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Metal ions release from metallic orthopedic implants exposed to tribocorrosion and electrochemical corrosion conditions in simulated body fluids: Clinical context and *in vitro* experimental investigations

Henryk Matusiewicz [1,*] and Magdalena Richter [2]

[1] *Faculty of Chemical Technology, Poznan University of Technology, Poznań, Poland.*
[2] *Department of Orthopedics and Traumatology, Poznan University of Medical Sciences, Poznań, Poland.*

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Abstract

The use of metallic biomaterials in the medical implant devices has become increasingly prevalent over the past few decades. Patients find themselves being exposed to metals in a variety of ways, ranging from external exposure to instruments such as medical devices to internal exposure *via* surgical devices being implanted in their bodies. Potential health risks are associated with metallic wear debris in the form of nanoparticles *in situ* generation and the release of *in vitro* metal ions into human biological specimen's circulation. In combination with mechanically accelerated electrochemical processes, this can lead to the release of toxic metal ions, nanoparticles or micro scaled debris in the surrounding tissues and body fluids bring about relevant health issues and contribute to implant loss of failure. Metal ions release from metallic materials; stainless steel, cobalt-chromium alloys, titanium and its alloys, and nickel-titanium alloys implanted into the human body in orthopedic surgery is becoming a major cause of concern. The degradation mechanisms of the metallic implants lead to implants failure and tissue inflammation. However, the levels of metallic ions in those fluids can vary depending on the involved degradation mechanism of the implant. After briefly recalling the clinical context, this article analyses the *in vitro* studies on metals and metallic alloys corrosion, tribocorrosion and electrochemical corrosion under simulated inflammation conditions. Specifically, the present review reported the results of *in vitro* experimental investigations on the release of metal ions from metallic orthopedic implant materials, subjected to different electrochemical corrosion and tribocorrosion conditions, into simulated human body fluids.

Keywords: Metallic orthopedic implants; Tribocorrosion; Electrochemical corrosion; *In vitro* metal ions release; Simulated body fluids; *In vitro* tests

1. Introduction

Developing prosthetic implantology faces more and newer challenges and involves research in a wide variety of fields associated both with clinical and basic sciences. The development of research on materials used extensively in medical implantology reveals new data on the possible adverse side effects of metal release from metallic biomaterials into the human body. Among the various biomaterials available, metallic-based implant materials (pure metals and metal alloys) can provide scaffolds for excellent tissue/bone/organ repair that are needed to save and prolong the human being's life. The most commonly employed metal alloys used in biomedical implants include stainless steel, cobalt-chromium alloys, titanium and its alloys, and nickel-titanium alloys. All these alloys contain in different amounts metals such as nickel, vanadium, aluminum, chromium and molybdenum. A large amount of released metal ions could be harmful to human health and may eventually lead to severe complications, implant failure and tissue inflammation. However, the levels of metallic ions in physiological fluids can vary depending on the involved degradation mechanism of the metal implants

* Corresponding author: Henryk Matusiewicz
Faculty of Chemical Technology, Poznan University of Technology, Poznań, Poland.

and prostheses used in the human body: corrosion [1] or tribocorrosion [2]. All these degradation phenomena result in metal ion release to the body fluids arising from the metallic parts. Metal ion release from implants has been reported *in vitro* as well as *in vivo*. The mechanisms of metal ion release from metallic orthopedic implants *in vitro* need to be understood in order to discuss the safety and biocompatibility of implant materials. Therefore, it is rather urgent and important to have a better understanding of the metal ion release processes for metallic orthopedic implant materials. Having a tool able to identify the origin of the metal ion concentration in patients using metal implants could allow faster and easier clinical treatments and failure detection. Since the release of metal ions depends on electrochemical rules, much effort has been made to analyze the electrochemical processes and investigate metal ion release. Metal ion release from surgical implants probably results primarily from corrosion. Another source of metal ions is passive films, which are thick and not stable in body fluids. As an implant corrodes, electrochemically or mechanically accelerated electrochemical processes (fretting corrosion, stress corrosion or corrosion fatigue), metal ions, metal complexes of implant-derived or implant-derived particles, in the nanometer range, are release. A major concern of modern implantology seems to be the possibility of release of toxic metal ions and nanoparticles through corrosion or wear processes of metallic orthopedic implants used in the production of implanted metal devices into the human body physiological environment. An understanding of the origin of metal ions is really important in order to design alloys for reduced ion release.

After briefly recalling the clinical context, this paper presents an overview of ongoing research regarding the potential related adverse biological effects associated with local nanotoxicity attributed to elevated metal ions and metal wear micro- and nanoparticles released from metal surgical implants, based on the available *in vitro* experimental clinical studies. Thus, the development of *in vitro* metal ion analysis is critical to investigating the complex problems associated with the chemical nature that underlies metallic orthopedic implants function. Recently, *in vivo/ex vivo* hip-simulator studies provided substantial evidence to prove that the debris generated using serum has similar characteristics to that of patient tissue samples [3]. In addition to the clinical practice, physiological solutions are often used for *in vitro* laboratory research as a biofluids simulation medium. Physiological fluids of the human body are solutions of complex chemical composition, which contain different amounts of inorganic salts, amino acids, sugars, proteins, and other components, and their content depends on many factors. Therefore, *in vitro* testing, under simulated inflammation conditions (artificial solutions), of the *in vitro* corrosion of metallic implants with a combination of electrochemical and mechanical tribo-corrosion conditions for assessing the bio-corrosion properties of metals used in implanted metal appliances was considered. In particular, in selecting suitable materials on the basis of used conditions and durations, the behavior of metal release from each base and alloying element constituting the metallic biomaterials was examined using various solutions simulated human body fluids. Finally, a collection of published experimental data on *in vitro* released metal ions from metallic orthopedic implant materials is included.

In vitro testing analyses of the toxicity, immunological, and gene expression effects of alloy wear debris and metal ions derived from metallic orthopedic implants on human cell cultures was not considered.

2. Tribocorrosion behaviour and ions release from metallic orthopedic implants

Chemically speaking, corrosion is the visible destruction of a metal. Electrochemical reactions during which the surface of a metal is deteriorated *via* ion release are called *corrosion*. Corrosion of metals and alloys used as implants in the body is a complex process that the metal is challenged within the body due to changes of the pH, body fluids, exposure to cellular processes, *etc.* and is due to the chemical environment of the body fluids [4,5]. A metallic "foreign body", such as metallic implants, may interfere with oxidation-reduction reactions that are the basis of metabolic and growth processes. Red-ox reactions occur at the metal surface and can cause denaturation of the tissue that is in contact with metallic implants. The metals and alloys used as surgical implants achieve passivity by the presence of a protective surface layer. This film inhibits corrosion and keeps current flow and the release of corrosion products at a very low level [6]. Corrosion plays a major role in the release of metal ions, however, both wear (mechanical) and corrosion (chemical) act synergistically (tribocorrosion) in the presence of protein rich synovial fluid. Tribocorrosion is defined as an irreversible transformation of a material resulting from simultaneous physico-chemical and mechanical interactions occurring in a tribological contact. This interaction results in the generation of complex degradation products. The degradation of metal-on-metal (MoM) artificial joints has been the object of an increasing number of scientific *in vitro* investigations. Aspects of such lubrication, clearance, wear patterns and mechanical *in vitro* testing methods have been throughout analyzed. Even so, metal ions from orthopedic metallic implant materials are eventually generated and released into the body by electrochemical corrosion of metal surfaces, chemical dissolution, *in vitro* wear debris, or mechanically accelerated electrochemical processes, such as fretting corrosion, stress corrosion or corrosion fatigue [7].

