Short Communication

Enhanced biocompatibility of a Ni–Cr alloy prepared by selective laser melting: a preliminary in vitro study

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\textbf{Abstract}
Against a general consensus regarding the high toxicity and low biocompatibility of cast nickel–chromium (Ni–Cr) alloys, the in vitro biocompatibility of selective laser melting (SLM)-fabricated Ni–Cr alloys was investigated and compared with that of cast alloy. Using a single Ni–Cr alloy powder, two SLM alloys with high (SLM,a) and low (SLM,b) porosity were prepared by adjusting the laser process parameters in addition to a cast alloy. The alloys were studied in terms of their microstructure, metal ion release, and cell response. All three alloys were composed of only the \textgamma\ (face-centered cubic) phase. However, both SLM alloys showed more homogeneous dispersion of Ni, Cr, and molybdenum elements and finer grain formation than the cast alloy. Immersion test results indicated that both SLM alloys (in particular, SLM,b) exhibited a significantly lower Ni ion release than the cast alloy ($P<0.001$). A water-soluble tetrazolium salt-8 assay also showed that the viability of L929 mouse fibroblasts was significantly higher for both SLM groups (in particular, SLM,b) than for the cast alloy group ($P<0.001$). Thus, the combined results of this in vitro study suggest that the SLM-processed Ni–Cr alloys exhibited greatly enhanced biocompatibility in comparison with the cast alloy.

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1. Introduction

In dentistry, metal-ceramic restorations are still a popular choice for fixed partial dentures because of their excellent esthetic and mechanical properties [1,2]. Traditionally, the metal frameworks are manufactured by lost-wax casting, and then the veneering ceramic is fused to the metal. Nickel–chromium (Ni–Cr) and cobalt–chromium (Co–Cr) alloys are the most commonly employed dental cast base-metal alloys for such use [3].

Although Ni–Cr alloys have advantages, including high acid/alkali resistance as well as great toughness and strength at high temperatures [4], they pose higher biologic risk than other alloys because of the common hypersensitivity to Ni [5]. Moreover, Ni–Cr–beryllium (Be) alloys corrode far more than their non-Be counterparts [5], although the addition of Be improves casting manipulation and enhances porcelain bonding [3,6]. Therefore, Co–Cr alloys are the most common base-metal alternative for patients who are allergic to Ni [3,5]. However, Co–Cr alloys have the highest melting ranges of the casting alloys, with the exception of titanium-based alloys, making laboratory manipulation (casting, finishing, and polishing) of these alloys difficult [3,5,7]. In addition, the surface oxide of Co–Cr alloys is difficult to mask, and the thermal expansion coefficients of these alloys are not often as compatible with the porcelains as Ni–Cr alloys [5,8]. Co is also the second-most common metal allergen [5].

Selective laser melting (SLM), an additive manufacturing technique, has attracted increasing attention as an effective alternative to traditional casting. In comparison with traditional casting, SLM can avoid errors made by technicians, prevent casting defects, and reduce the extent of impurities [9]. In addition, SLM alters the microstructures which determine the characteristics of an alloy in biological environments where there is direct contact with body fluids [9,10]. Xin et al. [9] demonstrated that a Co–Cr alloy prepared by SLM showed lower ion release and better cell response than cast alloy because of the homogeneous and compact microstructure formation. A similar tendency can also be expected for SLM-fabricated Ni–Cr alloys. To the best of our knowledge, however, little research has been conducted to investigate the biocompatibility of Ni–Cr alloys fabricated via SLM. The purpose of this preliminary in vitro study was to evaluate metal ion release from, and the cell response to, Ni–Cr alloys prepared by either SLM or casting.

2. Experimental

A non-Be-containing SLM alloy powder (Metal Player Co., Ltd.), with a composition of Ni 68 wt%, Cr 22 wt%, and molybdenum (Mo) 9 wt%, was used to prepare disk-shaped specimens (10 mm in diameter and 3 mm in thickness). The average particle size of the powder was less than 40 μm. In the CAST group, wax patterns were prepared, embedded in an investment material (BC-VEST P-1000, Bukwang), and finally cast using the Ni–Cr alloy in a casting apparatus (Centrifico, Kerr Corp.). In the SLM groups, two different specimens (SLM.a and SLM.b) were prepared by adjusting two laser process parameters (scan speed and laser power). An SLM device (MetalSys150, Winforsys Co., Ltd, Korea) with an ytterbium fiber laser beam (IPG YLR-200 CW) was operated under a nitrogen gas atmosphere (flow rate: 5 L/mm). The scan speeds for the SLM.a and SLM.b alloys were 600 and 1000 mm/s, respectively. The laser power levels for the SLM.a and SLM.b alloys were 125 and 175 W, respectively. For both SLM alloys, the scan-line spacing, layer thickness, and laser spot size were 0.10 mm, 35 μm, and 0.08 mm, respectively. The building direction was perpendicular to the radial direction of the disk specimens. The densities of the two SLM alloys were measured by the Archimedes method.

