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CYTOKINES SECRETION IN RESPONSE TO WEAR PARTICLES OF THERMALLY OXIDISED TI6AL4V ALLOY

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INTRODUCTION: Aseptic loosening of orthopaedic implants is a serious problem that leads to pain, loss of function, and the need for revision surgery. This problem is caused by a multistep process that includes generation of wear particles from the implants, activation of macrophages and other cells by wear particles, production of cytokines that stimulate osteoclast differentiation and or osteolysis due to the increased number of osteoclast [1]. IL-6 (interleuquin-6) and TNF-α (tumour necrosis factor-α) are the main cytokines that provoke local bone loss and modulate the release of bone resorbing factors from other cells [2]. Titanium (Ti) alloys are well known for their superior mechanical properties as well as for their good biocompatibility, making them desirable as surgical implant materials. However, these alloys have been proven to behave poorly in friction since wear particles were often detected in tissues and organs associated with Ti implants [3]**.** Thermal oxidation treatments of TAValloy aimed to obtain "in situ" ceramic coatings, mainly based on rutile, can offer thick, highly crystalline oxide films with very good protective performances. When this Ti alloy is implanted long term wear results in the accumulation of rutile particles from the surface [4]. The purpose of the current study was to evaluate the inflammatory effects of wear particles released from the oxide layer produced on TAValloy in primary culture of human osteoblasts and macrophages.

METHODS: Mean size of comercially pure Ti and rutile (TiO_2) particles (Johnson Matthey, Ward Hill, MA) was 1 µm. Human osteoblastic cells were derived from fresh trabecular bone explants from knee obtained during arthroplasty procedures in patients aged 69±5. Human peripheral blood monocytes were obtained from healthy donors and isolated by Ficoll sedimentation and adhrence to plastic. Osteoblast and macrophages (200000 cells/well) were seeded in six-well plates in the absence of particles (control group) or with different concentrations of Ti and TiO_2 particles (4.5×10^7, 4.5×10^8, 4.5×10^9 particles/well) for 24 hours. Medium was collected and used to determinate IL-6 secretion for osteoblastic cells and macrophages and TNF-α for macrophages. Cytokines secretion was measured by a solid phase sandwich Enzyme Linked-Inmunosorbent Assay (ELISA; IL-6 Biosource, Camarillo, CA; TNF-α, CLB, Amsterdam, The Netherlands). Aliquots of cell lysates were used for protein determinations by BCA protein assay kit (Pierce, Rockford, IL). Cytokines secretion was corrected by protein total amounts for all the samples tested. Cell toxicity was stimated by the lactate dehydrogenase (LDH) release assay using the Cyto Tox 96 citotoxicity kit (Promega, Madison, WI). Experimental medium as well as different particle solutions were tested for endotoxin using the limulus amoebocyte assay (Sigma, St. Louis, MO).

RESULTS: Osteoblast exposed to Ti particles at a concentration of 4.5×10^9 particles/well produced significantly more IL-6 than osteoblast exposed to TiO_2 particles or unexposed. Macrophages exposed to Ti particles produced much more IL-6 than TiO_2 at the three concentrations tested, being this increase maximum at 4.5×10^9 particles/well. No differences were observed when macrophages were exposed to TiO_2 particles compared to control group. Exposure of macrophages to Ti particles highly induced TNF-α secretion at the three concentrations tested, being this increase maximum at 4.5×10^9 particles/well. Exposure of cells to TiO_2 or Ti particles was not toxic, as reflected by release of the intracellular enzyme lactate dehydrogenase.

DISCUSSION & CONCLUSIONS: The developed surface thermal oxidation treatment seems to increase TAVbiocompatibility. This is highlighted by the lower impact of this treatment on the release of proinflammatory and pro-resortive cytokines. This study demonstrates that wear debris derived from thermal oxidation treatments of prosthetic materials can elicit different biological responses. Understanding these responses may help to identify materials better suited for prostheses.

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