Magnesium Technology 2011
Edited by: Wim H. Sillekens, Sean R. Agnew, Neale R. Neelameggham, and Suveen N. Mathaudhu
TMS (The Minerals, Metals & Materials Society), 2011

CORROSION, SURFACE MODIFICATION, AND BIOCOMPATIBILITY OF MG AND MG ALLOYS

Sannakaisa Virtanen[1], Ben Fabry[2]
[1]University of Erlangen-Nuremberg, Institute for Surface Science and Corrosion, Department of Materials Science (WW-4), Martensstrasse 7, 91058 Erlangen, Germany
[2]University of Erlangen-Nuremberg, Center for Medical Physics and Technology, Department of Physics, Henkestraße 91, 91052 Erlangen, Germany

Keywords: Magnesium, corrosion, biodegradation, biocompatibility

Abstract

This manuscript summarizes recent studies on the corrosion behavior of Mg and Mg alloys in simulated biological environments, as well as interactions between corroding Mg (alloy) surfaces and cells. The influence of different types of simulated body solutions on the corrosion behavior of Mg is discussed. The effects of different types of chemical surface treatments on cell adhesion and spreading is presented. Moreover, possible routes to further optimize the corrosion and the biological performance of Mg alloys by surface modification are discussed.

Introduction

Mg-based materials corrode in aqueous biological environments and are of growing interest for use as biodegradable implants (see e.g. reviews [1,2]). For a successful and biologically safe application, a thorough understanding of the corrosion behavior in (simulated) physiological environments is required. In spite of the increasing number of publications in this field, many open questions still exist. In particular, detailed mechanisms of the effects of proteins and cells on Mg corrosion are unknown. Concerning the effects of proteins on the dissolution rate of Mg alloys, contradicting effects have been reported. For the Mg alloy AZ31, albumin addition has been found to show relatively little effect on the electrochemical behavior, but for pure Mg and the alloy LAE442 albumin addition led to strongly accelerated anodic dissolution [3]. On the other hand, corrosion inhibition by albumin addition has been reported for the AZ91 alloy in SBF [4], as well as for Mg-Ca alloy in water and in NaCl solutions [5]. These seemingly contradicting findings could be due to different experimental approaches, but may also indicate that albumin effects on corrosion are alloy-dependent.

Moreover, the effect of Mg dissolution on the biological environment needs to be characterized, as the corrosion reaction is coupled with H_2 gas liberation and surface alkalization. An increasing number of *in vitro* cell culture studies on corroding Mg alloy surfaces have been recently reported (see e.g., [6-11]. However, as different alloys and different cell lines have been used in these studies, it is not easy to compare the results from different studies or to draw general conclusions on the critical Mg alloy corrosion induced effects on the biological performance.

The aim of our studies is to further elucidate the interactions between corroding Mg (alloy) surfaces and cells, by an interdisciplinary approach of electrochemistry, surface modification and analysis, and cell culture testing. Of special interest is the development of novel chemical and biological surface functionalization methods, for tailoring the corrosion rate and biocompatibility.

Experimental

Experiments were carried out on cp-Mg (99.9%, Chempur) as well as on commercial WE43 and AZ91 Mg alloys. Corrosion behavior of the materials was studied in simulated body fluids (SBF 5[12]) and in cell culture medium [Dulbecco's Modified Eagle's cell culture medium (DMEM) with addition of 10% fetal bovine serum (FBS)].

Corrosion rates and modes were studied by Mg ion release measurements (atomic absorption spectroscopy), H_2 gas collection, pH measurements of the medium, electrochemical impedance spectroscopy, and various surface characterization methods.

Different types of surface treatments, including simple chemical passivation in 1 M NaOH or soaking in simulated body fluids, were explored regarding their ability to optimize the corrosion behavior and the biocompatibility. Moreover, functionalization of Mg surface by protein layers was explored. Surface coating with bovine serum albumin (BSA) was achieved by silanization of the surface with 3-aminopropyltriethoxysilane (APTES) and using an ascorbic acid (vitamin C) linker for attachment of BSA. For comparison, magnesium discs were directly incubated in aqueous BSA solution for 24 h at room temperature under gentle stirring of the solution. Details to the experimental procedures for preparation of the protein layers can be found in Ref. [13].

