Effect of acid-etching on the production of inflammatory cytokine in 3D-printed titanium alloy implant

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INTRODUCTION:

In total joint arthroplasty, various surface modification techniques such as hydroxyapatite coating [1] and three dimensional (3D) porous structures [2, 3] have been developed to improve osseointegration. The successful osseointegration of implant depends on the orchestrated activities of both bone and immune cells. The acid-etching is a chemical surface modification that has been demonstrated to enhance the alkaline phosphatase activity [4]. However, the effect of the acid-etching on immune responses was not fully understood. In this study, we evaluated the inflammatory responses induced by the acid-etched surfaces using macrophage cell. The cellular metabolic activity and inflammatory cytokine secretion were investigated. Since the acid-etching has been widely applied to remove residual particles on the surface of 3D printed implants, we used 3D printed titanium alloy.

METHODS:

Ti-6Al-4V samples (φ14 mm × 2 mm) were additively manufactured via electron beam melting (EBM) technique. The fabricated samples were ultrasonically cleaned with the alkaline solution (Control-group), followed by treatment with a solution of fluorine nitric acid and hydrogen peroxide (Acid-group). The surface topography was observed using field-emission scanning microscope (FE-SEM) at an accelerated voltage of 5 kV. The surface roughness of the fabricated specimens was analyzed using three dimensional (3D) laser scanning microscope. Five different areas of each specimen were scanned, and the arithmetical mean height (*Sa*) was measured. All samples were sterilized with gamma irradiation prior to the cell test.

The mouse macrophage cell line RAW264 at a density of 1.0×10^5 cells/well were seeded on the samples. The cells were incubated in D-MEM containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37 ˚C and in 5% CO_2 for 1 and 3 days. The metabolic activity of cells were assessed using Alamar Blue assay according to the manufacturer's protocol. The fluorescent intensity (excitation: 570 nm, emission: 595 nm) was measured using a microplate reader. The supernatants after 1 and 3 days of culture were collected, and the secretion level of TNF-α (tumor necrosis factor-alpha) was quantitatively evaluated by enzyme-linked immunosorbent assay (ELISA).

For statistical analysis, Student's t-test was used. $P < 0.05$ was considered significant.

RESULTS:

3D laser scanning microscope analysis showed no significant differences in *Sa* between Control-group and Acid-group (data not displayed). FE-SEM images of each sample were shown in Figure 1. The surface of the Acid-group was confirmed signs of etching, and the beta phase structures were observed. Figure 2 shows the result of the cellular metabolic activity on the samples. There were no significant differences between Control-group and Acid-group in the fluorescence intensity. The levels of TNF-α secretion after cell cultivation are represented in Figure 3. Acid-group exhibited significantly lower levels of TNF-α in comparison to the Control-group.

DISCUSSION:

In the present study, we elucidated the macrophage response to the acid-etched 3D printed Ti-6Al-4V surfaces. No statistical differences in terms of cellular metabolic activity between Control and Acid groups were shown, indicating that acid etching had no effect on the cell adhesion and proliferation. Notably, the secretion levels of TNF-α was significantly reduced by acid-etching process. TNF-α is known as a pro-inflammatory cytokine, and leads to facilitate osteoclast differentiation and bone resorption [5]. This implies that the acid etching has the potential to suppress the bone resorption around 3D printed titanium alloy surfaces, and might lead to accelerate osseointegration of the implant. Further experiments are necessary to clarify the underlying mechanism through which acid-etching inhibits inflammatory cytokines.

SIGNIFICANCE:

Our finding could provide a surface modification strategy of 3D printed titanium alloy for orthopedic implant development.

REFERENCES: [1] Greesink RG et al, *Orthopedics*, 12 (1989) 1239-1242. [2] Bobyn JD et al., *JBJS Br*, 81 (1999) 907-914. [3] Dall'Ava L et al., *Metals*, 9 (2019) 729. [4] Zhao G et al, *J. Biomed. Mater. Res*, 74A (2005) 49-58. [5] Luo G et al., *Mol Med Rep*, 17 (2018) 6605-6611.

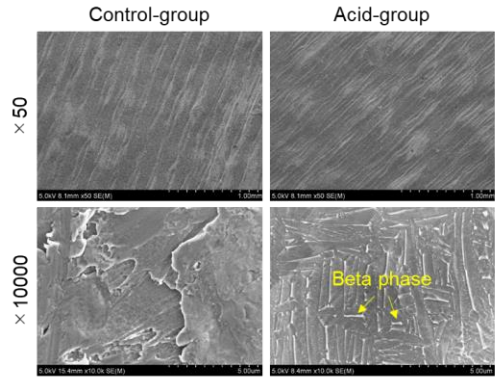

Figure 1. FE-SEM images of each sample at magnifications of 50× and 10000×. Yellow arrows indicate beta phase pits of titanium alloy.

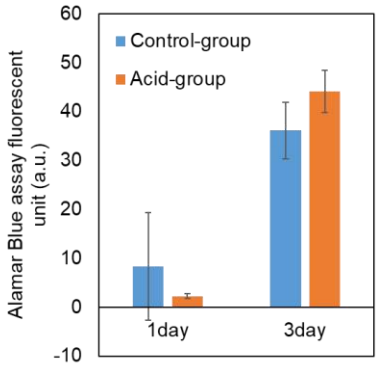

Figure 2. The result of Alamar Blue assay. There were no significant differences between Control-group and Acid-group.

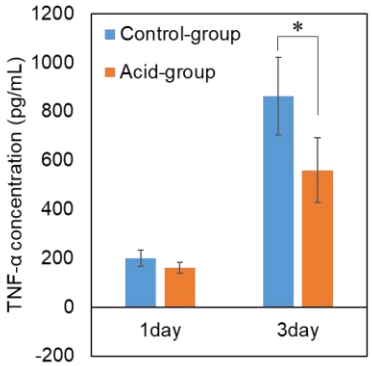

Figure 3. Quantitative results of TNF-α secretion using ELISA analysis. * $P < 0.05$