Effects of chromium ion implantation voltage on the corrosion resistance and cytocompatibility of dual chromium and oxygen plasma-ion-implanted biodegradable magnesium

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Abstract

Magnesium is modified by chromium ion implantation at different voltages followed by oxygen ion implantation to improve the corrosion resistance and cytocompatibility. All the implanted samples exhibit improved corrosion resistance and the ones implanted at a lower voltage yield better results. The chromium-rich layer with chromium in the metallic state beneath the protective oxide film may undermine the electrochemical stability by inducing galvanic effects which lead to poorer corrosion resistance. Although dual Cr–O plasma immersion ion implantation promotes osteoblast adhesion and proliferation on the magnesium samples and produces a more favorable environment for osteoblast growth, optimal results require careful selection of the ion implantation voltage.

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1. Introduction

Biodegradable implants are revolutionary [1] because they may reduce risks inherent to permanent implants such as irritation and inflammatory responses [2]. As one of the biodegradable materials, magnesium and its alloys have attracted much attention because magnesium is one of the essential elements and exists in bone tissues [3,4]. Moreover, the Young’s modulus of magnesium is close to that of natural bones thus rendering it more suitable for load-bearing fracture stabilization [5,6]. However, the poor corrosion resistance of Mg and Mg alloys hinders many clinical applications. Magnesium corrodes rapidly in an aqueous environment accompanied by evolution of hydrogen bubbles and high local pH [7]. Since rapid corrosion of magnesium results in early loss of mechanical stability before tissue healing [6], the surface corrosion resistance must be enhanced, especially in the initial stage after surgery [8].

Surface treatment is a common approach to improve the corrosion resistance of magnesium and its alloys and examples of techniques include electrodeposition [9–13], microarc oxidation [14–17], physical vapor deposition [18–21], and ion implantation [22–28]. Plasma immersion ion implantation (PIII) [29,30], which modifies selective surface characteristics and reduces the degradation rate without adding a foreign coating and altering the bulk properties, is especially useful to surgical implants with a complex shape. PIII produces oxide or other compounds on the surface of magnesium and magnesium alloys [29] to enhance the corrosion resistance. Chromium (Cr) plays a protective role in improving the corrosion resistance of stainless steels. Electrochemical and immersion tests in different solutions have confirmed the initial improved corrosion resistance of pure magnesium after Cr and oxygen (O) ion implantation [22,23]. Nevertheless, the influence of implantation parameters on the corrosion behavior and cytocompatibility of chromium and oxygen ion implanted magnesium and associated mechanism are not well known. In this study, pure magnesium blocks are implanted with chromium and oxygen at different voltages to determine the effects on the corrosion resistance and the cytocompatibility before and after dual Cr and O plasma ion implantation is evaluated by monitoring the behavior of rat calvaria osteoblasts.

2. Materials and methods

As-cast pure magnesium blocks (99.95% pure; 10 × 10 × 5 mm3) were mechanically ground with #400, #600, #800, and #1200 water proof diamond paper sequentially and polished with 1 μm alumina powder. Before PIII, all the samples were ultrasonically washed in pure ethanol and dried in air. Some pure magnesium samples

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Accelerated voltage (kV)</th>
<th>Decelerated voltage (kV)</th>
<th>Implantation time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mg-15</td>
<td>15</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Mg-20</td>
<td>20</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Mg-40</td>
<td>40</td>
<td>1.3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Untreated Mg</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1 Chromium ion implantation conditions.
(untreated Mg) were randomly selected to be implanted with chromium first and oxygen afterwards. Metal ion implantation was carried out on a HEMII-80 ion implanter manufactured by Plasma Technology Ltd. The base pressure in the vacuum chamber was $10^{-6}$ Pa and the other important ion implantation parameters are listed in Table 1. Oxygen PIII was performed on the GPI-100 ion implanter. The base pressure in the vacuum chamber was $10^{-3}$ Pa and 99.99% oxygen was bled into the chamber at a flow rate of 25 sccm. The pulsed voltage was 30 kV, pulse width was 30 μs, and pulsing frequency was 100 Hz. The oxygen plasma was ignited by 1 kW radio frequency (RF) and implantation lasted for 1 h.

Atomic force microscopy (AFM) was utilized to examine the morphology of the surface before and after ion implantation. The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI 5802). Al Kα irradiation was employed and the estimated sputtering rate was 7 nm/min.

