Biodegradable Mg and Mg based alloys for biomedical implants

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Mg and its alloys become natural biomaterials as the elemental Mg is found in the human body in abundance and their mechanical properties being akin to the natural bone as well as due to their inherent bioabsorbable/bioresorbable property. This paper discusses the development of new Mg alloys and their corrosion characteristics in detail. The latest advancements in coating of Mg alloys to control their degradation rate are also reviewed along with the future challenges that need to be addressed.

Keywords: Magnesium alloy, bio-degradable material, orthopedic implant

This paper is part of a special issue on biodegradable materials

Introduction

Biomedical implants play a very crucial role in the society as they are extensively used to replace or repair the fractured or diseased parts of human and animals, as well as improve the quality of life.[1–3] Implants are required either for a shorter period or as long as the patient is alive based on the tissue or organ that has failed. Temporary implants are commonly used for a shorter period until the fractured bone tissue grows or to overcome deformation. On the other hand, when the orthopaedic joints such as hip, knee, spinal, shoulder and ankle are affected by arthritis or are fractured severely, the patient suffers from severe pain and immobility and is often treated using permanent implants by performing arthroplasty surgeries, which involve the replacement of the entire joints. In addition, the uses of cardiac implants such as stent and valves for people who are affected by heart diseases have sky-rocketed owing to increase in the aged population.[4] The major requirements of an implant to serve successfully are sufficient mechanical strength, high wear and corrosion resistance, adequate hemocompatibility and complete acceptance by the human body termed as biocompatibility. In the design of an implant, the most crucial factor to be considered is that the materials should not elicit any type of toxicity in the short or long term. Commonly used materials for implant are metal and alloys (such as 316 stainless steel, cobalt–chromium, titanium and its alloys and magnesium and its alloys), polymers (such as ultrahigh molecular weight polyethylene), ceramics[5] and some composites.

Implant materials can be classified into two types, degradable or non-degradable (permanent implant), based on their short or long term existence in the human body. The common problems encountered with permanent implants are physical irritation, chronic inflammatory local reactions, thrombogenicity and endothelial dysfunction (for cardiovascular applications), inability to integrate with bone during growth in younger patients, stress shielding effect due to higher Young's modulus when compared with bone and over and above the material issues like wear, corrosion and bacterial formation.[6–8] The maximum service period of the permanent implants is around 12–15 years owing to the above said causes. Biodegradable materials are considered to be an alternative to overcome the above mentioned problems encountered with the permanent implants. Biodegradable materials are required to maintain sufficient mechanical properties until the organ/tissue grows in the implanted region and degrade/corrode in very controlled manner. The dissolved/corroded particles are expected to be removed by excretion without causing any side effects or toxicity. Although not for all applications, degradable/resorbable materials have emerged to greater extent in the fields of bone fixation devices and tissue engineering scaffolds. Polymers such as polyglycolic acid (PGA), poly-L-lactic acid, poly-DL-lactic acid, PGA/trimethylene carbonate copolymers, poly-*p*-dioxanone and poly-beta-hydroxybutyric acid and Mg and its alloys are the most common biodegradable materials that are studied extensively.[9] The biodegradable polymers are used for surgical sutures, antibacterial coatings, drug delivery systems, fixation devices and tissue replacement components.

Magnesium as biodegradable material

Magnesium has been considered as a suitable biomaterial as it is the fourth most abundant element in the human body required for human metabolism. Magnesium, apart from being a cofactor for many enzymes, also stabilises the DNA and RNA structures. In addition, the superior physical property of magnesium such as low density and modulus of elasticity closer to bone with higher fracture toughness when compared to the conventional ceramic material (the hydroxyapatite) makes it an attractive alternative for implant applications, which is shown in

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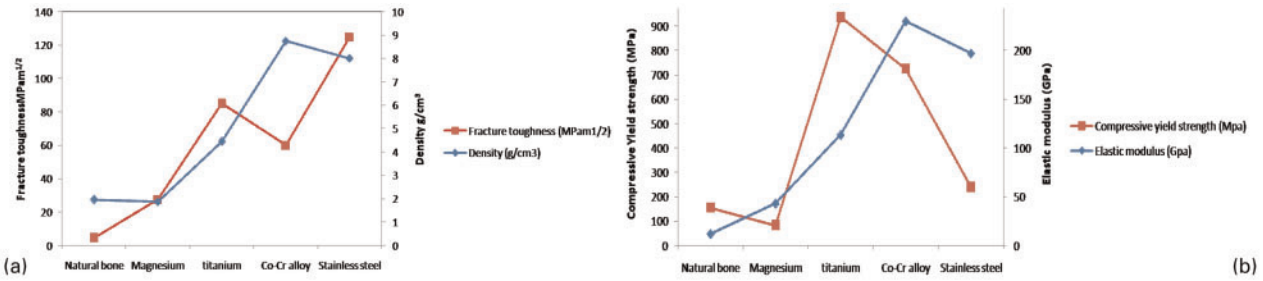


