Screening Potential Biomaterials of Ti- and Zr-Based Metallic Glasses Rapidly


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Abstract

Corrosion phenomenon is the important factor in the field of biomaterial applications. The corrosion resistance of the metallic glasses was investigated by the cyclic voltammetry and a low-voltage potential state test of the cell membrane potential simulation in the simulation body fluid Hank’s solution in this paper. The potential Ti- and Zr-based metallic glasses were screened rapidly to test the cytotoxicity of the metallic glasses. Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 of 9 metallic glasses composition showed superior corrosion resistance and electrochemical stability. The metallic glasses with the higher Cu content exhibited electrochemical current responses. Cytotoxicity of the metallic glasses and the medium of the metallic glasses after the potential state test were made a study by the in-vitro MTT test further. The cell viability test results showed the solid specimens and the medium after the potential state test of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 have no significant cytotoxicity for murine bone marrow stem cells. In this study, the metallic glasses with high Ti and Zr content (> 80%) and low Cu content (≦ 5%) for the cell tissue have excellent electrochemical stability and low cytotoxicity by rapid electrochemical tests and they are potential biomaterials for biomedical applications.

Keywords : metallic glasses, biomaterial, electrochemical stability, MTT test

1. Introduction

For biomaterial applications, titanium metal has been the best inserted or implanted materials timelessly because of excellent biocompatibility, physical, mechanical, and chemical properties. The metallic glasses with homogenous structure making it to form passive state have better corrosion resistance [1]. The corrosion behavior of biomaterials was needed to avoid reacting in the biomedical applications. When the biomaterial contact with cell tissues in the body, the metal ions with the corrosion behavior of the metallic glasses released into body inducing allergy, inflammatory, diseases or cancer [2-4]. Nevertheless, titanium with lower strength and hardness was not suited to use in the field of biomedical clinical applications. In the recent decades, many researchers made a study of the metallic glasses for biomaterial applications due to excellent mechanical properties and superior chemical stability. The metallic glasses with homogenate composition have high strength, high hardness, high elastic limit, and excellent corrosion resistance. Besides, there are not crystal structural deficiencies for the metallic glasses such as dislocations, twins, vacancies, or grain boundaries. The metallic glasses with amorphous structure have no grain boundaries of microstructural defects making that electrochemical corrosion is suppressed or eliminated [5]. In the other hand, cytotoxicity is important factor for biomedical application. The implanted materials might release toxicity ion into the body and result in necrosis and apoptosis of the cell tissue [6]. In the region of super-cooled liquid temperature, the metallic glasses have superplastic capabilities with the exception of excellent corrosion resistance and high mechanical strength. For biomedical applications, many properties of the metallic glasses suit to turn into biomaterial probably.
The Ti-based metallic glasses were developed particularly for biomedical applications because of excellent biocompatibility of titanium. Ti-Cu-Ni [7], Ti-Cu-Ni-Zr [8] and Ti-Cu-Ni-Zr-Nb(Ta) [9] metallic glasses with good glass-forming ability were fabricated to research. Al and V elements of Ti-6Al-4V metallic glasses were released into the solution [10]. But the metallic glasses with poisonous elements such as Ni, Al and V resulted in the limit of the biomedical applications. In addition, Al element was considered to lead to Alzheimer’s disease possibly [11]. Recently the metallic glasses of Ti-Zr-Cu-Pd systems were investigated because of better glass-forming ability, higher strength and lower Young’s modulus than TiAlV alloy [12-13]. The corrosion behavior of Ti40Cu36Pd14Zr10 metallic glasses and its crystalline without Ni was investigated and reported in the simulation body fluid Hank’s solution [14]. Ti40Cu36Pd14Zr10 of Zr-based metallic glasses is a potential biomaterial due to the higher strength and elastic modulus that is similar to bones.

In the 1990s, Zr-based metallic glasses have been reported in the literature such as Zr-Al-Ni-Cu [15], Zr-Ti-Cu-Ni-Be [16] and Zr-Cu-Ni-Al-Ti [17]. And they revealed good properties, for example high hardness, high strength, large elastic limit, low Young’s modulus and near-net-shape formability [18]. For biomaterial implantation applications such as pins, plates and screws, Mg60Zn35Ca5 is probed into the workability of the biomaterial [19]. The hydrogen evolution of the glassy Mg alloys during degradation might occur significantly. But Mg60Zn35Ca5 metallic glasses only exhibited sight hydrogen evolution and no inflammatory reaction significantly during degradation due to the increasing of Zn element. The practicality of the Au- or Pd-based metallic glasses is lower because of the high-priced cost. However, the component of the Ni-, Al- or Cu-based metallic glasses contains toxicity elements to cause damage to the cell tissue. Even though Mg- and Zr-based metallic glasses are potential material for biomaterial implantation applications, they contain the component of the toxicity elements such as Ni, Al, or Cu in order to form metallic glasses. So the cytotoxicity of metallic glasses must be analyzed by the cell viability test which was needed to test in long time and repeat several times.