The corrosion behavior of the control and implanted samples was evaluated by potentiodynamic polarization tests and electrochemical impedance spectroscopy (EIS) conducted on a Zahner Zennium electrochemical workstation at 37 °C. A three-electrode cell with the sample as the working electrode, saturated calomel electrode (SCE) as the reference electrode, and platinum electrode as the counter electrode was adopted. The working electrodes were covered by silicon and an area of 1 cm² was exposed to the solution. In the potentiodynamic polarization test, the scanning rate was 2 mV s⁻¹. The corrosion potential ($E_{corr}$) was derived from the curves and the corrosion current density ($i_{corr}$) was determined from the polarization curves by Tafel extrapolation. The impedance data were recorded from 100 kHz to 100 mHz with a 5 mV sinusoidal perturbing signal at the open circuit potential. All the electrochemical tests were carried out in the SBF solution with inorganic ion concentrations (in mM) similar to those of human extra fluids (142.0 Na⁺, 5.0 K⁺, 1.5 Mg²⁺, 2.5 Ca²⁺, 147.8 Cl⁻, 4.2 HCO₃⁻, 1.0 HPO₄²⁻, 0.5 SO₄²⁻ with a pH of 7.40) which was prepared by dissolving reagent grade (in mM) 137.4914 NaCl, 4.2258 NaHCO₃, 3.0181 KCl, 1.0122 K₂HPO₄ · 3H₂O, 1.5297 MgCl₂ · 6H₂O, 2.6311 CaCl₂, and 0.5068 Na₂SO₄ in distilled water and buffered at pH 7.4 with 50.5035 mM Trishydroxymethyl aminomethane (TRIS) and 1.0 M HCl [30].

The MC3T3-E1 cells were cultured in a Dulbecco’s modified Eagle medium (DMEM, Gibco, cat. no. 12100-046) supplemented with 10% fetal bovine serum (FBS, Gibco, cat. no. 10270-106). The osteoblasts were seeded at a density of 3 × 10⁴ cells per well on 24-well tissue culture plates and incubated in a humidified atmosphere of 5% CO₂ at 37 °C. Before cell culturing, the specimens were encapsulated with silicon and sterilized with 75% ethanol for more than 1 h. The specimens were untreated Mg and Mg-15 and the cell culture medium was refreshed every day. After culturing for 1, 3 and 7 days, the osteoblast-seeded samples were sputter coated with gold.

The morphology of the MC3T3-E1 cells was assessed by fluorescence staining. After incubation for 1 and 3 days, the osteoblasts were fixed with 2% paraformaldehyde and immunofluorescently stained with the cytoskeleton protein f-actin with phallolidin–fluorescein isothiocyanate (Sigma). The nuclei were counter-stained with 4′,6′-diadidino-2-phenylindole (DAPI). The pictures were acquired by a digital camera (Carl Zeiss Axio Observer Z1). To evaluate the effect of the solution on the osteoblasts, the cell morphology on the tissue culture plate was monitored after culturing for 3 days. A cell counting kit-8 (CCK-8 Sigma) was employed to quantitatively evaluate the cell viability. After culturing for 1, 3 and 7 days, the osteoblast-seeded samples were examined by field-emission scanning electron microscopy (FE-SEM, FEI/Philips XL30 Essem-FEG), and the samples were sputter coated with gold.
were rinsed twice with sterile PBS and transferred to fresh 24 well tissue culture plates with a culture medium with 10% CCK-8. After culturing for 4 h, the optical density (OD) was measured at 450 nm on a PowerWave microplate spectrophotometer (BioTek, USA). The statistical analysis was performed based on the one-way ANOVA analysis.

3. Results and discussion

The AFM images in Fig. 1 show that the surface morphology of the untreated Mg and Mg-20 is different. The surface of the untreated sample is characterized by uniformly distributed small cones with scratches produced during mechanical polishing (Fig. 1(a)). On the other hand, Fig. 1(b) shows that the surface of Mg-20 has uniformly distributed large dome-shaped islands. The root-mean-square (RMS) roughness values of the untreated Mg and Mg-20 are 12.8 and 11.1 nm, respectively. The implanted sample has a smoother surface probably due to surface restructuring at elevated temperature during ion implantation [31,32].

Fig. 2(a) displays the chromium depth profiles obtained from Mg-15, Mg-20, and Mg-40. A rich chromium rich layer is found from the implanted samples and the thicknesses are about 110 nm for 15 kV, 130 nm for 20 kV, and 170 nm for 40 kV. When the applied voltage is increased, the chromium ions have more energy and penetrate more deeply and the results are consistent with those reported by Tan et al. [33] and Liu et al. [34]. The oxygen depth profiles are shown in Fig. 2(b). A high concentration of oxygen is present on the near surface of the untreated and implanted samples and the oxygen concentration decreases gradually with depth. The oxygen distributions in Mg-15, Mg-20, and Mg-40 are very similar because of the same oxygen implantation parameters. The surface oxygen stems from natural surface oxidation, but compared to the untreated sample, the oxygen concentrations in the three implanted samples are higher.

