MG63 Cell Attachment on Micro-Arc-Oxidized and Microwave/Hydrothermally Treated Titanium Surface

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Introduction

In order to facilitate the bone formation, many researchers make great efforts in mimicking the bone mineral on titanium surface. By micro-arc-oxidization (MAO), the porous titania layer possess Ca and P ions in the less crystalline region though a high temperature plasma fusion process [1] and these ions would diffusion of calcium and phosphorus from the anodic oxide into HA crystals during the hydrothermal treatment [2]. HA nuclei are formed on the porous structure according to the following reaction [3]:

$$10Ca^{2+} + 6PO4^{3-} + 2OH^{-} \rightleftarrows Ca_{10}(PO_4)_6(OH)_2$$

It is noticeable that because the mechanism is based on the diffusion and ion exchange of Ca, P ions to the solid-liquid interface during hydrothermal treatments, thus the size of HA needles are hardly been controlled under nano-scale by traditional thermal convection method. Moreover, precipitate HA needles by diffusion of Ca and P from the surrounding could exhaust the Ca, P in the matrix oxide and resulting in Ca and P non uniform surface [4]. In our preliminary study [5], uniform and large number of nano-size CaP precipitates on titania micro-porous surface were obtained via microwave irradiation. However, their *in-vitro* biocompatibility is still unknown. In this study we present the initial cell attachment and cell viability of MG63 cells on MAO titanium followed by microwave and hydrothermal treatment with compared to MG63 cells on the MAO titanium surface.

Materials and Methods

Sample preparation:

The MAO titania porous surface was prepared by anodic oxidization (280V, 10mA/cm^2) of grade 2 commercial pure titanium discs in electrolytes composed of 0.2M calcium acetate and 0.04M β-glycerol phosphate disodium for 3mins. The MAO titanium discs were then microwave irradiated in diluted Ca/P solutions (0.00005M calcium hydroxide and 0.00003M ammonium dihydrogen phosphate) using a temperature control microwave MARS 5 Microwave Reaction System with XP-1500 vessels (MARS 5, CEM, Matthews, NC, USA) under 200^{o}C for 25 minutes. The MAO-hydrothermal treated samples were prepared by keep samples in a custom made hydrothermal device under 250^{o}C for 2.5hr.

Analysis:

The compositions of MAO, MAO-Hydrothermal, and MAO-Microwave samples were analysis by SEM/EDS (Quanta 200, FEI, USA).The cell attachment SEM morphology was obtained after 5×10^{-3} cells were seeded on each samples for 1 day followed by the critical drying process and gold coating process. The MG63 human osteosarcoma cell viabilities were measured by WST-1 assay after 5×10^{-3} cells were seeded on each samples for 1 day.

Table 1. EDS analysis Ca and P atomic percentage (at%) of the test samples.

Samples	Ca	P	Ca/P ratio
MAO	7.1	6.0	1.2
MAO- Hydrothermal	12.0	6.7	1.8
MAO- Microwave	10.2	6.6	1.5

Results

The microwave induced nano-precipitates on the MAO surface increase the Ca atomic percentage (10.2 at%) on the surface and the Ca/P ratio also increased to 1.5 when compared with the MAO sample (1.5 for MAO-Microwave, and 1.2 for MAO condition). The MAO-Hydrothermal possess highest Ca/P ratio (1.8) and Ca content (12.0 at%).

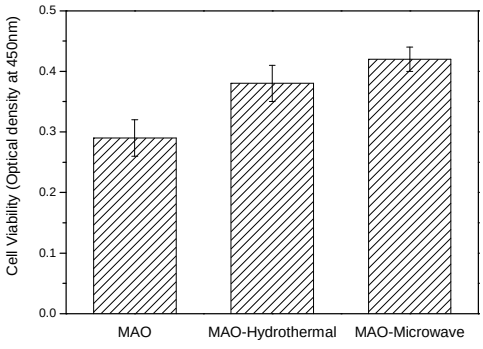

Fig.1. The MG63 cell viability of cells on MAO, MAO-Hydrothermal, and MAO-Microwave samples.

Figure 1 shows that MG63 cells culture on the MAO-hydrothermal and MAO-Microwave treated samples possess higher viability than the MAO samples. The one day viabilities of MG63 cells were similar between the MAO-hydrothermal and MAO-Microwave treated samples.

The MG63 cell revealed a more spread morphology on the MAO-Microwave sample (Figure 2C) when compared with cell cultured on the MAO (Figure 2A) and MAO-hydrothermal (Figure 2B) samples.

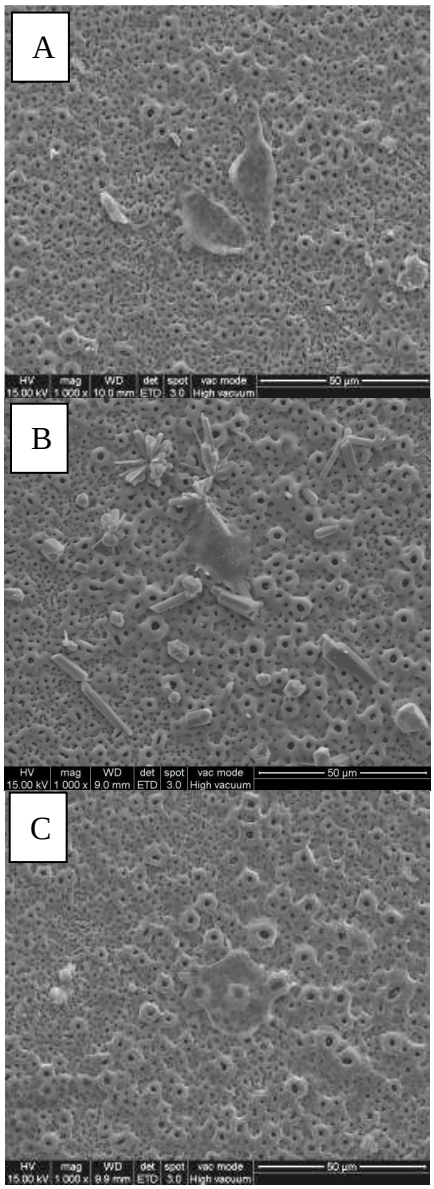

Fig.1. The MG63 cell attachment on MAO, MAO-Hydrothermal, and MAO-Microwave samples

Discussion

Chemical compositions and micro/nano morphology are important factors that would influence the long-term success of implants in the living body. In this study, we showed that higher Ca content and Ca/P ratio were obtained via a MAO-Microwave process. The MAO-Microwave surface also showed an excellent cytocompatibility and MG63 cells on the surface reveal a well spread character as compared to the MAO-Hydrothermal sample. This may because the MAO-Microwave surface was covered with nano- precipitates on the micro-roughened titania porous layer, which may provide more attractive site to protein resorption. Considerable further work will be necessary to evaluate the *in-vitro* bioactivity, and cell differential and minerization of this MAO-Microwave treated MAO titanium.

Conclusions

Microwave treated MAO titanium surface possess a comparable cell attachment character and cell viability with the hydrothermal treated MAO titanium surface, while the microwave process provide a shorter treatment time and uniform surface that could be benefit to their stability and bonding strength in vivo.

References

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