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METALLIC ION RELEASE FROM BIOCOMPATIBLE COBALT-BASED ALLOY

Article Highlights

- Metallic ion release from Co-Cr-Mo alloy in artificial saliva is examined
- The concentrations of released ions are determined using ICP-MS
- The effects of artificial saliva pH values and duration of immersion on metallic ion release are determined
- The released metallic ions concentrations are compared with literature data

Abstract

Metallic biomaterials, which are mainly used for the damaged hard tissue replacements, are materials with high strength, excellent toughness and good wear resistance. The disadvantages of metals as implant materials are their susceptibility to corrosion, the elastic modulus mismatch between metals and human hard tissues, relatively high density and metallic ion release, which can cause serious health problems. The aim of this study was to examine metallic ion release from Co-Cr-Mo alloy in artificial saliva. In that purpose, alloy samples were immersed into artificial saliva with different pH values (4.0, 5.5 and 7.5). After a certain immersion period (1, 3 and 6 weeks) the concentrations of released ions were determined using inductively coupled plasma-mass spectrometry (ICP-MS). The research findings were used in order to define the dependence between the concentration of released metallic ions, artificial saliva pH values and immersion time. The determined released metallic ions concentrations were compared with literature data in order to describe and better understand the phenomenon of metallic ion release from the biocompatible cobalt-based alloy.

Keywords: metallic biomaterials, cobalt-based alloy, ion release, artificial saliva, pH value.

Biomaterials are synthetic or natural materials that are used in contact with cells, tissues and/or body fluids without any negative effect on the human body [1]. A biomaterial can be defined as any substance or combination of substances that can be used for any period of time, as a whole or as a part of a system, which improves or replaces any tissue or organ in the human body [2-4]. Metallic biomaterials, which are mainly used for replacing damaged hard tissues, are materials with high strength, excellent wear resistance, as well as with good electrical and thermal conductivity [5-7]. The biocompatibility of the most metallic biomaterials is based on a protective passive oxide layer present on the metal surface [8]. The chemical composition of the passive oxide layers, their thickness and protection degree depend on the alloy composition, chemical environment, mechanical stresses, etc. [9]. In recent period, electrochemically obtained metallic powders were examined with the aim to determine the influence of deposition conditions on powder composition and possibilities for biomedical application [10]. The most extensively used metallic biomaterials are commercially pure (CP) titanium, titanium- and cobalt-based alloys, whilst stainless steels have been abandoned in recent years due to insufficient corrosion resistance and nickel-induced hypersensitivity of organism [11]. The implant materials are required to possess appropriate mechanical, physical, chemical and biological properties [12,13]. In addition to the good combination of mentioned pro-

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perties, corrosion resistance of these materials must be sufficiently high since the contact between implant materials and human body fluids results in corrosion appearance. The disintegration of the implant material and metallic ion release occur as a direct consequence of the corrosion process [14-16]. The corrosion stability of metallic biomaterials in simulated body fluids can be determined by electrochemical tests that measure the amount of released elements through the flow of the released electrons, static immersion test and/or tarnish test (cyclic immersion) that measure the released elements by spectroscopic methods [17-20]. The corrosion process progression is related to the material microstructure, surface roughness, alloy composition, exposure temperature and pH value of the chemical environment [21-24]. However, it should be mentioned that the amounts of released metallic ions do not reflect their relative weight contents in an alloy's composition [25].

pH value has a considerable influence on the concentration of released metallic ions from implant materials. The pH of the average human body fluids is usually within the range of 7.0–7.35 (although the pH value may fall to 4.0–5.0 range due to inflammation caused by surgery or injury) [26]. Generally, changes of pH in the human body are small due to buffering action of the body fluids. The hard tissues pH decreases to approximately 5.2 after implantation process and afterwards recovers to the value of 7.4 [27]. However, pH value changes are most commonly present in the oral environment due to acidic food consummation or bacteria metabolic activity and therefore dental implant materials have been tested in solutions with different acidity levels [28,29]. Human saliva pH is slightly acidic (~5.8). In the oral cavity, extremely low pH value (2.0-3.0) can occur in the case of pathological diseases of the oral cavity [30]. These oral diseases should be primarily treated with dental prosthetic treatment. In addition, diligent and constant oral hygiene (especially after meals) leads to the prevention of the spread of bacteriological activity and pH disbalance in the oral cavity. In healthy population, low pH value cannot be maintained for a long period because of the buffering action of the human saliva and such a low pH value was not considered in this study.

