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Review

The two faces of metal ions: From implants rejection to tissue repair/regeneration

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ABSTRACT

The paradigm of metallic ions as exclusive toxic agents is changing. During the last 60 years, knowledge about toxicological and immunological reactions to metal particles and ions has advanced considerably. Hip prostheses, namely metal-on-metal bearings, have prompted studies about excessive and prolonged exposure to prosthetic debris. In that context, the interactions of metal particles and ions with cells and tissues are mostly harmful, inducing immune responses that lead to osteolysis and implant failure.

However, in the last decade, new strategies to promote immunomodulation and healing have emerged based on the unique properties of metallic ions. The atom-size and charge enable ions to interact with key macromolecules (e.g. proteins, nucleic acids) that affect cellular function. Moreover, these agents are inexpensive, stable and can be integrated in biomaterials, which may open new avenues for a novel generation of medical devices.

Herein, orthopedic devices are discussed as models for adverse responses to metal ions, and debated together with the potential to use metal ions-based therapies, thus bridging the gap between unmet clinical needs and cutting-edge research. In summary, this review addresses the two "faces" of metallic ions, from pathological responses to innovative research strategies that use metal ions for regenerative medicine.

1. Introduction

The mechanical and electrical properties of metals have prompted their use in biomedical engineering, namely as implantable medical devices. Metals are present in almost all orthopedic devices but metal-on-metal (MoM) bearings raise the most concern, due to potential risk of adverse biological reactions elicited by excessive generation of metallic particles and ions [1–7]. In spite of widespread application of metallic components in joint replacement, these foster major concerns related to their safety, when tissues and body fluids are exposed to high levels of elements present in metal alloys [8–10].

On the other hand, it is well known that metal elements are vital for cells, where they play structural, catalytic and signaling roles. In fact, cells are able to interact with metal ions at non-toxic concentrations in the tissue microenvironment, but also at systemic level. Cells are sensitive to ions and their mechanisms and behavior may, at least in part, be modulated by these inorganic species [11–13]. Therefore, while metal ions are promising tools, a deeper understanding about the interactions between them and living systems is still needed, to identify the frontiers that limit their safe and therapeutic application.

In the last decade, metal ions have aroused attention, as tissue regeneration enhancers in bone tissue engineering strategies. These approaches promise to improve implant integration and tissue regeneration through metal ions release. A new generation of biomaterials aiming at a controlled degradation, and whose by-products induce healing processes are under intense investigation.

In this review, both potential adverse effects and healing properties of metal ions and particles released by biomaterials

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applied in Orthopedics, particular in hip prosthesis, will be addressed. While clinicians mainly perceive metal ions as toxic agents, scientists start to use their biological effects to improve the interaction of medical devices with surrounding tissues and cells. Thus, this review aims at brining together the two perspectives addressing the processes behind the origin of metal ions, their systemic dissemination, interaction with immune system and involvement in tissue regeneration.

2. Biological response to the degradation products of metallic hip implants

Despite advances in material design and surgical technique, a significant fraction of the high number of hip prostheses implanted every year still fail. MoM hip prostheses have raised concern among authorities and clinicians, due to the high cumulative revision rates registered at 5 years (3–6%), particularly when considering the 1.5% revision rate for metal-on-polyethylene (MoP) prostheses [1]. However, failed hip implants are an interesting model to enhance current knowledge about the biological response to the materials released by these medical devices [1]. Hip prostheses are mainly composed of metallic alloys, mostly CoCrMo (cobalt–chromium–molybdenum) and Ti_6Al_4V (titanium–aluminum–vanadium), but their bearing surface can be composed of different materials. The bearing surface refers to the superficial layer of each component at the articulation interface between the artificial femoral head and the acetabular cup [14]. The different categories of hip prosthesis are defined based on the materials that constitute their bearing surfaces. The most popular bearing surfaces used for total hip arthroplasty (THA) prostheses are MoP, MoM and ceramic-on-ceramic (CoC). Technological advances have contributed to minimize degradation of these materials. Nonetheless, wear at bearing surface is still responsible for the generation of the majority of prosthetic particles found in surrounding tissues [15]. In terms of amount, MoM bearings generate ten-fold more particles than MoP [16], and most of metallic wear particles are nanometric-sized (smaller than 100 nm) [16–21] while polymeric particles are around 100–10,000 nm [22]. Thus, metallic nanoparticles are known to be more reactive as their surface area per volume is higher than polymeric particles. As the majority of hip joint bearings are based on CoCrMo alloys, the particles released are mainly chromium oxide (Cr_2O_3) with an oval to round shape [19,21]. Also, titanium-based particles may be originated in different regions of hip implants, such as the femoral stem and/or acetabular cup [23].

Wear and corrosion are the mechanisms behind the generation of micro- and nanoparticles, as well as metallic ions. The failure of metallic prostheses is multifactorial, and likely related to adverse biological responses induced by the by-products that are released upon implantation. These agents are shed in synovial fluid, facing the synovial membrane as their first interface with the human body. Dimensions, quantity and chemical composition of the particles are important factors in assessing biological effects of wear debris, and are closely related to the biomaterial that suffers wear and corrosion [17,24,25]. Particles possessing diameters up to a few micrometers are phagocytosed by macrophages [17,26], while bigger particles can induce a foreign-body response [27]. The activation of macrophages upon phagocytosis has been pointed out as a trigger, activating the innate immune response, which may ultimately lead to implant failure, through a pathological response sometimes referred to as “particle disease” [28,29]. On the other hand, the physicochemical properties of metallic ions (eg. size and charge) allow these agents to reach different targets, such as DNA, producing toxic effects in a manner that is different from those caused by particles [30–32]. The local and systemic toxic effects and the mechanisms involved in the biological response against the most relevant metallic degradation products are discussed bellow.

2.1. Periprosthetic tissue response to MoM hip joints

The biological response behind aseptic loosening of hip implants is the cause of long-term failure for about 65% of hip prostheses [14] and depends on a set of immunological phenomena, including cell interactions and cytokine production. Despite MoM, MoP and CoC hip prostheses all potentially failing due to aseptic loosening, the molecular and cellular events underling those pathological response appear to be distinct. The immune response taking place around MoM hip prostheses is not as studied as the macrophage-mediated foreign body reaction to the polymeric wear particles released by MoP prostheses [33–35]. Metallosis, pseudotumors and aseptic lymphocytic vasculitis associated lesions (ALVAL) have all been associated to failed MoM hip implants [36–39]. The inflammatory response associated to the soft tissue lesions presented by some patients with MoM implants is not completely understood but thought to be related to the metal-rich periprosthetic environment. Thus, ARMD (Adverse Reaction to Metal Debris) [39,40] or ALTR (Adverse Local Tissue Reaction) [41] have been used as umbrella terms. In spite of ARMD being often associated to MoM hip prostheses, it occurs only in about 1% of MoM patients [39] and similar histological findings may be found in periprosthetic tissues of other types of hip implants, namely where modular metal–metal interfaces or impinged metallic components are present [42–44]. Metallic wear debris and ions may be released due to wear, corrosion or tribocorrosion processes [45]. Metallic particles are deposited in periprosthetic tissues giving them a gray color, which is usually named metallosis [46]. This microenvironment may lead to adverse local tissue responses such as pseudotumors and ALVAL as presented in detail in Fig. 1.

Pseudotumors are described as solid or cystic masses that may grow at the implant periphery. The prevalence of pseudotumors in MoM patients ranges from 20% to 61%, depending on the hip prostheses model [47,48]. Their etiology is not completely understood and pseudotumors are diagnosed in patients with a painful hip, or even asymptomatic patients [4]. Despite the fact that pseudotumors are neither malignant nor infectious they may provoke pain, due to the compression caused by these masses on other tissues (e.g. muscles or nerves) [4,49]. The continuous release of metallic particles may induce macrophage infiltration, which will later die (apoptosis) after phagocyting large amounts of toxic particles [39]. These particles will remain in tissues leading to new monocyte recruitment, chronic inflammatory response, and cytotoxicity, when their levels are high [50]. Thus, a stressful environment is created that may induce tissue destruction (necrosis), a significant feature in pseudotumors and failed MoM prostheses [50]. Moreover, the prevalence of pseudotumors was positively correlated with serum Co and Cr levels [51]. The excessive wear of MoM bearings is related with soft tissue lesion [39,40,51] but the extent of tissue destruction is not correlated with the amount of metallic particles [39]. These findings highlight the individual susceptibility of patients to metallic agents, particles and ions, which likely determines the magnitude and type of immune response. Sustained cytotoxic response, hypersensitivity to metallic degradation products, or both may be the mechanisms underlying pseudotumor formation [39,50,51].

Patients with ALVAL report pain as symptom [4], whose origin is reported as related to the swelling of the vascular endothelium, recurrent bleeding and extensive necrosis [52,53]. The extension of tissue destruction seems to be related with the thickness of lymphocytic perivascular cuff [39]. The histological features observed

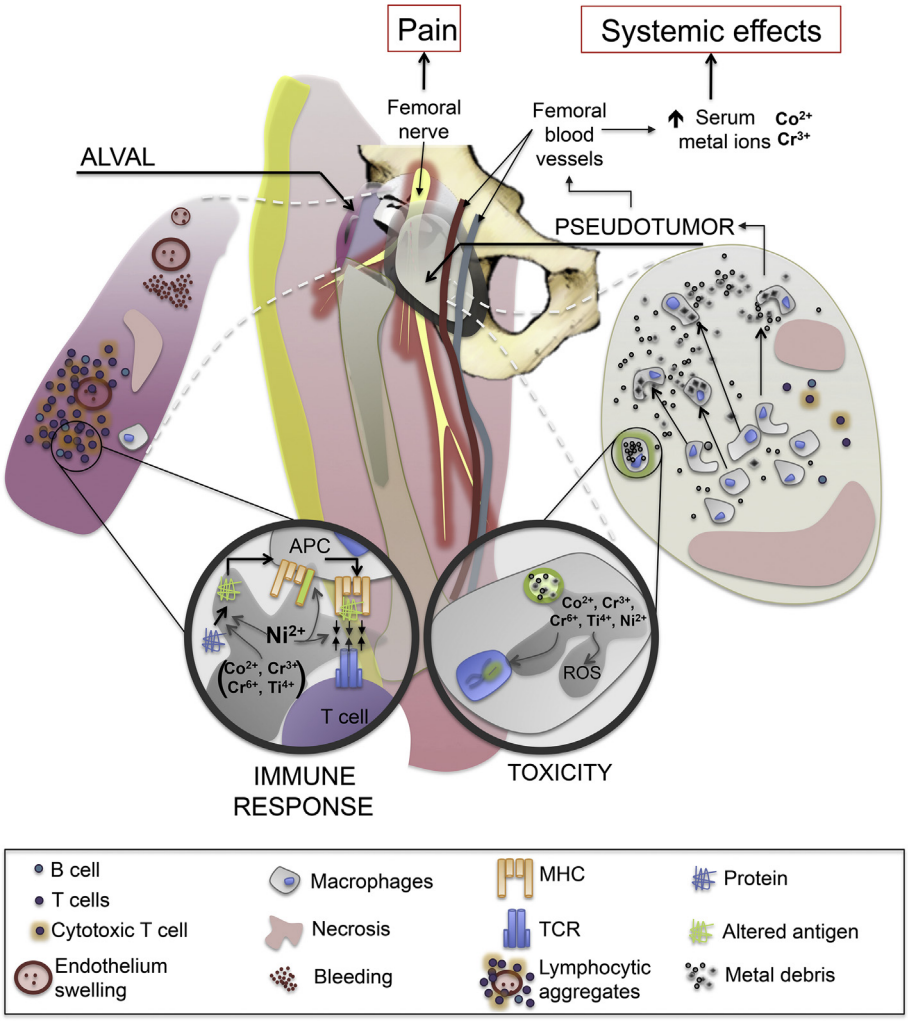

Fig. 1. Pathological responses to excessive exposure to metal particles and ions released by hip prostheses. Pseudotumors and Aseptic Lymphocyte-dominated Vasculitis-Associated Lesions (ALVAL) are the most relevant Adverse Reactions to Metal Debris (ARMD). The accumulation of high amounts of metal degradation products in hip joint space induce the formation of pseudotumors around the implant which leads to augmented serum metal ions levels. The mechanisms underlying pseudotumors formation appear to be related to the inability of macrophages to clean metal debris, despite the extensive recruitment of these immune cells induced by the prosthetic by-products. At intracellular level, high levels of metal particles and ions lead to toxic effects that ultimately result in a vicious cycle of cell death. Together with tissue necrosis, compression of femoral nerve are though to explain the pain suffered by patients with pseudotumors and ALVAL. Although ALVAL is related to metal exposure, it seems to depend more on immune system sensitivity to metal debris than on the amount of metallic particles and ions. Perivascular lymphocytic aggregates, where cytotoxic T cells (CD3+CD8+) appear to have a detrimental role, recurrent bleeding and tissue necrosis are the key events of ALVAL. In the context of delayed hypersensitivity response, several mechanisms were described regarding the influence of metal ions, namely Ni2+, on antigen T cell activation: producing immunogenic antigens by inducing conformational changes; causing structural changes in major histocompatibility molecules (MHC) on the surface of antigen presenting cells (APC) such as macrophages and dendritic cells; and boosting T cell activation via TCR-MHC interaction (superantigen).

in peri-implant tissues with a metal hypersensitivity reaction classify this immune response as ALVAL and may affect 1% of the patients with MoM bearings [4,52,54,55]. It is important to state that ALVAL and pseudotumors are not directly related and may occur independently [51,56]. ALVAL is characterized by dense perivascular lymphocytic aggregates of T cells ($CD3^+$), B cells ($CD20^+$) and some macrophages ($CD68^+$) [5,50,54,57]. Among lymphocytes, CD8+ T cells (cytotoxic) seem to be the prevalent lymphocyte population [42,50,53]. Diffuse lymphocytic infiltrations are also detected, and are composed by T cells only [50]. The potential role of metal degradation products on ALVAL is controversial. An association between the wear rate of MoM bearings and ALVAL has been successfully established [58], but doubts remain regarding the clinical correlation between blood and urine Cr and Co ions levels and the development of ALVAL [39,54]. Overall, the majority of pseudotumors seem to be related to excessive generation of metallic wear particles and macrophage infiltration, while cases of ALVAL are mostly correlated with unexplained pain or suspected hypersensitivity involving T cells [4].

Cases of metal hypersensitivity to implanted devices have been reported ever since their first use [59,60]. Presently, the immune response to metal implants is still unpredictable and results provided by metal sensitivity tests are controversial [61]. The prevalence of dermal metal hypersensitivity in healthy population is reported to be about 14% and nickel (Ni^{2+}) and cobalt (Co^{2+}) are the most described offenders [61,62]. Several reports suggest that an increasing proportion of patients, namely women, will develop hypersensitivity to metal implants, even in the absence of high wear [5,61,63,64].

