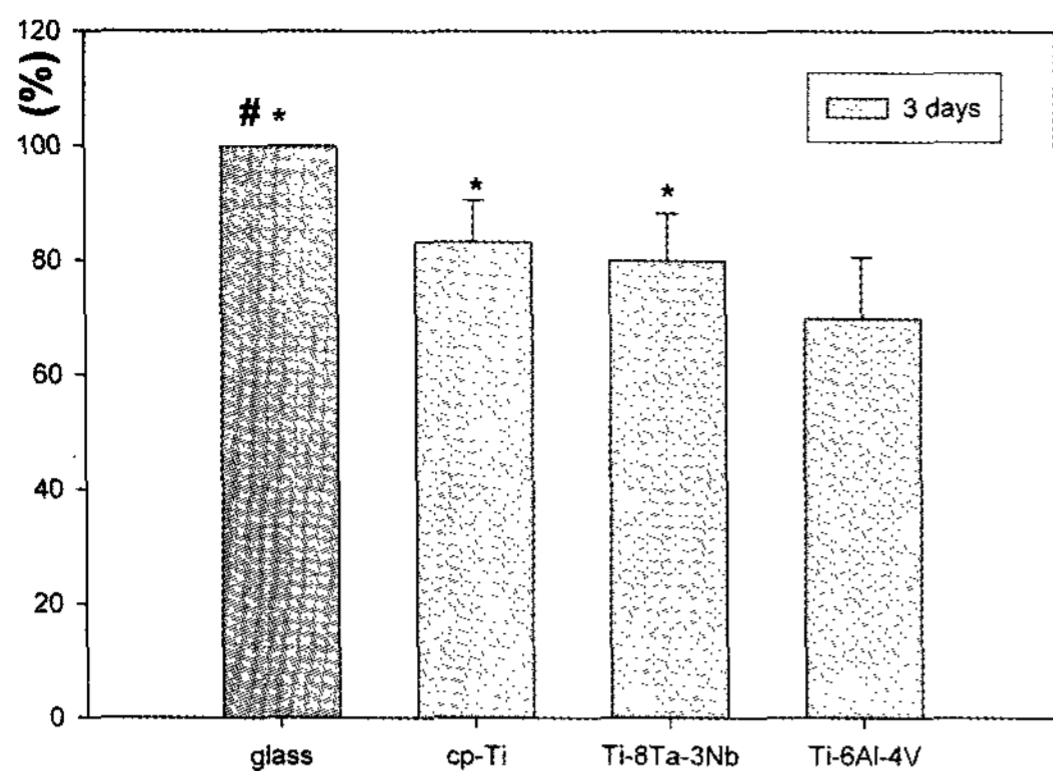


Figure 4. Cell proliferation assay after 3 days on pure Titanium (Ti), Ti-8Ta-3Nb and Ti-6Al-4V alloy. *: Significantly different than Ti-6Al-4V alloy ($p < 0.001$). #: Statistically significant differences between glass and all three metallic surfaces.