Co-Cr-Mo alloys have been widely applied as implant materials because of their excellent mechanical properties, corrosion resistance and biocompatibility [31,32]. Chromium, the main alloying element in Co-Cr-Mo alloys, is added to advance the formation of a stable passive oxide layer that contributes to corrosion resistance. Molybdenum is also frequently added to advance alloy resistance to pitting and crevice corrosion. The composition of Cr and Mo in commercial alloys is within the range of 11-25 mass% and alloys that contain lower amounts of Cr and Mo are found to be more susceptible to corrosion [33,34]. Although the metallic biomaterials are considered to have good corrosion resistance, numerous studies have shown that metallic ions can be released into the surrounding environment [35-37]. Consequently, the contact between Co-Cr-Mo alloys and body fluids leads to the metallic ion release [38]. Therefore, the aim of this study was to examine metallic ion release from Co-Cr-Mo alloy into artificial saliva with different pH values.

EXPERIMENTAL

The metallic ion release from Co-Cr-Mo alloy (Wironit® extra-hard produced by Bego, Germany) into artificial saliva with different pH values was investigated. The Co-Cr-Mo alloy in as cast condition was selected to be considered in this study since this type of implant alloy produced by casting is widely used in dental practice, mostly for manufacturing of crowns, bridges and denture bases [39,40]. The chemical composition of the examined alloy is given in Table 1, while the physicochemical properties of the investigated alloy are shown in Table 2.

Table 1. Chemical composition of the investigated Co-Cr-Mo alloy

Element	Weight content, %
Co	63.0
Cr	30.0
Mo	5.0
Fe	-
C	max 0.4
Other (Si, Mn)	-

Table 2. Physicochemical properties of the investigated Co-Cr-Mo alloy

Density	8.2 g/cm^3
Melting interval	1260-1305 °C
Casting temperature	1420 °C
Ductile yield	4.1%
Tensile strength R_m	910 MPa
Elongation limit $R_{p0,2}$	625 MPa
Modulus of elasticity	211000 MPa
Vickers hardness	375

Commercial Co-Cr-Mo alloy, produced in the cylindrically shaped pieces with 8.0 mm in diameter and 15.8 mm in height, were cut into the disc-shaped samples (8.0 mm in diameter and 3.2 mm in thickness) and polished. In order to eliminate surface contamination, the samples were cleaned in ultrasonic bath with ethanol for 10 min. After cleaning treatment, the samples were washed with distilled water and dried with sterile gauze. Subsequently, each sample was placed in a separate glass container with the testing solution (5 ml) and incubated at 37 °C. During testing the containers were hermetically closed in order to prevent the possible contamination and the evaporation of the testing solution.

The testing solution used in this paper was artificial saliva produced by Helvepharm AG, Switzerland with initial pH value of 6.8. Composition of the testing solution is shown in Table 3. The artificial saliva pH was lowered to a level of 5.5 and 4.0 by adding hydrochloric acid and increased to 7.5 by adding sodium hydroxide.

Table 3. Chemical composition of the artificial saliva

Component	Content, %
NaCl	0.0844
KCl	0.1200
$MgCl_2 \times 6H_2O$	0.0052
$CaCl_2 \times 2H_2O$	0.0146
Sorbitol	0.3000
KH_2PO_4	0.0342
Carboxymethylcellulose sodium	0.1000
Water	99.3416
pH	6.8

At the end of the immersion period (1, 3 and 6 weeks) a certain amount of artificial saliva was removed from the glass container in order to measure the cobalt, chromium and molybdenum ion concentrations that were released from the alloy. The concentrations of released ions were determined using an inductively coupled plasma-mass spectrometer (Agilent ICP MS 7500).