An immunomodulatory role of T cells in periprosthetic tissue response has been reported, but many open questions remain regarding the mechanisms behind the interactions with metallic nanoparticles and ions [5,31,54,65–67]. The molecular mechanisms that influence the intensity of histologic features observed in MoM

surrounding tissues are yet to be revealed [5,31,54]. On the other hand, the role of each T cell subpopulation on the biological response against this type of artificial joint and the influence of particulate and ionic degradation products on these immune cells are still unknown [65–67]. The increased proportions of T cells in relation to B cells in MoM periprosthetic tissues led the scientific community to believe that a type IV cell-mediated immune response was behind the lesions observed [7]. T cells may represent 10% of cells at bone-implant interface [67,68], with the helper population ($CD3^+CD4^+$) being more frequent in periprosthetic tissues than the cytotoxic one ($CD3^+CD8^+$) [67]. T helper (Th) 1 cells seem to dominate over the Th2 population, indicating a more cell-mediated immune response, with the participation of macrophages [67,69,70]. At systemic level, Hart et al. have identified in MoM patients a positive correlation between increased cobalt ions and reduced circulating CD8+ T cells [71]. In line with these findings, decreased number of circulating CD3+, CD4+ and CD8+ cells, together with increased serum interferon-gamma (IFN-γ), indicative of a Th1 response, and concomitant decrease of interleukin 4 (IL-4) levels, related to a Th2 response, were found in patients two years after large-head MoM THA [69].

The mechanisms underlying T cell hypersensitive response are though to be related to antigen processing and presentation in the context of adaptive immunity. Conformational alterations at protein/antigens level [31,72,73], major histocompatibility complex (MHC) and superantigen effect [74] have been pointed as potential action mechanisms of metallic ions involved in hypersensitivity, namely to Ni^{2+}. In fact, Ni^{2+} is the most concerning allergen and its effects at major steps of antigen T cell activation have been reported [61,62,74,75]. Although nickel is many times considered a trace chemical element in metals used for hip prostheses, nickel content (percentage by weight) can be up to 0.5%, 0.1% and 0.05% in CoCrMo (ASTM F75), Ti alloys (ASTM F136) and pure Ti (ASTM F67), respectively.

The microenvironment resulting from corrosion of prostheses may promote protein conformational changes and metal ions released may act as haptens, contributing to the formation of metalloproteins [31,72]. Haptens are small agents that can complex with "self" proteins, inducing an immune response. Since most proteins are negatively charged at physiological pH, their potential to interact with the positively charged metal ions is very high [76]. This hapten effect reported for metallic ions appears to be ion-specific and preferential molecular targets have been described. Previous studies show that serum proteins with 68 kDa and 180–330 kDa are the most frequently modified by Cr [31]. On the other hand, it has been reported that Ti binds preferentially to transferrin [32]. Also, Ti^{4+} and Co^{2+} binding sites have been identified in albumin (approx. 68 kDa) [77,78]. While no conformational changes were associated to Ti^{4+}, it was verified that Co^{2+} binding leads to a conformational transition of albumin that exposes more binding sites for Co^{2+} ions [77,78]. Proteins with molecular weight between 180 and 250 kDa, probably immunoglobulins, are associated with greater lymphocyte reactivity [31,72]. Moreover, the association of metallic ions with plasmatic proteins may facilitate their cellular uptake [32].

The involvement of some metallic ions in antigen-driven T cell activation seems to be related to antigen presentation. It is possible that antigen presenting cells' (APCs) like macrophages and dendritic cells can internalize hapten-modified proteins, process them and present as antigens, through human leukocyte antigen molecules, such as $HLA\text{-}DR^+$ [66,75,79]. HLA-DR is a MHC class II receptor, present on the surface of APCs, together with co-stimulatory receptors CD80/CD86 and CD40, that can present antigens to T cells, activating them through their counterparts CD28, CD3 and CD40L on the surface of T cells [7]. Interestingly, a previous study has shown increased expression of CD86 and HLA-DR on peripheral blood monocytes together with a decrease of T cell markers CD3 and CD28 in patients with MoM bearings for more than 30 years [68].

2.2. Interaction of metallic particles and ions with immune cells

The findings reported at the vicinity of hip prostheses have prompted both clinical and fundamental research, aimed to clarify the interactions between metal particles (and ions) and living systems. In the following sections current evidence at research and clinical levels is discussed, focusing on cobalt chromium alloys and titanium alloys. As these families of alloys are widely applied in artificial joints, this review will address the most representative and studied prosthetic degradation products. Although when these metal alloys corrode, they release Co^{2+},Cr^{3+},Cr^{6+} and Mo^{5+} (CoCrMo) or Ti^{4+}, Al^{3+} and V^{5+} (Ti_6Al_4V) into the human body, the literature has been mostly addressing the metallic ions present in higher percentages in those alloys. Thus, toxicity and other effects of metallic particles and ions (Co^{2+},Cr^{3+},Cr^{6+} and Ti^{4+}) on the immune response and bone homeostasis will be discussed bellow, from cellular to tissue and systemic levels.

2.2.1. Cobalt and chromium alloys

2.2.1.1. Research evidence – in vitro and other pre-clinical data. CoCr particles and Co^{2+}, Cr^{3+} and Cr^{6+} ions are the main degradation products of CoCrMo alloys studied this far. Molybdenum (Mo^{5+}) is not so widely studied, likely due to its lower amount in CoCrMo alloys, 5–7% of the weight (ASTM F75), and mild toxicity in comparison to Co^{2+} [70]. Cobalt and chromium potential effects on biological systems are in part related with their capacity to be internalized by cells and bind vital molecules such as DNA. Competing for targets used to interact with other ions such as Ca^{2+} or Mg^{2+}, Co^{2+} and Cr^{3+} can change mechanisms that lead to cellular death [80]. The potential cytotoxic effects or the role of cobalt and chromium ions in immune system sensitization is described bellow. The cellular uptake of metallic ions is the first step of this interaction. Co^{2+} and Cr^{6+} -more than Cr^{3+}- can cross the cytoplasmic membrane trough a non-specific anionic transporter or via an endosomal route [76,81–83]. Inside the cell, Cr^{6+} and Co^{2+} may exert time and concentration-dependent toxicity or even carcinogenic effects, through similar mechanisms [46,81,82,84]. The reduction of these species to Cr^{3+} and Co^{3+} respectively generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) [82], which saturate the cell antioxidant mechanisms and damage cytosolic proteins, lipids or even DNA. Additionally, Cr^{3+} can cross the nuclear membrane and bind to DNA [76] while Co^{3+} accumulates in cell nucleus and surrounding structures [80].

Co^{2+} has toxic effects on macrophages at lower concentrations (8000 μg/L) than Cr^{3+} (350,000 μg/L) [85]. Higher toxic thresholds, 30,000 μg/L (equivalent to 0.5 mM) for Co^{2+} and 590,000 μg/L for Cr^{3+} (equivalent to 10 mM), were observed for primary human lymphocytes [70]. Moreover, Cr^{6+} is highly toxic, as quite low concentrations (520 μg/L) significantly impair viability and proliferation of human lymphocytes [86]. An experiment with mouse macrophages showed induction of apoptosis at 24 h of incubation with Co^{2+} or Cr^{3+} and cellular necrosis at 48 h, but at very high concentrations (6000–10,000 μg/L for Co^{2+} or 150,000–500,000 μg/L for Cr^{3+}) [87]. Necrosis has been reported on some ALVAL observations, but such high levels of Co^{2+} and Cr^{3+} were never observed in periprosthetic tissues. Metal type, concentration and time of exposure seem to be the major factors for macrophage mortality induced by metal ions [17]. Reduction of the cytotoxicity induced by CoCr particles after phagocytosis by

monocytes and fibroblasts has been observed [24]. CoCr particles that underwent phagocytosis by monocytes have also presented a reduced genotoxicity, with the surface of the particle appearing to be a critical factor in this process [24].

Nevertheless, sub-lethal levels of Co and Cr ions seem to play a role in inflammation. In mouse macrophages, Co^{2+} and Cr^{3+} induced a concentration- and time-dependent increase in tumor necrosis factor-α (TNF-α) secretion [85]. CoCrMo microparticles as well as sub-millimolar Co^{2+} levels (4000–5900 μg/L) induce primary human macrophages to produce proinflammatory cytokines, namely IL-1β, IL-6, and TNF-α, together with ROS and vascular endothelial growth factor (VEGF) [88,89]. Macrophages are reported to sense CoCrMo particles via Toll-like receptor 4 (TLR4)-MyD88 signaling pathways, promoting nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) by reducing its antagonist I-kB, which results in overexpression of IL-1β, IL-6, IL-8 and TNF-α [90,91]. In addition, CoCrMo particles and Co^{2+} ions, at concentration from 0.01 mM (590 μg/L) to 0.3 mM (17,700 μg/L), lead to an increase in hypoxia-inducible factor (HIF-1α) protein expression what in turn augments NF-kB signaling [89]. Co^{2+} ions were demonstrated to directly activate TLR-4 promoting the secretion of IL-8 and (C-X-C motif) ligand 10 (CXCL10), chemokines that are involved in the recruitment of other immune cells [92]. This may also occur in ARMD, since the mRNA levels of both cytokines were reported to be 18-fold and 10-fold higher in peri-implant tissues of metallosis cases [93]. Furthermore, Co^{2+} may act at endothelial cells level easing the migration of immune cells to the periprosthetic environment. At 1 mM, Co^{2+} directly activates endothelial cells leading to increased secretion of IL-8 and monocyte chemoattractant protein-1 (MCP-1) and also intercellular adhesion molecule 1 (ICAM-1) overexpression, which may justify the augmented lymphocyte binding and transmigration [94]. Thus, chronic inflammatory response may be supported by the proinflammatory role of Co and Cr ions.

Delayed hypersensitivity reaction is likely to occur in presence of CoCrMo particles and ions released by implants. The Th lymphocyte population is though to play a major role in this inflammatory response. T cell activation (up-regulated CD69 expression) has been reported after incubation of peripheral blood mononuclear cells (PBMCs) from patients with loosening artificial hip joint with Cr ions (36.8 μg/L) [96]. Exposure to CoCrMo alloys appears to lead to T cell sensitization, as primary lymphocytes isolated from patients with well functioning hip prostheses present increased reactivity, both frequency and magnitude, to Cr^{3+} when compared to lymphocytes from healthy donors [70]. The same study showed that among the tested metallic ions, Co^{2+}, Cr^{3+}, Ni^{2+} and Ti^{4+}, only Cr^{3+} significantly promoted the production of IFN-γ, often secreted by the Th1 subpopulation. Other molecules involved in T cells stimulation (CD80, CD86 and ICAM-1) were up-regulated after incubation with Co^{2+} (4000 μg/L) [88]. On the other hand, a single study has reported reduced CD3-induced cell proliferation after 48 h incubation with CoCr nanoparticles at concentrations from 2500 μg/L to 25,000 μg/L. Under the same testing conditions, DCs and B cells were not activated and no direct cytotoxicity to T or B cells has assigned to CoCr nanoparticles [97]. When exposed to CoCrMo particles, T cells showed overexpression of TNF-α, IL-1β, IL-13, GM-CSF and IL-10 [95]. Interestingly, up-regulation of the anti-inflammatory cytokine IL-10 is significantly higher in women than in men [95]. Overall, the effects of metal prosthetic debris on immune cells seems to be highly influenced by the individual susceptibility [70,95].

Pathological bone resorption is the major biological event underlining aseptic loosening and implant failure. The activity of bone-producer cells (osteoblasts) seems to be reduced at 48 h of exposure to low doses of Co^{2+} (10 μg/L) whereas the production of proinflammatory molecules, such as prostaglandin E2 (PGE_2), cyclooxygenase-1 (COX-1), COX-2, IL-8 and MCP-1, is up-regulated [98].

As high systemic levels of metal ions have been reported in THA patients [99], their cytotoxicity towards vital organs has also been assessed. However, the concentrations of Co or Cr generally tested *in vitro* experiments tend to be higher than the ones found in clinical setting. Furthermore, short exposure time to metallic ions, undetermined local concentrations (oral administration) and incomplete protein and ions content of culture media may limit the relationship of these results with clinical findings.

Lung, intestine, liver and kidney cell lines and primary mouse dendritic cells (DCs) exposed to Co^{2+} ions or Co nanoparticles at concentrations higher than 12,000 μg/L for 48 and 72 h. Lung cells were the most Co^{2+} sensitive cell line, presenting signs of toxicity at lower concentration (<3000 μg/L) and after 72 h of incubation. On the other hand, DCs were the most resistant to ions, and one of the most resistant to nanoparticles [30]. The viability of macrophages and fibroblasts was reduced by more than 95% after 5 days of incubation with Co^{2+} and Cr^{3+} levels higher than 300 μg/L. At lower concentration of Co^{2+} and $Cr^{3'}$ (about 50 μg/L), fibroblast viability was more affected (−50%) than that of macrophages (−20%) [17].

The systemic effects underlying the dissemination of metallic particles and ions have been addressed in animal experiments [100–103]. Differential deposition of ions seems to depend on time of tissue exposure to ions/particles, element and tissue. Despite Co accumulating more than Cr, both ions levels showed an increase in liver, kidney, spleen, lung, heart, brain, testes and serum 48 h after CoCr particles implantation in the air pouch model in mice [100]. Long-term exposure (9 months) to CoCrMo implant led to reduced animal growth and demonstrated the accumulation of Cr and Mo but not of Co in the liver, which was likely excreted in urine [101]. Moreover, differences on Co and Cr distribution and accumulation were associated to intramuscular or peritoneal implantation of a CoCrMo wire [101]. It has been reported that the accumulation of metal particles and ions leads to oxidative stress, namely in the liver, spleen and kidneys. In these tissues the expression of antioxidant enzymes was altered, which correlates with the increased ROS production and DNA damage [100,102]. The toxic effects of Co and Cr seem to persist even after their concentration at systemic level decreases [101,102]. In line with *in vitro* observations, the implantation of CoCr particles induce early immune response with increased production of IL-1β and macrophage infiltration [103].

2.2.1.2. Clinical evidence. According to Hallab et al., more case reports of hypersensitivity reactions are associated with CoCrMo alloy implants than with Ti alloy components [104]. Levels of metallic ions in serum have been used for monitoring artificial MoM joints in terms of wear generation, risk of ARMD and implant functional outcome [36,37,56,66,105–108]. In summary, increased concentrations of metallic ions in body fluids are observed following implantation of MoM implants, specially Co^{2+}, even when compared to MoP THA [99].

