Journal of Orthopaedic Translation (2013) 1, 41—48

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Surface coating reduces degradation rate of magnesium alloy developed for orthopaedic applications

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Received 18 June 2013; accepted 28 June 2013
Available online 5 September 2013

KEYWORDS
Biodegradation;
Electrophoresis;
Magnesium alloy;
Microarc oxidation

Summary Magnesium (Mg) or its alloys have shown great potential as promising biocorrosive or biodegradable implantation materials and/or internal fixators, owing to their good biocompatibility and osteoinductive potential. However, poor anticorrosion property or rapid biodegradation has limited their clinical applications where initial mechanical stabilisation is required. One of the practical approaches for decreasing its biodegradation is to introduce a coating on Mg or its alloys. The current study compared the two most widely used coating techniques, i.e., microarc oxidation (MAO) and electrophoresis deposition (EPD), for coating onto the Mg—Zr pin surface, both *in vitro* and *in vivo*, to determine which method can prevent Mg—Zr alloy degradation better. *In vitro* pH measurement and *in vivo* microcomputed tomographic evaluation were used for determining its degradation rate. Our *in vitro* and *in vivo* testing results indicated that EPD demonstrated better corrosion resistance than MAO, implying the potential of electrochemical technology for surface modification of Mg or its alloys developed for orthopaedic applications.

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http://dx.doi.org/10.1016/j.jot.2013.06.003

Introduction

Magnesium (Mg) and its alloys have high electronegative potentials and can, therefore, degrade in an aqueous environment via an electrochemical reaction, which produces Mg hydroxide and hydrogen gas [1,2]. Especially in physiological conditions where a high chloride concentration exists, Mg alloys present a faster degradation rate because Mg hydroxide can rapidly convert into highly soluble Mg chloride [3]. As Mg or its alloys possess a Young's modulus closer to bone ($E = 3–20$ GPa), it is logical to conclude that Mg and its alloys have great potential to become desirable biodegradable or biocorrosive materials of medical implants for clinical applications, such as orthopaedic implants and cardiovascular stents [4–6]. In recent years, much endeavour has been made to develop appropriate biodegradable implants of Mg-based alloys for bone fracture fixation at the initial stage and then gradually degrade with healing towards an accelerated fracture repair. Yet, rapid degradation of Mg and its alloys *in vivo* may affect mechanical integrity of the healing complex [7–13]. Controlling Mg or its alloys' degradation rates *in vivo* remains a technical or conceptual obstacle to overcome prior to their clinical validation and applications. Although alloying elements and processing technology may decrease the degradation rate significantly, surface coating still remains an effective way to increase degradation resistance of Mg or its alloys [14–16].

Microarc oxidation (MAO), also known as plasma electrolytic oxidation, has been a commonly used industrial technology for metal surface coating. Briefly, application of high voltages (exceeding the dielectric breakdown voltage of the oxide) is involved in the treatment of metals in an alkaline electrolyte, leading to the formation of electric discharges/sparks locally. The plasma induced by local high temperature will contribute to reactions between metal substrate and ingredients in electrolytes. Therefore, ceramic coatings with higher corrosion and wear resistance will be adhered to the substrate tightly [17]. Basically, properties of coatings depend on electrolytic parameters and composition of electrolytes [17]. Many researches focused on the use of MAO in biological applications to decrease degradation rates of Mg or its alloys *in vivo* [18,19]. Generally, anodised layers can protect substrates from corrosion efficiently in the initial immersion stage. However, during implantation, media can still penetrate into the substrate through the channels connected by micropores, subsequently accelerating degradation of the substrate [20]. Thus, optimisation of electrolytic parameters and alteration of electrolyte ingredients have become the major factors affecting the potential of MAO technology in medical applications. Apart from the MAO method, electrophoresis deposition (EPD) is also a widely used anodised process [21]. As no evaporated gas is involved in EPD, pores can be avoided during the coating process. The current study was designed to compare MAO and EPD, the available surface coating techniques for protection of Mg or its alloys against corrosion, as no efforts had so far been made to explore their potential application in controlling degradation rates of Mg or its alloys *in vivo*. The methodological approaches for evaluation and findings of the current study may lay down a foundation to understand the application potential of relevant surface-coating techniques developed for enhancing degradation resistance of Mg or its alloys prior to their clinical validation and applications.

Materials and methods

Pretreatment of substrate

Mg–Zr (Magnesium–Zirconium) alloy (nominal concentration: 0.8 wt% Zr) pins, with a diameter of 0.5 mm, were prepared according to relevant previous publications [22]. Commercially pure Mg (99.9%) and highly pure Zr powder (99.9%) were melted and cast under a mixed atmosphere of sulfur hexafluoride (SF_6) and carbon dioxide (CO_2). The as-cast binary magnesium alloy was further cut into rods of diameter 0.5 mm. The surface of Mg–Zr alloy was polished using an abrasive paper of 500–1000 mesh and then washed ultrasonically with acetone and deionised water for three times.

Preparation of MAO specimens

Surface coatings of Mg–Zr pins were fabricated using the MAO process [21]. The electrolytic solution was composed of sodium silicate (5 g/L), potassium fluoride (8 g/L), and potassium hydroxide (11 g/L). The key fabrication parameters were as follows: voltage 600 V, frequency 600 Hz, and treatment time 10 min.

