

# The Release of Elements from Dental Casting Alloy into Cell-Culture Medium and Artificial Saliva

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## ABSTRACT

**Objectives:** The biocompatibility of dental casting alloys is a critical issue because these alloys are in long-term intimate contact with oral tissues. Since the biocompatibility of alloys is not completely known; the release of elements from the alloys has been studied. The aim of this study was to compare the elemental release from dental casting alloy during exposure to artificial saliva and cell-culture medium.

**Materials and Methods:** Twenty specimens made from Ni-Cr alloy were provided in the form of 5 mm diameter discs, 2 mm in thickness with a 7 mm stem attached to one face to facilitate handling. Ten of twenty samples were polished separately using a conventional technique. The remaining ten samples were left sandblasted with 50 µm Al<sub>2</sub>O<sub>3</sub>. Ten samples (5 polished, 5 sandblasted) were separately placed into cell-culture wells with Dulbecco's Modified Eagle's Medium. The other ten samples were placed separately into cell-culture wells with artificial saliva. The samples were subjected in contact with these medium for 30 days. These medium were collected every 7 days. The cell-culture medium and artificial saliva without alloy samples were subjected to elemental analyses as a control. At the end of the exposure time, Atomic Absorption Spectrometry (AAS) was used to determine the release of elements from the alloys into all collected medium. Statistical analyses were assessed with two-way ANOVA.

**Results:** In general, the elemental release occurred with in all medium. The elemental releases of sandblasted alloys were higher than polished alloys. Artificial saliva was found to cause more release from the samples. In both media, Ni released from polished and sandblasted alloys were higher than Cr and Mo.

**Conclusions:** The results suggest that the release of elements from the alloys might have correlated with the environments and the surface of dental alloy. (Eur J Dent 2007;2:86-90)

**Key Words:** Dental alloy; Ni-Cr alloy; Elemental release; Biocompatibility; Cell-culture medium; Artificial saliva.

## INTRODUCTION

Dental casting alloys with high and low noble metal contents are widely used in dentistry.

Corrosion of alloys occurs in oral environment. The biocompatibility of dental casting alloys is a critical issue because these alloys are in long-term intimate contact with oral tissues. The biocompatibility may correlate with elements of dental casting alloys.<sup>1-3</sup> The release of elements from dental casting alloys is directly related to adverse biological effects. Certain environmental conditions around the alloy will affect the release of elements. A reduction in pH will increase elemental release from dental alloys. This effect is especially pronounced for nickel-based alloys. The elemen-

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tal release from casting alloys has also been studied extensively in vitro in short and long term experiments.<sup>4-8</sup>

Studies have shown that the release of elements from the alloys may correlate with different factors. Wataha and other many investigators<sup>2-4,7-9</sup> have been studied about the affects of the ions released. They have shown that the relationships between elemental release and toxicity are complex. Several statements can be said about release of elements from dental alloys. In the mouth, alloys may be exposed to transient pH changes either from foods or plaque. The reduced pH would increase elemental release because it acts like corrosive medium.

The purpose of this study was to compare the elemental release from polished and sandblasted dental casting alloy during its exposure to artificial saliva and cell-culture medium.

## MATERIALS AND METHODS

### Sample preparation

Twenty specimens made from nickel–chromium (Ni–Cr) alloy (Cr 22.5, Ni 65, Mo 9.5, Fe 0.5, Si 1.0, C 0.02, Ce 0.5, Nb 1.0, weight %) (Wiron 99, Bego, Germany) were provided in the form of 5 mm diameter discs, 2 mm in thickness with a 7 mm stem attached to one face to facilitate handling, similar to the study of Wataha and Lockwood.<sup>9</sup>

Ten of twenty samples were polished separately using a conventional technique. The remaining ten samples were left sandblasted with 50 µm Al<sub>2</sub>O<sub>3</sub>. Each sample was then cleaned by a soft toothbrush, rinsed in distilled water, and ultrasonically treated in 99% ethanol for 15 min.