1 ***a*** **strength versus natural bone and other alloys and** ***b*** **density and fracture toughness versus natural bone and other alloys[10,11]**

Fig. 1. In spite of the fact that the use of magnesium based materials as biodegradable materials was envisaged as early as 1878 owing to their outstanding biocompatibility combined with the optimum physical and mechanical properties,[11] other metallic materials were considered to be superior due to the high cost of production of magnesium and its rapid corrosion characteristics. However, in recent times, the possibility of magnesium based implants is being revisited in order to overcome the revisional surgeries encountered with other metallic implants. Since magnesium is a constituent of human body, it could be a well accepted implant and can overcome the various biocompatibility issues associated with the conventionally used implant materials like 316 SS, Co and Ti based alloys, where Al, V, Ti, Co, Cr and Ni are used as alloying addition. The higher propensity for corrosion renders Mg and its alloys to be very promising biodegradable materials as they are very well adapted in human body and can be dissolved when they are no longer needed. These outstanding properties are very useful especially for younger patients suffering from congenital heart diseases or adult patients with retenosis. In fact, Mg as biodegradable materials is already used to treat any blockage in the coronary artery or any other circulatory systems as well as to correct the angular deformities of long bones, to overcome the limb length discrepancies and to treat patients suffering from cleft (F) palate.

Properties of Mg and its alloys to be used as biodegradable materials

Corrosion

The human body consists of very aggressive environment with pH 7 at a temperature 37°C, and an implanted metallic material should possess high corrosion resistance in these conditions. Ti and its alloys are considered to possess high corrosion resistance among other metallic biomaterials such as 316 SS and Co–Cr alloys owing to the formation of adherent thin passive oxide layer. However, the Ti debris is observed near an implant, resulting in blackening of the tissues due to wear accelerated corrosion.

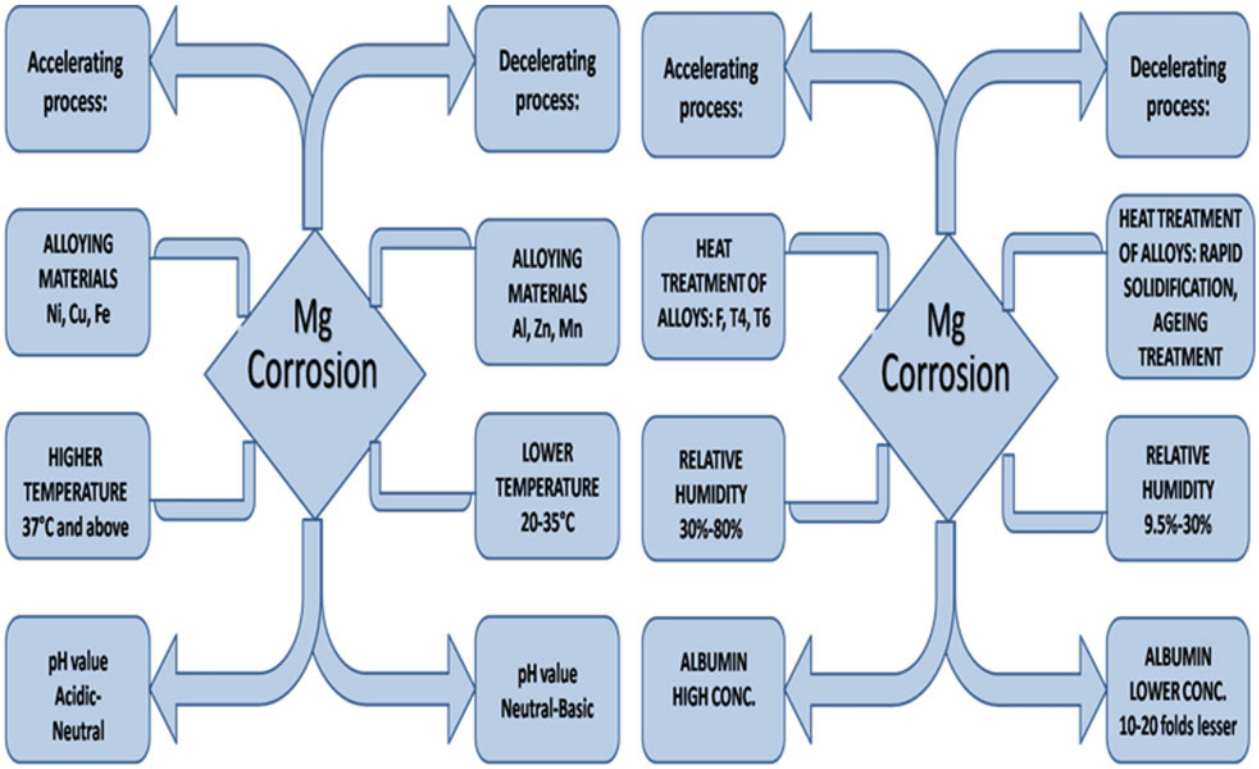
Rapid corrosion of magnesium implant used to secure fracture involving bones was observed as early as 1907 in *in vivo* condition along with the production of large amount of gas beneath the skin.[12] In continuation of this work, various Mg alloys such as Mg–Cd, Mg–Al and Mg–Al–Mn were tried to fuse the fractured bones. All these studies demonstrated clearly that the Mg alloys corrode more rapidly than the wound healing time, which is at least 12 weeks.[13] Various factors such as pH, temperature and the presence of certain blood plasma and proteins were observed to influence the corrosion of pure Mg in the tests carried out using simulated body fluids.