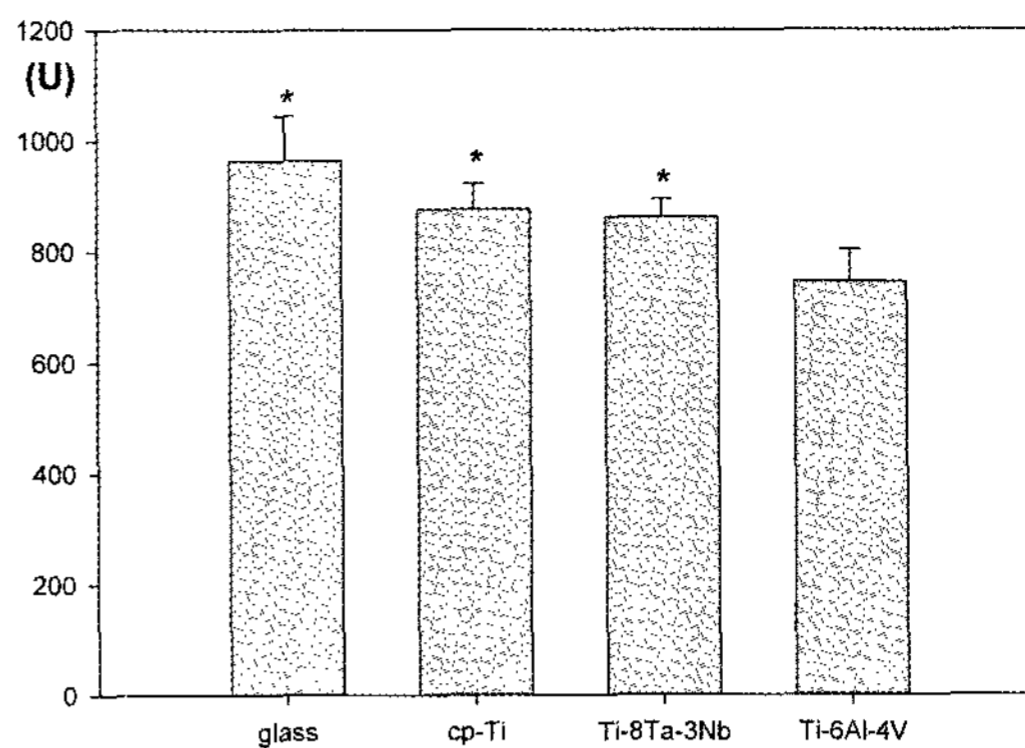


Figure 5. Alkaline phosphatase activity of primary rat calvarial cells on the pure Titanium (Ti), Ti-8Ta-3Nb and Ti-6Al-4V surfaces at day 7 (U/ 100,000 cells). *: Significantly different than Ti-6Al-4V alloy ($p < 0.001$).

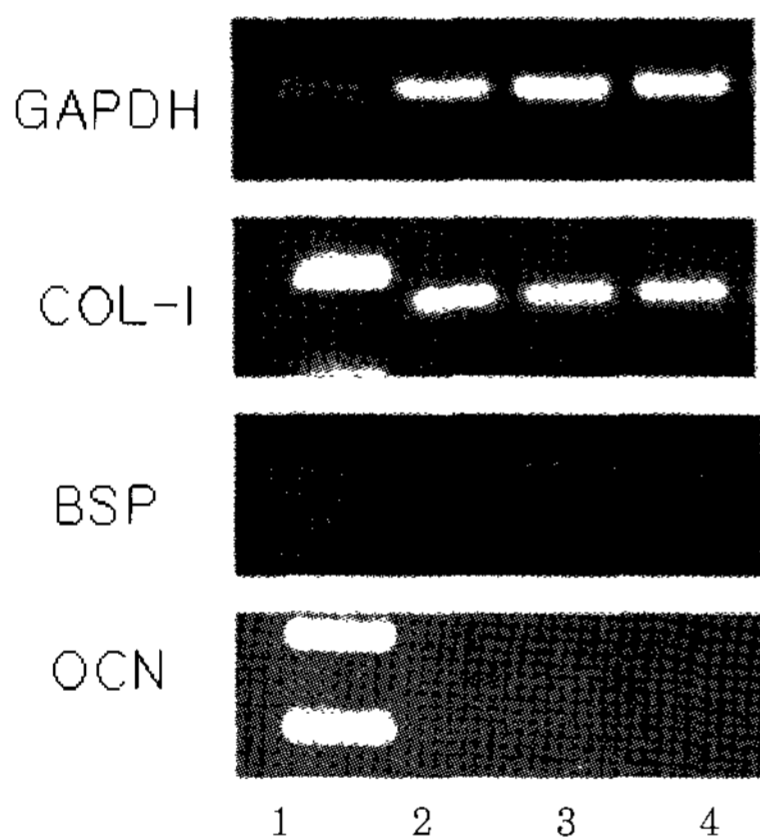


Figure 6. Reverse transcription-polymerase chain reaction (RT-PCR) analysis of collagen type I (COL-I), bone sialoprotein (BSP), osteocalcin (OCN), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression in rat calvarial cells (lane 1: DNA ladder, lane 2: glass, lane 3: cp-Ti, lane 4: Ti-8Ta-3Nb).

5. RT-PCR gene expression analysis

After 7 days culture, the expression of type I collagen gene (COL-I), bone sialoprotein (BSP), and osteocalcin (OCN) 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 glass surfaces, cp-Ti, and Ti-8Ta-3Nb. For COL-I, the expression level of COL-I mRNA was not different on glass, cp-Ti, and Ti-8Ta-3Nb. The level of BSP mRNA expression was not different on glass, cp-Ti, and Ti-8Ta-3Nb. OCN mRNA was not expressed on glass, cp-Ti, and Ti-8Ta-3Nb (Figure 6).