Serum Co and Cr levels in healthy control subjects were usually bellow 0.29 μg/L and 0.34 μg/L, respectively. Nonetheless, levels below 2 μg/L are considered normal by several authors, for well-functioning MoM implants [43,51,56,72,106,109,110]. The concentrations of both ions usually vary from one to few dozens of μg per liter, in all tested human fluids (serum, blood, whole blood, urine) [39,51,99,111]. Clinically, the average serum Co and Cr levels after MoM prostheses implantation are often reported to be with in the range of 1–7 μg/L but failing MoM hip implants, some pseudotumors cases [51], and extensive corrosion on the modular MoP stem-neck interface [43] often present concentrations in the order of hundreds of μg/L [36–38,51,56,66,105–108,110]. There is no

international consensus on toxic threshold limits for metallic ions concentrations found in the blood and urine of patients with hip prostheses, namely with large head MoM bearing (≥36 mm). Moreover, it remains unclear the true meaning of these levels. US Food and Drug Administration (FDA) has not defined Co or Cr threshold values for MoM management, due to the lack of scientific evidence linking serum or whole blood metal ions levels to excessive wear or failing of MoM hip implants. Nonetheless, in Europe is recommended that MoM patients presenting serum concentration of Co from 2 to 7 μg/L should be followed-up by imaging techniques to evaluate ARMD [109]. Serum Co or Cr levels above 7 μg/L may be a sign of ARMD and should be considered in discriminating well-functioning from failed MoM hip prostheses [112]. Revision surgery should be performed if abnormal radiological findings are observed, metal ions levels are steadily rising or serum concentration of Co surpasses 20 μg/L [109,113]. The levels of Co ions in serum were demonstrated to reduce to normal levels (10.96 μg/L vs 1.61 μg/L) after removal of their source [110].

Despite the fact that metallic ions reach the circulatory system, the majority of the particles remain entrapped in nearby tissues. Joint capsule is the tissue where higher Co, Cr and Ti concentrations have been found, ranging in the thousands of μg/L, what constitutes a 100–1000-fold increase after hip replacement [43,72]. This is an expectable finding since synovial fluid and membrane, capsule and bone constitute the closest biological environment to the origin of the metallic debris. Thus, local toxicity may occur, inducing a local biological response that provokes swelling in the hip region and impairs the functioning of the implant. The mechanisms underlying the accumulation of metallic particles and ions in the tissues and their potential role in regeneration remains vastly unknown.

The adverse effects of metallic particles and ions at systemic level are a major concern, namely in the long-term. Numerous small blood and lymphatic vessels are present in synovial membranes [114], allowing particles and metallic ions to travel to distant parts of the body. Lymphatic vessels transport molecules and ions from the synovial fluid and allow the trafficking of immune cells such as macrophages between the synovial membrane and the lymph nodes or other secondary lymphoid tissues, such as spleen. The capacity of macrophages, also present in synovial fluid, to phagocyte small particles may be responsible for particle deposition in lymph nodes, bone marrow, liver, lungs and spleen [115–117]. Lymph nodes and spleen are involved in the filtering of lymphatic fluid and blood, respectively. Thus, it is expectable that higher levels of metallic particles are found in those tissues in a free form, inside macrophages or in organometallic complexes [115,117,118]. Para-aortic lymph nodes are the most affected by metallic particles deposition and in severe cases tissue necrosis has been reported [115,116]. The fate of wear particles in other organs such as liver, spleen or heart is unknown. Probably, they remain trapped and continue to be corroded intracellularly, due to the corrosive environment of the phago-lysosome, potentially inducing higher local levels of metal ions, even after implant removal [72,119]. However, environmental sources can also be responsible for increased levels of Co, Cr and Ti species in the body [115].

One of the most worrying aspects of long-term exposure to increased levels of metallic particles and metallic ions is cancer risk. Overall, the incidence of prostate cancer and melanoma seems to be higher among THA patients [120,121], while lung cancer incidence is decreased compared to general population [120]. The period of ten years following THA appears to register an increased risk of cancer, namely kidney, likely related to the run-in wear period, where the amount of metallic debris is often very high, challenging renal clearance capacity. Augmented risk of chronic renal disease was observed in a 9-year follow-up of MoM patients [122]. In the period between 10 and 20 postoperative years, the general cancer incidence seems not to be increased in MoM or MoP THA patients [120]. Nonetheless, there is not enough evidence associating metallic debris and increased cancer incidence, so there is a need for longer follow-up periods (more than 20 years) and further epidemiological studies.

Significant increases in mortality due to cardiovascular diseases were verified in patients with MoP or MoM bearings, 20 years after implantation [123]. A case report of fatal cardiomyopathy secondary to severe wear of CoCr alloy femoral head was previously described [124]. The patient presented very high Co levels in the blood (6521 μg/L) and in the heart (3.85 μg/L), when compared to Co concentration in heart tissue of healthy individuals, which ranges from 0.1 to 0.4 μg/L. Cobalt toxicity at mitochondrial level with subsequent fibrosis of cardiac tissue is the most plausible cause of death.

Since artificial hip joints such as MoM bearings are being applied in younger patients, new problems may arise. For example, the possibility of transplacental transfer of metal ions during pregnancy was evaluated. Increased levels of Co^{2+} and Cr^{3+} have been detected in the umbilical cord of pregnant women with MoM bearings, when compared to controls [107]. However, these metal ion concentrations have been lower than 1 μg/L, with the placenta appearing to play a modulatory role.

2.2.2. Titanium and titanium alloys

2.2.2.1. Research evidence – in vitro and other pre-clinical data. Titanium particles and ions are considered less toxic than those derived from cobalt [125]. However, Ti ions are prone to bind and induce damage to phosphorus-rich molecules (e.g. RNA, DNA and phospholipids) [126,127], leading to aneuploidy events *in vitro* and *in vivo* [46]. Although other elements present in titanium alloys are highly toxic (vanadium) or known allergen (aluminum), few studies addressing them in the context of implant/prostheses are available.

Particulate and ionic Ti debris are reported to influence the immune system. In macrophages, Ti ions appear to increase the production of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) with concomitant downregulation of TGF-β expression [128]. Ti ions activate T cells, leading to increased expression of CD69, CCR4 and RANK-L in a concentration-dependent manner [126,127]. Moreover, the ability of Ti ions to promote T cell proliferation seems to be affected by pre-existing inflammatory conditions, since rheumatoid arthritis patients present augmented reactivity against Ti ions [129]. Recent *in vitro* evidence indicates that Ti^{4+} ions promote adaptive immune responses, the authors show that human dendritic cells (DCs) exposed to Ti^{4+} are able to select T cells specific for Ti^{4+}, that are not reactive against Ti^{3+} ions [79]. Moreover, Ti^{4+} ions influence cytokine and surface receptors prolife on DCs, towards a Th1 mediated-inflammatory response [126]. Ti-induced delayed hypersensitivity may occur due to chronic exposure to Ti ions or TiO_2 particles, but it seems that Ti allergy is confined to a reduced number of patients [129,130] even considering that TiO_2 particles are widely used in food, cosmetics and medicines. Moreover, a proteomic evaluation of draining lymph nodes of skin challenged with TiO_2 nanoparticles found that the expression of proteins related to mRNA processing, immune response and antimicrobial activity, and lipid metabolism was significantly decreased [131]. Many details related to metal sensitivity are still unclear, since the individual contribution of each element that constitutes metal alloys is not fully known and the role of the host susceptibility to allergic response is only partially understood [61].

Similarly to other types of particles, Ti microparticles may be phagocytosed by macrophages. This process is reported to involve scavenger receptors [132], it affects enzymes that manage intracellular ROS level [133] and leads to changes on the surface

chemistry of Ti particles, inactivating them [134]. In a study comparing human primary macrophage response to different types of prosthetic debris, Ti_6Al_4V, CoCr, alumina and PE microparticles, Ti was found to elicit higher expression of proinflammatory cytokines [135]. While CoCr particles induced increased secretion of TNF-α, IL-6 and IL-8, exposure to Ti_6Al_4V led to higher concentration of those cytokines and also IL-1β, IL-1a, MCP-1, granulocyte macrophage colony-stimulating factor (GM-CSF), but also IL-10 [135]. Concentration of Ti particles is critical as IL-1β production was found to be concentration-dependent, in both human and mouse macrophages [136]. Moreover, the interaction between Ti microparticles and macrophages involves the cellular receptors TLR-2,3,4 and 9, and the intracellular adaptors MyD88, TIR-domain-containing adapter-inducing interferon-β (TRIF) and NF-kB, whose involvement in particle-induced inflammation has been reported [137]. It is known that activation of NF-kB increases secretion of proinflammatory cytokines, such as TNF-α, IL1-β and IL-6 [137–139].

Some *in vitro* studies have addressed the effect of Ti microparticles on bone remodeling. Osteoblasts incubated with very high concentrations of Ti microparticles (30,000 μg/L) for 48 h led to increased production of osteoclastogenesis factors, such as receptor activator of nuclear factor kappa-B ligand (RANKL) and CSF-1 [140]. Additionally, bone marrow stromal/stem cells (BMSCs) exposed to Ti microparticles presented impaired osteogenic differentiation and increased production of IL-8 [141], a chemokine that is involved in the recruitment of neutrophils and is overexpressed in peri-prosthetic tissues of aseptic loosening patients [142].

Although Ti ions may diffuse systemically, most of the studies evaluate the local biological response to Ti particles. This fact may be related with Ti alloys susceptibility to degradation by wear, generating particles, accompanied by a high resistance to corrosion [143]. The distribution and accumulation of TiO_2 microparticles, TiO_2 nanoparticles and Ti^{4+} ions *in vivo* shows distinct patterns. Ti microparticles accumulated mostly in the lungs, while TiO_2 nanoparticles and Ti^{4+} ions diffused systemically and are found in several different organs, namely spleen, kidneys, lungs, liver, heart, blood and brain [144,145]. TiO_2 nanoparticles are small enough to overcome the blood–brain barrier (BBB) but further studies are required to clarify the neurotoxicity reported by some *in vitro* and *in vivo* studies [145]. Although Ti exhibits thrombogenic properties [146] cases of orthopedic patients reporting altered clothing due to exposure to Ti were not found. This feature seems to be more concerning for the performance of other medical devices, such as Ti-based heart valves [147].

Animal experiments have recapitulated the Ti particle-induced immune response and associated osteolysis. Intraperitoneal injection of Ti microparticles in mouse caused at short-term acute inflammation with signs of neutrophil recruitment, IL-1β production and activation of NALP3 inflammasome [103,136]. Fibrous tissue formation and bone resorption were also observed one week after Ti microparticles having been injected [148,149]. Osteolysis was also found using the murine calvaria bone defect model [137,150]. In this context, decreased bone density and volume with increased RANKL/osteopontegrin (OPG) ratio, number of osteoclasts, and also augmented TNF-α and IL-1β were locally found, in presence of Ti microparticles [149,150]. Interestingly, epithelial cells upon recognizing bacteria via TLR4 receptor in the presence of Ti ions, were reported to increase the RANKL/OPG ratio and (C–C motif) ligand 2 (CCL2) production, which highlights the potential modulatory effect of Ti ions during host–pathogen interactions and provides an explanation for increased osteolysis in the case of bacterial infection [151]. Another study has found increased serum IFN-γ levels in rats two weeks after implantation of Ti plates in muscle tissue, which is positively correlated with peri-implant macrophages [152].

2.2.2.2. Clinical evidence. Uncemented acetabular and femoral stem components are often made of Ti alloys. Although these components do not suffer much wear as materials applied on head-cup interface, Ti alloys at particular micromovements, like the interface with bone or other metallic prosthetic parts, suffer corrosion releasing metallic ions to surrounding tissues [153]. Soft tissues surrounding failed Ti_6Al_4V femoral stem presented high concentrations of Ti and Al ions, when compared to the levels of Co and Cr ions found in patients with both cemented and uncemented CoCrMo alloy femoral stems [153]. Locally, the accumulation of Ti debris may also occur in the synovial capsule (39,400 μg/g) as well as in synovial fluid (556 μg/L) together with Al (654 μg/L) and V (62 μg/L) [72]. Ti is more soluble than V but less than Al, so its concentrations in serum and synovial fluid are intermediate [154,155]. Serum Ti ions concentrations are from the same order of magnitude as those of Co and Cr ions and may achieve less than 4 μg/L for well-functioning hip prostheses and 8 μg/L for failed implants [37,115]. Moreover, the concentration of Ti in urine seems to significantly increase after MoM and MoP hip replacement [156]. Nevertheless, in the clinical setting, hip implants management is not based on Ti levels.

3. Therapeutic approaches using metallic ions

In the previous section, pathological responses that may be induced by the uncontrolled exposure to degradation products of CoCrMo and Ti-based alloys were discussed. In fact, metallic materials reviewed above were designed to be bionert, circumventing immune response and capsule formation. Avoiding wear and corrosion has been a major concern in the development of metallic materials for orthopedic applications [143]. Research and industry have been improving and creating new solutions with reduced generation of prosthetic debris, to provide long-term bone fixation and proper load transfer. Highly cross-linked PE [157] incorporating antioxidant agents (e.g. vitamin E) [158,159], Ti fiber-mesh cups [160], tantalum acetabular components [161], oxidized zirconia femoral heads [162] and metal-carbon fiber composite femoral stems [163] are the most promising technological advances in joint replacement.

More recent works have nonetheless been reflecting a paradigm shift, from “fighting” to “modulating” the immune response to biomaterials [164]. Similarly, the prevalent idea that ions released from metal-based implants induce negative effects on biological systems is also changing. Depending on the element, duration of exposure and tissue microenvironment, ions can modulate tissue response promoting regeneration or, conversely, leading to cell toxicity, oxidative stress and death. New strategies using metal ions are being developed focusing on different medical fields. The toxicity of some metal ions at low concentrations has been explored for application in Oncology. A bioglass incorporating vanadium was engineered to release this metal ion in a controlled fashion, to induce apoptosis of cancer cells [166]. Additionally, metal corrosion processes may be tuned to control tumor growth or infection, using systems such as galvanically coupled Mg–Ti particles [167]. Metal ions can be applied to improve the bioselectivity of surfaces used in medical devices, promoting tissue repair in bacterial-challenged environments [168,169]. The interaction between metal ions and proteins may generate metalloproteins such as organic vanadium compounds. In this form, the toxicity of inorganic vanadium is diminished, allowing the vanadium insulin-mimetic effect to improve bone fracture healing, by accelerating angiogenesis and chondrogenesis [170]. Besides vanadium, aluminum is another metal element present in Ti_6Al_4V alloy that has been applied in

vaccine development. Al is currently applied as a vaccine adjuvant, due to its capacity to induce a humoral response involving Th2 lymphocytes, boosting the production of antibodies (mainly IgG1) [171]. Alternatively and as described above, the metal hypersensitivity response to CoCrMo alloys is closer to a Th1 profile, which has prompted the application of cobalt nanoparticles as alternative adjuvants, when IgG2c antibodies (Th1) are also required [172].

