Titanium in medicine: electrochemical treatments for bacteria proliferation control

C. Giordano[1], L.Visai[2] , R. Chiesa[1], MP. Pedeferri[1], E. Saino[2], A. Cigada[1]

[1]Department of Chemistry, Materials and Chemical engineering "Giulio Natta" -Politecnico di Milano – Via Mancinelli, 7 – 20131 Milano, Italy

[2]University of Pavia, Department of Biochemistry, Medicine Section, Via Taramelli, 3/B-27100 Pavia-Italy

Statement of Purpose: Titanium and Ti6Al4V alloy based implants and osteosynthesis devices have achieved a remarkable success in clinical applications. Bacterial adhesion and colonization of tissue-implant interface might negatively effect the clinical performance resulting, in the most severe cases, in the medical device failure and removing. The chemical composition and structure of titanium (alloys) surface can be modified by several techniques in order to improve the *in vivo* performance of temporary and permanent implantable devices. In the recent years, at Politecnico di Milano we investigated several treatments based on electrochemical process aimed to modify the thickness and structure of the oxide passivation film [1-2]. The formation of a crystalline titanium oxide layer enriched in anatase, known to have antibacterial properties, operated by electrochemical technique, was found particularly promising [3-4]. In this work we investigated a new electrochemical based process capable to modify the crystalline structure of the surface oxide layer with no or little modification of the surface morphology at super-micrometric scale. Chemical-physical characterization and *in vitro* biological evaluation were carried out using selected cell lines and several bacterial species to investigate cytocompatibility and bacterial colonization.

Materials and Methods: Commercially pure grade 2 titanium and Ti6Al4V coupons (10x10x1 mm^3) were used. Electrochemical treatments were performed in strong acid solutions, by anodic polarization in DC at different voltages, in order to obtain two test conditions, i.e. an amorphous and a crystalline film structure, for each material:

- A = Ti grade 2, not treated (control)
- B = Ti grade 2 anodised at 90V (amorphous film)
- C = Ti grade 2 anodised at 130V (crystalline film)
- D = Ti6Al4V, not treated (control)
- E = Ti6Al4V anodised at 100V (amorphous film)
- F = Ti6Al4V anodised at 120 V (crystalline film)

The film structure was analyzed by thin film x ray diffractometry (XRD, Phillips pw 1710). For the biological evaluations, sample were previously sterilised by ethanol immersion and UV (235 nm) illumination. *In vitro* cell morphology, adhesion (by scanning electron microscopy) and proliferation activity (Alamar™ blue test) were assessed using human osteosarcoma (MG63) and murine fibroblast (L929) cells. Bacterial colonization was analyzed *in vitro* using 4 bacterial strains: *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans and Porphyromonas gengivalis*. Total Viable Count (TVC) was performed after 3h and 24h of bacterial incubation onto sample surfaces.

Results: Anodization treatment resulted in the formation of a thickened coloured oxide layer. The interference colour was associated to the film thickness, and consequently to the anodization voltage applied. The XRD results showed that the anodization performed at higher voltage induce the formation of a crystalline anatase-type structure oxide film. XRD analyses on anodization performed at lower voltage did not show the presence of any anatase structured film.

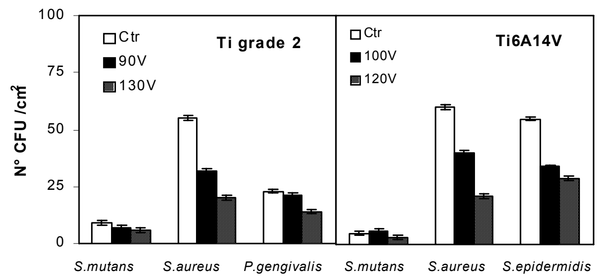

Figure 1 - In vitro bacterial adhesion

In vitro cellular study confirmed the good biocompatibility of all the considered materials with both the cell lines. After 3h of incubation, the bacterial adhesion was considerably reduced onto Ti grade 2 anodised at 130V and Ti6Al4V anodised at 120 V (Fig.1). Lower voltage anodization still showed some bacteria adhesion reduction, in comparison to untreated substrata.

Conclusions: The collected results showed that bacterial colonization can be reduced onto anodized titanium surface without negative effects on both fibroblast- and osteoblast-like cells. Although bacteria reduction was found particularly effective on titanium surfaces anodized at higher voltage, i.e. with anatase structured oxide film, some effect was found also on materials treated at lower voltage, characterized by an amorphous structured oxide film. The anodization treatment, also inducing the colorization of medical devices, may therefore open useful practical applications, for instance for medical device identification and colour coding associated to bacterial reduction capability.

References:

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