IV. DISCUSSION

One of the most important properties of biomaterials is safety. Metal and alloys are widely used as biomedical materials in medical and dental devices, and the biocompatibility of a metallic alloy is closely associated with the interaction of the alloy with the surrounding environment.

Among biomaterials, commercially pure titanium and titanium alloys are widely used. The biocompatibility of these materials is predom-

inantly due to passive oxide film formed on the metallic surface, which is thermodynamically stable, chemically inert, and shows low solubility in the body fluid¹⁻³⁾. Among titanium alloys employed as metallic implant materials, Ti-6Al-4V has been widely used in dental and medical fields. Although this alloy is still widely used in dental and medical fields, some concerns such as toxicity of V and Al and high elastic modulus, have been reported⁶⁻⁸⁾. New β -type titanium alloys composed of non-toxic elements like Ta, Zr, Mo and Nb with lower moduli of elasticity compared with that of Ti-6Al-4V alloy and good mechanical properties have been developed recently⁹⁻¹⁴⁾.

In this study on biocompatibility, a new β -type titanium alloy Ti-8Ta-3Nb, cp-Ti and Ti-6Al-4V alloy samples were put in direct contact with FRCC, in order to assess the reaction of the cells to these materials.

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 lifespan¹⁶⁾. Established cell lines, on the other hand, have an aneuploid chromosome pattern, tend to multiply rapidly and, if appropriately subcultured, have unlimited lifespan. An advantage of using a permanent cell line is that it provides phenotypically consistent and stable cell populations, large enough for biochemical analysis¹⁶⁻¹⁹⁾. However, it may not always be possible to extrapolate results from osteosarcoma cell cultures because immortalization can affect cellular behavior.

Established cell lines have been used in previous studies investigating cell morphology and cy-

tototoxicity in the presence of various titanium alloys. Primary cell strains derived from living tissues are necessary and have been recommended by the ISO for specific testing to simulate the in vivo situation¹⁷⁾.

In this study, primary osteoblasts were obtained from fetal rat calvaria. These are 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 phenotype²⁰⁾. The primary osteoblasts, used in this study are for assessment of biocompatibility of newly developed Ti-8Ta-3Nb alloy. The biocompatibility test was done by assessment of cell proliferation and differentiation. In this study, fetal rat calvarial cells were used to assess the biocompatibility of Ti-8Ta-3Nb alloy by cell proliferation, alkaline phosphatase activity, and RT-PCR analysis.

Surface roughness and topography can greatly affect the proliferation and protein synthesis of osteoblast cells that are cultured on a metal substrate characteristics in the healing of bone^{21,22)}. Many studies have demonstrated that roughness has a great influence on cell responses. According to this respects, this study showed that all the three test disk surfaces had a similar roughness after they were polished (figure 2).

For each specimen, cell morphology was examined by the SEM. The SEM study showed more cell spreading and higher cell densities on glass than titanium or titanium alloys. Cells had spread extensively and flattened on glass, cp-Ti, and Ti-8Ta-3Nb, Ti-6Al-4V surface.

However, cells grew under orientation of the residual machining grooves (figure 3). The absence of significant morphological modification means to the cytocompatibility of these metals. These appearances of cells were identical to those observed in other studies^{9,23)} involving plastics or titanium and tantalum metals. However, the cells spherical in shape on Ti-6Al-4V surface were detected occasionally (data unshown).

Measurement of cell proliferation were considerably greater on glass than on cp-Ti, Ti-8Ta-3Nb alloy, and on Ti-6Al-4V alloys. Cell proliferation on cp-Ti and Ti-8Ta-3Nb surfaces were not significantly different (figure 4). No reports compared the cell proliferation on cp-Ti with Ti-8Ta-3Nb or with Ti-6Al-4V alloy, this result is similar with other studies^{9,23,24)} showed that cytocompatibility on cp-Ti and β -type Ti alloy was not significantly different. However, the interesting finding of this study is that cell proliferation rate was lower on the Ti-6Al-4V surfaces than on other test surfaces. Okazaki et al²⁵⁾ reported that metallic ions such as Ti, Zr, Sn, Nb, and Ta had evidently no harmful effects on the growth ratios of cells however, Al and V ions presented lower rate as compared to that of titanium ion. Hallab et al²⁶⁾ also showed that cobalt (Co) and V released from Co- and Ti-based alloys respectively, could mediate cell cytotoxicity in the joint periprosthetic milieu. In the regards of these reports, Ti-6Al-4V alloy showed low cell proliferation in this study because of leaching of cell toxic ions such as Al or V into cell culture medium. So this result reflects that the cell proliferation may be affected by Ti chemical composition.