Amongst the wide potential applications of metallic ions, tissue engineering is certainly a promising field, where metals ions may be generated by the biomaterial used or added as a supplement. There is an increasing number of studies reporting successful therapies based on metallic ions, highlighting the role of these ionic agents as new tools for tissue engineering [165]. Depending on the application, the template/biomaterial may be designed to be durable or biodegradable. In the context of joints, application of complete biodegradable biomaterials is not yet feasible as the current technologies are not able to promote regeneration of extensive defects of bone and cartilage tissues. However, it is tempting to speculate that the pro-regenerative effects of some metal ions may be used at the interface of metal implants and surrounding tissues [173]. The trend in tissue engineering seems to be towards biodegradable materials that may incorporate growth factors, drugs or cells. On the other hand, the fabrication of biodegradable biomaterials is still a hot topic since there is no effective solution to avoid the foreign body response against implants often registered at long-term. The development of "smart" biomaterials that are able to degrade at controlled rates for *synchronized* tissue regeneration is at an early stage. Similarly, in Cardiology, the durable metallic stents are being substituted by biodegradable drug-eluting stents, aiming to reduce the risk of stent thrombosis and to restore vascular physiology [174].

The application of metal ions in the development of orthopedic biomaterials is related to their positive effect in angiogenesis and osteogenesis. A new generation of biomaterials aiming at controlled release of metal ions to induce bone healing has been developed, as summarized in Fig. 2 [12,13,175,176]. Embedding ions (e.g. magnesium, strontium, cobalt, calcium, among others) into ceramic or metallic matrices has been a concept underlying new regenerative strategies. In the next section, the application of magnesium and cobalt-based strategies for cartilage and bone repair will be discussed as both ions are released by orthopedic implants already available in clinical practice. Moreover, the storylines of magnesium and cobalt can be used to exemplify the importance of the balance between the elicited immune response and the effect of a biomaterial on tissue regeneration/repair. While magnesium is safe, may have a regulatory effect on immune cells and promote bone tissue healing, cobalt is still perceived as a toxic agent and its angiogenic potential trough hypoxia-mimetic effect is thus far limited by the immune system.

3.1. Magnesium

Magnesium and magnesium alloys have been tested for biomedical applications, namely in Cardiology, as biodegradable cardiovascular stents [177], or in Orthopedics as screws, fixation devices and bone filler materials [178,179]. Biodegradable magnesium-based metal implants are groundbreaking, providing a temporary solution that can fill the gap between the corrosion-resistant metallic devices with long-term problems (e.g. infections or hypersensitivity response) and the polymeric biodegradable materials with poor biomechanical performance. The biodegradability of magnesium and magnesium alloys resides on their corrosion in physiological environment, releasing mostly Mg^{2+} and H_2. However, controlling the degradation rate of magnesium alloys has been challenging, with implants degrading faster than tissue is able to repair, or releasing high amounts of H_2 that remains entrapped in periprosthetic tissues destabilizing bone-implant interface [179]. Thus, several magnesium alloys have been developed, to overcome the described drawbacks, these can be grouped in three families [180]: 1-pure magnesium with traces of other elements, 2-Al-containing alloys (e.g. AZ91, AZ31 or LAE442) and 3-Al-free alloys (e.g. WE43, WZ21 and Mg–Ca alloy) [178,179,181–183]. Combining magnesium with other elements such as aluminum (A), zinc (Z), manganese (M), yttrium (W), calcium (Ca), strontium (Sr) and rare earth elements (E), leads to improved mechanical properties and corrosion behavior. Nonetheless, envisaging orthopedic applications further optimization is still required [184]. Moreover, research has been improving the biological response to pure Mg and Mg alloys creating hybrid biomaterials combining this metal with polymers [185], ceramics [13], ECM proteins and cells [183].

Once implanted in animal models, pure Mg and Mg alloys promoted higher bone formation and complete fracture healing, overcoming the limited mechanical properties of polymeric biodegradable models (eg. polylactic acid) [178,179,183,186–191]. Moreover, Mg-based screws supported improved reconstruction of rabbit anterior cruciate ligament, with fibrocartilaginous tissue regeneration, when compared to Ti screws [191]. These findings prompted a controlled clinical pilot study where MAGNEZIX® screws, a Mg alloy similar to WE43, were successfully implanted in 26 patients that underwent orthopedic surgery [181]. Mg alloys biodegradability and mechanical properties similar to bone are believed to be the major factors underlying their success [192]. However, the ionic magnesium (Mg^{2+}), released upon corrosion of magnesium-based implants, may have a critical role in the induced biological response. Indeed, Mg^{2+} is crucial for health and life and is involved in stabilizing key biological molecules (e.g. ATP, DNA), acting as a co-factor of enzymes, in cell signaling, and cell migration, among other processes that guarantee the proper function of tissues such as muscles, heart or nervous system [193–195]. Although not in Orthopedics, Mg^{2+} is already applied in clinics through administration of magnesium sulfate ($MgSO_4$) for neuroprotection [196], muscle relaxing and treatment of pre-eclampsia [197].

Therapeutic concentrations of Mg^{2+} have been tested in bone and cartilage tissue engineering [198,199]. Exposing human BMSCs to milimolar levels of Mg^{2+}, from Mg-containing salts, pure Mg or Mg alloys extracts, increased cell proliferation and osteogenic-guided differentiation [12,191,199,200]. After 3 days in Mg^{2+}-enriched environment, molecules involved in osteogenesis such as TGFβ-1, BMP-2, SMAD4, VEGF and collagen I were found increased, while condrogenesis (SOX-9) and pluripotency (SOX-2) markers were downregulated [12]. Interestingly, after 14 days, another study has also reported the overexpression of VEGF, BMP-2 and COL2A1 but accompanied by an up-regulation of SOX-9, which was likely related to the regeneration of fibrocartilaginous tissue observed *in vivo* [191]. In this context, the most described cellular pathways affected by Mg^{2+} are TGF-β1/SMAD4, HIF-2a and PGC-1a but the effect of Mg^{2+} on osteogenesis depends on the differentiation status of human BMSCs [12,199]. In fact, the cellular demand for Mg^{2+} increases during osteogenesis, at reported for rat BMSCs overexpressing Mg^{2+} selective transporter (MagT1), whose knockdown led to decreased osteogenic differentiation [201]. Additionally, the deposition of bone matrix elements, calcium and collagen type X, were augmented in human BMSCs exposed to Mg^{2+} concentrations of 5 and 10 mM [199]. Despite concentrations of Mg^{2+} up to 15 mM improving osteoclast function [202], animal studies testing Mg alloys for bone repair indicate that Mg balances bone remodeling towards repair instead of osteolysis. Moreover, Mg^{2+} accelerate the regeneration of cartilage [198]. The underlying

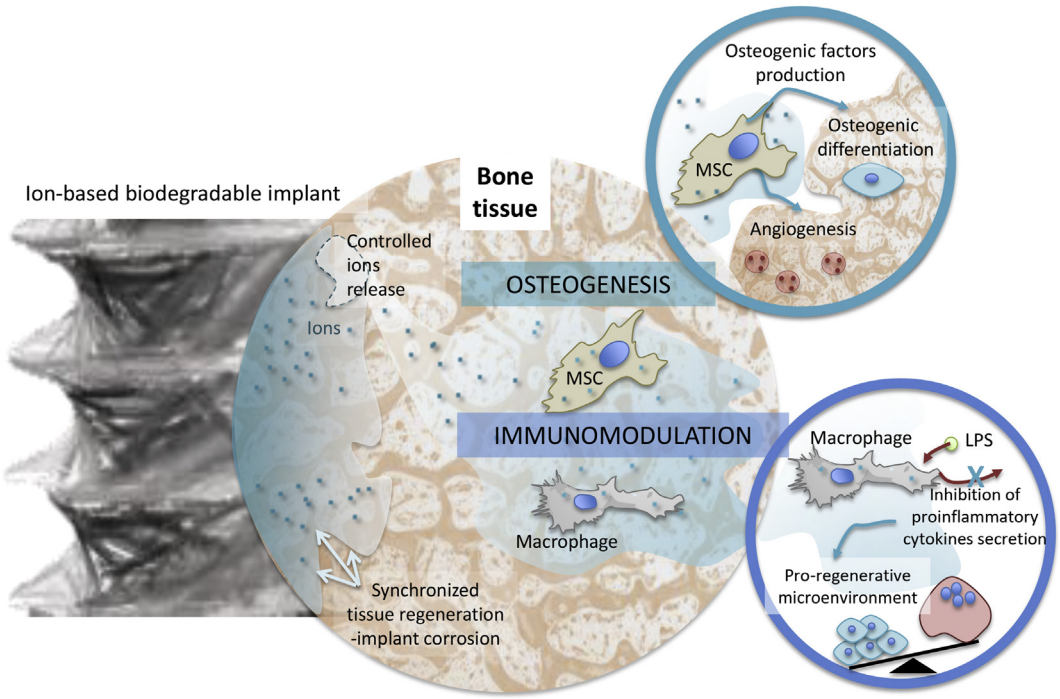

Fig. 2. Application of biodegradable materials for controlled release of metal ions in regenerative medicine. In bone tissue context, ions are able to promote osteogenesis coupled with a pro-regenerative immune response. The ion-enriched environment may induce osteogenic differentiation of mesenchymal stem/stromal cells (MSCs) as well as improve angiogenesis. Moreover, metal ions may modulate the response of immune cells to proinflammatory stimuli such as lipopolysaccharide (LPS), modifying the expression of cytokines such as TNF-α, IL-1β, IL-6 and PEG2, which ultimately affect the regenerative capacity of the tissues.

mechanism is though to be linked to integrins, ion-sensitive proteins that mediate the cellular adhesion to extracellular matrix [198,203]. However, Mg^{2+} concentrations above 20 mM negatively affect cartilage mechanical properties by reducing the production of collagen and glycosaminoglycans [204].

In addition to the pro-regenerative stimuli on BMSCs, Mg-based strategies can modulate the immune response to support tissue healing (Fig. 2). Thus, it is vital to understand the role of Mg^{2+} on cells, namely immune cells, where most of the knowledge stems from studies involving Mg-deficient models [205–207]. In hypomagnesemia conditions, cells suffer oxidative stress and the ability of the immune system to be challenged (e.g. infection) is reduced, leading to ineffective or uncontrolled responses [11,205,207]. On the other hand, Mg^{2+} supplementation was demonstrated to have an immunomodulatory role [11,208,209]. The production of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and PEG_2 after immune cells stimulation with lipopolysaccharide (LPS) was demonstrated to be reduced in the presence of increased concentrations of Mg^{2+} [208–210]. In an osteoarthritis rat model, injections of $MgSO_4$ reduced cartilage degeneration, nociception and synovial inflammation, while increasing chondrocyte survival [211]. The action of Mg^{2+} is believed to be NF-kB-dependent, a central regulator underlying proinflammatory cytokine production [209].

3.2. Cobalt

Most of the studies so far address Co as a toxic agent. Environmental sources (e.g. mining activity) or failed Co-containing biomaterials provide cases of massive human exposure to this element, which may lead to allergic reactions, tissue destruction and ultimately death [212,213]. Nevertheless, sub-toxic concentrations of Co may promote angiogenesis, which may contribute for tissue regeneration.

Novel strategies for bone tissue engineering have been investigated, taking advantage of the hypoxic-mimicking conditions generated by Co [214–216]. Biologically, Co signals cells for low O_2 pressure by stabilizing HIF-1α, which induces the secretion of factors that support cell survival in hypoxia environment and promote the formation of new blood vessels.

A possible strategy consists in pre-conditioning cells, namely stem cells using high doses of Co [217,218]. Enhanced vascularized bone formation was achieved through implantation of constructs with human BMSCs pre-treated with media containing 5900 μg/L of Co (equivalent to 0.1 mM Co) in rat bone defects [217]. Alternatively, Co-containing ceramics and bioglasses have been developed aiming to mimic hypoxia through Co action [219–222]. Cobalt was shown to promote angiogenesis by increasing the production of VEGF and enhancing the osteogenic differentiation of human BMSCs [219,221–223], which is in line with the, tube-like structures formed *in vitro* by endothelial cells (HUVECs) when treated with Co [219,221]. A recent work presented a polymeric core–shell scaffold with sustain release of Co^{2+} and BMP-2 that successfully promoted early angiogenesis and improved bone formation in rat calvaria defect model [216].

Notwithstanding, cobalt may be a double-edge sword. The history of Co as pro-regenerative agent may be spoiled by the immune system response. Immune cells are sensitive to Co exposure, macrophage activation and ROS production was found to take place at Co concentration used for cell pre-conditioning (0.1 mM) [92]. Exposing human BMSCs to conditioned media from macrophages treated with extracts from Co-containing ceramic led to decreased osteogenesis while osteogenic differentiation was observed when these cells were directly treated with the extracts [223]. Of note, the most part of the promising results using cobalt-inspired strategies have been obtained *in vitro* or using the immunocompromised animal model SCID mice [217], which could imply an immune system-related impairment of the regenerative potential of Co.

However, a very recent study reports pro-regenerative results obtained using immunocompetent animals and implanting core–-shell scaffolds that release small amounts of Co^{2+} (maximum of 14 μM at 6 days) [216].

In summary, the success of Co^{2+} for regenerative purposes seems to be highly dependent on the doses used. The current solutions using Co may lead to high concentrations of Co at the periphery of implants, such as levels of 20,000 μg/L that were reported *in vitro* at 7 days, decreasing then to 1000 μg/L [221]. The frontiers for therapeutic, immunological and toxic Co concentrations are still unclear. The role of Co in immune response associated to bone repair/regeneration, the identification of pro-healing Co levels and the development of biomaterials that ensure desirable Co levels and timing should be further investigated.

4. Conclusion and future perspectives

Until recent years, the development and application of metal alloys in the clinical setting have been driven by wear and corrosion proof-of-concepts, due to the risk of implant failure induced by the release of particles and ions. New materials, implant designs, and surgical techniques have been allowing the implantation of prostheses in younger and more active patients. Therefore, it is desirable that implanted biomaterials do not cause harmful reactions in the human body but instead promote regenerative microenvironments and restore tissue function.

MoM hip joints constitute a good clinical model and together with laboratorial studies shed light on cellular mechanisms underlying metal ions toxicity and immune response in presence of high levels of metal particles and ions. Although the need to control the amount of metal debris produced by MoM hip joints persists, this type of implant is effective since applied in specific patient populations. Nevertheless, future research should address the long-term effects of these metal degradation products, the impact of the individual susceptibility to metal-induced immune responses, and the development of therapies to control particle-induced immune response should be extended to the lifetime of prostheses.