2.1. *In vitro* metal ions release from stainless steels orthopedic implants

Stainless steel (SS) is the general name for a number of different steels used mainly because of its resistance to a wide range of corrosive agents. SS (primarily type 316L, iron based) is a widely used cost-effective orthopedic implant materials for internal fixation because of its mechanical strength and the possibility of bending and shaping the implant to create a custom fit in the operating room. It possesses good mechanical properties but suffers from poor biocompatibility. Stainless steel implants are used as temporary implants to help bone healing, as well as fixed implants such as for artificial joints. Typical temporary applications are bone plates and screws, nails and pins, joints for ankles, elbows, fingers, knees, hips, shoulders and wrists. However, major disadvantages of SS are well-documented. Upon prolonged contact with human tissue surface corrosion phenomena takes place resulting in a high rate of locally released corrosion products. Release of large amount of certain metal ions may lead to harmful deceases. The ions released from SS are mostly of iron, nickel and chromium. Specially nickel is recognized as a strong immunological reaction medium and may cause hypersensitivity reactions, contact dermatitis, asthma, and moderate cytotoxicity [8].

Mechanisms of metal ion release from metal implants have been recently summarized [9]. This paper described the behavior of stainless steel bone plates in simulated physiological conditions. A shift in pH value affects the metal ion release. The quantity of Ni and Fe released from SUS316L stainless steel gradually decrease linearly with increasing pH. The quantities of Cr and Mo released from SUS316L stainless steel were smaller at a pH of approximately 4 or higher. However, for SS (SUS316L) acidification induces an increased metal ion release, especially below pH~4. The *in vivo* results were qualitatively in good agreement with the *in vitro* experiments, among different simulated physiological solutions.

The influence of surface finishing (polishing and passivation) on the release of Cr, Fe, and Ni from the stainless steel 316 implant materials to Hanks solution with or without H_2O_2 (simulating a body inflammatory response) was investigated [10]. The total metal ions release rates are more than 10 times higher in the presence of H_2O_2, independently of the surface finishing. In general, it can be noted that iron as the main constituent of the steel is preferentially released from all the surfaces independently of finishing procedures. The relative release rates for the metal ions are Fe>Ni>Cr for the Hanks solution, whereas in the presence of H_2O_2 they change to Fe>Cr>Ni. In the absence of H_2O_2, formation of a surface layer consisting mainly of $Ca_2(PO_4)_2$ was observed, most likely it was responsible for the observed decrease of the release rates.

Cadosch et al. [11] investigated whether human osteoclasts (OC) are able to grow on stainless steel (SS) and directly corrode the metal alloy leading to the formation of corresponding metal ions, which may cause inflammatory reactions and activate the immune system. The amount of bio-corrosion was quantified by measuring the levels of metal ions released into the culture supernatant after every incubation week. Significantly increased concentrations of Cr, Ni, and Mn were detected in the supernatant of OC cultured on SS discs when compared to the levels measured in the supernatant of human monocytic cells (MC) cultured on SS discs and control discs left in culture medium alone. The released ions induced the secretion of pro-inflammatory cytokines, which may lead to the formation of osteolytic lesions in the peri-prosthetic bone, contributing to the loosening of the implant. It is reasonable to assume that this process may take place at the bone-implant interface, representing an additional mechanism of metal corrosion *in vivo* and contributing to the levels of metal ions measured in the peri-prosthetic tissues and in the serum from total arthroplasty patients.

Brooks et al. [12] assessed the influence of a simulated inflammatory response on the corrosion of 316L stainless steel. Samples were immersed in an electrolyte representing either normal or inflammatory physiological conditions. After 24 h of immersion in the test electrolytes, analytical method was performed on aliquots of the tests solution to quantify the concentration of metal ions (Cr, Fe, Mn, Mo, and Ni) released into the test electrolyte. For each element, there was a statistically higher ion release measured when 316L was exposed to inflammatory conditions as compared to normal physiological conditions. Based on analytical results, it is possible that an inflammatory response may lead to an increase in the release of ions from an orthopedic device. In turn, this ion release may also be responsible for initiating a more aggressive inflammatory response.

Implants have very specific surface finishing requirements. Since polished surfaces exhibit enhanced corrosion resistance, in many cases, the implant surface is polished mechanically and/or electro-polished. Additionally, all the debris from the manufacturing process, as well, as microbiological contaminations have to be removed from the implant surface before application into the body. In the literature there are only a few reports on the influence of surface finishing on the corrosion resistance, metal ions release, surface cleanliness of SS [13,14].

Table 1 *In vitro* times and metal concentration in simulated body fluids following ion release from stainless steel orthopedic implants

Implant material	Implant type	Simulated body fluid analyzed	Immersion time	Metal ion(s) measured	Concentration (μg L^{-1})	Analytical technique	Obtained research results	Refs.
SS (SUS316L)	Metal plates	PBS	7 d (37 °C)	Fe Ni	0.4 0.1	ICP-MS GF AAS	The quantity of Ni released from SS gradually decreased with increasing pH. The quantities of Cr and Mo released from SUS316L stainless steel were smaller at a pH of approximately 4 or higher	[9]
SS (SUS316L)	SS grade 316 samples	Hank's solution Hanks solution with 100 mM H_2O_2	28 d (37 °C)	Fe Ni Cr Fe Ni Cr	0.2 0.15 2.8 22 4 5.5	F AAS GF AAS	The total metal release from stainless steel 316 to Hanks solution are more than 10 times higher in the presence of H_2O_2 simulating an inflammatory response on the surface finish	[10]
SS (UNS S1673)	Metal discs	OC	21 d	Cr Ni Mn Mo	17 15 8 <4	ICP-OES	Significantly increased concentrations of Cr, Ni, and Mn were detected in the supernatant of OC cultured on SS disks	[11]
SS (316L)	Metal discs	PBS (pH 7.2) H_2O_2 solution in PBS (pH 5.0)	24 h (37 °C)	Cr Fe Mn Ni Mo Cr Fe Mn Ni Mo	12 90 100 15 2 40000 70000 5000 10000 1000	ICP-MS	Sample examination following the immersion period showed evidence of severe localized corrosion in crevice susceptible areas on the 316L surface. Analytical technique was used to evaluate differences in corrosion behavior and ion release induced by the inflammatory conditions. For each element, there was a statistically higher ion release measured when 316L was exposed to inflammatory conditions as compared to normal physiological conditions	[12]

SS grade 304	Metal panels	ALF (pH 4.5)	8,168 h	Cr Fe Ni	(µg cm^{-2}) 0.4-1.1 9-15 0.2-0.4	ICP-MS	Higher release rates of iron compared to chromium and nickel and a time-dependent metal release process were observed for all surface finishes and exposure periods, but all release rates were very low for all three surface finishes. No direct correlation was observed between corrosion resistance and metal release rates	[13]
SS grade 316L	Metal sheets	ALF (pH 4.5)	8,24,48,168 h	Cr Fe Mo Ni	(µg cm^{-2}) 0.1-0.9 1-12 0.1-0.3 0.1-0.6	ICP-MS GF AAS	Total metal release rates from all grades of stainless steel investigated were low. Acidic environment of ALF resulted in high total metal release rates	[14]

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; GF AAS, graphite furnace atomic absorption spectrometry; F AAS, flame atomic absorption spectrometry; Abbreviations: SS, stainless steel; PBS, phosphate buffered saline; OC, osteoclasts; ALF, artificial lysosomal fluid; h-hours; d-days

Herting et al. [13] has shown that there is no simple quantitative relationship between the bulk alloy composition or corrosion resistance and metal release rates from stainless steel of various grades exposed to physiological media that are relatively mild from the point of view of corrosivity, though there may be some correlation with the degree of chromium enrichment of the outermost surface film of the alloy [13]. Reliable information on metal release rates is invaluable when assessing the potential for risk of adverse effects arising from exposure to metal constituents of stainless steel. No simple relationship exists between the corrosion rate and metal release rate, or between the bulk composition of the alloy and the relative release rates of individual alloy constituents [14]. Further X-ray photoelectron spectroscopy (XPS) examination elucidated that metal release from various grades of stainless steel into synthetic body fluids changes depending on the Cr content in the oxide film. The rate of metal release from stainless steel into artificial lysosomal fluid decreases with the increase in Cr/(Cr + Fe) ratio in the oxide film, which clearly shows that Cr is attributed to passive film formation [13,14].

