Regenerative influence of nanostructured bredigite (Ca$_7$Mg$_4$Si$_4$O$_{16}$)/anodic spark coating on biodegradable AZ91 magnesium alloy implants for bone healing

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Abstract

Magnesium has been recently introduced as a novel biodegradable material for bone healing. However, the fast degradation of this material results in the fast release of hydrogen which limits its clinical applications. In view of that, in the present study, we attempt to overcome this drawback using a bredigite (Ca$_7$MgSi$_4$O$_{16}$) coating. In our previous work, we have coated AZ91 magnesium implants with bredigite through the combination of anodic spark deposition (ASD) and electrophoretic deposition (EPD) techniques. As continuation to that work, in this paper, we have focused on the *in vivo* examination of the bredigite/ASD compared to the plain ASD coated and the uncoated AZ91 substrates. The results of the *in vivo* animal test in the greater trochanter of rabbits indicated improved regeneration of bone and less bone inflammation upon employing bredigite/ASD coated implants. In addition, an enhancement in *in vivo* biodegradation was observed by the reduction in magnesium ion released in the blood plasma. In summary, a surface treatment using bredigite on magnesium implants promotes their bone healing capabilities for future clinical applications.

**Key words:** Magnesium implant; Bredigite coating; Surface coating; Bone regeneration; Biocompatibility.

1. Introduction

Magnesium alloys have been recently introduced as a new biomedical metal material with good mechanical and biological properties [1-3]. However, magnesium is degraded too quickly in physiological solutions leading to the hydrogen release which in turn inversely influences the bone healing during the implantation [4-6]. Surface coatings using ceramics have been used as an effective method to advance the regenerative properties and biodegradation of implants in corrosive media [7-10].
Silicate bioactive ceramics are an appropriate group of biomaterial that induces the incidence of biological fixation and tissue in-growth at the interface of the tissue/implant [6, 11]. A relevant example is bredigite which is a calcium magnesium silicate compound in the CaO-MgO-SiO$_2$ ternary system with the chemical formula of Ca$_7$MgSi$_4$O$_{16}$ [12]. Recent studies have shown that this ceramic is highly bioactive, induces regeneration and possesses apatite-formation ability due to the existence of Ca, Mg, and Si in its composition [12, 13].

In our preceding work, we employed a combination of anodic spark deposition (ASD) and electrophoretic deposition (EPD) techniques for the surface modification of AZ91 magnesium alloy using the bredigite bioactive ceramic [14]. Regarding the coating performance, we observed an improvement in bioactivity, corrosion resistance and cytocompatibility which was presented in our previous paper [14, 15]. In this article, we have focused on the bone healing analysis and in vivo examination of this implant made by AZ91 magnesium alloy coated by nanostructured bredigite (Ca$_7$MgSi$_4$O$_{16}$).

2. Materials and methods

The rod samples with the diameter of 3 mm and the length of 6 mm were machined from an AZ91 magnesium alloy billet for the in vivo assessments. The entire coating process was explained in our prior paper [14]. Briefly, for ASD coating, an alkaline silicate electrolyte was used containing sodium silicate (200 g/L) and sodium hydroxide (200 g/L). The voltage was 60 V which applied for half an hour. To do the EPD process, bredigite powder suspensions (100 g/L) were prepared using methanol as solvent. The ASD sample was placed in the location of cathode and a graphite rod as the anode. The applied voltage was 100 Volts, the distance between the electrodes was 20 mm and the deposition time was 3 min.
A transmission electron microscope, TEM (JEOL JEM-2100), a Philips XL 30: Eindhoven scanning electron microscope (SEM) equipped with energy-dispersive X-ray spectroscopy (EDX) and a laser scanning electron microscope (Keyence, VK X100/X200) was used to investigate the microstructure of the samples. 

*In vivo* animal testing was performed agreeing to the University Ethics Committee. Adult rabbits with 3 kg weight were anaesthetized with 35 mg/kg Ketamine, 5 mg/kg Xylazine and 1 mg/kg Acepromazine. Then, the implants were placed into the greater trochanter of the rabbits. The X-ray radiography was performed of operation sites after 2 weeks post-operation. The serum magnesium in the blood was measured by a Hitachi 911 automatic hemocyte analyzer. The rabbits were sacrificed after 2 months and the bone samples were taken out. The bone samples were decalcified by nitric acid and were stained with Hematoxylin and Eosin (H&E) stain and histological evaluation was conducted using a light microscope. The corrosion products on the surface of samples were cleaned by immersing the samples into the chromic acid (200 g/L) for 5 min. The weight loss was calculated using the difference in the weight of the samples before and after the chromic acid cleaning.

