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Chapter 6

BIODEGRADATION OF METALLIC BIOMATERIALS: ITS RELATION WITH THE GENERATION OF REACTIVE OXYGEN SPECIES

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ABSTRACT

Some specific clinical problems, particularly those related to orthopedic trauma and some cardiovascular diseases need only temporary support for healing. This support can be provided by biodegradable metallic materials such as, Fe-, Mg- based alloys that avoid some of the side effects of traditional biomaterials. They are expected to support the healing process of a diseased tissue or organ with slowly degrading after fulfilling their function. However, the excess of metal ions may catalyze the formation of reactive oxygen and nitrogen species (ROS and RNS). An increase in the intracellular levels of free metal ions affects the normal balance ROS-antioxidant. ROS could cause lipid peroxidation with changes in the composition and fluidity of cell membrane and alterations in other macromolecules as proteins and DNA. Considering that the concentration of metal ions can reach high values in the biomaterial-tissue interface inducing ROS generation it is important to evaluate the possible adverse effects of the degradation products of biodegradable biomaterials.

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INTRODUCTION

Biomaterials can be defined as "materials (synthetic and natural) that are used in contact with biological systems" [1]. This general definition does not take into account the concept of biocompatibility defined by Williams [2] as ''ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response to that specific situation, and optimizing the clinically relevant performance of that therapy''. This last definition implies that the body reacts against the foreign material while simultaneously the biomaterial is modified by its interaction with the environment. The interface material-biological medium is dynamic and this interaction depends on many factors, such as the kind of tissue where the interface is created, the composition of the biological medium, surface roughness and topography of the biomaterial, patient health status, technique used, etc. Therefore, the presence of a biomaterial within the body induces reactions from the surrounding tissues that are known as ''host responses". A biocompatible biomaterial is expected to show minimum inflammatory and toxicity reactions both locally and systematically [3].

Before William´s definition, the prevailing view was that successful materials played largely "inert" roles in the body, as in the case of joint replacements (hip, knee), heart valves, bone plates, dental implants, intraocular lens, etc. Those devices are intended to remain a long time within the body. To achieve this goal, the materials employed are commonly metals (Co, Cr, Ti, Ni alloys, and stainless steels), ceramics, polymers, glasses, among others. However, the biological environment can lead to gradual breakdown of many biomaterials; thus, many materials are exposed to continuous or cyclic stress and abrasion and flexure may also take place and lead to failures. Biological environment interacts with degradation products since proteins adsorb to the material and enhance or delay the corrosion rate of metals. Additionally, cells secrete powerful oxidizing agents and enzymes that can digest the material. Degradative agents usually concentrate in the interface between the cells and the material and sometimes, adverse effects may be detected.

For decades, the concept of metallic biomaterials has been thought as materials resistant to corrosion once implanted in the human body. Recently, degradable metallic biomaterials (DMB) have been proposed for some specific applications, including orthopaedic and cardio-vascular applications. These materials are expected to disappear after providing structural support for an appropriate period that ensures the healing process. Once the tolerance of surrounding tissues and organs to the presence of degradation products is evaluated, the improvement of degradation rate and host response may be possible [4] and a biodegradable material becomes a reality.

Metals are mechanically interesting for load-bearing degradable implants such as, internal bone fixation screws and plates and coronary stents. When biodegradable metallic materials are necessary, two groups of metals have been proposed: Mg- and Fe-based alloys [4].

Mg-based biomaterials are one of the promising biodegradable metals for orthopedic applications because they exhibit low density and mechanical properties close to those of cortical bone and consequently are suitable for fracture repair of weight bearing bone [5]. Their degradability allows avoiding a second surgery intervention for implant removal, which

is necessary for other non-degradable implants. However, one of the main limitations of using these materials is its high degradation rate that leads to changes in physical and mechanical properties [6]. The high susceptibility to corrosion can be mitigated using techniques such as, surface coatings, anodizing and with incorporation of alloying elements [7]. Diverse Mg alloys have been explored in an effort to increase its applicability, such as the ZEK100, AX30 and also Mg-Mn, Mg-Al-Zn alloys [8-10]. In the last years, other different systems such as Mg-Zn-Se and Mg-Zn-Cu [11] have been developed. Different corrosion mechanisms detected in Mg alloys could locally induce time-dependent concentration gradients for the alloying elements (Zn, Al, Cu, etc). This alteration in the local concentration may contribute differentially to the generation of adverse effects on the nearest cells.