In vitro times and elevated total concentration of metals in the simulated body fluids following ion release from stainless steel orthopedic implants are presented in Table 1.

2.2. *In vitro* metal ions release from cobalt-chromium alloys orthopedic implants

The cobalt-chromium alloys show an extremely high degree of corrosion resistance even in chloride surroundings due to the spontaneous creation of a chromium oxide passive layer within the human body; they exhibit balance between biocompatibility and mechanical properties; both forms are somewhat better than stainless steel in corrosion resistance and strength but more costly to design. The cobalt-chromium based alloys used in total joint replacement surgery are cast CoCrMo alloy and wrought CoCrNi alloys (CoCrWNi and CoNiCrMo) have been used for many years due to their biocompatibility and their superior mechanical properties such as: high elastic modulus, high tensile strength, and superior wear and corrosion resistance properties. As in stainless steels, alloying cobalt with chromium greatly enhances corrosion resistance. The main drawbacks and medical concerns regarding the use of such alloys include both the decreased biocompability due to the release of metal ions in the body and the material loss caused by tribocorrosion (combined effect of wear and corrosion).

In spite of the excellent corrosion resistance of CoCrMo alloy, there is still a concern about metal ion release from orthopedic implants into the body fluids (serum, urine, *etc*.). Implant components fabricated from Co-Cr alloys have been reported to produce elevated Co, Cr and Ni concentrations in body fluids [9]. Preferential release of Co was observed from CoCrMo alloy. The amount of Co released from CoCrMo alloy was not affected by pH shift in the acid direction. A recent study investigating the corrosion products of CoCrMo and SS316L alloys found CoCrMo corrosion products to be more toxic than those of SS (316L). The most toxic ions were Cr, Ni, and Co [15]. In addition, there is concern that a slow accumulation of metal ions such as cobalt and chromium can lead to adverse clinical reactions.

There are various surface treatment methods which may prevent and/or reduce the release of potentially harmful metal ions from metallic orthopedic implant materials. One is to thicken the protective oxide layer already present on the surface of metallic biomaterials *via* a process known as passivation. Another method is to apply coatings or protective layers, to act as a physical barrier between the aggressive body environment and the biomaterial to be protected. Quite a few literature studies related to various types of coatings such as TiN, TiAlVCN/CN and Ti plasma spraying show that these coatings are better barriers to ionic diffusion than are the native surface oxides [16-19].

Türkan et al. [16] investigated the effectiveness of physical vapor deposition (PVD) coated TiN layers on medical grade CoCrMo alloy in preventing the dissolution, potentially harmful metal ions from CoCrMo based surgical implants into the simulated body fluid (SBF). The analytical results showed that the presence of the TiN coating prevented the release of cobalt and chromium metal ions from the substrate CoCrMo alloy whereas cobalt was preferentially dissolved from the as-polished material. The results suggest that the TiN coating as an effective barrier to metal ion release compared to the surface oxides on the substrate material (CoCrMo alloy). The metal ion release may be associated with both the film structure and film thickness. In Ref. [17], which is a similar metal ion release study to Türkan et al. [16], metal ion release was detected in saline solution, it is reported that the TiN coating (*via* PVD) CoCrMo surgical implant material substantially reduced the concentration of dissolved metal ions, particularly cobalt; however, the coating was less efficient for reducing molybdenum ion release than that of cobalt and chromium. This was explained by the porous structure of the film and incomplete surface coverage. Pores on a film can act as a channel for corrosive media and increase the metal ion release from the substrate. In Türkan et al. [16] study, the film structure is quite dense and no pores were observed on the surface of the film.

In the work by Alemón et al. [18], a TiAlVCN/CN multilayer coating has been employed to improve the tribocorrosion-resistance of the CoCrMo substrate. During the tribocorrosion test, with the sample immersed in SBF containing bovine

serum albumin (BSA), open-circuit potential measurements showed more noble potential as well as a reduction of both the friction coefficient and wear-rate during the sliding phase. Analytical results demonstrate that the multilayer coating effectively blocked the emigration of metallic ions (Co, Cr, Mo, Ti, Al and V). The ions release concentration levels were very low. Nevertheless, these results confirm that the multilayer provided a significant barrier against the ions release in the physiological simulated fluid.

The corrosion behavior of CoCrMo implants with rough titanium coatings, applied by different suppliers by vacuum plasma spraying (Ti VPS), has been evaluated and compared with uncoated material [19]. The Co, Cr and Ti ions released from the samples into the electrolyte during a potentiostatic extraction technique were analyzed at the end of the extraction tests experiment. The analytical results reveal high quantities of cobalt and chromium in the electrolyte. The concentration of titanium, in contrast, was found to be significantly lower, although it is this element that makes up the surface. The presence of cobalt and chromium in the electrolyte implies that the Ti-coatings are permeable and let pass the metal ions extracted at the CoCrMo surface underneath the coating.

Besides TiN, other types of coating were considered to be beneficial for preventing the metal ion release. Őztürk et al. [20] examined nitrogen (N) ion implantation as a means to form protective layers on the surface of medical grade cobalt-chromium-molybdenum (CoCrMo) alloys and investigate the effectiveness of N implanted layers in preventing and/or reducing the dissolution of metal ions into the SBF, released from CoCrMo orthopedic materials. Static immersion tests were performed to investigate metal ion release into SBF. Analytical analysis results showed that *in vitro* exposure of the N implanted surfaces resulted in higher levels of cobalt ion release into the simulated body fluid compared to the untreated, polished alloy. The results of the static immersion tests suggest that the N implanted layers may not have any beneficial effect for reducing the Co ion levels released into the simulated body fluid. The higher Co release from the N implanted specimens is attributed to the nature of the implanted layer phases as well as to the rougher surfaces associated with the N implanted specimens compared to the relatively smooth surface of the untreated material. Guo et al. [21] examined the nitrogen ion implantation as means of forming a protective layer on the CoCrMo alloys surface. Wet ability and the corrosion resistance of the implanted layer were investigated, along with the ability of nitrogen implanted samples to resist metal wear loss and ion release caused by the interactive effects of wear and corrosion. CoCrMo samples before and after nitrogen ion implantation were compared using corrosion resistance tests under wear (tribocorrosion tests) in newborn serum (NBS). The concentration of Co and Cr ions released into the NBS fluid from the specimens was determined. The concentration of realized cobalt ions is greater than the concentration of chromium ions. Co and Cr ion release rates initially increase with nitrogen ion implantation dosage. Subsequently, the concentration of the released ions goes down to a level lower than the concentration of the Co and Cr ions released from the CoCrMo alloy substrate. Bazzoni et al. [22] characterized the tribocorrosion behavior of pulsed plasma treated, in a N_2/H_2 atmosphere at temperatures below 500 ^oC), biomedical CoCrMo in simulated body fluid (0.9 wt% NaCl) by quantitatively assessing the contributions of the different mechanical and chemical degradation mechanisms. The materials were tested for corrosion and tribocorrosion performance in 0.9 wt% NaCl at room temperature under controlled electrochemical conditions. The concentration ratio of Co was higher than what was expected from the alloy stoichiometry. This can be explained by the fact that Co forms dissolved ions, while Cr rather precipitates as solid oxide. Part of the solid particles remained in the electrochemical cell after pouring the solution, which is thus expected to be enriched in Co. Indeed, debris particles adhering to the metal sample and to the ball were observed.

