Titanium Powder Coating Using Metal 3D Printing: A Novel Coating Technology for Cobalt–Chromium Alloy Implants

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Abstract

BACKGROUND: Three-dimensional (3D) printing with a direct metal fabrication (DMF) technology has been innovatively introduced in the field of surface treatment of prostheses. The purpose of this study was to determine whether such modifications on the surface of cobalt–chromium (CoCr) alloy by titanium powder coating using DMF improves the osseointegration ability of CoCr alloy.

METHODS: We compared the *in vitro* and *in vivo* ability of cells to adhere to DMF-coated CoCr alloy with machining. Biological and morphological responses to human osteoblast cell lines were examined by measuring cell proliferation rate and observing expression of actin filament. For *in vivo* study, we inserted different specimens in each medulla of the distal femurs of rabbit. After 3 months, the distal femurs were harvested, and a push-out test and histomorphometric analyses were performed.

RESULTS: The cell proliferation rate and cell adhesion in the DMF group were higher compared with those in the machined group. Human osteoblast cells on the DMF-coated surface were more strongly adhered and well-proliferated compared with those on the other surface. In the *in vivo* test, there was a significant difference in the ultimate shear strength between the DMF and machined groups (2.49 MPa vs. 0.87 MPa, respectively, $p = 0.001$). In the histomorphometric analysis, there was a significant difference in the mean bone-to-implant contact percentages between the DMF and machined groups ($72.3 \pm 6.2\%$ vs. $47.6 \pm 6.9\%$, respectively, $p < 0.001$).

CONCLUSION: Titanium coating of CoCr alloy with 3D metal printing provides optimal surface characteristics and a good biological surface both *in vitro* and *in vivo*.

Keywords Cobalt–chromium alloy · Direct metal fabrication · Osseointegration · Surface treatment · 3D printing

1 Introduction

Cobalt–chromium (CoCr) is the most extensively studied metallic biomedical implant due to its high strength, high corrosion resistance, flexibility, and good biocompatibility [1–3]. However, a lack of osseointegration has limited its application [4, 5]. Conventional CoCr alloy implants have been made by casting or forging, followed by a surface treatment to create porous structures such as plasma sprays, fiber metals, and bead coatings [2, 6, 7]. The disadvantages of these methods are: structural deformation and a decrease

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in fatigue strength, delamination of coating surfaces, and not obtaining genuine porous structures.

Direct metal fabrication (DMF) is a method for creating features via applying the additive through laser welding using sprayed titanium (Ti) powder, rather than a bottom bed [8]. This method is inferior for the fabrication of complex features, compared with powder-bed-fusion (PBF) and electron-beam processes; however, extremely high mechanical strengths similar to that of forged metal, make the additive on the surface of the complex feature suitable for knee, ankle, and hip implants [9, 10].

DMF is a metal three-dimensional (3D) printing method that is cheap, maintains mechanical strength, and can be used to fabricate porous structures [8]. Using this technology, the characteristics of the surface can be controlled to create ideal porous surfaces with maximum porosity, ideal pore size, and maximum roughness [8, 10]. In addition, a stable coating–substrate interface can be constructed even with different physical and chemical properties of the coating and the substrate.

Here, we investigated whether a Ti-powder coating on CoCr alloy using DMF could improve *in vitro* and *in vivo* responses as reflected by (1) cell morphology, (2) confocal microscopy images of RUNX2 and fibronectin, (3) interfacial shear strength (push-out biomechanical test), and (4) bone histomorphometry, compared to a machined CoCr alloy.

2 Materials and methods

2.1 Sample preparation and test groups

We studied two types of CoCr alloy surfaces (machined and DMF) *in vitro* and *in vivo* to compare cell morphology, confocal microscopy of actin filaments, interfacial shear strength by push-out biomechanical analyses, and bone histomorphometric characteristics. Two types of CoCr alloy discs (ISO 5832-12; diameter: 12 mm; thickness: 10 mm) were manufactured (n = 60): (1) machined CoCr alloy (n = 30) and (2) DMF CoCr alloy (n = 30) for *in vitro* study. Two types of CoCr alloy (ISO 5832-12) rods (diameter: 3.5 mm; length: 30 mm) were manufactured (n = 40): (1) machined CoCr alloy (n = 20) and (2) DMF CoCr alloy (n = 20) for *in vivo* study.

