

Nano-Surface Behavior of Osteoblast Cell-Cultured Ti-30(Nb,Ta) with Low Elastic Modulus

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ABSTRACT

Titanium and Titanium alloys have been used for dental implants and load bearing bone-anchored implants. The nano-surface behavior of osteoblast cell-cultured Ti-30(Nb,Ta) with low elastic modulus have been investigated using various electrochemical methods. Surfaces of Ti-30(Nb,Ta) samples were polished and sandblasted. Specimen surfaces were cultured with MC3T3-E1 cells for 2 days. Corrosion behavior was investigated using a potentiostat, and electrochemical impedance spectroscopy was performed (10 mHz to 100 kHz) in 0.9% NaCl solution at $36.5 \pm 1^\circ\text{C}$. The microstructures of the Ti-30(Ta, Nb) alloys had a needle-like appearance. In sandblasted Ti-30(Nb,Ta) alloy, the pitting potential decreased and in cell-cultured Ti-30(Nb,Ta) alloy, passive current density increased as R_a increased. The polarization resistance decreased as R_a increased, whereas, R_a for cell-cultured Ti alloys increased compared to non-cell-cultured Ti alloys.

Keywords: titanium and titanium alloys, osteoblast cell, pitting potential, surface roughness, polarization resistance

1 INTRODUCTION

Ti and Ti-alloys have good biocompatibility, appropriate mechanical properties and excellent corrosion resistance. However, the widely used Ti-6Al-4V is found to release toxic ions (Al and V) into the body, leading to undesirable long-term effects. Ti-6Al-4V has much higher elastic modulus (100-120GPa) than cortical bone (10-30 GPa) [1]. Therefore, titanium alloys with low elastic modulus have been developed as biomaterials to minimize stress shielding [2].

Recently, Ti-Nb and Ti-Ta based alloy systems have been studied and found to display both lower elastic moduli and higher tensile strengths than are common for metals and alloys. Nb, and Ta can be stabilized to β -phase of Ti alloy and β -phase structure exhibits about 60-80 GPa of Young's modulus [1]. Some researcher reported that the Ti-30%Ta alloy with martensite α'' -phase has the potential to become a new candidate for biomedical application due to its good combination of low modulus and high strength. To improve bone tissue integration on implant surfaces, various techniques have been used to increase the

roughness of the implant surfaces [3-4]. Cell adhesion and proliferation depend on surface roughness [5] and metal ion dissolution [6]. That is, cell adhesion is a very specific parameter and describes the relative adherence of a cell to its substrate, generally at an early stage of culture when the cells are directly in contact with the material surface [5]. Metal ion released from metallic materials implanted into human body is usually accelerated by chemical species in the body. Metal ion dissolution is inhibited by the surface oxide as a passive film [6], whereas toxicity elements act inhibitors against the cell adhesion and proliferation.

In this study, nano-surface behavior of osteoblast cell-cultured Ti-30(Nb,Ta) with low elastic modulus have been investigated using various electrochemical methods.

2 EXPERIMENTAL

Binary Ti-30Ta and Ti-30Nb alloys were prepared by using high purity sponge Ti (G&S TITANIUM, Grade. 4, USA), Ta and Nb sphere (Kurt J. Lesker Company, 99.95% wt.% in purity). Two kinds of Ti alloys prepared using the vacuum arc melting furnace. The weighed charge materials were prepared by the vacuum arc furnace, flowing the purified Ar gas into water cooling copper hearth chamber in vacuum atmosphere of 10^{-3} torr, and controlled atmosphere in chamber by method to keep vacuum again. Also, before melting, the constituents were cleaned with methanol to minimize oxygen quantity and surface contaminants in chamber and pure Ti was melted six times with purified argon gas. After that, melting treatment was carried out more than six times by reversing the alloy sponges in order to homogenize, and each time was held in the molten state for 5 min. in a high purity argon atmosphere by arc furnace with water-cooled copper hearth and solution treatment was carried out for 6hrs at 1000°C in Ar gas atmosphere.