For the cell culture experiments, Mg samples were sterilized under UV irradiation with a wavelength of 260 nm. Cells from a human cervical cancer cell line (HeLa) were cultured in an incubator (5% CO_2, 37°C, 95% relative humidity) for 24 h in 24-well plates containing the differently treated Mg samples. DMEM with addition of 10% FBS and 100 U/ml Penicillin/Streptomycin was used as a cell culture medium. After 24 h in the incubator, the cells were fixed in 2% paraformaldehyde and stained with Alexa red phalloidin to visualize the actin cytoskeleton of the cells, and with Hoechst 33342 to visualize the cell nucleus. Fluorescence microscopy of the stained cells was carried out with a Leica DMI 6000B microscope.

Results and Discussion

Time-dependent corrosion behavior

Corrosion behavior of cp-Mg as well as Mg alloys was studied in SBF solutions as well as in cell culture medium. In agreement with findings from other laboratories [14,15], significantly lower corrosion rates were observed in cell culture medium than in SBF. The good corrosion resistance in cell culture medium has been brought into context of surface passivation by Mg-carbonates

[14,15]; however, detailed mechanisms on the reactions taking place on Mg surface in SBF vs. cell culture medium still need to be elucidated.

In previous studies, different types of simulated physiological solutions have been explored regarding their effect on the corrosion behavior of Mg alloys with an emphasis on the biodegradation behavior. A critical summary of the influence of the experimental parameters, including the electrolyte used, on the corrosion behavior of Mg alloys has been recently presented in [16]. A large number of investigations on Mg corrosion has been carried out in non-buffered NaCl solutions, however, these data have only limited value for understanding the corrosion behavior of Mg alloys in biological environments because body fluids are buffered. For Mg alloys, solution buffering is more critical than for many other metals, as a very strong alkaline pH shift takes place in the vicinity of corroding Mg surfaces. In non-buffered solutions, the pH can reach values >10-11 (starting from a neutral solution). In this case, the pH value is approaching the region of passivity of Mg [17]. Even though the presence of chloride ions in the environment hinders the formation of a stable passive surface, the high pH value suppresses the active dissolution rate. In simulated body fluids, buffered to pH 7.4, less strong pH effects occur. Therefore, different type of surface reactions take place on Mg in buffered or in non-buffered solutions.

Of course, not only buffering is important for the type of surface reactions taking place, but also the exact solution composition, as already discussed above in the case of SBF solutions versus cell culture medium. The nature of the corrosion layers changes from crystalline $Mg(OH)_2$ to an amorphous hydrated, carbonated (Mg,Ca)-phosphate in SBF [18]. The corrosion product layers from Mg and Mg alloys in SBF solutions are not highly protective, and active dissolution of Mg takes place even though the corrosion rate is gradually decreasing with time, with increasing coverage of the surface by the corrosion product layer [18,19].

Bovine serum albumin (BSA) addition showed a surprisingly complex time-dependent influence on the corrosion rate: in the first 6 h of immersion, a higher polarization resistance value was measured in presence of BSA in the solution, but during longer immersion times the polarization resistance again decreased, and for longer exposure times remained even lower than the polarization resistance in SBF in absence of albumin (Fig. 1). The initial increase of the polarization resistance could indicate adsorption of BSA on the surface, which partially blocks the dissolution reaction. The non-steady time-dependence suggests that the albumin adsorption layer changes with time, which is not surprising considering that the Mg alloy surface dissolves with a relatively fast rate during the experiment. This could for instance lead to removal of albumin from the surface. However, it is not clear why in the longer-term experiment the R_p-values remain significantly lower in the presence of albumin in the solution than in albumin-free solution. Clearly, a detailed surface characterization after different exposure times is required to understand the effects of albumin on the electrochemical behavior of Mg.

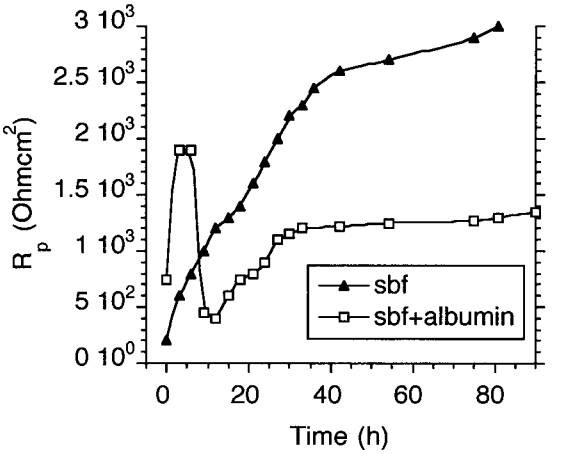

Fig. 1 Polarization resistance (R_p) values as a function of time in solution for the Mg alloy WE43. The R_p-values were determined from electrochemical impedance spectroscopy measurements in sbf or in sbf + 40 g/l bovine serum albumin (BSA) at 37°C.