With the advent of tissue engineering, novel solutions may be envisaged, but they still remain at the research phase. By rational design, these biomaterials may create ion-enriched environments that can, alone or in combination with drugs/molecules, promote regeneration and improve implant-tissue interfaces, and thereby implant survival. Metal-ion-based therapies are economically feasible, presenting lower costs and requirements (e.g. stability/storage conditions) than drugs. However, fundamental questions remain about the synchronized degradation of this class of biomaterials and the biological effects of all its elements.

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References

[1] A.J. Smith, P. Dieppe, K. Vernon, M. Porter, A.W. Blom, National Joint Registry of E, et al., Failure rates of stemmed metal-on-metal hip replacements: analysis of data from the National Joint Registry of England and Wales, Lancet 379 (2012) 1199–1204.
[2] M.S. Caicedo, R. Desai, K. McAllister, A. Reddy, J.J. Jacobs, N.J. Hallab, Soluble and particulate Co-Cr-Mo alloy implant metals activate the inflammasome danger signaling pathway in human macrophages: a novel mechanism for implant debris reactivity, J. Orthop. Res. 27 (2009) 847–854.
[3] P. Korovessis, G. Petsinis, M. Repanti, T. Repantis, Metallosis after contemporary metal-on-metal total hip arthroplasty. Five to nine-year follow-up, J. Bone Jt. Surg. Am. 88 (2006) 1183–1191.
[4] P. Campbell, E. Ebramzadeh, S. Nelson, K. Takamura, K. De Smet, H.C. Amstutz, Histological features of pseudotumor-like tissues from metal-on-metal hips, Clin. Orthop. Relat. Res. 468 (2010) 2321–2327.
[5] H.G. Willert, G.H. Buchhorn, A. Fayyazi, R. Flury, M. Windler, G. Koster, et al., Metal-on-metal bearings and hypersensitivity in patients with artificial hip joints. A clinical and histomorphological study, J. Bone Jt. Surg. Am. 87 (2005) 28–36.
[6] Y.T. Konttinen, J. Pajarinen, Adverse reactions to metal-on-metal implants, Nat. Rev. Rheumatol. 9 (2013) 5–6.
[7] P.A. Revell, The combined role of wear particles, macrophages and lymphocytes in the loosening of total joint prostheses, J. R. Soc. Interface/R. Soc. 5 (2008) 1263–1278.
[8] R.F. Coleman, J. Herrington, J.T. Scales, Concentration of wear products in hair, blood, and urine after total hip replacement, Br. Med. J. 1 (1973) 527–529.
[9] M.T. Clarke, P.T. Lee, A. Arora, R.N. Villar, Levels of metal ions after small- and large-diameter metal-on-metal hip arthroplasty, J. Bone Jt. Surg. Br. 85 (2003) 913–917.
[10] A.J. Smith, P. Dieppe, M. Porter, A.W. Blom, National Joint Registry of E, Wales, Risk of cancer in first seven years after metal-on-metal hip replacement compared with other bearings and general population: linkage study between the National Joint Registry of England and Wales and hospital episode statistics, BMJ 344 (2012) e2383.
[11] B. Chaigne-Delalande, F.Y. Li, G.M. O'Connor, M.J. Lukacs, P. Jiang, L. Zheng, et al., Mg2+ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D, Science 341 (2013) 186–191.
[12] R.W. Li, N.T. Kirkland, J. Truong, J. Wang, P.N. Smith, N. Birbilis, et al., The influence of biodegradable magnesium alloys on the osteogenic differentiation of human mesenchymal stem cells, J. Biomed. Mater. Res. Part A 102 (2014) 4346–4357.
[13] Z. Chen, X. Mao, L. Tan, T. Friis, C. Wu, R. Crawford, et al., Osteoimmunomodulatory properties of magnesium scaffolds coated with beta-tricalcium phosphate, Biomaterials 35 (2014) 8553–8565.
[14] S. Graves, National Joint Replacement Registry Annual Report 2011, Australian Orthopaedic Association, 2011.
[15] M.T. Mathew, M.J. Runa, M. Laurent, J.J. Jacobs, L.A. Rocha, M.A. Wimmer, Tribocorrosion behavior of CoCrMo alloy for hip prosthesis as a function of loads: a comparison between two testing systems, Wear 271 (2011) 1210–1219.
[16] P.F. Doorn, P.A. Campbell, J. Worrall, P.D. Benya, H.A. McKellop, H.C. Amstutz, Metal wear particle characterization from metal on metal total hip replacements: transmission electron microscopy study of periprosthetic tissues and isolated particles, J. Biomed. Mater. Res. 42 (1998) 103–111.
[17] M.A. Germain, A. Hatton, S. Williams, J.B. Matthews, M.H. Stone, J. Fisher, et al., Comparison of the cytotoxicity of clinically relevant cobalt-chromium and alumina ceramic wear particles in vitro, Biomaterials 24 (2003) 469–479.
[18] C. Brown, S. Williams, J.L. Tipper, J. Fisher, E. Ingham, Characterisation of wear particles produced by metal on metal and ceramic on metal hip prostheses under standard and microseparation simulation, J. Mater. Sci. Mater. Med. 18 (2007) 819–827.
[19] I. Catelas, J.D. Bobyn, J.B. Medley, J.J. Krygier, D.J. Zukor, O.L. Huk, Size, shape, and composition of wear particles from metal-metal hip simulator testing: effects of alloy and number of loading cycles, J. Biomed. Mater. Res. Part A 67 (2003) 312–327.
[20] F. Billi, P. Benya, A. Kavanaugh, J. Adams, H. McKellop, E. Ebramzadeh, The John Charnley Award: an accurate and extremely sensitive method to separate, display, and characterize wear debris: part 2: metal and ceramic particles, Clin. Orthop. Relat. Res. 470 (2012) 339–350.
[21] R. Pourzal, I. Catelas, R. Theissmann, C. Kaddick, A. Fischer, Characterization of wear particles generated from CoCrMo alloy under Sliding wear conditions, Wear 271 (2011) 1658–1666.
[22] J.L. Tipper, A.L. Galvin, S. Williams, H.M. McEwen, M.H. Stone, E. Ingham, et al., Isolation and characterization of UHMWPE wear particles down to ten nanometers in size from in vitro hip and knee joint simulators, J. Biomed. Mater. Res. Part A 78 (2006) 473–480.
[23] I. Milosev, M. Remskar, In vivo production of nanosized metal wear debris formed by tribochemical reaction as confirmed by high-resolution TEM and XPS analyses, J. Biomed. Mater. Res. Part A 91 (2009) 1100–1110.
[24] I. Papageorgiou, V. Shadrick, S. Davis, L. Hails, R. Schins, R. Newson, et al., Macrophages detoxify the genotoxic and cytotoxic effects of surgical cobalt chrome alloy particles but not quartz particles on human cells in vitro, Mutat. Res. 643 (2008) 11–19.
[25] J. Gallo, M. Slouf, S.B. Goodman, The relationship of polyethylene wear to particle size, distribution, and number: a possible factor explaining the risk of osteolysis after hip arthroplasty, J. Biomed. Mater. Res. B Appl. Biomater. 94 (2010) 171–177.
[26] J.A. Champion, A. Walker, S. Mitragotri, Role of particle size in phagocytosis of polymeric microspheres, Pharm. Res. 25 (2008) 1815–1821.
[27] J.M. Anderson, A. Rodriguez, D.T. Chang, Foreign body reaction to biomaterials, Semin. Immunol. 20 (2008) 86–100.
[28] J. Gallo, M. Raska, F. Mrázek, M. Petrek, Bone remodeling, particle disease and individual susceptibility to periprosthetic osteolysis, Physiol. Res. 57 (2008)