The corrosion process of Mg in a body is a very complex phenomenon, and the corrosion products such as magnesium hydroxide and hydrogen gas along with Mg particles are often encountered in the presence of water. Apart from hydrogen embitterment, H_2 bubbles delay healing at the surgical site, leading to necrosis of the surrounding tissue.[14] In the heart stent, excess gas bubbles formed during corrosion in serum could block the bloodstream, causing death,[15] and at times, the bubbles can accumulate in the surrounding tissues if formed in excess rate.

The most prominent corrosion mechanism in magnesium and its alloys are pitting and localised corrosion due to the presence of the second phase and impurities. Out of 31 Mg alloys tested, 29 were observed to undergo pitting and localised corrosion, while only 2 underwent uniform corrosion. The presence of intermetallics (second phase) is often required to enhance the mechanical strength of Mg alloys. Most of the alloying elements as well as impurities are more noble than Mg, and hence, the intermetallic particles act as a cathode, creating a potential difference between the second phase and the Mg matrix, which acts as an anode resulting in galvanic corrosion when exposed to aggressive environment. This kind of non-uniform corrosion is further dangerous for the device under the presence of mechanical load.

Apart from alloying element pH, temperature and the presence of proteins, surface/volume ratio of the solution used with respect to the alloys exposed plays a vital role in corrosion behaviour of Mg implants. Low volume/surface area ratio results in high pH. An ideal surface/volume ratio is recommended by ISO 10993-12 as 3 cm^2 mL^{-1}. A high SV/SA ratio of 6·7 is recommended to simulate the degradation of Mg stent in artery, while an SV/SA ratio of 0·67 in Hank's solution is recommended for simulating the degradation behaviour of Mg bone screw in a bone screw in cortical bone.[16]

Temperature also plays a very crucial role on the corrosion mechanisms of the Mg alloys. The studies carried out by Kirkland *et al.* on Mg alloys in minimal essential medium at 37°C exhibited 100% higher corrosion than those measured at 20°C. Thus, the body temperature often accelerates the electrochemical reactions and changes the corrosion mechanism than that which occurs at room temperature. Thus, studies conducted at room temperature underestimates the corrosion behaviour of Mg alloys. In addition to



2 Accelerating and decelerating factors of corrosion due to various factors in Mg based materials

temperature, pH at the site of implant also plays a significant role in Mg corrosion. The increase in pH due to Mg dissolution in buffered solutions (SBF) or cell culture medium and in non-buffered solution is often reported.[17,18] Although increase in pH (alkalisation) is beneficial in many applications as it forms stable $Mg(OH)_2$ surface layers and provides passivation, such shift to alkaline pH is expected to be harmful for the biological applications. If the pH increase is too high, cell death is speculated to take place.[19] However, the increase in pH can dramatically changed under dynamic flow conditions of surrounding liquid where there is mass transfer of the corrosion products from the site. Generally, the pH change in non-buffered solutions is higher than the buffered solutions resulting in lower corrosion rates due to the formation of the Mg hydroxide layers. Electrochemical impedance studies further corroborated this finding by presenting a higher R_p values in non-buffered NaCl owing to higher pH values. Further, the type of buffers used is also observed to have various influences on the corrosion behaviour of Mg alloys. The various parameters that play some role in accelerating and decelerating corrosion of magnesium alloys is depicted in Fig. 2.