Metallic materials in orthopedic implants must bear mechanical loads with resultant surface damage. In addition to mechanical load, the surface damage on the implants occurs due to electrochemical corrosion from the physiological environment *in vivo*. Mumme et al. [23] investigated *in vitro* experimental test bodies of four different standardized, frequently used, artificial implant alloys (TiAl6V4, Ti(Ti), CoCr29Mo, FeCrNiMoMnNbN). The test materials were immersed in serum (pH 7.4) by avoidance of any friction and mechanical load. Here, they were subject to electrochemical corrosion independently of mechanical influences when in contact with body liquids. It was found that up to 80% of the entire dissolution of each ion occurred within the first 24 h of immersion. Additionally, the dissolution of the metal ions depended on the proportional mix of the individual elements in respective alloys. Therefore, the highest percent of ion concentration was found for Co (FeCrNiMoMnNbN) followed by Fe (FeCrNiMoMnNbN) and Mo (FeCrNiMoMnNbN). The lowest percent of ion concentration were evident for Ti (Ti), Cr (CoCr29Mo) and Cr (FeCrNiMoMnNbN).

Rae et al. [24] used MoM hip simple joint simulators in tissue culture medium with serum to conduct serial analysis of the soluble metal content in the solution and analyzed the wear debris composition. The soluble metal concentrations measured at the end of each cycle contained significantly more Co ions than Cr ions, whereas analysis of the wear debris particulates showed significantly lower Co concentrations compared to Cr. Additionally, the parent alloy contained 60% Co, 28.5% Cr, and 6% Mo, which equates to a Co:Cr ratio of 2.1:1, however, the metal ion ratio in the lubricant fluid

contained a Co:Cr metal ion ratio of 4:1 suggesting that Co is preferentially dissolved, thus generating Cr-rich wear debris particles. No soluble metal was detected from a titanium alloy. From the biological standpoint, in view of the very much higher levels of soluble metal produced, metal against metal bearings should be avoided.

Hip resurfacing arthroplasty has become a popular alternative to conventional hip surgery. Increased failure rates of hip implants in patients are associated with smaller bearing diameters. *In vitro* hip simulator tests comparing 39 mm and 55 mm bearing sizes showed that the smaller bearings resulted in increased volumetric wear and the larger bearings reached steady state level for metal ion levels in serum lubricant solution earlier than bearings of smaller size. The ion levels measured suggest both bearing sizes have similar initial wear rates; however, the 55 mm bearings reach steady state wear more rapidly. Another words, simulated wear debris has a similar composition to that of the bulk alloy [25].

Maruyama et al. [26] evaluated the pin-on-flat type friction-wear behavior of nickel-free CoCrMo alloy in quasi-biological environment (simulated body fluid). Test solution containing wear debris was recovered after the friction-wear test. The solution was unfiltered or filtered to remove wear debris. Metals in the solutions unfiltered and filtered were quantified. No difference was observed among the concentrations of Co, Cr and Mo in unfiltered solution, those are main components of the alloy. Those of Co and Cr in filtered solution were 3 orders of magnitude lower than those in unfiltered solution. Concentration of Mo was 2 orders of magnitude higher than Co and Cr in filtered solution, indicating that Mo preferentially dissolved as ions. Ni was detected as the same order as Co and Cr. These results indicate that even trace elements in an alloy could not be negligible. This preferential dissolution of specific elements may be problem for metallic implants used under a friction.

It has been reported that 20%-30% material loss can be attributed to corrosion-related damage, and not only includes the pure corrosion process but also the wear induced/enhanced corrosion process that is defined as tribocorrosion. Mechanically assisted corrosion, also referred to as tribocorrosion, is an irreversible process that occurs on the surface causing deterioration of the material due to the combined wear and corrosion actions that simultaneously take place. Tribocorrosion, present at bearing surfaces and within modular taper connections between components of the arthroplasty device, has been proposed to be the primary process by which ions and particles are generated [2].

There is no standard method for generating particles for *in vitro* and *in vivo* studies. To accurately model the biological impact of wear debris, it is important to simulate the *in vivo* degradation products, both particles and ions, as closely as possible. To potentially mimic the *in vivo* joint conditions, 2nd generation toxicity evaluation researchers used a hip wear simulator for generating wear debris. Many studies have shown that *in vitro* simulator testing is an accurate methodology to predict the performance of joint replacements [27-34].

Yan et al. [27] attempted to separate wear debris and ions, produced by pin-on-plate testing in biological solutions, by using centrifugation (10 min, 14,000 rpm) prior to analysis. Cobalt was the ion in highest concentration among the three detectable elements followed by chromium and molybdenum released from CoCrMo alloys on both forms of apparati (synovial fluid and bovine serum). In the tested solution, albumin can accelerate the dissolution of Co from the wear debris. They found more than 92% concentration ratio of Co in a synovial fluid solution after one week digestion at 37 ^{o}C of wear debris particles generated in hip simulator. Wang et al. [28] tested nano-sized wear debris of CoCrMo alloy in a protein containing BSA medium. It was observed in this study that the crystalline wear debris was comprised of two constituent's instead of one, that is Cr oxide and the being crystalline Co with a close-packed hexagonal structure. It can be quantified that 43% Co and 99% Cr from the tested CoCrMo alloy were in wear debris after 24 h tests and the rest became ions in the solution. The release rates for Co and Cr from the wear debris were 5 μg L^{-1}/h and 0.6 μg L^{-1}/h, respectively. As the hip implant is designed for longer service life, the amount of ions from wear debris can be considerably great. Hesketh et al. [29] measured the metal ion release after instrumented hip simulator tests performed on a 36 mm diameter CoCrMo alloy. They investigated the combined effects of wear and corrosion on the performance, material loss caused by tribocorrosion, of MoM hip replacements. Corrosion related damage contributed significantly to material loss and to the formation of metal ions (Co, Cr and Mo), and varied throughout the tests. Slight variations may be attributable to the difference in solubility of the three metals, or their tendency to react with proteins and be removed in the sample preparation procedure. Changes in corrosion rate were attributed to the formation of Co(II) and Cr(III) in a ratio equal to their abundance in the alloy, tribochemical reaction layers. Moreover, Catelas et al. [30] carried out a comparative evaluation of the physicochemical properties of wear particulate extracted from tissue samples of patients who underwent CoCrMo implant revision surgery in comparison to wear particles generated from the hip simulator. It was revealed that the particles from metallic implants generated in the simulator were comparable to those found in the peri-prosthetic patient samples with regards to the chemical composition, size and shape. It is interesting to note that although wear debris from metal-metal patients can be found with Cr as the main constituent (free of Co and Mo); the authors noted that there were no particles found to be composed of only Co (free of Cr and Mo). The

oxidized Cr particles are likely generated from the passivation layer covering the implant materials, whereas the CoCrMo particles containing Co are likely generated from the bulk implant materials. This remains consistent with the characteristics of wear debris observed in patient studies and serum hip simulator experiments which primarily contained oxidized Cr particles and minimal Co content. Simoes et al. [31] developed a protocol for the analysis of metal ion levels in fluids extracted from CoCrMo alloy, MoM hip implant patients and also hip simulators. The size of wear debris particles produced by the hip simulator used here, were within the range reported for other hip simulator and also the size of wear debris generated *in vivo* [30]. Using this procedure, they present a new perspective on the release of metal ions from CoCrMo alloy implants, revealing significantly lower levels of metal ion release during tribocorrosion in hip simulators combined with the release of much higher percentages of molybdenum ions relative to cobalt and chromium. They have clarified the influence of nanoparticles on dissolution studies and hence revealed relatively high levels of molybdenum-containing ions that could be complexes with serum proteins. From the pre-clinical point of view, the benefit of this procedure is that it allows an effective discrimination between the three wear products produced during tribocorrosion: free (metal) ions, complexes (metal) ions and (metal) nanoparticles (where the metal in the latter is nominally in a zero valence state).

