Materials Science and Engineering C 77 (2017) 308–317

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# In-vivo investigations and cytotoxicity tests on Ti/Zr-based metallic glasses with various Cu contents

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ARTICLE INFO

*Article history:*
Received 13 September 2016
Received in revised form 1 November 2016
Accepted 24 March 2017
Available online 29 March 2017

*Keywords:*
Bulk metallic glass
BMG
Cytotoxicity
Copper content
In-vivo
Implantation
Osteoconduction

ABSTRACT

The Ti/Zr-based metallic glasses (MGs) with various Cu contents are prepared, with nominal compositions of $Ti_{45}Zr_{40}Si_{15}$ (Cu-free), $Ti_{45}Zr_{40}Si_{10}Cu_5$ (low-Cu), and $Ti_{45}Zr_{20}Cu_{35}$ (high-Cu). The mechanical properties, corrosion resistance, and in-vitro biocompatibility of these MGs are investigated by means of nano-indentation, electrochemical analyses, MTS assays and inductively coupled plasma mass spectrometry, as well as in-vivo biocompatibility in terms of scanning electron microscopy, micro-CT scans and histological observations. The results show that the electrochemical activity and biocompatibility of the MGs are sensitive to the Cu content. Following the electrocorrosion tests, an increase in ion concentration is observed in high-Cu MG. Eight independent in-vitro tests show that the higher ion concentration leads to a lower cell viability. The twelve-week in-vivo tests show that the Cu-free MGs can be a promising material for developing bio-implants. The high-Cu MG would release Ti and Zr ions with insignificant Cu ion following corrosion testing, enhancing an increased local osteoclast activity.

## 1. Introduction

Biomedical implants are widely used to replace missing biological structures (e.g., prostheses) or to support damaged biological structures as they heal (e.g., fixtures). However, in designing implants, particularly those which are intended to be placed permanently, an appropriate selection of the material composition is essential because of electrochemical reaction of material may result in adverse reactions to ensure the long-term integrity of the device and minimize the risk of infection and/or rejection. Implants are generally fabricated from steel, titanium (Ti), Co-based alloys, or precious metal alloys [1]. Among these various materials, Ti is one of the most commonly used due to its light weight good corrosion resistance, and excellent biocompatibility. However, pure Ti has a lower strength (300–500 MPa) and lower hardness (~1.5 GPa), which could be problematic for high loading conditions [2–4]. These alloys typically have a low wear resistance, and may therefore cause debris contamination following prolonged use. Furthermore, the Young's modulus of Ti implants is typically around 112 GPa, while that of human bone is just 3–20 GPa [5,6]. For implants attached directly to the bone, the large modulus-mismatch results in a significant stress shielding effect, which can lead to bone loss and, in extreme cases, bone fracture [7]. For implants, prolonged exposure to body fluids may lead to a corrosive reaction and result in the release of metal ions into the body. These ions may subsequently cause some negative effects [8,9]. Consequently, bio-inert materials such as Ti and Co alloys are most commonly preferred in the biomedical field [10,11]. The addition of Zr, Ta and Nb to Ti-based alloys has many mechanical advantages for biomaterial applications. However, the presence of multiple metallic components in the matrix increases the susceptibility of the alloy to electrochemical corrosion [12,13].

Metallic glasses (MGs) have an amorphous rather than crystalline structure, and therefore exhibit none of the dislocation, twin, vacancy and grain boundary properties of typical metals and alloys. The lack of grain boundaries also improves the electrochemical corrosion resistance [14,15]. Metallic glasses have been considered potential materials for biomedical applications for years [16,17]. The Ti-based metallic glass is a good candidate for implantation applications due to its improved corrosion and mechanical properties [18]. Ti-based MG exhibited passive behavior at the open-circuit potential with a low mean corrosion-penetration rate has been reported [19]. Alternatively, Zr-based MGs have also attracted a lot of intention for biomaterial applications [20–22]. The Ti/Zr-based metallic glass has many advantages as an implant

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http://dx.doi.org/10.1016/j.msec.2017.03.228
0928-4931/

material, including a light weight, good mechanical properties, high corrosion resistance, and good biocompatibility.

TiZr-based MGs have shown their potential for biomedical applications. However, the low glass forming ability (GFA) for TiZr-based MGs is an issue for practical fabrications processes. Therefore, in current Ti/Zr based MGs, Cu and Ni are almost universally added to an appreciable amount (~25–35 at.%) owing to their small atomic size (~0.125 nm in diameter) to match with the second empirical rule to improve the glass forming ability for MGs proposed by Inoue [23,24]. Although these two elements have been considered as harmful for human body, the literature contains many proposals for Ti/Zr-based Cu-containing MGs for biomedical applications, including Ti-Cu-Ni [25], Ti-Cu-Ni-Co [26], Ti-Cu-Ni-Zr [27], Ti-Cu-Ni-Zr-Be [28], and Zr-Cu-Ni-Al-Y [29] consequently. It is thus meaningful to examine systematically the in-vitro and in-vivo studies on the Cu content in the potential Ti/Zr-based MGs.

In order to exclude small atoms of Cu or Ni in MGs, Si has been proposed to replace Cu/Ni, such as the Ti-Zr-Si or Ti-Zr-Ta-Si, Ti-Zr-Nb-Si systems [30,31]. Si has even smaller atomic size (~0.110 nm in diameter), much smaller than Cu. A much lower amount of Si addition (such as 10–15 at.%) can already effectively randomize the atomic packing of the alloy, resulting in fully amorphous MGs. It has been shown that Ti-Zr-Ta-Si MG with 15% Ta addition has a higher yield strength (2390 MPa) than Ti-Zr-Si-Nb MG with 15% Nb addition (2200 MPa) [30]. Unfortunately, due to the ease in generating Si-containing nanocrystalline phases at high temperatures, Ti-Zr-Si MGs cannot be inherent with high GFA, and are difficult to be produced in bulk form. Thus, the developments of Ti-Zr-Si based MGs are intended to be designed into powders, followed by the powder metallurgy method to prepare porous foams for investigating their biocompatibilities [32]. The other route is to apply such Ti-Zr-Si MGs as coating layers on the commercial crystalline Ti based alloys to improve the wear and corrosion resistance. Both the powder and coating methods would avoid the disadvantage of lower GFA for such Ti-Zr-Si based MGs.