2.2 Manufacturing of DMF specimens

Pure Ti (grade 2, ASTM F1580) metal powders were melted and laminated using high-powered laser irradiation of the CoCr alloy surface. The porous structure was then manufactured using a 3-D computer-assisted design (CAD) program that created a sufficient fixation force by matching

the material to the properties of cancellous bone. The surface was irradiated with a laser power: 100 W; scan speed: 1.5 m/min; power delivery rate: 2.2 g/min by following a pre-programmed path along a grid, which formed a melted pool. Next, metal powders were sprayed and laminated onto the surface to create a coating layer (average thickness: 500 μ m) [11].

The surface roughness was measured using an optical interferometer (Accura 20001; Interplus Co, Seoul, Korea). The average surface roughness (Ra value) was 6.3 ± 0.18 μ m [mean $\pm$ standard deviation (SD)] for the DMF group and 0.2 ± 0.11 μ m for the machined group. The two surfaces were characterized using scanning electron microscopy (SEM; JEOL JSM-6700F; JEOL, Ltd, Tokyo, Japan) after the test specimens had been coated with platinum. EDS (JEOL JSM-6700F; JEOL, Ltd, Tokyo, Japan) was performed to evaluate composition of the CoCr alloy's surfaces of the two groups.

2.3 Culture and osteogenic differentiation of human mesenchymal stem cells (hMSCs)

Two passages of human bone marrow-derived mesenchymal stem cells (hMSCs; Catholic Master Cells) were obtained from the Catholic Institute of Cell Therapy (CIC, Seoul, Korea). The certificates of analysis for the hMSC phenotype confirmed negative markers CD31, 34, and CD45 and positive markers CD73 and CD90. hMSCs were cultured in Dulbecco's modified Eagle's medium (DMEM) (GE Healthcare Hyclone, Salt Lake City, UT, USA), 20% fetal bovine serum (FBS) (GE Healthcare Hyclone), with 1% penicillin/streptomycin (GibcoBRL, Grand Island, NY, USA) for five passages of hMSCs. The cells were maintained at 37 °C for 24 h in a humidified incubator with 5% CO₂.

Culturing hMSCs of passage 6 induced osteogenic differentiation using a Stem Pro Osteogenesis Differentiation Kit (GibcoBRL, Grand Island, NY, USA). The osteogenesis differentiation medium was osteocyte differentiation basal medium with osteogenesis supplement gentamicin reagent. hMSCs were seeded in a 6-well culture plate at a cell density of 3×10^4 cells per cm²; the media was replaced every 3–4 days, for a total incubation period of 21 days.

2.4 Cell morphology

The osteoblasts from hMSCs were seeded with 5×10^4 cells on DMF and CoCr alloy specimens. After 6 h of seeding of cells in each implant, the media was removed and then the cells were washed with PBS three times. After adding 2% glutaraldehyde-PBS solution, these cells were stabilized for 2 h. The cells were then washed with DW

three times. At 30-min intervals, the cells were dehydrated with 50–100% ethanol solutions. The ethanol was removed, and the cells were left at room temperature to allow for complete ethanol evaporation. The two surfaces were then characterized using SEM (JEOL JSM-6700F; JEOL, Ltd, Tokyo, Japan) after the test specimens had been coated with platinum.

2.5 Visualization of actin cytoskeleton using confocal microscopy

After 0.5 mL of osteoblasts (5×10^4 cells/mL) were seeded on a CoCr sample for 6 h, it was flooded with PBS 3 times and stabilized with 4% paraformaldehyde for 10 min. After the preserved cell was irrigated with PBS 3 times and treated with 0.1% Triton X-100 for 10 min, it was again irrigated with PBS. Rhodamine-phalloidin (Molecular Probes inc., Eugene, OR, USA) was diluted by 1:100 and left to react for 1 h at 37 °C avoiding any lights. It was sprayed with PBS 3 times and included by the aqueous mount. Using confocal and multiphoton microscope system (Bio-Rad Laboratories, Mississauga, ON, Canada), the actin filament structure (cytoskeleton of the cell) was observed.