The corrosion of the Mg and its alloys is highly influenced by the test solutions used for the study as blood is much more complex than the artificial salt solutions, which are as standards in corrosion testing. The difference in the composition results in different rates of corrosion and also variation in the release of potentially toxic corrosion products. *In vitro* tests are usually performed using either NaCl solution, Hank's solution SBF, artificial plasma or Dulbecco's modified Eagle's medium (DMEM). Chloride content in all these solutions is higher than that in human plasma. The cell culture medium often used has 10- to 20-fold lesser albumin compared to the blood, and this variation drastically changes the corrosion behaviour of the materials. A small addition of 0·1 g L^{-1} bovine serum in artificial plasma has led to an increase in corrosion current from 7–24 μm cm^{-2} for MgZn, while a fourfold increase was observed for $MgAl_3$ alloy. Mueller *et al.* have critically reviewed and presented the difference in corrosion behaviour of various Mg alloys in different simulated body conditions.[20] The study conducted by Mueller *et al.* proved that a small addition of albumin to phosphate buffer solution enhanced the anodic dissolution of pure Mg and LAE442, while AZ31 was least affected.[21] In contrast, R_p values of Mg with rare earth (RE) elements was initially high and later reduced with time, and this was attributed to the formation of barrier layer in the beginning and formation of metal protein complex after some time, resulting in dissolution of the implant.[22] Further corrosion behaviour of Mg varies between the cell culture medium and simulated body conditions (Hank's solution). Corrosion was much slower in the case of culture medium when compared to the SBF, clearly demonstrating the poor protective properties of the layer formed in SBF. In contrast to SBF, soaking in cell culture medium is observed to result in high carbonate layer on the surface of the implant resulting in superior passivation.[23,24]

Effect of alloying elements on corrosion of Mg materials

Amid different alloying elements Al, Zn, Mn, Si and Zr, RE elements have profound influence on the corrosion characteristics and mechanical strength of Mg alloys. Mg alloys such as WE43, LAE442, Mg–Gd, Mg–Dy and Mg–Nd–Zn–Zr are under consideration for biomedical applications. Among all the alloys, WE43 has already been subjected to clinical trials.[25] The presence of Li in Mg increases the pH and stabilises the hydroxide film on

the corroding surface, while the presence of 10Dy was reported to have higher corrosion resistance. Dy is one of the best tolerated RE elements and has very high solubility in Mg.[26] Solution treatment of Mg–10Dy results in a single phase matrix as the second phase dissolves completely in the Mg matrix. Lei *et al.* carried out extensive characterisation of the corrosion layer formed in this alloy and also their cytocompatibility in *in vitro* conditions. The corrosion tests were carried out by immersing the samples in cell culture medium, the DMEM solution for 28 days and also the characterised corroded products that were removed by immersing the corroded samples in chromic acid solution after 3, 7 and 14 days of immersion. Their work clearly demonstrated the corrosion rate of Mg–10Dy was lesser compared to the pure Mg; however, the difference was not high (0·75 mm/year for Mg and 0·56 mm/year for Mg–Dy). Further, the top surface of the corrosion layer was enriched with Dy oxides and hydroxides, while the concentration of Ca and P decreased gradually from the surface to the interface.

Between two extruded Mg–11·3Gd–2·5Zn–0·7Zr and Mg–10·2Gd–3·3Y–0·6Zr alloys, Mg–11·3Gd–2·5Zn–0·7Zr alloys exhibited higher corrosion resistance in Hank's solution due to formation of a compact film consisting of Mg, O, P and Ca elements with some bright particles with same composition with lesser Mg concentration. The corroded surface consisted of high pits in the case of Mg–10·2Gd–3·3Y–0·6Zr when compared to the other alloy.[27]

