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Biocompatibility and thermoplastic formability of Pt-based metallic glasses

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ABSTRACT

Pt-based metallic glasses are considered for biomedical applications, but the presence of Ni poses the cytotoxicity concerns. Here, we compare the in vitro cell response and thermoplastic formability of Ni-free and Ni-containing Pt-based metallic glasses. Three glass forming compositions, $Pt_{57.5}Cu_{14.7}Ni_{5.3}P_{22.5}$, $Pt_{58.7}Cu_{20.3}Ag_1P_{20}$, and $Pt_{57}Cu_{23}P_{20}$ were investigated. The Ni-free Pt-Cu-Ag-P metallic glass combines the best combination of biocompatibility and thermoplastic forming.

1. Introduction

Metallic glasses (MGs) are amorphous alloys which exhibit remarkable strength and elasticity due to absence of crystal defects [1]. Amorphous structure can be formed over a wide range of compositions, which offers flexibility in tailoring the MG for a specific application [2]. For example, Mg-Zn-Ca MGs are biodegradable [3], Pt-Cu-Ni-P MGs are catalytically active [4], and Co-Fe-Ta-B MGs are stronger than steel [5]. MGs are also attractive because of their thermoplastic forming in the supercooled liquid state below crystallization temperature [6]. Pt-based MGs are appealing because of their low processing temperatures (<300°C) and higher oxidation resistance [7,8]. Pt-based MGs can be molded into intricate shapes at moderate temperature and pressure in ambient conditions [6–8].

Recent trend in Pt-based MGs is shifting towards small-scale biomedical devices, which can harness the manufacturing potential without requiring significant material or ductility [9]. Material cost and limited ductility have been the major roadblocks for applications of MGs. Surgical tools, sensors, and microneedles have been produced by thermoplastic forming of Pt-based MG [9–11]. One of the issues for biomedical applications is the presence of Ni in Pt-based MGs. It remains unclear if Pt-based MGs cause toxicity, but Ni-free MGs are actively investigated [12–14]. The aim of this work is to study the effect of Ni on biocompatibility and thermoplastic formability of Pt-based MGs.

2. Materials and methods

$Pt_{57.5}Cu_{14.7}Ni_{5.3}P_{22.5}$, $Pt_{58.7}Cu_{20.3}Ag_1P_{20}$, and $Pt_{57}Cu_{23}P_{20}$ MGs were prepared in the form of 2 mm diameter cylindrical rods as reported elsewhere [6,8]. The thermal properties were studied using differential scanning calorimeter (DSC). Thermoplastic forming experiments were conducted using heating plates mounted on a mechanical tester. The formability was quantified using an approach proposed by Schroers [15]. Circular discs of comparable volumes (diameter = 3.45 ± 0.05 mm and thickness = 0.40 ± 0.05 mm) were heated from 220°C to 330°C under a constant load of 500 N and the final diameters were compared. Ability to replicate nanoscale features was tested by isothermal embossing at 270°C under 1000 N against nanoporous alumina containing pores of about 120 ± 25 nm diameters. The MG nanowires were imaged using scanning electron microscope (SEM).

In vitro cytotoxicity of MGs was evaluated by direct contact method following ISO 10993–5 guidelines [16]. Six samples of each MG were polished with 2400 grit paper and sterilized with 70% ethanol. The mouse fibroblast L-929 cells (ATCC) were grown in Eagle's Minimum Essential Medium (ATCC) supplemented with 10% fetal bovine serum (ATCC) and 1% penicillin–streptomycin (Gibco). The cells were cultured in 24-well plates (25000 cells/well) at 37°C with 5% CO_2 for 24 h. The MGs were carefully placed on the cell layer, and the plates were incubated for 24 h and 48 h. Subsequently, the media were removed, 500 ml MTT solution (0.5 mg/ml in PBS) was added to each well, and the plate was incubated at 37°C for 3 h. The MTT solution was removed, and the resulting formazan was dissolved in 500 ml DMSO solution. The

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MGs were removed, and the absorbance was measured at 570 nm using a microplate reader. The cell viability was calculated by the equation viability% = OD570s/OD570b × 100. The OD570s and OD570b are the average values of the measured optical density of the MG and the blank groups, respectively. A tested product has cytotoxic potential when the cell viability reduces below 70%.

To observe the cell morphology by fluorescence staining, L-929 cells were directly seeded on the MGs placed in the well and incubated under the same condition as described before. At about 80% confluency, the cells were fixed with 4% paraformaldehyde for 30 min and then stained with ActinGreen™ 488 ReadyProbes® Reagent and ProLong® Gold reagent (Thermo Fisher). The images were taken by an inverted fluorescence microscope.

3. Results and discussion

Fig. 1 shows the constant heating rate and isothermal DSC curves of Pt-based MGs. The glass transition (T_g) and the crystallization (T_x) temperatures are in the range of 220–240°C and 300–310°C, respectively (Fig. 1a). The ternary Pt-Cu-P MG shows a single exothermic crystallization peak, which has been attributed to coupled eutectic growth [17]. The Pt-Cu-Ni-P MG has the largest supercooled liquid temperature range of 75°C. Isothermal crystallization curves measured at 270°C are plotted in Fig. 1b. The onset time of crystallization is longer for the Pt-Cu-Ni-P MG which is consistent with the constant heating rate crystallization data. However, all three MGs remain in viscous state for more than 15 min, which is sufficient for forming operations.

