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BIODEGRADABLE MAGNESIUM ALLOYS: COMPARISON OF CORROSION RATE IN VITRO AND IN VIVO

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Recent years there has been an increased interest in Mg-based alloys as a promising biodegradable implant material. In this connection, comparison of in vitro and in vivo degradation of magnesium alloys has become topical. This article discusses in detail the features of corrosion behavior and compares the corrosion rate of magnesium with different purity and various magnesium alloys in simulated body fluids (SBF) solutions (in vitro) and directly in the body (in vivo). It has been shown that corrosion of biodegradable magnesium alloys in the aqueous environment of the body (in vivo) is not as straight forward as corrosion in various media, simulating a biological fluid (in vitro). Moreover, for Mg alloys, the in vivo corrosion rate is 1–6 times lower compared with in vitro corrosion rate in SBF solutions. It should be noted that pure Mg has the lowest average value of the corrosion rate in vitro. As for alloying, Mg alloys with rare earth elements (Mg-RE) showed the greatest corrosion resistance in vitro and in vivo. At the same time, the average corrosion rate of Mg-RE alloys in vivo is 6 times less than in vitro.

INTRODUCTION

For today, the creation of new generation of implants is a very important task. However, the development of treatment technology with the use of implants, which should function in the body for a long or limited period, caused an acute problem of creating materials with a wide range of requirements on chemical, mechanical and biological characteristics. The main requirements include the absence of undesirable chemical reactions with tissues and inter-tissue fluid, the absence of corrosion or dissolution at a controlled rate, high strength and plastic properties, crack resistance, resistance to delayed destruction, wear resistance, lack of reactions from the immune system (biocompatibility), integration of the implant in bone tissue (osteointegration), stimulation of bone formation (osteoconductivity) [1–12].

Of course, the choice of materials for creation different type of implants is very large. However, in this paper, the choice was made in favor of magnesium-based alloys. This can be explained by the following. Magnesium is a biodegradable and bioresorbable material with very light weight and, therefore, it is widely tested as a potential implant material [12]. Magnesium-based alloys are already successfully used in medical practice and are a promising direction in orthopedics and traumatology due to low corrosion resistance, or more exactly high solubility in biological tissues [1–4, 7, 10, 11, 13]. Biodegradable magnesium alloys have a great potential because they are completely biocompatible, have mechanical properties similar to the mechanical properties of natural bone, do not cause an inflammatory reaction, have the ability to osteointegration and stimulate the growth of new bone tissue [8–11]. Moreover, due to their ability to dissolve, there is no need for repeated surgery to remove the implant [7, 10, 11]. And, it should be noted that the threat of oversaturation with magnesium is low due to the effective excretion of the element with urine [8, 11].

PECULIARITIES OF CORROSION BEHAVIOR

The degradation of biodegradable metals in physiological settings occurs through a series of anodic and cathodic reactions (herein, the terms "degradation" and "corrosion" convey the same meaning) [11]. In general, the degradation of a metal occurs through corrosion involving conversion of the metallic material to a more stable ion. Contact with body fluid results in oxidization of the metal, with the generated electrons being consumed by cathodic reactions. These reactions lead to the release of hydrogen gas along with hydroxide, resulting in the formation of a protective metal oxide layer on the surface. For magnesium alloys, the high concentration of chloride ions in body fluid weakens this protective oxide layer and accelerates the degradation process. As this process proceeds, saturated calcium and phosphate in the body fluid and local alkalization lead to calcium phosphate deposition on the metal oxide layer, which allows cells to adhere on the surface to form tissues [11].

However, it should be noted that corrosion of biodegradable magnesium alloys in the aqueous environment of the body (in vivo) is not as straight forward as corrosion in various media (including infusion solutions for medical purposes such as saline solution), simulating a biological fluid (in vitro). This is due to the corrosion rate being influenced by a variety of other factors such as: the pH of body fluids; variations in the pH value; concentration of ions; the presence of proteins and protein adsorption on the orthopedic implant; the influence of the surrounding tissues [4]. For medical applications, the human body is too aggressive corrosive environment for pure magnesium. The body fluids are composed of water,

dissolved oxygen, proteins and electrolytic ions such as chloride and hydroxide. In this environment, magnesium with a negative electrochemical potential of -2.37 V, is very susceptible to corrosion and results in free ions migrating from the metal surface into the surrounding fluid environment. The migration of ions leads to the formation on the metal surface the layer of magnesium hydroxide $Mg(OH)_2$, which slows corrosion.

