**In vitro** studies of biomedical magnesium alloys in a simulated physiological environment: A review

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1. Introduction

Stainless steels, Co-based alloys and titanium alloys are widely used in hard-tissue implants, especially in load-bearing applications, owing to their high strength, ductility and good corrosion resistance. With regard to biomedical implants, such as plates, screws and pins, used to repair serious bone fracture, it is desirable to use materials that can degrade in the physiological environment so that a subsequent surgical procedure to remove the implants from the human body after the tissues have healed is not necessary [1,2]. Repeated surgery increases morbidity and health costs. Magnesium and its alloys which are chemically active can degrade naturally in the physiological environment by corrosion and are potential candidates in biodegradable hard-tissue implants. Mg2+ is the fourth most abundant cation in the human body and is largely stored mainly in bone tissues. It is vital to metabolism between natural bones and implants can be mitigated [1]. The unique mechanical properties of magnesium alloys also render them desirable hard-tissue implants. Magnesium alloys possess a density of ~1.7–2.0 g cm−3 that is close to that of natural bones (1.8–2.1 g cm−3) and the compressive strength and tensile strength are much higher than those of biodegradable polymers. Compared with Ti alloys (110–117 GPa), stainless steels (189–205 GPa), and Co–Cr alloys (230 GPa), the elastic modulus of magnesium alloys (41–45 GPa) is closer to that of natural bones. Hence, the stress shielding effect induced by serious mismatch in the elastic moduli between natural bones and implants can be mitigated [1].

Hard-tissue repair typically requires implantation of the fixture for at least 12 weeks [1]. In this respect, magnesium is undesirable, because it is very active chemically, with a standard potential ~−1.7 V (Mg/Mg2+, standard hydrogen potential). The native MgO and/or Mg(OH)2 surface layers are loose in nature and cannot provide sufficient protection to resist corrosion encountered in the physiological environment which contains a large amount of chloride ions (~104 mmol L−1) [10] Chloride ions can convert the surface Mg(OH)2 into more soluble MgCl2, and dissolution of Mg(OH)2 makes the surface more active, decreasing the protected area and promoting further dissolution of magnesium. The reactions are summarized as follows [1,10]:

\[
\text{Mg} + 2\text{Cl}^- \rightarrow \text{MgCl}_2
\]

(1)

\[
\text{Mg(OH)}_2 + 2\text{Cl}^- \rightarrow \text{MgCl}_2
\]

(2)

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\[
\text{Mg} + 2\text{H}_2\text{O} \rightarrow \text{Mg}^{2+} + 2\text{OH}^- + \text{H}_2 \quad (3)
\]

The buffering agents consume the generated \( \text{OH}^- \) quickly in turn, expediting the conversion from \( \text{Mg} \) to \( \text{Mg}^{2+} \). It has been demonstrated that inorganic components as well as proteins and amino acids influence the degradation rate. As a result, Mg-based biomedical implants can lose the necessary mechanical integrity before the tissue has sufficient time to heal completely.

Many in vitro studies have been carried out to evaluate the degradation characteristics of magnesium and fathom the associated mechanism. Compared with \textit{in vivo} and animal/human studies, \textit{in vitro} experiments are convenient and can provide quick and reasonable feedback on the efficacy. The results are typically used to complement \textit{in vivo} and clinical studies. However, the accuracy of the results acquired from in vitro studies depends on various experimental factors, and both positive and negative (conflicting) results have been reported. This review summarizes the recent progress in \textit{in vitro} assessment of biomedical Mg alloys and future research trends.

### 2. \textit{In vitro} degradation of biomedical magnesium alloys in simulated physiological environment

#### 2.1. Materials selection and in vitro biocompatibility

Alloying elements play important roles in magnesium alloys, and the mechanical properties are usually the primary consideration when introducing alloying elements to the materials. Moreover, in biomedical engineering, factors such as biocompatibility and the rate of degradation are crucial. Good biocompatibility is essential in that materials released from the implants to body tissues and fluids must not be toxic, and this is especially important for degradable implants. In fact, the large amount of magnesium and potentially harmful alloying elements released during corrosion may lead to cytotoxicity, and the degree of toxicity highly depends on the dissolution rate. Evaluation of the biocompatibility is typically performed in two ways, namely by direct contact and indirect evaluation using extracted test solution. In direct contact experiments, cells are seeded on the samples directly. One of the problems is the changes in the materials surface during fast degradation in the early stage. The dynamic surface modifies cell attachment, making it difficult to carry out cell culture experiments. Hence, indirect immersion tests are often performed. The samples are first exposed to a suitable solution for different durations and then the solution is extracted and used for cell cultures.

