INTRODUCTION

Bone plates are commonly used in bone fracture healing for internal fixation. They primarily provide mechanical support at the location of bone fracture before bone reunion has completed. Bone plates can be either non-degradable or degradable. Non-degradable or permanent bone plates are made of metallic materials such as Ti alloys and stainless steel. These materials have a high mechanical strength and are used in high load-bearing parts. In addition, they possess an excellent corrosion resistance and will remain inside the human body after bone reunion has completed. After the healing stage, the presence of a permanent bone plate results in a number of adverse effects, the most serious one being the occurrence of osteoporosis in the neighboring bone tissues due to the mismatch in elastic modulus and hence stress shielding [1, 2]. For young patients, permanent bone plates also restrict bone growth [3]. In view of these adverse effects, a second surgery to remove the bone plate is usually recommended.

However, second surgery is accompanied by risk, economic burden, and psychological stress. Whereas, the degradable bone plates would gradually degrade and disappear inside the body. In this case, no second surgery is required, and the retention-removal dilemma in using permanent bone plates is solved. However, the degradable bone plates currently in use are made of polymeric materials, which are inherently of lower mechanical strength and are suitable for low load-bearing parts only. Development of degradable bone plates for high load-bearing parts only. Development of degradable bone plates would gradually degrade and disappear inside the body. In this case, no second surgery is required, and the retention-removal dilemma in using permanent bone plates is solved. However, the degradable bone plates currently in use are made of polymeric materials, which are inherently of lower mechanical strength and are suitable for low load-bearing parts only. Development of degradable bone plate for high load-bearing applications is thus a challenging problem in biomedical materials science.

Magnesium is potentially a wonderful implant material for its non-toxicity to the normal adult stem cells and this means that the magnesium AZ31 alloy with NA is distinguished for its good biocompatibility in a cell culture in vitro. Magnesium is potentially a wonderful implant material for its non-toxicity to the normal adult stem cells and this means that the magnesium AZ31 alloy with NA is distinguished for its good biocompatibility in a cell culture in vitro. The coating of nicotinic acid (NA), sodium fluoride (SF) and sodium fluoric - nicotinic acid (SF-NA) deposited from a sodium fluoric solution with subsequent treatment in nicotinic acid on magnesium alloy AZ31 has been investigated. The coating of nicotinic acid (NA), sodium fluoride (SF) and sodium fluoric - nicotinic acid (SF-NA) deposited from a sodium fluoric solution with subsequent treatment in nicotinic acid on magnesium alloy AZ31 has been investigated.

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Keywords: magnesium alloys; conversion coatings; corrosion; degradable implant material, cytotoxicity.
EXPERIMENTAL DETAILS

Samples of dimensions 10 mm × 40 mm × 2 mm were cut from commercial AZ31 magnesium alloy. The AZ31 samples were mechanically polished to a 2000 grit finish, degreased with acetone and were rinsed in distilled water.

The coating of sodium fluoride was deposited from 0.3 M sodium fluoride for 2 h (pH 8.5) on magnesium alloy AZ31 [12]. The coating of nicotinic acid was deposited at a concentration of 0.05 M for 2 h (pH 2.1). And the coating of sodium fluoride and nicotinic acid was deposited from a sodium fluoride solution (1 h) with subsequent treatment in nicotinic acid (1 h) on magnesium alloy AZ31. Samples of untreated AZ31 and sodium fluoride and/or nicotinic acid-coated AZ31 were immersed in a simulated body fluid (SBF) solution [4]: 8.0 g/l NaCl, 0.4 g/l KCl, 0.14 g/l CaCl₂, 0.35 g/l NaHCO₃, 1.0 g/l CH₂O₆ (glucose), 0.2 g/l MgSO₄·7H₂O, 0.1 g/l KH₂PO₄, 0.06 g/l Na₄HPO₄·7H₂O at 37 °C ±1 °C (pH 7.0).

The potentiodynamic polarization experiments were carried out in naturally aerated SBF at 37 °C using a PGSTAT302 AUTOLAB(The Netherlands). A saturated Ag│AgCl│KCl electrode was used as reference. A platinum foil served as a counter electrode. Potentiodynamic polarization experiments were performed at a low scan rate of 2 mV/s. The corrosion current density (i corr) was estimated by linear fit and Tafel extrapolation to the cathodic and anodic parts of the polarization curves.