Preparation of EPD specimens

The EPD solution included three reagents with a ratio of electrophoretic paint (Datong chemistry Co., Ltd, Dongying, China):solvent (Datong Chemistry Co., Ltd):pure water = 1:1:10; the parameters of electrophoresis were set as voltage 150–160 V and treatment time 75–80 s.

In vitro pH tests of immersion process

Mg–Zr pins undergoing EPD and MAO processes were cut into 1 cm long pieces, and then washed ultrasonically with acetone and distilled water for three times. Each surface-coating group included three samples, and all the samples were immersed in 5 mL Dulbecco's Modified Eagle Medium (DMEM, Gibco, Invitrogen, Califorlia, USA) based on the requirement of minimum solution volume-to-specimen area according to the standard of American Society of Testing Materials (ASTM)-G31-72 [23]. In an incubator (MCO-20AIC, Sanyo, Osaka, Japan) at 37 °C and 5% CO_2, pH values of media were measured with a pH meter (S209, Mettler Toledo, Columbus, Ohio, USA) at various time points over a period of 11 days.

In vivo degradation

Sterilised pins were inserted into surgically predrilled bone tunnels from the distal femur of 3-month-old male mice obtained in animal house of the Chinese University of

Hong Kong. Animal ethics approval was obtained from the Chinese University of Hong Kong (Ref. No. 10/049/MIS). Mice were divided randomly into MAO and EPD groups ($n = 4$ for each group). Animal surgery for implantation was conducted according to the previously described protocol [9,24]. The mice were anaesthetised with a combination of ketamine (75 mg/kg) and xylazine (10 mg/kg) through intraperitoneal injection. Then, an incision was made on the skin of the distal femur for exposure of the left knee under general anaesthesia. Osseous tunnel was predrilled along the axis of the bone shaft from the middle point of femoral condyles. Pins (0.5 mm in diameter and 5 mm in length) were then inserted into the bone tunnel through the hole made at the distal femur prior to closing the wound with a suture. The mice were observed closely for 48 h immediately after surgery in the animal house. A general inspection of the operated distal femur was made weekly for 4 weeks postoperatively by observing the radiographs taken by a digital X-ray machine (Faxitron MX-20, Faxitron Bioptics, Tucson, Arizona, USA) operating at 30 keV for 10 s. An *in vivo* microcomputed tomograph (micro-CT; Viva CT40, Scanco Medical AG, Brüttisellen, Switzerland) with a voxel size of 20 μm was used to monitor degradation behaviour of pins at Week 1, Week 2, Week 3, and Week 4 postoperatively. Basically, a three-dimensional volume of interest of the implanted pins was reconstructed with an appropriate threshold value (i.e., 125) to evaluate density changes of pins [using hydroxyapatite (HA) as the reference] in both groups.

Statistical analysis

SPSS version 16.0 (IBM, Armonk, New York, USA) was used for statistical analysis. Differences between groups were analysed using one-way analysis of variance (ANOVA), followed by *post hoc* Tukey's test. A p value of <0.05 was considered statistically significant.

Results

Micromorphology of coated pins

After Mg—Zr pins were coated using the MAO and EPD methods, cross-sections of magnesium alloys were observed with a scanning electron microscope (SEM; Fig. 1). Results showed that both methods provided a protective layer on the surface of magnesium alloys. In case of both coatings, homogenous layers, approximately 6 μm in thickness, were obtained.

In vitro immersion tests

Experimental results of immersion of magnesium alloys *in vitro* are summarised in Fig. 2. The pH values of media for both groups increased fast in the first 24 h, followed by a gradual decrease. The highest mean pH value of MAO and EPD specimens was 8.2 and 7.7, respectively, indicating better protective effects of EPD coatings. In the later stage of immersion, the gap between pH values remained nearly constant (i.e., 0.5).

In vivo experiments

X-rays

Radiographic images in Fig. 3 do not demonstrate any sign of osteomyelitis of the operated femora postoperatively, indicating excellent biocompatibility of both coated Mg alloys. Hydrogen bubbles resulting from the degradation of Mg could be observed only around the implanted tissue of the MAO group within the initial 2 postoperative weeks.

Micro-CT analysis

After a Mg—Zr pin was implanted into the distal femur of mice, the initial pin insertion and its placement were confirmed by micro-CT immediately after implantation surgery (Fig. 4). Recently, we established a monitoring protocol to compare volume changes of the Mg alloy implants to assess their corrosion rates *in vivo* [25]. However, deposition of corrosion products in the micropores in MAO coatings would confound the calculation of volume of Mg alloy and its changes in the initial stage of implantation. As shown in Fig. 4, it is difficult to conclude which group has a slower or faster degradation rate by comparing only the apparent volume of the implanted Mg—Zr pins, as no remarkable changes in volume were found within the first 4 weeks of implantation. Thus, it is also appropriate to compare the densities of implants, as the corroded portion has lower substrate density than its residual part. As HA is the dominant ingredient of bone minerals, changes in its densities were plotted based on reference of HA in Fig. 5. Both MAO and EPD groups showed a linear decrease in mineral density of Mg—Zr pins after its implantation *in vivo*, with around 35% reduction in the MAO group as compared to only 15% reduction in the EPD group, over an implantation period of 4 weeks ($p < 0.01$).

