Effect of rotary swaging and subsequent aging on the implant-relevant properties of magnesium alloy WE43

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Abstract. The magnesium alloy WE43 were pre-strained by rotary swaging (RS) with a final temperature of 350°C. RS led to a significant grain refinement with the formation of a predominantly subgrain structure. The average size of the structural elements decreased up to 450±50 nm after RS. In addition, the RS-induced precipitation of intermetallic Mg41Nd5 particles with an average size of 210±13 nm was observed. Subsequent heat treatment caused the average size of structural elements and intermetallic particles to grow to 781±88 nm and 494±51 nm, respectively. After RS, the ultimate tensile strength (UTS) of the alloy rose to 363±2 MPa with tensile elongation of 11.4±0.6%. After heat treatment, the UTS of the alloy increased to 376±9 MPa, with some drop of tensile elongation to 7.6±0.4%. Interestingly, the biocorrosion resistance of the alloy was not compromised by RS, while subsequent heat treatment of the swaged alloy reduced its biodegradation rate. No significant differences in the biocompatibility of the alloy WE43 between different microstructural states were observed. It was found that RS with subsequent aging of the alloy WE43 reduces the adhesion of yeast to its surface, which in the long term may reduce the risk of infectious complications after orthopedic surgery.

Keywords: magnesium alloys; rotary swaging; mechanical properties; biodegradation; biocompatibility in vitro

1. Introduction
Magnesium alloys are among the most promising alloys considered for bioresorbable orthopedic implants [1 – 3]. They are able to degrade without the release of toxic degradation products into the patient's body, have good biocompatibility and a Young's modulus close to that of the cortical bone [4]. However, improvement of the strength characteristics of magnesium alloys is often required for their
successful application in orthopedics. This may be challenging, as it is necessary to select the type and schedule of mechanical treatment that would lead to an increase in the strength of the alloy without sacrificing its corrosion resistance. Refining the microstructure of magnesium alloys to an ultrafine-grained (UFG) state, notably by severe plastic deformation (SPD), has come to the fore as a viable option for this purpose [5 – 7]. However, SPD processing is still technologically challenging and costly for it to be employed at industry scale. Therefore, one has to look for opportunities to obtain an UFG structure in magnesium alloys by more traditional methods of metal forming, e.g. swaging, rolling, etc. Rotary swaging (RS) is a promising technology for processing metallic materials imparting to them an ultrafine-grained structure [8 – 14]. Furthermore, the main advantage of this method is its applicability to large-scale manufacturing at relatively low cost. In view of the promising aspects of the RS technology, we conducted a study of its effect, in conjunction with subsequent heat treatment, on the structure, mechanical properties, biocorrosion resistance and biocompatibility in vitro of magnesium alloy WE43.

In the past, we looked into the effect of RS on the behavior of this alloy, but for the RS regime chosen the resulting UFG microstructure led to a catastrophic deterioration of the corrosion resistance because of the formation of deformation twins [14]. For the present investigation we decided to carry out RS at a higher temperature (350 °C), followed by a heat treatment. We were guided by the expectation that a higher deformation temperature will inhibit the formation of deformation twins thus having a positive effect on the corrosion resistance of the alloy. We also believed that the annihilation of RS-induced dislocations owing to the thermal recovery during subsequent heat treatment would also promote the corrosion resistance. The results reported below demonstrate that this processing strategy did bear fruit.

2. Experimental

The magnesium alloy WE43 (Mg-3.46%Y-2.20%Nd-0.47%Zr) investigated in this work, was obtained by melting in an induction furnace. The alloy was homogenized at a temperature of 525 °C for 8 hours and cooled in air at ambient temperature. Rotary swaging (RS) was carried out on rods with a diameter of 20 mm and a length of 20 cm on a rotary swaging machine RKM 2129.02 (see [15] for details). The deformation was carried out in two stages with a stepwise decrease in the treatment temperature from 400 to 350 °C and concurrent increase in the cumulative strain to \( \varepsilon = 1.39 \) (\( \varepsilon = \ln(A_0/A_f) \), where \( A_0 \) and \( A_f \) are the initial and the final cross-section areas of the billets, respectively). The study of the microstructure in the initial state was carried out using Axio Observer D1m light microscope (Carl Zeiss, Germany). The microstructure of the alloy after RS was studied by transmission electron microscopy (TEM) using a JEM-1400 electron microscope (JEOL, Japan) operating at a voltage of 120 kV. Foils for TEM were mechanically thinned to 180 μm and then subjected to ion bombardment in a GATAN 600 unit. The size of the structural elements was measured by random intercept method using Image Expert Professional 3 software. The mechanical properties of the alloy before and after deformation were studied by uniaxial tensile tests in an Instron 3382 testing machine on flat specimens with a gauge length of 5.75 mm and a cross-sectional area of 1 mm × 2 mm. The study of the corrosion resistance of the alloy was carried out by the weight loss measurements after immersion of alloy samples (at least 3 samples per point in time) in 2 mL of fetal bovine serum (FBS; PanEco, Russia) at 37 °C in an atmosphere of 5% CO2 for 2, 3, and 4 weeks. After immersion, the samples were cleaned in a mixture of Cr2O3, AgNO3, Ba(NO3)2 and reagent water for 1 min (ASTM_G1-03-E). The weighing of the samples before and after corrosion tests was carried out on an electronic scale GR 200 with an accuracy of four decimal places. The mass loss of the alloy was calculated as an indicator of degradation according to the formula