The microstructures of the alloys were investigated using optical microscopy (MM-40/2U, Nikon) and X-ray diffractometry (XRD, X’Pert PRO, PANalytical). The surfaces were polished with silicon carbide papers and then diamond suspensions until a 0.05-μm surface finish was achieved. Phase identification was carried out by XRD, using Cu Kα radiation (λ = 0.1541 nm) at an accelerating voltage of 40 kV, a beam current of 30 mA, a 2θ-angle scan range of 20–100°, a scanning speed of 2°/min, a sampling pitch of 0.02°, and a preset time of 0.6 s.

To analyze the elemental composition, the specimens for each alloy were examined using field emission-scanning electron microscopy (FE-SEM, Merlin, Carl Zeiss AG) with energy dispersive X-ray spectroscopy (EDS, Oxford Instruments) under an accelerating voltage of 20 kV. To determine the crystallographic orientation, electron backscattered diffraction (EBSD) scans were performed on the FE-SEM equipped with a Nordlys Nano EBSD detector (Oxford Instruments) under an accelerating voltage of 20 kV.

To evaluate the amounts of Ni, Cr, and Mo ions released from the alloys, the specimens were immersed in either Roswell Park Memorial Institute (RPMI) 1640 culture medium or acidic (pH 2.3) artificial saliva. The experimental protocols have been described elsewhere [9,11]. Briefly, each specimen was immersed in 7.5 mL of solution and stored in an oven at 37°C. After 7-day immersion, the solutions (n = 5) were tested using inductively coupled plasma mass spectroscopy (Nex Ion 3000X, PerkinElmer), based on matrix-matched standards. The concentrations of Ni, Cr, and Mo ions were converted to units of μg/cm².

The effect of ion release on the viability of L929 mouse fibroblasts was evaluated by a water-soluble tetrazolium salt-8 (WST-8) assay. The experimental protocols have also been described elsewhere [9,11]. Briefly, the extracts were prepared by immersing the specimens in the RPMI 1640 culture medium at a ratio of 3:1 volume of solution to specimen surface area for 7 days. L929 mouse fibroblasts were seeded in 96-well plates at 4 × 10^3 cells/mL and incubated for 24 h [12]. The medium in each well was then replaced with each extract. After 1, 3, and 7 days of culture, the absorbance of each solution was measured using a spectrophotometer at 450 nm (n = 9), according to the WST-8 protocol. The medium itself and a 10% dimethyl sulfoxide-containing medium were used as negative and positive controls, respectively [11,12]. The cell morphology at 1 day of culture was also examined using a phase-contrast microscope after Giemsa staining [11].
The results were analyzed using one-way analysis of variance, followed by Tukey’s post hoc test to determine whether there were significant differences among groups (α = 0.05). If appropriate, the data were transformed before analysis using a square root transformation to meet homogeneity of variance.

3. Results and discussion

Ni–Cr alloys with Cr contents of over 20 wt% have superior corrosion resistance qualities [5,13]. In addition, Mo is an effective hardening element for Ni–Cr alloys and has the added benefit of influencing the coefficient of thermal expansion [3]. The pitting corrosion resistance of Ni–Cr alloys in an acid medium is dramatically improved with increased Mo content [13]. In this study, a single Mo-containing Ni–Cr alloy, composed of Ni 68 wt%, Cr 22 wt%, and Mo 9 wt%, was subjected to the three different manufacturing processes (one casting and two SLM) to prepare three different alloys.

Fig. 1 shows the optical microscopy images and XRD patterns of the alloys produced by casting or SLM processes. The CAST and SLM_b specimens showed less pore formation on the surface than the SLM_a specimen. The XRD patterns indicate that the CAST, SLM_a, and SLM_b alloys were composed of only γ (face-centered cubic, fcc) phase, which presents a solid solution of Ni, without any precipitates.