Metal-on-metal total hip resurfacing arthroplasty is increasingly being performed in young and active patients. Preclinical *in vitro* testing of implants is usually performed with use of hip simulators, and the serum metal ion concentration is determined for the purpose of monitoring the patients. Heisel et al. [32] investigated the early running-in period *in vivo* and *in vitro* by characterizing metal ion levels. The simulator study allowed an exact characterization of the running-in period and showed a delayed onset of running-in wear. In contrast, the clinical data showed a slow increase in measured ion concentrations. Specifically, it was found that the released Co levels continuously increased during the running-in period and showed a significant decrease thereafter. Hodgson et al. [33] investigated the electrochemical properties of the oxide layer on CoCrMo alloy under simulated biological conditions. It was found that the passive film on CoCrMo alloy changed in composition and thickness with both potential and time. The mass transport controlled process has been observed on CoCrMo alloys. Authors also observed a drastic increase of the dissolution rate and a high accumulation of Co in the electrolyte when polarizing the sample from cathodic (simulating electrochemical depassivation) to anodic (simulating electrochemical repassivation) potentials.

Espallargas et al. [34] investigated the degradation mechanisms which cause metal ion release of a CoCrMo biomedical alloy subjected to different electrochemical and tribocorrosion conditions in simulated body fluids at the human body conditions of 37 ^{o}C. The total metal ion release measured *in vitro* was higher for the tribocorrosion testing conditions compared to corrosion due to the active dissolution caused by depassivation. Indeed, in tribocorrosion the degradation mechanism is governed by depassivation (mainly cobalt dissolution) and mechanical detachment of wear particles. Cobalt is always present in the highest ratio, molybdenum has the lowest ratio and chromium is always in between. Despite the few works reporting metal ion release after *in vitro* tests of biomedical CoCrMo alloys, the metal ion concentration ratios obtained by other authors are in well agreement with the results obtained in this work [18,22,27,29]. The presence of albumin drastically increases the amount of Mo release in electrochemical corrosion conditions. Whereas in tribocorrosion, albumin slightly increases the chromium and molybdenum concentrations. Despite the very dissimilar testing conditions (electrolytes, contact pressures, different tribometer configurations and different alloy compositions and manufacturers) the results of metal ion release ratios where in all cases in the same range. This shows that *in vitro* tests are the powerful and reliable tool for assessing the biotribocorrosion properties of metals for biomedical applications.

In vitro times and elevated total concentration of metals in the simulated body fluids following ion release from cobalt-chromium alloys orthopedic implants are presented in Table 2.

Table 2 *In vitro* times and metal concentration in simulated body fluids following ion release from cobalt-chromium alloys orthopedic implants

Implant material	Implant type	Simulated body fluid analyzed	Immersion time	Metal ion(s) measured	Concentration ($\mu g\ L^{-1}$)	Analytical technique	Obtained research results	Refs.
CoCrMo alloy (TiN coated)	Metal discs	SBF (pH 7.4)	1,3,7,15,30, 60,90,120,150 d (37 °C)	Co Cr Ni Mo Ti	<0.5 <0.5 <0.5 30 20	GF 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 (CoCrMo alloy)	[16]
CoCrMo alloy (PVD TiN coated)	Femoral hip components	NaCl, EDTA (pH 6.2)	550 h (37 °C)	Co Cr Mo Ti	0.65 0.2 0.2 1.2	F AAS	Ti coating off the polished substrate resulted in a dramatic reduction in the release of Co and Cr, whilst some reduction in the Mo release was also observed	[17]
CoCrMo alloy (TiAlVCN coated)	Metal discs	SBF, BSA (pH 7.4)	1,3,6,24 h (36.5 °C)	Co Cr Mo V	0.02-0.01 0.07-0.06 0.09-0.12 0.2-0.11	ICP-OES	Analytical comparison results demonstrate that the multilayer coating effectively blocked the emigration of metallic ions	[18]
CoCrMo alloy (Ti coating)	Tibial and femoral components	Artificial bone fluid	48 h (37 °C)	Co Cr Ti	1700-2240 555-470 4.9-3.9	ICP-MS	The analytical results reveal high quantities of Co and Cr in the electrolytes. The concentration of Ti, in contrast, was found to be significantly lower, although it is this element that makes up the surface	[19]
CoCrMo alloy (Nitrogen implanted layer)	Metal discs	SBF (pH 7.4-7.6)	1,3,7,15,30, 150 d (37 °C)	Co Cr Ni Mo	25-50 <0.5 <0.5 <20	GF AAS ICP-MS	Analytical analysis showed that *in vitro* exposure of the nitrogen implanted surfaces resulted in higher levels of Co ion release into the simulated body fluid compared to the untreated, polished alloy	[20]
CoCrMo alloy	Metal discs	NBS	5 h	Co Cr	70 19	ICP-MS	Metal ion release is mainly from the friction contact area and the wear-induced debris. These results provide	[21]

(Nitrogen ion implanted)							evidence for the effect of the ion implantation process on improving the corrosion and tribocorrosion performance of the CoCrMo alloy. The detrimental effects of the specimen implanted at higher doses was attributed to the formation of the nanocrystals CrN in the amorphous matrix	
CoCrMo alloy	Metal discs	SBF (0.9 wt% NaCl)	85 m	Co Cr Mo	7.75 0.025 0.005	ICP-MS	The concentration ratio of Co is higher than what is expected from the alloy stoichiomatry; Co forms dissolved ions, while Cr precipitates as solid oxide	[22]
CoCrMo alloy	Metal pins and flats	PBS (pH 7.5)	n/a	Co Cr Mo Mn Ni Fe	701 318 20 <1 4 <1	ICP-OES	These results indicate that even trace elements in an alloy could not be negligible from the viewpoint of metal ion release	[26]
CoCrMo alloy	Metal pins and plates	BSF (pH 7.4)	4 h (37 °C)	Co Cr Mo	2200 900 200	ICP-OES	Cobalt ions were the most abundant ions released from CoCrMo alloys on both forms of apparati. Wear debris can also be corroded and release ions	[27]
CoCrMo alloy	Metal discs and bars	BSA media (pH 7.4) PBS media (pH 7.4)	24 h	Co Cr Mo Co Cr Mo	10.99 0.817 1.715 3.05 1.53 2.032	ICP-MS	This results demonstrated the evolutionary process of different elements (Co,Cr,Mo) from matrix to wear particles or lubrication under simulator wear in different test media (BSA and PBS)	[28]
CoCrMo alloy	Hip bearing surfaces	FBS, PBS (pH 7.4)	~14 d	Co,Cr,Mo	**Ions mass** 1.1 mg	ICP-MS	Cumulative metal ion release was determined from Co, Cr and Mo ion concentrations	[29]