The osteoblastic cells undergo a temporal sequence of phase during the development of their completely differentiated phenotype: proliferation, differentiation, and mineralization²⁷⁾. 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 that play a fundamental role in bone remodelling and represents the specific markers of final differentiation of osteoblasts.

In general, cell synthesis activity is sensitive to the type of material²⁸⁾. Cells grown on glass showed alkaline phosphatase levels showed significantly higher level than on cp-Ti, Ti-8Ta-3Nb, and Ti-6Al-4V ($p < 0.001$, figure 5). Among three metallic test materials, both cp-Ti and Ti-8Ta-3Nb alloy exhibited the similar activity, but showed significantly higher than on Ti-6Al-4V alloy. This may be in case of Ti-6Al-4V alloy, due to the release of Al and/or V ions in culture medium. In particular, vanadium is known to cytotoxic, this might affect cells to protein synthesis. This result means that Ti chemical composition interfered with alkaline phosphatase activity.

In the 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 Ti-8Ta-3Nb alloy was consistent. This means

that cp-Ti and Ti-8Ta-3Nb alloy did not inhibit mineralized matrix expression of FRCC. It is known that BSP and OCN are secreted by osteoblasts and regulate calcification. Surface topography has a direct impact not only on attachment, but also on migration, proliferation and differentiation of osteoblasts. Kawahara et al.²⁹⁾ demonstrated that increased osteogenic effects for cells cultured on Ti with increased surface roughness. They showed that bone growth protein (Gla-OCN) production was reached the highest peak within 10 days on rough surfaces however, BGP production on the plain surfaces was detected by 21 days after culture. The experimental time period in this study was up to 7 days, which might be short for FRCC to differentiate into functional osteoblasts on the smooth cp-Ti and Ti-8Ta-3Nb surfaces. This might be the reason why the expression of OCN was not detected in cp-Ti and Ti-8Ta-3Nb alloy.

In this study, Ti-8Ta-3Nb alloy has similar biocompatibility to cp-Ti as evaluated by rat osteoblast proliferation and differentiation. These properties could make Ti-8Ta-3Nb alloy a useful candidate for the preparation of medical device. However, more animal and human studies will be needed before using this alloy in medical and dental use.

V. CONCLUSION

β -titanium alloys are useful in biomedical application because of their excellent mechanical properties. Titanium-8 Tantalum-3 Niobium (Ti-8Ta-3Nb) has recently developed as a new implant material. Ti-8Ta-3Nb alloy is composed of non-toxic elements and has better

mechanical properties than commercially pure titanium (cp-Ti) or the other $\alpha + \beta$ titanium alloys. However, its biocompatibility has not been adequately investigated.

In this study, the biocompatibility of a new β -type Ti-8Ta-3Nb alloy was assessed by scanning electron microscopy (SEM), cell proliferation, alkaline phosphatase analysis, and reverse transcription polymerase chain reaction (RT-PCR) method.

The results are as follows;

1. Proliferation rate on Ti-8Ta-3Nb alloy was equivalent to cp-Ti alloy and showed better results than on Ti-6Al-4V alloy.
2. In comparison to Ti-6Al-4V alloy, cp-Ti and Ti-8Ta-3Nb alloy enhanced alkaline phosphatase activity in vitro ($p < 0.05$).
3. In RT-PCR analysis, COL-1 and BSP mRNA were expressed in cp-Ti and Ti-8Ta-3Nb alloy.

These results suggest that no differences in aspect of biocompatibility between cp-Ti and Ti-8Ta-3Nb alloy. These properties could make Ti-8Ta-3Nb alloy a new implant material for medical and dental fields.

