In vitro and in vivo analysis of the biodegradable behavior of a magnesium alloy for biomedical applications

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The present study was designed to investigate the biodegradation behavior of Mg alloy plates in the maxillofacial region. For in vitro analysis, the plates were immersed in saline solution and simulated body fluid. For in vivo, the plates were implanted into the tibia, head, back, abdominal cavity, and femur and assessed at 1, 2, and 4 weeks after implantation. After implantation, the plate volumes and the formed insoluble salt were measured via micro-computed tomography. SEM/EDX analysis of the insoluble salt and histological analysis of the surrounding tissues were performed. The volume loss of plates in the in vitro groups was higher than that in the in vivo groups. The volume loss was fastest in the abdomen, followed by the head, back, tibia, and femur. There were no statistically significant differences in the insoluble salt volume of the all implanted sites. The corrosion of the Mg alloy will be affected to the surrounding tissue responses. The material for the plate should be selected based on the characteristic that Mg alloys are decomposed relatively easily in the maxillofacial region.

Keywords: Biodegradation, Magnesium, Corrosion, Blood flow, Wound healing

INTRODUCTION

For substitution of stainless steel and titanium and titanium alloys used internal fixation of fractures\(^{1,2}\), various biodegradable materials have been developed to avoid a second surgery to remove metal devices after healing\(^9\). However, the currently available biodegradable polymers have unsatisfactory load bearing capabilities, and, therefore, limited applications\(^9\). The potential suitability of magnesium (Mg) alloys as biodegradable materials has been focused because Mg alloys have physical and mechanical properties close to those of bone\(^5,6\) and biocompatibility\(^7,8\).

In the maxillofacial region, polymeric materials are used for the treatment of bone fractures since the load applied to the region represented by occlusal force is relatively small compared to that to the limb. Mg alloys are applied in the limited regions of the body for limited treatment options because the strength of Mg alloys are lower than that of other metal alloys at the present; however, Mg alloys are expected to be applied in the maxillofacial region prior to using in the entire body since they show higher-strength compared to polymeric materials. The application of Mg alloys in the maxillofacial region requires them to possess an aesthetic function, and also the alloys should rapidly corrode and disappear in accordance with the period of the healing of a bone fracture. Thus, it is necessary to understand the characteristics of degradation behaviors of devices made of Mg alloys in the maxillofacial region for selecting an appropriate Mg alloy from various kinds of Mg alloys.

The corrosion rate of Mg alloy is difference by composition and processing method of alloy. So, the corrosion rate of Mg must be controlled corresponding to healing period in clinical uses. Mg or Mg alloys, which corrode in aqueous solutions by several oxidations—reduction reactions, are also prone to in vivo corrosion in tissue fluid environment. Therefore, tissue fluid environment to corrode Mg alloy will be reflected by the in vivo environment of vasculature in association with blood flow and tissue fluid circulation to the implanted site. Because blood flow varies among different anatomical sites, biodegradable behavior was predicted to differ at each implanted site in association with a site-specific flow\(^7,9,11\).

The corrosion rate of Mg alloy is subject to influence to the circulation of surrounding tissue fluids, which is exudate from blood vessels. So, the contact with exudate will depend on the amount of surrounding blood vessels, and the coating of substance produced on the surface of Mg alloy. During the corrosion process, Mg is formed \(H_2\) gas and \(OH^-\) in aqueous solutions\(^12\). In this process, Mg hydrate and Mg oxide are precipitated on the surface of Mg alloy, and additionally Mg phosphate and calcium phosphate was deposited\(^13\). These insoluble
salts formation may constrain the contact between Mg alloys and tissue fluid. On the other hands, the implant procedure evokes injury to the surrounding tissue and activates inflammatory and wound healing response. So, the implanted Mg alloy is surrounded by generated capsular tissue, which progress to fibrous tissue containing less blood vessels in the process of healing. As a consequence, the fibrosis of surrounding tissue may inhibit the corrosion of Mg alloy. Here, the present study was designed to widely analyze in vitro and in vivo the hypothesis that the corrosion of Mg alloys may progress more rapidly in surrounding regenerated tissue with greater primary blood flow and may be synergistically accelerated by high mobility.