Discussion

This comparative study was designed to identify a better coating method for slowing down the degradation of a Mg-based alloy developed for potential orthopaedic applications. Determination of biodegradation or biocorrosion *in vitro* and *in vivo* was an essential methodological approach for the establishment of guidelines for testing biocorrosive metals for their potential adaptation in medical applications.

Calculation of corrosion or biodegradation rate

For the calculation of *in vitro* corrosion rates of Mg or its alloys, several methods have been proposed, including quantification of pH values, Mg ion release, hydrogen evolution, mass or volume changes, potentiodynamic polarisation, and electrochemical impendence [26]. Apart from *in vitro* measurements, choice of media is also a critical parameter that must be taken into account. phosphate buffer solution (PBS), hanks balanced salt solution (HBSS), and simulated body fluid (SBF) solutions are the most commonly used inorganic media for *in vitro* tests, as their inorganic ingredients are similar to those of plasma [27]. However, more and more research revealed that organic substances in blood should not be ignored, so cell culture

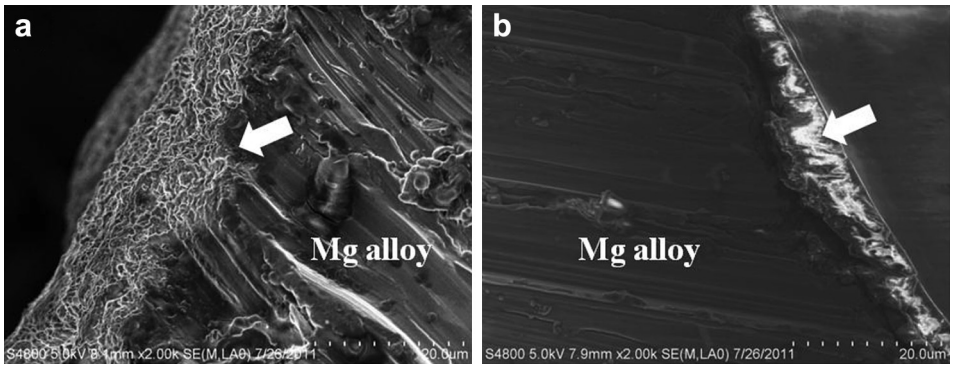

Figure 1 Representative cross-sectional scanning electron microscope images of Mg—Zr alloys coated with (A) microarc oxidation or (B) electrophoresis. Layers can be observed clearly (white arrows). Mg = magnesium; Zr = zirconium.

media (e.g., DMEM) with a composition closer to that of the physiological environment has been recommended widely [28]. Among the abovementioned methods, pH measurement is the most user-friendly and quickest qualitative technique for monitoring corrosion behaviour of Mg or its alloys through the determination of OH^- concentration in extracts. Dissolution of Mg is accompanied by the release and precipitation of OH^-. Thus, the peak value of the pH curve can reflect corrosion rates of samples in the initial degradation stage, and the height of the smooth trend means stable corrosive behaviour. In most of the cases, to know only the *in vitro* comparison results of Mg or its alloys is sufficient; more details on the selected superior alloys can be obtained through precise and validated quantitative measurements. Nevertheless, pH values in extracts could also be a valid prescreening method reflecting cell contacting environments, which would be beneficial for our better understanding of *in vivo* biological evaluations developed for biodegradable Mg-based implants. Our present study showed that Mg—Zr pins coated using EPD had lower peak pH values and showed a more stable trend than

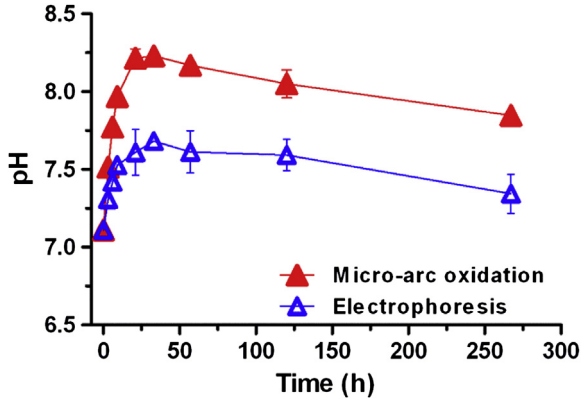

Figure 2 Changes in pH as a function of immersion time and an *in vitro* index of biocorrosion or degradation of the coated Mg—Zr alloy pins. Rapid increase in pH was detected in Dulbecco's Modified Eagle Medium in which Mg—Zr alloy pins underwent (A) microarc oxidation or (B) electrophoresis. Mg = magnesium; Zr = zirconium.

samples coated using MAO technique, implying better protection effects of EPD coating that prevented penetration of electrolytes. More interestingly, effects of CO_2 on the corrosion behaviour of Mg—Zr pins was also taken into account to mimic the real physiological environment due to the presence of CO_2 in our blood ($(CO_2)_{eq}$ = 22—25 mM) [13]. The gradual decrease of pH values after the attainment of peak values can be ascribed to the dissolution of CO_2 in the media.