These films of $Mg(OH)_2$ are slightly soluble in water, however severe corrosion occurs in aqueous physiological environments where chloride ions are present at levels on the order of 150 mmol/l, as $Mg(OH)_2$ reacts with Cl^- to form highly soluble magnesium chloride and hydrogen gas [8]. Pitting of magnesium is observed for Cl^- concentrations exceeding 30 mmol/l [8]. The continuous migration of ions from the metal surface into the solution leads to the further formation of an oxide layer. When the oxide layer fully covers and seals the metal surface, it forms a kinetic barrier or passive layer that physically limits or prevents further migration of ionic species across the metal oxide solution interface.

An important property of the oxide layer is its ability to remain fixed to the metal surface during a variety of mechanical loading situations. If the oxide layer ruptures during mechanical loading it will expose the pure Mg substrate to body fluids which will result in further corrosion. The clinical repercussion of the corrosion process is the loss of mechanical strength and the ultimate failure of the implant [4].

COMPARISON OF IN VITRO AND IN VIVO DEGRADATION

However, to compare the corrosion rate of magnesium in vitro and in vivo, it is necessary to understand in more detail the peculiarities of corrosion in various electrolytes and in the body. The corrosive environment of the human body consists of a 0.14 M NaCl solution with small amounts of other inorganic species, such as Ca^{2+}, PO_4^{3-}, and HCO_3^- [5]. The presence of chloride ions typically leads to accelerated corrosion, whereas phosphates and carbonates may promote the formation of protective or partially protective corrosion product layers. The body temperature of 37 °C can somewhat accelerate the corrosion reactions compared to the room temperature, but the temperature also has an influence on precipitation of various types of Ca-phosphates from the body fluids. In addition to temperature, the pH of various solutions simulating the body's environment and the change in pH during the dissolution process has a great influence on the corrosion rate of magnesium [5]. The normal pH of blood is 7.4, buffered by the CO_2/HCO_3^- system. However, local variations of the pH value can occur in the process of dissolving. In addition to the variety of inorganic components of body fluids, the presence of organic components such as biomolecules, proteins, cells, or bacteria, can further influence corrosion reactions. These different factors show a variety of interactions with the corroding Mg alloy surface and with each other, making the corrosion scenario highly complex. When analyzing the processes occurring during the interaction of the corrosive surface of magnesium alloys and the biological environment, the following factors should be considered [5]:

1. Mg corrosion leads to release of Mg^{2+}, H_2 (g) generation and alkalization.
2. OH^- ions produced by the cathodic reaction associate with Mg dissolution is detrimental to cells.
3. The pH increase promotes precipitation of Ca-phosphate on the alloy surface.
4. The increased alkalinity can kill bacteria.
5. Carbonates are incorporated in the growing surface layers.
6. Chloride ions attack the alloy surface, and $MgCl_2$ forms.
7. Cells and proteins adhere to the Mg surface. Cells on the surface produce lactic acid.
8. Proteins in solution can complex Mg^{2+} cations.

A large number of experiments have been carried out to study the corrosion of pure magnesium and various Mg-based alloys in SBF solutions (in vitro) and directly in the body (in vivo). Tables 1 and 2 compare the corrosion rates of pure magnesium and various magnesium alloys in vitro and in vivo. In Table 1, the corrosion rate is given in units taken from the original articles, and in Table 2 the rate is recalculated into the same units ($mg \cdot cm^{-2} \cdot day^{-1}$) to enable comparison of data from different authors.

Table 1

Corrosion rate of pure magnesium and magnesium alloys in various electrolytes and in the environment of the body (units of measure are taken from the original source)

Material	In vitro corrosion (electrolyte)			In vivo corrosion (in the environment of the body)
	Hanks Solution/ Nor's solution*	Simulated Body Fluid	NaCl	
Pure Mg (HP is high-purity, LP is low-purity, UP is ultra-high-purity)	0.011 ($mg \cdot cm^{-2} \cdot h^{-1}$) 99.95% Mg [4] 0.9* (mm/year) (HP) [14] 0.3* (mm/year) (HP) [6] 0.5* (mm/year) (HP) [6] 0.4* (mm/year) (UP) [6]	0.038 ($mg \cdot cm^{-2} \cdot h^{-1}$) 99.95% Mg [4]	6.4 ($mg \cdot cm^{-2} \cdot day^{-1}$) (99.99% Mg, 1 M NaCl) [5] 0.8 (mm/year) (HP Mg, 0.1 M NaCl) [6] 18 (mm/year) (pure Mg, 3.5 wt.% NaCl) [15] 0.6 (mm/year) (HP Mg,	1.0 (mm/year) (HP Mg, 30 days) [14] 0.7 (mm/year) (HP Mg, 60 days) [14]