Various types of magnesium alloys as well as pure magnesium are proposed for biomedical applications and many \textit{in vitro} studies have been performed to study the degradation rate and mechanism. The compositions and phase constituents of representative alloys are summarized in Table 1. Typical examples of Mg–Al–Zn alloys used are AZ91D, AZ31 and AZ63. Compared with pure magnesium, the introduction of Al not only modifies the mechanical properties, but also enhances the corrosion resistance [2]. It has been demonstrated that, in the Mg–Zn–Al system, the corrosion resistance in simulated body fluids (SBF) drops with increased Al content [24]. In fact, both Mg(OH)\(_2\) and Al\(_2\)O\(_3\) will form in a corro-}

![Image](https://via.placeholder.com/150)

<table>
<thead>
<tr>
<th>Family</th>
<th>Representative alloys</th>
<th>Alloying elements (wt.%)</th>
<th>Main phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Mg</td>
<td>Mg [11]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
</tr>
<tr>
<td>Mg–Al–Zn</td>
<td>AZ31 [11,12]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<td></td>
<td>AZ91 [11,13]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
</tr>
<tr>
<td>Mg–Ca</td>
<td>Mg–xCa [11,14,15]  (x = 1, 2, 3, 4, 5, \ldots)</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
</tr>
<tr>
<td>Mg–Zn–Ca</td>
<td>Mg–xZn–1Ca [16]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td>Mg–Zn–Mn–Ca</td>
<td>Mg–xZn–2.0n–1.2Mn–1Ca [17]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td>Mg–Si–Ca</td>
<td>Mg–xSi–Ca [11]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td>Mg–Zn</td>
<td>Mg–xZn [11,13,18,19]  (x = 1, 3, 10)</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td>Mg–Zn–Mn–Mn</td>
<td>Mg–xMn–1Mn [20]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td>Mg–Mn</td>
<td>Mg–xMn [11]</td>
<td>3Al 1Zn</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
</tr>
<tr>
<td>RE containing magnesium alloy</td>
<td>LAE442 [11]</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td></td>
<td>WE43 [8]</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<td></td>
<td>ZK41 [11,13]</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<td></td>
<td>AE44 [11]</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td></td>
<td>Mg–xGd [21] (x = 5, 10, 15, \ldots)</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<td></td>
<td>WZ21 [22]</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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<tr>
<td></td>
<td>Mg–BY [23]</td>
<td>4Al 2RE</td>
<td>Mg; Mg(<em>{17})Al(</em>{12})</td>
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In extruded Mg–Mn alloys, Mn is usually present in the form of pure Mn. Mn is an essential trace element (<0.8 \( \mu \)g L\(^{-1}\) in blood serum), but high concentrations may induce neurotoxicity [2]. Cell culture studies on Mg–1Mn indicate that the extracted media with a Mn concentration of 1.8 ± 0.5 \( \mu \)mol L\(^{-1}\) induce significant cytotoxicity to several cell lines [25,26].

Mn does not affect the mechanical properties of magnesium alloys significantly, but can increase the yield strength slightly. The most important function of Mn lies in the improved corrosion resistance by converting iron and other metal elements into relatively harmless intermetallic compounds [9]. In extruded Mg–Mn alloys, Mn is usually present in the form of pure Mn. Mn is an essential trace element (<0.8 \( \mu \)g L\(^{-1}\) in blood serum), but high concentrations may induce neurotoxicity [2]. Cell culture studies on Mg–1Mn indicate that the extracted media with a Mn concentration of 1.8 ± 0.5 \( \mu \)mol L\(^{-1}\) induce significant cytotoxicity to several cell lines [25,26].

Rare earth (RE) elements can improve the mechanical characteristics, corrosion properties and creep resistance of Mg alloys. For example, yttrium has a high solid solubility in magnesium and is often incorporated into magnesium alloys together with other RE elements to enhance the creep resistance at high
temperature. Y has also been reported to benefit corrosion resistance. Various RE-doped magnesium alloys such as WE43, Mg–4Y, Mg–5Gd and LAE 442 have been proposed. In WE43 alloy, Mg12YNd and Mg14YNd2 are the main precipitated phases. As for Mg–Gd alloy, Mg0.5Gd can precipitate both in grains and at grain boundaries. In the Mg–Al–RE system, RE tends to form an intermetallic with Al as Al11RE3 and Al12RE. While most of these RE elements retard in vivo corrosion of magnesium alloys [2], the use of RE elements in magnesium alloys for biomedical purposes should also be studied from the perspective of their potential cytotoxicity. Many RE elements possess anti-carcinogenic properties, but some studies have also discussed cytotoxicity for an intraperitoneal LD50 dose of GdCl3 of 550 mg kg\(^{-1}\) in mice. GdNO3 induces acute toxicity at a concentration of 300 mg kg\(^{-1}\) in mice and 230 mg kg\(^{-1}\) in rats [28,29]. Excess yttrium can change the expression of some rat genes and impose adverse effects on DNA transcription factors [19,30,31]. In spite of these scattered studies, systematic in vitro studies of RE elements dissolved from magnesium alloy are still rare. Hence, it is important to investigate systematically the potential cytotoxicity of dissolved RE elements from biomedical Mg alloys in the future.