Hydrogen gas formation during Mg alloy corrosion

In an *in vivo* situation, production of hydrogen gas is accompanied by Mg corrosion ($Mg + 2H_2O \rightarrow Mg^{2+} + 2OH^- + H_2$). Basically, 1 mol of hydrogen gas will be produced with dissolution of 1 mol of Mg. Previous *in vivo* studies have shown that hydrogen bubbles are accumulated in local host tissue if corrosion rates of Mg or Mg alloy implants are too high. Actually, an increase in local pressure caused by bubbles affects cell adhesion to implants adversely. As in the MAO group, bubbles could be observed only around the implantation site in the initial 2 weeks, our current comparative study suggested higher *in vivo* degradation rates of Mg—Zr pins coated using MAO in comparison to those coated by EPD. In the following 2 weeks, bubbles were not observed around the host tissue in the MAO group, indicating a slower rate of release of hydrogen from the implant that was dissolved mainly in the local tissue fluid and/or entered the blood circulation [29].

Corrosion reflected more sensitively by changes in Mg alloy mineral density than by changes in its volume

In our *in vivo* micro-CT 3D reconstruction of Mg—Zr implants, both coatings were shown to protect Mg—Zr pins efficiently from corrosion in physiological environments, as their shape and volume remained nearly unchanged during the 4 weeks. Considering that the deposited products may confound volume measurement of pins significantly, we logically proposed to compare their material densities and found that MAO coating did not protect Mg from corrosion,

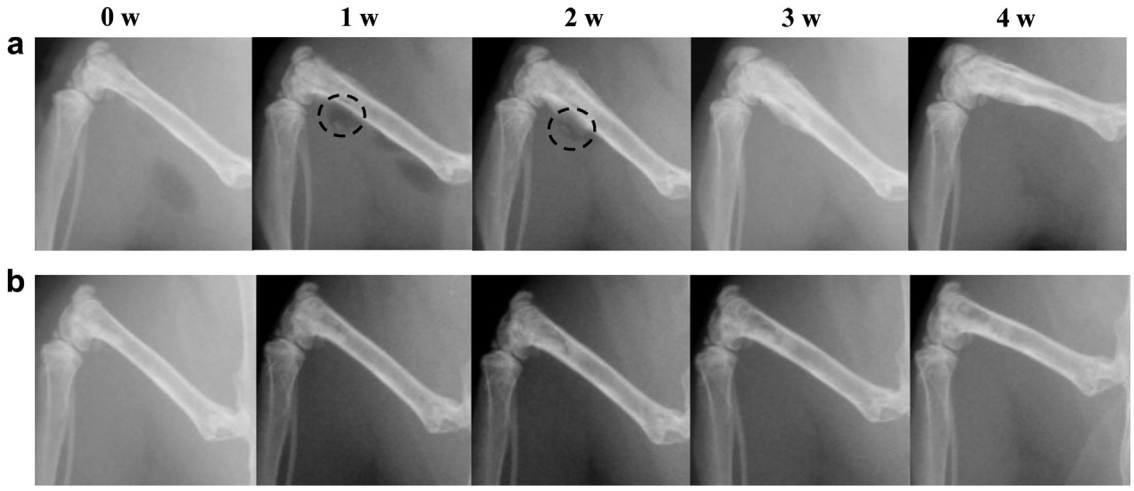

Figure 3 Radiographs of distal femora in mice implanted with Mg—Zr pins coated by (A) microarc oxidation and (B) electrophoresis. The broken circles indicate hydrogen bubbles formed during pin degradation. Mg = magnesium; Zr = zirconium.

with a 30% decrease in material density as compared with only 15% reduction in the case of EPD coating *in vitro*.

Indeed, an electrochemical impendence study of alloys coated by MAO clearly showed that the layers of substrate consisted of a porous ceramic coating and an inner barrier layer [30]. Transmission electron microscopy confirmed an interconnection among micropores in the outer ceramic coating [31]. The proposed corrosion mechanism of MAO coatings on pure Mg or its alloys is illustrated in Fig. 6. With increasing immersion time, electrolytes would penetrate into the substrate surface, leading to the formation of corrosion cracks. At the same time, accumulation of corrosion products around the implant surface would facilitate the sealing of holes on MAO coatings both *in vitro* and *in vivo*. Sealing of holes on the coating surface has a predominant role in reducing degradation of Mg or its alloys in the initial phase after implantation. In terms of EPD coating technique, our *in vivo* radiographic observation implied that, during implantation, the hydrogen bubbles were either not accumulated or absorbed by surrounding tissues over time, indicating an excellent degradation resistance by EPD coating, especially attributed to significantly fewer number of holes or less surface contact between tissue fluid and Mg—Zr pins.

