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Features of *in vitro* and *in vivo* behaviour of magnesium alloy WE43

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ABSTRACT

Biocompatibility of magnesium alloy WE43 was investigated under *in vitro* and *in vivo* conditions. The biodegradation of WE43 samples *in vitro* was found to occur very rapidly. The evolution of a significant amount of hydrogen as a product of biodegradation prevented the attachment of cells to the sample surface and caused their likely damage. A tendency to the lysis of red blood cells and a drop in the index of survival of mesenchymal stromal cells were observed. By contrast, *in vivo*, after subcutaneous implantation to mice, the biodegradation rate of WE43 was significantly lower and no sizeable hydrogen generation occurred. An intimate contact between the sample surface and the surrounding tissue was formed without damage to the tissue, with the occurrence of neoangiogenesis in the contact area. Importantly, morphological studies showed that no major detrimental systemic effects were associated with the implantation of WE43 in mice.

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1. Introduction

Magnesium alloys are becoming attractive as candidate materials for biodegradable implants, including cardiovascular stents and fixation pins, screws, wires, sheets, and plates [1]. Indeed, biodegradation of a magnesium implant concurrent with the healing process can be used to avoid a repeat surgery for implant removal. According to literature reports, many magnesium alloys have excellent biocompatibility without any negative effects of implantation [1–3]. Alloy WE43 (Mg-Y-RE-Zr) is considered as one of the most promising magnesium alloys for biomedical purposes. The rare earth (RE) metals contained in the alloy provide good mechanical properties and improved corrosion resistance, thus reducing the biodegradation rate [4]. Cardiovascular stents and orthopedic screws fabricated from alloy WE43 and similar alloys have been successfully tested *in vivo* on animals and humans and are currently used in clinical practice [5–7]. It has been shown that the full degradation period for WE43-based implants ranges from 12 to 24 months. By contrast, in an *in vitro* test the WE43 alloy exhibits a much more rapid degradation [8].

While it is no longer commonly believed that *in vitro* studies can provide a valid prediction of the behavior of an implant material *in vivo*, we saw it as an important goal to test that for alloy WE43 in a more systematic way. The aim of the present study was thus to compare the results of biocompatibility tests for the WE43 alloy under *in vitro* and *in vivo* conditions.

2. Experiment

2.1. WE43 samples

Hot extruded alloy WE43 with the chemical composition (in weight %) of Mg–3.56%Y–2.20%Nd–0.47%Zr, was homogenised for 8 h at 525 °C. Disk-shaped samples with a diameter of 5 mm and thickness of 2 mm were prepared for testing by grinding on abrasive paper (P2000) and subsequent polishing on a wet cloth without abrasives. Before testing, all specimens were sterilised for 6 h in 70% ethanol.

2.2. Hemolysis testing

The hemolytic activity was investigated according to Fischer et al. [9]. A suspension of erythrocytes (V = 2 ml) with one WE43 sample was incubated on shaker in atmosphere 37 °C, 5% CO_2 for 2.5 h. The release of hemoglobin was determined after centrifugation

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(800g for 10 min) by photometric analysis of the supernatant in triplicate at the wavelength of 540 nm on a Labsystems Multiskan MS plate reader.

2.3. Cytotoxicity testing

Peripheral mononuclear lymphocytes (ML) were isolated from blood of a healthy volunteer according to M'Bemba-Meka et al. [10]. Multipotent mesenchymal stromal cells (MSC) were isolated from bone marrow of dog and cultured until third passage according to Yaochite et al. [11]. 1 ml of ML or MSC in RPMI-1640 medium (PanEco, Russia) supplemented with 10% fetal bovine serum (Thermo Scientific, USA), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (all Gibco, USA) were cultivated in a 24-well tissue culture plate (Nunc, USA) with one sample (n = 6) for 3 or 5 days at 37 °C with 5% CO_2. Control cells were treated only with RPMI 1640. The index of survival (IS) of cells was determined using the colorimetric test with MTT (3-[4,5-dimethylthia zol-2-yl]-2,5-diphenyl-tetrazolium bromide) according to Bounous et al. [12]. After transferal of this solution to triplets of a 96-well microculture plate (Nunc, USA), the optical density (OD) was measured spectrophotometrically at the wavelength of 540 nm on a Labsystems Multiskan MS plate reader. The index of survival was calculated by applying the following formula: IS = [(OD of cells + WE43 sample)/OD of control cells] × 100%.