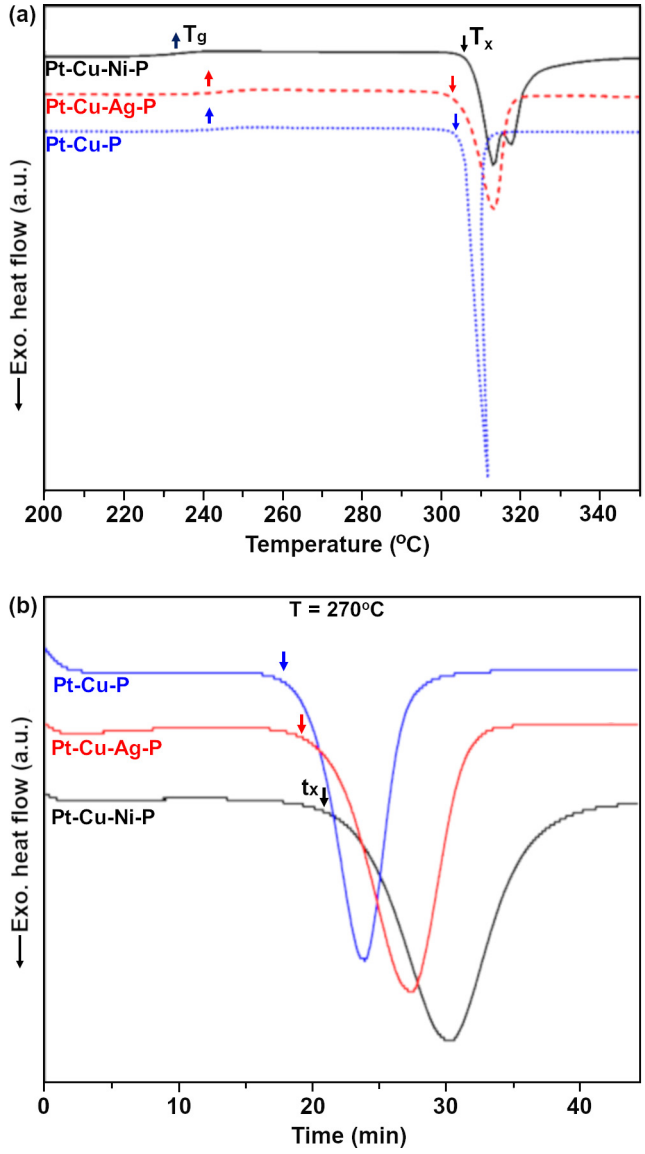

Fig. 1. (a) Constant heating rate (20 °C/min) and (b) isothermal DSC curves at 270 °C for Pt-based MGs.

Fig. 2 shows the thermoplastic forming results for three MGs. The upper panel compares the final diameters after deforming the same volume through the entire supercooled temperature range. The Pt-Cu-P MG shows the better formability with a diameter of 8.6 mm compared to 7.8 mm for Pt-Cu-Ni-P MG. The results show that the supercooled liquid temperature range is not a measure of thermoplastic deformability. The large formability of Pt-Cu-P MG is attributed to its ultrahigh fragility [17]. It has been shown that fragile MGs can exhibit high thermoplastic formability despite their narrow supercooled liquid temperature range [18]. The strong temperature dependent viscosity compensates the detrimental effect of short crystallization time. Fig. 2 (lower panel) compares the SEM images of MG nanowires imprinted by isothermal embossing. Nanowires with an aspect-ratio of 5 were formed on all samples. The variation in diameter of nanowires stems from the alumina templates which contain nonuniform pores. The ability to flow at nanoscale suggests that the interfacial and oxidation properties of three MGs are similar.

The cell viability after direct contact was quantified using MTT assays [19]. Mitochondria of viable cells reduce the tetrazolium compound into an insoluble formazan, which is spectrophotometrically analyzed after dissolution in DMSO. Fig. 3a shows the viability of L-929 cells cultured for 24 h and 48 h on MG surfaces. The cell viability on all three MGs remains greater than 70% in comparison with the blank group, which suggests that there is no potential cytotoxicity in these MGs. Surprisingly, the Ni-free Pt-Cu-P MG shows lower cell viability of 74.5% compared to 82% for the Ni-containing Pt-Cu-Ni-P MG. The cell viability drops to 70% for Pt-Cu-P MG after 48 h, which suggests that the ternary alloy has higher cytotoxicity. This is likely due to the higher weight concentration of Cu in Pt-Cu-P, which results in more Cu ions released into the medium. Heavy metal ions can induce toxic effects once they exceed the normal range [20]. The quaternary Ni-free Pt-Cu-Ag-P MG retains more than 80% cell viability after 48 h. These findings provide a preliminary biocompatibility assessment of Pt-based MGs though in vivo analysis is required for clinical applications.

