Materials Letters 178 (2016) 300–303

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Comparison of in vitro biocompatibility of a Co–Cr dental alloy produced by new milling/post-sintering or traditional casting technique

Hae Ri Kim [a], Young Kyung Kim [b], Jun Sik Son [c], Bong Ki Min [d,*], Kyo-Han Kim [e], Tae-Yub Kwon [e,*]

[a] Department of Dental Science, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea
[b] Department of Conservative Dentistry, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea
[c] Korea Textile Development Institute, Daegu 703-712, Republic of Korea
[d] Center for Research Facilities, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[e] Department of Dental Biomaterials, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea

ARTICLE INFO

Article history:
Received 12 March 2016
Received in revised form
29 April 2016
Accepted 7 May 2016
Available online 9 May 2016

Keywords:
Biomaterials
Metallurgy
Co–Cr alloy
Microstructure
Metal ion release
Cell viability

ABSTRACT

The purpose of this study was to evaluate metal ion release of a cobalt–chromium (Co–Cr) alloy produced by using both the new milling/post-sintering (MPS) and traditional casting (CAST) techniques and the influence of ion release on cell response. The concentration of released ions from the specimens immersed in artificial saliva (pH 5.3 and 2.3) and cell culture medium was measured by inductively coupled plasma-mass spectroscopy. Cell (L929 mouse fibroblasts) response to the specimens was evaluated by water-soluble tetrazolium salt-8 assay (1, 3, and 7 days). The microstructure of the MPS specimen was characterized by fine grain size and predominant ε (hexagonal close-packed) phase. The MPS specimens showed consistently significantly smaller releases of Co ions than the CAST specimens ($P < 0.05$) regardless of the immersion solutions used. Although the cell morphology was normal in both groups, cell viability was consistently significantly higher in the MPS group than in the CAST group ($P < 0.05$). These findings suggest that the MPS-fabricated Co–Cr alloy showed better in vitro biocompatibility than the cast one.

1. Introduction

The casting method based on the investment casting process has traditionally been used to fabricate dental metallic restorations. Alternatively, cobalt-chromium (Co–Cr) dental restorations can be fabricated using either of the two main approaches based on computer-aided design/computer-aided manufacturing (CAD/CAM) processing. Subtractive manufacturing can be categorized as either hard machining and/or soft machining. The hard machining of Co–Cr alloy blanks can minimize the formation of casting-induced flaws and porosities. However, increased tool and machine wear caused by the high rigidity of the solid blank and high acquisition and maintenance costs pose significant disadvantages [1]. A recent development in the fabrication of Co–Cr dental restorations is “soft” machining, in which pre-sintered blank is dry-milled followed by a final sintering to full density in a furnace [2].

The biocompatibility of alloys used in dental crowns and fixed partial dentures is critical because these materials are generally in long-term intimate contact with oral tissues. The metallic restorations in the oral cavity should have a low release of toxic ions to avoid harmful tissue reactions such as allergies. However, metal ion release from, and the cell response to, Co–Cr alloys produced by soft machining has not been reported. The purpose of this in vitro study was to compare the toxic metal ions release from a Co–Cr alloy prepared by either soft machining or casting technique. The effect of ion release on cell morphology and viability was also assessed.

2. Materials and methods

A commercial Co–Cr alloy developed for soft machining (Soft Metal™, LHK, Korea), with a composition of Co 63 wt%, Cr 29 wt%, molybdenum (Mo) 6 wt%, and trace amounts of silicon, was used. Disc-shaped specimens (10 mm in diameter and 3 mm in thickness) were prepared via both casting (CAST group) and soft machining (milling/post-sintering, MPS group) technique. In the CAST group, wax patterns were embedded in an investment material

* Corresponding authors.
E-mail addresses: bkmin@ynu.ac.kr (B.K. Min), tykwon@knu.ac.kr (T.-Y. Kwon).