\[
\text{Mass loss} = \left( \frac{M_f - M_0}{M_0} \right) \times 100\% \tag{1}
\]

where \( M_0 \) is the initial and \( M_f \) the final mass of the sample.
In order to study the biocompatibility, the samples of the alloy in the initial state and after processing were sterilized by immersion in 70% ethanol for 20 h. Before an experiment, the samples were pre-incubated in a complete growth medium overnight at 37 °C in an atmosphere of 5% CO₂. Then the samples were placed one by one on the bottom of a well of a 24-well plate (Corning Costar). Human multipotent mesenchymal stromal cells (MMSCs) from the collection of the N.N. Blokhin National Medical Research Center of Oncology were used as a cell model. The cells in logarithmic growth stage were re-suspended in a complete growth medium based on Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich, St. Louis, MO, USA), supplemented by 10% FBS (HyClon, Thermo Fisher, Loughborough, Leicestershire, UK), 2 mM glutamine, and 100 mg/mL penicillin-streptomycin (both PanEco, Moscow, Russia). 20 μL of a suspension containing 9.2 × 10^5 cells was carefully added to each sample, as well as to the wells void of samples used as Control. Plates with samples and cells were incubated for 15 min at 37 °C in an atmosphere of 5% CO₂. Then, 1.98 mL of the complete growth medium was carefully added to each well of the plate. Samples of the alloys were incubated with cells at 37 °C in an atmosphere of 5% CO₂ for 24 h. Then the lactate dehydrogenase (LDH) activity of the cells was determined using the Pierce LDH Cytotoxicity Assay Kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer’s instructions. Optical density (OD) was measured on an MS Multiscan plate reader (Labsystems, Thermo Fisher) using a 492 nm filter.

To study the adhesion of microorganisms, alloy samples (three samples of each type) were sterilized as described above and dried in a sterile atmosphere. The yeast *S. cerevisae* (from the collection of the N.N. Blokhin NMRC of Oncology) was used as a model for microorganisms. The yeast in logarithmic growth stage was re-suspended in a sterile Phosphate-Buffered Saline (PBS) (PanEco, Russia) in the concentration of 6.3 × 10^5 cells per 1 mL. 20 μL of the suspension was carefully added to each sample and to the bottom of the wells without samples (Control). Plate with alloy samples and yeast were incubated for 30 min at 37 °C in an atmosphere of 5% CO₂. The surface of the samples was subsequently washed with PBS. The amount of yeast on the surface of each sample and in Control was counted with the Invitrogen™ LIVE/DEAD™ FungaLight™ Yeast Viability Kit (Thermo Fisher Scientific, USA) in a light microscope (Selena, Logos, Korea).

For statistical analysis, the Statistica 6.0 software (StatSoft package; version 6.0, Tulsa, OK, USA) was used. Comparative analysis was performed using Dunn’s test. Differences were considered significant at p < 0.05.

The cell test protocols used in this work were evaluated and approved by the local Ethics Committee of N.N. Blokhin NMRC of Oncology.

### 3. Results and Discussion

Figure 1 shows the results of the investigation of the microstructure of the alloy in different structural states. After homogenization, the microstructure was composed of grains of a supersaturated solid solution (SSSS) with an average grain size of 51.4 ± 1.9 μm. No second phase particles were detected (Figure 1 a). The subsequent RS led to a significant refinement of the microstructure and partial decomposition of the SSSS with the precipitation of Mg₄₁Nd₅ phase particles [14]. As a result of RS, a predominantly sub-grain structure developed, as indicated by diffuse reflections in the rings of the electron diffraction pattern, with an average size of structural elements (sub-grains) of 450 ± 50 nm. The average size of the precipitated particles was 210 ± 13 nm (Figure 1 b). The microstructure of the alloy after RS and subsequent heat treatment is shown in Figure 1 c. The heating was carried out at a temperature of 200 °C, which is a typical aging temperature of magnesium alloys containing rare earth elements, including the alloy WE43 [16-17]. The choice of the heating time (1 hour) was dictated by the requirement that the intended relaxation of residual stresses and the reduction of the dislocation density would not provoke a significant grain growth. Inspection of the ensuing microstructure showed that this heat treatment did lead to grain coarsening and growth of Mg₄₁Nd₅ particles, but this undesirable effect, with the average grain size of 781 ± 88 nm and particle size of 494 ± 51 nm, was moderate and thus tolerable. The aging resulted in a developed ultra-fine grain (as opposed to sub-grain) structure as
corroborated by the occurrence of predominantly point reflections in the electron diffraction pattern in Figure 1c.