MATERIALS AND METHODS

Implant materials
In the present study, commercially available Mg alloy foil having a thickness of 0.1 mm, AZ 31 (96% Mg, 3% Al, 1% Zn; mass%) was cut and prepared as an implant material, and the height was 3 mm and the width was 2 mm. The burr in the cut edges was removed by silicon carbide (SiC) abrasive papers (# 180, # 400 grit), and finally full surfaces were grinded by SiC abrasive papers (# 2000 grit) before implantation. The plates were ultrasonically cleaned in 2% formic acid for 5 min and acetone for 2 min, and then air dried.

Micro-computed tomography (CT) analysis before implantation
The plates were visualized using micro-CT to detect metal artifacts (ScanX-mate-E90, Comscan Tecno, Kanagawa, Japan), and the volumes were measured using three-dimensional (3D) software (VG Studio Max 2.0, Volume Graphics, Heidelberg, Germany). The X-ray source was set at a voltage of 60 kV, current of 70 mA, and a pixel size of 11.04 nm.

Scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDX) analysis before implantation
Before implantation, surfaces of the samples were observed using a high performance SEM/EDX (JEOL, Tokyo, Japan) with an acceleration voltage of 15 keV and a working distance of 10 mm using a focused electron beam. The EDX spectra were acquired from a spot size of 60 nm on the selected regions with a collection time of 30 min.

Immersion test of saline and simulated body fluid (SBF)
After micro-CT and SEM/EDX sample analysis, the samples were tested using immersed test. The saline (0.9% NaCl) and SBF solution in the amount of 60% of a rat’s body weight per sample were prepared. A total of 24 samples were prepared and immersed in the saline solution and SBF in 40 mL plastic vials and incubated in an oven at 37.0°C. Considering the urination and water feed per day, a part (10% of total volume) of each solution was exchanged to fresh solution every day. Four groups with and without the exchange of saline and SBF were prepared as follows: (1) saline with no exchange group [saline (−)]; (2) saline with exchange group [saline (+)]; (3) SBF with no exchange group [SBF (−)]; and (4) SBF with exchange group [SBF (+)]. After immersion for 1, 2, and 4 weeks, the samples were visualized using micro-CT data (ScanX-mate-E90, Comscan Tecno, Kanagawa, Japan), and the sample volumes were measured. The surface structure of samples was analyzed using SEM combined with EDX.

Animal models
All protocols for the animal experiments were reviewed and approved by the Animal Care and Use Committee of Tohoku University (Approval number: 2011DnA-080). For experiments using animals, adult male Wistar rats (mean body weight, 346.1±4.85 g) were randomized into three groups of six animals each, with a study interval of 1, 2, and 4 weeks after implantation. Before implantation, the AZ31 plates were ultrasonically cleaned in 2% formic acid for 5 min and acetone for 2 min, and then air dried. All implantations were performed under general
Table 1  Comparison of water content in tissues from animals without blood and the average blood flow to the specific organ\(^{12}\)

| Tissue   | Water content (%) | Blood flow (mL/min/dg) |
|----------|------------------|-----------------------|
| Heart    | 79.0±0.2         | 39                    |
| Muscle   | 75.6±0.3         | 33                    |
| Brain    | 78.8±0.2         | 0.75±0.09*            |
| Liver    | 70.5±0.7         | 29.4±2.0              |
| Spleen   | 77.1±0.4         | 1.19±0.9\(^a\)        |
| Intestine| 74.9±0.7         | 2.23±0.3              |
| Adipose  | 18.3±1.7         | 9.8±1.3               |
| Skin     | 65.1±0.7         | 18.9±1.4              |
| Bone     | 44.6±1.7         | 2.3±2.0               |