			3.5 wt.%. NaCl) [16] 0.9 (mm/year) (HP Mg, 3 wt.% NaCl) [17] 1.1 (mm/year) (HP Mg, 1 M NaCl) [18] 50 (mm/year) (LP Mg, 1 M NaCl) [18] 0.25 (mm/year) (UP Mg, 3.5 wt.% NaCl) [19] 0.3 (mm/year) (UP Mg, 3.5 wt.% NaCl) [6] 0.4 (mm/year) (HP Mg, 3.5 wt.% NaCl) [6]	
Mg	0.36 (mm/year) (as-cast Mg) [20] 0.22 (mm/year) (as-rolled Mg) [20]	1.94 (mm/year) (as-cast Mg) [20] 0.84 (mm/year) (as-rolled Mg) [20]	2.5 (mm/year) (as-cast Mg, 9 g/l NaCl) [21]	
Mg–1Al	0.40 (mm/year) (as-cast Mg) [20] 3.90 (mm/year) (as-rolled Mg) [20]	0.36 (mm/year) (as-cast Mg) [20] 0.22 (mm/year) (as-rolled Mg) [20]		
Mg6Al			4 (mm/year) (3.5 wt.% NaCl) [16]	
AZ31	0.0065 ($mg \cdot cm^{-2} \cdot h^{-1}$) [4] 0.35 ($mg \cdot cm^{-2} \cdot h^{-1}$) [22]		102 ($mg \cdot cm^{-2} \cdot h^{-1}$) (3.5 wt.% NaCl) [23] 11.6 (mm/year) (3.5 wt.% NaCl) [16]	1.17 ($mg \cdot mm^{-2} \cdot year^{-1}$) [4]
AZ91	0.0028 ($mg \cdot cm^{-2} \cdot h^{-1}$) [4] 6* (mm/year) [14]	-0.267 (mm/year) [17]	8 (mm/year) (3 wt.% NaCl) [16]	1.38 ($mg \cdot mm^{-2} \cdot year^{-1}$) [4] $3.516 \cdot 10^{-4}$ (mm/year) [7] 0.7 (mm/year) (30 days) [14] 0.7 (mm/year) (60 days) [14]
LAE442		5.535 (mm/year) [7]		0.39 ($mg \cdot mm^{-2} \cdot year^{-1}$) [4] $1.205 \cdot 10^{-4}$ (mm/year) [7]
WE43		0.085 ($mg \cdot cm^{-2} \cdot h^{-1}$) [4] 38.41 (mm/year) (as-cast) [24]	5.84 (mm/year) (as-cast, 1 M NaCl) [25] 2.97 (mm/year) (extrusion, 1 M NaCl) [25] 0.26 ($mg \cdot cm^{-2} \cdot h^{-1}$) (3.5 wt.% NaCl) [26] 0.4 (mm/year) [27]	1.56 ($mg \cdot mm^{-2} \cdot year^{-1}$) [4]
Elektron21			1.43 ($mg \cdot cm^{-2} \cdot h^{-1}$) (3.5 wt.% NaCl) [26]	

*Nor's solution (CO_2-bicarbonate buffered Hanks' solution).

Table 2

Corrosion rate of pure magnesium and magnesium alloys in various electrolytes and in the environment of the body (units of measure are converted to $mg \cdot cm^{-2} \cdot day^{-1}$)***

Material	In vitro corrosion (electrolyte)			In vivo corrosion (in the environment of the body)
	Hanks Solution/ Nor's solution*	Simulated Body Fluid	NaCl	
Pure Mg (HP is high-purity, LP is low-purity, UP is ultra-high-purity)	0.264 (99.95% Mg) [4] 0.429* (HP) [14] 0.143* (HP) [6] 0.238* (HP) [6] 0.19* (UP) [6]	0.912 (99.95% Mg) [4]	6.4 (99.99% Mg, 1 M NaCl) [5] 0.381 (HP, 0.1 M NaCl) [6] 8.57 (pure Mg, 3.5 wt.% NaCl) [15] 0.286 (HP, 3.5 wt.% NaCl) [16] 0.429 (HP, 3 wt.% NaCl) [17] 0.524 (HP, 1 M NaCl) [18] 23.81** (LP, 1 M NaCl) [18] 0.119 (UP, 3.5 wt.% NaCl)[19] 0.143 (UP, 3.5 wt.% NaCl) [6] 0.190 (HP, 3.5 wt.% NaCl) [6]	0.476 (HP, 30 days) [14] 0.333 (HP, 60 days) [14]
Mg	0.171 (as-cast Mg) [20] 0.105 (as-rolled Mg) [20]	0.924 (as-cast Mg) [20] 0.4 (as-rolled Mg) [20]	1.19 (as-cast Mg, 9 g/L NaCl) [21]	
Pure Mg The average value of corrosion rate	1.018			0.405
Mg-1Al	0.190 (as-cast Mg) [20] 1.857 (as-rolled Mg) [20]	0.171 (as-cast Mg) [20] 0.105 (as-rolled Mg) [20]		
Mg6Al			1.905 (3.5 wt.% NaCl) [16]	
AZ31	0.156 [4] 8.4 [22]		2448** (3.5 wt.% NaCl) [23] 5.524 (3.5 wt.% NaCl) [16]	0.321 [4]
AZ91	0.0672 [4] 2.857* [14]	-0.127** [17]	3.809 (3 wt.% NaCl) [16]	0.379 [4] $1.674 \cdot 10^{-4}$** [7] 0.333 (30 days) [14] 0.333 (60 days) [14]
Mg-Al alloys The average value of corrosion rate	2.277			0.340
LAE442		2.636 [7]		0.106 [4] $0.573 \cdot 10^{-4}$** [7]
WE43		2.04 [4]	2.781	0.427 [4]