In general, both *in vitro* and *in vivo* measurements proved that EPD coating can protect the substrate better than MAO coating. The difference in corrosion resistance may be ascribed to differences in coating structure, which can be clearly seen from SEM images in Fig. 1, where the micropores are fully distributed on MAO coatings [19]. However, no cracks or pores can be observed on the surface of EPD coatings. Although MAO coating can suppress infiltration of electrolyte in the short term, during implantation, interconnected pores will allow penetration of culture media or body fluid over time. Therefore, controlling pore size and its number on the coating is one of the key parameters to be considered while developing an appropriate MAO coating for surface modification of Mg or its alloys.

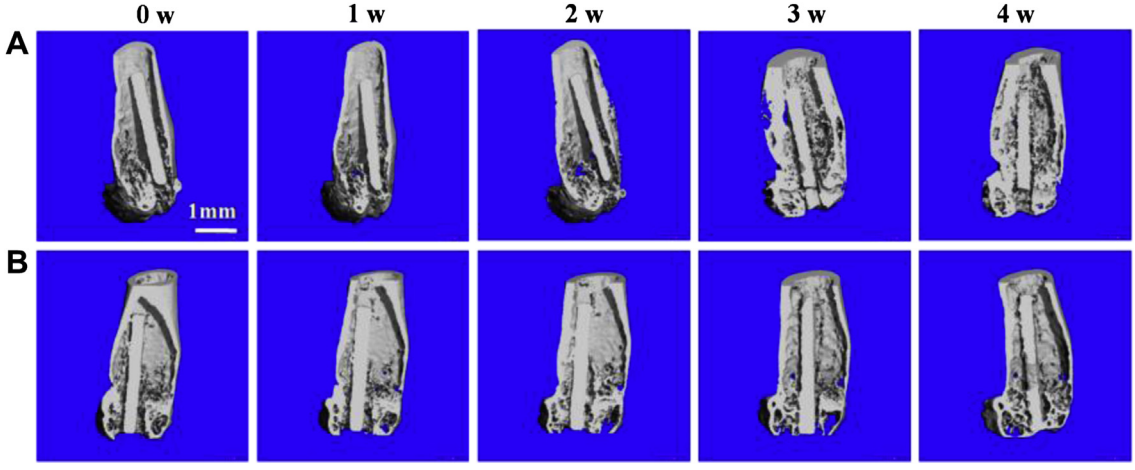

Figure 4 Representative micro-computed tomography midsagittal cuts at distal femora of mice implanted with Mg—Zr pins coated with (A) microarc oxidation or (B) electrophoresis. Mg = magnesium; Zr = zirconium.

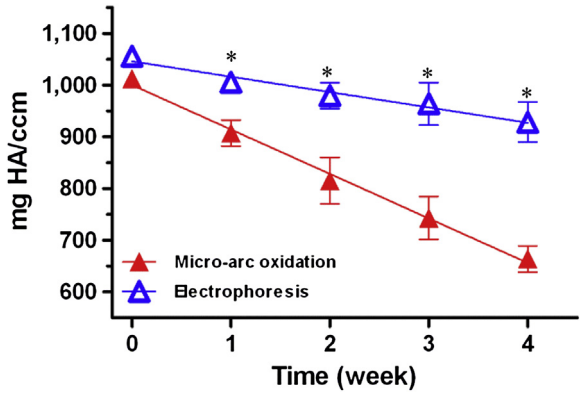

Figure 5 Changes in density of the implanted Mg—Zr alloy pins and its hydroxides after implantation of Mg—Zr alloy into the distal femora of mice for 4 weeks. Using hydroxyapatite as the reference, the relative average density of pins was measured by micro-computed tomography and then processed with linear fits for quantifying the pin mineral density; the density decline of Mg—Zr alloy coated by microarc oxidation shows a more negative slope (−86.03) than that coated by electrophoresis (−29.73). *$p < 0.01$. Mg = magnesium; Zr = zirconium.

Perspectives and future efforts

The current experimental study proposed that EPD coating provided better anticorrosion ability than MAO coating. However, findings of the current preclinical study were not adequate for us to conclude that EPD is a better coating method and possesses potential for enhancing degradation resistance of either pure Mg or Mg-based alloys that are used as biocorrosive internal fixators in clinical applications. Although poor corrosion resistance of currently available Mg alloys may be the most challenging issue affecting their wide orthopaedic applications, other coating-related issues, such as properties of binding between coatings and substrates, biocompatibility, long-term biosafety, and wear resistance, should also be evaluated systemically.

According to previous published works, insufficient adhesion of coatings is the main obstacle of the EPD technique and its application despite favourable surface contact and formation of uniform layers. Frequent friction is known to cause easy peeling off of EPD coatings, especially in load bearing skeletons, inducing a dramatic increase of corrosion rates. As a contrast technique, MAO can fabricate porous coatings with strong adhesion to the substrate, but tiny pores on coatings are not able to suppress the