2.6 Cell proliferation assay

The osteoblasts were seeded with 5×10^4 cells on DMT and CoCr alloy samples and incubated for 24, 48, 72, and 96 h. The medium was replaced with fresh medium before measuring cell proliferation using the Cell Titer 96 Nonradioactive Cell Proliferation Assay (Promega Corp, Madison, WI, USA), according to the manufacturer's instructions. Cell proliferation assay is a colorimetric method for determining the number of viable cells. In this study, the number of viable cells was measured at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Tek Instruments, Inc, Winooski, VT, USA) (Fig. 1).

2.7 Implantation of coated rod in distal femur of rabbits

Twenty full-grown rabbits (> 3.2 kg) were assigned as the experimental subjects. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Catholic University of Korea (CUMC-2014-0109-03). The rabbits were anesthetized intramuscularly with ketamine 35 mg/kg and rompun 5 mg/kg. In the supine position, the legs were incised longitudinally from 4 cm above the knee joint to 2 cm below the knee joint. From the superomedial side of the patella, vastus medialis muscle was incised through the medial side patella and patella tendon to proximal of the tibia tuberosity. This allowed exposure of the femoral condyle with eversion of

the patella to the lateral side. A hole in the femoral condyle was created with a 3.5-mm drill bit, taking care that the hole was gently reamed. A DMF-coated rod was placed in the distal femur and a CoCr rod was positioned in the contralateral femur. Reduction of the patella and repair of the incised structure with Vicryl 2-0 was performed (Fig. 2). Three months later, the distal femur was harvested, and the specimen underwent a push-out test and histological examination.

2.8 Push-out test

Each harvested distal femur was sliced at the two ends of the rod, and foreign substances were removed. With the jig of a universal testing machine (Daekyung tech DTU-900MH30kN, Incheon, Korea) positioned vertically along the long axis of the rod, a push-out test was performed at a push rate of 1 mm/min (Fig. 3). The push strength was recorded until the rod became dissociated with the femur or breakage of the femur occurred.

2.9 Bone histomorphometry

The harvested bone tissue was dehydrated with alcohol in stages and soaked in Technovit 7200 resin (Heraeus Kulzer, Morphisto, Frankfurt, Germany). The soaked tissue was embedded in paraffin for curing via a light system (Exakt Technologies Inc., Oklahoma City, OK, USA). The block was sliced into 200- μ m-thick sections with a hard tissue slicer (Struers, Willich, Germany). These sections were then stained with hematoxylin and eosin (H&E; Sigma-Aldrich, St. Louis, MO, USA). Microscopy images were obtained by X12.5, X100 (BX51, Olympus, Tokyo, Japan). The specimens from each implant were analyzed by determining the percentage of direct contact between mineralized bone and the CoCr alloy surface from intersection counting, using an integrative eyepiece with parallel sampling lines at a magnification of 100 $\times$.

2.10 Statistical analysis

We compared the mean interfacial shear strength and bone-to-implant contact percentage of the two different surfaces using a Wilcoxon signed-rank test. Statistical analysis was performed using SPSS[®] 20.0 software (SPSS, Chicago, IL, USA).

3 Results

SEM results indicated different surface characteristics (Fig. 1). Compared with the machined surface (Fig. 1A, B), the DMF surface had a remarkably porous structure

Fig. 1 A–C SEM images of the surfaces of **A** machined ($\times 50$), **B** machined ($\times 1000$), **C** DMF-coated ($\times 30$), **D** DMF-coated ($\times 1000$), **E** cross-sectional image of DMF-coated ($\times 30$), and **F** EDS spectra of machined, and **G** EDS spectra of DMF-coated CoCr alloy specimens showed different surface characteristics. **A**, **B** Compared to the machined surface, **C**, **D** the DMF-coated surface showed

a remarkable porosity of 200–500 μm , and **E** the cross sectional image that the boundary about coating–substrate interface was unclear. That means that a stable coating–substrate interface was made between Ti and CoCr alloy. In spectra of EDS, the DMF groups showed a completely different surfaces, with high peaks of Ti (**G**) compared to the machined group (**F**)

(average pore size in the coating layer: 200–500 μm ; average porosity: $65 \pm 5\%$; coating thickness: $500 \pm 100 \mu\text{m}$) (Fig. 1C, D). In a cross-sectional image, the boundary of the coating–substrate interface was unclear (Fig. 1E), which indicated a stable coating–substrate interface between Ti and the CoCr alloy. In spectra of EDS, the DMF groups showed a completely different surfaces, with high peaks of Ti (Fig. 1G) compared to the machined group (Fig. 1F).