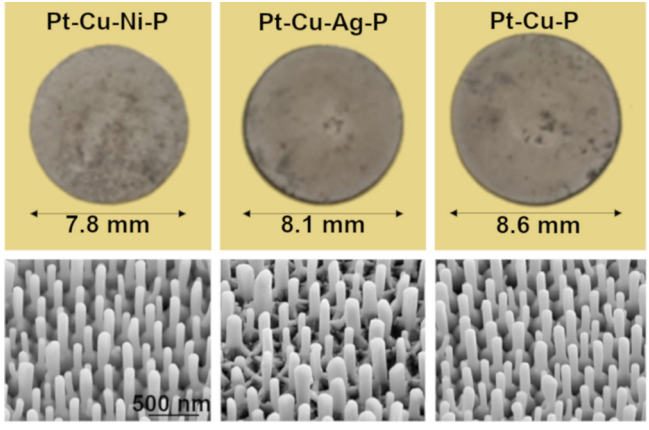

Fig. 2. The sample diameters after pressing through the entire supercooled range (upper panel) and nanowires (lower panel) imprinted by isothermal embossing for Pt-based MGs.

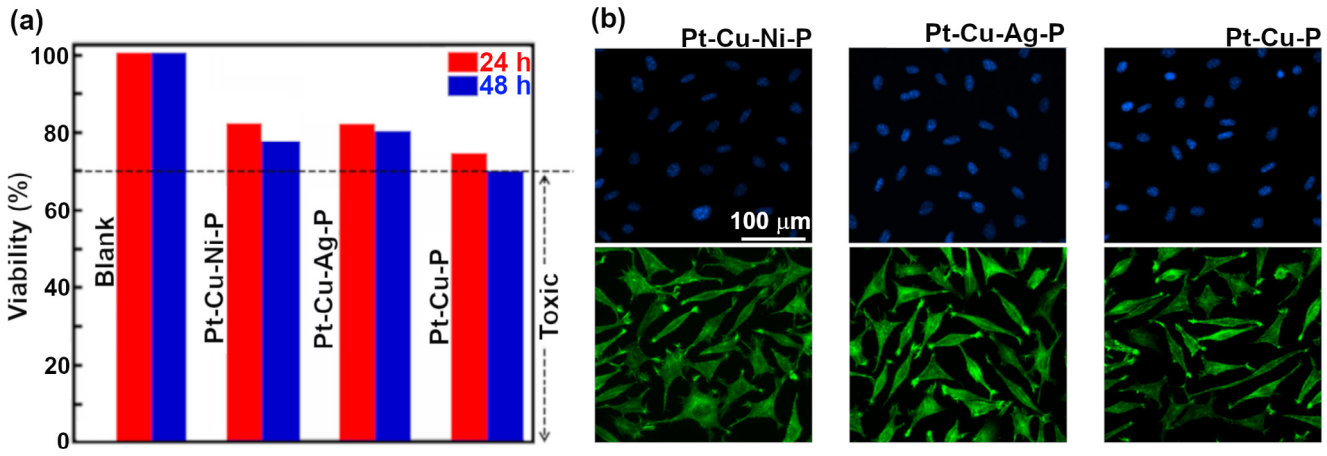

Fig. 3. (a) Viability of cells cultured on MGs after 24 h and 48 h. (b) The fluorescent images of L-929 cells cultured for 48 h on MGs and stained with ProLong™ Gold Antifade Mountant with DAPI (nuclei, blue) and ActinGreen™ 488 ReadyProbes™ Reagent (F-actin, green).

The formazan absorbance is not an absolute measure of the cell number because a change in metabolic state of cells (i.e., metabolic deactivation or activation) can affect the results. An exposure to higher concentrations of certain metal ions may not necessarily lead to cell death or reduced cell proliferation, but to a reduced cell metabolism. Therefore, we have also studied the morphology of cells directly seeded on the MGs after 48 h. The attachment and growth of L-929 cells on MG surfaces was studied by imaging the cells after staining with fluorescent dyes. Fig. 3b shows the cell nucleus' morphology stained with ProLong™ Gold Antifade Mountant with DAPI (upper panel) and cytoskeleton fiber F-Actin with ActinGreen™ 488 ReadyProbes™ Reagent (lower panel). The results demonstrate smooth nuclear membrane, evenly distributed chromatin, uniform cell size and shape, firm adhesion without intracytoplasmatic granules. The observations indicate that the cells are in a healthy condition, and no visible difference was observed among the three compositions.

4. Conclusions

In summary, we studied the thermoplastic forming capability and in vitro biocompatibility of three Pt-based metallic glass forming compositions. All three alloys can be thermoplastially molded into nanoscale features below 300°C in air. The Pt-Cu-P shows larger deformation due to higher fragility while its biocompatibility is inferior compared to the Pt-Cu-Ni-P and Pt-Cu-Ag-P metallic glasses. About 77% cells remain viable on Pt-Cu-Ni-P after 48 h despite the presence of Ni. The Pt-Cu-Ag-P metallic glass retains cecombines the best combination of thermoplastic forming and biocompatibility.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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