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The effects of handling and storage on magnesium based implants — First results

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ABSTRACT

The present work aimed to investigate the influence of acetone and formalin as well as the duration and type of storage on magnesium based implants by means of microscopic, μ-computed tomographic, scanning electron microscopic, EDX and metallographic investigations.
In contrast to storing in acetone, storage in formalin led to an increase in surface to volume ratio, and a decrease of the volume and the density. The various types of storage exerted no differing effects on the implants but with increasing storage duration, a spreading of oxygen rich areas on the surface, increased precipitations and a decrease in grain size could be observed.

1. Introduction

Osteosynthetic materials which are to be employed in living organisms must be tested according to specific ISO standards prior to their use (for example, ISO 14971 [1], ISO 13485 [2], ISO 14155 [3] or ISO 10993 [4]). Furthermore, they have to be cleaned of possible residues which remain on the implant following the manufacturing process [2, 5, 6]. Magnesium based alloys are the objects of current research as new resorbable implant materials in which the implant is cleaned with acetone for use in in vivo tests [7–10]. Amongst other things, acetone is eminently suitable as a cleaning medium for removing grease [11–13]. Moreover, it is legally stipulated that medical products must not change their characteristics and performances during their storage prior to their intended use taking into account the manufacturer's data (Guideline 93/42/EWG from 1993 [14], implemented into, amongst others, ISO 9000 [15] and DIN EN ISO 13485 [2]). Little is known about the influence of the magnesium implant's type and duration of storage. One only knows that, for magnesium implants, surface damage leads to an increase in corrosion both in vitro [16] as well as in vivo [17]. An unsuitable type of storage could damage the surface and thereby have an unfavourable effect on the corrosion resistance. Moreover, it is known that magnesium forms an oxide/hydroxide layer on its surface subject to atmospheric conditions [18, 19]. Both the type and duration of storage could therefore exert an influence on this layer's formation, particularly when additives such as silica gels are added, which should reduce the humidity [20, 21]. Other materials, such as polymers, also exhibit oxidation processes as well as increased surface roughness following storage at room temperature in air and, over and above this, incipient hydrolysis also occurs [5, 22]. These processes are described as pronounced for the surface and as marginal for the material's interior [22].

Hitherto, nothing has been found in the accessible literature concerning the influence of formalin on magnesium alloys or its surfaces. However in various in vivo studies, bone-implant combinations are frequently fixed in formalin in order for them to then be histologically examined with regard to bone-implant contact [23–25]. Since, in this way, the implant also comes into contact with the formalin solution, it is very important to investigate those influences which the formalin exerts on the magnesium alloy's surface. Magnesium reacts with water in the form of an anodic ($Mg \rightarrow Mg^{2+} + 2e$) and cathodic reaction ($2H_2O + 2e \rightarrow 2OH^- + H_2$) according to the formula; $Mg + 2H_2O \rightarrow Mg(OH)_2 + 2H_2$ [26, 27]. Formalin is the aqueous solution of formaldehyde [28]. Thus, corrosive processes, which had not occurred during the in vivo implantation by means of the degradation processes, could occur on the implant during the bone-implant's fixing in the formalin solution.

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Since, as yet, there are no investigations of the influence of different chemicals, such as acetone or formalin, regarding magnesium materials in the accessible literature and no studies exist on the influence of different types and durations of storage on the implants' surface and structure, the objective of this work is to investigate these influences by using an in vitro study.

2. Materials and methods

2.1. Implant material

For this study, LANd442 magnesium alloy cylindrical implants (2.5 × 25 mm) were used. All implants were manufactured by die-casting and subsequent direct extrusion as described by Ullmann et al. [29] and Seitz et al. [30]. They were designated in accordance with the ASTM Standard B275-05 [31]. In accordance with this, the 50 magnesium implants used contained 4 wt.% lithium, 4 wt.% aluminium and 2 wt.% neodymium.

2.2. Experimental set-up

2.2.1. Storage in acetone and formalin

A total of 18 LANd442 implants were weighed prior to the experiment and their surfaces were both descriptively assessed using a microscope (Imager.Z1, Carl Zeiss AG, Oberkochen, Germany; 5–20× magnification) as well as quantitatively evaluated using μ-computed tomography (μCT) (μCT80, Scanco Medical, Zürich, Switzerland).

Three implants each were subsequently stored in a dipping bath of acetone (60 ml, Aceton reinst, AppliChem GmbH, Darmstadt, Germany (acetone high purity Ph. Eur., NF. specification: content (GC) min 99.5%, Ba, Cd, Co, Cr, Fe, Mg, Mn, Ni, Pb, Zn max 0.00001% each, Na max 0.00002%, Al, Ca max 0.00005% each, water max 0.25%, acidity/alkalinity max 0.0005 meq/g)) for 30, 45 or 60 min. Another three implants each were stored in 4% formalin (60 ml, prepared of 6.5 l 37% formalin (Merck KgaG, Darmstadt, Germany), 53.5 l Aqua dest, 240 g $NaH_2PO4 * H_2O$ and 650 g Na_2HPO_4 (waterfree), pH 7.0) in plastic tubes (Sarstedt, Nümbrecht, Germany) in a darkened cabinet for two, four and eight weeks. Following this, the implants were weighed and microscopically and μ-computed tomographically analysed in accordance with the assessments prior to storage.