339–349.
[29] S. Landgraeber, M. Jager, J.J. Jacobs, N.J. Hallab, The pathology of orthopedic implant failure is mediated by innate immune system cytokines, Mediat. Inflamm. 2014 (2014) 185150.
[30] L. Horev-Azaria, C.J. Kirkpatrick, R. Korenstein, P.N. Marche, O. Maimon, J. Ponti, et al., Predictive toxicology of cobalt nanoparticles and ions: comparative in vitro study of different cellular models using methods of knowledge discovery from data, Toxicol. Sci. 122 (2011) 489–501.
[31] N.J. Hallab, J.J. Jacobs, A. Skipor, J. Black, K. Mikecz, J.O. Galante, Systemic metal-protein binding associated with total joint replacement arthroplasty, J. Biomed. Mater. Res. 49 (2000) 353–361.
[32] Y. Nuevo-Ordonez, M. Montes-Bayon, E. Blanco Gonzalez, A. Sanz-Medel, Titanium preferential binding sites in human serum transferrin at physiological concentrations, Metallomics 3 (2011) 1297–1303.
[33] P.G. Ren, A. Irani, Z. Huang, T. Ma, S. Biswal, S.B. Goodman, Continuous infusion of UHMWPE particles induces increased bone macrophages and osteolysis, Clin. Orthop. Relat. Res. 469 (2011) 113–122.
[34] T.R. Green, J. Fisher, M. Stone, B.M. Wroblewski, E. Ingham, Polyethylene particles of a 'critical size' are necessary for the induction of cytokines by macrophages in vitro, Biomaterials 19 (1998) 2297–2302.
[35] E. Ingham, J. Fisher, The role of macrophages in osteolysis of total joint replacement, Biomaterials 26 (2005) 1271–1286.
[36] D.J. Langton, S.S. Jameson, T.J. Joyce, J.N. Gandhi, R. Sidaginamale, P. Mereddy, et al., Accelerating failure rate of the ASR total hip replacement, J. Bone Jt. Surg. Br. 93 (2011) 1011–1016.
[37] P.A. Vendittoli, A. Roy, S. Mottard, J. Girard, D. Lusignan, M. Lavigne, Metal ion release from bearing wear and corrosion with 28 mm and large-diameter metal-on-metal bearing articulations: a follow-up study, J. Bone Jt. Surg. Br. 92 (2010) 12–19.
[38] D.H. Williams, N.V. Greidanus, B.A. Masri, C.P. Duncan, D.S. Garbuz, Prevalence of pseudotumor in asymptomatic patients after metal-on-metal hip arthroplasty, J. Bone Jt. Surg. Am. 93 (2011) 2164–2171.
[39] D.J. Langton, T.J. Joyce, S.S. Jameson, J. Lord, M. Van Orsouw, J.P. Holland, et al., Adverse reaction to metal debris following hip resurfacing: the influence of component type, orientation and volumetric wear, J. Bone Jt. Surg. Br. 93 (2011) 164–171.
[40] D.J. Langton, S.S. Jameson, T.J. Joyce, N.J. Hallab, S. Natu, A.V. Nargol, Early failure of metal-on-metal bearings in hip resurfacing and large-diameter total hip replacement: a consequence of excess wear, J. Bone Jt. Surg. Br. 92 (2010) 38–46.
[41] C.A. Engh, H. Ho, C.A. Engh, W.G. Hamilton, K.B. Fricka, Metal-on-metal total hip arthroplasty adverse local tissue reaction, Semin. Arthroplasty 21 (2010) 19–23.
[42] C. von Domarus, J.P. Rosenberg, W. Ruther, J. Zustin, Necrobiosis and T-lymphocyte infiltration in retrieved aseptically loosened metal-on-polyethylene arthroplasties, Acta Orthop. 82 (2011) 596–601.
[43] I.P. Gill, J. Webb, K. Sloan, R.J. Beaver, Corrosion at the neck-stem junction as a cause of metal ion release and pseudotumour formation, J. Bone Jt. Surg. Br. 94 (2012) 895–900.
[44] A.J. Walsh, V.S. Nikolaou, J. Antoniou, Inflammatory pseudotumor complicating metal-on-highly cross-linked polyethylene total hip arthroplasty, J. Arthroplasty 27 (324) (2012) e5–8.
[45] Y. Yan, A. Neville, D. Dowson, S. Williams, J. Fisher, Effect of metallic nanoparticles on the biotribocorrosion behaviour of Metal-on-Metal hip prostheses, Wear 267 (2009) 683–688.
[46] B. Daley, A.T. Doherty, B. Fairman, C.P. Case, Wear debris from hip or knee replacements causes chromosomal damage in human cells in tissue culture, J. Bone Jt. Surg. Br. 86 (2004) 598–606.
[47] A.J. Hart, K. Satchithananda, A.D. Liddle, S.A. Sabah, D. McRobbie, J. Henckel, et al., Pseudotumors in association with well-functioning metal-on-metal hip prostheses: a case-control study using three-dimensional computed tomography and magnetic resonance imaging, J. Bone Jt. Surg. Am. 94 (2012) 317–325.
[48] K.T. Hwang, Y.H. Kim, Y.S. Kim, J.A. Ryu, Prevalence of a soft-tissue lesion after small head metal-on-metal total hip replacement: 13- to 19-year follow-up study, Bone Jt. J. 96-B (2014) 1594–1599.
[49] P. Harvie, H. Giele, C. Fang, O. Ansorge, S. Ostlere, M. Gibbons, et al., The treatment of femoral neuropathy due to pseudotumour caused by metal-on-metal resurfacing arthroplasty, Hip Int. 18 (2008) 313–320.
[50] G. Mahendra, H. Pandit, K. Kliskey, D. Murray, H.S. Gill, N. Athanasou, Necrotic and inflammatory changes in metal-on-metal resurfacing hip arthroplasties, Acta Orthop. 80 (2009) 653–659.
[51] Y.M. Kwon, S.J. Ostlere, P. McLardy-Smith, N.A. Athanasou, H.S. Gill, D.W. Murray, "Asymptomatic" pseudotumors after metal-on-metal hip resurfacing arthroplasty: prevalence and metal ion study, J. Arthroplasty 26 (2011) 511–518.
[52] H. Pandit, S. Glyn-Jones, P. McLardy-Smith, R. Gundle, D. Whitwell, C.L. Gibbons, et al., Pseudotumours associated with metal-on-metal hip resurfacings, J. Bone Jt. Surg. Br. 90 (2008) 847–851.
[53] P. Aroukatos, M. Repanti, T. Repantis, V. Bravou, P. Korovessis, Immunologic adverse reaction associated with low-carbide metal-on-metal bearings in total hip arthroplasty, Clin. Orthop. Relat. Res. 468 (2010) 2135–2142.
[54] T.S. Watters, D.M. Cardona, K.S. Menon, E.N. Vinson, M.P. Bolognesi, L.G. Dodd, Aseptic lymphocyte-dominated vasculitis-associated lesion: a clinicopathologic review of an underrecognized cause of prosthetic failure, Am. J. Clin. Pathol. 134 (2010) 886–893.
[55] I. Kosukegawa, S. Nagoya, M. Kaya, K. Sasaki, M. Sasaki, T. Yamashita, Revision total hip arthroplasty due to pain from hypersensitivity to cobalt-chromium in total hip arthroplasty, J. Arthroplasty 26 (978) (2011) e1–e3.
[56] M. Hasegawa, K. Yoshida, H. Wakabayashi, A. Sudo, Pseudotumor with dominant B-lymphocyte infiltration after metal-on-metal total hip arthroplasty with a modular cup, J. Arthroplasty 27 (493) (2012) e5–e7.
[57] W.C. Witzleb, U. Hanisch, N. Kolar, F. Krummenauer, K.P. Guenther, Neocapsule tissue reactions in metal-on-metal hip arthroplasty, Acta Orthop. 78 (2007) 211–220.
[58] Y.M. Kwon, S. Glyn-Jones, D.J. Simpson, A. Kamali, P. McLardy-Smith, H.S. Gill, et al., Analysis of wear of retrieved metal-on-metal hip resurfacing implants revised due to pseudotumours, J. Bone Jt. Surg. Br. 92 (2010) 356–361.
[59] E.M. Evans, M.A.R. Freeman, A.J. Miller, B. Vernonro, Metal sensitivity as a cause of bone necrosis and loosening of prosthesis in total joint replacement, J. Bone Jt. Surg. – Br. Vol. B 56 (1974) 626–642.
[60] G.C. Brown, M.D. Lockshin, E.A. Salvati, P.G. Bullough, Sensitivity to metal as a possible cause of sterile loosening after cobalt-chromium total hip-replacement arthroplasty, J. Bone Jt. Surg. – Am. Vol. 59 (1977) 164–168.
[61] M.M. Wood, E.M. Warshaw, Hypersensitivity reactions to titanium: diagnosis and management, Dermatitis 26 (2015) 7–25.
[62] J.P. Thyssen, S.S. Jakobsen, K. Engkilde, J.D. Johansen, K. Soballe, T. Menne, The association between metal allergy, total hip arthroplasty, and revision, Acta Orthop. 80 (2009) 646–652.
[63] N. Hallab, K. Merritt, J.J. Jacobs, Metal sensitivity in patients with orthopaedic implants, J. Bone Jt. Surg. Am. 83-A (2001) 428–436.
[64] D. Granchi, E. Cenni, D. Tigani, G. Trisolino, N. Baldini, A. Giunti, Sensitivity to implant materials in patients with total knee arthroplasties, Biomaterials 29 (2008) 1494–1500.
[65] S.B. Goodman, Wear particles, periprosthetic osteolysis and the immune system, Biomaterials 28 (2007) 5044–5048.
[66] N.P. Hailer, R.A. Blaheta, H. Dahlstrand, A. Stark, Elevation of circulating HLA DR(+) CD8(+) T-cells and correlation with chromium and cobalt concentrations 6 years after metal-on-metal hip arthroplasty, Acta Orthop. 82 (2011) 6–12.
[67] B. Hercus, S. Saeed, P.A. Revell, Expression profile of T cell associated molecules in the interfacial tissue of aseptically loosened prosthetic joints, J. Mater. Sci. Mater. Med. 13 (2002) 1153–1156.
[68] P.M. Whittingham-Jones, E. Dunstan, H. Altaf, S.R. Cannon, P.A. Revell, T.W. Briggs, Immune responses in patients with metal-on-metal hip articulations: a long-term follow-up, J. Arthroplasty 23 (2008) 1212–1218.
[69] Z. Chen, Z. Wang, Q. Wang, W. Cui, F. Liu, W. Fan, Changes in early serum metal ion levels and impact on liver, kidney, and immune markers following metal-on-metal total hip arthroplasty, J. Arthroplasty 29 (2014) 612–616.
[70] N.J. Hallab, M. Caicedo, A. Finnegan, J.J. Jacobs, Th1 type lymphocyte reactivity to metals in patients with total hip arthroplasty, J. Orthop. Surg. Res. 3 (2008) 6.
[71] A.J. Hart, J.A. Skinner, P. Winship, N. Faria, E. Kulinskaya, D. Webster, et al., Circulating levels of cobalt and chromium from metal-on-metal hip replacement are associated with CD8+ T-cell lymphopenia, J. Bone Jt. Surg. Br. 91 (2009) 835–842.
[72] N.J. Hallab, K. Mikecz, C. Vermes, A. Skipor, J.J. Jacobs, Orthopaedic implant related metal toxicity in terms of human lymphocyte reactivity to metal-protein complexes produced from cobalt-base and titanium-base implant alloy degradation, Mol. Cell. Biochem. 222 (2001) 127–136.
[73] Y. Zhang, D. Wilcox, Thermodynamic and spectroscopic study of Cu(II) and Ni(II) binding to bovine serum albumin, J. Biol. Inorg. Chem. 7 (2002) 327–337.
[74] K. Gamerdinger, C. Moulon, D.R. Karp, J. Van Bergen, F. Koning, D. Wild, et al., A new type of metal recognition by human T cells: contact residues for peptide-independent bridging of T cell receptor and major histocompatibility complex by nickel, J. Exp. Med. 197 (2003) 1345–1353.
[75] B. Van Den, Heffler, L. Tengvall, Nilsson, Karlberg, Scheynius, Direct Ni2+ antigen formation on cultured human dendritic cells, Immunology 96 (1999) 578–585.
[76] A. Sargeant, T. Goswami, Hip implants – paper VI – ion concentrations, Mater. Des. 28 (2007) 155–171.
[77] H. Liang, J. Huang, C.Q. Tu, M. Zhang, Y.Q. Zhou, P.W. Shen, The subsequent effect of interaction between Co2+ and human serum albumin or bovine serum albumin, J. Inorg. Biochem. 85 (2001) 167–171.
[78] A.D. Tinoco, E.V. Eames, A.M. Valentine, Reconsideration of serum Ti(IV) transport: albumin and transferrin trafficking of Ti(IV) and its complexes, J. Am. Chem. Soc. 130 (2008) 2262–2270.
[79] E. Chan, D. Cadosch, O.P. Gautschi, K. Sprengel, L. Filgueira, Influence of metal ions on human lymphocytes and the generation of titanium-specific T-lymphocytes, J. Appl. Biomater. Biomech. 9 (2011) 137–143.
[80] R. Ortega, C. Bresson, A. Fraysse, C. Sandre, G. Deves, C. Gombert, et al., Cobalt distribution in keratinocyte cells indicates nuclear and perinuclear accumulation and interaction with magnesium and zinc homeostasis, Toxicol. Lett. 188 (2009) 26–32.
[81] K. Jomova, M. Valko, Advances in metal-induced oxidative stress and human disease, Toxicology 283 (2011) 65–87.
[82] D. Bagchi, S.J. Stohs, B.W. Downs, M. Bagchi, H.G. Preuss, Cytotoxicity and oxidative mechanisms of different forms of chromium, Toxicology 180

(2002) 5–22.
[83] K. Merritt, S.A. Brown, Release of hexavalent chromium from corrosion of stainless steel and cobalt-chromium alloys, J. Biomed. Mater. Res. 29 (1995) 627–633.
[84] K. Salnikow, S.P. Donald, R.K. Bruick, A. Zhitkovich, J.M. Phang, K.S. Kasprzak, Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress, J. Biol. Chem. 279 (2004) 40337–40344.
[85] I. Catelas, A. Petit, D.J. Zukor, J. Antoniou, O.L. Huk, TNF-α secretion and macrophage mortality induced by cobalt and chromium ions in vitro-Qualitative analysis of apoptosis, Biomaterials 24 (2003) 383–391.
[86] M. Akbar, J.M. Brewer, M.H. Grant, Effect of chromium and cobalt ions on primary human lymphocytes in vitro, J. Immunotoxicol. 8 (2011) 140–149.
[87] I. Catelas, A. Petit, H. Vali, C. Fragiskatos, R. Meilleur, D.J. Zukor, et al., Quantitative analysis of macrophage apoptosis vs. necrosis induced by cobalt and chromium ions in vitro, Biomaterials 26 (2005) 2441–2453.
[88] M.S. Caicedo, P.H. Pennekamp, K. McAllister, J.J. Jacobs, N.J. Hallab, Soluble ions more than particulate cobalt-alloy implant debris induce monocyte costimulatory molecule expression and release of proinflammatory cytokines critical to metal-induced lymphocyte reactivity, J. Biomed. Mater. Res. Part A 93 (2010) 1312–1321.
[89] L. Samelko, M.S. Caicedo, S.J. Lim, C. Della-Valle, J. Jacobs, N.J. Hallab, Cobalt-alloy implant debris induce HIF-1alpha hypoxia associated responses: a mechanism for metal-specific orthopedic implant failure, PLoS One 8 (2013) e67127.
[90] P.A. Potnis, D.K. Dutta, S.C. Wood, Toll-like receptor 4 signaling pathway mediates proinflammatory immune response to cobalt-alloy particles, Cell. Immunol. 282 (2013) 53–65.
[91] Shweta, K.P. Mishra, S. Chanda, S.B. Singh, L. Ganju, A comparative immunological analysis of CoCl2 treated cells with in vitro hypoxic exposure, Biometals 28 (2015) 175–185.
[92] H. Lawrence, D. Deehan, J. Holland, J. Kirby, A. Tyson-Capper, The immunobiology of cobalt: demonstration of a potential aetiology for inflammatory pseudotumours after metal-on-metal replacement of the hip, Bone Jt. J. 96-B (2014) 1172–1177.
[93] U. Dapunt, T. Giese, F. Lasitschka, J. Reinders, B. Lehner, J.P. Kretzer, et al., On the inflammatory response in metal-on-metal implants, J. Transl. Med. 12 (2014) 74.
[94] J.T. Ninomiya, S.A. Kuzma, T.J. Schnettler, J.G. Krolikowski, J.A. Struve, D. Weihrauch, Metal ions activate vascular endothelial cells and increase lymphocyte chemotaxis and binding, J. Orthop. Res. 31 (2013) 1484–1491.
[95] M.J. Pearson, R.L. Williams, H. Floyd, D. Bodansky, L.M. Grover, E.T. Davis, et al., The effects of cobalt-chromium-molybdenum wear debris in vitro on serum cytokine profiles and T cell repertoire, Biomaterials 67 (2015) 232–239.
[96] D. Granchi, G. Ciapetti, L. Savarino, S. Stea, F. Filippini, A. Sudanese, et al., Expression of the CD69 activation antigen on lymphocytes of patients with hip prosthesis, Biomaterials 21 (2000) 2059–2065.
[97] B. Ogunwale, A. Schmidt-Ott, R.M. Meek, J.M. Brewer, Investigating the immunologic effects of CoCr nanoparticles, Clin. Orthop. Relat. Res. 467 (2009) 3010–3016.
[98] J.M. Queally, B.M. Devitt, J.S. Butler, A.P. Malizia, D. Murray, P.P. Doran, et al., Cobalt ions induce chemokine secretion in primary human osteoblasts, J. Orthop. Res. 27 (2009) 855–864.
[99] A. Hartmann, F. Hannemann, J. Lutzner, A. Seidler, H. Drexler, K.P. Gunther, et al., Metal ion concentrations in body fluids after implantation of hip replacements with metal-on-metal bearing–systematic review of clinical and epidemiological studies, PLoS One 8 (2013) e70359.
[100] G.A. Afolaranmi, M. Akbar, J. Brewer, M.H. Grant, Distribution of metal released from cobalt-chromium alloy orthopaedic wear particles implanted into air pouches in mice, J. Biomed. Mater. Res. Part A 100 (2012) 1529–1538.
[101] S.S. Jakobsen, G. Danscher, M. Stoltenberg, A. Larsen, J.M. Bruun, T. Mygind, et al., Cobalt-chromium-molybdenum alloy causes metal accumulation and metallothionein up-regulation in rat liver and kidney, Basic Clin. Pharmacol. Toxicol. 101 (2007) 441–446.
[102] M.S. Madejczyk, C.E. Baer, W.E. Dennis, V.C. Minarchick, S.S. Leonard, D.A. Jackson, et al., Temporal changes in rat liver gene expression after acute cadmium and chromium exposure, PLoS One 10 (2015) e0127327.
[103] P.H. Wooley, R. Morren, J. Andary, S. Sud, S.-Y. Yang, L. Mayton, et al., Inflammatory responses to orthopaedic biomaterials in the murine air pouch, Biomaterials 23 (2002) 517–526.
[104] N.J. Hallab, J.J. Jacobs, Biologic effects of implant debris, Bull. NYU Hosp. Jt. Dis. 67 (2009) 182–188.
[105] P.R. Kim, P.E. Beaule, M. Dunbar, J.K. Lee, N. Birkett, M.C. Turner, et al., Cobalt and chromium levels in blood and urine following hip resurfacing arthroplasty with the conserve plus implant, J. Bone Jt. Surg. Am. 93 (Suppl. 2) (2011) 107–117.
[106] R.K. Hallows, C.E. Pelt, J.A. Erickson, C.L. Peters, Serum metal ion concentration: comparison between small and large head metal-on-metal total hip arthroplasty, J. Arthroplasty 26 (2011) 1176–1181.
[107] H. Ziaee, J. Daniel, A.K. Datta, S. Blunt, D.J. McMinn, Transplacental transfer of cobalt and chromium in patients with metal-on-metal hip arthroplasty: a controlled study, J. Bone Jt. Surg. Br. 89 (2007) 301–305.
[108] G.A. Afolaranmi, J. Tettey, R.M. Meek, M.H. Grant, Release of chromium from orthopaedic arthroplasties, Open Orthop. J. 2 (2008) 10–18.
[109] K.P. Gunther, J. Schmitt, P. Campbell, C.P. Delaunay, H. Drexler, H.B. Ettema, et al., Consensus statement "Current evidence on the management of metal-on-metal bearings" – April 16, 2012, Hip Int. 23 (2013) 2–5.
[110] H.J. Cooper, C.J. Della Valle, R.A. Berger, M. Tetreault, W.G. Paprosky, S.M. Sporer, et al., Corrosion at the head-neck taper as a cause for adverse local tissue reactions after total hip arthroplasty, J. Bone Jt. Surg – Am. Vol. 94a (2012) 1655–1661.
[111] E. Dunstan, A.P. Sanghrajka, S. Tilley, P. Unwin, G. Blunn, S.R. Cannon, et al., Metal ion levels after metal-on-metal proximal femoral replacements: a 30-year follow-up, J. Bone Jt. Surg. Br. 87 (2005) 628–631.
[112] A.J. Hart, S.A. Sabah, A.S. Bandi, P. Maggiore, P. Tarassoli, B. Sampson, et al., Sensitivity and specificity of blood cobalt and chromium metal ions for predicting failure of metal-on-metal hip replacement, J. Bone Jt. Surg. Br. 93 (2011) 1308–1313.
[113] Agency MaHPR, Medical Device Alert MDA/2010/033: All Metal-on-metal (MOM) Hip Replacements, 2010.
[114] H. Xu, J. Edwards, S. Banerji, R. Prevo, D.G. Jackson, N.A. Athanasou, Distribution of lymphatic vessels in normal and arthritic human synovial tissues, Ann. Rheum. Dis. 62 (2003) 1227–1229.
[115] R.M. Urban, J.J. Jacobs, M.J. Tomlinson, J. Gavrilovic, J. Black, M. Peoc'h, Dissemination of wear particles to the liver, spleen, and abdominal lymph nodes of patients with hip or knee replacement, J. Bone Jt. Surg. Am. 82 (2000) 457–476.
[116] C.P. Case, V.G. Langkamer, C. James, M.R. Palmer, A.J. Kemp, P.F. Heap, et al., Widespread dissemination of metal debris from implants, J. Bone Jt. Surg. Br. 76 (1994) 701–712.
[117] C.E. Anderson Jr., A.K.D. Moore, T.N. Vinh, D.C. Washington, G.A. Engh, V. Alexandria, Titanium prosthetic wear debris in remote bone marrow. a report of two cases, J. Bone Jt. Surg. 79 (1997) 1721–1725.
[118] P. Campbell, R.M. Urban, I. Catelas, A.K. Skipor, T.P. Schmalzried, Autopsy analysis thirty years after metal-on-metal total hip replacement. A case report, J. Bone Jt. Surg. Am. 85-A (2003) 2218–2222.
[119] M. Lundborg, R. Falk, A. Johansson, W. Kreyling, P. Camner, Phagolysosomal pH and dissolution of cobalt oxide particles by alveolar macrophages, Environ. Health Perspect. 97 (1992) 153–157.
[120] T. Visuri, P. Pulkkinen, P. Paavolainen, E. Pukkala, Cancer risk is not increased after conventional hip arthroplasty, Acta Orthop. 81 (2010) 77–81.
[121] P. Wagner, H. Olsson, L. Lidgren, O. Robertsson, J. Ranstam, Increased cancer risks among arthroplasty patients: 30 year follow-up of the Swedish Knee Arthroplasty Register, Eur. J. Cancer 47 (2011) 1061–1071.
[122] S.E. Chandran, N.J. Giori, Nine-year incidence of kidney disease in patients who have had total hip arthroplasty, J. Arthroplasty 26 (2011) 24–27.
[123] T. Visuri, H. Borg, P. Pulkkinen, P. Paavolainen, E. Pukkala, A retrospective comparative study of mortality and causes of death among patients with metal-on-metal and metal-on-polyethylene total hip prostheses in primary osteoarthritis after a long-term follow-up, BMC Musculoskelet. Disord. 11 (2010) 78.
[124] M.G. Zywiel, J.M. Brandt, C.B. Overgaard, A.C. Cheung, T.R. Turgeon, K.A. Syed, Fatal cardiomyopathy after revision total hip replacement for fracture of a ceramic liner, Bone Jt. J. 95-B (2013) 31–37.
[125] Y.M. Kwon, Z. Xia, S. Glyn-Jones, D. Beard, H.S. Gill, D.W. Murray, Dose-dependent cytotoxicity of clinically relevant cobalt nanoparticles and ions on macrophages in vitro, Biomed. Mater. 4 (2009) 025018.
[126] E.P. Chan, A. Mhawi, P. Clode, M. Saunders, L. Filgueira, Effects of titanium(iv) ions on human monocyte-derived dendritic cells, Metallomics 1 (2009) 166–174.
[127] D. Cadosch, M. Sutanto, E. Chan, A. Mhawi, O.P. Gautschi, B. von Katterfeld, et al., Titanium uptake, induction of RANK-L expression, and enhanced proliferation of human T-lymphocytes, J. Orthop. Res. 28 (2010) 341–347.
[128] J.Y. Wang, B.H. Wicklund, R.B. Gustilo, D.T. Tsukayama, Titanium, chromium and cobalt ions modulate the release of bone-associated cytokines by human monocytes/macrophages in vitro, Biomaterials 17 (1996) 2233–2240.
[129] V. Stejskal, T. Reynolds, G. Bjorklund, Increased frequency of delayed type hypersensitivity to metals in patients with connective tissue disease, J. Trace Elem. Med. Biol. 31 (2015) 230–236.
[130] K. Muller, E. Valentine-Thon, Hypersensitivity to titanium: clinical and laboratory evidence, Neuro Endocrinol. Lett. 27 (Suppl. 1) (2006) 31–35.
[131] Y. Gao, N.V. Gopee, P.C. Howard, L.R. Yu, Proteomic analysis of early response lymph node proteins in mice treated with titanium dioxide nanoparticles, J. Proteomics 74 (2011) 2745–2759.
[132] D.S. Rakshit, J.T. Lim, K. Ly, L.B. Ivashkiv, B.J. Nestor, T.P. Sculco, et al., Involvement of complement receptor 3 (CR3) and scavenger receptor in macrophage responses to wear debris, J. Orthop. Res. 24 (2006) 2036–2044.
[133] W. Chen, Z. Li, Y. Guo, Y. Zhou, Z. Zhang, Y. Zhang, et al., Wear particles promote reactive oxygen species-mediated inflammation via the nicotinamide adenine dinucleotide phosphate oxidase pathway in macrophages surrounding loosened implants, Cell. Physiol. Biochem. 35 (2015) 1857–1867.
[134] Z. Xing, L.P. Schwab, C.F. Alley, K.A. Hasty, R.A. Smith, Titanium particles that have undergone phagocytosis by macrophages lose the ability to activate other macrophages, J. Biomed. Mater. Res. B Appl. Biomater. 85 (2008) 37–41.
[135] A.M. Kaufman, C.I. Alabre, H.E. Rubash, A.S. Shanbhag, Human macrophage response to UHMWPE, TiAlV, CoCr, and alumina particles: analysis of

