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Effect of surface pre-treatments on biocompatibility of magnesium

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Abstract

This study reports the influence of Mg surface passivation on the survival rate of human HeLa cells and mouse fibroblasts in cell culture experiments. Polished samples of commercially pure Mg show high reactivity in the cell culture medium, leading to a pH shift in the alkaline direction, and therefore cell adhesion and survival is strongly impaired. Passivation of the Mg surface in 1 M NaOH can strongly enhance cell survival. The best initial cell adhesion is observed for Mg samples incubated in simulated body fluid (M-SBF), which leads to the formation of a biomimetic, amorphous Ca/Mg-phosphate layer with high surface roughness. This surface layer, however, passivates and seals the Mg surface only partially. Subsequent Mg dissolution leads to a significantly stronger pH increase compared to NaOH-passivated samples, which prevents long-term cell survival. These results demonstrate that surface passivation with NaOH and M-SBF together with the associated changes of surface reactivity, chemistry and roughness provide a viable strategy to facilitate cell survival on otherwise non-biocompatible Mg surfaces.

Keywords: Magnesium; Corrosion; Cell culture; Passivation

1. Introduction

Magnesium and its alloys are of growing interest for biomedical applications, in particular as a biodegradable material for cardiac or orthopedic implants [1–16]. Bioabsorbable Mg implants might provide a solution for a number of problems associated with permanent metallic implants such as permanent physical/mechanical irritation, and inability to adapt to growth and other ongoing shape changes in the human body. Bioabsorbable Mg implants might also be able to prevent problems associated with long-term release of metallic ions and/or particles through corrosion or wear processes.

To reliably predict the behavior of Mg implants in the human body, the in vivo corrosion behavior as well as the biological interactions with the Mg surface need to be characterized. Corrosion behavior of commercial Mg alloys has been widely studied in typical environments the alloys may encounter in different applications, e.g. in NaCl solutions. General information on the electrochemical corrosion behavior of Mg and its alloys have been reviewed in Ref. [17,18]. Mg corrosion takes place under H_2 gas formation, and leads to alkalization of the surroundings. On the one hand, both factors could strongly impair the biocompatibility of Mg, e.g. by preventing cell adhesion on the implant surface. On the other hand, release of Mg ions by implant dissolution is not expected to lead to toxic reactions, as the concentration of Mg in the body is controlled by homeostatic mechanisms [6].

Even though the interest in Mg in medicine is increasing, only a few studies on cell behavior on Mg alloy surfaces can be found in the literature. It has been reported that osteoblasts and human bone derived cells adhere, prolifer-

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ate and survive on the corroding surface of the Mg alloy AZ91D [19]. However, in comparison to tissue-culture-treated plastic surfaces, a reduction in cell viability was observed which was attributed either to an increase in pH (+0.39) or to the release of ions into the medium. Another study reported that the presence of commercially pure Mg samples in the cell culture medium had no inhibitory effect on cell growth in murine bone marrow cells [20]. A very recent paper reported that cells do not survive on untreated Mg alloy AZ91 for longer than 1 day, whereas samples coated with hydrogenated amorphous silicon show good biocompatibility [21]. An evaluation of binary Mg–Ca alloys using L-929 cells showed no cytotoxic effects; instead the viability of cells cultured in the alloy extraction medium was even better than under control conditions [22]. A similar study was carried out on fibroblasts and osteoblasts grown on a wide range of binary Mg alloys [23]. In that study, however, the cell viability was shown to depend on the type of corrosion products produced by the dissolution of the different binary alloys.

In conclusion, the effect of Mg corrosion on cell behavior is ambivalent, as reports indicating either survival or death of cells on corroding Mg surfaces can be found in the literature. The type of alloys and cell lines used for these experiments vary widely, and therefore it is not possible to draw any generalizing conclusions on the critical factors influencing cell behavior on Mg alloy surfaces.