Biocompatibility and toxicity of Mg and its alloys

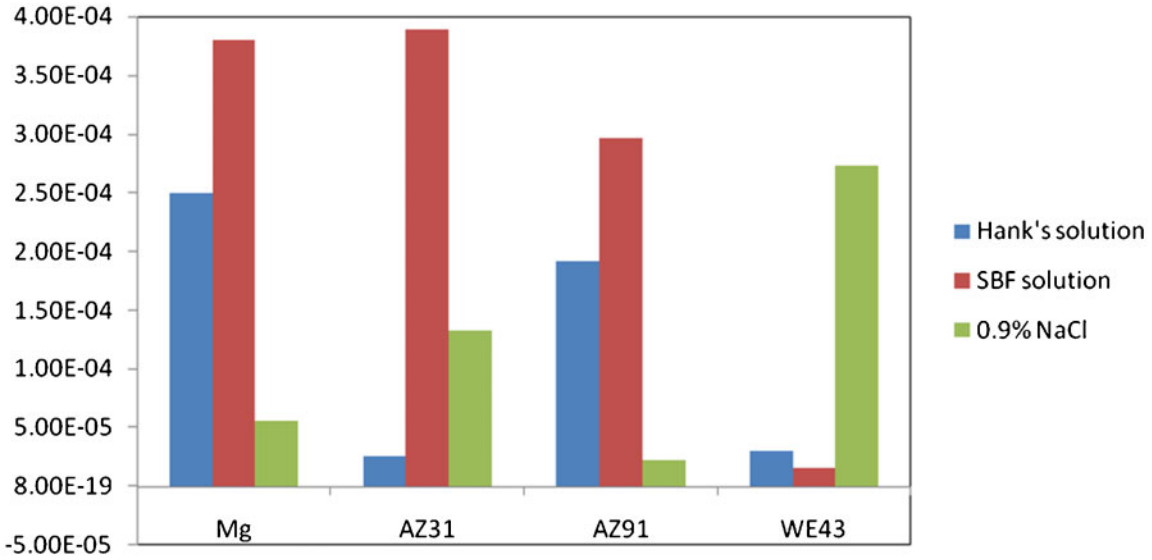
The presence of excess of Mg due to its corrosion response causes various effects. Mg plays a vital role in bone formation. The presence of Mg in hydroxyapatite (HaP), along with alumina, is reported to result in increased interfacial strength of implants.[28,29] Enrichment in $\alpha_5\beta_1$ and increased expression of collagen I intracellular matrix protein are observed in the presence of Mg.[30] However, Serre *et al.* reported the toxic effect of magnesium on bone cells *in vitro*.[31] The presence of Mg in the range $>1{\cdot}05$ mmol L^{-1} in serum is associated with muscular paralysis, hypotension and respiratory distress and even leads to cardiac arrest when its level in serum is as high as 6–7 mmol L^{-1}.[32,33] The corrosion products of Mg, such as hydrogen (H_2), hydroxyl groups (OH) and magnesium hydroxide [$Mg(OH)_2$], have been detected to lead to several negative effects. Evolution of hydrogen increases with increase in anodic polarisation of Mg, and the stress caused by hydrogen pressure results in hydrogen embitterment, leading to brittle fracture of the implants. Further, hydrogen gas, which evolves in the rate of 1 mL for every 1 mg of Mg, is difficult to be released from corrosion sites and also results in toxic effect to the tissue. Further, the presence of hydroxyl groups increases the pH in the surrounding tissue and inhibits cell proliferation and tissue formation. This effect is very severe in laboratory testing as the conditions are static over there where pH can rise up to 10.[12] The biocompatibility of the few Mg alloys such as MgZn, $MgAl_3$, $MgAl_9$, $MgNd_2$, MgY_4, $MgAl_3Zn$, $MgAl_9Zn$ and $MgDy_4Nd_2$ was investigated for cytotoxicity, cell proliferation, metabolic activity and corrosion in bovine serum with different extracts by Scheideler *et al.*[34] Their studies clearly revealed the test condition that cell culture medium and serum resulted in different ranking for toxicity of the alloys and hence clearly demonstrated that a serum solution which simulates clinical conditions can lead to better predictability of the *in vivo* corrosion behaviour and biocompatibility of Mg based biomaterials. Among all the alloys, $MgAl_9$ showed severe toxicity under both extraction conditions.

In vivo tests were conducted using rods of AZ31, AZ91 and WE43. LAE442 in guinea pigs revealed that LAE442 corroded much slower than other alloys, while the other alloys degraded in similar rates.

