Biological and Biomechanical Properties of Chemically Modified SLA Titanium Implants \textit{In Vitro} and \textit{In Vivo}

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Abstract. The objective of this study was to evaluate the interface shear strength and the responses of osteoblast-like cells to titanium implants with a sandblasted and acid-etched surface modified by alkali and heat treatments (SLA-AH). The implants with machined and SLA surface served as controls. Each type of implant was characterized by scanning electron microscopy (SEM) and energy-dispersive x-ray (EDX) analysis. In vitro assays were made using human osteoblast-like cell culture on different surfaces. The rectangle plates were also transcortically implanted into the proximal metaphysis of New Zealand White rabbit tibiae. After 4, 8 and 12 weeks implantation, mechanical and histological assessments were performed to evaluate biomechanical and biological behavior \textit{in vivo}. By SEM examination, SLA surface combined with AH treatments revealed a macro-rough surface with finely microporous structure. The \textit{in vitro} assays showed that the SLA-AH surfaces exhibited more extensive cell deposition and improved cell proliferation as compared with controls. Pull-out test demonstrated that the SLA-AH treated implants had a higher mechanical strength than the controls at all interval time after implantation. Histologically, the test implants revealed a significantly greater percentage of bone-implant contact when compared with controls. The results of this study suggest that a useful approach by combined processes could optimize implant surfaces for bone deposition and produce distinct biological surface features.

Introduction

Titanium (Ti) and its alloys have widely proved to have good mechanical properties and to be biocompatible as orthopedic and dental implants, but data on Ti implant failures acquired over the past decade suggest that some osseointegration and bone-bonding problems still exist. To solve these problems, there have been many studies on the surface modification to produce rough titanium surfaces\textsuperscript{[1]} or to coat bioactive ceramics\textsuperscript{[2]}. It has been shown that surface blasting and acid etching can increase the rate and amount of bone formation on the implant surface\textsuperscript{[3]} and that the sandblasted and acid-etched (SLA) surface demonstrated higher removal torque values in biomechanical testing\textsuperscript{[4,5]}. Besides surface topography, surface bioactivity is another key variable for bone apposition to implants\textsuperscript{[6]}. The most usual method is to coat bioactive ceramics like hydroxyapatite by plasma spraying technique. It has shown to enhance bioactivity of Ti implants\textsuperscript{[7]}. However, the coated hydroxyapatite is so brittle that it may causes many problems including degradation in a living body due to the inhomogeneous composition distribution\textsuperscript{[8]}. Recently, it has been demonstrated that Ti implants with alkali- and heat- treatments (AH) remarkably enhanced bone bonding ability and early bone formation\textsuperscript{[9]}.
In this study, we verified the hypothesis that the surface topographies introduced by combination of chemical processes could be more effective in enhance bone apposition than surfaces produced by topography alone. Biological behavior and biomechanical properties of a newly SLA-AH treated Ti implant were investigated to examine the effects of surface topographies with chemical process on interface shear strength and cell responses.

Materials and methods

Implants preparation. Plates of commercially pure titanium (10x12x2mm) were made and divided into three groups of treatments: Group A was untreated; Group B was sandblasted and then etched with mixed acid of 1:1 HCL and H\textsubscript{2}SO\textsubscript{4}; Group C was SLA treated followed by immersion in 6N NaOH solution at 60 °, then heated at 600 ° for 1 h. The SLA and SLA-AH surface were characterized by scanning electron microscopy (SEM) and energy-dispersive x-ray (EDX) analysis.

Cell culture. Human osteoblast-like cells MG-63 were used for in vitro assays. The cell culture was performed on implants in 6-well cell culture plates. Cells were plated at 9300 cells cm\textsuperscript{-2} in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and 0.5% antibiotics (diluted from a stock solution containing 5000 Uml\textsuperscript{-1} penicillin, 5000 Uml\textsuperscript{-1} streptomycin) and cultured at 37°C in an atmosphere of 100% humidity and 5% CO\textsubscript{2}.

Surgical procedures. The rectangle implants were sterilized conventionally with ethylene oxide. The plates were trans cortically implanted into the proximal metaphyses of bilateral tibiae of mature New Zealand white rabbits (2.6 ~ 3.2 kg). The rabbits were anesthetized by an intravenous injection of pentobarbital sodium (0.5 ml kg\textsuperscript{-1}) and local administration of 0.5% lidocaine. In each procedure, a 3 cm skin incision was made on the medial side of the knee, and the fascia and periosteum were also cut and retracted to expose the tibia. Using a dental bur, a 10×2 mm hole was made from the medial to the lateral cortex parallel to the longitudinal axis of the tibial metaphysis. After irrigating the holes with saline, the titanium plates were implanted in the frontal direction. Five rabbits of each group were killed at 4, 8 and 12 weeks after the operation.