Calcium contributes to the solid solution and precipitate strengthening. It also acts as a grain refining agent to some extent and additionally contributes to grain boundary strengthening. Zn improves the strength of magnesium alloys, owing to solid solution strengthening and castability [2]. Zn and Ca are both biologically benign elements, and Zn- and/or Ca-containing magnesium alloys such as Mg–32Zn, Mg–12Zn–1Ca and Mg–5Ca have been proposed as candidates for biodegradable implants [15,32,33]. In the Mg–Ca system, Mg2Ca is the only second phase besides α-Mg and distributes around grain boundaries. In the Mg–Zn family, MgZn, Mg2Zn3 and Mg2Zn6 are mostly present as second phases, while, in magnesium alloy containing both Ca and Zn, α-Mg, Mg2Ca and Ca3Mg2Zn6 generally constitute the main phases. Cytotoxicity tests on Mg–Zn–Ca, Mg–6Zn, and Mg–Ca alloys suggest similar cytotoxicity levels of 0–1 [32–34]. Good cell attachment has also been observed from Mg–Zn, Mg–Ca and Mg–Zn–Ca alloys according to direct cell cultures. However, a statistically significant decrease in the alkaline phosphatase activity and total proteins are observed in Mg–0.5Ca after culturing for 8 days [32–34]. The reason is not yet clear.

The initial surface states play a critical role in the establishment of cell–surface bonding. Rapid dissolution accompanied by fast evolution of hydrogen bubbles leads to a very dynamic surface and interface, which can dramatically hinder cell attachment. Hence, control of the surface reaction pertaining to initial cell reaction is important [35]. With regard to cell spreading and long-term survival, surface chemistry and morphology are key factors. In cytotoxicity studies of biomedicall magnesium alloys, tetrazolium salt-based assays, including MTT and XTT assays, are not suitable. The dissolved Mg in the extracted media can convert the tetrazolium salts to formazan, leading to a higher background and falsifying the results of cell viability. Another test, the BrdU assay, is found to be immune from the influence of Mg ions and is probably more appropriate for cytotoxicity evaluation of Mg-based materials [36]. Current evaluation of the cytotoxicity of magnesium alloys is preliminary, and more systematic and insightful understanding is needed from both scientific and technical standpoints.

### 2.2. Degradation characteristics of Mg-based implants

#### 2.2.1. Corrosion types

General corrosion, pitting corrosion and localized corrosion are the main corrosion mechanisms of magnesium alloys in simulated physiological fluids. The corrosion behavior of common magnesium alloys in the minimum essential medium (MEM) is discussed in Ref. [11], and the results are summarized in Table 2. These measurements are carried out in an incubator with ambient temperature 37 °C and 5.0% CO2. Fig. 1 depicts the typical morphology of the three types of corrosion occurring on magnesium alloys in a simulated physiological environment. Fig. 1a shows the typical morphology of AZ91 magnesium alloy suffering from localized corrosion, characterized by inhomogeneous corrosion on the surface and severely corroded sites. In fact, a metastable and partially protective film usually forms on magnesium alloys naturally, and selective attack tends to occur at these vulnerable sites when exposed to the solution. Continuous localized corrosion at these vulnerable sites gives rise to the non-uniformly corroded surface [10].