CoCrMo alloy	Hip bearing surfaces	BSA (pH 7.2) PBS (pH 7.2) SSF (pH 7.2)	24 h (37 °C)	Co Cr Mo Co Cr Mo Co Cr Mo	33.1 0.6 76.7 174.4 0.3 35.6 157 0.8 52.9	ICP-MS	The release of metal ions from CoCrMo alloy implants revealing significantly lower levels of metal ion release during tribocorrosion in hip simulators, combined with the release of much higher percentages of molybdenum ions relative to cobalt and chromium	[31]
CoCrMo alloy	Metal rods and discs	SBF (pH 7.4)	1 h (37 °C)	Co Cr Mo	12.00 4.50 0.61	ICP-MS	Drastic increase of the dissolution rate and a high accumulation of Co in the electrolyte (0.14 M NaCl) when polarizing the sample from cathodic (simulating electrochemical depassivation) to anodic (simulating electrochemical repassivation) potentials was observed. The clinical data showed a slow increase in measured ion concentrations. Active dissolution behavior is mainly dominated by the alloying element Co	[32]
CoCrMo alloy	Metal discs	NaCl (9 g L^{-1}) PBS	10 m (37 °C)	***Corrosion test*** Co Cr Mo Co Cr Mo	 2727 992 175 3249 1268 222	ICP-MS	The total metal ion release measured *in vitro* was higher for the tribocorrosion testing conditions compared to corrosion due to the active dissolution caused by depassivation. Active dissolution behavior is mainly dominated by the alloying element Co	[33]

		PBS + Albumin		Co Cr Mo	2500 800 107			
		NaCl (9 g L^{-1}) PBS PBS + Albumin		***Tribocorrosion test*** Co Cr Mo Co Cr Mo Co Cr Mo	 672 32 68 419 19 26 510 50 45			

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; GF AAS, graphite furnace atomic absorption spectrometry; Abbreviations: SBF, simulated body fluid; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; NBS, newborn bovine serum; PBS, phosphate buffered saline; BSF, bovine synovial fluid; FBS, fetal bovine serum; m-minutes; h-hours; d-days

2.3. *In vitro* metal ions release from titanium and its alloys orthopedic implants

Titanium and its alloys: ά+ß alloys, Ti6Al4V, TiAlNb, ß-Ti alloys and NiTi alloys typically used in biomedical applications, are the most biocompatible materials as the atoms, molecules and particles have been perceived as less toxic. Titanium high inertia due to formation of a thin surface titanium oxide layer, light weight and excellent biocompatibility make it the ideal material for load-bearing applications, such as hip and knee arthroplasties (the two most common surgical operations involving implants). The problems with poor mechanical strength of Ti were overcome by the addition of 6% aluminum and 4% vanadium to commercially pure (cp-Ti) resulting in an alloy (TiAl6V4) with mechanical properties similar to those of stainless steel or Co-Cr alloys. At higher concentrations the metal ions were observed in the tissues around the implants, in urine, in serum, and in remote tissue positions. Other Ti-based alloys are (TiAl6Nb7) where V is substituted by niobium (Nb) and (TiZr15Nb4Ta4) where vanadium (V) is substituted by niobium (Nb) or tantalum (Ta) and Al is substituted by zirconium (Zr). The pure Ti and Ti-based alloys have inherent corrosion resistance attributed to the spontaneous formation of a strong passivating oxide layer. The extent of this corrosion resistance dictates their biocompability, useful lifetime and often their functional ability [35].

In this section, the focus is mainly laid on the *in vitro* investigations of the degradation behavior of titanium and titanium alloys under simulated inflammation conditions, with an emphasis on metal ions generated by metallic implants and their wear debris, during electrochemical and tribocorrosion tests.

The release of metal ions from titanium materials into pseudo-body fluid, considerable research has been conducted [36-47].

How much of these metal ions are released is a function of the corrosion resistance of the metal, the physiological conditions (pH, chloride ion concentration, temperature, *etc*.), mechanical factors (pre-existing cracks, surface abrasion and film adhesion), electrochemical effects (galvanic effects, pitting or crevices) and the dense cell concentrations around implants [36].

Mumme et al. [37] investigated *in vitro* experimental test bodies of four different standardized, frequently used, artificial implant alloys. The test materials were immersed in serum (pH 7.4). Here, they were subject to electrochemical corrosion independently of mechanical influences when in contact with body liquids. It was found that up to 80% of the entire dissolution of each ion occurred within the first 24 h of immersion. Additionally, the dissolution of the metal ions depended on the proportional mix of the individual elements in respective alloys. Therefore, the highest percentage of ion concentration was found for Co in CoCr29Mo and low percentages of ion concentration were evident for Ti (cp-Ti), Cr (CoCr29Mo) and Cr (FeCrNiMoMnNbN). The lowest percent of ion concentrations were evident for Ti (Ti), Cr (CoCr29Mo) and Cr (FeCrNiMoMnNbN).

Okazaki and Gotoh [38] compared the release of metallic elements among Ti6Al4V, vanadium-free Ti6Al7Nb and Ti15Zr4Nb4Ta alloys in pseudo-body fluids. For the Ti-based alloys, mostly Ti was released, but also significant amounts of Al and a small amount of V (for the Ti6Al4V alloy) could be detected. The amounts of Ti and Al release were similar for the two Ti-Al-(V,Nb) alloys. However, the quantity of Ti released from the Ti15Zr4Nb4Ta alloy was much smaller than the quantity released from the TiAl-(V,Nb) alloys. This may reflect the very stable passivity of the alloy containing only valve metals as alloying elements.

Wisbey et al. [39] investigated the influence of the surface oxide on the dissolution of the substrate material in saline solution. It was demonstrated that a substantial reduction in the release of metal ions may be achieved by ageing the surface oxide in boiling distilled water or by thermal oxidation; this was discussed in terms of the structure of the oxide film.

Hip replacement stems manufactured from Ti6Al4V titanium alloy were surface treated in one of four ways and tested for dissolution resistance in bovine serum [40]. Those stems treated thermally were found to have significantly lower metal ion release compared with those receiving standard commercial treatments. The improved dissolution behavior is associated with a change in the surface oxide structure from mixed titanium oxides to a more stable rutile structure. It has been reported that metallic materials with strong passive films exhibit lower quantities of metal ions released from implants. That is, the quantity of a metal released changes depending on the nature and strength of the metal-oxide bond, structure, role of alloying elements, composition and thickness of oxide films. In Al-containing alloy, a significant Al ion release was registered because of a great driving force for ion migration combined with smaller, more mobile aluminum ions. The effect of passivation and pitting corrosion on higher metal ion release was reported.

Höhn and Virtanen [41] studied Ti, Al and V ion release and calcium phosphate formation of anodized and as-received Ti6Al4V in DMEM (Dulbecco's Modified Eagle Medium) under normal (pH 7.4) and inflammatory conditions (pH 5 and presence of H_2O_2), reporting marked increase of the ion liberation from both materials at pH <5. However, the release of metals was strongly reduced with the spin-coated films. The anodized TiO_2 coating exhibited the poorest protective properties and was completely dissolved after 14 days in presence of H_2O_2. Moreover, it was found that inflammatory conditions led to a delayed formation of Ca-P compounds.

The anti-corrosion performance of plasma electrolytic oxidation (PEO) coatings was investigated by Matykina et al. [42]. This type of porous Ca- and P-containing coatings was designed to promote osseointegration. The solution was a near-neutral (pH 7.4) simulated body fluid with an addition of 100 mM H_2O_2. The release of titanium in the solution up to 8 weeks was markedly reduced by the coatings on both cp-Ti and Ti6Al4V alloy. However, for the latter, the release of aluminum and vanadium was more important for the coated samples than for the PEO coating formed on Ti6Al4V alloy initially contained *ca.* 3-4 at% of aluminum and vanadium. The excess of release of potentially harmful aluminum and vanadium elements was not desired and the optimization of the coating fabrication should be carried on in order to avoid this issue. The way the microstructure evolved with time in H_2O_2-containing electrolyte was not documented.