Among biodegradable materials, Cu, main component of intrauterine devices (IUD), should also be considered. Cu-based IUDs are commonly used as a reversible contraception method by over 150 million women (about 15% of the world´s women in reproductive age). They based its contraceptive action on the release of Cu ions from a Cu wire [12]. The biological response depends on the concentration of ions released and the exposure time, among other factors. In this sense, during insertion Cu-IUD probably represents a dangerous combination of variables since the metallic device is in intimate proximity with local tissue for a long period and a high amount of Cu ions is released, particularly in the first period after insertion (burst release). In fact, cellular and biochemical changes occurring in the endometrium and uterine fluid after Cu-IUD insertion [13, 14], as well as inflammatory response enhancement by cupric ions together with an increase of Cu ions in plasma, were reported [15-17]. On the other hand, it is worth mentioning that several hundreds of Cu alloys are also employed in odontology for prosthodontic restorations [18]. Biocompatibility analysis shows some apparent inconsistencies between several authors [19-20]. Some of them found that cellular functions were not altered in response to ions released from the alloys and to their salts. They highlighted that salt solutions are not adequate to represent alloy cytotoxicity because ions release from these alloys is a complex process and when salts or extracts are used to simulate the effect of ion release in cell cultures, the concentration is uniform without the concentration gradients characteristic of the *in vivo* situation. Whereas, when the evaluation of the alloy is made *in situ*, within the culture, cytotoxic effects were observed [21].

Otherwise, nanotechnology has provided new materials for medical and dental applications. They show interesting properties due to their large surface area to volume ratios. However, are also involved in adverse effects. Some of them are effective growth inhibitors against various microorganisms and thereby are applicable to diverse medical devices such as catheters, bandages for burn healing, and dressing materials for wound repair [22]. They are also used as active antibacterial ingredient for dental materials and as topically applied agents in the control of oral infections [23]. Ag nanoparticles are effective biocides that are biodegradable in the biological fluid and release Ag ions and/or are internalized by the bacteria or cells. It has been reported that they cause ROS formation in the cells, a reduction in their cell viability and mitochondrial membrane potential (MMP), an increase in the proportion of cells in the sub-G1 (apoptosis) population, S phase arrest and down-regulation of the cell cycle associated proliferating cell nuclear antigen (PCNA) protein, in a concentration time-dependent manner [24]. Overall, biodegradable metallic materials in the macro or nano-scale may provide interesting properties as biomaterials but may also cause adverse effects, frequently associated to ROS generation.

BIODEGRADABLE STENTS

In the last decade, the study of degradable biomaterials has become one of the most revolutionary topics in the field of biomaterials. So-called *biodegradable stents* provide support for the temporary opening of the blood vessel permitting tissue remodeling with the simultaneous gradual dissolution of the stent. Degradability avoids the problems of traditional permanent stents: restenosis [25, 26], thrombosis [27, 28] and the need for prolonged antiplatelet therapy [29], besides they are specially intended for children during growth.

Stent degradation should ideally start at a low speed in order to maintain the mechanical integrity required for tissue remodeling process. Tissue remodeling requires an estimated period of 6 to 12 months[30, 31]. As mechanical integrity decreases as a consequence of the degradation process, corrosion should take place at controlled rate without causing excessive accumulation of degradation products in the area close to the site of implantation. It is considered that a period of 12 to 24 months after implantation is adequate to achieve complete degradation of the stent. The effects of changes during stent dissolution on the cells in contact with the implant are described in Figure 1.

FE IONS RELEASE IN RELATION WITH THE MASS AND SURFACE OF THE STENT

When experiments related to Fe-based biodegradable materials are designed one of the first steps is to estimate the rate of the ions release and the probable local concentrations at the biomaterial surroundings to evaluate cytotoxicity and oxidative damage by Fe ions and pH changes.