RESULTS AND DISCUSION

The concentrations of ions released from the investigated Co-Cr-Mo alloy into the artificial saliva with different pH values after 1 week-long immersion are shown in Figure 1. Even though the weight content of Mo in the composition of examined alloy is low, the concentration of Mo ions released into the simulated body fluid was the highest among all investigated after 1 week-long immersion. The results obtained in the present study are in accordance with the earlier reported findings that the alloying elements present in traces can be released in larger quantities [25]. Similarly, Okazaki and Gotoh [41] showed that the quantity of released Mo ions from Co-Cr-Mo cast alloy was higher than the quantity of released Cr ions in 0.9% NaCl solution, phosphate-buffered saline (PBS) solution and artificial saliva after 1 week, although the weight content of Cr in alloy's composition (28.36 mass%) was higher than the weight content of Mo (6.1 mass%). Conversely, the quantity of released Mo ion was lower than the quantity of released Cr ion in 1% lactic acid and 1.2% L-cysteine. Therefore, the metallic ion release depends on the composition of immersion solution, but does not depend directly on weight content of this element in the examined alloy. An early study by Geis-Gerstrorfer *et al.* [42] showed that Co-Cr-Mo alloys were more corrosion resistant as compared to Ni-Cr-Mo alloys, which might be related to the immersion solution, such as cell culture media, artificial saliva, saline solution, diluted acids, etc. Accordingly, it can be concluded that the composition of immersion solution has a significant influence on the concentration of released metallic ions from implant materials. Furthermore, results showed that the pH value decrease resulted in the released metallic ions concentrations increase.

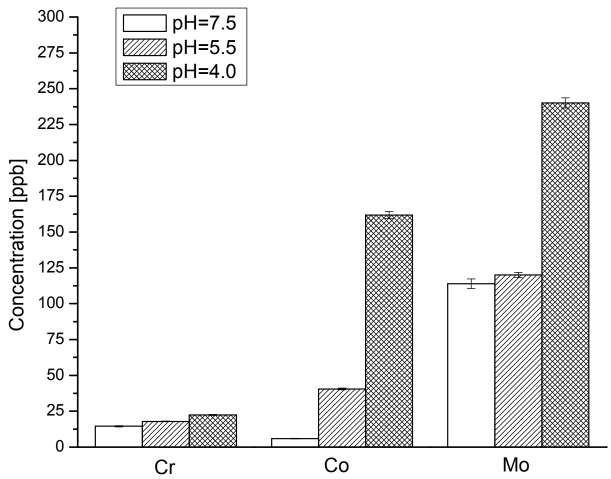


Figure 1. Concentration of ions released from the Co-Cr-Mo alloy into artificial saliva with different pH values after 1 week.

The concentrations of released metallic ions from Co-Cr-Mo alloy into the artificial saliva with different pH values (4.0, 5.5 and 7.5) after 1, 3 and 6 weeks are shown in Figure 2.

As can be seen from the diagrams presented in Figures 1 and 2, the metallic ion release is dependent on the pH value of the artificial saliva in which the alloy was immersed, as well as on the immersion duration. The obtained results indicate that the metallic