In-vitro study of the morphologic assessment of the cells after 6 h of incubation using SEM showed that the machined CoCr alloy (Fig. 4A) was covered with small, slender osteoblast cells, whereas DMF (Fig. 4B) surfaces were largely covered with lamellipodia from the osteoblast cells. Additionally, the lamellipodia coverage was

extensive, and thin cytoplasmic projections (filopodia) extended into the interior of DMF surface pores.

Actin filament differentiation and organization using Rhodamine phalloidin (Molecular Probes inc., Eugene, OR, USA) staining was distributed. On the surface of DMF-coated specimen, the actin filaments were more intensively distributed than machined (Fig. 5A, B).

In cell proliferation rate, the DMF group increased 5.8 times from 24 h to 96 h, but machined group did not see any difference. However, there is statistically insignificant difference between the two groups ($p = 0.076$) (Fig. 6).

In the push-out biomechanical test, the measurement of interfacial shear strength showed that DMF group (2.49 MPa) was 2.9 times greater than the machined (0.87 MPa) group ($p = 0.001$) (Table 1).

Fig. 2 **A** With medial para-patellar approach, DMF-coated rod was inserted into the medullary canal of the right femur and machined CoCr alloy rod was inserted into the left femur. **B** After surgery, X-rays were taken to check the status of the femur

Fig. 3 **A, B** After 3 months, both femurs were harvested. Push out mechanical strength test is done with harvested distal femur with placing in Jig vertically. Push strength is recorded until the rod is dissociated with femur or breakage of femur occurred

Fig. 4 SEM images show osteoblast cells after 6 h of incubation on **A** machined ($\times 1000$), **B** DMF-coated CoCr alloy specimens ($\times 1000$). The **A** machined surface was weakly covered with small, slender osteoblast cells (arrows). **B** The DMF-coated surface was

largely and strongly covered with healthy lamellipodia of the osteoblast cells, and a thin cytoplasmic process branched out from the filopodia to enter the inside of a pore

Fig. 5 Confocal microscopy examination of differentiation and organization of action filament using Rhodamine phalloidin staining: **A** machined- ($\times 200$) and **B** DMF-coated ($\times 200$) CoCr alloy

Fig. 6 Results (mean + SD) of osteoblast cell proliferation assays at 24, 48, 72 and 96 h for DMF-coated and machined specimens. The cell proliferation assay was insignificantly different statistically between DMF and machined groups ($p = 0.076$). However, the DMF group increased 5.8 times from 24 h to 96 h

In the histomorphometric analysis, the mean bone-to-implant contact percentages of DMF groups (Fig. 7B) ($72.3 \pm 6.2\%$) was 1.5 times greater than the machined group (Fig. 7A) ($47.6 \pm 6.9\%$) ($p < 0.001$). The DMF group (Fig. 7D) was tightly attached compared with the machined group (Fig. 7C) in high resolution images ($\times 100$).

specimens. On the surface of DMF-coated specimen, the actin filaments were more intensively distributed than machined

Table 1 Push-out biomechanical test results

Group	F (N) $\pm$ STD	τ (MPa)
Machined group	302.3 ± 184.5	0.87
DMF group	864.0 ± 252.7	2.49

STD standard deviation

τ (MPa) = $F(N)/\text{Area (mm}^2\text{)}$

Area of Machined group = 357.1 mm^2 , area of DMF group = 364.2 mm^2

4 Discussion

To enhance the potential for CoCr alloy to osseointegrate and make a stable coating–substrate interface between Ti and CoCr alloy, we introduced a Ti powder coating using metal 3D printing deposition, to create an ideal porous structure without delamination of the coating surface. We hypothesized that this coating process could improve the morphology, proliferation, and differentiation ability of osteoblast cells to CoCr alloy *in vitro*, as well as the interfacial shear strength and bone-to-implant contact percentage of rabbit *in vivo*, compared with machined CoCr alloy.