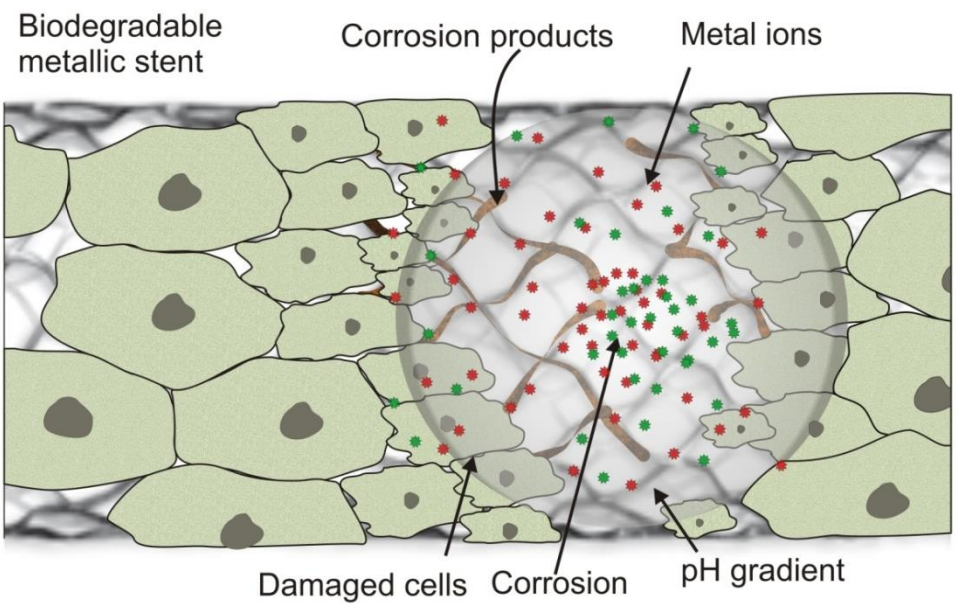

Figure 1. Schematic representation of biological and physicochemical changes during stent dissolution and its possible effects on cells in contact with the implant.

For this purpose one may assume that the amounts of Fe released from implanted coronary or femoral stents were related with their weights (weight: 41 mg for a 4 mm diameter x 20 mm long stent, weight: 750 mg for a 7 mm diameter x 200 mm long stent, respectively). Thus, 41-750 mg of degradation products should be released by the stent in c.a. 12 months. This means that the amount released per day (assuming a constant dissolution rate) is between 0.11 and 2 mg day^{-1}. A fraction of this material is removed by blood but some may diffuse and be retained in the endothelium-stent interface. It is not easy to know the fluid volume present at the interface in which Fe ions dissolve to calculate a real local concentration. If 0.5 ml of biological fluid are considered as a rough estimation of the volume of biological medium in contact with the stent, concentrations between 3.9 and 71.0 mM could be reached in the interface. Moravej et al. [32] evaluated the degradation rate of pure Fe in simulated biological fluids under static conditions and found a corrosion rate of c.a. 0.4 mm $year^{-1} \cong 3.44$ mg day^{-1}. Corrosion rates close to 1.8 mg day^{-1} were found by Zhu et al. [33] in simulated body fluids, with 2 cm^2 samples. However, it must be taken into account that the corrosion rate of the metal is strongly dependent on the electrolyte and on the environmental properties and, frequently, *in vivo* results are lower than the results obtained by *in vitro* assays [34]. Anyway, an increasing accumulation of Fe degradation products in the vessel wall adjacent to the stent strut over time was found *in vivo* by Peuster et al. [35].