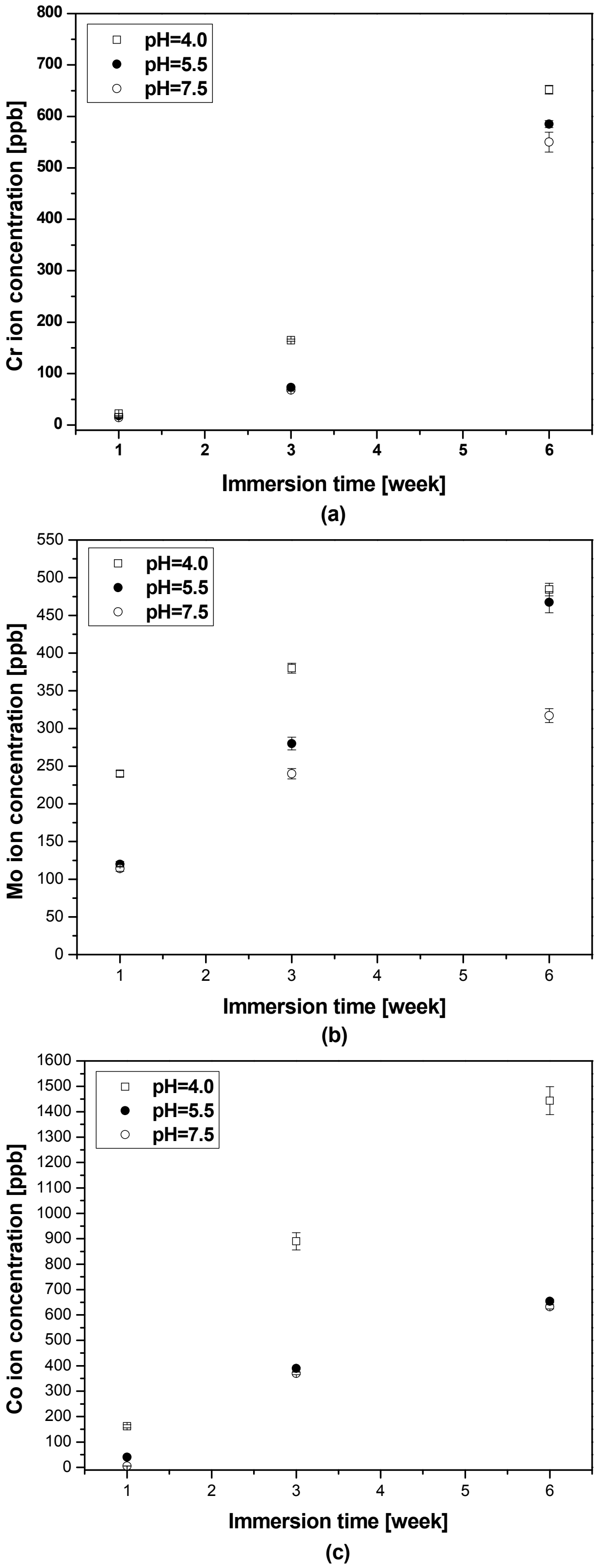


Figure 2. Concentration of ions released from the Co-Cr-Mo alloy into artificial saliva with different pH values over different immersion periods: a) chromium; b) molybdenum; c) cobalt ions.

ion release from the examined alloy is directly proportional to the immersion time. Namely, with the increase of time period, during which the alloy was exposed to artificial saliva, the concentrations of all released metallic ions were increased. Accordingly, the increase of the metallic ion release is associated with the corrosion susceptibility of the Co-Cr-Mo alloy. As mentioned above, the pH value also has a great influence on the quantity of released metallic ions. With the decrease of solution pH value to 4.0, the concentrations of released metallic ions were proportionally increased (see Figure 1). At pH 4.0 the released ions concentrations were rapidly increased and these results are in agreement with the literature data. Namely, Okazaki and Gotoh [41] denoted pH 4.0 as a critical pH value, obtained by testing multiple biomaterials in several solutions of different acidity levels. Despite their metallic ion release, the Co-Cr-Mo alloys can be considered as corrosion resistant. Likewise, Puškar *et al.* [43] investigated element release from Co-based alloy in artificial saliva and concluded that the concentrations of released metals were far under the permitted level. Corrosion resistance and inertness of these alloys are above all achieved through the formation of a protective surface chromium oxide layer.