http://dx.doi.org/10.1016/j.matlet.2016.05.053
0167-577X/

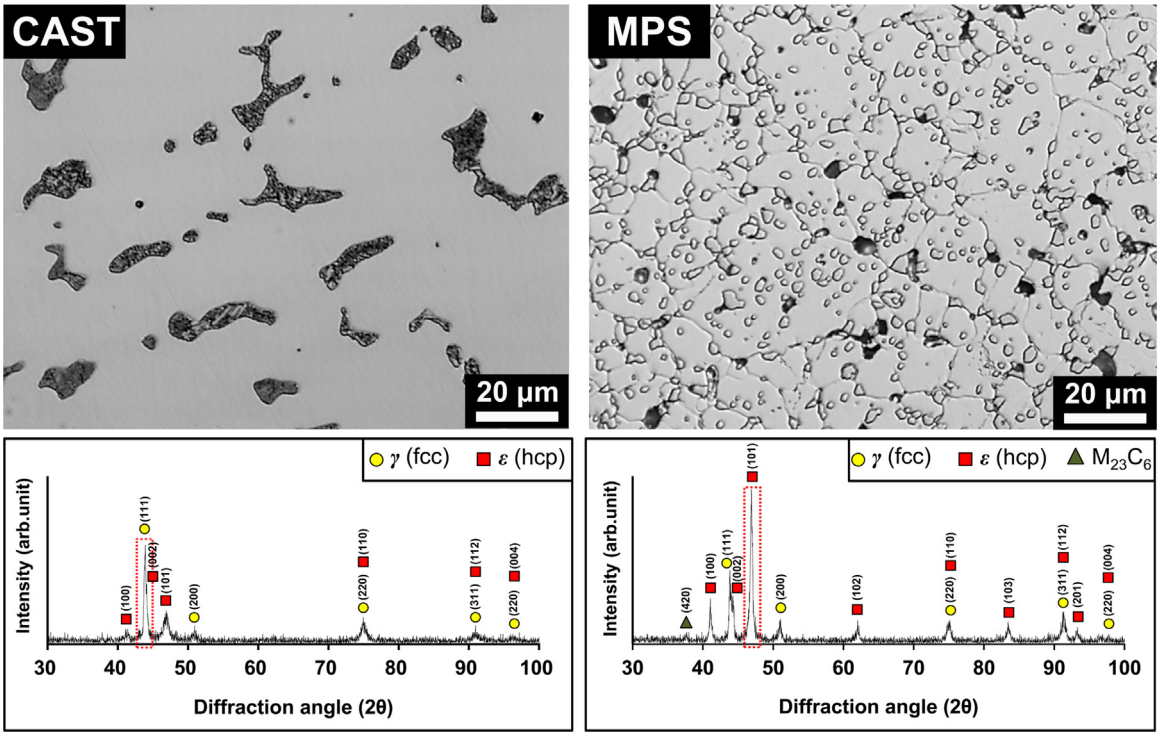

Fig. 1. Optical microscopy images and corresponding XRD patterns of the CAST and MPS specimens. The Co-based γ (face-centered cubic, fcc) and ε (hexagonal close-packed, hcp) matrix phases were identified with ICDD cards no. 15-806 and no. 05-727, respectively. The peaks indexed as $M_{23}C_6$ (M=Cr, Co, Mo) metal carbides with a cubic structure were identified by ICDD card no. 35-783.

Table 1
Metal ion release (mean (SD), μg/cm^2) in each immersion solution (n=5).

Immersion solution	Group	Co	Cr	Mo
Artificial saliva (pH 5.3)	CAST	1.951 (0.182)	0.003 (0.001)	0.224 (0.050)
	MPS	0.338 (0.011)	0.002 (0.0004)	0.010 (0.001)
		$P<0.001$	$P=0.242$	$P=0.001$
Artificial saliva (pH 2.3)	CAST	3.068 (0.403)	0.140 (0.042)	0.265 (0.105)
	MPS	0.646 (0.023)	0.101 (0.005)	0.023 (0.001)
		$P<0.001$	$P=0.101$	$P=0.007$
Culture medium	CAST	0.417 (0.052)	0.006 (0.002)	0.061 (0.022)
	MPS	0.220 (0.012)	0.006 (0.001)	0.007 (0.001)
		$P=0.001$	$P>0.99$	$P=0.005$

and then cast using the alloy in a casting apparatus (Centrifico, Kerr, USA). In the MPS group, a soft block was dry-milled using a machine (Zenotec T1, Wieland, Germany) to form disc-shaped specimens, which were finally sintered in a furnace (SinTagon, Denstar, Korea) under an argon gas purge (flow rate=0.6 L/min) at 1350 °C for 1 h.

Optical microscopy (OM) analysis and X-ray diffractometry (XRD) were used to investigate the microstructures of the disc specimens. The flat surfaces were polished with silicon carbide papers and then with a 1 μm diamond suspension. For OM analysis, the surfaces were further electropolished. Phase identification was performed on a specimen from each group by XRD (MAXima_X XRD-7000, Shimadzu, Japan), using Cu K_α radiation (λ=0.1541 nm) at an accelerating voltage of 30 kV, a beam current of 30 mA, a 2θ angle scan range of 30–100°, a scanning speed of 2°/min, a sampling pitch of 0.02°, and a preset time of 0.6 s

Immersion tests were performed to evaluate the amount of metal ions released from the Co–Cr alloy specimens immersed in three different solutions. An artificial saliva solution with pH 5.3 was prepared [3]. A more acidic artificial saliva solution with pH 2.3 was additionally prepared by adding lactic acid [4]. Roswell Park Memorial Institute (RPMI) 1640 culture medium was also used as an immersion solution. Each specimen was immersed in 7.5 mL of one of the immersion solutions in sterile polypropylene centrifuge tubes (50 mL) and stored in an oven at 37 °C [5]. After 7 days, the solutions were tested by inductively coupled plasma-mass spectroscopy (NexIon 300X, PerkinElmer, USA), using matrix-matched standards. The concentrations of Co, Cr, and Mo ions were finally converted to units of μg/cm^2 (n=5).

The effect of ion release on cell viability was assessed by water-soluble tetrazolium salt-8 (WST-8) assay. L929 mouse fibroblasts, a popular cell line in material biocompatibility testing, were cultured in RPMI 1640 culture medium at 37 °C in a humidified atmosphere of 5% CO_2. The CAST and MPS extracts were prepared by immersing the corresponding specimens in the medium at a ratio of 3:1 volume of solution to specimen surface area for 7 days [5,6]. Cells were seeded in 96-well cell culture plates at 4×10^4 cells/mL and incubated for 24 h to allow attachment [7]. The medium in each well was then replaced with CAST or MPS extract. The cells were incubated for 1, 3, and 7 days, during which the extracts were replaced once every 3 days. 10 μL of WST-8 solution were added to each well and cells were incubated for 4 h. The absorbance of each solution was measured with a spectrophotometer at the wavelength of 450 nm (n=9). RPMI 1640 medium was used as negative control, whereas the medium containing 10% dimethyl sulfoxide served as positive control [7]. The cell morphology at 1 day of culture was further observed using a phase-contrast microscope after washing the cells with phosphate-buffered saline, fixing with methanol, and staining with Giemsa.

The results of the metal ion release between the CAST and MPS groups were statistically compared by Student's t test. The results of the cell viability assay (including the positive control) were analyzed using one-way ANOVA and the Tukey *post hoc* test to determine significant differences among groups. The significance level was set at 0.05.