Immersion tests of AZ31 samples coated with NA, SF-NA and SF were carried out in SBF solution at pH 7.0, kept at 37 °C in open air. The volume of solution was calculated based on a volume-to-sample area ratio of 50 mL/cm², which well exceeded the minimum ratio required by ASTM G31 [13]. After the immersion test for 4 h, the samples were then cleaned for removing the corrosion products formed on the samples using a standard procedure in growth medium as negative control. Cytotoxicity tests were carried out by indirect contact [15]. Primarily, the NA samples were sterilized in a laminar camera with UV radiation, both sides for 15 min. The extracts of test samples were prepared using a cell growth medium as the extraction medium with the surface area of extraction medium ratio of 1.25 ml/cm² and incubated for 48 h [16].

After each procedure the sample-extract was withdrawn and diluted 1:9 in the growth medium. Such examples of diluted extracts were used for indirect cytotoxicity tests. The control cell groups involved the use of regular growth medium as positive control and 0.64 % phenol in growth medium as negative control.

In the study of cytotoxicity, primary adult myogenic stem cell line was applied. It was prepared from an adult rabbit muscle [17]. The cells were grown in Iscove’s modified Dulbecco’s medium (IMDM, Gibco) supplemented with 10 % of fetal calf serum (Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were maintained at 37 °C in humidified atmosphere with 5 % CO₂ and passaged twice a week detaching cells from the plate by a 0.25 % (w/v) trypsin/EDTA solution (Gibco).

The cells were inoculated into 96-well tissue culture test plates (Orange Scientific) at a density of 3 × 10⁵ cells per 100 μl in the growth medium in each well and incubated for 24 h. Then, the medium was replaced with 100 μl of prepared extracts and incubated for 24 h or 72 h. After that, the culture medium was removed and 10 μl of MTT was added to each well. MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, Sigma) test is a colorimetric method for the quantitative evaluation of cell proliferation and viability in vitro. The cell samples were incubated with MTT for 1 h at 37 °C, the solution was removed and insoluble formazan was dissolved in the ethanol. The absorbance of colored solution was quantified by measuring at a 570 nm wavelength by a microplate reader (Tecan Infinite 200).

A qualitative analysis of cell viability was assessed in neighboring samples using an inverted optical and fluorescence microscope (Nicon). Cell visualization was accomplished by using a dye-mix solution of 100 μg/ml acridine orange (AO, Molecular probes) and 100 μg/ml ethidium bromide (EB, Sigma). A volume of 3 μl of this dye mixture was added to 50 μl of growth medium in the well. AO is taken up by viable cells. It intercalates into double-stranded DNA and makes it appear green. EB is only taken up by nonviable cells; it intercalates into DNA, making it appear orange [18].

RESULTS AND DISCUSSION

The polarization curves of the coatings obtained from different solutions are demonstrated in Fig. 1 and the corresponding corrosion potential and corrosive current are tabulated in Table 1. The corrosion potential (E corr) of NA coating have shown a negative shift to 70 mV as compared with that of magnesium alloy substrate with SF. The corrosion current density (i corr) decreased sharply from 70.1 × 10⁻⁶ A/cm² of the substrate with SF coating to 20.4 × 10⁻⁶ A/cm² for NA coating.

Fig. 2 reveals the morphology of the conversion coatings on magnesium alloy after various treatments. Fig. 2, a, b, displays the morphology of AZ31 after 2 h treatment using the NA (0.05 M). A protective layer with “dry-mud” morphology is seen. Fig. 2, c, d, shows coatings on the magnesium alloy treated for 1 h in the SF solution with subsequent one-hour treatment in the NA solution. Network-like cracks as those in Fig. 2, a, b, are uniformly distributed all over the surface of the conversion-coated layer. It can be seen that some irregular cracks are formed on the surface. The conversion coatings were no longer continuous with some patches on the magnesium alloy surface. These cracks may possibly be due to hydrogen evolution during the conversion treatment and/or the

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dehydration of the surface layer after treatment. While as Fig. 2, e, f, presents the morphology of magnesium alloy after treatment in SF (0.3 M) without any cracks.

![Polarization curves of AZ31 in SBF: 1 – SF-NA; 2 – SF; 3 – NA](image)

**Fig. 1.** Polarization curves of AZ31 in SBF: 1 – SF-NA; 2 – SF; 3 – NA

**Table 1.** Corrosion potentials and corrosion current densities obtained from the electrochemical potentiodynamic polarization curves

<table>
<thead>
<tr>
<th>Sample</th>
<th>(-E_{\text{corr}}) (V(_{\text{Ag/AgCl}}))</th>
<th>(i_{\text{corr}}) (A/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZ31 treatment by immersing in nicotinic acid solution</td>
<td>1.43</td>
<td>20.4 \times 10^{-6}</td>
</tr>
<tr>
<td>AZ31 treatment by immersing in Na(_2)F solution and in nicotinic acid solution</td>
<td>1.39</td>
<td>48.6 \times 10^{-6}</td>
</tr>
<tr>
<td>AZ31 treatment by immersing in Na(_2)F solution</td>
<td>1.36</td>
<td>70.1 \times 10^{-6}</td>
</tr>
</tbody>
</table>

