

Effects of Ca-Ozone Treatment on Initial Cell Attachment to Titanium

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Introduction: Improvement of osteoconductivity of titanium (Ti) is strongly desired to allow faster bone loading. Alkali&heat (AH) treatment increased the degree of osteoconductivity and its clinical use was approved in Japan as AHFIT[®]. However, further improvement of osteoconductivity is still strongly required. It is reported that Ca is bonded on the surface of AH treated Ti when implanted, and Ca plays important role for the improved osteoconduction. If so, modification of Ti with Ca is thought to be effective since larger amount of Ca can be bonded on the Ti surface since harder condition can be applied to the Ti outside the body. In fact, hydrothermal treatment of Ti in the presence of CaCl₂ result in the fabrication of Ca modified Ti and the Ti showed improved osteoconductivity. In the present study modification of Ti with larger amount of Ca was studied based on ozone (O₃) treatment in the presence of CaCl₂. Also, its effect on initial cell attachment was studied using osteoblasts.

Methods: Mirror-polished pure Ti discs were ultrasonically washed in acetone followed by distilled water (DW) for 10 min each. These discs without post-treatment were denoted as "Ti". In order to form titanium oxide layer (TiO₂), Ti discs were heated at 400°C for 1hr under an ambient condition. They were denoted as "HT". HT discs were treated in O₃-dissolved DW or 10 mM CaCl₂ at 25°C for 24 hrs. They were denoted as "HT+O₃+DW" and "HT+O₃+Ca", respectively. For comparison, HT discs were soaked in DW or 10 mM CaCl₂ without O₃ at 25°C for 24 hrs. They were denoted as "HT+DW" and "HT+Ca", respectively. After the treatment, Ti discs were sufficiently rinsed with DW. Surface topography of non-treated and treated discs was observed under field emission-scanning electron microscope (FE-SEM). Element analysis of non-treated and treated discs was performed by X-ray photoelectron spectrometer (XPS). MC3T3-E1 osteoblast-like cells were cultured in α modified Eagle's medium (α -MEM) supplemented with 10% fetal bovine serum (FBS), 1% streptomycin. In order to measure initial cell attachment, a suspension of the cells (1×10^4 cells/cm³) were seeded on the non-treated and treated discs, and incubated in an atmosphere containing 5% CO₂ at 37°C for 3hrs. After that, the cells were rinsed with phosphate buffer saline (PBS) and were detached from specimens with 0.25% trypsin and 0.02% EDTA for 5 min incubated at 37°C. The amount of initial attached cells on Ti disc was measured by a hemocytometer and the morphology of initial attached cells on Ti disc was observed by FE-SEM.

Results: There was little morphological change before and after the treatment at FE-SEM level. Figure 1 shows XPS spectra for Ti discs before and after the treatments.

No Ca peaks were detected even for HT+Ca; soaked in 10 mM CaCl₂ solution without O₃ at 25°C for 24 hrs. In contrast, Ca2p peaks were detected only on HT discs after treated in O₃-dissolved 10 mM CaCl₂ solution at 25°C for 24 h (HT+O₃+Ca). These results demonstrated that Ca can be bonded on Ti surface when treated both O₃ and CaCl₂. Figure 2 shows the initial attachment of MC3T3-E1 cells on Ti discs before and after treatment after 3hrs. The initial attachment of MC3T3-E1 cells on HT and HT+O₃+DW discs were higher than that on non-treated Ti. These results might be caused by newly formed hydroxyl groups after heat treatment and subsequent O₃ treatment. The initial attachment of MC3T3-E1 cells on HT+O₃+Ca disc was the highest among samples. Moreover, the attached cells on HT+O₃+Ca surface revealed polygonal shape with filopodial extensions which was the good sign for the attachment. These results could be affected by Ca-attached Ti surface.

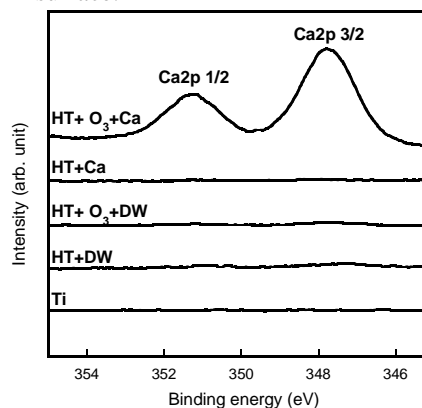


Figure 1. XPS spectra of Ti before and after treatment

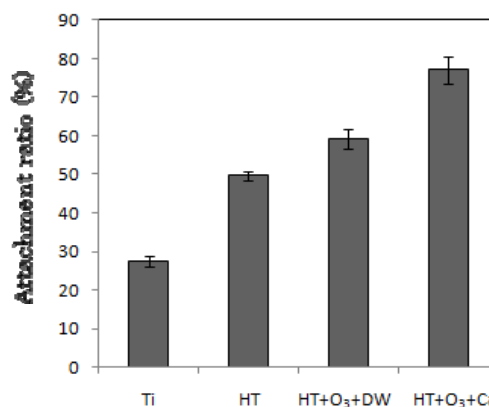


Figure 2. Percentage of attached MC3T3-E1 cells after 3h culture on prepared Ti surface.

Conclusions: Oxon treatment of Ti in CaCl₂ solution is expected to be an effective method for the fabrication of osteoconductive Ti.