With the purpose of decreasing experiment time and cost, the cyclic voltammetry was used to investigate the electrochemical behavior of the metallic glasses in the simulation body fluid. To exclude the metallic glasses with significant electrochemical current response by the cyclic voltammetry screen. When the cyclic voltammetry current exhibited significant variations, the metallic glasses with corrosion behavior might release some toxicity ions and be instability for the biomedical applications. In addition, to simulate the membrane potential of the cell applying the potential of 80 mV in simulated body fluid in order to test the electrochemical stability of the metallic glasses. It is rapid to foresee the electrochemical corrosion and stability of the metallic glasses in the shorter time by two methods.

2. Experimental

2.1 MGs materials preparation and characterization

In this study, the Ti-based and Zr-based metallic glasses with nominal composition of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15, Zr50Ti35Si15, Ti40Cu36Pd14Zr10, Ti45Cu35Zr20, Zr61Cu17.5Ni10Al7.5Si4, Zr53Cu30Ni9Al8 and Zr53Cu30Al8Pd5Nb4 were fabricated by melt spinning method. The pure elements of Ti (99.99 wt%), Cu (99.99 wt%), Pd (99.9 wt%), Al (99.9 wt%), Zr (99.9 wt%), Ni (99.9 wt%), Si (99.9 wt%), Nb (99.99 wt%), Ta (99.9 wt%) were heated to liquid states, then quench to the solid state rapidly via single roller melt-spinning under argon atmosphere for producing the 1 x 10 mm2 MG ribbons with 0.1 mm thickness. The amorphous structure of the melt spinning MG ribbons were determined by using Bruker D8 X-ray diffractometry (XRD) with a mono-chromatic Cu-Kα radiation (λ = 1.5406 Å) and operated at 40 kV and 40 mA. The thermal properties of the MG ribbons were characterized by Perkin-Elmer Diamond DSC differential scanning calorimeter (DSC) at a constant rate of 0.67 K/s. Furthermore, the JEOL JSM-6330 scanning electron microscopy (SEM) equipped with energy dispersive spectrometry (EDS) is selected to check whether the compositions of the MG ribbons are mixed homogeneously.

2.2 Electrochemical activity test

The electrochemical behavior of the metallic glasses was investigated using a commercial electrochemical measurements (CHI 611c, CH Instruments Inc., USA). Electrochemical analyzer was connected to a three-electrode electrochemical cell with platinum wire counter electrode and standard Ag/AgCl reference electrode. The electrolyte was typical simulation body fluid Hank’s solution that was at 310K open to air. The composition of Hank’s solution is 0.137 M of NaCl, 5.4 mM of KCl, 0.25 mM of Na2HPO4, 0.44 mM of KH2PO4, 1.3 mM of CaCl2, 1.0 mM of MgSO4, 4.2 mM of NaHCO3. To observe the electrochemical behavior between the specimen and solution by a standard cyclic voltammetry (CV) with the scanning potential from 1 V to -1 V and the scan rate of 0.1 V/s. In addition, materials used in medical usually contact cell tissue of the body. The membrane potential of cell in the body is about 80 mV, the electrochemical test of low-voltage (80 mV) was used to predict the electrochemical corrosion condition between the metallic glasses materials and cell tissue. Experiment time was 30 min at 310K.