![Figure 1. Structure of the alloy WE43 (a) in the initial state, (b) after RS and (c) after RS and subsequent aging at 200 °C for 1 h](image)

The mechanical properties of the alloy in the various states are summarized in Table 1. The refinement of the microstructure of the alloy by RS led to an increase in its strength characteristics. The yield stress (YS) was increased from 156 ± 8 MPa in the homogenized state to 332 ± 2 MPa in the post-RS state. At the same time, the ultimate tensile strength (UTS) increased from 227 ± 9 MPa in the initial (homogenized) condition to 363 ± 2 MPa after RS. It is interesting to note that the tensile elongation (El) of the alloy after RS did not change within the measurement error (9.8 ± 1.1% and 11.4 ± 0.6% in the homogenized and swaged states, respectively), despite the pronounced microstructure refinement. This may be attributable to texture effects, and also to a concurrent decrease in the degree of supersaturation of the SSSS due to its partial decomposition. A gain in mechanical strength was achieved by subsequent heat treatment: aging at 200 °C for 1 h led to an increment of the yield stress (to 348 ± 11 MPa) and ultimate tensile strength (to 376 ± 9 MPa), despite an increase in the average grain size. This additional strengthening may be related to finely dispersed Mg41Nd5 particles precipitated as a result of aging. Unfortunately, strengthening was achieved at the cost of tensile elongation, which dropped to 7.6 ± 0.4% upon aging. However, this level of tensile ductility may still be acceptable in the bone implantology context.

| Treatment                     | UTS, MPa | YS, MPa | El, %  |
|-------------------------------|----------|---------|--------|
| Homogenization                | 227 ± 9  | 156 ± 8 | 9.8 ± 1.1 |
| Rotary Swaging (RS)           | 363 ± 2  | 332 ± 2 | 11.4 ± 0.6 |
| RS + aging at 200 °C for 1 h  | 376 ± 9  | 348 ± 11| 7.6 ± 0.4 |

The mass loss data for the different structural states of the alloy after incubation for 2, 3, and 4 week immersion in FBS are shown in Figure 2. RS is seen to accelerate degradation over two weeks of incubation in FBS. Specifically, the mass loss of samples in the homogenized state was 3.26 ± 1.07% after 2 weeks of incubation, while after RS it amounted to 5.29 ± 1.08%. On a positive side, it should be mentioned that with further immersion time, the mass loss of the RS-processed samples nearly stagnated, staying at a level of 5.37 ± 1.09% after 3 weeks of immersion – in contrast with the rapidly progressing mass loss in the homogenized state (4.81 ± 0.85% vs. 3.26 ± 1.07% after 2 week immersion). The magnitude of the mass loss of the alloy measured after 4 weeks of immersion in FBS was very close for both conditions (5.18 ± 0.80% and 5.82 ± 0.83% for homogenized and swaged alloy, respectively). A fortunate effect of subsequent heat treatment is that degradation rate slows down. Indeed, the mass loss was decreased compared with that of the non heat treated swaged alloy and the homogenized one.
The figures for the mass loss of samples of the alloy after combined RS and aging treatment were as follows: 1.39 ± 0.53% after 2 weeks, 3.32 ± 0.56% after 3 weeks, and 3.92 ± 0.41% after 4 weeks of immersion. A plausible reason for this deceleration of biodegradation may be a decrease in the density of defects caused by the heat treatment and the formation of a grain structure. It should also be noted that RS did not lead to a deterioration of the corrosion resistance relative to the initial homogenized state, at least over 4 weeks of immersion in FBS. This is at variance with the earlier studies showing that RS carried out at 325 °C, which led to the formation of deformation twins worsens the corrosion resistance of the alloy WE43 quite significantly. (In that case, the mass loss after 4 weeks of immersion in FBS was as high as 53.22 ± 1.85%) [14]. It can be conjectured that serious deterioration of the corrosion resistance could be avoided thanks to the suppression of twinning processes due to higher swaging temperature.