Surfaces of test samples were treated as follows: $0.3\mu\text{m}$ polished; $25\mu\text{m}$, $50\mu\text{m}$ and $125\mu\text{m}$ sandblasted. Specimen surfaces were cultured with MC3T3-E1 cells for 2 days. Average surface roughness (R_a) and morphology of specimens were determined using a surface profilometer, OM, and FE-SEM.

In order to investigate the corrosion behavior, potentiodynamic test (potential range of $-1500 \sim 2000$ mV) was carried out using a potentiostat(EG&G PARSTAT 2273) test at scan rate of 1.67 mV/sec in 0.9% NaCl solution

at $36.5 \pm 1^\circ\text{C}$. Electrochemical impedance spectroscopy was performed (10 mHz to 100 kHz) in 0.9% NaCl solution at $36.5 \pm 1^\circ\text{C}$. All electrochemical characteristics were performed in a standard three-electrode cell having specimen as a working electrode and a high carbon as counter electrode. The potential of working electrode was measured against a saturated calomel electrode (SCE) and all given potentials were referred to this electrode. After electrochemical corrosion tests, the surfaces of each specimen were investigated by using FE-SEM.

3 RESULTS AND DISCUSSION

Figure 1 is OM and SEM micrographs showing the microstructure of homogenized Ti alloy surface. Figure 1(a) and (c) show the microstructure of Ti-30Nb alloy. Figure 1(b) and (d) show the microstructure of Ti-30Ta alloy, respectively. The microstructures of the Ti-30(Ta,Nb) alloys had a needle-like appearance. It is consistent with pre-researched results[7,8] that the quenched alloys exhibit lamellar martensite α' structure at a Ta content below 20wt%, needle-like orthorhombic martensite α'' structure at a Ta content from 30 to 50wt%. These photos show the two phases, α and β -phase, and it is estimated that the white part was α phase and black part was β -phase. The α -phase, white image in figure, decreases as the amount of Ti and Nb increases[8]. We confirmed that the microstructure changes from α phase to β -phase through XRD and β phase increases according to the amount of Nb added[8]. With these results, we could make an analogical inference that Nb and Ta is the β stabilizing element as the β -phase increases according to the addition of Nb and Ta.

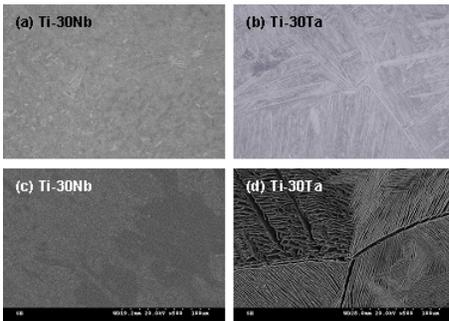


Figure 1: OM and SEM micrographs showing the microstructure of homogenized Ti-30Nb(a,c) and Ti-30Ta(b,d) alloy surface(X 500). (a),(b) OM (c),(d) SEM.

Figure 2 shows SEM micrographs of the non-cell cultured Ti-30Nb surface. Figure 2 (a) is polished, (b) is 25 μm sandblasted, (c) is 50 μm sandblasted, and (d) is 125 μm sandblasted surface of samples. It is confirmed that the surface roughness increased as size of aluminum oxide particle increased. The R_a of polished Ti-30Ta and Ti-30Nb alloys was lower than that of the sandblasted Ti alloy from Table 1.

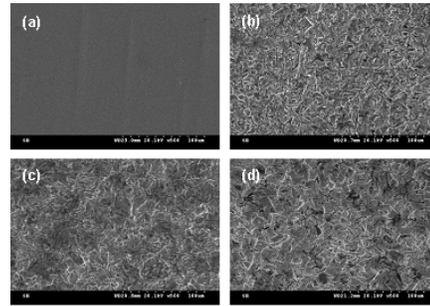


Figure 2: SEM micrographs showing the non-cell cultured Ti-30Nb surface. (a) polished (b) 25 μm sandblasted (c) 50 μm sandblasted (d) 125 μm sandblasted.