In the present work, we study the behavior of human HeLa cells and mouse fibroblasts in cell cultures in the presence of commercially pure Mg, either without or with specific surface modifications, which were used to control the reactivity, chemical composition and/or roughness of the surface. The results demonstrate the importance of surface treatment for initial cell adhesion and for cell survival on Mg surfaces.

2. Materials and methods

For all experiments, samples were obtained by cutting a commercially pure Mg rod (25.4 mm diameter, 99.9% purity, ChemPur) into 2 mm slices. The samples were mechanically ground on a microcut paper disc (600 grit soft), then polished with diamond paste (6 μm) using an ethanol/glycerol (3:1) mixture as lubricant. Subsequently the samples were cleaned by sonicating in pure ethanol for 3 min, and air dried.

To modify the corrosion resistance, chemical composition and morphology of the Mg surface, the samples were either passivated by soaking in 1 M NaOH for 24 h, or by soaking in simulated body fluid (M-SBF) [24] at 37 °C for 5 days. Table 1 shows the composition of the M-SBF solution used in this study. Soaking in 1 M NaOH reduces the surface reactivity due to the formation of a thin $Mg(OH)_2$ passive layer. Soaking in M-SBF leads to the formation of mixed Ca/Mg-phosphate layers on the surface (e.g. [25–27]).

After these surface pre-treatments, the samples were rinsed with ethanol and dried in air. The morphology and composition of the sample were characterized by field-emission scanning electron microscopy (FE-SEM, Hitachi S4800) and energy-dispersive X-ray (EDX) analysis. The wettability of the surface was determined by optically measuring the water droplet contact angle. A UBM Microsoft Expert laser profilometer with a wavelength of 780 nm was used to determine the roughness of the differently treated surfaces.

Table 1
Composition of the simulated body fluid (M-SBF) [24] used in this study. All concentrations are given in mmol l^{-1}.

Ion	M-SBF
Na^+	142.0
K^+	5.0
Mg^{2+}	1.5
Ca^{2+}	2.5
Cl^-	103.0
HCO_3^-	10.0
HPO_4^{2-}	1.0
SO_4^{2-}	0.5
HEPES	75.0

pH changes that were induced by Mg dissolution were optically monitored in situ in 2 ml of Eagle's minimum essential cell culture medium incubated at 37 °C, 5% CO_2 and 95% humidity in order to ensure environmental conditions identical to those used for the cell culture tests. The cell culture medium contains phenol red as a pH indicator; the color changes from red at pH 7.4 to pink at pH 9.0 were monitored with an RGB CCD camera (Canon PowerShot G5). A calibration curve was determined in the pH range 7.4–9.0. The pH values in the presence of the Mg samples were then calculated colorimetrically from the ratio of the red-to-blue channel intensities.

For cell culture testing the Mg samples were sterilized under UV irradiation with a wavelength of 260 nm. Human HeLa cells were cultured in the dish with the differently treated Mg specimens under 5% CO_2 in air atmosphere at 37 °C, 95% relative humidity for 24 h in an incubator. Eagle's minimum essential medium with addition of 10 vol.% fetal bovine serum (MEM + FBS) was used as a cell culture medium. About 1,000,000 cells were seeded on the dish. Three dishes were measured for each condition. Under selected conditions, additional experiments were carried out with GSP-C12 mouse fibroblasts stably transfected with EGFP-actin to study the time-series of their attachment and spreading. Both cell lines are standard cell lines and are used in many laboratories worldwide; they show robust growth under a range of conditions, and they form a dense monolayer when grown onto a flat substrate which makes them suitable for cell attachment and spreading area quantification.

After 24 h in the incubator, the cells were fixed with a 3% paraformaldehyde/0.3% Triton-X100 solution in PBS and stained with Alexa red phalloidin to visualize the actin cytoskeleton of the cells, or with Hoechst 33342 to visualize the cell nucleus. Fluorescence microscopy of stained cells was carried out with a Leica DMI 6000B microscope.

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