2.2.2. Dry-storage under different circumstances

After cleaning with acetone for 45 min (15 min of the total time in an ultrasonic bath), four implants each were stored at room temperature under different types of storage for a time period of three months. The storage types were as follows: 1st individually in Eppendorf tubes or in special sterilisation bags (Krauth and Timmermann, Isernhagen, Germany) and sterilised using γ-radiation (29.3 kGy, BBF Sterilisationsservice, Kernen, Germany), 2nd and 3rd with and without silica gel, respectively and 4th individually packed in a commonly used needle casket made of surgical steel (Wirtschaftsgenossenschaft deutscher Tierärzte eG, Garbsen, Germany). Furthermore, eight implants each were stored for a time period of one and six months respectively.

2.3. Evaluation of the different types of storage

2.3.1. Weight analysis

The weight of every implant was measured prior to the experiments using precision scales (Metler Toledo AB204-S/Fact, Giessen, Germany). The weight of each group was determined and the mean value was calculated using Excel®, Version 2003 (Microsoft Corporation, Redmond, Washington, USA).

2.3.2. Microscopy evaluation

Prior to their storage in acetone or formalin, the implants' surfaces were microscopically (Imager.Z1, Carl Zeiss AG, Oberkochen, Germany) examined using a magnification of ×5–×20. Images with extended depth of focus (Axio CamMRC, Carl Zeiss AG, Oberkochen, Germany) were recorded. By means of the programme Z-Stapel (Carl Zeiss AG, Oberkochen, Germany), the images were merged in order to generate one image with a high degree of sharpness. The evaluation of the surface differences prior to and after storage was performed descriptively.

2.3.3. μ-Computed tomography

Approx. 300 slices at the end and in the middle of each implant stored in acetone and formalin respectively were analysed using μCT having a resolution of 10 μm at 55 kV and 72 μA and an integration time of 500 ms. The surface as well as the volume and the density were measured by means of the software V6.1 (Xtreme CT, Scanco Medical, Zürich, Switzerland) after manually contouring the implants using a threshold of 138. Using the same software, the implants' evenness was calculated by virtually filling the implants with overlapping spheres of maximum diameter according to former studies [9]. The percentage of the volume per slice, the density, surface to volume ratio changes and the evenness in comparison to the initial values were subsequently calculated by using Excel®, Version 2003.

2.3.4. Scanning electron microscopy

The implants stored at room temperature under different types of storage were examined using SEM (LEO 1455VP, Zeiss, Oberkochen, Germany, resolution: 5 nm) with RBS (Rutherford Backscattering Spectroscopy). In order to evaluate the surface, images from the entire length of the implants were considered. At selected areas, an energy dispersive analysis (EDX) (EDAX Genesis, EDAX, Mahwah, USA) was performed in order to identify oxygen rich regions. These are displayed as dark regions in RBS due to the low density of oxygen. The images were analysed by means of the programme GSA ImageAnalyser (GSA Rostock Bansemer und Scheel GbR, Rostock, Germany) using a threshold of 140. This calculated the percentage of the dark regions determined in EDX as oxygen rich compared to the total evaluated surface of the 2D SEM images.

2.3.5. Metallographic examination

One implant of each of the implants' time groups for the dry-storage was embedded in Demotec 70 (Demotec Metallografie, Nidderau, Germany) and examined as metallographically polished sections in order to identify possible structural changes (for example, the grain size and depositions). The grain sizes were determined subsequent to etching (3 g picric acid, 20 ml acetic acid, 50 ml ethanol, 20 ml water) by means of the DIN EN ISO 643 [32] at six locations on the polished lateral and cross-sections of the respective implants. By doing this in a defined circle, whole grains and the grains cut by the circle were counted and calculated according to the following formula: $K = \sqrt{(A_{circle} / (K_{whole} + (K_{cut} / 2)))}$ [32]. Here, K is the average grain size, A is the area of the defined circle, K_{whole} is the whole grains counted and K_{cut} is the cut grains counted.

2.4. Statistical analysis

The statistical analysis was done by means of the programme Microsoft Office Excel®, Version 2003 (Microsoft Corporation, Redmond, Washington, USA) and SPSS® Version 17.0 (IBM, Armonk, New York, USA). All groups were tested for a normal distribution. The SEM results for the different storage types were tested with ANOVA, the results over different periods of storage time were tested using a *t*-test for independent samples. All μ-CT results were tested using a *t*-test for connected samples. Comparisons between groups as well as comparisons of the grain sizes were carried out using a univariant Variance analysis with subsequent post-hoc tests (Tukey and Games Howell respectively). The weight analysis was tested using Wilcoxon tests. Values of $p \leq 0.05$ were evaluated as significant, values of $p \leq 0.01$ as highly significant.