2.3 Cell viability test
The standard MTT assay(50 μg MTT in100 μl in medium) was used to observe cell viability experiment of the metallic glasses. The vitro cell culture test of specimen was investigated using pluripotent mesenchymal cells. Took the Balb/c mouse bone marrow stem cells and cloned D1 (ATCC) bone marrow stem cells in low glucose Dulbecco’s modified Eagle’s medium (DMEM). The composition of DMEM is 10% fetal bovine serum (FBS), 1.5 g/L sodium bicarbonate, 1% NEAA, 1% Vitamine C and 1% penicillin and streptomycin. The D1 cell with the concentration of 5×10⁴ cells/ml was prepared in a 6-well cell culture dishes. The test was prepared at 37°C in the humidified atmosphere with 5% CO2. The 75% alcohol was used to wash the metallic glasses and pure Ti with the size of 25 mm² for sterilization, the specimen immersed into 5 mL cultured medium for 72 hr. Furthermore, The low-voltage electrochemical ions release test of the metallic glasses in the medium with the D1 cells was investigated. The precipitate in the medium was separated by the 0.2 μm Minisart® NML syringe filter and the 200 μL medium added into 5 mL cultured medium for 24 hr. The cell viability of the D1 cells was evaluated by MTT assay after the cell culture test. Before the optical density measurement, cell incubation was performed at 37°C for 4 hr and putted in 100 μl DMSO solution. The crystals were dissolved by slow shaking for 5 min. The OD values of the cell viability was measured by reading and reference wavelength of 570 nm and 630 nm.

3. Results and discussion

3.1 Structural characterization of MGs

Before the test of electrochemical activity and cell viability, the amorphous structure of 9 MGs ribbons has to be determined and ensured by the XRD and DSC. Figure 1a shows all the XRD patterns of MG ribbons reveal the diffuse hump, and no obvious crystalline peak can be found. The thermal properties of the MG ribbons are shown in Figure 1b, the glass transition and crystallization temperature can be precisely defined by DSC. Hence, both of the XRD and DSC results demonstrated the Ti-based and Zr-based MGs ribbons fabricated by the melt spinning process are all in amorphous state.

3.2 Electrochemical activity of produced MGs

Cyclic voltammetry that is a fast measurement was used to measure the electrochemical behavior of the metallic glasses materials. The cyclic voltammetry result of pure titanium and the metallic glasses in Hanks’ solution at 310K are presented in Figure 2. The electrochemical behavior of the metallic glasses with the composition of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 is smaller, but the electrochemical responses or decompose of the metallic glasses with other composition was observable during the cyclic voltammetry measurement. Especially, the composition of the metallic glasses contains higher the Cu content that revealed observable the electrochemical behavior. The metallic glasses with the higher Cu content demonstrated stronger oxidation reaction and corrosion in the simulation body fluid environment with salt-rich. The metallic glasses with lower electrochemical responses would agree with biomaterials applications by the cyclic voltammetry measurement.

Figure 3 displays enlarged CV curves to show the current responses of the four metallic glasses with the composition of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 was lower than the titanium in the cyclic voltammetry measurement. Low current response for the metallic glasses with high Ti and Zr content (≥ 80%) and low Cu content (≤ 5%). The CV curves illustrated the excellent electrochemical stability of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 in the simulation body fluid with high ionic environment. The potential biomedical metallic glasses were sifted rapidly by the cyclic voltammetry measurement.

When the materials were used in the implanted biomedical applications, the materials suffered from the electric potential between the interior and the exterior of the cell membrane. The values of membrane potential for contacting with cell tissue range about –40 mV to –80 mV typically. In order to make a study of the electrochemical stability of the metallic glasses in the simulation body fluid, the specimens were immersed into simulation body fluid in the low-voltage potential state test with 80 mV for 30 min. Figure 4 displays the amperometric i-t records of the metallic glasses and the reference metal Ti in the electric potential test of 80 mV. The current response of metallic glasses with the composition of Ti40Cu36Pd14Zr10, Ti45Cu35Zr20, Zr61Cu17.5Ni10Al7.5Si4, Zr53Cu30Ni9Al8 and Zr53Cu30A18Pd5Nb4 was observable under the potential state test of 80 mV. Results showed that noticeable electrochemical behavior of the five metallic glasses for 30 min, indicating electrochemical instability for Ti40Cu36Pd14Zr10, Ti45Cu35Zr20, Zr61Cu17.5Ni10Al7.5Si4, Zr53Cu30Ni9Al8 and Zr53Cu30A18Pd5Nb4. Particularly, the current response of Zr53Cu30Ni9Al8 decreased little by little as time goes by. As time passes by, the decomposition of Zr53Cu30Ni9Al8 caused that the surface area was immersed into simulation body fluid of the specimen decreased by degrees. The current response of the specimen decreased as the surface area decreased.