DEGRADATION OF IRON: DIFFERENT SPECIES INVOLVED

It was previously mentioned that both, degradation rate and mechanical integrity, depend not only on the characteristics of the biomaterial but on the conditions of the implantation site. Consequently, it is necessary to investigate the interaction of degradation products with the surrounding tissue [36]. It is well known that toxicity of elements depends on their physicochemical forms and their excess may have serious implications in living organisms. Among degradable materials for stent applications, pure Fe is a good option due to its moderate degradation rate, mechanical properties comparable with those of stainless steel as well as probably, good biocompatibility because of the role of Fe as essential element for human body [32, 37]. The study of Fe is particularly interesting because its ions (Fe^{2+} and Fe^{3+}) and its several oxidation products represent an additional complexity. Degradation of Fe in a chloride medium such as simulated biological fluids occurs through the following reactions that, in most cases, are pH dependent.

$$Fe \rightarrow Fe^{2+} + 2\ e- \quad (1)$$

Some of Fe^{2+} can be oxidized to Fe^{3+} under neutral conditions and oxygen environment and $Fe(OH)_3$ is produced

$$½\ O_2 + H_2O + 2e- \rightarrow 2OH^- \quad (2)$$

$$Fe^{2+} + 2\ OH^- \rightarrow Fe(OH)_2 \quad (3)$$

$$Fe^{2+} \rightarrow Fe^{3+} + e^- \quad (4)$$

$$Fe^{3+} + 3\ OH^{-} \rightarrow Fe(OH)_3 \quad (5)$$

In the presence of O_2 and chloride ions, $Fe(OH)_3$ is hydrolyzed and goethite (α-FeO(OH)) precipitates according to

$$2\ Fe(OH)_3 + H_2O \rightarrow 2\ FeO(OH)_{(s)} + 2\ H+ \quad (6)$$

Whereas Fe^{2+} is extremely water soluble, Fe^{3+} is quite insoluble in water (at pH 7, $[Fe^{3+}]=10^{-18}$ M) and significant concentrations of water-soluble Fe^{3+} species can be attained only by strong complex formation [38]. Since the maximal coordination number of Fe is six, a chelator molecule that binds to all six sites of the Fe ion completely deactivates the "free Fe". Such chelators are termed "hexidentate" [38]. Free metal ions which are released during degradation could bind to various metal chelators such as adenosine 5´-diphosphate (ADP), histidine, ethylenediaminetetraacetic acid (EDTA), citrate, etc. These chelators form complexes which catalyze the formation of ROS with different efficiency through the Fenton reaction [39].

TRANSPORT OF THE RELEASED METAL IONS WITH EMPHASIS IN FE

An important issue to be considered when the toxicity of degradation products is analyzed is the variation of local levels of metal ions concentrations [40,41] because high concentration of corrosion by-products could become trapped at the stent/tissue interface leading to cytotoxicity and migration of the ions through the *tunica intima*. On this respect, mass transport theory developed for drug eluting stents may provide some information about the movement of degradable mass of metals. The elution of the drug is the key issue in the drug-eluting stents but metal ions release is critical for biodegradable stents. Mass transport within the human vasculature can be broken up into two types. One of them is blood side mass transport, related to species transport within the vessel lumen which is subject of haemodynamics.

The second, and most important mode in relation to toxicity studies of bioabsorbable stents, is the transport within the wall of the artery, frequently referred as wall side mass transport (WSMT). The situation is complex because coronary arteries are usually heavily diseased and even a thin layer of plaque between the stent strut and the wall can inhibit WSMT. Simulations of the drug concentrations through the depth of the artery wall showed that concentrations can vary in one order with respect to the bulk within 0.04 mm depth. Moreover, after implantation, a clot will immediately develop once the strut becomes covered by the plasma proteins, altering the diffusion of ions. Thus, concentration distribution along the stent is heterogeneous, with important accumulation of ions in some places which may lead to cytotoxic effects.

In the bloodstream, serum transferrin has the specific role of transporting Fe from sites of absorption and haem degradation to sites of utilization and storage. This protein is able to bind tightly (affinity constant= 10^{19} - 10^{20} M^{-1}), but reversibly, two Fe^{3+} ions with concomitant binding of two carbonate anions. *In vitro*, Fe can be released from serum transferrin by acidification. Great number of other metals can bind to transferrins in addition

to Fe^{3+}, including Al^{3+}, Ga^{3+}, Cr^{3+}, Cu^{2+}, Mn^{3+}, Co^{3+}, Cd^{2+}, Zn^{2+}, among others. Apotransferrin binds Fe rapidly and seems to be quite able to oxidize Fe^{2+} and incorporate it in the Fe^{3+} form. Fe cellular cycle involves the endocytosis of diferric transferrin bound to its receptor, which leads to Fe release within the endosome at pH values below 6, followed by recycling of apotransferrin and the transferrin receptor. In essentially all proliferating (both normal and malignant), differentiating and haemoglobin synthesizing mammalian cells, Fe uptake is mediated by transferrin receptors [38].

