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Magnesium degradation under physiological conditions – Best practice

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ABSTRACT

This review focusses on the application of physiological conditions for the mechanistic understanding of magnesium degradation. Despite the undisputed relevance of simplified laboratory setups for alloy screening purposes, realistic and predictive *in vitro* setups are needed. Due to the complexity of these systems, the review gives an overview about technical measures, defines some caveats and can be used as a guideline for the establishment of harmonized laboratory approaches.

1. Introduction

Research about degradable magnesium alloys is of increasing interest for material scientists, biologists and clinicians. As the first products are already clinically available [1], a higher awareness of this topic has been achieved. Unfortunately, not too many research groups are interested in getting a mechanistic insight into the underlying processes due to the high complexity of the degradation process under physiological conditions. A main reason for this is the comparably aggressive environment – salt-containing fluids, the presence of sugars and proteins and the application of cell culture conditions. Moreover, one additional quite difficult aspect is to keep sterility of the systems.

1.1. Why are physiological conditions so important?

The easiest and most astonishing experiment to prove this specific importance is the direct observation of the sample morphology after immersion in pure/deionized water and cell culture conditions (Fig. 1). While the samples under atmospheric conditions exhibit a black surface, typically consisting of $Mg(OH)_2$, the samples immersed under cell culture conditions show many precipitates, which could be identified as $MgCO_3$ [2,3]. Additionally, the introduction of cell culture conditions accelerates the degradation rate of all materials, as monitored by the increase of osmolality.

Despite the fact that cell-based experiments are conducted under physiological conditions, the environment has a considerable influence on the degradation behaviour of various materials. This is not only applicable to degradable metals (i.e. magnesium, iron, zinc, tungsten), but also to degradable polymers. As it was stated in a leading opinion paper [4], even for general testing of materials physiological conditions should be applied when using, e.g. simulated body fluids.

In the case of degradable materials, this is even more interesting, as a continuously changing interface between material and cells is developing over time. To understand this development is of utmost importance in this research area. Also, the biological clues (e.g. cellular communication, the interaction of various cell types, material – protein interactions) have to be analysed, as they additionally will have an impact on the material degradation.

This manuscript aims to give an overview about physiological experimental setups, state some caveats and to help harmonising laboratory approaches.

2. Simulated or not? The question of appropriate conditions

To choose a suitable physiological solution, it is a critical point to

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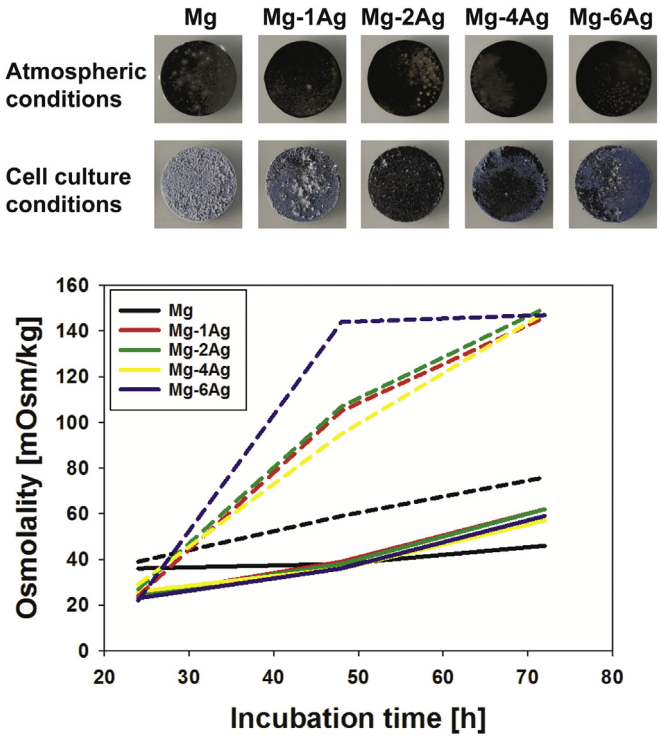

Fig. 1. Upper panel: optical morphology of different materials (10 mm diameter) after 72 h incubation in distilled water. **Lower panel:** Measurement of the change of osmolality during the 72 h immersion. Solid lines: experiment performed under atmospheric condition, dashed lines: cell culture conditions (PhD thesis Die Ti, unpublished results).

evaluate the degradation of Mg alloys and to obtain comparable *in vitro* results to *in vivo* tests. Simulated physiological solutions with increasing complexity were used to determine the degradation of Mg: from 0.9% NaCl solution, Hanks balanced salt solution (HBSS), simulated body fluid (SBF), to cell culture medium. Different simulated solutions used result not only in different degradation rates of Mg [5], but also different degradation products [6,7], suggesting different degradation pathways and degradation mechanism. Therefore, the choice of a suitable solution for the evaluation of Mg degradation is of utmost importance.