After cleaning, 10 samples (5 polished, 5 sandblasted) were separately placed into cell-culture well. Then the cell-culture medium was added in this well (0.5 ml Dulbecco's Modified Eagle's Medium-Ham's F-12, pH 7.4, Sigma, USA) that containing penicillin (100 units/mL), streptomycin (100 mikrog/mL), amphotericin B (2,5 mikrog/mL) and sodium bicarbonate (1.2 g/L) in a 37°C, humidified 5% CO<sub>2</sub> atmosphere. The other ten samples were placed separately into cell culture wells; then 0.5 ml artificial saliva (0.017 mol NaCl, 0.02 mol Na<sub>2</sub>HPO<sub>4</sub>, 0.02 mol NaH<sub>2</sub>PO<sub>4</sub>, pH 3.5, 37°C) was added.<sup>10</sup>

The samples were subjected in contact with

this medium for 30 days. The cell-culture medium and artificial saliva without alloy samples were analyzed to elemental analyses as a control for the presence of metal elements. The cell-culture and artificial saliva were collected aseptically every 7 days and new medium were placed in the cell-culture wells.

### Atomic absorption spectrometry analysis

Atomic absorption spectrometry analysis (AAS) (Varian 30/40 Model, GTA 96, Australia) was used to determine the release of elements from the alloys into collected the cell-culture medium and artificial saliva solutions. Nickel (Ni), Chromium (Cr), and Molybdenum (Mo) elements were selected for analysis based on previous research which showed that they were released from dental casting alloy. The other elements in the Ni-Cr alloys were not checked because they are trace elements in the compositions. In the AAS, there are certain measurement ranges each element. For this reason, standard solutions were used for calibration of each element. Standard solutions were prepared by dilution of purchased 1000 ppm stock solutions with double-distilled water. To check the accuracy of the standards, the calibration graphics were obtained for each element. The specific parameters (wavelength, slit width, standard concentration) used for detection of each element for AAS technique, are listed in Table 1.

Statistical analyses were assessed with two-way ANOVA. At the same time, Kolmogorow-Smirnow and repeated measures of ANOVA were used.

## RESULTS

The release amount of elements in both artificial saliva and cell-culture medium were deter-

**Table 1.** Atomic absorption spectrometry parameters.

	Wavelength (nm)	Slit width (mA)	Standart concentration (ng/ml)		
Ni	232	4	20	40	60
Cr	357.9	7	5	10	5
Mo	313.9	7	10	20	30

mined to ng/ml=mikrogram/L=ppm. These data were obtained like this: (the experimental result-the control result). The results are illustrated in Tables 2,3 and Figure 1.

Medium, polished and sandblasted alloys and interaction between them were analyzed. Although each elements show different release, it was clear from the data that extraction solution and the surface altered the release of elements.

In every medium, the effects of polished and sandblasted alloys were different. In cell-culture medium, the release of sandblasted alloy was more than polished alloy for Ni and Mo. The amount of released Cr was very low. In artificial saliva, the pattern of release was same with the other medium, but the amount of release was more than cell-culture medium. The release of Ni was statistically significant ( $P<.001$ ) according to medium, sandblasted and polished alloys and the interaction of medium and surfaces in every medium. The release of Mo was not statistically significant for every medium ( $P>.05$ ). At the other hand it was statistically important for surface ( $P<.01$ ) and interaction between medium and surface ( $P>.05$ ). The release of Cr was very low. Its correlation with medium, surface and interaction between them was less important ( $P<.001$ ).

### DISCUSSION

Dental alloys are subjected to a variety of chemical environment in the mouth. The dynamic natures of intraoral conditions cause corrosion. The release of an element from alloy depends on some factors such as the nature of element, alloy composition, multiple phases of alloy the metallurgical environment around these alloy and polished alloy. Therefore some of metal elements have an inherently higher tendency to be released from dental alloys. The biological liabilities may be related to the released elements.

Many studies have been performed about the release of metallic elements from dental alloys. The release of elements from dental casting alloys has been investigated with many different material and methods by different researchers.<sup>2-4,6-9,11</sup>