Table 1: Average surface roughness values(R_a : μm) of surface modified Ti alloys.

Samples	Alloys	Ti-30Nb	Ti-30Ta
polished		0.1199	0.1131
25 μm sandblasted		0.3902	0.3875
50 μm sandblasted		0.6063	0.5824
125 μm sandblasted		0.7923	0.7810

Figure 3 shows SEM micrographs of MC3T3-E1 cell cultured on Ti-30 Nb surface. Figure 3(a) is polished, (b) is 25 μm sandblasted, (c) is 50 μm sandblasted, and (d) is 125 μm sandblasted specimen surface. On polished and 25 μm sandblasted surface, the cell were more widely spread with lamellipods[9] demonstrating cell migration and no particular orientation of cells was observed. Cell growth and size increased and more cytoplasmic prolongations were observed at rough surfaces. Cultured cells displayed round shapes. Cells were well-cultured on the polished surfaces compared to sandblasted alloy samples.

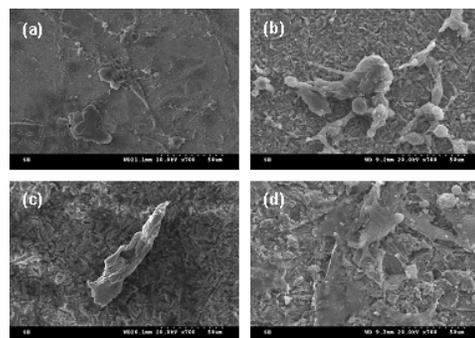


Figure 3: SEM micrographs showing MC3T3-E1 cell cultured on Ti-30 Nb surface. (a) polished (b) 25 μm sandblasted (c) 50 μm sandblasted (d) 125 μm sandblasted.

Figure 4 and 5 show the potentiodynamic polarization curves of non-cell and cell cultured Ti-30Nb(a) and Ti-30Ta(b) alloys after potentiodynamic test in 0.9% NaCl solution at $36.5 \pm 1^\circ\text{C}$. It could see that the corrosion

potentials(E_{corr}) of Ti-30Nb and Ti-30Ta show similar potential(-400~-500mV), but current density in passive region largely decreased as R_a increased. It is confirmed that the pit formed by aluminum oxide particles increased on the surface and this pit caused site to be more susceptible to aggressive ion like Cl^- [3,8]. Whereas polished surface shows a stable passive region in anodic polarization curves because Ti combines with oxygen and forms a stable immunity such as the TiO_2 film on the surface. Anodic polarization curves of cell-cultured Ti alloys showed unstable behavior in the passive region compared to non-cell-cultured alloys.

In sandblasted Ti-30(Nb,Ta) alloy, the pitting potential(E_{pit}) decreased and passive current density(I_{pass}) of cell-cultured Ti-30(Nb,Ta) alloy increased as R_a increased because covered cell on the surface has better corrosion resistance than non-cell cultured Ti alloy. It is assumed that the cell plays a role to improve the corrosion resistance like passive film and it prohibits the active corrosion site formed by sandblasting treatment. The presence of cells on Ti-30(Nb,Ta) alloy alloy leads to an increase in passivation of metals. Table 2 shows the polarization test results and also shows E_{pit} and I_{pass} value. In case the Ti-30Nb alloy, E_{pit} is higher than that of Ti-30Ta alloy.

Table 2: The passive current density(I_{pass}) and pitting potential(E_{pit}) of MC3T3-E1 cell cultured Ti-30Nb and Ti-30Ta alloys from anodic polarization curves.