anesthesia with an intra-abdominal injection of 25–35 mg/kg pentobarbital sodium salt (Tokyo Kasei, Tokyo, Japan). An injection of 2% lidocaine (Astra Zeneca, Osaka, Japan) containing 1:80000 epinephrine was administered into the surgical site. Five sample plates were implanted into the following five different sites in each rat: tibia (under periosteum), head (cephalic) (under peristium), back (into adipose tissue), abdominal cavity, and femur (intramuscular) (Fig. 1). The five implantation sites were selected with reference to local primary blood flow, water content, and local mobility (Table 1)\(^7\). Postoperative monitoring included daily evaluations of surgical incisions and well-being of the rats over the 4-week follow-up period.

**Serum and urine analyses**

The three animal groups were euthanized using pentobarbital at 1, 2, and 4 weeks after implantation, respectively. Blood from the right ventricle after laparotomy and urine from the bladder were obtained from all rats. The blood samples were centrifuged to obtain serum and blood corpuscle fractions. The Mg concentrations of serum and urine were analyzed.

**Micro-CT analysis after implantation**

The implanted samples were extracted from each implantation site and the surrounding tissues were carefully removed. The extracted samples were imaged before implantation under the same conditions using the ScanX-mate-E90 micro-CT system, and the remnant volume of each sample was measured using 3D software. The X-ray source was set at a voltage of 60 kV, current of 70 mA, and a pixel size of 11.04 nm. The volume loss for each plate from each animal was calculated. The percentage volume change (ΔV) was calculated as follows:

\[
\Delta V[\%]=\Delta V_0-(\Delta V_1+\Delta C)/V_0 \times 100
\]

where \(\Delta V_0\) is the initial volume, \(\Delta V_1\) is the volume for each time point and \(\Delta C\) is corrosion products.

The insoluble salts of the dense areas on the CT images were quantitatively measured. All data are presented as means±standard deviations. Statistical differences were analyzed by two-way analysis of variance and the Tukey’s test using JSTAT 12.5 software, a statistical library written in JavaScript for advanced statistical operations (http://www.jstat.org/). A probability (\(p\)) value<0.05 was considered statistically significant.

**SEM/EDX analyses after implantation**

SEM/EDX analysis was performed to evaluate the phase of corrosion products, the corrosion layer on the implant surfaces, and the spread of elements within the samples via SEM/EDX (JSM-6390LA, JEOL). The acceleration voltage was set at 15 keV and a working distance of 10 mm using a focused electron beam. EDX spectra were acquired from a 60-nm\(^2\) spot size from the selected regions at a collection time of 30 min.

**XPS analysis after implantation**

Deposits on the Mg alloy were analyzed by X-ray photoelectron spectroscopy (XPS; PHI X-tool, ULVAC-PHI, Chigasaki, Japan) after a few minutes of sputtering with an argon ion beam at 2 kV and 7 mA. The XPS instrument was equipped with a monochromated Al K\(\alpha\) X-ray source operating at 25 W and 15 kV. The analyzed spot diameter was approximately 100 µm. The pressure of the XPS chamber during the measurement was 5×10\(^{-7}\) Pa.

**Histological analysis**

Histological analysis of the tissues surrounding the plates was performed to evaluate local lesions (i.e., inflammation, tissue necrosis, and granulation) related to the implantation site. Histological observations were performed at 1, 2, and 4 weeks after implantation. The tissues surrounding the samples were fixed in 10% buffered formaldehyde for 24–48 h and embedded in paraffin following standard protocols. All soft tissue blocks were cut into 4-µm sections using a microtome and plated on glass slides. For staining of each sample, three serial sections were cut at a distance of 100 µm from each other and stained with hematoxylin and eosin (H&E) and Elastica-Masson (EM) stains.
Histopathological grading of tissues surrounding the implanted plates
Histopathological evaluation of the organization and fibrosis of the regenerated tissues in the healing wounds surrounding the implants was assessed using the following grading system: grade 1, initial exudation (score 4); grade 2, immature granulation tissue (score 3); grade 3, mature granulation tissue (score 2); and grade 4, fibrous tissue (score 1) (Table 2).