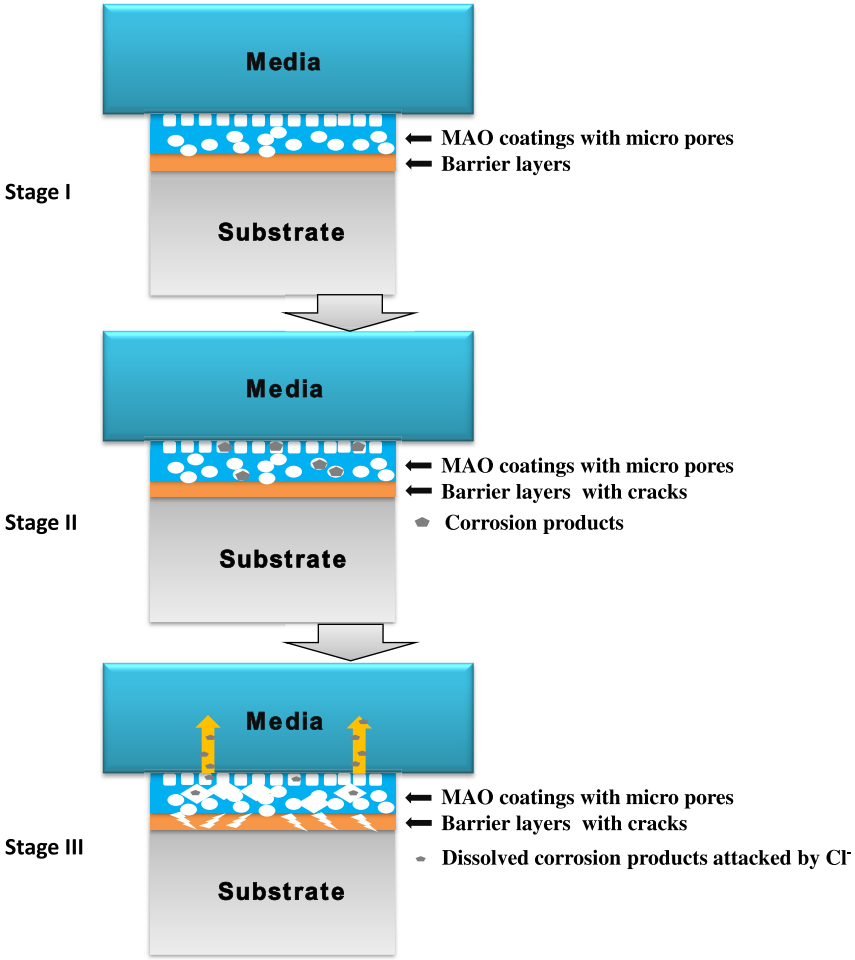

Figure 6 Schematic presentation of the proposed corrosion mechanisms of MAO-coated pins at different stages of a physiological environment. MAO = microarc oxidation.

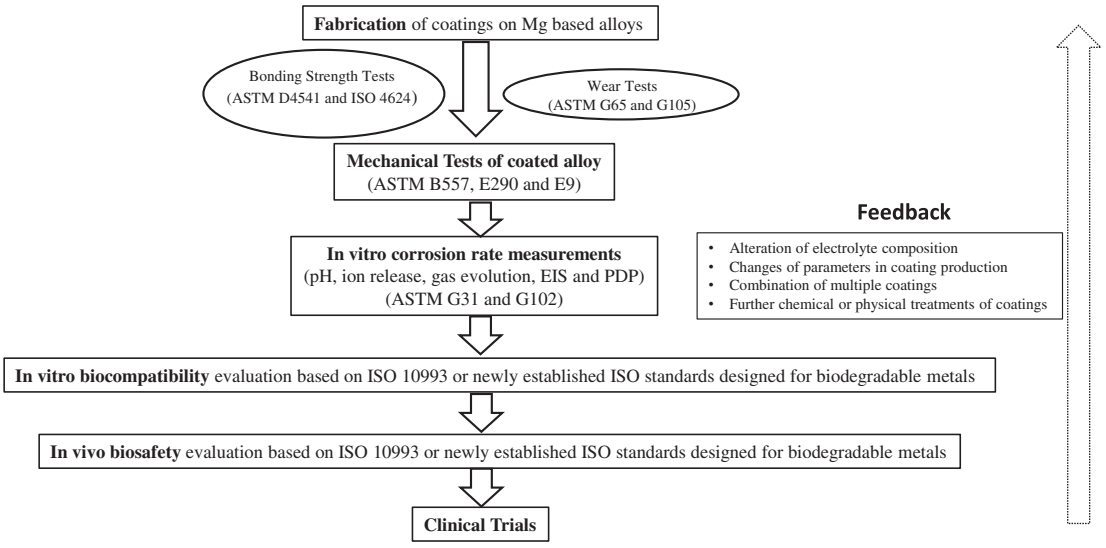

Figure 7 Proposed roadmap for clinical translation of pure Mg or Mg alloys with relevant surface coating to meet regulatory requirements. EIS = electrochemical impedance; Mg = magnesium; PDP = potentiodynamic polarisation.

penetration of media over time during implantation. Either other potential coatings with stable surface physical properties or composite coatings would help reduce corrosion rate. Indeed, Chen and his colleagues [19] used both MAO and EPD methods in their previous study, and their *in vitro* experiments showed that composite coatings have superior bonding strength and corrosion resistance than single EPD or MAO coating.