Fig. 2 shows the SEM, EDS, and EBSD images of the three prepared alloys. The EDS maps of both SLM groups show a more homogeneous dispersion of the metal elements than those of the CAST group, indicating superior corrosion resistance of the SLM alloys [14]. The EBSD images show that the samples of the SLM groups were composed of finer grains, probably due to rapid solidification by a very high cooling rate [8], than the CAST group. Such small grains of the SLM groups...
also could potentially enhance corrosion resistance under acidic condition [11,15]. In addition, the CAST alloy showed a compositional segregation of the Cr and Mo elements during solidification [16]. The measured density of the CAST alloy was 8.31 g/cm³. Within the SLM groups, the SLM_b alloy showed a more dense and compact structure, with less porosity than the SLM_a alloy (measured densities 8.52 and 7.11 g/cm³, respectively) (Figs. 1 and 2). Such porosity may increase susceptibility to corrosion, such as crevice corrosion and pitting corrosion [17].

Table 1 shows the results of the Ni, Cr, and Mo ions released from the alloy specimens. In both immersion solutions, a greater release of Ni ions was detected in comparison with that of the other ions. The concentrations of Ni and Mo ions released from the SLM alloys (in particular, in SLM_b) were significantly lower than those from the CAST alloy (P < 0.001). Acetic artificial saliva was also used as an immersion solution because the corrosion of Ni–Cr alloys may be particularly evident in acidic environments [5,11]. Overall, the acidic artificial saliva aggravated the toxic metal ion release from the alloy specimens in comparison to the culture medium [11]. In particular, the release of Cr ions was greater in the acidic saliva than in the culture medium. It was assumed that relatively low proportion of Cr (22 wt%) in the alloys caused slight breakdown and repassivation of the surface in the saliva and, as a result, an acceleration of Cr ion release [18].

The phase-contrast microscopy and cell viability results (Figs. 3 and 4) were paralleled by the metal ion release results (Table 1). The SLM groups exhibited greater cell density and viability than the CAST group (P < 0.05). Moreover, the SLM_b group exhibited superior cell viability (approaching that of the negative control) in comparison to the other two groups (P < 0.05). The findings of this in vitro biocompatibility test suggest that the Ni–Cr alloy prepared via an optimally controlled SLM process (SLM_b) potentially possesses much lower biological risk than cast Ni–Cr alloy. Even the SLM_a alloy, which had much higher porosity than the cast alloy (Fig. 1), yielded more favorable results than the cast alloy. Thus, the combined results of the present study suggest that the application of the SLM technique for Ni–Cr alloys may have potential to overcome the toxicity problem of cast Ni–Cr alloy. However, further comprehensive studies are required to confirm the enhanced in vitro and in vivo biocompatibility of SLM-fabricated Ni–Cr alloys.

### Table 1 – Metal ion release (μg/cm²) in the immersion solutions.

<table>
<thead>
<tr>
<th>Immersion solution</th>
<th>Group</th>
<th>Ni</th>
<th>Cr</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture medium</td>
<td>CAST</td>
<td>0.663 ± 0.019 a</td>
<td>0.009 ± 0.002 a</td>
<td>0.101 ± 0.010 b</td>
</tr>
<tr>
<td></td>
<td>SLM_a</td>
<td>0.199 ± 0.014 b</td>
<td>0.008 ± 0.001 a</td>
<td>0.064 ± 0.009 b</td>
</tr>
<tr>
<td></td>
<td>SLM_b</td>
<td>0.018 ± 0.001 c</td>
<td>0.008 ± 0.001 a</td>
<td>0.006 ± 0.001 c</td>
</tr>
<tr>
<td>Artificial saliva (pH 2.3)</td>
<td>CAST</td>
<td>0.932 ± 0.117 a</td>
<td>0.118 ± 0.023 a</td>
<td>0.270 ± 0.030 a</td>
</tr>
<tr>
<td></td>
<td>SLM_a</td>
<td>0.361 ± 0.025 b</td>
<td>0.126 ± 0.022 a</td>
<td>0.102 ± 0.015 b</td>
</tr>
<tr>
<td></td>
<td>SLM_b</td>
<td>0.175 ± 0.029 c</td>
<td>0.117 ± 0.011 a</td>
<td>0.022 ± 0.004 c</td>
</tr>
</tbody>
</table>

For each solution, the same superscripted lowercase letters within each column indicate statistically similar means (P > 0.05).
4. Conclusions

The results of this in vitro study suggest that the Ni–Cr alloys fabricated by SLM were superior to the traditional-cast Ni–Cr alloy in terms of biocompatibility, mainly due to the more homogeneous dispersion of Ni, Cr, and Mo and finer grain formation within the alloys. Moreover, the SLM_b alloy, which had a highly dense structure with less porosity, resulted in enhanced cell viability in comparison to the cast alloy due to further reduction in the amount of toxic metal ions released. The SLM technique thus merits consideration for the fabrication of Ni–Cr dental prostheses with low health risks.

Conflicts of interest

The authors declare no conflicts of interest.

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References


