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Responses of osteoblast to surface modified bulk metallic glass[†]

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1. Introduction

Metallic glass has many attractive properties as a biomaterial, such as high strength, high elastic limit, low Young's modulus and high corrosion resistance. Surface modifications of the metallic materials have been tried due to insufficiency of biocompatibility. One of the techniques is a combination of femtosecond laser and hydrothermal-electrochemical treatment (Ref. 1,2). The modified surfaces are expected to obtain bioactive property since apatite forming ability in the simulated body fluid has been demonstrated. To evaluate biocompatibility in vitro, the objective study was to examine cellular behavior responding to the surface modified metallic glass. The attachment, spread, and adhesion of osteoblasts on the materials are investigated.

2. Materials and Methods

1. Fabrication of surface modified Ti-based bulk metallic glass

a) Ti-based ($Ti_{40}Zr_{10}Cu_{36}Pd_{14}$) bulk metallic glass (BMG) samples

The $Ti_{40}Zr_{10}Cu_{36}Pd_{14}$ alloy ingots (compositions are given in nominal atomic percentages) were prepared by arc melting mixtures of pure Ti, Zr, Cu, and Pd metals (>99.9 mass%) in a high purity argon atmosphere purified using Zr getter. Bulk cylindrical rods with a diameter of 5 mm were prepared by melting of the master ingots followed by tilt casting into copper molds.

The disc samples with 5 mm in diameter and 2 mm in height were cut out from the rods, then polished. The glassy structure of as-prepared samples was examined by X-ray diffractometry in reflection with monochromatic Cu $K\alpha$ radiation. The thermal stability was examined by differential scanning calorimetry (DSC) under an argon atmosphere at a heating rate of 0.67 K/s.

b) Femtosecond (Ti:sapphire) laser

A femtosecond (Ti:sapphire) laser was employed in this experiment. The wavelength, pulse duration and repetition rate of the femtosecond laser were 775 nm, 150 fs and 1

kHz, respectively. The laser beam was focused onto the Ti-based BMG ($Ti_{40}Zr_{10}Cu_{36}Pd_{14}$) surface by a lens with a 100 mm focal length. The Gaussian laser beam had a diameter of 60 μm (at the $1/e^2$ intensity points) on the Ti-based BMG surface. Ti-based BMG's surface was irradiated with the femtosecond laser at the average laser fluence of 0.15 and 1.0 J/cm^2 , respectively. The laser beam was scanned on the Ti-based BMG's surface in the area of $5 \times 5 mm^2$ using the XY stage. Then, laser scanning speed was 1 mm/sec and hatching distance was 20 μm .

c) Hydrothermal-electrochemical (H-E) treatment

After femtosecond laser irradiated, Ti-based BMG was subjected to H-E treatment at 90 °C for 120 hours in 5 mol/L NaOH solutions to attain the surface bioactivity. The cylindrical Ti-based BMG ($Ti_{40}Zr_{10}Cu_{36}Pd_{14}$) specimen, 5 mm in diameter was used as the working electrode facing the circular surface to the counter electrode of platinum sheet, $10 \times 50 \times 0.5 mm^3$. The constant dc voltage of 1.4V was applied between these electrodes keeping their distance at 4 cm during H-E treatment.

2. Cell culture and evaluations of biological responses

a) Cell culture

Osteoblast-like MG63 cells were seeded directly onto the Ti-based BMG's surface irradiated with the femtosecond laser at the average laser fluence of 0.15 and 1.0 J/cm^2 and subjected H-E treatment (designated 0.15 and 1.0 J/cm^2 surface, respectively).

b) Cell attachment assay and morphology analysis

The attachment of cells were fixed and stained with fluorescent dye, rhodamine phalloidin and DAPI, and analyzed using a microscopic image-based observation.

c) Vinculin expression analysis

The expression and localization of focal adhesion protein, vinculin, were analyzed using a microscopic image-based observation and densitometry.

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3. Results and Discussion

The number of osteoblasts attached to the two surfaces after 3h of incubation was not significantly different (**Fig. 1**).

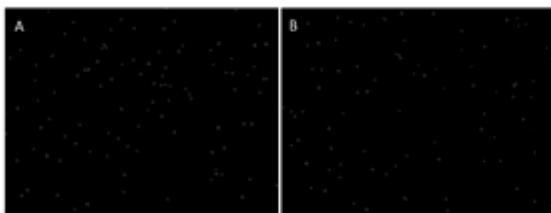


Fig. 1 Representative microscopic images of cells stained with DAPI for nuclei (blue) on (A) 0.15 and (B) 1.0 J/cm² surface.

Microscopic images of cells stained with rhodamine phalloidin showed that the cells were larger on the 1.0 J/cm² surface than on the 0.15 J/cm² surface where a majority of cells were rounded (**Fig. 2**).

The expression of vinculin was observed in cells both on 0.15 and 1.0 J/cm² surfaces. However, the localization of vinculin at the tip of stretching cytoplasmic projections was seen apparently on 1.0 J/cm² surface compared to 0.15 J/cm² surface (**Fig. 3**).

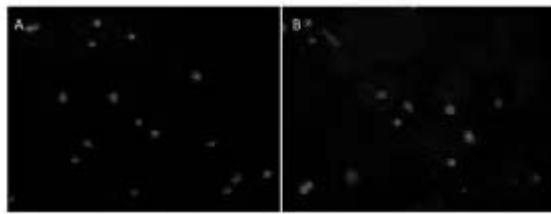


Fig. 2 Representative microscopic images of cells stained with rhodamine phalloidin for actin filaments (red) and DAPI (blue) on (A) 0.15 and (B) 1.0 J/cm² surface.

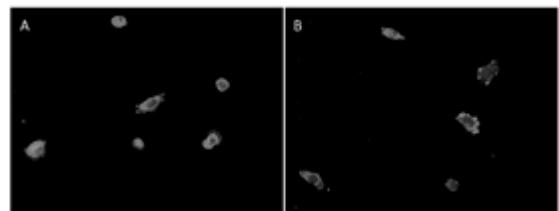


Fig. 3 Representative microscopic images of cells stained with anti-vinculin (green) and DAPI (blue) on (A) 0.15 and (B) 1.0 J/cm² surface.

4. Conclusions

This study addressed the initial cell-material interaction occurring on a surface-modified BMG. The conclusions of this study are summarized as follows.

- (1) The number of osteoblasts attached to both surfaces is nearly equal. The spread and adhesion of osteoblasts was enhanced on 1.0 J/cm² surface compared to 0.15 J/cm² surface, which was supported by the increased expression of focal adhesion protein, vinculin.
- (2) The interactions between cells and the surface-modified BMG at an early stage may indicate the potential as future biomaterials.

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