RESULTS

In vitro immersion analysis using micro X-ray CT
The implanted plates at all groups corroded to about 50% at a week, and in saline solution group, the plates largely corroded at 2 weeks after implantation. The saline groups had higher corrosion than SBF groups, and comparing the exchange groups with the no exchange groups, the exchange groups had high corrosion (Fig. 2). The plate volume average before immersion was 0.57±0.0037 mm³. At week 2, the saline (+) group completely corroded, whereas at week 4 the SBF group corroded to about 80% (Fig. 3). The volume loss in all groups at week 4 significantly increased compared with that at a week (p<0.05).

1. In vitro SEM/EDX analysis
In the present study, SEM/EDX analysis was performed to confirm the surface morphology of the plates and retention of AZ31 in the composites. The SEM images clearly showed that all the implants had smooth surfaces. As shown in Fig. 4, only Mg and a small amount of elemental Zn and Al were detected by EDX. Overall, the concentration of Mg was highest before immersion (Figs. 4

Table 2  Histological grading scale for implants

| Histopathological grading         | Tissue fluid | Vascular number | Collagen fibers | Score |
|-----------------------------------|--------------|-----------------|-----------------|-------|
| Grade 1: initial exudation        | ++           | +               | −               | 4     |
| Grade 2: immature granulation tissue | +            | ++              | −               | 3     |
| Grade 3: mature granulation tissue | −            | +               | +               | 2     |
| Grade 4: fibrous tissue           | −            | −               | ++              | 1     |

The environment predisposing corrosion around the implants was scored by the macroscopic appearance of wound healing in the implantation sites: Grade 1=Score 4, Grade 2=Score 3, Grade 3=Score 2, and Grade 4=Score 1.

Fig. 2  In vitro micro-computed tomography 2-dimensional (2-D) and 3-D images of an implant during corrosion at 1, 2, and 4 weeks after implantation.

Fig. 3  The volume loss of samples by several corrosion processes at 1, 2, and 4 weeks in immersion test. Four groups with and without the exchange of saline and SBF were prepared as follows: (1) saline with no exchange group [saline (−)], (2) saline with exchange group [saline (+)], (3) SBF with no exchange group [SBF (−)], (4) SBF with exchange group [SBF (+)]. The volume loss during the immersion period is shown on the Y-axis. Note the different volume loss independent of the solution. The standard deviations are shown. *indicates a significant difference from the initial value and between time points (p<0.05).
Fig. 4 SEM images and EDX analysis of surface morphology and composition of the corrosion products before implantation.
(A) SEM image. (B) SEM image indicating the area of EDX analysis. (C) EDX mapping data of surface before implantation: (I) Mg, (II) Al, (III) Zn. Scale bar=500 µm.

4B and C).

Figures 5A and B revealed the surface morphologies and EDX results for the surface insoluble salts on AZ31 at 4 weeks in SBF (+) group. A number of cracks were observed on the samples’ surface after immersion for 4 weeks (Fig. 5A). EDX results revealed that the surface morphology and composition (triangular area in Fig. 5A) were rich in P, Ca, O, and a small amount of element Mg, Al, and C (Fig. 5B). Elemental mapping images of the sample surface revealed the predominant constituents of insoluble salts at 4 weeks (Fig. 6). A greater amount of Ca and P were present when the insoluble salts formed, while P and Ca were uniformly distributed in the formed precipitate.