Fig. 3 depicts the high-resolution Cr 2p XPS spectra acquired from the implanted samples. As the sputtering time increases, the oxidized state (Cr³⁺) shifts to the metallic state (Cr⁰) and the peak intensity decreases gradually, suggesting the formation of chromium oxide near the

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### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Untreated Mg</th>
<th>Mg-15</th>
<th>Mg-20</th>
<th>Mg-40</th>
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</thead>
<tbody>
<tr>
<td>E&lt;sub&gt;corr&lt;/sub&gt; (V vs. SCE)</td>
<td>−2.90</td>
<td>−1.57</td>
<td>−1.51</td>
<td>−1.52</td>
</tr>
<tr>
<td>i&lt;sub&gt;corr&lt;/sub&gt; (A/cm²)</td>
<td>3.954 × 10⁻⁴</td>
<td>3.804 × 10⁻⁵</td>
<td>1.038 × 10⁻⁴</td>
<td>2.534 × 10⁻⁴</td>
</tr>
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</table>

Fig. 4. Potentiodynamic polarization curves obtained from the untreated and implanted samples.

Fig. 5. Nyquist plots of the untreated and implanted samples.
surface. The thickness of the oxidized layer is about 70 nm and about the same in all three implanted samples. The thicknesses of the layers with the metallic state are about 40 nm in Mg-15, 60 nm in Mg-20, and 100 nm in Mg-40.

The potentiodynamic polarization curves acquired from the untreated and implanted samples in SBF at 37 °C are displayed in Fig. 4 and the corrosion potential ($E_{corr}$) and corrosion current density ($i_{corr}$) are summarized in Table 2. In our experiments, $E_{corr}$ of the untreated sample is about $-2.0$ V and increases to $-1.5$ V versus SCE after ion implantation. All the implanted samples have distinctly lower $i_{corr}$ than the untreated sample and in particular, $i_{corr}$ of Mg-15 is significantly reduced to $3.804 \times 10^{-5}$ Å/cm², which is nearly an order of magnitude smaller than that of the untreated sample. Generally, the smaller the corrosion current density, the lower the corrosion rate and the better the corrosion resistance [35–38]. These results imply that the degradation rate of magnesium is indeed retarded after dual ion implantation and furthermore, the sample implanted with chromium at a smaller voltage exhibits better corrosion resistance.

Fig. 5 depicts the EIS spectra of the untreated and implanted samples in SBF at 37 °C. A capacitive loop can be observed from the higher frequency region in all the curves. After ion implantation, the capacitive loop is evidently enlarged indicating a reduced corrosion rate and the data are consistent with ones obtained previously [23,39,40]. Different chromium ion implantation voltages give rise to different corrosion properties. Mg-15 implanted using the smallest voltage shows the largest capacitive loop and the best corrosion resistance. The conclusion is similar to that drawn from the polarization data.
The chromium oxide film on the implanted samples plays a protective role [22,23]. It is believed that a thicker oxide film provides better corrosion, but based on XPS, the chromium oxide and magnesium oxide layers in the different samples have a similar thickness but different corrosion resistance. Chromium in the metallic state can induce severe galvanic effects by serving as the cathode with magnesium being the anode when an aggressive medium penetrates the protective layer [22,41,42]. As a result, the thinner metallic chromium layer in Mg-15 actually yields a smaller corrosion rate but the thicker layer in Mg-40 produces a larger corrosion rate. Our results reveal that the implantation voltage is crucial to the corrosion resistance.

Fig. 6 shows the morphology of MC3T3-E1 osteoblasts on the untreated Mg and Mg-15 at different magnifications. The number of cells on Mg-15 after culturing for 1 day is significantly larger than that on the untreated sample. Most osteoblasts on Mg-15 have an irregular and flattened morphology with many pseudopodia and the spreading suggests better initial cell anchorage [43]. A cracky and corroded surface is observed from the untreated Mg but the surface of Mg-15 appears to be intact after culturing for 1 day. The data provide evidence of enhanced cytocompatibility in the early stage.