Sample		$I_{pass}(A/cm^2)$	$E_{pit}(mV)$
Ti-30Nb	polished	9.63×10^{-7}	-
	25 μm sandblasted	1.43×10^{-6}	1339
	50 μm sandblasted	3.45×10^{-6}	1335
	125 μm sandblasted	5.95×10^{-6}	1331
Ti-30Ta	polished	5.19×10^{-7}	-
	25 μm sandblasted	2.06×10^{-6}	1328
	50 μm sandblasted	2.23×10^{-6}	1318
	125 μm sandblasted	3.06×10^{-6}	1310

Figure 6 and 7 show Bode plot of non-cell and cell cultured Ti-30Nb(a) and Ti-30Ta(b) alloys after AC impedance test in 0.9% NaCl solution at $36.5 \pm 1^\circ C$. The polarization resistance(R_p)of non-cell cultured alloy is lower than that of cell cultured alloy. From impedance tests of sandblasted and cell-cultured alloys, the polarization resistance decreased as R_a increased, whereas, R_a for cell-cultured Ti alloys increased compared to non-cell-cultured Ti alloys as shown in Table 3 and 4. The high value of R_p implies a high corrosion resistance of alloy, that is, a low rate of released metallic ion into the electrolytic solution or cell on the surface. The Bode plot results indicated that the corrosion behavior of alloy in solution was under charge-transfer controlled because of the local variation of aggressive ion like Cl^- , preferentially attacking or damaging the oxide film and covered cell. The Bode plots for all the alloys showed near capacitive response in the lower and middle frequency region which was characterized by slope ≈ -1 in the $\log|z|$ vs. $\log(f)$ curve[10]. It can also be observed that the $|z|$ values in high frequency region increased as R_a decreased and cell proliferation increased. In conclusion, the R_p of Ti and Ti-6Al-4V alloy cultured with cells increases with the cell growth, including adhesion, spreading, and proliferation.

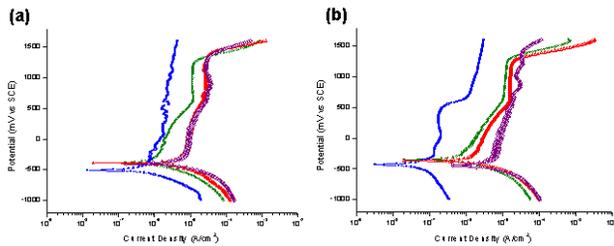


Figure 4: Potentiodynamic polarization curves of non-cell cultured Ti alloys after potentiodynamic test in 0.9% NaCl solution at $36.5 \pm 1^\circ C$. (a) Ti-30Nb (b) Ti-30Ta. (■ 0.3 μm , ● 25 μm , ▲ 50 μm , ◆ 125 μm)

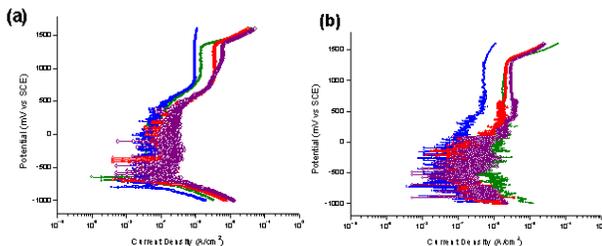


Figure 5: Potentiodynamic polarization curves of MC3T3-E1 cell cultured Ti alloys after potentiodynamic test in 0.9% NaCl solution at $36.5 \pm 1^\circ C$. (a) Ti-30Nb (b) Ti-30Ta. (■ 0.3 μm , ● 25 μm , ▲ 50 μm , ◆ 125 μm)

