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ANALYSIS OF METAL ION RELEASE FROM BIOMEDICAL IMPLANTS

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Abstract

Metallic biomaterials are commonly used for fixation or replacement of damaged bones in the human body due to their good combination of mechanical properties. The disadvantage of metals as implant materials is their susceptibility to corrosion and metal ion release, which can cause serious health problems. In certain concentrations metals and metal ions are toxic and their presence can cause diverse inflammatory reactions, genetic mutations or even cancer. In this paper, different approaches to metal ion release examination, from biometallic materials sample preparation to research results interpretation, will be presented. An overview of the analytical techniques, used for determination of the type and concentration of released ions from implants in simulated biofluids, is also given in the paper.
Keywords: metallic implants, biofluid, corrosion, ion release, analytical techniques

Introduction

Metals and metal alloys used in medicine and dentistry are commonly known as metallic biomaterials or biometallics [1]. Biomaterials should function in contact with cells, tissues and/or body fluids without any negative effect on the human body. Most commonly, these materials are used to replace or upgrade the structural components of the human body in order to compensate certain existing defects or damages [2]. Therefore, biomaterials are widely used in dental, orthopaedic, cardiovascular and reconstructive surgery [3]. Medical implant materials must possess properties such as: biocompatibility (high cell affinity to the surface of metallic implant materials), high corrosion resistance, durability (high corrosion fatigue strength and corrosion fretting

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fatigue strength, as well as low release of fretting wear particles), low Young's modulus and high strength and toughness [4]. Except specified physical and chemical properties, biomaterials should be non-toxic and non-carcinogenic, they should not cause immunological reactions and should be good thermal and electric insulators, while sterilization of these materials and their manufacturing in adequate geometric shapes should also be enabled [5]. Radiographic images of different implant types after surgical implantation procedure are given in Fig. 1.

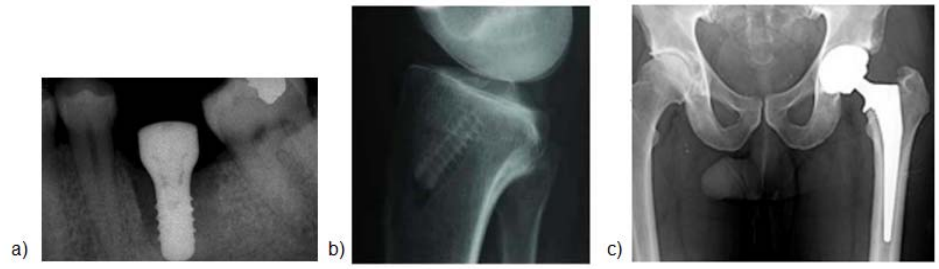

Figure 1. Postoperative radiographs showing: a) dental implant, b) screw placement in knee joint, c) hip implant [2, 6].

Metals are commonly used for fixing or replacing damaged bones and structural components of the human body. Disadvantages of metallic biomaterials are their small biocompatibility, susceptibility to corrosion, high strength (compared to soft and hard tissues) and relatively high possibility of metal ion release [7]. Having all that in mind improvement of biometallics biocompatibility and non-toxicity is of prime concern for further development of this group of materials. To achieve the optimal material properties, alloying is used as technological process that can lead to the required properties improvement and, in the same time, unfavourable properties minimization or elimination [8]. Comprehension of the type and concentration of the released elements from the metallic implants is very important for prediction and assessment of their local and systematic effects on the human body.

Effect of human body fluids on the implant material

Physiological solutions are solutions which can be entered into the blood stream without harmful effects to the living organism. These solutions, more or less, mimic the internal environment of the human body where living cells retain their normal shape and function. They can be divided into two categories:

- simple solutions, which satisfy isotonicity (ex. 0.9% NaCl, 5% glucose, 10% fructose), and
- complex solutions, which should be isotonic (the same concentration of electrolytes as in the plasma), isohydric (the same concentration of hydrogen ions as in the plasma) and isothermal (the same temperature as the temperature of the human body) [9,10].

In addition to the clinical practice, physiological solutions are often used for *in vitro* laboratory research as a biofluid simulation medium.