Macroscopic observation in implanted sites
The implanted plates at all five implantation sites were well tolerated. The surgical head (cephalic) wounds of all rats showed severe swelling and wound reactions, which occurred after a few days and lasted up to 2 weeks. Simultaneously, hematomas developed but resolved 4 weeks after implantation. All surgical wounds, except those in the heads of all rats, showed minor swelling and mild wound reactions, all of which completely resolved no later than 2 weeks after implantation. There were no signs of suture intolerance or wound infection during the follow-up period after implantation. The performance and behavior of all rats after implantation appeared normal with no signs of ill health.
Fig. 7  In vivo micro-computed tomography 2-D and 3-D images of an implant during corrosion at 1, 2, and 4 weeks after implantation.

1. In vivo micro-CT analysis and corrosion performance
   Figure 7 shows a detailed sequence of 3D images of the corrosion process. As shown, the plates underwent biodegradation with duration. However, the order of corrosion differed among the implant sites in the order of tibia, head, back, abdomen, and femur. Corrosion of the implants was fastest in the abdomen, followed by the head, back, tibia, and femur, as demonstrated by the specific implant sites. As shown in Fig. 7, the corrosion patterns varied and were not uniform.

2. Volume loss
   Figure 8 reveals the volume loss process evaluated as time-dependent change in five sites. There were significant differences in volume loss at 4 weeks compared with that at 1 and 2 weeks (p<0.05), and in that at 2 weeks and 4 weeks for the implants in four sites except for abdominal site (p<0.05). The volume loss in abdominal cavity showed significant differences compared with tibia, femur and back (p<0.05). The volume loss in head showed significant difference compared with femur and back (p<0.05).

3. The volume of insoluble salts
   As shown in Fig. 9, the volume of insoluble salts was observed a significant increase in the insoluble salts of the abdominal plates at 1 and 4 weeks (p<0.05).
4. *In vivo* SEM/EDX analysis
In the present study, SEM/EDX analysis was performed to confirm the surface morphology of AZ31 plates in the composites. The surface morphology of the residual plate at 4 weeks after implantation is shown in Fig. 10, which revealed rough surface covered with a thick layer of insoluble salts containing large amounts of Ca, P, and O (Fig. 10). As shown in Fig. 11, elemental mapping images of a representative sample surface revealed that most of the insoluble salts formed 4 weeks after implantation. The Ca and P were present in the formed insoluble salts, while Ca and P were not uniformly distributed in the formed precipitate.

5. *In vivo* XPS analysis
The XPS wide-scan spectrum was shown in Fig. 12 (A). The magnified binding energies determined for P 2p and Mg 2p were also shown in Figs. 12 (B) and (C) respectively. The peak shift of P 2p demonstrated that phosphorus detected in EDX existed as phosphate on the surface after implantation. The detailed spectrum of Mg 2p indicated that the samples were covered by not only soluble Mg(OH)₂ but also stable Mg carbonate and Mg phosphate.

6. Histopathological findings
Histopathological examination revealed a normal wound healing process. As shown in Fig. 13, the optical microstructures of the tissues surrounding the implants were stained by HE and EM at 1, 2, and 4 weeks after implantation. At 1 week after implantation, hemorrhage and exudative change were observed (Figs. 13A and B). At 1–2 weeks after implantation, immature granulation as well as infiltration by inflammation-associated cells was revealed in the tissues (Figs. 13C and D). At 2–4 weeks after implantation, the granulation tissue matured and the wounds revealed well developed granulation tissues and a markedly pronounced proliferation of fibroblasts and capillary growth as well as formation of collagen fibers (Figs. 13E and F). At 4 weeks after implantation, the reduction of the capillaries and fibroblasts and the appearance of large areas of collagen fibrils were observed (Figs. 13G and H).

7. Graded analysis of wound healing
Figure 14 showed the quantitative scoring of tissue
response in head, tibia, back and femur. Histological scoring at 1 week was the highest among all sites, and afterward the score increased. There were significant differences in histological score at 1 week ($p<0.05$) compared with that at 2 and 4 weeks in four implantation sites. There were no statistically significant differences among four implantation sites ($p>0.05$).