Figure 5 displays enlarged amperometric i-t curves to show the close-up current responses of the pure Ti and the metallic glasses with the composition of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15. In the low-voltage potential state test, the current curves of the four metallic glasses were slighter than the other metallic glasses. The current

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values of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 were close to the reference metal Ti. And the measured current values of the four metallic glasses kept in 30 min, illustrating that the electrochemical reaction of the specimen didn’t almost occur. The electrochemical stability of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 was considered excellent further.

3.3 Cell viability test
Cytotoxicity is the quality of toxicity to the cell tissue that would result in the occurrence of necrosis for biomedical applications. TO make a study cytotoxicity of the metallic glasses and pure Ti with cultured D1 cells is an important experiment. The cell viability test result of the metallic glasses and pure Ti with culturing for 72 hrs and the medium of the metallic glasses and pure Ti with culturing for 24 hrs after the low-voltage potential state test presented in Figure 6. The electrochemical decomposition of the metallic glasses during the potential state test might cause that the metal ions of the metallic glasses released into the simulation body fluid, these metal ions might be toxicity to the cell tissue. To test the toxicity of the released metal ions by adding the cultured medium (4% in volume) and the cultured D1 cells into the medium after the potential state test. The pure Ti was used as the standard in the cell viability test. The blue bar showed that the cell viability of the specimen with culturing D1 cells for 72 hrs, the cell viability of each specimen was higher than 85%. Results indicated that the cytotoxicity of the specimen without applied low-voltage electrical potential was no significant. The red bar showed that the cell viability of the medium after the low-voltage potential state test with culturing for 24 hrs, the metallic glasses of Ti60Cu30Pd10Zr10, Ti45Cu35Zr20, Zr50Cu17.5Ni10Al7.5Si4, Zr50Cu17.5Ni10Al7.5Si4 and Zr50Cu17.5Ni10Al7.5Si4 displayed noticeable cytotoxicity. The cell viability of these metallic glasses was lower than 70%, the cell viability of Zr50Cu17.5Ni10Al7.5Si4, Zr50Cu17.5Ni10Al7.5Si4 and Zr50Cu17.5Ni10Al7.5Si4 was lower than 50% particularly. The released Ni, Al or Cu ions of the metallic glasses might be toxicity to the cell tissue, and resulting in apoptosis. However, the cell viability of Ti60Zr40Si15Cu5, Ti60Zr40Si15Cu5, Ti60Zr40Si15Cu5 and Zr50Ti35Si15 was similar to the pure Ti, indicating the four metallic glasses were potential materials for biomaterial applications.

4. Conclusions
In this study, the potential materials for biomaterial applications were tested and screened rapidly by the method of experiment. The tested results of Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15 and Zr50Ti35Si15 approximated to the pure Ti in the CV test, the potential state test and the cell viability test. Electrochemical activity and electrochemical response of the metallic glasses with high content of Cu were significant especially. The metallic glasses with the composition of Ni, Al and Cu metal had noticeable corrosion response and cytotoxicity. Ni, Al and Cu content of the metallic glasses materials for the biomedical applications are better to be decreased during the compounds manufacturing. This method with rapid test reduces the time of screening potential biocompatible materials and the costs of the experiment. The summary of the study, the metallic glasses with high Ti and Zr content (> 80%) and low Cu content (≤ 5%) have better electrochemical stability and biocompatible for the biomedical applications.

5. Acknowledgment
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6. References

7. Chart arrangement

Fig 1 (a) XRD patterns and (b) DSC scans of MGs ribbons

Fig 2 The comparison of the electrochemical (CV) responses for pure Ti, Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15, Zr50Ti35Si15, Ti40Cu36Pd14Zr10, Ti45Cu35Zr20, Zr61Cu17.5Ni10Al7.5Si4, Zr53Cu30Ni9Al8 and Zr53Cu30Al8Pd5Nb4 MGs

Fig 3 Closed-up view of the electrochemical (CV) for pure Ti, Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15, Zr50Ti35Si15 in Hank’s solution
Fig 4 The comparison of the i-t curves for pure Ti and various MGs in the low-voltage (80 mV)

Fig 5 Closed-up view of the i-t curves for pure Ti, Ti40Zr40Si15Cu5, Ti42Zr40Ta3Si15, Ti45Zr40Si15, Zr50Ti35Si15 in the low-voltage (80 mV)

Fig 6 The comparison of MTT tests for pure MGs with culturing for 72 hrs and the medium with culturing for 24 hrs after the potential state test