multiple cytokines using protein arrays, J. Biomed. Mater. Res. Part A 84 (2008) 464–474.
[136] C.A. St Pierre, M. Chan, Y. Iwakura, D.C. Ayers, E.A. Kurt-Jones, R.W. Finberg, Periprosthetic osteolysis: characterizing the innate immune response to titanium wear-particles, J. Orthop. Res. 28 (2010) 1418–1424.
[137] G.A. Obando-Pereda, L. Fischer, D.R. Stach-Machado, Titanium and zirconia particle-induced pro-inflammatory gene expression in cultured macrophages and osteolysis, inflammatory hyperalgesia and edema in vivo, Life Sci. 97 (2014) 96–106.
[138] T. Cheng, G.Y. Zhang, C.J. Guo, X. Zhang, Effects of NF-kappaB inhibitor on titanium particulate-induced inflammation in a murine model, J. Surg. Res. 162 (2010) 225–230.
[139] B. Baumann, J. Seufert, F. Jakob, U. Noth, O. Rolf, J. Eulert, et al., Activation of NF-kappaB signalling and TNFalpha-expression in THP-1 macrophages by TiAlV- and polyethylene-wear particles, J. Orthop. Res. 23 (2005) 1241–1248.
[140] D.P. Pioletti, A. Kottelat, The influence of wear particles in the expression of osteoclastogenesis factors by osteoblasts, Biomaterials 25 (2004) 5803–5808.
[141] H. Haleem-Smith, E. Argintar, C. Bush, D. Hampton, W.F. Postma, F.H. Chen, et al., Biological responses of human mesenchymal stem cells to titanium wear debris particles, J. Orthop. Res. 30 (2012) 853–863.
[142] E. Jamsen, V.P. Kouri, J. Olkkonen, A. Cor, S.B. Goodman, Y.T. Konttinen, et al., Characterization of macrophage polarizing cytokines in the aseptic loosening of total hip replacements, J. Orthop. Res. 32 (2014) 1241–1246.
[143] M. Geetha, A.K. Singh, R. Asokamani, A.K. Gogia, Ti based biomaterials, the ultimate choice for orthopaedic implants – a review, Prog. Mater. Sci. 54 (2009) 397–425.
[144] A. Sarmiento-Gonzalez, J.R. Encinar, J.M. Marchante-Gayon, A. Sanz-Medel, Titanium levels in the organs and blood of rats with a titanium implant, in the absence of wear, as determined by double-focusing ICP-MS, Anal. Bioanal. Chem. 393 (2009) 335–343.
[145] B. Song, J. Liu, X. Feng, L. Wei, L. Shao, A review on potential neurotoxicity of titanium dioxide nanoparticles, Nanoscale Res. Lett. 10 (2015) 1042.
[146] J. Hong, A. Azens, K.N. Ekdahl, C.G. Granqvist, B. Nilsson, Material-specific thrombin generation following contact between metal surfaces and whole blood, Biomaterials 26 (2005) 1397–1403.
[147] S.J. Phillips, Thrombogenic influence of biomaterials in patients with the Omni series heart valve: pyrolytic carbon versus titanium, ASAIO J. 47 (2001) 429–431.
[148] E.M. Schwarz, A.P. Lu, J.J. Goater, E.B. Benz, G. Kollias, R.N. Rosier, et al., Tumor necrosis factor-alpha/nuclear transcription factor-kappaB signaling in periprosthetic osteolysis, J. Orthop. Res. 18 (2000) 472–480.
[149] L.M. Childs, J.J. Goater, R.J. O'Keefe, E.M. Schwarz, Efficacy of etanercept for wear debris-induced osteolysis, J. Bone Miner. Res. 16 (2001) 338–347.
[150] X. Liu, S. Zhu, J. Cui, H. Shao, W. Zhang, H. Yang, et al., Strontium ranelate inhibits titanium-particle-induced osteolysis by restraining inflammatory osteoclastogenesis in vivo, Acta Biomater. 10 (2014) 4912–4918.
[151] T. Wachi, T. Shuto, Y. Shinohara, Y. Matono, S. Makihira, Release of titanium ions from an implant surface and their effect on cytokine production related to alveolar bone resorption, Toxicology 327 (2015) 1–9.
[152] A. Hoene, M. Patrzyk, U. Walschus, B. Finke, S. Lucke, B. Nebe, et al., Systemic IFNgamma predicts local implant macrophage response, J. Mater. Sci. Mater. Med. 26 (2015) 131.
[153] K. Kmiec, M. Synder, P. Kozlowski, M. Drobniewski, M. Sibinski, Metal debris concentrations in soft tissues adjacent to loosened femoral stems is higher in uncemented than cemented implants, BMC Musculoskelet. Disord. 15 (2014) 267.
[154] B.F. Shahgaldi, F.W. Heatley, A. Dewar, B. Corrin, In vivo corrosion of cobalt-chromium and titanium wear particles, J. Bone Jt. Surg. Br. 77 (1995) 962–966.
[155] P.A. Lalor, P.A. Revell, A.B. Gray, S. Wright, G.T. Railton, M.A. Freeman, Sensitivity to titanium. A cause of implant failure? J. Bone Jt. Surg. Br. 73 (1991) 25–28.
[156] X. Qu, X. Huang, K. Dai, Metal-on-metal or metal-on-polyethylene for total hip arthroplasty: a meta-analysis of prospective randomized studies, Arch. Orthop. Trauma Surg. 131 (2011) 1573–1583.
[157] H.M. Kremers, J.L. Howard, Y. Loechler, C.D. Schleck, W.S. Harmsen, D.J. Berry, et al., Comparative long-term survivorship of uncemented acetabular components in revision total hip arthroplasty, J. Bone Jt. Surg. Am. 94 (2012) e82.
[158] P. Bracco, E. Oral, Vitamin E-stabilized UHMWPE for total joint implants: a review, Clin. Orthop. Relat. Res. 469 (2011) 2286–2293.
[159] S.L. Rowell, O.K. Muratoglu, Investigation of surgically retrieved, vitamin E-stabilized, crosslinked UHMWPE implants after short-term in vivo service, J. Biomed. Mater. Res. B Appl. Biomater. (2015), http://dx.doi.org/10.1002/jbm.b.33465.
[160] T. Baad-Hansen, S. Kold, P.T. Nielsen, M.B. Laursen, P.H. Christensen, K. Soballe, Comparison of trabecular metal cups and titanium fiber-mesh cups in primary hip arthroplasty: a randomized RSA and bone mineral densitometry study of 50 hips, Acta Orthop. 82 (2011) 155–160.
[161] E.D. Batuyong, H.S. Brock, N. Thiruvengadam, W.J. Maloney, S.B. Goodman, J.I. Huddleston, Outcome of porous tantalum acetabular components for Paprosky type 3 and 4 acetabular defects, J. Arthroplasty 29 (2014) 1318–1322.
[162] S.S. Jassim, S. Patel, N. Wardle, J. Tahmassebi, R. Middleton, D.L. Shardlow, et al., Five-year comparison of wear using oxidised zirconium and cobalt-chrome femoral heads in total hip arthroplasty: a multicentre randomised controlled trial, Bone Jt. J. 97-B (2015) 883–889.
[163] D.B. Bennett, J.C. Hill, J. Dennison, S. O'Brien, J.L. Mantel, G.H. Isaac, et al., Metal-carbon fiber composite femoral stems in hip replacements: a randomized controlled parallel-group study with mean ten-year follow-up, J. Bone Jt. Surg. Am. 96 (2014) 2062–2069.
[164] P.M. Mountziaris, P.P. Spicer, F.K. Kasper, A.G. Mikos, Harnessing and modulating inflammation in strategies for bone regeneration, Tissue Eng. Part B Rev. 17 (2011) 393–402.
[165] V. Mourino, J.P. Cattalini, A.R. Boccaccini, Metallic ions as therapeutic agents in tissue engineering scaffolds: an overview of their biological applications and strategies for new developments, J. R. Soc. Interface/ R. Soc. 9 (2012) 401–419.
[166] N. Kilcup, S. Gaynard, U. Werner-Zwanziger, E. Tonkopi, J. Hayes, D. Boyd, Stimulation of apoptotic pathways in liver cancer cells: An alternative perspective on the biocompatibility and the utility of biomedical glasses, J. Biomater. Appl. (2015), http://dx.doi.org/10.1177/0885328215621663.
[167] J. Kim, J.L. Gilbert, Cytotoxic effect of galvanically coupled magnesium-titanium particles, Acta Biomater. 30 (2016) 368–377.
[168] I. Burghardt, F. Luthen, C. Prinz, B. Kreikemeyer, C. Zietz, H.G. Neumann, et al., A dual function of copper in designing regenerative implants, Biomaterials 44 (2015) 36–44.
[169] G. Jin, H. Qin, H. Cao, Y. Qiao, Y. Zhao, X. Peng, et al., Zn/Ag micro-galvanic couples formed on titanium and osseointegration effects in the presence of S. aureus, Biomaterials 65 (2015) 22–31.
[170] D.N. Paglia, A. Wey, A.G. Park, E.A. Breitbart, S.K. Mehta, J.D. Bogden, et al., The effects of local vanadium treatment on angiogenesis and chondrogenesis during fracture healing, J. Orthop. Res. 30 (2012) 1971–1978.
[171] V. Aimanianda, J. Haensler, S. Lacroix-Desmazes, S.V. Kaveri, J. Bayry, Novel cellular and molecular mechanisms of induction of immune responses by aluminum adjuvants, Trends Pharmacol. Sci. 30 (2009) 287–295.
[172] W.S. Cho, K. Dart, D.J. Nowakowska, X. Zheng, K. Donaldson, S.E. Howie, Adjuvanticity and toxicity of cobalt oxide nanoparticles as an alternative vaccine adjuvant, Nanomedicine (Lond.) 7 (2012) 1495–1505.
[173] J. Zhang, X. Ma, D. Lin, H. Shi, Y. Yuan, W. Tang, et al., Magnesium modification of a calcium phosphate cement alters bone marrow stromal cell behavior via an integrin-mediated mechanism, Biomaterials 53 (2015) 251–264.
[174] H. Kitabata, R. Waksman, B. Warnack, Bioresorbable metal scaffold for cardiovascular application: current knowledge and future perspectives, Cardiovasc. Revasc. Med. 15 (2014) 109–116.
[175] M. Schumacher, A. Lode, A. Helth, M. Gelinsky, A novel strontium(II)-modified calcium phosphate bone cement stimulates human-bone-marrow-derived mesenchymal stem cell proliferation and osteogenic differentiation in vitro, Acta Biomater. 9 (2013) 9547–9557.
[176] J.P. Cattalini, A. Hoppe, F. Pishbin, J. Roether, A.R. Boccaccini, S. Lucangioli, et al., Novel nanocomposite biomaterials with controlled copper/calcium release capability for bone tissue engineering multifunctional scaffolds, J. R. Soc. Interface/R. Soc. 12 (2015) 0509.
[177] M. Moravej, D. Mantovani, Biodegradable metals for cardiovascular stent application: interests and new opportunities, Int. J. Mol. Sci. 12 (2011) 4250–4270.
[178] A. Chaya, S. Yoshizawa, K. Verdelis, N. Myers, B.J. Costello, D.T. Chou, et al., In vivo study of magnesium plate and screw degradation and bone fracture healing, Acta Biomater. 18 (2015) 262–269.
[179] A. Bondarenko, N. Angrisani, A. Meyer-Lindenberg, J.M. Seitz, H. Waizy, J. Reifenrath, Magnesium-based bone implants: immunohistochemical analysis of peri-implant osteogenesis by evaluation of osteopontin and osteocalcin expression, J. Biomed. Mater. Res. Part A 102 (2014) 1449–1457.
[180] F. Witte, N. Hort, C. Vogt, S. Cohen, K.U. Kainer, R. Willumeit, et al., Degradable biomaterials based on magnesium corrosion, Curr. Opin. Solid State Mater. Sci. 12 (2008) 63–72.
[181] H. Windhagen, K. Radtke, A. Weizbauer, J. Diekmann, Y. Noll, U. Kreimeyer, et al., Biodegradable magnesium-based screw clinically equivalent to titanium screw in hallux valgus surgery: short term results of the first prospective, randomized, controlled clinical pilot study, Biomed. Eng. Online 12 (2013) 62.
[182] K. Pichler, T. Kraus, E. Martinelli, P. Sadoghi, G. Musumeci, P.J. Uggowitzer, et al., Cellular reactions to biodegradable magnesium alloys on human growth plate chondrocytes and osteoblasts, Int. Orthop. 38 (2014) 881–889.
[183] S. Yoshizawa, A. Chaya, K. Verdelis, E.A. Bilodeau, C. Sfeir, An in vivo model to assess magnesium alloys and their biological effect on human bone marrow stromal cells, Acta Biomater. 28 (2015) 234–239.
[184] A.H. Martinez Sanchez, B.J. Luthringer, F. Feyerabend, R. Willumeit, Mg and Mg alloys: how comparable are in vitro and in vivo corrosion rates? A review, Acta Biomater. 13 (2015) 16–31.
[185] A. Brown, S. Zaky, H. Ray Jr., C. Sfeir, Porous magnesium/PLGA composite scaffolds for enhanced bone regeneration following tooth extraction, Acta Biomater. 11 (2015) 543–553.
[186] F. Witte, H. Ulrich, C. Palm, E. Willbold, Biodegradable magnesium scaffolds: part II: peri-implant bone remodeling, J. Biomed. Mater. Res. Part A 81 (2007) 757–765.
[187] F. Witte, V. Kaese, H. Haferkamp, E. Switzer, A. Meyer-Lindenberg, C.J. Wirth, et al., In vivo corrosion of four magnesium alloys and the associated bone