Passivation of Ti6Al4V alloy and cp-Ti implants was used with the intention of reducing their surface reactivity, and consequently the corrosion potential, in the highly corrosive biological milieu [43]. The authors detected trace element release from solid, mill-annealed, Ti6Al4V alloy and cp-Ti into serum-containing culture medium. Their detected significantly greater levels of Ti, Al, and V in the presence of passivated compared to nonpassivated Ti6Al4V alloy. In contrast, nitric acid passivation did not influence Ti release from mill-annealed Ti-based metals. Dissolution of oxide layer occurs during nitric acid passivation treatment resulting in a thinner, less stable oxide.

Høl et al. [44] determined the fretting corrosion of the contact areas between screws and plates made of different metals used for internal fixation of bone fractures. The plates were fixed to a bone-simulating material and subjected to tensile and compressive forces in both human serum and Hank's solution. Serum was chosen because proteins affect the corrosion resistance of titanium alloy. The outcome variables included in the analyses were weight loss, and release of Ti, Cr, Ni and Mo to the different media. Lower concentrations ranging from 3 to 21 μg were reported for plate-screw connections. The main finding was that the galvanic combination of titanium and stainless steel did not accelerate the corrosion in the plating system. Based on metal release in an *in vitro* test, it appears that combination of stainless steel and titanium palting components does not pose a clinical risk.

Kretzer et al. [45] analyzed and compared the corrosion effects to evaluate the particle and ion release of the modular neck hip implants. Five different implant designs were investigated in an experimental set-up. *In vivo* conditions were simulated and the long-term titanium release was measured. Very low concentrations of titanium release were found. No differences between the different designs in total titanium were seen. Compared to *in vivo* and other *in vitro* studies extremely low concentrations of titanium release were seen. However, the surface roughness of the taper connection seems to affect the total titanium release of the implants tested. From the authors' point of view the measured titanium concentrations are within a clinically non-critical range.

Browne and Gregson [46,47] analyzed *in vitro* the degree of metal ion dissolution from Ti6Al4V alloy hip replacement stems subjected to various mechanical and chemical surface pre-treatments. High-dissolution rates were observed for nitric acid passivated samples that had been mechanically surface treated to increase the implant surface area. Significantly lower ion release levels were observed for mechanically treated samples which had been aged in de-ionized water. This study concentrated on aluminum ion release. The standard surface mechanical pre-treatment of grit blasting results in an increase in aluminum ion release of up to 90% compared with polished titanium alloy hip stems. It was reported that Al is present throughout the oxide film, but more enriched at the oxide/liquid interface.

Cp-Ti and Ti6Al4V alloy are the most utilized types of titanium alloy for medical implants, however, there are still many unsolved questions regarding the effect of its alloying components. Although, the Ti6Al4V alloy exhibit excellent corrosion properties, the metal ions released by corrosion or wear processes may induce aseptic loosening after long-term implantation. The Ti-Nb system is particularly promising regarding release of metal ions as a fraction of the corrosion resistance of the metal alloy [9,48-50].

Table 3 *In vitro* times and metal concentration in simulated body fluids following ion release from titanium and its alloys orthopedic implants

Implant material	Implant type	Simulated body fluid analyzed	Immersion time	Metal ion(s) measured	Concentration (µg L^{-1})	Analytical technique	Obtained research results	Refs.
cp-Ti	Metal plates	L-medium 0.01% HCl	7 d (37 °C)	Ti Ti	600 1700	ICP-MS GF AAS	The quantity of Al released from the Ti6Al4V or Ti6Al7Nb alloys gradually decreased with increasing pH. The quantity of Ti released from the Ti15Zr4Nb4Ta alloy was smaller than those released from the Ti6Al4V and Ti6Al7Nb alloys in all the solutions. Therefore, Ti15Zr4Nb4Ta alloy with its low metal release is considered advantageous for long-term implants	[9]
Ti6Al4V		L-medium 0.01% HCl		Ti Al V	1000 90 30			
Ti6Al7Nb		L-medium 0.01% HCl		Ti Al Nb	800 70 10			
Ti15Zr4Nb4Ta		L-medium 0.01% HCl		Ti Zr	300 10			
cp-Ti	Metal cylindrical materials	Human serum (pH 7.4)	24,72,120,168, 720 h	Ti	5002.5	GF AAS	It was found that up to 80% of the entire dissolution of each ion occurred within the first 24 h of immersion. Additionally, the dissolution of the metal ions depended on the proportional mix of the individual elements in respective alloys	[37]
Ti6Al4V				Ti Al V	5786.5 1559.2 801.3			
FeCrNiMoMnNbN				Fe Cr Mo Ni	24964.7 88.6 802.7 874.9			
Ti6Al4V	Metal plates	1.2% cysteine	7 d (37 °C	Ti Al V	1070 90 30	ICP-MS	The quantities of Ti ions released from the titanium alloys into PBS, L-medium and fetal bovine serum were very small. The quantities of Ti ions released from the Ti15Zr4Nb4Ta alloy into fetal bovine serum	[38]

Ti6Al7Nb Ti15Zr4Nb4Ta		0.05% HCl 1.2% cysteine 0.05% HCl 1.2% cysteine 0.05% HCl		Ti Al V Ti Al Nb Ti Al Nb Ti Zr Ti Nb	1590 120 60 770 70 20 1120 90 20 260 10 380 20		and acidic solutions were approximately 30% of those of Ti ions released from the Ti6Al4V alloy. The total quantity of Zr, Nb and Ta ions released from the Ti15Zr4Nb4Ta alloy was much smaller than that of elements released from the Ti6Al4V and Ti6Al7Nb alloys	
Ti6Al4V	Metal discs	DMEM (pH 5)	1,7,14,22, 56,84 d (37.5 °C)	Ti Al V	1.6 0.8 0.15	ICP-MS	Simulated inflammatory conditions (DMEM) drastically increase release of Ti, Al and V from bare and coated Ti6Al4V samples. In all cases, the concentration ratios of the ions released from the alloy do not reflect the composition of the substrate material (Ti6Al4V: 86.4 at % Ti, 10 at % Al and 8.6 at % V)	[41]
cp-Ti Ti6Al4V	Titanium foils	SBF (pH 7.4)	1,4,8 w (37 °C)	Ti Ti Al V	500 2400 900 100	ICP-MS	All coatings reduce the long-term liberation of Ti ions into the SBF by five to six times, PEO-coatings on Ti6Al4V alloy contain Al and V ions, which are chemically lixiviated into SBF during long- and short-term exposure so that the cumulative Al and V ions release from PEO-coated Ti6Al4V alloy exceeds that from the non-coated alloy	[42]