VI. REFERENCES

1. Park JB, Lakes RS. Biomaterials; an introduction, 2nd ed. New York: Plenum Press;1992:89-92.
2. Rae T. Cell biochemistry in relation to the inflammatory response to foreign materials. CRC Fundamental Aspects of Biocompatibility, Vol. 1, CRC press, Boca Raton, FL, 1981: 159-181.

3. Garcia-Alonso MC, Saldana L, Valles G et al., In vitro corrosion behaviour and osteoblast response of thermally oxidised Ti6Al4V alloy. *Biomaterials* 2003;24:19–26.
4. Semlitsch MF, Weber H, Streicher RM, Schon R. Joint replacement components made of hot-forged and surface-treated Ti–6Al–7Nb alloy. *Biomaterials* 1992;13:781–788.
5. Sykaras N, Lacopino AM, Marker VA, Triplett RG, Woody RD. Implant materials, designs, and surface topographies: Their effect on osseointegration. A literature review. *Int J Oral Maxillofac Implants* 2000; 15:675–690.
6. Okazaki Y, Gotoh E, Manabe T, Kobayashi K. Comparison of metal concentration in rat tibia tissues with various metallic implants. *Biomaterials* 2004;25:5913–5920.
7. Okazaki Y, Gotoh E. Comparison of metal release from various metallic biomaterials in vitro. *Biomaterials* 2005;26:11–21.
8. Hallb NJ, Anderson S, Caicedo M, Brasher A, Mikecz K, Jacobs JJ. Effects of soluble metals on human peri-implant cells. *J Biomed Mater Res* 2005;74A:124–140.
9. Gordin DM, Gloriant T, Texier G, Thibon I, Ansel D, Duval JL, Nagel MD. Development of a β -type Ti–12Mo–5Ta alloy for biomedical applications: cytocompatibility and metallurgical aspects. *J Mat Sci* 2004;15: 885–891.
10. Niinomi M. Recent metallic materials for biomedical applications. *Metal Mater Trans* 2002;33A:477–486.
11. Li SJ, Yang R, Li S, Hao YL, Cui YY, Niinomi M, Guo ZX. Wear characteristics of Ti–Nb–Ta–Zr and Ti–6Al–4V alloys for biomedical applications. *Wear* 2004;257:869–876.
12. Sakaguchi N, Niinomi M, Akahori T, Takeda J, Toda H. Relationships between tensile deformation behavior and microstructure in Ti–Nb–Ta–Zr system alloys. *Mat Sci Eng C* 2005;25:363–369.
13. Park BS. A study on biocompatibility of Ti–8Ta–3Nb alloys with surface modification. MS Thesis, Chonnam National University. 2004:1–75.
14. Cui DZA, Vang MS, Yoon TR. Evaluation of cytotoxicity and biocompatibility of Ti–Ta–Na alloy. *J Korean Acad Prosthodont* 2006;44:250–263.
15. Bretaudivere JP, Spillman T. Alkaline phosphatase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analysis*, vol 4. Weinheim: Verlag Chemica 1984:75–92.
16. Freshney R. *Culture of animal cells: a manual of basic technique*, 3rd edition, NY: Wiley–Liss, Inc. 1994.
17. Schmalz G. Use of cell cultures for toxicity testing of dental materials—advantages and limitations. *J Dent* 1994;22(suppl 2):6–11.
18. Lang H, Mertens T. The use of cultures of human osteoblast-like cells as an in vitro test system for dental materials. *J Oral Maxillofac Surg* 1990;48:606–611.
19. Naganawa T, Ishihara Y, Iwata T et al. In vitro biocompatibility of a new titanium–29niobium–13tantalum–4.6zirconium alloy with osteoblast-like MG63 cells. *J Periodontol* 2004;75:1701–1707.
20. Luben RA, Wong GL, Cohn DV. Biochemical characterization with parathyroid hormone and calcitonin of isolated bone cells: provisional identification of osteoclasts and osteoblasts. *Endocrinology* 1976;99:526–534.
21. Postiglione L, Di Domenico G, Ramaglia L, Lauro AE, Di Meglio F, Montagnani S. Different titanium surfaces modulate the