response, Biomaterials 26 (2005) 3557–3563.
[188] P.R. Cha, H.S. Han, G.F. Yang, Y.C. Kim, K.H. Hong, S.C. Lee, et al., Biodegradability engineering of biodegradable Mg alloys: tailoring the electrochemical properties and microstructure of constituent phases, Sci. Rep. 3 (2013) 2367.
[189] E. Marukawa, M. Tamai, Y. Takahashi, I. Hatakeyama, M. Sato, Y. Higuchi, et al., Comparison of magnesium alloys and poly-l-lactide screws as degradable implants in a canine fracture model, J. Biomed. Mater. Res. B Appl. Biomater. (2015), http://dx.doi.org/10.1002/jbm.b.33470.
[190] O. Charyeva, U. Thormann, K.S. Lips, L. Heimann, U. Sommer, G. Szalay, et al., Histological comparison of new biodegradable magnesium-based implants for maxillofacial applications, J. Maxillofac. Oral Surg. 14 (2015) 637–645.
[191] P. Cheng, P. Han, C. Zhao, S. Zhang, H. Wu, J. Ni, et al., High-purity magnesium interference screws promote fibrocartilaginous entheses regeneration in the anterior cruciate ligament reconstruction rabbit model via accumulation of BMP-2 and VEGF, Biomaterials 81 (2015) 14–26.
[192] M.P. Staiger, A.M. Pietak, J. Huadmai, G. Dias, Magnesium and its alloys as orthopedic biomaterials: a review, Biomaterials 27 (2006) 1728–1734.
[193] B. Chaigne-Delalande, M.J. Lenardo, Divalent cation signaling in immune cells, Trends Immunol. 35 (2014) 332–344.
[194] J.H. de Baaij, J.G. Hoenderop, R.J. Bindels, Magnesium in man: implications for health and disease, Physiol. Rev. 95 (2015) 1–46.
[195] J.J. Grzesiak, M.D. Pierschbacher, Shifts in the concentrations of magnesium and calcium in early porcine and rat wound fluids activate the cell migratory response, J. Clin. Investig. 95 (1995) 227–233.
[196] L.W. Doyle, C.A. Crowther, P. Middleton, S. Marret, Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus, Cochrane Database Syst. Rev. (2007), http://dx.doi.org/10.1002/14651858.CD004661.pub2. CD004661.
[197] L. Duley, A.M. Gulmezoglu, D.J. Henderson-Smart, D. Chou, Magnesium sulphate and other anticonvulsants for women with pre-eclampsia, Cochrane Database Syst. Rev. (2010), http://dx.doi.org/10.1002/14651858.CD000025.pub2. CD000025.
[198] M. Shimaya, T. Muneta, S. Ichinose, K. Tsuji, I. Sekiya, Magnesium enhances adherence and cartilage formation of synovial mesenchymal stem cells through integrins. Osteoarthritis and cartilage/OARS, Osteoarthr. Res. Soc. 18 (2010) 1300–1309.
[199] S. Yoshizawa, A. Brown, A. Barchowsky, C. Sfeir, Magnesium ion stimulation of bone marrow stromal cells enhances osteogenic activity, simulating the effect of magnesium alloy degradation, Acta Biomater. 10 (2014) 2834–2842.
[200] P. Han, P. Cheng, S. Zhang, C. Zhao, J. Ni, Y. Zhang, et al., In vitro and in vivo studies on the degradation of high-purity Mg (99.99wt.%) screw with femoral intracondylar fractured rabbit model, Biomaterials 64 (2015) 57–69.
[201] J. Zheng, X. Mao, J. Ling, C. Chen, W. Zhang, Role of magnesium transporter subtype 1 (MagT1) in the osteogenic differentiation of rat bone marrow stem cells, Biol. Trace Elem. Res. (2015), http://dx.doi.org/10.1007/s12011-015-0459-4.
[202] L. Wu, B.J. Luthringer, F. Feyerabend, A.F. Schilling, R. Willumeit, Effects of extracellular magnesium on the differentiation and function of human osteoclasts, Acta Biomater. 10 (2014) 2843–2854.
[203] J.J. Grzesiak, M. Bouvet, Divalent cations modulate the integrin-mediated malignant phenotype in pancreatic cancer cells, Cancer Sci. 99 (2008) 1553–1563.
[204] C.K. Hagandora, M.A. Tudares, A.J. Almarza, The effect of magnesium ion concentration on the fibrocartilage regeneration potential of goat costal chondrocytes, Ann. Biomed. Eng. 40 (2012) 688–696.
[205] A. Mazur, J.A. Maier, E. Rock, E. Gueux, W. Nowacki, Y. Rayssiguier, Magnesium and the inflammatory response: potential physiopathological implications, Arch. Biochem. Biophys. 458 (2007) 48–56.
[206] S. Ferre, E. Baldoli, M. Leidi, J.A. Maier, Magnesium deficiency promotes a pro-atherogenic phenotype in cultured human endothelial cells via activation of NFkB, Biochim. Biophys. Acta 1802 (2010) 952–958.
[207] F.Y. Li, B. Chaigne-Delalande, C. Kanellopoulou, J.C. Davis, H.F. Matthews, D.C. Douek, et al., Second messenger role for Mg2+ revealed by human T-cell immunodeficiency, Nature 475 (2011) 471–476.
[208] C.Y. Lin, P.S. Tsai, Y.C. Hung, C.J. Huang, L-type calcium channels are involved in mediating the anti-inflammatory effects of magnesium sulphate, Br. J. Anaesth. 104 (2010) 44–51.
[209] J. Sugimoto, A.M. Romani, A.M. Valentin-Torres, A.A. Luciano, C.M. Ramirez Kitchen, N. Funderburg, et al., Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism, J. Immunol. 188 (2012) 6338–6346.
[210] E.W. Son, S.R. Lee, H.S. Choi, H.J. Koo, J.E. Huh, M.H. Kim, et al., Effects of supplementation with higher levels of manganese and magnesium on immune function, Arch. Pharmacal Res. 30 (2007) 743–749.
[211] C.H. Lee, Z.H. Wen, Y.C. Chang, S.Y. Huang, C.C. Tang, W.F. Chen, et al., Intra-articular magnesium sulfate (MgSO4) reduces experimental osteoarthritis and nociception: association with attenuation of N-methyl-D-aspartate (NMDA) receptor subunit 1 phosphorylation and apoptosis in rat chondrocytes. Osteoarthritis and cartilage/OARS, Osteoarthr. Res. Soc. 17 (2009) 1485–1493.
[212] J.H. Kim, H.J. Gibb, P.D. Howe, Cobalt and Inorganic Cobalt Compounds, World Health Organization, 2006.
[213] S.S. Tower, Arthroprosthetic cobaltism: neurological and cardiac manifestations in two patients with metal-on-metal arthroplasty, J. Bone Jt. Surg. 92 (2010) 2847–2851.
[214] H.K. Kim, H. Bian, J. Aya-ay, A. Garces, E.F. Morgan, S.R. Gilbert, Hypoxia and HIF-1alpha expression in the epiphyseal cartilage following ischemic injury to the immature femoral head, Bone 45 (2009) 280–288.
[215] Y. Huang, K.M. Du, Z.H. Xue, H. Yan, D. Li, W. Liu, et al., Cobalt chloride and low oxygen tension trigger differentiation of acute myeloid leukemic cells: possible mediation of hypoxia-inducible factor-1alpha, Leukemia 17 (2003) 2065–2073.
[216] R.A. Perez, J.H. Kim, J.O. Buitrago, I.B. Wall, H.W. Kim, Novel therapeutic core-shell hydrogel scaffolds with sequential delivery of cobalt and bone morphogenetic protein-2 for synergistic bone regeneration, Acta Biomater. 23 (2015) 295–308.
[217] W. Fan, R. Crawford, Y. Xiao, Enhancing in vivo vascularized bone formation by cobalt chloride-treated bone marrow stromal cells in a tissue engineered periosteum model, Biomaterials 31 (2010) 3580–3589.
[218] K.M. Ng, Y.C. Chan, Y.K. Lee, W.H. Lai, K.W. Au, M.L. Fung, et al., Cobalt chloride pretreatment promotes cardiac differentiation of human embryonic stem cells under atmospheric oxygen level, Cell. Reprogr. 13 (2011) 527–537.
[219] E. Quinlan, S. Partap, M.M. Azevedo, G. Jell, M.M. Stevens, F.J. O'Brien, Hypoxia-mimicking bioactive glass/collagen glycosaminoglycan composite scaffolds to enhance angiogenesis and bone repair, Biomaterials 52 (2015) 358–366.
[220] N. Ignjatovic, Z. Ajdukovic, V. Savic, S. Najman, D. Mihailovic, P. Vasiljevic, et al., Nanoparticles of cobalt-substituted hydroxyapatite in regeneration of mandibular osteoporotic bones, J. Mater. Sci. Mater. Med. 24 (2013) 343–354.
[221] M. Zhang, C. Wu, H. Li, J. Yuen, J. Chang, Y. Xiao, Preparation, characterization and in vitro angiogenic capacity of cobalt substituted beta-tricalcium phosphate ceramics, J. Mater. Chem. 22 (2012) 21686–21694.
[222] C. Wu, Y. Zhou, W. Fan, P. Han, J. Chang, J. Yuen, et al., Hypoxia-mimicking mesoporous bioactive glass scaffolds with controllable cobalt ion release for bone tissue engineering, Biomaterials 33 (2012) 2076–2085.
[223] Z. Chen, J. Yuen, R. Crawford, J. Chang, C. Wu, Y. Xiao, The effect of osteoimmunomodulation on the osteogenic effects of cobalt incorporated beta-tricalcium phosphate, Biomaterials 61 (2015) 126–138.