2.4. Statistics

The results are presented as a mean ± standard deviation (SD). Comparisons between the two groups were made using Student's *t*-test. The data with $p < .05$ were considered as statistically significant.

2.5. In vivo test

As experimental animals, C57Bl/6 mice were used (male, 3–4 months old, weight 22–24 g). The animals were housed in polypropylene cages inside a well-ventilated room, provided with chow pellets and water *ad libitum* and maintained under a 12-hour light/dark cycle. Before implantation, the skin of the mouse was disinfected with 40% ethanol. The samples (n = 5) were subcutaneously implanted into intracapsular region of the back of a mouse. Each mouse underwent the implantation of a single sample, so five mice were used in a test. Two months after implantation, the animals were sacrificed by cervical dislocation followed by the extraction of the implant with surrounding tissue. The tissue samples were fixed in 10% buffered formalin (pH = 7.4), dehydrated in alcohol and embedded in paraffin. Sections were stained by haematoxillin and eosin (HE).

2.6. Ethics statement

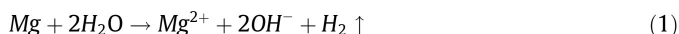
The use of animals and blood sampling procedures for this study was approved by the Ethics Committee of N.N. Blokhin Russian Cancer Research Center. All surgery was performed under ketamine and xylazine anesthesia, and all efforts were made to minimise suffering of the animals tested.

3. Results and discussion

3.1. Research in vitro

Incubation of WE43 with erythrocytes for 2.5 h induced the lysis of part of the cells: the level of hemolysis was 2 ± 1.02%. The study of cytotoxicity showed no statistically significant change of the survival of human mononuclear leukocytes after co-incubation with WE43 in RPMI-1640 medium for 3 days compared with intact cells in control. On the 5th day of co-incubation we observed a minor reduction of survival of MSCs with faint signs of the sample surface colonisation by the cells (Fig. 1).

Magnesium is known to dissolve in an aqueous solution according to the reaction



$$Mg + 2H_2O \rightarrow Mg^{2+} + 2OH^- + H_2 \uparrow \quad (1)$$

but, of course, the physiological environment complicates the corrosion process [13].

During *in vitro* experiments, after immersion of a sample in a cell suspension noticeable formation of hydrogen bubbles on the sample surface was observed. By the end of the experiment, the appearance of the samples was changed significantly. Signs of degradation of the WE43 sample surface were clearly visible already two hours after the start of the experiment and were pronounced after a longer exposure to the cell suspension (Fig. 2).

3.2. Research in vivo

Morphological examination of organs of mice with a WE43 sample implanted subcutaneously for two months did not show any macroscopically visible signs of toxic effects of the implants. Neither in the area of implantation, nor in the internal organs of the animals, including heart, lung, kidney, liver and spleen, were there any local or systemic symptoms of purulent inflammation, edema, or hemorrhage.

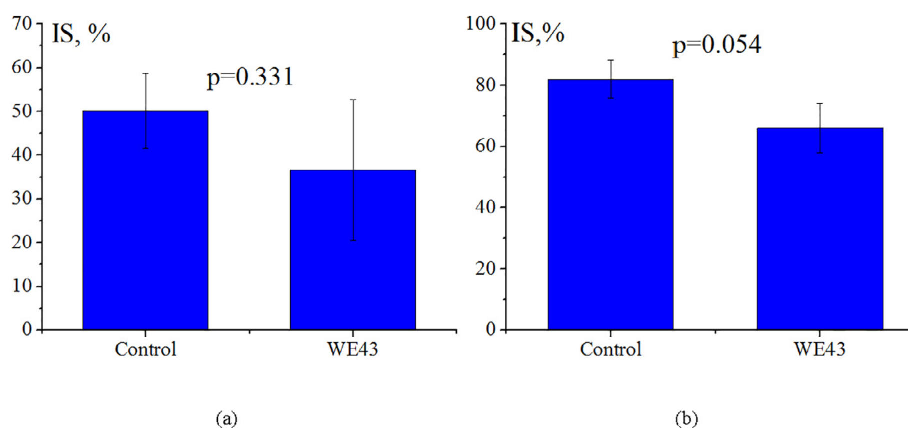


Fig. 1. Survival of human cells after co-incubation with WE43: (a) ML, 3 days; (b) MSC, 5 days.

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