Fig. 7 reveals the double stained osteoblast morphology on the untreated Mg and Mg-15 after culturing for 1 and 3 days. Most osteoblasts on Mg-15 show an irregular and flattened morphology with many pseudopodia and the spreading suggests better initial cell anchorage [43]. A cracky and corroded surface is observed from the untreated Mg but the surface of Mg-15 appears to be intact after culturing for 1 day. The data provide evidence of enhanced cytocompatibility in the early stage.

The plasma modified layer is composed by a protective chromium oxide layer and an interlayer with chromium in the metallic state. The thickness of the layer increases with chromium ion implantation voltage due to the higher ion energy as well as elevated temperature [50,51]. However, the location where the transition from the oxidized to metallic states occurs does not depend significantly on the ion implantation voltage and therefore, the thickness of the oxide layer in the implanted samples is more or less the same. This is attributed to the same implantation conditions for oxygen. As a result of the chromium oxide protective layer, all the implanted samples show higher corrosion potentials and lower corrosion current densities than the control but interestingly, our data disclose that a smaller Cr implantation voltage is actually better from the perspective corrosion resistance. The thickness of the chromium interlayer increases with higher ion implantation voltage and this metallic chromium interlayer appears to be much larger than that on the untreated after the first and third day. The cell density on Mg-15 after the third day is larger than that after the first day indicative of normal osteoblast proliferation. On the other hand, large bubbles labeled with white arrows can be observed from the control suggesting fast corrosion. To evaluate the effects of the solution, the osteoblast morphology on the tissue culture plate is examined after culturing for 3 days (Fig. 8). Similar to the aforementioned results, more osteoblasts with irregular polygon shapes spread well in the extracted medium of the implanted sample, thereby furnishing evidence of a more favorable environment for cell proliferation.

Good cell adhesion and spreading not only promote cell—material interactions, but also produce positive effects on the subsequent cell performance [44]. Fig. 9 displays the osteoblast viability after culturing for 1, 3, and 7 days. The OD value on Mg-15 is higher than that on the untreated Mg at each time point, suggesting improved osteoblast attachment and growth throughout the period. Osteoblast growth is fast during the initial 3 days but slows thereafter and this observation is consistent with that by Stein et al. [45] and Wang et al. [44]. Stein et al. [46,47] indicated that the proliferation genes were down-regulated by the functionally coupled relationship between osteoblast proliferation and differentiation in initial differentiation.

Pll alters both the physical and chemical characteristics of magnesium, especially the surface morphology. The surface roughness and topography are affected by the implantation voltage. Tian et al. [48] and Liu et al. [34] have shown that the surface morphology of stainless steel and titanium alloys varies substantially with implantation voltages. Lampin et al. have observed that a rough polymer surface has positive effects on cell adhesion due to better anchorage of cells to the substrate [49]. In general, a rough surface offers a larger surface area for better cell attachment. However, based on this study, the surface roughness does not appear to be the dominant factor affecting osteoblast adhesion.

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the culprit that degrades the corrosion property. The galvanic effect occurring between the chromium interlayer and magnesium substrate [22,52] is accentuated by a thicker metallic chromium interlayer. The results reveal that proper conditions must be adopted in order to improve the corrosion resistance.

Cells are very sensitive to a fluctuated environment and a high pH often leads to fatal effects from the viewpoint of cell viability [51,53,54]. In contact with the culture media, corrosion on the untreated magnesium produces cracks, hydrogen gas, and higher pH in the vicinity [55–57]. These reasons account for the poor osteoblast adhesion and proliferation [57] observed from the untreated magnesium. On the other hand, Mg-15 exhibits good corrosion resistance and no obvious surface corrosion and hydrogen bubbles can be observed. The osteoblasts also possess a good morphology and our results reveal no apparent cytotoxicity on the implanted samples.

4. Conclusion

Magnesium is surface modified by chromium ion implantation at different voltages and then oxygen plasma immersion ion implantation. The dual plasma implantation process produces a surface chromium oxide layer which improves the corrosion resistance as inferred from the higher $E_{corr}$ and lower $i_{corr}$ compared to the untreated control samples. Among the samples implanted at different voltages, Mg-15 implanted with Cr at the smallest voltage exhibits the best electrochemical stability. The thickness of the metallic state chromium layer beneath the oxide film in Mg-15 is the smallest and this layer which induces galvanic effects appears to be the reason for the degraded corrosion resistance among the implanted samples. The MC3T3-E1 cell cultures on Mg-15 indicate that the osteoblasts attach and proliferate better on the plasma-implanted sample. Although dual Cr and O plasma ion implantation can improve the corrosion resistance and cytocompatibility of Mg, optimal results require careful selection of the implantation voltage.

Acknowledgments

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