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Effects of surface treatment of Ti–6Al–4V titanium alloy on biocompatibility in cultured human umbilical vein endothelial cells

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Abstract

Among the titanium alloys employed as implant materials, the Ti–6Al–4V alloy is still widely used. Ti–6Al–4V titanium alloy samples, in untreated state and subjected to treatments in air by furnace or glow-discharge processes, were put in contact with human umbilical vein endothelial cells (HUVEC) in order to evaluate their effects on biocompatibility. In HUVEC kept for 48 h in the presence of the three sample types neither cell proliferation nor protein content nor lactate dehydrogenase release in the culture medium are affected, while apoptosis is induced after 48- and 96-h contact of the cells with the untreated sample type, and after 96-h contact with the plasma treated one, the furnace treated sample type being ineffective. The expression of two adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) was also studied. The incubation of HUVEC with the three sample types for 48 or 96 h induces a significant increase in ICAM-1 protein levels, in comparison with control cells, while VCAM-1 expression is not detectable. In the same way, TNF-α release in the culture medium, assayed after 48- and 96-h contact of the cells with the three sample types, is significantly higher, in comparison with control, even if the highest values are registered in the presence of the untreated samples. Taken together, these data indicate that, although Ti–6Al–4V alloy samples, and in particular the treated ones, show a good biocompatibility, attention must be given to the first signs of inflammation.

Keywords: Titanium alloy; Surface treatment; Biocompatibility; Endothelial cells; Adhesion molecules

1. Introduction

Metals and alloys are the most common materials used as surgical implants, and titanium alloys in particular show properties which make them suitable as surgical implant materials [1,2]. The high degree of biocompatibility of titanium and its alloys is also intimately connected with the passive oxide film formed on the metallic surface [3,4]. In fact, the high oxygen affinity of titanium promotes, at room temperature, the formation of a surface oxide layer, which is thin, very stable, continuous and adherent, and able to protect the material in many aggressive environments. However, the low hardness and poor tribological properties of titanium alloys may become a critical factor when wear phenomena are involved. Among the titanium alloys employed as implant materials, the Ti–6Al–4V alloy is still widely used. However, it is difficult to avoid the release of titanium, aluminium or vanadium into tissues and body fluids associated with Ti–6Al–4V implants [5]; this has also been reported by Okazaki and Gotoh [6], who compared the metal release from various alloys in vitro. If wear phenomena occur, the debris produced are likely to cause the release of high

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amounts of inflammatory mediators implicated in osteolysis [7]. Moreover, although titanium is an inert, biocompatible material, there is evidence to suggest that its surface may activate the complement system [8], platelets, and the coagulation cascade [9,10]. Wang et al. [11] investigated the cytotoxicity of titanium, cobalt and chromium, and reported that the proliferation of human peripheral blood mononuclear cells and the release of interleukin-2 (IL-2) and interleukin-6 (IL-6) were significantly inhibited in the presence of the studied materials, while titanium did not alter IFN-gamma production. These authors suggested that patients with an extensive exposure to the studied metals may develop immune dysfunctions, with an increased risk of infection.

Therefore, in order to obtain a high degree of biocompatibility, surface treatments of titanium have been widely applied. The surface modification of titanium alloys is usually performed by means either of coatings, obtained by techniques like chemical vapour deposition (CVD), physical vapour deposition (PVD) or plasma spray, or of modified surface layers, obtained by laser-assisted treatments, ion implantation or diffusion treatments, which take advantage of the high reactivity of titanium with respect to carbon, nitrogen or oxygen [12].

In vitro cytocompatibility studies of diamond like carbon (DLC) coatings on titanium on cell lines of mouse fibroblasts, human osteoblast and human umbilical vein endothelial cells (HUVEC) have demonstrated an improved cytocompatibility of DLC coated titanium in comparison with the bare Ti alloy [13]. As regards the nitrogen-based surface treatments, Bordji et al. [14] investigated glow-discharge nitrogen implantation, plasma nitriding by plasma diffusion treatment (PDT) and deposition of TiN layer by plasma-assisted CVD additionally to PDT, in order to improve the wear resistance and the hardness of Ti–6Al–4V and Ti–5Al–2.5Fe alloys. The effects of such treatments on the cytocompatibility of these materials were studied in vitro on human dermal fibroblasts and trabecular bone osteoblasts, and it was demonstrated that Ti–5Al–2.5Fe alloy was as cytocompatible as the Ti–6Al–4V alloy and that, after the two nitriding treatments, cell proliferation and viability appeared to be significantly reduced, osteoblast phenotype expression and protein synthesis capacity being not affected. Leng et al. [15] showed that Ti O Ti N duplex coating on Ti–6Al–4V alloy had better blood compatibility and mechanical properties than low temperature isotropic pyrolytic carbon LTIC, which is currently used for artificial heart valves. Alloying with oxygen is the base of other important surface modification techniques used for improving the biocompatibility of titanium alloys by means of thickening the natural surface oxide film. Eisenbarth et al. [16] investigated the interactions between cells and the titanium surface, in order to establish whether an artificial oxide layer would be able to protect the cells from toxic alloying elements in metallic biomaterials. These authors put the samples into direct contact with Vero-fibroblasts and with MC3T3-E1 murine osteoblasts and, after seven days, the reaction of the cells was examined, testing proliferation, MTT, morphology and actin staining. It was shown that the sol gel-produced titanium oxide layer shielded the cells from toxic alloying elements, so that the cell reaction was influenced only by the titanium oxide surface layer and not by the composition of the bulk material. Eisenbarth et al. [16] concluded that biocompatibility and cell adhesion depend on the vanadium content in the surface oxide layer.

The effect of titanium passivation on the response of rat bone marrow cells has also been studied, and no effect of this surface treatment was shown on in vitro biocompatibility of titanium as evaluated by osteoblast attachment, proliferation and differentiation [17]. The influence of surface finishing on the biocompatibility of titanium alloys was also studied and various surface textures have therefore been created and used to positively affect cell and tissue responses [18–20].

Tambasco de Oliveira and Nanci [21] examined cells obtained by enzymatic digestion of newborn rat calvaria and grown for periods of 6 h, 1 day, and 3 days on commercially pure (c.p.) titanium and Ti–6Al–4V alloy discs with nanotextured or machined surfaces. These authors demonstrated that nanotexturing of titanium-based surfaces upregulates the early expression of bone sialoprotein and osteopontin in osteogenic cell cultures.

The biocompatibility of implanted materials is influenced by interactions at the tissue–implant surface. During inflammation, as a response to an implanted material, leukocytes migrate into tissues, macrophages become involved in maintaining a chronic inflammatory response [22], and the cells secrete proinflammatory cytokines, such as interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α), which contribute to inflammation. In particular, TNF-α is a pleiotropic regulator that controls the expression of a number of inflammatory and immune genes through activation of the nuclear transcription factor kappa B (NF-kB), which may, in turn, promote adhesion molecule expression [23,24]. On endothelial cells, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are members of a group of immunoglobulin-like molecules involved in recognition and adhesion. E-selectin, an adhesion molecule expressed on activated endothelium, has a terminal lectin domain, which binds carbohydrate ligands expressed on leukocytes in the first phase of migration [25]. Studies on E-selectin function have shown that this molecule can support the adhesion of neutrophils and monocytes [26,27]. The expression of ICAM-1, VCAM-1 and E-selectin on the endothelial cell