		18.29** (as-cast) [24]	(as-cast, 1 M NaCl) [25] 1.414 (extrusion, 1 M NaCl) [25] 6.24** (3.5 wt.% NaCl) [26] 0.19 [27]	
Elektron21			34.32** (3.5 wt.% NaCl) [26]	
Mg-RE alloys The average value of corrosion rate	1.812			0.267

*Nor's solution (CO_2-bicarbonate buffered Hanks' solution).
**Corrosion rates, which were not taken into account when calculating the average value of corrosion rate.
***The conversion of units was carried out taking into account the standard density of magnesium 1.738 g/cm^3 [28].

DISCUSSION

As we see, various types of simulated body fluids were used to study the biodegradation of Mg alloys in vitro. Unfortunately, there is no clear agreement on which chemical composition of the solution is the optimal medium for simulating in vivo conditions [5]. In experiments, it has often been observed that the corrosion rate of Mg alloys significantly varies depending on the type of simulated body fluid. In addition, even the type of buffer used can play a crucial role in determining the corrosion rate. This makes it difficult to directly compare various data in the literature.

Unfortunately, the role of various inorganic and organic compounds that are present in various modeled solutions and are involved in the process of magnesium biocorrosion is also not well understood and has not yet been clearly clarified [5]. It is clear that more complex corrosion products, such as different types of (Ca/Mg) phosphates and carbonates, form in simulated body fluids than in simple saline solutions. These corrosion products not only influence the corrosion rate, but for sparingly soluble corrosion products, complete degradation of the implant will not take place. Instead, a gradual conversion of the metallic material into corrosion products occurs.

Because the different simulated body fluids contain slightly different amounts of Ca^{2+}, carbonates/hydrocarbonates and phosphates, and the Mg^{2+} concentration will depend on the dissolution rate and mass transport, the specific conditions at the surface such as the local pH value can vary and different stoichiometries of corrosion product layers can precipitate [5]. These different types of precipitates can be expected to have different protection properties, which in turn will have a fairly strong effect on the dissolution rate of the implant.

As already noted, the corrosion of magnesium alloys in simulating biological solutions is a complex process. Some of the parameters to be considered include temperature, pH, buffer type, surrounding gas atmosphere, a high number of inorganic ions, amino acids, proteins, cells, hydrodynamic conditions [5]. Many studies have been carried out with the aim of exploring the influence of one of these parameters, but determination of the mechanisms is complicated by the fact that these parameters are not independent of each other. Moreover, many of the factors influencing Mg alloy corrosion may have multiple operative mechanisms. For example of proteins can both adsorb on the surface and/or complex metal cations. Depending on the system at hand, one of the operative mechanisms can be more dominant than the other.

The situation becomes even more complex when considering in vivo corrosion. At present, a limited amount of data is available on the in vivo behavior of Mg alloys, and especially systematic and direct comparisons of in vitro and in vivo degradation. An attempt was made to carefully analyze the existing literature in a recent review [5]. Interestingly, the in vivo corrosion rates showed a smaller range of values (between 0 and 1.5 mm/year (between 0 and 0.7 mg·cm^{-2}·day^{-1})) than in vitro corrosion rates (between 0.1 and 2 mm/year (between 0.05 and 1 mg·cm^{-2}·day^{-1})) [5]. In addition, for all alloys the corrosion rates were 1–6 times lower for in vivo than in vitro [5].