Another potential source of Fe for cells is a receptor-independent uptake of Fe from transferrin. Furthermore, evidence indicates the existence of a transferrin-independent cellular Fe-uptake system and a tissue-distribution pattern that depends on the presence or absence of transferrin.

Non-transferrin bound iron (NTBI) are found in serum mainly complexed to citrate. Strictly speaking, NTBI corresponds to Fe which is not only unbound to transferrin but also does not correspond to heme or ferritin Fe. This Fe is thought to be much more reactive and available than transferrin-bound Fe, and to pose a greater potential toxicity. NTBI uptake may involve more than one transport system [38].

Thus, Fe enters the cell, via de transferrin receptor 1 pathway, through endocytotic vesicles and is released into de cytosol. Ferritin- bound Fe represents the major form of storage Fe, with each molecule of ferritin being capable of storing up to 4500 Fe atoms. Another form of intracellular Fe is the the transit iron pool or labile iron pool (LIP). It corresponds to the Fe species exerting a pivotal role between the vesicular storage, and functional Fe compartments. This pool of Fe consists of chemical forms of Fe that can participate in redox cycling and are associated with oxidative stress [42].

METAL IONS- MEDIATED ADVERSE REACTIONS

Specific differences between the toxicity of the components of metallic biomaterials may be related to differences in solubility, adsorbability, transport, chemical reactivity and the complexes that are formed in the biological medium [43]. Fe, Cu, Cr, V and Co undergo redox-cycling reactions. A second group of metals, Hg, Cd and Ni, the primary route for toxicity is depletion of glutathione and bonding to sulfhydryl groups of proteins. Arsenic (As) is thought to bind directly to critical thiols. However, the unifying factor in determining toxicity and carcinogenicity for all these elements is the generation of ROS and RNS. Common mechanisms involving the Fenton reaction, generation of the superoxide radical ($O_2^{\bullet -}$) and the hydroxyl radical ($HO^{\bullet}$), appear to be involved for Fe, Cu, Cr, V and Co primarily associated with mitochondria, microsomes and peroxisomes [44].

Metal-mediated formation of free radicals causes various modifications to DNA bases and proteins, enhances lipid peroxidation, and alteres calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adduct.

Reactive radical species include a wide range of oxygen-, carbon-, sulfur- radicals, originated from $O_2^{\bullet -}$ radical, H_2O_2 and lipid peroxides but also from chelates of amino-acids, peptides, and proteins complexed with the toxic metals.

METAL IONS AND ROS GENERATION

Fe ions

It is well known that Fe is an important component of proteins such as hemoglobin, myoglobin and cytochrome and also participates in the exchange of oxygen and carbon dioxide and promotes the transport of lipids in blood [45, 46]. As we mentioned above, major portion of Fe in circulation is associated with transferrin which prevent the existence of free Fe. Almost all forms of life require Fe but this element, under certain conditions, has unfavorable chemical properties that lead to the formation of insoluble ferri-hydroxide polymers and toxic free radicals. Molecules having one or more unpaired electrons are termed free radicals: they are generally very reactive, and will act as chain carriers in chemical reactions. Thus, the hydrogen atom, with one unpaired electron, is a free radical, as are most transition metals and the oxygen molecule itself [38]. When a single electron is accepted by the ground-state O_2 molecule, it must enter one of the p∗ antibonding orbitals, to form the $O_2^{\bullet -}$. Addition of a second electron to $O_2^{\bullet -}$ gives the peroxide ion (O_2^{2-}) with no unpaired electrons. At physiological pH, O_2^{2-} will immediately protonate to give H_2O_2. The third reactive oxygen species found in biological systems is $HO^{\bullet}$. Two $HO^{\bullet}$ can be formed by homolytic fission of the O–O bond in H_2O_2, either by heating or by irradiation. However, as Fenton first observed in 1894 [47], a simple mixture of H_2O_2 and Fe^{2+} salt also produces the $HO^{\bullet}$ radical (equation 7):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-} \quad (7)$$

In the presence of trace amounts of Fe, $O_2^{\bullet -}$ can then reduce Fe^{3+} to molecular oxygen and Fe^{2+}.