**Body weight**
There were no significant differences in weight gain in all the rats implanted with the Mg alloy (Fig. 15A). At the implantation sites, no gas accumulations were observed at any time.
Fig. 14 Quantitative scoring of tissue response to implants during wound healing.

Scoring in the abdominal was not examined.

* indicates a significant difference from the initial value and between time points (p<0.05).

Fig. 15 General condition.

(A) Rat body weights after implantation. (B) Serum magnesium (Mg) levels at 1, 2, and 4 weeks after implantation. (C) Urine Mg level at 1, 2, and 4 weeks after implantation.

1. Blood and urine biochemical analyses

The blood and urine biochemical parameters of Mg obtained at 1, 2, and 4 weeks after implantation are shown in Figs. 15B and C. There were no significant differences in longitudinal changes in the serum and urine Mg concentrations. The results showed that in most cases, serum Mg levels were basically within normal values for rats (2.15–3.45 mg/dL)\(^{17}\), while in some, the levels were higher than normal values.

**DISCUSSION**

Witte *et al.*\(^7\) stated that the differences in local corrosion patterns due to various anatomical locations or different mechanical loading situations may clarify on the underlying corrosion mechanism of the Mg alloy *in vivo*. This study was designed to investigate the differences of biodegradable behavior in various tissues, which have probability of contact with Mg device in clinical use. In each implanted site, Mg plates were assumed to contact with the periosteum and the bone in tibia and head, for application of bone fixation device, and the adipose tissue in back, and the muscle in femur, which indicates the high mobility and more blood flow. The intraperitoneal implantation was performed for confirmation of biodegradation without the encapsulation by regenerated tissue.

Willbold *et al.*\(^11\) discussed that differences in the volume loss of Mg alloy in the implanted sites was dependent on the Mg alloy properties and highly dependent on local blood flow. Although our results showed the different of corrosion rate in each implanted site, there was no correlation between blood flow in existing tissue and corrosion rate for 4 weeks after implantation. However, the intraperitoneal implantation group and *in vitro* group showed the faster degradation. These results will represent to progress the degradation of Mg alloy in implanted site without regenerated capsular tissue. On the other hand, muscle implantation group, in which the degradation has possibilities to affect from high mobility and the blood inflow during exercise, represented the slow degradation of Mg alloy. In this study, the plates implanted into muscle were surrounded by the regenerated tissue. So, the presences of regenerated capsular tissue eliminate the effect of blood flow to surrounding tissues by isolation of the implanted Mg alloy.

The histological observation at the implantation sites showed typical tissue responses to biodegradable materials\(^18\). The regenerated tissue around the Mg plates showed organization, which was consistent with observations of other Mg alloy\(^3,19\). The sequence
of tissue changes in implanted areas is as follows: vascular connecting granulation of tissues that contain many interconnecting capillaries, fibrous granulation tissue, which is infiltrated with fibroblasts that synthesize collagen, and finally formation of collagenous scar tissue containing abundant collagen fibers. Therefore, the initial biodegradation behavior of Mg alloys will depend on the volume of exposed interstitial fluid or exuding blood serum caused by inflammation and/or hemorrhage. Eventually, the regenerated tissue proceeds by a process known as organization and fibrosis, which present as a decrease in blood vessels and interstitial fluid in the implanted area. Hence, the enclosure by fibrous connective tissue containing a little interstitial fluid will affect the biodegradation behavior of Mg alloys. Although the histological evaluation by histological grade showed statistically no significant differences among four implanted sites, the advanced corrosion of the back (dorsal) and head (cephalic) implants may be explained by the delayed organization of regenerated tissue surrounding the Mg alloys. The effect of surrounding tissue may be clarified by the objective assessment of the number and amount of blood vessels.