Contact between implant material and human body fluids results in corrosion appearance. Corrosion is a general term for gradual degradation of materials, especially

metals, under the influence of diverse chemical and physical factors [11]. Implant materials are prone to various types of corrosion (pitting, crevice, galvanic, stress and fretting corrosion), depending on the alloy composition and the environment conditions [12]. Corrosion occurs when the passive film, present on the metallic material surface, is damaged by friction and micro-movements [13]. The metal ion release from the implant material occurs as a direct consequence of the corrosion process. Release of metal ions can cause local and systemic health problems, due to the ions diffusion through the whole body. Ion release is favoured by certain material microstructures or by surface roughness [14]. Corrosion rate, other than specified factors, depends on the composition, temperature and pH of the chemical environment [15]. With decreasing pH, metal ions concentration is increasing [16, 17]. Also, corrosion leads to degeneration of implant materials such as aesthetic appearance (important for dental implants), strength and biocompatibility [18].

Immersion test

Immersion test can be conducted in static or dynamic conditions. Static immersion test is a test in which the sample is exposed to a corrosive solution with minimum relative motion between sample and solution. On the other hand, during dynamic immersion test the sample exposed to a corrosive solution is in relative motion with solution [19]. In this paper static immersion test was analysed.

Sample preparation

Metallic materials corrosion behaviour testing is complicated because of the investigated materials diversity, as well as the diversity of their applications and environments to which they are exposed. In order to examine metal ion release, the samples of metallic implant materials could be prepared in different ways and can be present in various shapes and sizes. The implant material samples, subjected to the same testing conditions, should be same size and shape, in order to obtain comparable results. According to EN ISO 10271 standard, the total surface area of the investigated samples has to be at least 10 cm^2 [19]. Sample preparation can be done in many different ways, but even so it always includes some basic procedures. In order to eliminate surface contamination, the samples are after grinding and polishing treatments subjected to intensive cleaning, which includes samples immersion into ultrasonic bath with alcohol or acetone for 20 min. After cleaning treatment, the samples are washed with distilled water and dried by hot air [14].

Each sample is then placed in a separate container with a medium that simulates biofluids and incubated at 37°C using a thermostat. The containers are hermetically closed in order to prevent the possible contamination and the evaporation of the testing solution. The most commonly used containers are made of polypropylene (PP) or polystyrene (PS). The glass containers, with a rubber stopper, are also used frequently. At the end of the immersion period, a certain amount of eluent is removed from each testing solution in order to analyse the released ions type and concentration (Fig. 2).

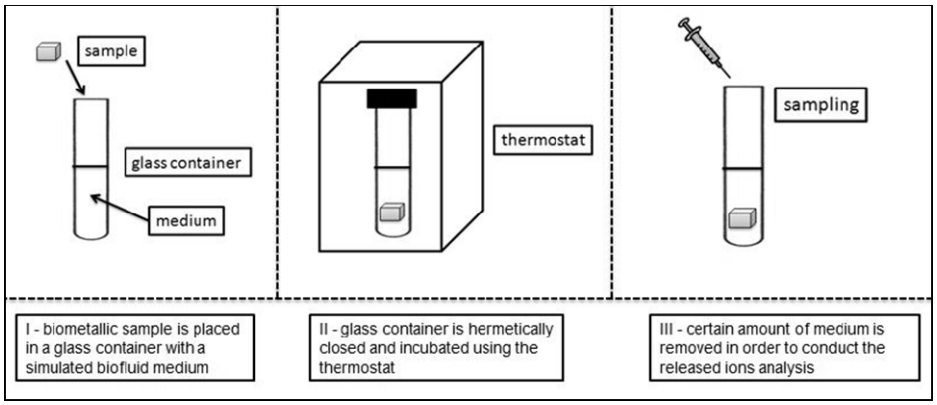

Figure 2. Schematic overview of the static immersion test.

Detailed overview of the metal ion release research results already published in the literature is given in Table 1.

Table 1. In vitro metal ion release from the implant material [14-18, 24, 33-42].

