

## THERMALLY OXIDISED Ti6Al4V ALLOY ENHANCES OSTEOBLASTIC CELLS BEHAVIOUR

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**INTRODUCTION:** Ti6Al4V is among the most commonly used implant materials due to its high corrosion resistance, good mechanical properties and low toxicity. Thermal oxidation can offer thick, highly crystalline oxide films with very good protective performances of both wear and corrosion properties. Surface characteristics of materials play an essential role in osteoblast adhesion on biomaterials. The initial interaction of bone cells with the biomaterial influence the cell capacity to proliferate and to differentiate itself on contact with the implant [1]. In our previous study, we have demonstrated that thermal oxidation treatment of Ti6Al4V at 500°C and 700°C for 1 hour protect the material and do not decrease the high in vitro corrosion resistance of the Ti6Al4V alloy [2]. The purpose of the present study was to evaluate the biocompatibility of surface modification after thermal oxidation treatments at two different temperatures in comparison to the polished as-received state in primary culture of human osteoblastic cells.

**METHODS:** The material used in this research was Ti6Al4V (TiAlV) (wt%) alloy. The oxidation treatment was performed at 500 °C (TiAlV500) and 700 °C (TiAlV700) for 1 hour, in air. Human osteoblastic cells were derived from fresh trabecular bone explants from knee obtained during arthroplasty procedures in patients aged 75±5. Kinetic of cell attachment was determined for quantification by spectrofluorimetry using BCECF-AM. Actin cytoskeletal organization was examined by labeling with rhodamine phalloidin. The morphology of cells was tested using acridine orange which simultaneous stain for DNA and RNA. Alamar blue staining was used to determine the cell viability while Hoechst 33342 staining was used to evaluate cell proliferation as a correlation of DNA content.

**RESULTS:** Temporal profile of the osteoblastic cell attachment showed that the percentage of bound cells increased with time until 3 hours on all tested substrates. The percentage of adhesion after 1 and 2 hours was significantly greater on TiAlV700 than time matched TiAlV500. To evaluate the actin cytoskeletal organization we have used the criteria of Sinha which classified the degree of cytoskeletal organization into three classes [3]. Each class represents a different stage in degree of cytoskeletal reorganization, with Type I the least organized, Type II intermediate and Type III the most organized. The relative percentage of each morphologic cell type was determined as a function of time and substrate. The percentage of Type III cells increased with time on all substrates tested. After 3 hour, thermal oxidation significantly increased the percentage of Type III cells. Thus, TiAlV700 showed

maximal number of this type of cells when compared to TiAlV500 and TiAlV untreated. Data of cytoskeletal organization correlate well with the enhanced cell attachment observed for TiAlV700 when compared to TiAlV500. Microscopical examination of cell morphology after 24 hours in culture revealed well spread, confluent and viable osteoblasts on all substrates tested. No obvious differences were visually found between the different substrates.

Hoechst staining showed that, in all cases, the cells proliferated between day 3 and day 7. After 3 days in culture, a statistically significant increase in proliferation was measured for osteoblasts on TiAlV and TiAlV500 as compared to TiAlV700. At 7 days there was no significant difference in the proliferation on any of the material. This pattern was repeated for cell viability measured by Alamar Blue staining.

**DISCUSSION & CONCLUSIONS:** Thermal oxidation treatments do not affect the material biocompatibility. Moreover, our data on early adhesion events such as cell attachment and cytoskeletal organization indicate that TiAlV700 improves osteoblast response as compared to TiAlV500. Experiments aimed to study cell proliferation and viability indicate that TiAlV700 do not alter the osteoblast long term properties. Taken together, these results indicated that thermal oxidation improves osteoblast behaviour in comparison to the polished as-received state.

**REFERENCES:**<sup>1</sup> K. Anselme (2000) *Biomaterials*. 21:667-681 <sup>2</sup> MC García-Alonso, L. Saldaña, G. Vallés et al (2003) *Biomaterials* 24:19-26. <sup>3</sup> RK Sinha, F. Morris, SA Shah et al (1994) *Clin. Orthop* 305:258-272.

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