Osteoblast compatibility of 3D printed Co–Cr–Mo alloys with different building direction

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Abstract: Three-dimensional (3D) metal printing is an attractive technique for fabricating biomedical devices. The microstructures and mechanical properties of the fabricated 3D metal printed products show anisotropy because the grains grow along the z-axis direction. However, the effects of different printing directions on the cytocompatibility of the products are unknown. In the present study, cobalt–chromium–molybdenum (Co–Cr–Mo) alloy cylinders were fabricated along the z-axis (vertical) and x-axis (horizontal), respectively, and the effect of build direction on the cytocompatibility was evaluated. The build direction of 3D printed Co–Cr–Mo alloy changed grain anisotropy; however, there was no statistical difference in grain size. The wettability, protein adsorption, cell attachment, and cell proliferation on the disks of vertical and horizontal products were similar to those on cast alloy; they were significantly lower than those of commercially pure titanium and tissue culture polystyrene. The build direction of 3D printed Co–Cr–Mo alloy does not affect osteoblast compatibility.

1 Introduction

Cobalt–chromium–molybdenum (CCM) alloys are widely used for biomedical applications, such as in the dentistry or orthopaedics fields because the alloys exhibit high mechanical strength and great wear resistance [1–4]. However, the fabrication of CCM alloys is usually difficult owing to their high melting points and hardness [5]. Recently, 3D printing technologies using selective laser melting (SLM) and electron beam melting have been developed to fabricate medical devices based on CCM alloys [6–14]. These technologies could overcome the limitations of the CCM fabrication process. Interestingly, the mechanical properties of 3D printed CCMs are better than those of cast alloy and satisfy the type 5 criteria in ISO22764, and their metal elution is lower than that of cast alloy [9].

In the 3D printing process, the products are produced in several build directions such as the horizontal (along the x-axis) and vertical (along the z-axis) directions [9, 11, 15]. The build direction relates to the tensile strength of the 3D metal printed product. Vertical products of 3D printed CCM show higher yield strength and lower elongation than horizontal products. In addition, the build direction also affects grain size and microstructure of the alloy [9, 11]. Especially, grains grow along the build direction of the products. Grain size and grain boundaries of metal influence surface properties of protein adsorption and cell adhesion to its surface [16–20]. Therefore, clarifying the cytocompatibility of the different build directions of 3D metal printed products is important. However, the effect of different printing directions on the cytocompatibility of 3D printed CCM products is unknown.

The aim of this study is to investigate the effect of build direction, which affects grain anisotropy, on the osteoblast compatibility of 3D printed CCM alloys. The wettability, protein adsorption, osteoblast adhesion, and osteoblast proliferation on 3D printed CCM alloys with different build directions were evaluated. Our findings will provide important information for the design and creation of CCM alloy-based 3-D printed biodevices for medical application.

2 Materials and methods

2.1 3D printed CCMs

CCM cylinders (8 mm in diameter) were fabricated using the SLM process along either the z-axis or x-axis direction using an EOSINT M250 (EMO GmbH, Krailling, Germany) under laser power of 200 W, scan spacing of 0.2 mm, and stacking thickness of 0.05 mm. EOS MP1 powder (Co–28Cr–6Mo; EOS) was used in this study. The definition of the build direction is shown in Fig. 1a. To prepare the test specimens, the cylinders were cut into 2-mm-thick disks. The test specimens, which were cut from cylinders fabricated along the either vertical or horizontal direction, were named ‘CCM-A’ or ‘CCM-B’, respectively (Fig. 1a). The microstructure of 3D printed CCM was characterised using field emission scanning electron microscopy and electron backscattered diffraction (Quanta 200 3-D SEM-TSL) analysis. The specimens were polished by #1500 silicon carbide (SiC) polishing paper to obtain similar surface roughness values among the specimens because surface roughness leads to changes in cellular function [21–23]. After polishing, the specimens were ultrasonically rinsed in methanol and ultrapure water for 15 min, in this order. The hydrophilicity of the CCMs and commercially pure titanium (cpTi) surface was evaluated by measuring the contact angle of 1 μl of water placed on the disks. Photographs of water drops on the disks were captured using Image J software (NIH, Bethesda, MD, USA).

2.2 Protein adsorption

Bovine serum albumin (Thermo Fisher Scientific, Waltham, MA, USA) was dissolved in 0.1 M phosphate-buffered saline (PBS) at a concentration of 1 mg/ml. The disks were immersed in the protein solution at 37°C for 1 h. Proteins were recovered from the disks by sonication in 5% sodium dodecyl sulphate (Wako Pure Chemical, Osaka, Japan) and quantified using a micro-BCA assay kit (Thermo Fisher Scientific). Absorbance at 562 nm was
measured using an ultraviolet–visible (UV–vis) spectrophotometer (SmartspecPlus spectrophotometer, Bio-Rad, Hercules, CA, USA).

