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Cytocompatibility of pure metals and experimental binary titanium alloys for implant materials

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ABSTRACT

Objective: This study was performed to evaluate the biocompatibility of nine types of pure metal ingots (Ag, Al, Cr, Cu, Mn, Mo, Nb, V, Zr) and 36 experimental titanium (Ti) alloys containing 5, 10, 15, and 20 wt% of each alloying element.

Methods: The cell viabilities for each test group were compared with that of CP-Ti using the WST-1 test and agar overlay test.

Results: The ranking of pure metal cytotoxicity from most potent to least potent was as follows: Cu > Al > Ag > V > Mn > Cr > Zr > Nb > Mo > CP-Ti. The mean cell viabilities for pure Cu, Al, Ag, V, and Mn were 21.6%, 25.3%, 31.7%, 31.7%, and 32.7%, respectively, which were significantly lower than that for the control group ($p < 0.05$). The mean cell viabilities for pure Zr and Cr were 74.1% and 60.6%, respectively ($p < 0.05$). Pure Mo and Nb demonstrated good biocompatibility with mean cell viabilities of 93.3% and 93.0%, respectively. The mean cell viabilities for all the Ti-based alloy groups were higher than 80% except for Ti–20Nb (79.6%) and Ti–10V (66.9%). The Ti–10Nb alloy exhibited the highest cell viability (124.8%), which was higher than that of CP-Ti. Based on agar overlay test, pure Ag, Cr, Cu, Mn, and V were ranked as 'moderately cytotoxic', whereas the rest of the tested pure metals and all Ti alloys, except Ti–10V (mild cytotoxicity), were ranked as 'noncytotoxic'.

Significance: The results obtained in this study can serve as a guide for the development of new Ti-based alloy implant systems.

1. Introduction

Recently, commercially pure titanium (CP-Ti) has become widely used as a biomaterial for dental implants, orthopaedic implants, cardiovascular appliances, and implant-supported dental crowns because of outstanding characteristics that include high specific strength, high resistance to corrosion, greater biocompatibility, low modulus of elasticity, and high capacity to be osseointegrated with bone.[1–3] However, the use of unalloyed CP-Ti requires further improvement to overcome its limitations including strength, hardness, wear resistance, fatigue strength, and poor grindability. It is desirable also to decrease its elastic modulus as close as possible to that of bone

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tissue.[4–11] Reports have focused on improved properties of CP-Ti for use as implant materials. In a study of Ti–Ag and Ti–Cu alloys,[12] Ti–20% Ag and Ti–5% Cu alloys exhibited better grindability than pure Ti, which is a desirable property for dental CAD/CAM alloys. The results from a previous study utilizing experimental Ti–Au (5–20 wt% Au), Ti–Ag (5–20 wt% Ag), and Ti–Cu (2–10 wt% Cu) alloys suggested that their hardness and tensile strength were higher than these properties for pure Ti.[13] In another study, Ti–Cr alloys exhibited greater flexure strength than CP-Ti, which was associated with the strengthening effect of the ω phase.[5] The flexure strength of the Ti–20Cr alloy was about 80% greater than that for CP-Ti, and the elastic recovery capability was 460% greater than that of CP-Ti. The lower elastic modulus of CP-Ti and Ti alloys, compared with 316L stainless steel and Cr–Co alloys, is potentially advantageous for preserving the surrounding bone by minimizing the reduction in physiological stress within bone and a reduction in bone density. This occurs because of a more favourable match of elastic modulus between the implants and bone.[14–18] The elastic moduli of recently developed β-Ti alloys range from 55 to 85 GPa, which are much lower than those of 316L stainless steel, Cr–Co alloys, and CP-Ti.[2] Manganese (Mn), molybdenum (Mo), and niobium (Nb) have been investigated as β stabilizers for Ti alloys and studies have suggested that they can decrease the elastic modulus and increase other important mechanical properties of Ti-based alloys.[19–27] Biocompatibility tests for dental alloys including Ti alloys have been performed in various studies.[19,28–31] When metallic materials are implanted inside a body, they may corrode and/or wear. The released metal ions and/or debris can be toxic or irritating to surrounding tissues. The release of metallic ions during the destruction of the passive film can cause side effects in the body. Even though the Ti–6Al–4V alloy is an established implant material in orthopaedics, it is reported that ions associated with Ti–6Al–4V alloy inhibit the normal differentiation of bone marrow stromal cells to mature osteoblasts in vitro.[32] If biologically relevant molecules are released from the metallic biomaterial and interact with biologically relevant molecules, biologically active organo-metallic and metallic salts can be formed.[30,33–40] Thus, the biocompatibility of the metallic materials used for implant treatment should be evaluated during the development of Ti alloys.