The results of the presented examinations indicate that the metallic ion release could be described by third-degree polynomials, as dependence on the pH value and the immersion duration. However, it is rather difficult to estimate and predict a trend with the data obtained so far, hence the future work will include longer periods of immersion (at least six months) in order to predict the tendency of metallic ion release from Co-based alloys.

Generally, the metallic ion release is difficult to describe and predict by equations, since it depends on numerous factors such as alloy composition, sample dimensions, type, composition, temperature, pH value and volume of testing solution, etc. [21-25,28,29]. Beck *et al.* [44] analyzed elemental release from Co-Cr alloy containing palladium (Pd) and concluded that Co-Pd-Cr alloy released a significantly greater amount of respective ions (Co, Cr, Mo and total ions) compared to the traditional Co-Cr alloy. A study by Peter *et al.* [45] showed that addition of titanium and zirconium to Co-based alloy had a positive effect on the alloy hardness and metallic ion release. Can *et al.* [46] emphasized that the release of metallic ions from dental cast alloys has correlated with the surrounding environmental conditions and the surface of dental alloy. The results of the above mentioned study showed that the release of metallic ions from sandblasted alloys was higher than from polished alloys. Also, artificial saliva intensified element release more than cell-culture medium. Okazaki [47] pointed out that the metal release depends on the chloride concentration and pH of the testing solution, but the effect of chloride concentration on the quantity of released metallic ions was small. Also, Denizoglu *et al.* [48] highlighted pH of the immersion solution as a very important factor for metallic ion release. Joseph *et al.* [49] determined the pH effects on metallic release from the Co-Cr-Mo alloy in three different conditions: as cast, annealed and hot-forged and concluded that the trends of the pH effect on Co, Cr and Mo release were nearly the same. Actually, the results indicated that alloy heat treatment and hot forging had negligible effect on metallic ion release.

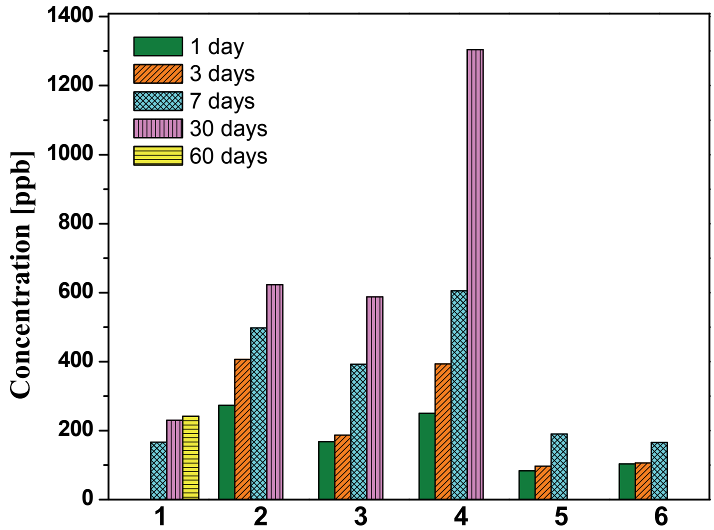
Dependence of the cobalt ion release from Co-Cr-Mo alloys on the mentioned factors can be properly observed by comparing research findings published in the literature, Figure 3.

Literature data confirm that with decreasing pH and increasing immersion time, the concentrations of released metallic ions are increasing [50-52]. Moreover, it can be observed that concentration of released ions from Wironit was significantly higher when compared to other Co-Cr-Mo alloys.

It should be mentioned that released metallic ions can accumulate in organs and tissues and cause local and systemic health problems due to the ions diffusion through the entire body. Any metal or alloy implanted in the human body is a potential source of toxicity [53]. Nevertheless, metallic ions released into the human body do not always damage the body. Namely, if released metallic ions are combined with biomolecules, then they can cause cytotoxicity, allergy or other harmful effects. However, if metallic ions are combined with water molecules or anions, then they will form oxide, hydroxide or inorganic salt [26]. Therefore, the type of the molecules that react with released ions, as well as type and concentration of the released ions and their toxicity, must be considered in order to determine the safety of metallic biomaterials.

