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Effects of alloying elements on the cytotoxic response of titanium alloys

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ABSTRACT

Titanium alloys, especially β-type alloys containing β-stabilizing elements, constitute a highly versatile category of metallic materials that have been under constant development for application in orthopedics and dentistry. This type of alloy generally presents a high mechanical strength-to-weight ratio, excellent corrosion resistance and low elastic modulus. The purpose of this study is to evaluate the cytotoxicity and adhesion of fibroblast cells on titanium alloy substrates containing Nb, Ta, Zr, Cu, Sn and Mo alloying elements. Cells cultured on polystyrene were used as controls. *In vitro* results with Vero cells demonstrated that the tested materials, except Cu-based alloy, presented high viability in short-term testing. Adhesion of cells cultured on disks showed no differences between the materials and reference except for the Ti–Cu alloy, which showed reduced adhesion attributed to poor metabolic activity. Titanium alloys with the addition of Nb, Ta, Zr, Sn and Mo elements show a promising potential for biomedical applications.

1. Introduction

Metallic materials are commonly used in various areas of biomedicine. In orthopedics, they are applied as plates, pins and fixing screws for bone fractures and in some complex devices as parts for total hip prostheses or as femoral and tibial components in total knee arthroplasty [1].

Among the metallic materials used in implants, titanium and its alloys possess properties that render their performance superior to that of Co–Cr alloys and stainless steels. Ti–6Al–4V alloy and CP–Ti (commercially pure) are the most important Ti-based materials used in the orthopedic implant industry. Ti–6Al–4V alloy was initially developed to meet the demands of the aerospace industry, and due to its interesting properties, it has been applied in the biomedical field since the 1960s. The use of metallic devices for the replacement of damaged parts of the human body requires not only mechanical compatibility, which is achieved by a combination of a low elastic modulus, high mechanical strength and fatigue resistance, but also biological compatibility, since the material will be in contact with body fluids and should therefore be atoxic to cells.

Williams [2] defines "biocompatibility" as follows: "Biocompatibility is the ability of a material to develop its functions with adequate tissue response in a specific application." Several studies have highlighted the high biocompatibility of titanium and its alloys [3–6]. However, there are some concerns regarding the biocompatibility of Al and V elements in Ti–6Al–4V alloy. Several studies have shown that such elements are toxic and can cause neurological disorders and Alzheimer's disease [7,8], as well as accumulation of particulates in adjacent tissues [9].

The abovementioned problems have motivated the constant development of new titanium alloys with nontoxic and nonallergenic elements such as the β-stabilizing elements Nb, Ta, Zr, Mo, Pt, Sn [10–15]. These elements can stabilize the titanium body-centered cubic crystal structure (β phase) at room temperature and the resulting alloys may represent the future for titanium alloys insofar as orthopedic applications are concerned. Beta-phase stabilization through the addition of the aforementioned elements yields titanium alloys with low elastic modulus and high mechanical strength, as well as optimized electrochemical and biological performance.

Some metallic materials used in implants may not be toxic, but the presence of dissolved metal ions, corrosion products and wear particles may lead to some level of toxicity when combined with certain types of biomolecules and cells [16]. For instance, wear particles can cause osteolysis due to the natural defense mechanism called fagocytosis [17,18].

The biocompatibility of a material can be evaluated from *in vitro* and *in vivo* tests. Because *in vivo* tests take longer, *in vitro* tests can be considered preliminary assays to determine certain toxicity-related parameters such as cell death, adhesion on substrate surfaces, changes in cell morphology, cell proliferation and biosynthetic activity.

With regard to *in vitro* tests, the cell culture technique is an important methodology in biomaterials research because it allows for the rapid evaluation of biological performance. According to the protocol of the ISO 10993-5 standard [19] for biological evaluations of medical devices, one of the recommended cytotoxicity tests is the MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium

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bromide]. This procedure allows for a quantitative evaluation by measuring the mitochondrial activity of cells after their exposure to the surrounding toxins, revealing information about the material's cytotoxicity and the functionality of cells on its surface [20].

After eliminating the possibility of cytotoxicity, cell adhesion is another relevant factor that indicates cell/material interaction, mainly in applications that integrate the prosthesis to the bone or articulation. Cell adhesion indicates, in an early stage, the adhesion of cells to substrate, which is essential for cell proliferation and differentiation and the formation of neo-tissue [20]. Moreover, characteristics of the material such as roughness, chemical composition and surface free energy are essential for adhesion and affect cell morphology and function. Evidence has revealed that cell morphology can regulate cell growth, protein secretion, differentiation, proliferation and death. In the case of titanium alloys, polished surfaces (with low roughness) help cell fixation and flattening [1].

Cytocompatibility studies of new titanium alloys develop by biomedical application have been investigated by several researches [21,22]. Koike and co-workers observed slightly elevated mitochondrial activity in Cu–Cr and Cu–Si alloys with highest copper dissolution using Balb/c 3T3 mouse fibroblast. In the same study, the biocompatibility of Ti–6Al–4V, Ti–1Fe, Ti–5Al–11Fe and Ti–16Mo–3.2Nb alloys was evaluated [21]. The results showed similar behavior when compared to pure titanium used as control. According to Watanabe et al., Ti–10Cu alloy showed high level of released copper [22]. Their results also showed slight suppression of mitochondrial activity in the Ti–6Al–7Nb alloy, which could be associated to the release of Al into the medium [22]. Again, separated studies showed that Ti–Au alloys exhibits 100% cell viability [23]. Similarly, Mn addition to titanium-based alloys in concentration up to 8% (wt) results in acceptable cytocompatibility [24].

The main objective of this work is to evaluate the effects of alloying elements on the cytotoxicity of titanium alloys for biomaterial applications by ascertaining their *in vitro* cytocompatibility.