Element distribution of the coating was analyzed using electronic probe microanalysis. Fig. 3 shows the EDS X-ray maps of magnesium, aluminum, zinc, fluorine, carbon, nitrogen and oxygen at the same place of the coating. The distribution images of the elements resembled the micrograph obtained by SEM which also presented a net-like structure. The distribution of the net coincided with the cracks in the conversion coatings. The quantities of magnesium, fluorine and zinc were increased while aluminum was sensed at the same sites. The carbon and oxygen elements mainly concentrated on the islands surrounded by the cracks.

So, the results of morphological studies seemingly contradict the ones of polarization studies. In our opinion the reason is that the protective coating of Mg alloy is formed of two layers. The former being unsoluble MgF\(_2\) (in the cracks), and the latter being formed by NA and compounds of magnesium alloy elements (in islets). Thus, the two-layer coating is distinguished for its barrier protection of magnesium alloy.

The morphology of the sample surface is shown in the SEM micrographs in Fig. 4. It could be observed that all samples are similar in their corrosion behavior. To characterize the corrosion resistance of immersion samples of AZ31 we exploited the method of intersections lines based on the Saltykov method [19]. This method consists in calculating of the length of intersections across uncorroded areas in relation to the unit of intersection length. A linearly size \((L)\) was calculated according to the equation:

\[
L = \frac{l_1 + l_2 + \ldots + l_n}{l},
\]

where \(l_n\) is the length of intersections on uncorroded areas, mm; \(l\) is the total length of all intersections lines, mm.

According to (1), \(L\) decreases in the following order: NA > SF-NA > SF or 0.237>0.227>0.129, respectively. Thus, the immersion test has shown that the corrosion resistance of coatings NA is higher than that of SF coatings.

![SEM micrograph of AZ31 alloy after immersion in solution at 25 °C](image)

**Fig. 2.** SEM micrograph of AZ31 alloy after immersion in solution at 25 °C: (a, b) NA (2 h), (c, d) 0.3 M SF (1 h) and NA (1 h), (e, f) 0.3 M SF (2 h). Magnitude: a, c, e – ×400; b, d, f – ×2000

The experimental results suggest that 10 % of tested extracts did not indicate cytotoxic activity in normal adult stem cell culture. Fig. 5 shows the results of the indirect cytotoxicity test for magnesium alloy AZ31 samples. Qualitative analysis of the cell monolayer had no signs of lesion (Fig. 5, a – control culture, c – monolayer after the treatment). The study of cell viability certified the same cell viability level: after AO&EB staining the cells appeared green (viable) both in control and in tested cell cultures (Fig. 5, b and d). MTT quantitative analysis of the cells after 24 h and 72 h treatments (Fig. 5, e and f) demonstrated the same proliferative activity in the tested and control (negative) cultures. So, it can be concluded that these materials did not liberate any toxic components which could inhibit cell growth or provoke cell death.
Fig. 3. Mapping pictures Mg, Al, Zn, F, C, N, O elements distribution of NaF-NA coating obtained from solutions 0.3 M SF (1 h) and 0.05 M NA (1 h). Magnitude: a – h – ×400

Fig. 4. SEM micrographs surface morphology after immersion in SBF at 37°C, in open air, for 4 h, with corrosion products removed according to ASTM G1-90: (a, b) NA –coated, (c, d) (SF – NA) and (e, f) SF. Magnitude: a, c, e – ×400; b, d, f – ×2000

CONCLUSIONS

A comparison polarization tests results for conversion coatings have shown a sharp reduction in corrosion current density from SF to NA, $70.1 \times 10^{-6}$ A/cm$^2$ to $20.4 \times 10^{-6}$ A/cm$^2$ respectively.

The morphology results revealed that the NA coatings possess cracks. Despite the presence of cracks, the NA coatings protect the AZ31 magnesium alloy against corrosion better than SF coatings.
Immersion tests recorded a significant increase in corrosion resistance due to the nicotinic acid conversion coating.

The cytotoxicity tests have shown that AZ31 magnesium alloy with NaF-NA conversion coatings in 10% of the tested extracts did not indicate cytotoxic activity in a normal adult stem cell culture. This means that the magnesium AZ31 alloy with NaF-NA conversion coatings is distinguished for its good biocompatibility in cell culture in vitro.

Nicotinic acid is an efficient and environment-friendly inhibitor for magnesium alloy AZ31 in a simulated body fluid (SBF).

REFERENCES