Pure Mg has slower corrosion rate than Mg, with harmful impurities such as Fe and Ni whose tolerance limits are 170×10^{-6}, 1000×10^{-6} and 5×10^{-6} respectively. The effect of various alloying elements on the corrosion behaviour of Mg was critically reviewed by Li *et al*.[35] Owing to high corrosion rate and high hemolysis rate, pure Mg may not be a proper material for biodegradable vascular stents; however, pure Mg shows the ability of inducing the formation of new bone in spite of their poor mechanical properties. The addition of Ca, Zn, Si, Sr and RE is found to improve the corrosion resistance of Mg and biocompatibility. The bone formation is speeded up in the presence of both Ca and Mg as Ca incorporation is aided by the presence of Mg. Among these elements, Ca also helps in grain refinement in Mg alloys, and its solubility in Mg is 1·3 wt-%. Similarly, addition of Zn, which exists in human body tissues, is also found to impart superior biocompatibility with degradation rate of 2·32 mm $year^{-1}$ in *in vivo* studies when added up to 6 wt-%. Addition of Y into magnesium alloys further increases the solubility of Zn in Mg matrix, enhances both the tensile strength and elongation and therefore slows down the corrosion rate, while addition of Zr acts as a grain refiner in Mg alloys. Apart from Y and Zr addition of Mn, a non-toxic element is found to enhance the mechanical properties of Mn–Zn alloy when added up to 3 wt-% by refining grain size while reducing the corrosion rate when added up to 1 wt-%.

Similar to Mn addition, Ca addition to Mg–Zn alloy is found to have high influence in both the mechanical properties and corrosion resistance. The distribution of nanosized second phase in Mg–2Zn–0·24Ca alloy subjected to high pressure torsion decreased the corrosion rate remarkably.[36] Although Si is another vital element in the diet, when added to Mg even at low concentration it exhibits low ductility. On the other hand, Sr, which belongs to IIA group of periodic table similar to Ca and Mg, resembles the latter elements in chemical, biological and metallurgical properties. In vivo experiments conducted by Gu *et al.*[37] with Mg–2Sr revealed the bone mineralisation and new bone formation around the implant without any adverse effects.[38]

Currently, the addition of RE element based Mg–RE alloys is considered to be much superior when compared to Mg alloyed with other elements. Among different RE elements, Gd and Dy have higher solubility than Y, while Eu, Nd and Pr (with relatively lower solubility in Mg) are also considered to be suitable. Although Mg based orthopaedic alloys are in preclinical trial stage, cardiovascular based Mg alloys have already entered clinical trials.



3 Mg and alloys in different solutions versus I_{corr} value (units)[43]

Coating on Mg alloys for corrosion inhibition and bone cell attachment

Recent interest in biodegradable Mg alloys has led to development of various surface coatings on the Mg and its alloys to control the corrosion process and enhance the bone growth. From the above discussion, it is evident that evolution of H_2 gas, formation of anodic and cathodic sites on the implants, and pit formation are the major reasons for the rapid degradation of Mg based materials in aggressive body environment. An extensive review on coatings on Mg alloys is provided by Hornberger *et al.*[39]. Two major approaches to combat corrosion are discussed in this present article: tailoring of microstructure by processing technique or alloying additions and developing surface treatments or coatings using bioceramics, polymers and composite layers. Conversion coatings are formed by specific reaction on the surface, and the environment and deposition of specific materials are the two ways by which coatings are developed. The most widely used conversion coatings are passivation, anodisation, calcium phosphate or fluoride layer deposition, and oxide coatings using plasma electrolytic oxidation technique, while deposition coatings involve formation of metallic coatings using ion implantation, creation of inorganic coatings using plasma spraying and laser application, development of diamond-like carbon coatings using chemical vapour deposition cathodic and sol–gel deposition of HaP, and phosphate coatings in addition to spin coatings, dipping and immersion of organic molecules are being attempted by several researchers. Although most of these coatings have improved the corrosion resistance, the degradation of the coatings with time has also been demonstrated[40,41] in *in vivo* conditions. Similar to the formation of nanotubes on Ti, the formation of nanoporous or nanoxides is also reported to have beneficial effects in Mg.[42] According to Chen *et al.*, pretreatment plays a major role in the development of an appropriate coating with required functions than the coatings itself. The corrosion behaviour of the coatings is studied using immersion tests, polarisation and impedance spectroscopy studies using various test conditions, and hence, it is very difficult to compare the results, thus making it difficult to arrive at the best coatings for long term applications. Hence, it is essential to develop a long term database on short term and longer corrosion behaviour of the coatings in both *in vitro* and *in vivo* conditions to understand the degradation behaviour of the coatings. The corrosion rates of various coatings developed using different techniques are shown in Fig. 3.