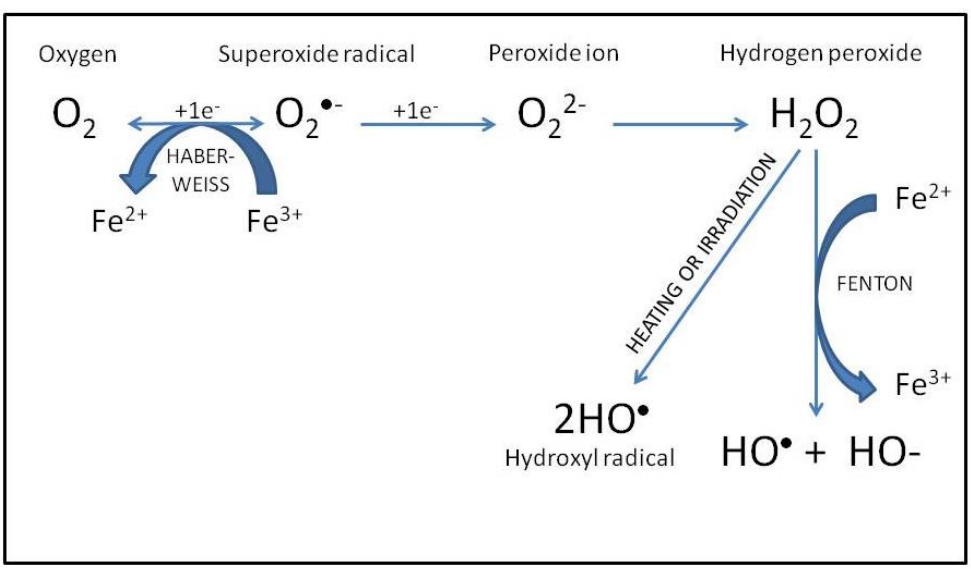

Figure 2. Reactive species formation.

The sum of this reaction (equation 8) plus the Fenton reaction (equation 7) produces O_2 plus $HO^{\bullet}$, plus OH^- from $O_2^{\bullet-}$ and H_2O_2, in the presence of catalytic amounts of Fe, the so-called Haber–Weiss reaction [48] (equation 9).

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2 \quad (8)$$

$$O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + HO^{\bullet} + OH^- \quad (9)$$

The generation of the mentioned reactive species and the role of Fe in these reactions is schematized in Figure 2.

It should be noted that this proposed sequence requires that the reaction would occur under standard conditions reaching equilibrium state, which is rarely the case for biological systems. A simple example will illustrate the problem, whereas under standard conditions, reaction 8 has a redox potential of −330 mV (at an O_2 pressure= 1 atm), *in vivo* with $[O_2] = 3.5 \times 10^{-5}$ M and $[O_2^{\bullet-}] = 10^{-11}$ M the redox potential is +230 mV [38].

Thus, "free" Fe^{2+} may catalyze a variety of free radical oxidative reactions which in turn lead to various degenerative changes (lipid peroxidation, changes in the composition and fluidity of cell membrane proteins and DNA alterations) [49]. Accordingly, when toxicity of metal ions from bioadsorbable materials is assessed, degradation rate, ion transport and possible accumulation in human vasculature should be taken into account. However, it is worth noting, that the biological damage is not only owed to the presence of ions but also to other parameters such as pH changes.

Other Biodegradable Metals

As previously mentioned, one of the attractive features of biodegradable metal materials is their ability to serve as a temporary scaffold for biological tissue growth and degrade thereafter [50]. Several metallic materials have emerged as a potential alternative to permanent metal devices, because they possess the ability of degrading at physiological environment. In addition to Fe, extensively described previously, Mg-based materials are other of the promising biodegradable metals [7]. Diverse Mg alloys have been explored in an effort to control their degradation rate to increase their applicability [8-10], but in some cases chemical and biological effects at biomaterial-tissue interface, were observed.