Reference	Implant material	Medium	Immersion time	Analytical techniques	Obtained research results
F.J. Gil, et al. [14]	Ti alloy Au alloy Pd alloy Ni-Cr alloy	Artificial saliva	1, 3, 5, 10, 24, 120, 168, 360 and 560 h	ICP-MS	Ni-Cr alloy shows the highest rate of ion release
N. Rinčić, et al. [15]	Co-Cr-Mo alloy (Wironit)	Phosphate buffer (pH=6,0; pH=3,5) Lactic acid (pH=3,5)	1, 2, 3, 4, 5, 6, 7, 14, 21 and 30 days	ICP–AES	The releasing of metal ions from the tested Co-Cr-Mo alloy in the solution depends on both the nature of the solution where the alloy was immersed and the duration of the immersion
L.A. Joseph, et al. [16]	CP Ti Ti-6Al-7Nb	Hank's solution WBS (Whole Blood Serum) PBS (Phosphate Buffered Saline)	1 day and 1, 2, 4, 8, 12, 20 weeks	GFAAS	Variations in pH and chloride ions of the test medium has a significant effect on the amounts of Ti ions, while increase in chloride ions concentration significantly elevates the release of Al ions into the biofluids
Y. Okazaki, et al. [17]	SUS 316L Co-Cr-Mo CP Ti (grade 2) Ti-6Al-4V Ti-6Al-7Nb Ti-15Zr-4Nb-4Ta	PBS α-MEM calf serum 0,9% NaCl artificial saliva 1,2% L-cysteine 1% lactic acid 0,01% HCl	7 days	ICP-MS GFAAS	Ti-15Zr-4Nb-4Ta shows the lowest rate of ion release; With decreasing pH, concentration of metal ion is increased (pH=4.0 – critical value)

F. Nejatidanesh, et al. [18]	Ni-Cr alloys (Minalux, Supercast) Co-Cr alloys (Minalia, Wironit)	Artificial saliva	1, 3, 7 days	ICP-AES	Ni-Cr alloys were more resistant to corrosion in artificial saliva as compared to Co-Cr alloys; Wironit was the most susceptible to metal ion release; The amount of element release is increased with increasing immersion time
L. Mutlu-Sagesen, et al. [24]	Co-Cr-Mo alloy (Wirobond C) Ni-Cr-Mo alloy (Wiron 99) Ti alloy (Rematitan CP Ti) Au alloy (PontoStar)	Artificial saliva (pH=2,3; pH=6,5) 0,9% NaCl (pH=7,3)	7, 15, 30 and 60 days	AAS	Ion release from alloys was pH-dependent; The metal ion release is increased with length of immersion period
A.J. Ortiz, et al. [33]	Stainless steel Ti alloy Ni-free alloy	Biofluid	30 days	ICP-MS	Ti alloy shows the lowest rate of ion release
M. Mikulewicz, et al. [34]	Stainless steel	Artificial saliva	30 days	ICP-MS	Ion release is not proportional to ion content in an alloy
S. Tamilselvi, et al. [35]	Ti-6Al-7Nb Ti-6Al-4Al ELI	SBF (simulated body fluid)	0, 120, 240 and 360 h	EIS SEM EDAX	After prolonged immersion in SBF solution, material exhibits a two-layer structure composed of a dense inner layer and a porous outer layer
T.H. Huang, et al. [36]	Stainless steel	Artificial saliva (pH=4,0; pH=7,0)	1 h and 48 weeks	GFAAS	Ion release depends on immersion time; With decreasing pH, concentration of metal ion is increased
J.C. Wataha and P.E. Lockwood [37]	Au-Pt Au-Pd Pd-Cu-Ga Pd-Ag Au-Cu-Ag Au-Ag-Cu Ag-Pd Ni-Cr	Dulbecco's Modified Eagle's Medium	10 months	FAAS	Ni-Cr alloy shows the lowest rate of ion release (about 6 $\mu g/cm^2$)
C. Yfantis, et al. [38]	Ni-Cr alloy (Wiron99); Co-Cr alloy (Wirobond C)	Artificial saliva pH=2,3	28 days	ICP-AES	The corrosion rates of Co-Cr alloy were lower compared to Ni-Cr alloy
J. Stipetić, et al. [39]	Au-Pt Ni-Cr Co-Cr-Mo	Phosphate buffer (pH=6,0; pH=3,5) Lactic acid (pH=3,5)	1, 2, 3, 4, 5, 6, 7, 14, 21 and 30 days	ICP–AES	The greatest increase in Ni ions release was noted in the PBS at pH=6,0 where it exceeded the approved daily dietary intake of this element
U. Turkan, et al. [40]	TiN coated Co-Cr-Mo alloy	SBF (pH=7,4)	1, 3, 7, 15, 30, 60, 90, 120 and 150 days	AAS ICP-OES	The presence of the coating substantially reduces the concentration of dissolved metal ions; The TiN coating can be an effective barrier for reducing the potentially harmful ions, in particular Co from the substrate material (Co-Cr-Mo alloy)

