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Preliminary Cell Culture Study of Medical Grade Beta Titanium Alloys for Next Generation Orthopaedic Applications

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ABSTRACT

The word "osseointegration" was originally termed by Professor Brånemark in the late 1950s. The integration of titanium and bone was observed after the interfacial remodelling between bone and implant⁽¹⁾. Ideal osseointegration can be achieved if the biomaterial surface offers⁽¹⁾ good and tight initial adhesion⁽²⁾, supports cell attachment and viability and⁽³⁾ elicits a positive influence upon differentiation⁽²⁾. In this study samples were heat-treated between 400°C and 700°C, and the microstructure was characterised using optical microscopy and X-ray diffraction (XRD) techniques. Results showed that recrystallisation occurred at ~700°C. A preliminary cell viability study was also conducted on the control samples. Results indicated that the samples were biocompatible as evidenced by the elongated fibroblasts attached to the surface.

Introduction

The metallic materials used in orthopaedic devices must possess an elastic moduli in the same range with human bone (7-30GPa)⁽³⁾ to avoid a shielding effect which will result in bone loss in the long term. Beta titanium (β Ti) has an elastic modulus of 55GPa, making it an excellent choice of material to reduce stress shielding and aseptic loosening.

Heat treatment (HT) is a simple and fast process to enhance the material's surface and mechanical properties. In this study, it serves as the preliminary work to understand the material preparation process and microstructure analysis.

The purpose of this study was to heat treat β Ti at various temperatures and study the effect on microstructure and phase composition. Control samples were used for cell culture.

Results and Discussion

Microstructure

The samples consisted of equiaxed grains that vary in both shape and size throughout individual samples. The samples after HT at 400°C and 700°C had an average grain size of 0.6 μ m and 0.78 μ m, respectively. The slight increase in grain size indicated recrystallisation has occurred which is also proven through the XRD results.

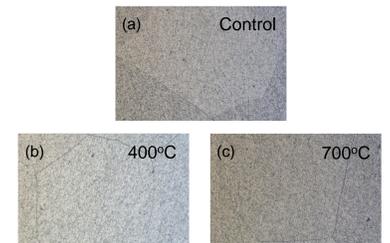


Figure 2. Microscope images at 100 magnification of β Ti (a) control sample (b) 400°C and (c) 700°C (the lowest and highest heat treatment temperatures used).

Methodology

Material Characterisation

β Ti (Ti-35Nb-7Zr-6Ta) samples were pre-processed using selective sanding method to obtain consistent surface roughness. Characterisation was performed using optical microscopy and XRD to examine the surface microstructure and phase composition.

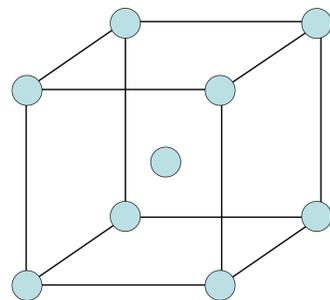
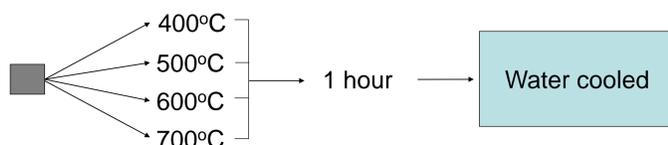


Figure 1. β titanium body centred (BCC) cubic structure

Heat Treatment

β Ti samples (4 x 3 x 2mm³) underwent HT in a furnace at varying temperatures in accordance with literature. Depending on temperatures, the HT process can result in the following stages: precipitation, recovery, recrystallization and grain growth. Recovery removes the dislocations within the material's structure. Recrystallization allows new grains to grow and replace those deformed by internal stresses.



Metallographic Preparation

Samples were ground to achieve a consistent finish using a sandpaper grit progression of 300 - 1500. The samples were then polished using 9 μ m and 3 μ m diamond paste. The samples were chemically etched using a strengthened Kroll's reagent; 8% hydrofluoric acid, 15% nitric acid and 77% H₂O for 90 seconds.

XRD

Analysis was performed in accordance with laboratory protocol to compare the resulting spectra's of samples that underwent HT versus the control and to determine phase composition and possible changes.

Cell Culture

Human fibroblasts 153 at passage 6 were seeded onto two control β Ti samples at 1x10⁴ cells/cm². After 24 hours (37°C and 5% CO₂) cells were stained with DAPI nuclei stain. Imaging was performed using confocal scanning laser microscopy.

XRD Analysis

The β Ti phase in the XRD spectra showed the presence of [110], [200], [211] and [220] diffraction lines. Additional Titanium oxide (Ti₆O) peaks were present in the 700°C heat treated sample at ~ 41 and 54 2 θ position.

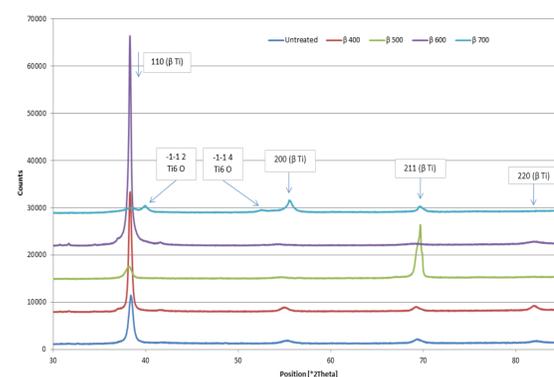


Figure 3 (left). XRD analysis of untreated and heat treated β titanium alloy. Precipitation occurs between 400°C and 600°C while recrystallisation occurs at ~700°C and above.

Cell Culture Viability Study

The degree of cell response varies from one type of cell to another. Generally, osteoblasts prefer rough surfaces while fibroblasts prefer smooth surfaces⁽⁴⁾.

DAPI highlighted the cell cytoplasm rather than just the nucleus. Slightly elongated cells can be seen in Figure 4, suggesting the cells were alive and non-spherical prior to staining. Optical microscopy highlighted the presence of debris, as a result the protocol will be revised to eliminate this from future studies.

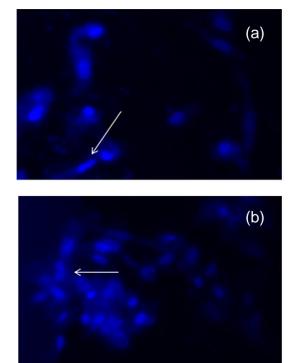


Figure 4. Untreated β Ti with DAPI nuclei stain (a) sample 1 showing elongated shape typical of fibroblast and (b) sample 2 showing large cluster of cells present on material surface.

Conclusions

The optical micrograph and XRD results showed that recrystallisation has occurred proving that the HT has an effect on the microstructure. The cell culture test using fibroblasts showed that the untreated samples were biocompatible. Further cell culture work will be required to continuously image the cells at multiple time points. Mesenchymal stem cells (MSC) will be used in further studies.

Future Work

Larger study culturing heat treated samples. Laser is one of the widely-used methods to modify the surface properties of metal, because it is a clean, fast and highly repeatable process. The effect of laser on the responses of different cells will be explored and compared with the HT samples.