Certain environmental conditions around the alloy affect release of elements. Cell culture or different solution such as normal saline, bovine serum solution, artificial saliva, tissue culture media and diluted acids are used to evaluate the

corrosion.<sup>2,4,6-11</sup>

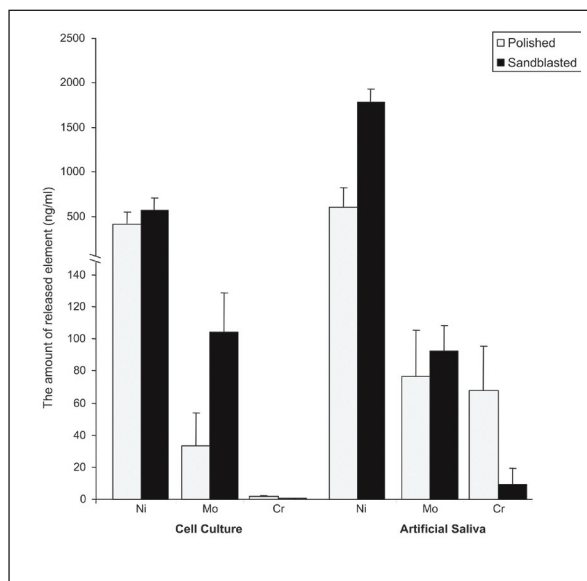
A reduction in pH increases elemental release from dental alloys. This effect is especially pronounced for Ni-based alloys.<sup>8,11-14</sup> Although, for the Ni-based alloy, pH appeared to be the dominating factor and the composition of the solution is less important. Liability of Ni element from Ni-based alloys in acidified saliva was confirmed by Covington.<sup>11</sup> He showed that; especially Ni tends to be released from these types of alloys in acidified saliva. Wataha et al,<sup>12</sup> in a various solution with pH

**Table 2.** Elemental release from dental casting alloy in cell culture medium. Values are mean±sd. \*  $P<.01$  when compared with sandblasted.

	Polished	Sandblasted
Ni	425.66±119.89	566.06±136.41
Cr	1.45±1.15	0.57±0.16
Mo	33.27±20.33*	104.42±24.22

**Table 3.** Elemental release from dental casting alloy in artificial saliva. Values are mean ±sd. \*  $P<.05$  when compared with sandblasted.

	Polished	Sandblasted
Ni	768.75±228.91*	1539.0±543.93
Cr	33.95±30.41	2.72±3.15
Mo	105.94±67.82	115.66±62.30



**Figure 1.** The release amount of Ni, Cr, Mo from polished and sandblasted Ni-Cr alloy in cell culture medium and artificial saliva.

ranging between 1-7 simulating intraoral dynamic conditions, investigated the release of elements from Ni-based alloys in 30 minutes. The authors found that the elemental release was increased in pH 1-4. Wataha<sup>9</sup> measured the release of elements from dental alloys at monthly intervals for 10 months. They hypothesized that element release should decrease as a function of time of exposure to the medium and that the cytotoxic effects of the alloys should also decrease. Also, they stated that the initial release rates were the highest.<sup>13</sup>

Our results revealed that Ni was more active than Cr and Mo in both mediums and polished and sandblasted alloys. As expected, reduced pH dramatically increased Ni release from the Ni-based alloy in the present study. This result is essentially identical to trends seen in previous studies performed in a 72 hours period. The release of elements in a cell-culture medium, were generally less than those in artificial saliva.<sup>14,15</sup>

Previous studies support the idea that composition of the alloy surface is critical to elemental release behavior for base-metal alloys.<sup>5,8,16</sup> The elemental release may be caused by a change in surface composition of the alloys. This type of change was observed in Pd-Cu alloys. Cu released initially leaving of the alloy before Pd.

The results of our study showed that the release of Ni from sandblasted Ni-Cr dental alloy was identical to the previously reported observations.<sup>14,15</sup> The release of Mo was less than that of Ni because it is not an active metal. We expected that release of Cr would be minimal since chromium oxide layer formed prevents corrosion. In fact, our findings confirmed this assumption.

Nelson et al<sup>17</sup> evaluated the elemental release and cytotoxicity of casting alloys in three types solutions. They stated that cell-culture medium was much more complex than biological medium. However, in our study the elemental release from dental casting alloy into the cell-culture medium was not affected as much as in artificial saliva. Our hypothesis was supported by the other studies.<sup>18,20</sup> When the cell-culture medium was compared to the artificial saliva, the data showed clearly that the release of Ni, Cr and Mo were lower in cell-culture medium.

Messer and Lucas<sup>19</sup> showed that a number cellular functions were altered in response to ions released from Ni-Cr alloys. Their results demon-

strated ionic release from alloy was a complex process depended on variables including ion chemistry, ion valence and dose-time dependence.

## CONCLUSIONS

The cytotoxicity of the alloys is complex and remains incompletely understood. The biocompatibility of dental materials is a critical concern with the development of new product.

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