In this study, the TiZr-based amorphous systems with different Cu contents are systematically explored. Three different MGs with the nominal compositions of $Ti_{45}Zr_{40}Si_{15}$ (no-Cu or Cu-free), $Ti_{45}Zr_{40}Si_{10}Cu_5$ (low-Cu), and $Ti_{45}Zr_{20}Cu_{35}$ (high-Cu) are included. The reason we did not use the $Ti_{45}Zr_{40}Cu_{15}$ but apply the $Ti_{45}Zr_{20}Cu_{35}$ as the high-Cu MG is owing to the fact the 15 at.% Cu cannot result in the alloy to be fully amorphous. It needs to a higher level of 35 at.% Cu to disorder the hexagonal close-packed (HCP) crystalline structure of Ti and Zr. The mechanical properties, corrosion resistance, and in-vitro biocompatibility of the three MGs are investigated by means of nano-indentation, electrochemical corrosion tests, MTS assays and inductively coupled plasma mass spectrometry (ICP-MS). In addition, the in-vivo biocompatibility of these MGs is investigated by implanting MG ribbons (corroded and non-corroded) into the tibia on the right side of Sprague-Dawley rats and then performing SEM, micro-CT and histological observations.

## 2. Materials and methods

Fig. 1 shows the experimental design for the in-vivo studies and cytotoxicity tests performed in the present study. Before this, the in-vitro evaluation is firstly conducted for the Cu effects on the mechanical, electrochemical and biocompatibility characteristics of the TiZr based MG systems. Moreover, in evaluating the electrochemical corrosion properties of the MGs, commercially pure titanium (CP Ti) was used as a benchmark. The details of the material preparation process, cytotoxicity tests and implantation tests are described in the following sections.

### 2.1. MG preparation and characterization

Three bulk MGs with atomic compositions of $Ti_{45}Zr_{40}Si_{15}$ (Cu-free), $Ti_{45}Zr_{40}Si_{10}Cu_5$ (low-Cu), and $Ti_{45}Zr_{20}Cu_{35}$ (high-Cu) were prepared. The MG specimens were prepared using pure metals of Ti (99.99 wt%), Cu (99.999 wt%), Zr (99.9 wt%) and Si (99.99 wt%). The MGs were produced using a melt spinning method in a vacuum chamber. For each MG, an amorphous metal structure was formed by rapidly quenching the molten metal onto a copper roller in an argon atmosphere. (Note that full details of the melt spinning process are available in a previous study by the current group [33].) The spun MGs had a ribbon-like shape with a thickness of approximately 0.1 mm. The MGs were cut into pieces with dimensions of 3.00 mm × 6.0 mm (width × length) for subsequent electrochemical and cytotoxicity tests and

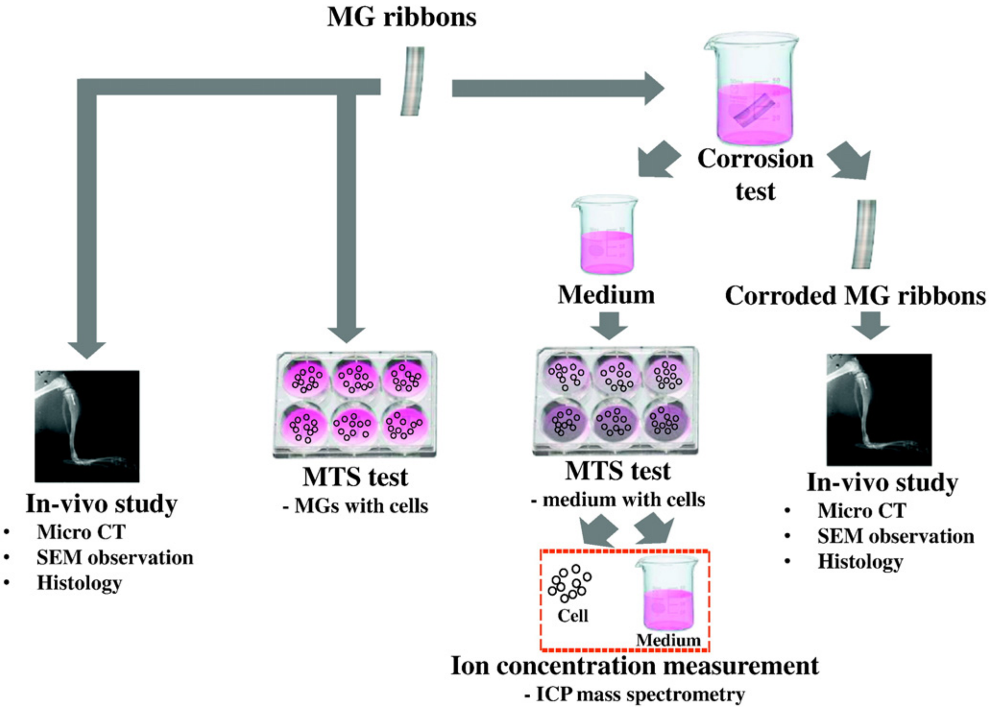

**Fig. 1.** Experimental design for in-vivo studies and cytotoxicity tests.