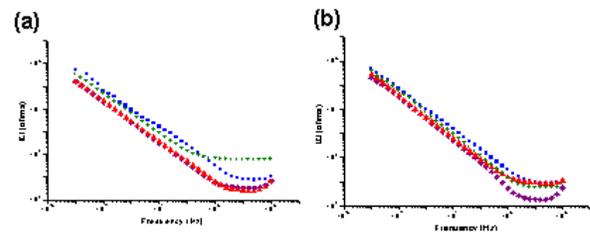


Figure 6: Bode plot of non-cell cultured Ti alloys after AC impedance test in 0.9% NaCl solution at $36.5 \pm 1^\circ C$. (a) Ti-30Nb (b) Ti-30Ta. (■ 0.3 μm , ● 25 μm , ▲ 50 μm , ◆ 125 μm)

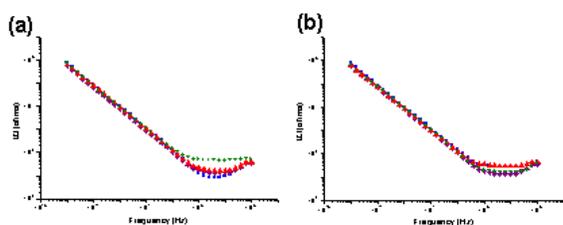


Figure 7: Bode plot of MC3T3-E1 cell cultured Ti alloys after AC impedance test in 0.9% NaCl solution at 36.5 ± 1 °C. (a) Ti-30Nb (b) Ti-30Ta. (■) 0.3 μm , (●) 25 μm , (▲) 50 μm , (◆) 125 μm

Table 3: EIS parameters of non-cell cultured Ti implant alloys.

Sample		$R_p(\Omega\text{cm}^2)$	$R_s(\Omega\text{cm}^2)$
Ti-30Nb	polished	473,762	7.48
	25 μm sandblasted	354,042	7.87
	50 μm sandblasted	159,287	2.55
	125 μm sandblasted	155,827	3.25
Ti-30Ta	polished	521,413	8.40
	25 μm sandblasted	366,975	5.43
	50 μm sandblasted	277,332	8.41
	125 μm sandblasted	197,258	1.78

Table 4: EIS parameters of MC3T3-E1 cell cultured Ti implant alloys.

Sample		$R_p(\Omega\text{cm}^2)$	$R_s(\Omega\text{cm}^2)$
Ti-30Nb	polished	794,832	27.16
	25 μm sandblasted	647,284	15.93
	50 μm sandblasted	611,169	20.84
	125 μm sandblasted	583,424	29.24
Ti-30Ta	polished	797,973	28.04
	25 μm sandblasted	719,516	34.38
	50 μm sandblasted	630,216	14.27
	125 μm sandblasted	592,343	16.65

4 CONCLUSIONS

The microstructures of Ti-30Ta and Ti-30Nb alloys showed the needle-like structure, especially in Ti-30Ta alloy. The R_a of polished Ti-30Ta and Ti-30Nb alloys was smoother than that of sandblasted Ti alloy. In the case of sandblasted Ti-30Ta alloy, R_a decreased in compared with Ti-30Nb alloy. MC3T3-E1 cells cultured on the Ti-30Nb and Ti-30Ta alloy were a round shape. In the case of polished sample, cells were well cultured on the surface of Ti-30Nb and Ti-30Ta alloy in compared with sandblasted alloy. Cell growth and size increased and more cytoplasmic prolongations were observed at rough surfaces. E_{pit} decreased and I_{pass} increased as surface roughness increased. Anodic polarization curves of cell cultured Ti alloy showed

unstable behaviors in passive region in compared with non-cell cultured Ti alloy due to covered cells. R_p decreased as R_a increased, whereas, R_p of cell-cultured Ti alloy increased in compared with non-cell cultured Ti alloy.

In conclusion, corrosion resistance decreases as surface roughness increases. Cell cultured surface shows the good corrosion resistance against Cl^- ion like halides.

ACKNOWLEDGEMENTS

“This work was supported by research funds from Chosun University(2004), KEMCO and LG Yonam Foundation.”

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