ADHESION, GROWTH AND DIFFERENTIATION OF HUMAN OSTEOBLAST-LIKE CELLS ON THERMALLY OXIDIZED Ti AND TiNb SUBSTRATES

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Abstract

Metallic materials are essential for construction of load-bearing bone implants, such as replacements of hip, knee and other joints. For these applications, modern materials used in advanced tissue engineering, e.g. resorbable porous or fibrous polymeric and ceramic scaffolds are mechanically insufficient, even if these materials enable the ingrowth of bone cells and bone tissue formation. Therefore, searching for new metallic materials and their surface modifications improving their biocompatibility and osseointegration is still desirable.

As first metallic materials for bone implantation, AISI 316L stainless steel and Co-Cr alloys were used. In the 1950's, the Ti-6Al-4V alloy was developed. These materials are still frequently used for construction of implants because of their relatively low price [1]. However, these materials are biomechanically incompatible with the bone tissue, because their Young's modulus is markedly higher (110-220 GPa) than that of the bone (10-40 GPa). Implants with high stiffness take over a considerable part of load from the bone. This phenomenon, referred as "stress-shielding effect", can then cause the bone resorption and loosening of the implant [1]. Also chemical compositions of the mentioned metallic materials limit their biocompatibility, because they contain harmful elements as V, Al, Co and Cr, which can act as cytotoxic, catabolic, immunogenic or even carcinogenic agents [2,3], and can also cause serious neurological problems [4]. Due to these adverse reactions, new types of Ti-alloys have been developed, namely low-rigidity β-type Ti alloys, containing non-toxic and non-allergenic elements (Nb, Ta, Zr etc.) and having good mechanical properties and workability [4,5].

The goal of this study was to evaluate the adhesion, growth and differentiation of osteoblast-like MG-63 and Saos-2 cells on titanium-niobium alloys after their surface modification by thermal oxidation at two different temperatures (165°C and 600°C). Pure titanium (treated at 165°C and 600°C) and polystyrene culture dishes (PS) were used as control materials. Possible immune activation of the cells was tested by the levels of TNF-alpha secreted to the cell culture media by murine macrophage-like RAW 264.7 cells cultured on the tested materials.

On samples treated at 165°C, the number of initially adhered MG-63 and Saos-2 cells was on an average higher on TiNb than on Ti or PS. On day 3 after seeding, the trend of the cell numbers remained similar, with the highest cell density found on TiNb. Similar results were obtained on samples treated at 600°C, where the difference in cell number between TiNb and Ti samples became more apparent. This cell behavior could be attributed to a less negative zeta potential on TiNb samples. In samples treated at 165°C, the zeta potential of TiNb surfaces was on the average less negative than on Ti surfaces, but this difference was not significant. However, in samples treated at 600°C, this difference became much more pronounced, which was probably due to the formation of T-Nb_2O_5 phase on the surface of the TiNb samples. This phase was of a crystalline structure, while at 165°C, the structure of Nb_2O_5 was amorphous. In addition, both Ti and TiNb samples treated at 600°C contained rutile, while the samples treated at 165°C contained anatase in their surface layer. It has been shown that rutile films deposited on PEEK enhanced the adhesion and growth of osteoblasts more than anatase films [6]. This phenomenon was explained by an increase in the material surface wettability, and particularly to the presence of –OH- groups on the rutile films.

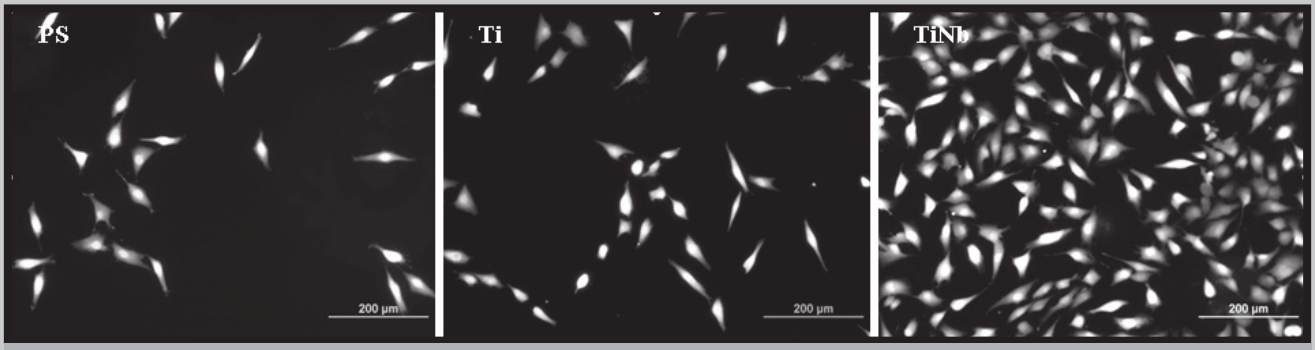

FIG.1. Human osteoblast-like MG 63 cells on day 1 after seeding on polystyrene dishes (PS), and Ti or TiNb subjected to thermal oxidation at 600°C. Cells stained with LIVE/DEAD viability/cytotoxicity kit for mammalian cells. Olympus IX 51 microscope, DP 70 digital camera. Obj. 10x, bar=200 µm.

The expression of collagen I and osteocalcin, i.e. an early and late marker of osteogenic cell differentiation, respectively, was higher on Ti than on TiNb samples, and this difference was more apparent in samples treated at 165°C. At the same time, no considerable immune activation of the cells on all tested samples was found. The production of TNF-α by RAW 264.7 cells was very low in comparison with cells grown in the presence of bacterial lipopolysaccharide, and also significantly lower than on untreated samples.

These results indicate that TiNb substrates increased the proliferation of human bone cells, while pure Ti rather supported the cell differentiation. The effect on cell proliferation was more apparent in samples treated at the higher temperature (600°C), while the effect on cell differentiation was more pronounced at the lower temperature (165°C). None of the tested samples induce significant cell proinflammatory activation. Thus, all tested samples are suitable as carriers for bone cells; only an appropriate application (i.e., requiring either proliferation or quick differentiation of osteogenic cells) should be selected.

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