When a Mg alloy is immersed in a physiological medium, the contact between the fresh surface and an electrolyte-containing aqueous medium lead to higher initial corrosion rates. This process involves the release of hydrogen and the alkalinization of the environment as the net reaction shows:

$$\textit{Anodic reaction}: \quad Mg \rightarrow Mg^{2+} + 2e^{-} \tag{1}$$

$$\textit{Cathodic reaction}: \quad 2H_2O + 2e^{-} \rightarrow 2OH^{-} + H_2\uparrow \tag{2}$$

$$\textit{Overall reaction}: \quad Mg + 2H_2O \rightleftharpoons Mg^{2+} + 2OH^{-} + H_2\uparrow \tag{3}$$

The formed $Mg(OH)_2$ is the first product in the degradation process and readily precipitates because of its low solubility of 12 mg/L in water. The strengthening and dissolution of this layer depend further on the other elements present in the electrolyte and the time of immersion. However, it has been shown that MgO, $Mg(OH)_2$ and $MgCO_3$ are the main degradation products formed with the application of HBSS, SBF and Dulbecco's modified eagle medium (DMEM) [3,8]. Additionally, the solubility of the various phases is dependent on environmental factors like temperature, pH and magnesium dissolution [2].

A suitable simulated solution, therefore, should contain three essential parts: appropriate inorganic ingredients, a buffering system and organic components. The detailed compositions of several common simulated physiological solutions and plasma are compared in Table 1 with the blood plasma composition. There are some reports to study the degradation of Mg in physiological saline (0.9% NaCl) solution [9,10], but they will not be discussed, as the results obtained with physiological saline solutions are far away or even contradictory from that obtained under physiological conditions [11]. To gain closer physiological conditions results, simulated body fluids (SBF) and Hanks' solution are widely used to determine the degradation rate of Mg, as they have a similar inorganic ion composition compared to plasma. SBF was developed as a solution for *in vitro* measurement of apatite-forming ability on implant materials and several improved recipes are available (Technical Committee ISO/TC150) [12,13]. Therefore it is of utmost importance to state the exact composition or to cite the original publication in the materials and methods part as a guide for the readers [14]. Compared with other solutions showed in Table 1, SBF has a closer composition to plasma. However, a significant amount of Ca and Mg ions present in plasma is bound to proteins, which should be taken into consideration due to the absence of organic compounds in SBF. Moreover, Ca^{2+} ions in combination with a high concentration of HCO_3^- can largely affect the degradation behaviour of Mg under cell culture condition [15].

Another critical parameter is the buffering system. A good simulated body solution should possess the similar buffering capacity to that of body plasma. Blood pH is regulated by (a) the open system HCO_3^-/CO_2 adjusted by the respiration via the lungs, (b) plasma protein buffers (HPr/Pr^-) and (c) a low concentration of phosphate [12]. However, the most common buffers for simulated body fluids used are (a) 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), (b) Tris-HCl, (c) $CO_2/NaHCO_3$ and (d) phosphate. HEPES and TRIS were introduced in the 1960's by Good et al. [32] for systems without CO_2-buffering.

The phosphate buffering contribution in human body is low and only significant in the urine and in the intracellular fluid, due to its low concentration. The too high concentration of phosphate alters the chemical properties of the corrosion layer, as they can produce insoluble salts with magnesium ions and eventually precipitating on the surface, thereby leading to a different degradation performance compared to *in vivo* conditions [31,33]. Therefore, PBS is not suitable to simulate or predict degradation behaviour of Mg alloys under *in vivo* conditions. In addition, PBS also should be avoided for live/dead experiment or critical point drying due to the change of surface condition, as shown in Fig. 2.

Under the same conditions, HEPES increases the corrosion rate of pure Mg by a factor of up to four times compared with $NaHCO_3$ buffering alone not only in simple salt solutions but also in EBSS and DMEM [7,25,34–36]. For WZ21 alloy, this factor increased to approximately 60 in SBF buffered with HEPES (100 mmol/L) compared with that buffered with $CO_2/NaHCO_3$ [18]. Moreover, HEPES in testing solutions reduces the formation of calcium phosphate and carbonate in the degradation layer by influencing the nucleation processes [25,34]. Therefore, HEPES destabilises the protective layer, generating a less dense degradation layer and allowing the progressive diffusion of aggressive ions like Cl^- [37]. Also on glass-ceramics it was shown that HEPES leads to a selective dissolution of Ca-containing phases and is therefore also for this class of degradable materials not recommended [38].

Tris is also one common buffering used in simulated body fluid, which also accelerates the degradation rate of pure Mg by a factor of ten during earlier stage exposure due to the consumption of OH^- [39]. Moreover, when Tris-HCl is present in SBF, pure magnesium is