There are two methods of orthopedic implant fabrication via metal 3D printing. One is the powder-bed-fusion (PBF) method involving laser irradiation of metal powder such as Ti; the metal is melted to the material for structure assembly layer by layer [12]. The other method is to generate structures using an electron beam to create the desired features [13]. The advantages of these techniques

Fig. 7 Light microscope images shows bone-to-implant contact of **A** machined ($\times 12.5$), **B** machined ($\times 100$), **C** DMF ($\times 12.5$), and **D** DMF ($\times 100$). The percentage of bone-to-implant contact was

calculated from the analysis of eight specimen sections. The DMF group (**B**, **D**) ($72.3 \pm 6.2\%$) was 1.5 times greater than machined group (**A**, **C**) ($47.6 \pm 6.9\%$)

over traditional manufacturing methods are that they can be used to construct complex structures with the intended porous surfaces. However, metal 3D printing still has the disadvantages of long manufacturing times, high fabrication costs, and low mechanical strength for weight bearing (even with heat treatment) so as to prevent use in hip and knee implants. Here, we introduce a DMF coating method for implant surfaces to compensate for the disadvantages of PVF and e-beam processes [11].

The cell morphology indicated that all of the surfaces examined were cytocompatible, consistent with many studies indicating Ti and CoCr alloy as biocompatible for cell culturing [14–16]. We noticed that osteoblast cells on the surfaces of the DMF specimens spread and formed lamellipodia on mirror-polished CoCr alloy surfaces. These findings indicate that the DMF coating process enhances cell integration on a CoCr alloy surface.

The cell proliferation assay indicated all the surfaces examined were cytocompatible. This is consistent with many studies indicating CoCr alloy and the surface of DMF are

biocompatible substrates for cell culturing [17]. In our study, cell proliferation was elevated on DMF group compared with the machined group in 96 h. This finding indicates that the DMF coating process enhances cell proliferation on a CoCr alloy surface. DMF group showed smaller at first 24 and 48 h than the machined group, then larger after 96 h, and DMF group was five times increases from 24 to 96 h. These results are not typical, so further studies are needed.

Roughened CoCr surfaces are effective in enhancing the interfacial biomechanical properties of bone-anchored implants by providing a mechanical interlock [18]. The interfacial bone formation may also be promoted by roughened surfaces as a significantly greater percentage of bone-to-implant contact has been observed in micro-roughened DMF surfaces in comparison to machined CoCr alloy surfaces [19]. The results of this study confirm that the DMF coating process makes the CoCr alloy surface rougher and increases the bone-to-implant contact areas in *in vivo* studies. Thus, the DMF coating process facilitates osseointegration in CoCr alloy.

We note several limitations of our study. First, a DNA study was not performed. A DNA study could enhance the impact of these findings by also assessing the levels of Type I collagen and osteocalcin. Further study is needed. The Second, we did not compare our experiments with other porous-coated implants, such as plasma spray. The third, vivo studies were conducted with different cross section's shape (the machined groups were square, and the DMF groups were a circle.) If the cross-section of the rod is small and ring, we could hardly perform DMF coating. Therefore, we use the different shape's specimens.

Surface modification of CoCr alloy was evaluated in *in vitro* and *in vivo* settings. The DMF surface treatments have the potential to enhance peri-implant bone healing mechanisms. In summary, a Ti-powder coating on CoCr alloy via 3D metal printing technology provided optimal surface characteristics and good biological outcomes both *in vitro* and *in vivo*. This technique could be applied to cementless knee arthroplasty components (total/unicompartamental knee arthroplasty) or to CoCr-based acetabular cup fabrication for total hip replacements. Therefore, this method should contribute significantly to advancements in orthopedic implants.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement The animal experiment procedures were approved by the institutional animal care and use committee of The Catholic University of Korea (CUMC-2014-0109-03).

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