2. Materials and methods

2.1. Preparation of titanium alloys

Titanium alloys with nominal compositions of Ti–35Nb, Ti–35Nb–7.5Ta, Ti–35Nb–4Sn, Ti–25Nb–15Zr, Ti–25Nb–8Sn, Ti–6Mo, Ti–7.1Cu, Ti–6Al–4V and Ti–CP (wt.%) were placed in copper crucibles and prepared in an arc furnace under argon atmosphere. The alloys were then homogenized at 1000 °C for 24 h in an inert atmosphere, after which they were subjected to plastic deformation through swaging, solution-treated following water quenched and milled to their final dimension of 5 mm diameter. They were then cut into 5 mm × 200 μm disks using a Buehler cutter (IsoMet 4000). The samples were prepared metallographically by sanding with #800 and #1500 grit sandpaper, followed by polishing through tumbling. This process was carried out in a revolving cylindrical drum at 45 rpm for a period of 12 h, with 8 mm size particles immersed in an aqueous solution of B-5 (Roger Química Ltda) and PL-4 (Roger Química Ltda) tensoactives.

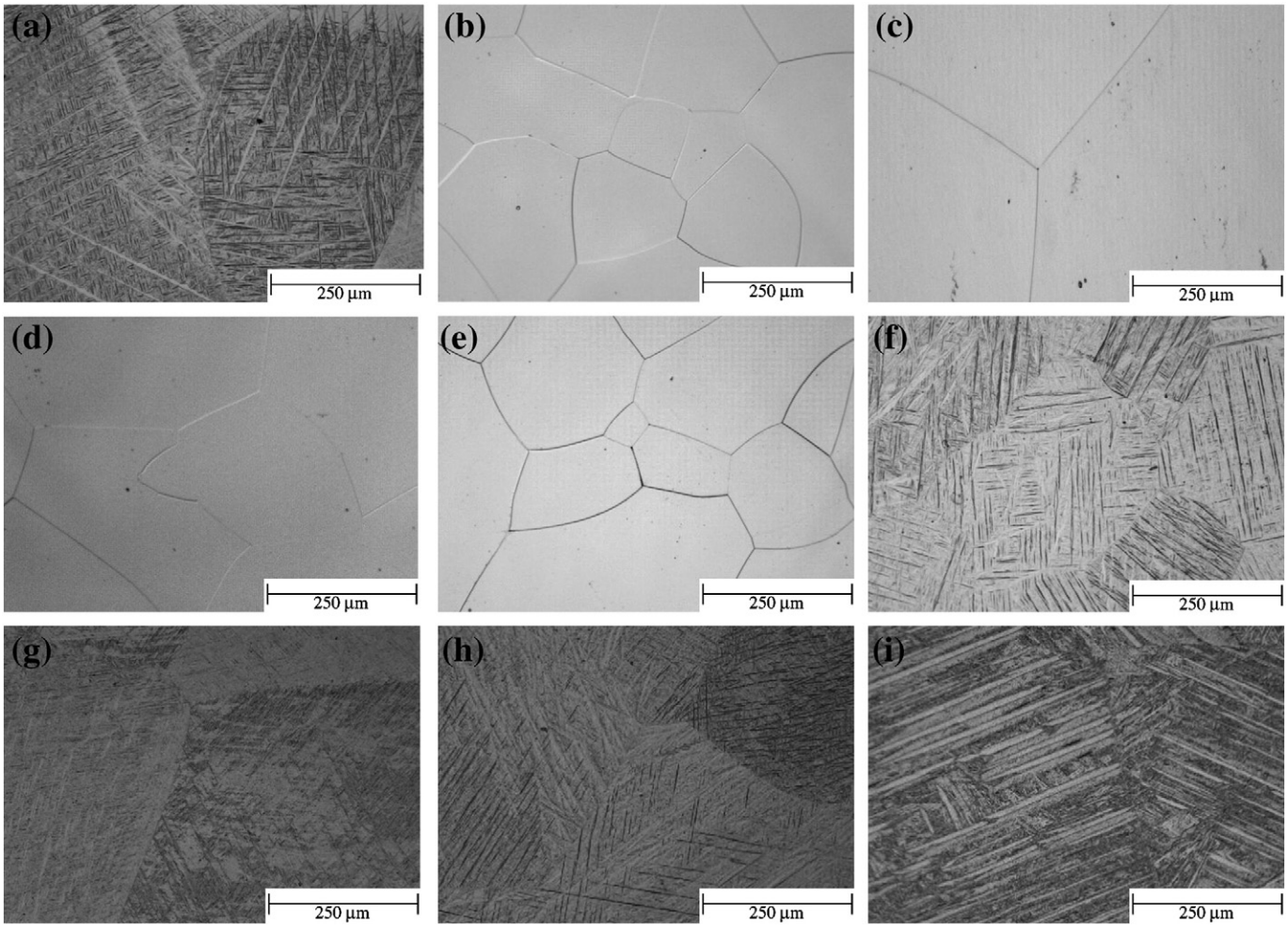

Fig. 1. (a) — Optical micrograph of Ti–35Nb alloy. (b) — Optical micrograph of Ti–35Nb–7.5Ta alloy. (c) — Optical micrograph of Ti–35Nb–4Sn alloy. (d) — Optical micrograph of Ti–25Nb–8Sn alloy. (e) — Optical micrograph of Ti–25Nb–15Zr alloy. (f) — Optical micrograph of Ti–6Mo alloy. (g) — Optical micrograph of Ti–7.1Cu alloy. (h) — Optical micrograph of Ti–6Al–4V alloy. (i) — Optical micrograph of Ti–CP.