Challenges and future of biodegradable materials

It is inevitable to accurately simulate the corrosion test of Mg alloys similar to the process occurring in cardiovascular situations by performing the test in a solution containing the appropriate concentrations of chloride, phosphate and protein and also providing the right mechanical stimulus and flow cell environment. However, one should understand that the increase in flowrate increases the corrosion of implants and the introduction of mechanical stimulus often results in stress corrosion of Mg, pit formation and fatigue corrosion, and hence, the design of the Mg implant by choosing appropriate alloying elements that would prevent the above said failures is very crucial. Several strategies such as smart biomaterials, which can control the degradation rate based on the requirement or application of cathodic protection that controls corrosion by using an external electronic circuit, are recommended to avoid the failures of the implants.

The toxicity of all the alloying elements including the RE elements (Ce, La, Pr, Nd, Y, Gd and Gy) as well as Li and Zr should be tested and screened for their long term exposure hazards. A controlled biodegradation can be achieved by an appropriate coating, and all these coatings should be tested in highly simulated conditions for them to be used as a final product. It has become crucial to establish a database of all the published results with their experimental parameters in order to predict the performance of an alloy by numerical methods such a neural network.

There is a large variation in the cell compatibility response under static and dynamic conditions. Results

of cell culture tests show variation in cell adhesion behaviour of untreated Mg owing to complex interactions between corroding Mg and cells. New protocols should be carefully evaluated[42] for the static conditions as well as dynamic conditions.

Nanoscale distribution of surface heterogeneities might result in more uniform corrosion. There is an urgent need to develop a standard protocol that will be uniformly followed for testing the corrosion behaviour of coated and uncoated Mg alloys along the appropriate characterisation to understand the corrosion process in body conditions and develop a long lasting Mg based implant.