It is seen that in all types of in vitro tests, the corrosion rate is higher than biocorrosion in the body [6]. At the same time, biocorrosion rate significantly depends on the composition of the corrosive environment. This indicates that the corrosive environment and the mechanism of biocorrosion during in vitro tests do not fully correspond to the processes occurring in the body [6].

As we can see from Table 2, the purity of the material has a great effect on corrosion, thus ultra-pure magnesium has the lowest corrosion rate of 0.19 mg·cm^{-2}·day^{-1} in the Nor's solution [6] and 0.119 mg·cm^{-2}·day^{-1} in 3.5% NaCl [19]. In this case, in general, pure magnesium has the lowest average value of the corrosion rate in vitro 1.018 mg·cm^{-2}·day^{-1}. It should be noted (see Table 2) that the average corrosion rate of pure magnesium in vivo is 2.5 times less (0.405 mg·cm^{-2}·day^{-1}). Alloying magnesium alloys with

aluminum leads to increased corrosion rates in vitro (2.277 $mg \cdot cm^{-2} \cdot day^{-1}$). At the same time, the average corrosion rate of Mg-Al alloys in vivo is 6 times less (0.340 $mg \cdot cm^{-2} \cdot day^{-1}$). Alloys with rare earth elements showed the greatest corrosion resistance in vitro and in vivo (1.812 and 0.267 $mg \cdot cm^{-2} \cdot day^{-1}$, respectively). At the same time, the average corrosion rate of Mg-RE alloys in vivo is 6 times less than in vitro. The data in Table 2 are in good agreement with the review data [5].

The correlation between the in vitro and in vivo corrosion rates also strongly depends on the electrolyte chosen for the laboratory studies. While some possible reasons for the lower corrosion rates observed in vivo can be identified, such as a lower chloride concentration in blood plasma as compared to most synthetic media, or the presence of proteins and cells, many unknowns remain. The authors emphasized [5], that the anatomical location of the implant as well as the surgical procedure can also influence the subsequent degradation behavior. Hence, there is lack of information about the detailed in vivo conditions that would enable understanding of the origin of the different corrosion rates observed.

RESULTS

Summarizing, we can conclude that:

1. There is no clear agreement on which chemical composition of the solution is the optimal medium for simulating in vivo conditions. Data from laboratory studies of in vitro corrosion of magnesium alloys, firstly, significantly vary depending on the composition of the selected corrosive environment and, secondly, can overestimate the measured corrosion rate several times in comparison with experiment in the body (in vivo).

2. For magnesium alloys, the in vivo corrosion rate is 1–6 times lower compared with in vitro corrosion rate in SBF solutions.

3. The purity of the material has a great effect on corrosion, namely, the higher the purity of magnesium, the lower its corrosion rate. Thus ultra-pure magnesium has the lowest corrosion rate of 0.19 $mg \cdot cm^{-2} \cdot day^{-1}$ in Nor's solution and 0.119 $mg \cdot cm^{-2} \cdot day^{-1}$ in 3.5% NaCl. The greatest corrosion resistance of magnesium alloys with rare earth elements in vitro and in vivo are 1.812 $mg \cdot cm^{-2} \cdot day^{-1}$ and 0.267 $mg \cdot cm^{-2} \cdot day^{-1}$, respectively.

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БІОРОЗЧИННІ СПЛАВИ МАГНІЮ: ПОРІВНЯННЯ ШВИДКОСТІ КОРОЗІЇ IN VITRO ТА IN VIVO

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В останні роки спостерігається підвищений інтерес до сплавів на основі магнію як перспективного біорозчинного матеріалу для імплантатів. У зв'язку з цим актуальним стало порівняння деградації магнієвих сплавів in vitro та in vivo. Детально розглядаються особливості корозійної поведінки та порівнюється швидкість корозії магнію різної чистоти та різних магнієвих сплавів у розчинах (in vitro) модельованих рідин організму (МРО) та безпосередньо в організмі (in vivo). Було показано, що корозія біорозчинних магнієвих сплавів у водному середовищі організму (in vivo) не є настільки однозначною, як корозія в різних середовищах, що імітують біологічну рідину (in vitro). Крім того, для магнієвих сплавів швидкість корозії in vivo в 1–6 разів нижча, як порівняти зі швидкістю корозії in vitro в розчинах МРО. Слід зазначити, що чистий Mg має найнижче середнє значення швидкості корозії in vitro. Що стосується легування, то магнієві сплави з рідкісно-земельними елементами (Mg-РЗ) показали найбільшу корозійну стійкість in vitro та in vivo. При цьому середня швидкість корозії сплавів Mg-РЗ in vivo в 6 разів менша, ніж in vitro.