Indeed, micro-CT *in vivo* can be used to monitor biocorrosive behaviour of Mg or its alloys. However, noninvasive technology mainly concentrates on mineral structure (e.g., bone and materials). Actually, both the "remaining" [i.e., visible two dimensional (2D) or 3D structure] and the "left" (i.e., released ions) parts of implants should be tracked carefully. Generally, blood circulation, urinary excretion, and intestinal output contribute to Mg ion homeostasis in the human body. Therefore, ion concentrations in blood (plasma and erythrocytes), urine, and faeces should be determined to evaluate health risks of Mg-based implants, such as hypo- or hypermagnesaemia.

If biomaterial scientists and bioengineers can fabricate Mg-based alloys with tightly adherent coatings and excellent corrosion resistance, many aspects associated with the biosafety of coated biocorrosive implants will require our immediate attention, such as cell—coating material interaction, how the local host tissue responds to the coating materials, and mechanisms of degraded coating materials; for example, whether the "debris or wear of the coating" is embedded in the newly formed bone or eliminated via circulation becomes a safety concern, and further investigations are highly desirable. Generally, the coatings prepared either by MAO or by EPD are constituted of several substances. Firstly, these ingredients of coatings should be tested *in vitro* and *in vivo* to ensure their biological safety while applying Mg-based medical devices. Secondly, long-term friction may lead to a production of debris in the tissue. Thus, the potential risks of debris-induced inflammation should be evaluated carefully based on ISO 10993 standards, which is shown in Fig. 7. In this study, it was insufficient to measure *in vitro* pH values to assess potential changes of the acid—base microenvironment. *In vivo* pH values could be monitored using florescence dyes to evaluate the efficiency of coatings in corrosion resistance. Additionally, osmolality changes in local tissue should be also assessed carefully, as pH and osmolality are the two key factors contributing to the biocompatibility of implants. Results of pH and osmolality tests can provide further details of the biosafety of Mg-based implants with or without coating.

Although the study of corrosion resistance was the primary objective in the current study, it should be noted that an increase in corrosion resistance may apply to all orthopaedic indications, as the degradation or corrosion rate is skeletal site dependent, e.g., weight-bearing versus non-weight-bearing (stress-dependant), or trabecular bone versus cortical bone (contact surface dependent, i.e., more fluid contact in porous trabecular bone and faster corrosion of Mg alloys as compared with compact cortical bone with less fluid contact to Mg alloys). In addition, cell—material interactions were not considered in the current study to evaluate potential underlying mechanisms. This study established a simple *in vivo* testing model in distal tibial bone marrow cavity of rabbits without involving mechanical and skeletal site-specific variations. This implies that orthopaedic application is the key variable influencing corrosion rate of the Mg implants, and *in vivo* validation and optimisation of implants made of pure Mg or its alloys shall serve the standard for relevant applications in the translational roadmap.

Conclusions

In vitro pH measurement of immersion culture media, *in vivo* radiographic and micro-CT-based images, as well as density changes of Mg—Zr pins indicated that, as compared with MAO, EPD can produce a better protective coating for Mg alloys against corrosion. However, more specifically

designed studies are desirable to understand how the physiological environment alters corrosion behaviours, because this is an essential step towards dedicated and targeted improvement of biocorrosive or biodegradable medical implants that may meet the orthopaedic applications with initial mechanical stability for fixation and subsequent degradation towards better healing without undergoing a removal surgery required for nondegradable implants or fixators.

Conflicts of interest

All the authors declare no conflicts of interest.

Acknowledgements

This work was supported by a Guangdong Innovation Team Grant on Biodegradable Magnesium and Medical Implants (Ref. 201001C0104669453).