Examination of the sample surface after degradation tests revealed localization of corrosion in the form of pits for the homogenized state and after RS (without additional aging) (Figure 3). Compared with the alloy in the homogenized state, pitting was more pronounced for the swaged alloy immersed in FBS for 2 weeks (Figure 3 a). However, the difference became less significant with an increase of the immersion time to 4 weeks (Figure 3 b, c). The sample surface for the alloy after RS and additional aging (‘Ag.’) exhibited only minor signs of localization of degradation after 3 and 4 weeks of immersion in FBS (Figure 3 a-c).
To assess the effect of alloy processing on the biocompatibility, we evaluated the change in the LDH activity of human MMSCs on the surface of the samples of the studied alloy after 2 days of incubation (Figure 4). The results obtained showed no significant difference between the level of LDH activity of cells on the sample surface in comparison with the control, where cells were incubated at the bottom of the plate well ($p > 0.05$). This result can be considered as evidence for the preservation of cell viability during contact with the surface of all types of tested samples. Thus, we have found that the deformation of the alloy WE43 by rotary swaging and subsequent heat treatment did not impair its biocompatibility.

**Figure 3.** The images of the surface of the samples before the removal of degradation products following immersion in FBS for 2 (a), 3 (b) and 4 (c) weeks. (Red arrows indicate the sites of localized corrosion)

**Figure 4.** Study of the cytotoxicity of the alloy WE43 in the various structural states. The results of assessing the LDH activity of human MMSCs on the surface of the alloy samples are juxtaposed with the control, where the cells were incubated at the bottom of the wells, after 2 days of incubation, $p > 0.05$ (OD stands for ‘optical density’).
To evaluate the effect of the processing of the alloy WE43 on the adhesion of microorganisms, we incubated a suspension of *S. cerevisae* yeast, used as a model for microorganisms, on the sample surface for 30 minutes (Figure 5). The results showed that the adhesion properties of the sample after RS did not change in comparison with the homogenized alloy (percentage of adhering microorganisms in relation to the control: 12 ± 1.1% and 12 ± 2.4%, respectively). By contrast, drastically reduced adhesion, down to 6 ± 1.5% (p = 0.02) was observed after RS with subsequent aging. Considering the biodegradation rate results given above, the most probable reason for the mentioned effect is a longer preservation of a smooth (non-pitted) surface of the swaged+aged alloy in comparison with the surfaces of the other samples that rapidly erode during the immersion. This is a very favorable outcome of the thermomechanical treatment (RS + aging), as it promises antifungal properties of biodegradable implants made from the processed alloy WE43.

**Figure 5.** Adhesion of *S. cerevisae* microorganisms on the surface of the samples of the alloy WE43 in the various structural states in relation to the control

The results obtained are of practical interest from the viewpoint of the prospects for using alloy WE43 in implants and submersible fasteners for orthopedic surgery. The occurrence of local infectious complications in the early postoperative period is one of the most serious problems of reconstructive therapy [18]. Rapid adhesion of microorganisms trapped in the wound cavity during the surgery hinders their removal by washing with antiseptic solutions before suturing. The next stages in the evolution of infection are the formation of a film by microorganisms on the surface of the implanted device and their systemic spreading. Considering this, RS with subsequent aging of the alloy is able to reduce the incidence of infectious complications in the early postoperative period of orthopedic reconstructive surgeries by preventing the adhesion of microorganisms.

**4. Conclusions**

1. Rotary swaging of alloy WE43 led to the formation of a predominantly sub-grain structure with an average size of structural elements (predominantly grains) of 450 ± 50 nm and the precipitation of intermetallic Mg41Nd5 particles with an average size of 210 ± 13 nm. Further aging of the swaged alloy at 200 °C for 1 h increases the average size of structural elements and particles to 781 ± 88 nm and 494 ± 51 nm, respectively.

2. Through the use of rotary swaging, it was possible to increase the UTS of the alloy to 363 ± 2 MPa without lowering its ductility (El = 11.4 ± 0.6%). Subsequent aging additionally strengthens the alloy (UTS = 376 ± 9 MPa) with a slight decrease in ductility (El = 7.6 ± 0.4%).

3. The microstructure induced by RS did not impair the biocorrosion resistance over 4 weeks of immersion in FBS, while additional aging of the swaged alloy reduced the biodegradation rate.

4. No significant differences in the biocompatibility of the alloy WE43 in homogenized, swaged and swaged with additional aging were observed.
5. RS with subsequent aging of the alloy reduced the propensity of microorganisms for adhesion to the surface, which may potentially reduce the risk of infectious complications of orthopedic surgery.

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