CONCLUSION

The metallic ion release from Co-Cr-Mo alloy into artificial saliva with different pH values was



No.	Author	Alloy	Testing solution	Immersion time
1	L. Multu-Sagesen *et al.* [50]	Wirobond C (61Co, 26Cr, 6Mo, 5W, 1Si, 0.5Fe, 0.5Ce, 0.02C)	Artificial saliva + lactic acid (pH=2.3)	7, 30, 60 days
2	N. Rinčić *et al.* [51]	Wironit (64Co, 29Cr, 5Mo, 1Si, 1Mn, 0.5C)	Phosphate buffer (pH=6.0)	1, 2, 3, 4, 5, 6, 7, 14, 21, 30 days
3	N. Rinčić *et al.* [51]	Wironit (64Co, 29Cr, 5Mo, 1Si, 1Mn, 0.5C)	Phosphate buffer (pH=3.5)	1, 2, 3, 4, 5, 6, 7, 14, 21, 30 days
4	N. Rinčić *et al.* [51]	Wironit (64Co, 29Cr, 5Mo, 1Si, 1Mn, 0.5C)	Lactic acid (pH=3.5)	1, 2, 3, 4, 5, 6, 7, 14, 21, 30 days
5	F. Nejatidanesh *et al.* [52]	Minalia (62Co, 31Cr, 5Mo)	Artificial saliva	1, 3, 7 days
6	F. Nejatidanesh *et al.* [52]	Wironit (64Co, 28Cr, 5Mo)	Artificial saliva	1, 3, 7 days

Figure 3. Cobalt ion release from Co-Cr-Mo alloys [50-52].

examined in this study and the following conclusions were drawn:

1. The concentrations of released metallic ions do not reflect their weight contents in the alloy's composition.

2. With increasing immersion time the concentrations of released metallic ions were proportionally increased.

3. Decrease of the pH value lead to the increase of the concentrations of released metallic ions.

4. pH 4.0 is a critical pH value since at this value the concentrations of released metallic ions begins to increase rapidly.

5. The metallic ion release is difficult to describe and predict using the mathematical equations, because this phenomenon depends on many different factors.

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NAUČNI RAD

OTPUŠTANJE METALNIH JONA IZ BIOKOMPATIBILNE LEGURE KOBALTA

Metalni biomaterijali, koji se najčešće koriste za zamenu oštećenih čvrstih tkiva u organizmu čoveka, su materijali velike čvrstoće, žilavosti i otpornosti prema habanju. Nedostaci metala, kao implantnih materijala, su njihova podložnost koroziji, neusklađenost modula elastičnosti metala i čvrstih ljudskih tkiva, velika gustina i otpuštanje metalnih jona koje može da izazove ozbiljne zdravstvene probleme. Cilj ovog rada je bio da se ispita otpuštanje metalnih jona iz Co-Cr-Mo legure u rastvoru veštačke pljuvačke. Uzorci legure su potopljeni u rastvor veštačke pljuvačke različite pH vrednosti (4,0, 5,5 i 7,5). Nakon određenog vremena izlaganja legure veštačkoj pljuvačci (1, 3 i 6 nedelja) određene su koncentracije otpuštenih jona primenom indukcije spregnute plazme sa masenom spektrometrijom (ICP-MS). Dobijeni rezultati su iskorišćeni u cilju definisanja zavisnosti između koncentracije otpuštenih jona, pH vrednosti veštačke pljuvačke i dužine potapanja legure u rastvor veštačke pljuvačke. Osim toga, utvrđene koncentracije otpuštenih metalnih jona iz ispitivane legure su upoređene sa podacima dostupnim u literaturi u cilju što boljeg opisivanja i razumevanja fenomena otpuštanja metalnih jona iz biokompatibilne Co-Cr-Mo legure.

Ključne reči: metalni biomaterijali, legure kobalta, otpuštanje jona, veštačka pljuvačka, pH vrednost.