References

[1] Witte F, Hort N, Vogt C, Cohen S, Kainer KU, Willumeit R, et al. Degradable biomaterials based on magnesium corrosion. Curr Opin Solid State Mater 2008;12:63—72.
[2] Staiger MP, Pietak AM, Huadmai J, Dias G. Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials 2006;27:1728—34.
[3] Song GL, Atrens A. Corrosion mechanisms of magnesium alloys. Adv Eng Mater 1999;1:11—33.
[4] Mani G, Feldman MD, Patel D, Agrawal CM. Coronary stents: a materials perspective. Biomaterials 2007;28:1689—710.
[5] Griffiths H, Bakhai A, West D, Petrou M, De Souza T, Moat N, et al. Feasibility and cost of treatment with drug eluting stents of surgical candidates with multi-vessel coronary disease. Eur J Cardiothorac Surg 2004;26:528—34.
[6] Zberg B, Uggowitzer PJ, Loffler JF. MgZnCa glasses without clinically observable hydrogen evolution for biodegradable implants. Nat Mater 2009;8:887—91.
[7] Chang JW, Guo XW, Fu PH, Peng LM, Ding WJ. Effect of heat treatment on corrosion and electrochemical behaviour of Mg—3Nd—0.2Zn—0.4Zr (wt.%) alloy. Electrochim Acta 2007;52:3160—7.
[8] Zeng RC, Dietzel W, Witte F, Hort N, Blawert C. Progress and challenge for magnesium alloys as biomaterials. Adv Eng Mater 2008;10:B3—14.
[9] Wang YB, Xie XH, Li HF, Wang XL, Zhao MZ, Zhang EW, et al. Biodegradable CaMgZn bulk metallic glass for potential skeletal application. Acta Biomater 2011;7:3196—208.
[10] Xie XH, Wang XL, Wang YB, Zhang G, He YX, Zheng YF, et al. Ca—Mg—Zn metallic glass as degradable biomaterials developed for potential orthopaedic applications. Bone 2010;47:S425.
[11] Kannan MB, Raman RKS. Evaluating the stress corrosion cracking susceptibility of Mg—Al—Zn alloy in modified-simulated body fluid for orthopaedic implant application. Scripta Mater 2008;59:175—8.
[12] Wan G, Wu BL, Zhao YH, Zhang YD, Esling C. Strain-rate sensitivity of textured Mg—3.0Al—1.0Zn alloy (AZ31) under impact deformation. Scripta Mater 2011;65:461—4.
[13] Willumeit R, Fischer J, Feyerabend F, Hort N, Bismayer U, Heidrich S, et al. Chemical surface alteration of biodegradable magnesium exposed to corrosion media. Acta Biomater 2011;7:2704—15.
[14] Zhang E, Xu LP, Yang K. Formation by ion plating of Ti-coating on pure Mg for biomedical applications. Scripta Mater 2005;53:523—7.
[15] Wong HM, Yeung KWK, Lam KO, Tam V, Chu PK, Luk KDK, et al. A biodegradable polymer-based coating to control the performance of magnesium alloy orthopaedic implants. Biomaterials 2010;31:2084—96.
[16] Friedrich H, Mordike B. Magnesium technology. Heidelberg: Springer; 2006.
[17] Guo XH, Du KQ, Ge H, Guo QZ, Wang Y, Wang FH. Good sensitivity and high stability of humidity sensor using micro-arc oxidation alumina film. Electrochem Commun 2013;28:95—9.
[18] Qi ZR, Zhang Q, Tan LL, Lin X, Yin Y, Wang XL, et al. Comparison of degradation behavior and the associated bone response of ZK60 and PLLA in vivo. J Biomed Mater Res A 2013. http://dx.doi.org/10.1002/jbm.a.34795.
[19] Chen S, Guan SK, Li W, Wang HX, Chen J, Wang YS, et al. *In vivo* degradation and bone response of a composite coating on Mg—Zn—Ca alloy prepared by microarc oxidation and electrochemical deposition. J Biomed Mater Res B 2012;100B:533—43.
[20] Wang JL, Tang J, Zhang P, Li YD, Wang J, Lai YX, et al. Surface modification of magnesium alloys developed for bioabsorbable orthopedic implants: a general review. J Biomed Mater Res B 2012;100B:1691—701.
[21] Zhang J, Dai CS, Wei J, Wen ZH. Study on the bonding strength between calcium phosphate/chitosan composite coatings and a Mg alloy substrate. Appl Surf Sci 2012;261:276—86.
[22] Gu XN, Zheng YF, Cheng Y, Zhong SP, Xi TF. *In vitro* corrosion and biocompatibility of binary magnesium alloys. Biomaterials 2009;30:484—98.
[23] American Society for Testing and Materials. ASTM-G31—72: standard practice for laboratory immersion corrosion testing of metals. In: Annual book of ASTM standards. Philadelphia, PA: ASTM; 2004.
[24] Li HF, Xie XH, Zhao K, Wang YB, Zheng YF, Wang WH, et al. *In vitro* and *in vivo* studies on biodegradable CaMgZnSrYb high-entropy bulk metallic glass. Acta Biomater 2013 in press.
[25] Gu XN, Xie XH, Li N, Zheng YF, Qin L. *In vitro* and *in vivo* studies on a Mg—Sr binary alloy system developed as a new kind of biodegradable metal. Acta Biomater 2012;8:2360—74.
[26] Kirkland NT, Birbilis N, Staiger MP. Assessing the corrosion of biodegradable magnesium implants: a critical review of current methodologies and their limitations. Acta Biomater 2012;8:925—36.
[27] Yamamoto A, Hiromoto S. Effect of inorganic salts, amino acids and proteins on the degradation of pure magnesium *in vitro*. Mat Sci Eng C 2009;29:1559—68.
[28] Tie D, Feyerabend F, Hort N, Willumeit R, Hoeche D. XPS studies of magnesium surfaces after exposure to Dulbecco's Modified Eagle Medium, Hank's buffered salt solution, and simulated body fluid. Adv Eng Mater 2010;12:B699—704.
[29] Erdmann N, Bondarenko A, Hewicker-Trautwein M, Angrisani N, Reifenrath J, Lucas A, et al. Evaluation of the soft tissue biocompatibility of MgCa0.8 and surgical steel 316L *in vivo*: a comparative study in rabbits. Biomed Eng Online 2010;9:63—79.
[30] Wen L, Wang YM, Liu Y, Zhou Y, Guo LX, Ouyang JH, et al. EIS study of a self-repairing microarc oxidation coating. Corros Sci 2011;53:618—23.
[31] Zhang P, Nie X, Hu H, Liu Y. TEM analysis and tribological properties of plasma electrolytic oxidation (PEO) coatings on a magnesium engine AJ62 alloy. Surf Coating Tech 2010;205:1508—14.