Cytotoxicity of a biomaterial can be examined using either a monolith of the material,[41] a particulate form,[42–44], or extracted solutions, which may contain several types of released metallic compounds.[45–48] Most of the biocompatibility reports for metallic elements have been based on the use of metal salts or on extracted solutions containing the metal cations.[45,49,50] Because the cytotoxicity evaluations of metallic compounds are performed at different places by various methods using several kinds of cell lines, the results cannot be compared directly to each other. Moreover, the mass release of a particular element is not expected to be proportional to its atomic percentage in the alloy, and the lability of an element can be altered by other elements in the alloy.[51,52] Thus, when developing new Ti alloys, cytotoxicity assays for the fabricated alloys and the constituent pure metals are useful to study the effect of each component on the behaviour of cells. The in vitro cytotoxicity tests are relatively fast and they can be standardized relatively easily. Thus, the in vitro tests can provide highly reliable data and reproducible measurements even though their relevance to clinical practice is not always consistent.[53]

The evaluation of the bulk alloy cytotoxicity in a biological environment should be performed initially. However, to date, there is scant information about the cytotoxicity of Ti-alloy ingots.

The aim of this study was to evaluate the biocompatibility of candidate Ti-alloys using well-characterized fibroblast-like cell lines. Candidate alloying elements with Ti (Ag, Al, Cr, Cu, Mn, Mo, Nb, V, and Zr) were evaluated. Titanium alloys with varying elemental contents of alloying elements were evaluated for their cytotoxicity, consistent with the aim of developing new Ti-alloy systems as dental implant materials.

2. Materials and methods

2.1. *Sample preparation*

Binary Ti–A alloys that varied in the concentrations of element A (where A was Ag, Al, Cr, Cu, Mn, Mo, Nb, V, and Zr), in concentrations of 5, 10, 15 or 20 wt% in the Ti alloys, were fabricated using vacuum arc melting under a high purity argon atmosphere on a water-cooled hearth (Table 1). To homogenize the alloys, the prepared ingots were melted seven times, and the alloy specimens were treated for 4 h at temperatures 150 °C below the respective solidus temperatures and furnace cooled at an approximate rate of 10 °C/min to 600 °C in a high purity argon atmosphere followed by air cooling to room

Table 1 – Materials used in the study.

Raw material	Specification	Lot no.	Manufacturer
CP-Ti (Grade 2)	Rod, 10 mm dia. ASTM B265	04RB-10	Daido Steel Co. Ltd., Nagoya, Japan
Titanium	Sponge 3 mm and down 99.9%	129Q001	Alfa Aesar, Ward Hill, USA
Aluminium	Foil, 1.0 mm thick, annealed, 99.99%	40762	Alfa Aesar, Ward Hill, USA
Chromium	Pieces, 2–3 mm thick, 99.995%	38494	Alfa Aesar, Ward Hill, USA
Copper	Shot, 13 mm dia., 99.99%, oxygen free	36686	Alfa Aesar, Ward Hill, USA
Manganese	Granules, 0.8–10 mm, 99.98%	K21T034	Alfa Aesar, Ward Hill, USA
Molybdenum	Foil, 1.0 mm thick, 99.95%	36215	Alfa Aesar, Ward Hill, USA
Niobium	Sheet, 99.9%	SH-NB04	GMH Stachow-Metall GmbH, Goslar, Germany
Silver	Silver shot, 1–5 mm, Premion®, 99.99%	12186	Alfa Aesar, Ward Hill, USA
Vanadium	Pieces, 99.7%	42775	Alfa Aesar, Ward Hill, USA
Zirconium	Foil, 0.02 mm thick Annealed, 99.8%	44752	Alfa Aesar, Ward Hill, USA