Cell culturing on corroding Mg surfaces

For conventional cell culture experiments with direct adhesion of the cells on the sample surface, the surface of cp-Mg, without any surface modification was far too reactive for cell adhesion and survival. Even though strong retardation of the corrosion rate takes place for longer immersion times of Mg in cell culture medium, the initial corrosion rate was very high. Immediate hydrogen gas liberation and alkalinization of the culture medium could be observed. Cytotoxicity testing indicated that high Mg-concentrations - corresponding to the dissolution of the samples in the cell culture medium - did not reduce cell survival [20]. Rather, the single most important cause for reduced cell adhesion and survival was the increased pH in the cell culture medium due to Mg corrosion [21].

A viable strategy to achieve cell adhesion on corroding Mg (alloy) surfaces under *in vitro* cell culture conditions is to reduce the initial surface reactivity [22]. For instance, Figure 2 shows the effect of simple Mg surface passivation by soaking in 1 M NaOH. On the NaOH passivated surface, good spreading of the cells can be seen (2a). This in contrast to the polished Mg sample, in this case only very few non-spread cells were seen on the surface (2b). Although the $Mg(OH)_2$ passive film formed on Mg in NaOH is not stable in cell culture medium, it provides sufficient short-term protection to enable initial cell adhesion.

In general, all surface treatments that reduced the initial reactivity of the surface also increased the cell survival rate in our studies, but distinct differences were observed between different types of surface treatments as they altered not only surface reactivity but also chemistry, roughness, and wettability. For example, Mg samples incubated in SBF showed a biphasic behavior. During incubation in SBF, the Mg surface was covered by an amorphous hydrated, carbonated (Mg,Ca)-phosphate layer. Monitoring the cell behavior on such a surface as a function of time showed that initially strong cell spreading took place, but over time cell death

occurred. These findings indicate that the surface chemistry and roughness of coatings formed by soaking in SBF are initially beneficial for cell adhesion and spreading, but after longer times the high reactivity of the surface induces cell death, presumably due to the local surface pH increase as these coatings are porous and hence not highly protective. The best coating, until now, in view of an efficient reduction of the corrosion rate and a good biocompatibility was the layer formed by incubating the Mg sample in cell culture medium [20].

Electrochemical experiments (open-circuit potential measurements and impedance spectroscopy EIS) of Mg surfaces were carried out in the absence and presence of a cell adhesion layer on the surface [21]. The electrochemical response correlated with the spreading of the cell layer on the surface. The formation of a cell layer on the Mg alloy surface significantly reduced the corrosion rate, as indicated by a reduced pH increase with time for Mg samples covered with a cell layer.

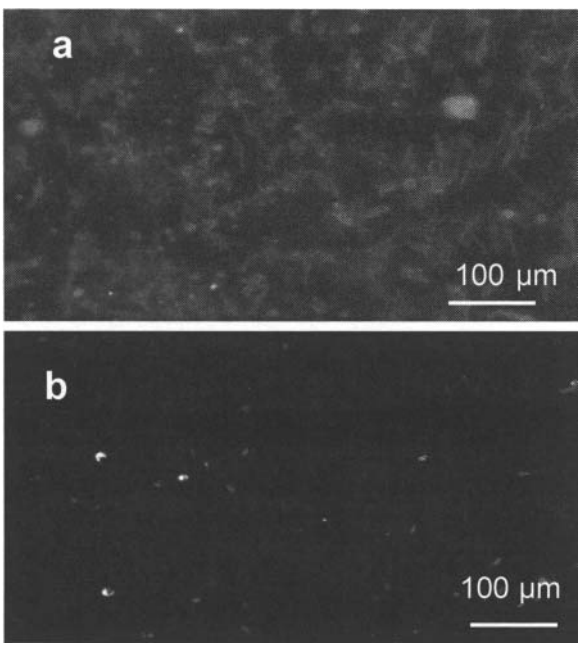

Fig. 2 Actin staining of HeLa cells on cp-Mg surfaces after 24 h culture. a: Mg surface passivated for 24 h in 1 M NaOH prior to cell culture testing; b: polished Mg surface.