Biomechanical testing. Interfacial shear strength of implants with different surfaces was measured by pull-out testing. After the rabbits were scarified with an overdose of pentobarbital sodium, the segments of the proximal tibial metaphyses containing the implanted plates were cut out and prepared for the pull-out test. The bone tissue surrounding the plates was removed with a dental burr. Steel wire was used for holding plates through the hole at the top midst of implants (Fig.3).

The thickness of the cortical bone in contact with the implant was measured for each pull-out sample. The thickness was used to determine the contact area according to the following formula: interface area=2×{implant width +2mm}× cortical thickness. The shear strength at the interface was calculated by dividing the load at failure by the interfacial area. All data were expressed as mean ± standard deviation (SD) and assessed using a one-way ANOVA and Fisher’s PLSD method as a post hoc test. Differences of $P<0.05$ were considered to be statistically significant.

Histological examination and SEM observation. For histological examination, the undecalcified sections were ground to a thickness of about 80 μm. After toluidine blue staining, the sections were examined by light microscopy. A SEM was used to examine metal surfaces after cell culture and pull-out testing.

Results and Discussion

The SEM examination revealed that a macro-rough surfaces structure was formed after the sandblasted and dual acid treatments. After alkali and heat treatment, the implant surface showed finely irregular microporous structure (Fig.1).
Cell culture on the SLA-AH surfaces showed that the osteoblast-like cell had spread more extensively and flattened on the SLA-AH plate surface at 5 days as compared with controls (Fig.2). The result of the cells proliferation indicated that the number of cells deposited on the SLA-AH surface was higher than that of SLA or untreated surfaces (p< 0.05).

The results of the pull-out test are summarized in Table1. At 4 weeks, the untreated implants showed low shear strengths. All of the SLA- and SLA-AH treated implants showed significantly higher shear strengths than the untreated implants, and so as to SLA-AH than SLA. At 12 weeks, the shear strengths of all implants had increased as compared with the 4-week assessment, but the value of both group A and B are still lower than that of group C.

<table>
<thead>
<tr>
<th>Groups</th>
<th>4weeks</th>
<th>8weeks</th>
<th>12weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.37±0.33</td>
<td>0.74±0.66</td>
<td>0.94±0.81</td>
</tr>
<tr>
<td>B</td>
<td>0.48±0.31</td>
<td>1.29±0.71</td>
<td>1.42±0.94</td>
</tr>
<tr>
<td>C</td>
<td>3.45±1.87</td>
<td>3.68±1.95</td>
<td>3.79±1.98</td>
</tr>
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Table 1. The results of pull-out testing (Sheer strength MPa)

| p-value | **<0.0001; *<0.001 | **<0.0001, *<0.001 | **<0.0001, *<0.001 |

Values are means ± SD; n=5. **means group C vs. group A; *means group C vs. group B.
No foreign body or inflammatory reaction was noted with every implant throughout the study. For the SLA-AH treated implants, new bone formed in the gap created at the implantation site within 4 weeks, and the new bone was in direct contact with the implant. In the groups of the untreated and SLA treated implants, an intervening fibrous layer or a small amount of bone was observed. At 12 weeks, the untreated and SLA-treated implants had more amount of new bone, but in the meanwhile, the fibrous tissue at the interface between the bone and the implant was much thicker than that at 4 weeks. Scanning electron microscopy revealed almost the same findings as those noted in the toluidine blue staining samples (Fig. 4).

It was considered that sandblasting prior to acid etching may lead to a superimposed macroroughness on top of the acid produced micro-roughness. The added bone anchorage may be gained by the sandblasting step. Our results suggested that sandblasting before acid etching has a beneficial effect on the interfacial shear strength. However, the manner in which titanium interacts with bone is complex and not only dependent on surface topography. Other factors such as the chemical composition of the surface play a crucial role in early stages of bone formation. We developed SLA-AH surface and compared to those data, the results suggest that SLA surface following by alkali and heat treatments could result in a dramatic increase in bony bonding and osteoblast-like cells deposition, leading to better biological performances. It indicates that the surface topographies introduced by combination of chemical processes, exemplified here by alkali and heat treatments, can be more significantly and more effective in enhance bone-implant contact and cell apposition than surfaces produced by topography process alone.

**Conclusion**

Our results demonstrated that the SLA-AH surface promoted bone apposition during early stages of bone regeneration and provided a promising method for orthopedic and dental implants of clinical application. It also suggests that a systematic approach to the optimization of Ti surfaces for bone-bonding ability should consider utilizing combinations of processes each of which modifies the topography and bioactivity of the implant surfaces.

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**References**