S. Spriano, et al. [41]	Ti-6Al-7Nb	SBF	from 3 h to 60 days	ICP-GFAA	The samples induce a rapid increase in metal ion concentration during the first 3 days; After longer immersion period, a slow increase of metal ion concentration was found in SBF
M. Baučić, et al. [42]	Au-Pt alloy	Phosphate buffer	1, 2, 3, 4, 5, 6, 7, 14, 21 and 30 days	ICP–AES	Ion release behavior is a function of the immersion time

Immersion solution

Testing of metal ion release from implant materials can be performed *in vitro* and *in vivo*. *In vitro* tests contribute to a better understanding of the complex metal ion release phenomena that occurs in *in vivo* conditions, while *in vivo* tests can indicate local and systemic disorders (toxic, mutagenic, and immunogenic) that may occur in patients with surgically implanted medical devices. Corrosion of metals, subjected to the body fluids influence, is caused by Cl^- ions presence and the corrosive environment of the human body can be simulated by 0.9% NaCl solutions. Different types of solutions are used for simulation of complex biofluids in order to perform *in vitro* tests under conditions that are very similar to *in vivo* conditions. Commonly used mediums are Ringer's solution, Phosphate Buffered Saline (PBS), Hank's solution, Eagle's minimum essential medium (MEM) and α-MEM [20]. The medium can be used alone or with addition of glucose and/or chloride and fluoride ions [21]. Artificial saliva is mostly used for laboratory testing of dental implants [22]. Synthetic medium, such as artificial saliva, is often used in *in vitro* studies because of its greater accessibility and chemical stability compared to natural medium [23]. pH value has a great influence on the concentration of the released ions and because of that metallic implant materials are tested in the medium with different degrees of acidity. In normal conditions body fluids pH value is in the range from 7.0 to 7.35. Disruptions and disbalance in the human body, acidic food consummation or bacteria metabolic activity can lead to pH value variation. However, pH value changes are most commonly present in the oral environment and because of that dental implant materials are tested in mediums with different levels of acidity [24].

Immersion time

Immersion time is time during which metallic implant materials are submerged to simulated biofluid medium. Short immersion time testing (several hours to several days) is used in order to compare amounts of released ions from different implant materials. Medium immersion time during released ions testing (several days to several weeks) is the time during which the equilibrium stage is established. On the other hand, long immersion time (several weeks to several months) can lead to the violation of the protective layer formed on the implant material surface and the main problem that may occur in this type of testing is the crack initiation in the surface layers and implant material decomposition. Fig. 3 shows the results obtained in the literature for static short immersion tests, while Fig. 4 shows previously published results of other research groups which correspond to the amounts of ions released during static long immersion tests.

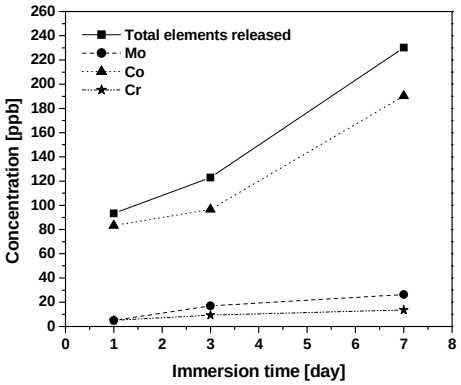

Figure 3. Release amounts of Co, Cr and Mo from Co-Cr-Mo alloy (WIRONIT) in artificial saliva [17].

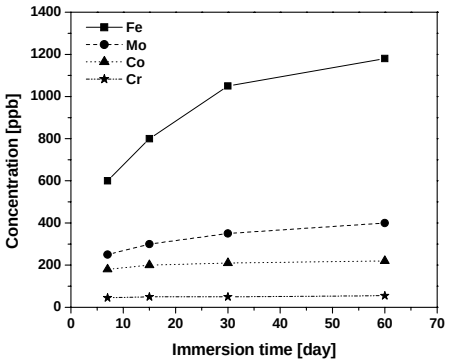

Figure 4. Release amounts of Co, Cr, Mo and Fe from Co-Cr-Mo alloy (WIROBOND C) in artificial saliva [23].