References

1. M. Geetha, A. K. Singh, R. Asokamani and A. K. Gogia: *Prog. Mater. Sci*., 2009, **54**, 397–425.
2. S. Ramakrishna, J. Mayer, E. Wintermantel and W. Leong Kam: *Compos. Sci. Technol.*, 2001, **61**, 1189–1224.
3. D. L. Wise: 'Biomaterials engineering and devices', 205–319; 2000, Berlin, Humana Press.
4. J. B. Park and J. D. Bronzino: 'Biomaterials: principles and applications', 1–241; 2003, Boca Raton, FL, CRC Press.
5. I. Clarke, H. Jones and D. Swinson: 'Biomaterials – an overview', http://www.azom.com/
6. D. R. Sumner, T. M. Turner, R. Igloria, R. M. Urban and J. O. Galante: *J. Biomech.*, 1998, **31**, 909–917.
7. S. Logothetidis: *Diamond Relat. Mater.*, 2007, **16**, 1847–1857.
8. K. Wang: *Mater. Sci. Eng. A*, 1996, **A213**, 134–137.
9. R. D. Blasier R. Bucholz, W. Cole, L. L. Wohnson and E. A. Makela: *Instr. Course Lect.*, 1997, **AAOS46**, 531–546.
10. F. Witte: *Acta Biomater.*, 2010, **6**, 1680–1692.
11. M. P. Staiger and A. M. Pietak: Biomaterials, 2006, **27**, 9, 1728–1734.
12. J. J. Jacobs, J. L. Gilbert and R. M. Urban: *J. Bone Joint Surg.*, 1998, **80**, 268–282.
13. F. Witte, V. Kaeseb, H. Haferkamp, E. Switzer, A. Meyer-Lindenberg, C. J. Wirth and H. Windhagen: *Biomaterials*, 2005, **26**, 3557–3563.
14. A. Meyer-Lindenberg, H. Windhugen and F. Witte: US 200410241036, 2006.
15. D. Willliams: *Med. Device Technol.*, 2006, **17**, 9–10.
16. L. Yang and E. L. Zhang: *Mater. Sci. Eng. C*, 2009, **C29**, 1691–1696.
17. N.-C. Quach, P. J. Uggowitzer and P. Schmutz: *C. R. Chim.*, 2008, **11**, 1043–1054.
18. C. Lorenz, J. G. Brunner, P. Kollmannsberger, L. Jaafar, B. Fabry and S. Virtanen: *Acta Biomater.*, 2009, **5**, 2783–2789.
19. S. Virtanen: *Mater. Sci. Eng. B*, 2011, **B176**, 1600–1608.
20. W. F. Mueller, M. F. L. de Mele, M. L. Nascimento and M. Zeddier: *J. Biomed. Mater. Res. A*, 2009, **90A**, 487–495.
21. W. D. Mueller, M. Lucia Nascimento and M. Fernández Lorenzo de Mele: *Acta Biomater.*, 2010, **6**, 1749–1755.
22. Y. T. Konttinen, M. Takagi, J. Mandelin, J. Lassus, J. Salo, M. Ainola, T.-F. Li, I. Virtanen, M. Liljeström, H. Sakai, Y. Kobayashi, T. Sorsa, R. Lappalainen, A. Demulder and S. Santavirta: *J. Bone Miner. Res.*, 2001, **16**, 1780–1786.
23. X. N. Gu, Y. F. Zheng and L. J. Chen: *Biomed. Mater.*, 2009, **4**, 1–8.
24. A. Yamamoto and S. Hiromoto: *Mater. Sci. Eng. C*, 2009, **C29**, 1559–1568.
25. H, Hermawan, D. Dubé and D. Mantovani: *Acta Biomater.*, 2010, **6**, 1693–1697.
26. L. Yang, N. Hort, D. Laipple, D. Höche, Y. Huang, K.-U. Kainer, R. Willumeit and F. Feyerabend: *Acta Biomater.*, 2013, **9**, 8475–8487.
27. X. Zhang. Y. Wu, Y. Xue, Z. Wang and L. Yang: *Mater. Lett.*, 2012, **86**, 42–45.
28. P. A. Revell, E. Damien, X. S. Zhang, P. Evans and C. R. Howlett: *Key Eng. Mater.*, 2004, **254–256**, 447–450.
29. H. Zreiqat, C. R. Howlett, A. Zannettino, P. Evans, G. Schulze-Tanzil, C. Knabe and M. Shakibaei: *J. Biomed. Mater. Res.*, 2002, **62**, 175–184.
30. Y. Yamasaki, Y. Yoshida, M. Okazaki, A. Shimazu, T. Kubo, Y. Akagawa and T. Uchida: *Biomaterials*, 2003, **24**, 4913–4920.
31. C. M. Serre, M. Papillard, P. Chavassieux, J. C. Voegel and G. Boivin: *J. Biomed. Mater. Res.*, 1998, **42**, 626–633.
32. 'Merck manual of diagnosis and therapy', Section 2, 'Endocrine and metabolic disorders', Chap. 12, 'Water, electrolyte mineral and acid/base metabolism'; 2011, Whitehouse Station, NJ, Merck.
33. N. L. Saris, E. Mervaal, H. Karppanen, J. A. Khawaja and A. Lewenstam: *Clin. Chim. Acta*, 2000, **294**, 1–26.
34. L. Scheideler, C. Füger, C. Schille, F. Rupp, H. P. Wendel, N. Hort, H. P. Reichel and J. Geis-Gerstorfer: *Acta Biomater.*, *Acta Biomater*., 2013, **9**, (10), 8740–8745.
35. N. Li and Y. Zheng: *J. Mater. Sci. Technol.*, 2013, **26**, (9), 489–502.
36. J. H. Gao, S. K. Guan, Z. W. Ren, Y. F. Sun, S. J. Zhu and B. Wang: *Mater. Lett.*, 2011, **65**, 691–693.
37. X. N. Gu, X. H. Xie, N. Li, Y. F. Zheng and L. Qin: *Acta Biomater*., 2012, **8**, 2360–2374.
38. H. S. Brar, J. Wong and M. V. Manuel: *J. Mech. Behav. Biomed. Mater.*, 2012, **7**, 87–95.
39. H. Hornberger, S. Virtanen and A. R. Boccaccini: *Acta Biomater*., 2012, **8**, 2442–2455.
40. L. Xu, F. Pan, G. Yu, L. Yang, E. Zhang and K. Yang: *Biomaterials*, 2009, **30**, 512–523.
41. F. Witte, J. Fischer, J. Nellesen, C. Vogt, J. Vogt, T. Donath and F. Beckmann: *Acta Biomater.*, 2010, **6**, 1792–1799.
42. W. F. Mueller, M. F. L. de Mele, M. L. Nascimento and M. Zeddier: *J. Biomed. Mater. Res. A*, 2009, **90A**, 487–495.
43. Z. Rong-chan, C. Jun, W. Dietzel, N. Hort, K. U. Kainer: Trans. Nonferrous Met. Soc. China, 2007, **17**, 166–170.