- bone phenotype of SaOS-2 osteoblast-like cells. *Eur J Histochem* 2004;48:213-222.
22. Bachle M, Kohal RJ. A systematic review of the influence of different titanium surfaces on proliferation, differentiation and protein synthesis of osteoblast-like MG 63 cells. *Clin Oral Implants Res* 2004;15:683-692.
 23. Lavos-Valereto IC, Wolyneec S, Deboni MC, Konig B Jr. In vitro and in vivo biocompatibility testing of Ti-6Al-7-Nb alloy with and without plasma-sprayed hydrox-yapatite coating. *J Biomed Mater Res* 2001;58:727-733.
 24. Niinomi M. Fatigue performance and cytotoxicity of low rigidity titanium alloy, Ti-29Nb-13Ta-4.6Zr. *Biomaterials* 2003;24:2673-2683.
 25. Okazaki Y, Rao S, Ito Y, Tateishi T. Corrosion resistance, mechanical properties, corrosion fatigue strength and cyto compatibility of new Ti alloys without Al and V. *Biomaterials* 1998;19:1197-1215.
 26. Hallab NJ, Anderson SA, Caicedo M et al. Effects of soluble metals on human peri-implant cells. *J Biomed Mater Res* 2005;74A:124-140.
 27. Lian JB, Stein GS. The developmental stages of osteoblast growth and differentiation exhibit selective responses of genes to growth factors and hormones. *J Oral Implantol* 1993;19:95-105.
 28. Lincks J, Boyan BD, Blachard CR. Response of MG63 osteoblast-like cells to titanium and titanium alloy is dependent on surface roughness and composition. *Biomaterials* 1998;19:2219-2232.
 29. Kawahara H, Soeda Y, Niwa K et al. In vitro study on bone formation and surface topography from the standpoint of biomechanics. *J Mat Sci* 2004;15:1297-1307.

백서 태자 두개관세포에서 Ti-8Ta-3Nb 합금의 생체적합성

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타이타늄은 기계적 특성이 우수하고 생체적합성이 뛰어나 의료용 장비의 주 재료로 사용되고 있으며 타이타늄 보다 기계적 특성이 더 우수한 타이타늄 합금들(주로 Ti-6Al-4V와 Ti-6Al-7Nb 합금)도 개발되어 치과와 의료용 임플란트로 사용되고 있다. 그러나 타이타늄 합금 성분들 중 알루미늄 (aluminum)과 바나듐 (vanadium)은 인체에 노출되면 세포손상과 신경계에 문제를 일으킬 수 있다. 따라서 인체에 독성이 없으면서 기계적 성질과 생체적합성이 우수한 타이타늄 합금의 개발이 필요하다. 최근 인체에 독성이 없는 성분들이 함유된 새로운 β -형태의 타이타늄 합금들이 개발되고 있는데, β -타이타늄 합금은 그 기계적 성질이 기존의 $\alpha + \beta$ 타이타늄 합금에 비해 우수하다고 알려져 있다. 최근 새로운 β -타이타늄 합금이 전남대학교 부설 타이타늄 연구소에서 개발되었다.

이 연구는 새로 개발된 β -타이타늄 합금의 생체 적합성을 세포 증식도, 알카리 인산 분해 효소 활성과 유전자 증폭법을 통해 알아보려고 하였다.

그 결과는 다음과 같다;

1. Titanium-6aluminum-4vanadium (Ti-6Al-4V) 합금 표면에서의 세포 증식율은 Titanium-8Tantalum-3Niobium (Ti-8Ta-3Nb) 합금과 순수 타이타늄 표면에 비해 유의하게 낮았다 ($p < 0.001$). Ti-8Ta-3Nb 합금 표면에서의 증식도는 순수 타이타늄 표면과 유사하였다.
2. Ti-8Ta-3Nb 합금과 순수 타이타늄에서 배양된 세포의 알카리 인산 분해 효소의 활성도는 Ti-6Al-4V 합금에서의 것보다 유의하게 높았다 ($p < 0.001$).
3. 유전자 증폭 분석 결과, Ti-8Ta-3Nb 합금과 순수 타이타늄에서 collagen type I과 bone sialoprotein mRNA 가 유사한 수준으로 발현되었다.

이상의 결과는 생체 적합성 측면에서 Ti-8Ta-3Nb 합금과 순수 타이타늄의 차이가 없음을 보여주며 따라서 Ti-8Ta-3Nb 합금이 의학 및 치의학 영역에서 새로운 임플란트 재료로 사용될 수 있음을 의미한다.