Analytical techniques

The type and concentration of ions released from the metallic implant materials can be determined using different analytical techniques such as Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) [25], while Scanning Electron Microscopy (SEM) is commonly used for the analysis of the implant material microstructure and the surface layer morphology before and after corrosion [26]. Brief overview of commonly used analytical techniques for ion release investigation is given in the following text.

Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy (AAS) is an analytical technique for determining the concentration of a particular chemical element present in the investigated sample by measuring absorbed optical radiation (light) with wavelength which is characteristic for every particular free atom in the ground state. The energy levels for an electron in an atom can be changed and excited state can be reached if the electrons of the atoms in the atomizer are transferred to higher orbitals for a short period of time by absorbing a defined quantity of energy. Since, each wavelength corresponds to only one element (the analyte) this technique enables their identification. For accurate analysis AAS technique requires the standard setting up using the known analyte content in order to establish the relation between the measured absorbance and the analyte concentration. This relation relies on Beer-Lambert Law:

$$A = log_{10} \frac{I_o}{I} = \varepsilon \cdot c \cdot l \tag{1}$$

where A represents absorbance, I_0 is intensity of the incident light, I is intensity of the transmitted light, ε is molar absorptivity (molar extinction coefficient), c is molar concentration and l is the path length [27].

Inductively Coupled Plasma - Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) is an analytical technique that performs elemental analysis with excellent sensitivity. It can be applied to solutions, solids and gases. This technique employs argon plasma, as the ionisation source, and a mass spectrometer to separate the produced ions. During ICP-MS analysis the investigated material is transferred by an argon flow into inductively coupled plasma in which an effective temperature results in atomisation and ionisation of the material. Subsequently, the ions are extracted into a mass spectrometer, using which the elemental composition of the material is determined [28]. ICP-MS, as an analytical technique, has many advantages in the laboratory usage for trace metals identification:

- almost all elements can be determined and identified,
- combination of high sensitivity and low background signal provide a very low detection limit and
- rapid analysis, which is a result of high-speed operation of the mass spectrometer (quadrupole) [29].

Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES)

Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) is one of the most commonly used techniques for elemental analysis. Its high specificity, multi-element capability and good detection limit results in the possibility of using this technique in a large variety of applications. All kinds of dissolved samples can be analysed. A plasma source is used to dissociate the sample into its constituent atoms or ions, exciting them to a higher energy level. They return to their ground state by emitting photons of a characteristic wavelength, depending on the element present. This light is recorded by an optical spectrometer [30, 31] and elemental identification is enabled.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is technique which is commonly used for the analysis of the different implant materials microstructure before and after corrosion, as well as for surface layer morphology examination. SEM uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron – sample interactions reveal information about the sample including external morphology (texture), crystalline structure and orientation of materials making up the sample. Also, SEM coupled with Energy Dispersive Spectrometer (EDS) can be used for the fine chemical analysis of the material surfaces and surface layers formed during corrosion [32].

Data processing and interpretation of results

The average dissolution rate (R_{avg}) (kgm^{-2}s), after relatively long immersion periods, can be calculated using the equation:

$$R_{avg} = \frac{\Delta C \cdot V}{\Delta t \cdot A} \tag{2}$$

where ΔC is concentration change (kg/L), V is volume of the medium (L), Δt is time difference (s) and A is the alloy exposed area (m^{-2}).

If the concentration is determined frequently or dissolution is monitored continuously, the dissolution rate at time t (R_t) is proportional to the slope of a tangent to the concentration - time curve:

$$R_t = \left(\frac{dC}{dt} \right) \cdot \frac{V}{A} \tag{3}$$

Therefore, the dissolution rate at time t can be obtained by differentiation of the quotient concentration - time, analytically, graphically or numerically [25].

Conclusion

When the development of new materials for medical usage is concerned, critical factors are biocompatibility and non-toxicity of the implant materials. Various technological processes are used in order to achieve the optimal material properties (improve the required properties and eliminate the hazardous ones). Insight into the type and concentration of the released elements from the metallic implants is very important for prediction and assessment of their local and systemic effects in the human body. Examination of metal ion release from biomedical implants starts by immersion of the prepared samples in the medium that simulates biofluid. After certain immersion time, the type and concentration of released ions are determined by appropriate analytical technique. The amount of released ions is increased with increasing immersion time until the adsorption-desorption equilibrium is reached.

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