Combination of cp-Ti and SS 316 L materials	Metal plates and screws	Human serum Hank's solution	5 d (37 °C)	Ti Ni Cr Mo Ti Ni Cr Mo	9.6 30.9 10.9 4.3 3.0 851.6 28.7 23.4	ICP-MS	Galvanic combination of titanium and stainless steel did not accelerate the corrosion in the plating system. Based on metal release in an *in vitro* test, it appears that combination of stainless steel and titanium plating components does not pose a clinical risk	[44]
Ti6Al4V	Stem-modular neck connection	Calf serum (pH 7.4)	n/a	Ti	12-44 µg	ICP-MS	Very low concentrations of titanium release were found. The measured titanium concentrations are within a clinically non-critical range	[45]
Ti6Al4V	Metal cylinders	Physiological saline solution	2,4,25 d	Al	27 µg	GF AAS	High Al ion dissolution rate have been recorded for the titanium alloy during *in vitro* abrasion testing. A ten-fold increase in aluminum ion release is observed at the lower loading condition whilst there was only a 33% increase in total particle surface area. The discrepancy may be due to the unique ion release behavior of Al in Ti6Al4V	[46]
Ti6Al4V	Femoral stems	Fetal calf serum	16 h (37 °C)	Al	60 µg	GF AAS	The standard surface mechanical pre-treatment of grit blasting results in an increase in aluminum ion release of up to 90% compared with polished titanium alloy hip stems	[47]
Ti6Al7Nb	Metal disks	SBF	From 3 h to 60 d (37 °C)	Ti Al	2 10	ICP-MS GF AAS	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	[48]

cp-Ti Ti6Al7Nb	Metal plates	Hank's solution WBS PBS Hank's solution WBS PBS Hank's solution WBS PBS	1 d and 1,2,4,8,12,20 w (37 °C)	Ti Ti Al	448 450 470 470 200 390 200 60 90	GF AAS	A variation 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	[49]
Ti40Nb4In	Metal rods	TBS (pH 7.6)	1 - 4 w (37 °C)	Ti In	mmol cm^{-2} $7x10^{-8}$ $4x10^{-7}$	ICP-OES	The release of ion indium species from the beta-type (Ti40Nb)-4In alloy in neutral and acid simulated body fluids remained below the quantification limit of the analytical method. This support the conclusion from potentiodynamic polarization test that passivation is the dominating process at the alloy surface	[50]

Analytical techniques: ICP-MS, inductively coupled plasma mass spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; GF AAS, graphite furnace atomic absorption spectrometry; Abbrevaitions: DMEM, culture medium; SBF, simulated body fluid; WBS, whole blood serum; PEO, plasma electrolytic oxidation; PBS, phosphate buffered saline; TBS, tris-buffered saline; n/a - data not available; h-hours; d-days; w-weeks

Okazaki and Gotoh [9] conducted *in vitro* metal release studies in different physiological media. The quantity of Ti released from the Ti15Zr4Nb4Ta alloy was smaller than those released from the Ti6Al4V and Ti6Al7Nb alloys in all the solutions. In particular, it was approximately 30% or smaller in 1% lactic acid, 1.2% L-cysteine and 0.01% HCl. The quantity of (Zr+Nb+Ta) released was considerably lower than that of (Al+Nb) or (Al+V) released. Therefore, Ti15Zr4Nb4Ta alloy with its low metal release is considered advantageous for long-term implants.

Ti6Al7Nb alloy samples, used for prosthetic implants, treated by innovative multi-step chemical and thermal processes were characterized in order to evaluate their surface properties and cell interaction [48]. Ion release tests in SBF were performed focusing on titanium and aluminum content. Titanium contents as high as three times respect to the usual serum values were registered in the case of patients with titanium hip prostheses. In Al-containing alloys, a significant Al ion release was registered because of a great driving force for ion migration combined with smaller, more mobile aluminum ions. Joseph et al. [49] investigated the effects of pH, chloride ions and nature of some bio-fluids on the amount of metal ions released from pure titanium (cp-Ti) and TiAl6Nb7 plates following incubation in actual and simulated bio-fluids over time. TiAl6Nb7 plate showed no releases of Ti ions were released from the cp-Ti plate from the 1 day immersion time. The release of measurable amount of Al ions from TiAl6Nb7 alloy was after 12 weeks of incubation. The rate of release of Ti and Al ions from the samples increased initially with incubation time and then stabilized due to adsorption-desorption equilibrium. The results showed that variations in pH and chloride ions of the test media has a significant effect on the amounts of Ti ions released, while increase in chloride ions concentration significantly elevates the release of Al ions into the bio-fluids.

Small indium additions to the beta-type Ti40Nb alloy are known to effectively improve its mechanical biofunctionality, and the impact on its biocompatibility was addressed in this study [50]. The release of ionic indium species from (Ti40Nb)-4In alloy is insignificant in a simulated body fluid (TBS) at neutral (7.6) and acidic (2.0) pH value levels. When in its homogeneously dissolved in the beta-phase, spontaneous surface passivation with Ti and Nb-oxides and formation of ionic complexes with In(III) ions may occur with dominance of the passivation processes. The metal ion releases from beta-type alloys were generally very low.

In vitro times and elevated total concentration of trace metals in the simulated body fluids following ion release from titanium and its alloys orthopedic implants are presented in Table 3.

3. Conclusion

The development of new metallic orthopedic implant materials is an extremely complicated and complex process that includes a number of necessary analyses confirming the quality and potential of these metallic biomaterials. Patients find themselves being exposed to metals in a variety of ways, ranging from external exposure to instruments such as medical metallic implant devices to internal exposure *via* surgical devices being implanted in their bodies. Insight into the type and concentration of the released elements, metal particles and ions, from metallic implants is very important for prediction and assessment of their local and systemic effects in the human body. *In vitro* methods are key tests used by the clinical orthopedists and commercial orthopedic laboratories and metallurgical manufacturers to generate high-quality information on the safety and efficacy of metallic orthopedic implant candidates. The purpose of this review was to describe the behavior of different metallic implant alloys in physiological and simulated physiological conditions. In addition to the clinical practice, physiological solutions are often used for *in vitro* laboratory research as a biofluids simulation medium. Different types of solutions were 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, Hank's solution, Eagle's Minimum Essential Medium (MEM) and L-MEM. Proteins, amino acids, serum, glucose and/or chloride and fluoride ions, or other substances, as appropriate, may be added as needed to these solutions. Characterization of corrosion products from orthopedic metal alloys and determining their implication for biocompatibility is a multi-faceted problem. Although *in vitro* corrosion investigations are important to value the biocompatibility of orthopedic materials the results must be regarded very carefully. Therefore, to truly understand the corrosion mechanisms at the implant/biology interface, and to fill the gap between *in vitro* and *in vivo* studies, joint efforts between medicine, biology, materials science and engineering should be aimed at. Only when compared with *in vivo* and clinical investigations the biocompatibility of a novel biomaterials that will extend the life of orthopedic implants can be valued. However, *in vivo* findings often do not correlate with *in vitro* results which may be very far from the chemical states of the *in vivo* degradation products generated from joint replacement implants. Therefore, it is important to emphasize the need for accurate studies that will determine the behavior of these new biomaterials before their clinical use and determining an approach to improve our understanding of the different biological reactions that occur immediately after implantation. Experimental models should be developed using wear debris, cells, culture media and environmental milieu which more closely simulate *in vivo* conditions. Nowadays, some metal implants have been replaced by ceramics and polymers due to their excellent biocompatibility and bio-

functionality. However, for implants which require high strength, toughness and durability, they are still made of metals. On the other side, clinical use of the promising research in using bioactive polymers and ceramics in regenerative medicine is still far away from practice. With further improvement on novel bio functionalities and revolutionary use of metal such as biodegradable implants, it is with a confidence to say that metals will continue to be used as biomaterials in the future. In the future, more holistic approaches should be adopted in evaluation of wear and corrosion of metallic orthopedic implants. More adequate simulations of activity involved in daily life, lubrications, time dependent changes to surface topography and bearing surface geometry affecting clearance are desirable. The combined experimental approaches to wear and corrosion should be coupled with elastohydrodynamic evaluation of likely bearing performance under mixed lubrication conditions.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflicts of interest associated with this manuscript.

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