Functionalization of Mg surface by proteins

As shown in Fig. 1, protein adsorption on Mg surfaces can have a significant effect on the corrosion behavior. We further studied protein adsorption on Mg surfaces, and also explored the possibility to use protein adsorption layers as a pre-treatment for tailoring the corrosion behavior and biocompatibility. First trials to simply coat Mg by directly soaking in BSA containing aqueous solutions were only partially successful; even though BSA adsorption took place, this was accompanied by corrosion (and H_2 liberation), and hence no homogeneous albumin layer could be prepared on the surface. To achieve a homogeneous protein coating on Mg surfaces, we therefore exploited silane coupling chemistry of a OH^- -terminated Mg surface. To enhance the OH^- termination, pre-passivation in NaOH is feasible, but the method works also for samples simply passivated in air (native oxide layers formed in air after surface polishing). The success of albumin-coating was verified by XPS (X-ray photoelectron spectroscopy) and ToF-SIMS (Time-of-flight Secondary Ion Mass Spectroscopy) measurements [13].

One advantage of such selective protein adsorption is to prevent non-selective protein adsorption from body fluids, and hence to condition the surface to the desired cell reaction. In our work, we are also interested in the effect of such protein coatings on the corrosion behavior. All experiments until now indicate that homogeneous protein coatings can be highly efficient in decreasing the corrosion rate. An example is shown in Fig. 3, illustrating the cumulative H_2 gas liberation as a function of time for albumin-coated Mg, in comparison to ground Mg surface. An efficient protection of the Mg surface by the albumin layer is observed, and the protective effect remains clearly detectable for a period of 5 days at least (longer-term experiments are running, results not shown). The exact amount of H_2 liberated from protein-covered samples varies, depending on the details of the surface pre-treatments. But it is also evident from Fig. 3 that no complete protection of the surface can be achieved, i.e., slow dissolution still takes place, which is desired for the material to be biodegradable.

This approach is currently being further explored on different proteins and different Mg alloys. Moreover, optimization of the protein coatings by a variation of surface pre-treatments, linker molecules, and soaking time is being carried out. Especially in the case of Mg alloys, specific pre-treatments of the heterogeneous multiphase alloy surface may be required, to ensure a homogeneous reactivity towards the linker molecules in the first coating step. For the optimized (homogeneous and well-structured) protein coatings, *in vitro* cell culture studies with suitable cell lines will be carried out in order to study the biological performance of the layers.

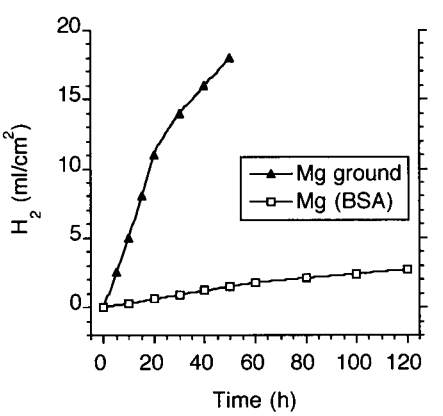

Fig.3 H_2 gas volume as a function of time for ground Mg sample and an albumin-coated Mg sample exposed to SBF.

References

1. M.P. Staiger, A.M. Pietak, J. Huadmai, G. Dias, Magnesium and its alloys as orthopedic biomaterials: a review, *Biomaterials* 27 (2006)1728-1734

2. F. Witte, N. Hort, C. Vogt, S. Cohen, K.U. Kainer, R. Willumeit, F. Feyerabend, Degradable biomaterials based on magnesium corrosion, *Current Opinion in Solid State and Materials Science* 12 (2008) 68-72.

3. W.-F. Mueller, M.F. L. de Mele, M. L. Nascimento, M. Zeddier, Degradation of magnesium and its alloys: Dependence on the composition of the synthetic biological media, *J. Biomed. Mater. Res.* 90A (2009) 487-495.

4. C. Liu, Y. Xin, X. Tian, P.K. Chu, Degradation susceptibility of surgical magnesium alloy in biological fluid containing albumin, *J. Mater. Res.* 22 (2007) 1806-1814.