In the corrosion process, Mg(OH)$_2$ or MgO layer is formed on the underlying Mg matrix in aqueous solution. However, if the Cl$^-$ concentration in the corrosive environment rises above 30 mM, Mg(OH)$_2$ starts getting converted into highly soluble MgCl$_2$. Pitting due to corrosion can be observed on Mg alloys in vivo if the Cl$^-$ content of the body fluid accumulates to approximately 150 mM, thereby explaining the irregular distribution of corrosion products and irregular corrosion patterns observed in this study. On the other hand, an insoluble salt is formed from Ca$^{2+}$, PO$_4^{3-}$ and CO$_3^{2-}$ in biological fluid. Although the insoluble salts deposited on the surface in SBF group containing inorganic ion, the implanted Mg alloy showed faster degradation than saline group. Their deposition on the surface of Mg was deduced to inhibit corrosion of the Mg alloys. In this study, the volume loss of the Mg alloy showed the increase of X-ray impermeability area, which is primarily composed of insoluble Mg$_3$(PO$_4$)$_2$ and Ca$_3$(PO$_4$)$_2$. The most deposition of insoluble salt was observed in intraperitoneal group. So, this increasing volume of soluble salt may be only a representation of deposition in degradation reaction. The significant relationship between insoluble salt deposition and corrosion rate cannot confirm in short-term examination of this study.

In this study, the implanted sites of femur were selected on the basis of mobility. A previous researcher considered that the Mg was corroded by the destruction of protective corrosion layer due to the free movement of the Mg alloy plate inside the gas cavity. Our results showed that Mg implanted into muscle did not result in a significantly high volume loss. Mg corrosion may be relatively insensitive to the mobility.

Miura et al. examined other Mg alloy showing relatively slow corrosion in same way. Mg alloy used in this study showed considerably faster corrosion and more insoluble salt formation than Mg alloy in previous report. However, our results shared its corrosion characteristics in different implanted site. Our results indicated the suppression of fibrosis of surrounding tissues. It may be caused by the change of surrounding tissue in shape associated with a volume of gas formation. So, tissue fluid reflux of each section of implanted sites in regeneration process will control the degradation of Mg alloy. The results of this study indicated that in the head region, Mg alloys were degraded in the relatively early period as shown by Miura et al. Rapid degradation of Mg alloys emits gas rapidly, which can affect the bone. Considering the above mentioned characteristic, it might be necessary to use the alloys that show slow degradation or that are mechanically prepared in order to select materials that lead to the healing of a cranial fracture.

Mg is an essential element and present in large amounts (the fourth most abundant cation) in the human body. Mg is almost contained in bone tissue and well-tolerated in vivo without inducing systemic inflammatory reactions or negatively affecting blood composition. The daily intake of Mg for a normal adult is about 300–400 mg and redundant Mg cations can be harmlessly and efficiently excreted in urine. Recent studies have also shown that the implantation of Mg devices resulted in minimal changes to blood composition without causing damage to organs, such as the liver and kidneys. Our results are in accordance with those of other studies. As Mg balance is maintained by renal regulation of Mg reabsorption, special attention should be paid to post-implantation renal function. Meanwhile dissolved metal ions may induce hypersensitivity and allergy. It is reported that some Mg alloys are nonallergic in an epicutaneous patch test in accordance with the International Organization for Standardization Guidelines.

CONCLUSIONS

The volume loss of the Mg alloy AZ31 was examined in different implant sites, which revealed different primary blood flow and local mobility. The corrosion of the Mg alloy was independent of primary local blood flow and local mobility, suggesting that it affected the amount of blood vessels in regenerated tissue around the Mg alloy. The insoluble salt did not cause the inhibition of Mg corrosion for 1 month after implantation. Because biological tissues have nonuniform environments and structures, the selection of an Mg alloy in consideration with the rate of wound healing is recommended. The Mg alloy that exhibits biodegradation and mechanical property in accordance with the healing period of a bone fracture should be selected since a Mg alloy is biodegraded relatively early in the maxillofacial region.

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