Al and Cu are some of the alloying elements frequently present in different Mg alloys. However, they may induce cellular damage by direct or indirect generation of free radicals through various mechanisms. Among these mechanisms, Fenton– and Haber–Weiss type reactions are the most common, leading to generation of the $O_2^{\bullet-}$ and $HO^{\bullet}$ radicals. Even though Al is in principle a non-redox metal, it is well known [51] that it can exert a significant pro-oxidant activity. An early hypothesis by Exley [52] established that central to this ability was the possibility of stabilization by Al^{3+} of $O_2^{\bullet-}$. This could eventually lead to the formation of various ROS either by a direct pathway with formation of the •OOH radical, either indirectly by influencing the redox equilibrium in the Fenton reaction.

Cu, the main component of Cu-based IUD, can induce oxidative stress by two mechanisms depending on its concentration level. It can directly catalyze the formation of

ROS via a Fenton-like reaction [53, 54] for low concentrations or can significantly decrease glutathione levels at higher levels [55].

Cu ions (cupric and cuprous) can act in both oxidation and reduction reactions. Cu^{2+} in the presence of $O_2^{\bullet-}$ (reaction 11) or biological reductants, such as ascorbic acid or reduced glutathione (GSH), can be reduced to Cu^{+} which is capable of catalyzing the formation of reactive $OH^{\bullet}$ through the decomposition of H_2O_2 via the Fenton reaction (reaction 10) [56-58].

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{-} \quad (10)$$

$$Cu^{2+} + O_2^{\bullet-} \rightarrow Cu^{+} + O_2 \quad (11)$$

The $OH^{\bullet}$ is extremely reactive and can further react with practically any biological molecules in the near vicinity. Cu is also capable of causing DNA strand breaks and oxidation of bases via ROS. Cu in both oxidation states (cupric or cuprous) was more active than Fe in enhancing DNA breakage induced by the genotoxic benzene metabolite 1,2,4-benzenetriol. DNA damage occurred mainly by a site-specific Fenton reaction [59].

GSH is a substrate for several enzymes that removes ROS and is also a powerful cellular antioxidant present in the cells in millimolar concentration. It has multiple functions in intracellular Cu metabolism and detoxification. GSH can suppress Cu toxicity by directly chelating the metal [60] and maintaining it in a reduced state making it unavailable for redox cycling. Disruption of Cu homeostasis resulting in elevated pools of Cu may contribute to a shift in redox balance towards more oxidizing environment by depleting GSH levels [61]. The depletion of GSH may enhance the cytotoxic effect of ROS and allow the metal to be more catalytically active, thus producing higher levels of ROS. The large increase in Cu toxicity following GSH depletion clearly demonstrates that GSH, is an important cellular antioxidant acting against Cu toxicity [62].

A new generation of biomaterials in the nanoscale has been developed in the last years. Inorganic nanomaterials from metals and derivatives are also potentially degradable biomaterials for biomedical applications. However, cyto- and genotoxicity have been detected for these nanoparticles, the origin of nanotoxicity have been frequently attributed to ROS generation and oxidative stress [63]. He et al. [64] provide direct evidence of ROS generation during decomposition of H_2O_2 assisted by Ag nanoparticles. Additionaly, Setyawati et al. [65] showed than ZnO nanoparticles induced cytotoxicity on several cellular systems by ROS way. Interesting, at low concentrations these nanoparticles induce ROS and p53 triggers expression of antioxidant genes to restore oxidative homeostasis while at higher concentrations apoptosis of cells due to the elevated level of intracellular ROS was found.

CONCLUSION

- Biodegradation of metals induces the accumulation of ions at the metal/tissue interface.
- Released ions are involved in conformational changes of biomolecules.

- Trace amounts of metals may catalyze the production of ROS by Fenton or Haber-Weiss reactions
- ROS, in turn, induce peroxidation of lipids, proteins and DNA. This situation is associated to alteration of membranes, enzymes and proteins that can result in cell injury and death.

Importantly, metal-induced and metal-enhanced formation of free radicals and other reactive species may be a common factor in determining metal-induced toxicity and carcinogenicity.

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