5. C.L. Liu, Y.J. Wang, R.C. Zheng, X.M. Zhang, W.J. Huang, P.K. Chu, In vitro corrosion degradation behaviour of Mg-Ca alloy in the presence of albumin. *Corrosion Science* 52 (2010) 3341-3347.

6. L. Li, J. Gao, Y. Wang, Evaluation of cyto-toxicity and corrosion behavior of alkali-heat-treated magnesium in simulated body fluid. *Surface & Coating Technology* 185 (2004) 92-98.

7. Y. Zhang, H.-R. Tao, Y.-H. He, G.-Y. Zou, Y. Jian, S.-X. Zhang, B.-L. Zhang, J.-N. Li, C.-L. Zhao, X.-N. Zhang, Cytotoxicity and hemolytic properties of biodegradable Mg-Zn alloy, *Journal of Clinical Rehabilitative Tissue Engineering* 12 (2008) 8162 – 8166.

8. X. Gu, Y. Zheng, Y. Cheng, S. Zhong, T. Xi, In vitro corrosion and biocompatibility of binary magnesium alloys, *Biomaterials* 30 (2009) 484-498.

9. Y.H. Yun, Z. Dong, D. Yang, M.J. Schulz, V.N. Shanov, S. Yarmalenko, Z. Xu, P. Kumta, C. Sfeir, Biodegradable Mg corrosion and osteoblast cell culture studies, *Materials Science and Engineering C* 29 (2009) 1814-1821.

10. E. Zhang, D. Yin, L. Xu, L. Yang, K. Yang, Microstructure, mechanical and corrosion properties and biocompatibility of Mg-Zn-Mn alloys for biomedical application, *Materials Science and Enigineering C* 29 (2009) 987-993.

11. S. Zhang, J. Li, Y. Song, C. Zhao, X. Zhang, C. Xie, Y. Zhang, H. Tao, Y. He, Y. Jiang, Y. Bian, In vitro degradation, hemolysis and MC3T3-E1 cell adhesion of biodegradable Mg-Zn alloy, *Materials Science and Engineering C* 29 (2009) 1907-1912.

12. L. Müller, F.A. Müller, Preparation of SBF with different HCO_3^- content and its influence on the composition of biomimetic apatites. *Acta Biomaterialia* 2 (2006) 181-189.

13. M. Killian, V. Wagener, P. Schmuki, S. Virtanen: Covalent functionalization of metallic magnesium with protein layers, *Langmuir* 26 (2010) 12044-12048.

14. X.N. Gu, Y.F. Zheng, L.J. Chen, Influence of artificial biological fluid composition on the biocorrosion of potential orthopedic Mg-Ca, AZ31, AZ91 alloys, *Biomedical Materials* 4 (2009) 1-8.

15. A. Yamamoto, S. Hiromoto, Effect of inorganic salts, amino acids and proteins on the degradation of pure magnesium in vitro, *Materials Science and Engineering C* 29 (2009) 1559-1568.

16. W.-D. Mueller, M. Lucia Nascimento, M. Fernández Lorenzo de Mele, Critical discussion of the results from different corrosion studies of Mg and Mg alloys for biomaterial applications, *Acta Biomaterialia* 6 (2010) 1749–1755.

17. M. Pourbaix (1974) *Atlas of Electrochemical Equilibria in Aqueous Solutions, 2nd ed.* NACE, Houston.

18. R. Rettig, S. Virtanen, Composition of corrosion layers on a magnesium rare-earth alloy in simulated body fluids, *J. Biomed. Mater. Res. 88A* (2009) 359-369.

19. R. Rettig, S. Virtanen, Time-dependent electrochemical characterization of the corrosion of the magnesium alloy WE43 in simulated body fluids, *J. Biomedical Materials Research A* 85A (2008) 167-175.

20. S. Keim, J.G. Brunner, B. Fabry, S. Virtanen, Control of magnesium corrosion and biocompatibility with biomimetic coatings, *J. Biomedical Materials Research B* (in press, 2010).

21. F. Seuss, S. Keim, M. Can Turhan, B. Fabry, S. Virtanen (to be submitted)

22. C. Lorenz, J. Brunner, P. Kollmannsberger, L. Jaafar, B. Fabry, S. Virtanen, Acta Biomaterialia 5 (2009) 2783-2789.