3. Results and discussion

Fig. 1a illustrates the morphology and size of the bredigite nanoparticles using TEM imaging. According to this Fig. the size of the bredigite nanoparticles are in the range of 50–100 nm. On the cross-sectional view of bredigite/ASD coating, Ca, Mg and Si elements can be detected in the line-scan analysis (Fig. 1b). According to this Fig., the intensity of Mg increased from bredigite/ASD coating to AZ91 substrate, while the Ca and Si elements are in the opposite trends. According to the laser scanning microscope images (Fig. 1c -1e), bredigite /ASD had a rough and porous morphology. Two and three-dimensional images demonstrated the existence of
islands with heights in the range of 100 – 400 µm on the coating morphology which can be
distinguished by red color on Fig. 1c, e. The area of these islands is in the range of $10^5$-$2\times10^5$
µm². Some submicron fluctuations are observed on the line scan profilometry analysis (Fig. 1e)
showing the presence of small scale roughness on the surface other than the large scale islands.
Fig. 2 shows the surgery images during the implantation of AZ91 (a), ASD (c), and
bredigite/ASD coated (e) samples. Clinical signs such as skin alteration and swelling were not
observed and good wound healing occurred after the surgery. Also, Fig. 2 shows the radiography
images of samples after 2 weeks post-operation for AZ91 (b), ASD (d) and bredigite/ASD coated
(f) samples. According to this Fig., gas bubbles can be seen around the samples. The AZ91
sample showed the highest gas formation, and gas formation for ASD was less than AZ91
sample. Almost no gas bubbles were found around the bredigite/ASD coated implant. Hydrogen
bubble formation is mostly owing to the biodegradation reactions of implanted samples [16].
The results of the histological evaluation of AZ91 (a), ASD (b) and bredigite/ASD (c) implants
were presented in Fig. 3. The bone surface area from the histology slides has been measured and
the results of image analyses in different samples reveal that the order of new bone formation has
been as follows: bredigite/ASD (36%) > ASD (31%) > AZ91 (27%), and the order of
inflammation is: bredigite/ASD < ASD < AZ91. Thus, the bredigite coating has increased the
regeneration of the bone approximately 5% in bredigite/ASD samples compared to the ASD ones
in similar condition. Since the ASD coating itself has enhanced the new bone formation by 4%,
the total improvement of bone regeneration in bredigite/ASD coated sample compared to an
uncoated one can be estimated to be 9%.

Before the surgery and 2 weeks, 1 and 2 months after the surgery, the changes in the serum
magnesium levels of blood samples of rabbits were evaluated and the results were presented in
Fig. 4. Before the surgery, the serum magnesium was the same for all rabbits, however after the implantation of samples this value increased. It is worth mentioning that the detected serum magnesium level is lower than 20 ppm which is in the normal range of magnesium levels in blood. Thus, the bredigite/ASD coated sample did not induce a great increase of serum magnesium because of the lower biodegradation of the bredigite/ASD coated samples compared to the uncoated samples. It is worth noting that the excess Mg ions are regulated in the kidney and excreted in the urine [17, 18].

After the rabbits were sacrificed, the weight loss of AZ91, ASD, and bredigite/ASD coated implants was measured. The weight loss for AZ91, ASD, and bredigite/ASD coated samples was about 25, 16, and 7 mg/cm², respectively. However, a significant increase in serum magnesium was not seen indicating the excretion of magnesium ions has occurred in the urine [19].

Removing the samples from the implanted site in vivo, we observed that the implants became smaller in both diameter and thickness. A white layer had precipitated on the surface of implants as corrosion products. Previous in vitro bioactivity evaluations on the same sample confirmed that the corrosion products have composed of bioactive minerals such as calcium phosphates and calcium magnesium phosphates [14]. After cleaning the corrosion products using chromic acid, the signs of corrosion defects were appeared on the surface of all implants. Severe pits existed on the surface of the uncoated AZ91 Mg alloy implant. Some smaller pits were observed on the surface of ASD sample. However, the pits on the surface of Bredigite/ASD coated implant were very minor, which indicates that this coating can act as a proper barrier, sealing the implant from exposure to the corrosive body fluid. According to our observation, none of the samples presented uniform degradation.
4. Conclusion

The clinical applications of magnesium implants have been restricted due to the high degradation rate of magnesium alloys. The results of the present research showed that the bredigite/ASD coated magnesium implants can induce better wound healing, enhance the new bone formation and decrease the bone inflammation. Thus, the AZ91 magnesium alloy implant coated with bredigite/ASD may be utilized as a suitable regenerative and biodegradable implant for future clinical applications.

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References


Figure captions

Fig. 1. TEM image of bredigite nanoparticles (a), EDS line scan analysis of different locations in the cross section of the implant from bredigite coating to AZ91 substrate (b), laser scanning microscope images including two-dimensional (2-D) (c), laser + optical (d), three-dimensional (3-D) (e) images and profilometry analysis of the surface of bredigite/ASD coated samples (f).

Fig. 2. Operation images during the implantation of AZ91 (a), ASD (c), and bredigite/ASD coated (e) implants into the greater trochanter of rabbits and the X-ray radiography taken from the operation site of AZ91 (b), ASD (d) and bredigite/ASD coated (f) samples implanted into the greater trochanter of rabbits.

Fig. 3. Histological analysis of the host tissue around the AZ91 (a), ASD (b) and bredigite/ASD coated (c) implants after 2 months post-operation.

Fig. 4. The amount of Mg ions released from the samples in the blood plasma of rabbits during the in vivo animal test.
Fig. 1.
Fig. 2.
Fig. 3.

(a) Severe inflammation

(b) Bone

(c) Mild inflammation
Fig. 4.