2.3 Cell culture and cytocompatibility assessment

Mouse osteoblasts (MC3T3-E1) were obtained from RIKEN BRC (Tsukuba, Japan). Cells were cultured in α-minimal essential medium (Wako) containing 10% foetal bovine serum (Biosera, Sussex, UK) supplemented with 1% penicillin/streptomycin (Nacalai Tesque Inc., Kyoto, Japan) in a 5% CO2 incubator at 37°C. To evaluate osteoblast adhesion, MC3T3-E1 cells were seeded onto the CCMs at a density of 20,000 cells/cm2 and cultured for 4 h. After cultivation, non-attached cells were removed by washing with PBS. The number of attached cells was calculated using a Cell-counting kit 8 (Dojindo Chemical Industries, Kumamoto, Japan). The absorbance of the samples was measured at 450 nm using a UV–vis spectrophotometer (Bio-Rad). The cell attachment activity was determined as the ratio of cells attached to the CCMs to those attached to tissue culture polystyrene (TCPS). To evaluate proliferation, cells were seeded on CCMs at a density of 5000 cells/cm2 and cultured for 1, 2, 4, and 7 days. The cells were also cultured on TCPS and cpTi under the same conditions as those for controls. Cell growth on CCMs was determined using Cell-counting kit 8.

2.4 Statistical analysis

Five samples (n = 5) were analysed for each experiment. Differences between groups were evaluated by Student’s t test. p < 0.05 was considered to indicate statistically significant differences between groups.

3 Results and discussion

Figs. 1b and c show the backscattered electron (BSE) images of each 3D printed CCM. The grains of CCM-B exhibited anisotropy along the z-axis direction. The grain size in diameter of CCM-A and CCM-B was 48.2 ± 30.1 and 108.4 ± 70.1 μm, respectively. The grain size of CCM-B tended to be larger than that of CCM-A. However, fine grains appeared both in CCM-A and CCM-B. The variation of data was large and calculated p value was >0.05 (p = 0.117), hence we decided there was no statistical difference in grain size.

The surface properties of the CCMs were evaluated by assessing their wettability and protein adsorption. The water contact angles were 71.6 ± 1.3°, 70.9 ± 3.0°, 68.5 ± 3.8°, and 56.2 ± 8.4° for CCM-A, CCM-B, cast CCM, and cpTi, respectively (Fig. 2a). Although CCMs exhibited lower wettability than cpTi, there was no statistical difference between the CCMs. The amount of

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**Fig. 1** Definition of build direction of
a 3D-printed CCMs
b BSE images of vertical-‘CCM-A’
c BSE images of horizontal-‘CCM-B’

**Fig. 2** Surface chemical properties of CCMs and cpTi
a Static water contact angle
b Amount of protein adsorption
adsorbed albumin was 4.6 ± 1.5, 4.2 ± 3.9, 4.8 ± 2.1, and 10.2 ± 2.0 μg/cm² for CCM-A, CCM-B, cast CCM, and cpTi, respectively (Fig. 2b). Albumin more easily adsorbed onto cpTi than that onto the CCMs. However, there were no differences in the protein adsorption between the CCMs.

The attachment activity of osteoblasts is shown in Fig. 3a. The percentage of attached cells compared with those attached to TCPS was 43.2 ± 9.2, 34.8 ± 2.4, 41.3 ± 5.7, and 68.3 ± 6.8%, for CCM-A, CCM-B, cast CCM, and cpTi, respectively. The number of attached cells on CCM-B was lower than that of cells on CCM-A; however, the p-value calculated from the statistical analysis between CCM-A and CCM-B was 0.203, we decided there was no statistical difference in CCM-A and CCM-B. Moreover, the p-value calculated from the statistical analysis between cast CCM and CCM-A was 0.774, and that between cast CCM and CCM-B was 0.142. Hence we decided there were no statistical differences in three CCMs. Osteoblast proliferation on CCM-A, CCM-B, cast CCM, and cpTi and TCPS is shown in Fig. 3b. The cell growth rate on CCMs was significantly slower than that on cpTi and TCPS. However, the cells continued to grow on CCMs for 7 days. According to a previous study, metal ion elution from cast CCM is lower than that onto the CCMs. However, there were no differences in the amount of metal ions eluted from cast CCM and CCM-B. Moreover, the number of attached cells compared with those attached to TCPS, cast CCM, and cpTi, respectively (Fig. 2a). Albumin more easily adsorbed onto cpTi than that onto the CCMs. However, there were no differences in the protein adsorption between the CCMs.

In summary, although CCM-A and CCM-B were fabricated with different build directions and exhibited different grain anisotropy, these differences had no effect on their surface biochemical properties and cytocompatibility.

4 Conclusion

In summary, although CCM-A and CCM-B were fabricated with different build directions and exhibited different grain anisotropy, these differences had no effect on their surface biochemical properties and cytocompatibility.

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