Pitting corrosion manifests as laterally spreading porous pits, as shown in Fig. 1b, and undermines the mechanical integrity. The sample is mechanical polished with 4000# waterproof diamond paper and is immersed in test solution with ambient temperature 37 °C. The pitting corrosion behavior is related to not only the inhomogeneous structure of the alloy, but also the composition of the solution. A second phase in the alloy usually leads to pitting corrosion. The second phase has a more positive potential than magnesium, resulting in the formation of a micro-galvanic couple. For instance, the AZ91 magnesium alloy has a large tendency towards pitting corrosion when exposed to common simulated body fluid (c-SBF) and 0.9 wt.% NaCl solution. As shown in Fig. 1b, severe porous pitting corrosion occurs on the AZ91 magnesium alloy after exposure to a 0.9 wt.% NaCl solution for 4 days. In AZ91 magnesium alloy, the second phase of Mg17Al12 has a more positive standard potential than Mg and exhibits passive behavior over a wide pH range. The formation of a micro-galvanic couple between the Mg matrix and Mg17Al12 phase leads to selective corrosion at the boundaries between the Mg17Al12 phase and Mg matrix. The Mg17Al12 phase usually delaminates from the surface and corrosion pits ensue [10]. These corrosion pits usually develop laterally from these sites, and a porous pitting corrosion morphology forms, owing to propagation of the pits and subsequent coalescence of the pit fronts, as shown in Fig. 2. Preferential dissolution of the second phase may sometimes produce porous corrosion pits. The Mg2Ca network at the grain boundary of the Mg–Zn–Mn–Ca alloy tends to dissolve preferentially during exposure to Hanks’ solution for 1 h, and deep irregular corrosion pits emerge [17]. The extent of pitting corrosion also strongly depends on the composition of the solution. In a solution with a high concentration of HCO3−, such as revised simulated body fluid (r-SBF) and Dulbecco’s modified eagle medium (DMEM), AZ91 is usually immune from pitting.
corrosion. High concentrations of hydrocarbonates have been observed to induce fast passivation on the surface, owing to quick precipitation of insoluble carbonates. Fig. 1c typically presents the corrosion morphology of pure magnesium suffering from general corrosion. A very smooth surface appears after exposure in the test solution.

2.2.2. Degradation rates

To measure the degradation rates in magnesium alloys, two techniques are usually employed, namely the weight loss method and the hydrogen evolution method. In the weight loss method, the degradation rates of the specimens are calculated as below:

\[
DR = \frac{W}{At}
\]

where \( DR \) refers to the degradation rate, \( W \) is the weight loss from the sample, and \( A \) and \( t \) represent the exposure area and exposure time in the solution, respectively. Before weighing, the sample is usually soaked in chromate acid (200 g L\(^{-1}\) CrO\(_3\) + 10 g L\(^{-1}\) AgNO\(_3\)) for 5–10 min to remove the corrosion products. Chromate acid can react with the corrosion products, but does not damage the Mg substrate.

The hydrogen evolution technique described in Fig. 3 is based on reaction (3). The amount of dissolved magnesium can be calculated from the volume of hydrogen generated from the reaction. This technique is reliable, easy to implement, and not prone to errors that are inherent to the weight loss method. In addition, the hydrogen evolution method allows the study of the variation in degradation rates vs exposure time. Experimental data have shown that the corrosion products do not influence the relationship between hydrogen emission and magnesium dissolution.

The degradation rates determined from magnesium alloys in MEM are presented in Fig. 3. The degradation rates of various magnesium alloys can vary by 3 orders of magnitude from \(0.06\) mg cm\(^{-2}\) day\(^{-1}\) to \(18\) mg cm\(^{-2}\) day\(^{-1}\), and AZ91 has the lowest degradation rate, while Mg–5Ca shows the highest corrosion rate. A lower Ca concentration in the Mg–xCa binary alloy...
Corrosion of Mg-based implants gives rise to four components: a corroded surface on the implant; dissolved magnesium ions and other alloying elements; a large amount of OH−; and hydrogen gas. Solutions such as SBF, DMEM and PBS used in in vitro studies contain large amounts of buffering agents such as HCO−3, HPO4−2, Tris–HCl and Hepes, which can consume the generated OH− and mediate abrupt changes in the pH. Therefore, although fast dissolution of magnesium occurs in these buffered solutions, the pH changes slowly. The dissolved magnesium ends up in two places: the solution and surface layer. For instance, after immersion in c-SBF for 10 days, more than half the dissolved magnesium precipitates in the surface layer on pure Mg [38].

The constituent in this surface layer after corrosion varies with the type of solution as well as the composition of the alloys. In solutions such as SBF, DMEM and Hanks’ solution with HCO−3 and HPO4−2 ions, insoluble phosphates and carbonates are usually present in this surface layer in addition to MgO and/or Mg(OH)2 [39,41,43]. Owing to the amorphous nature of these components in the layer, it is difficult to identify the exact substances [10,43,44]. However, some studies have disclosed the presence of crystalline phosphates and carbonates in the layer [20,44]. In the PBS solution, the main corrosion products are magnesium phosphates and Mg(OH)2, but insoluble carbonates are also found on samples exposed to PBS, possibly formed by the dissolved CO2 in the solution. The calcium-containing corrosion products on AZ91 magnesium alloy exposed to SBF and DMEM tend to aggregate at isolated regions [41]. Fig. 5a gives the morphology of AZ91 magnesium alloy after immersion in c-SBF for 1 day. Energy dispersive spectroscopy (EDS) indicates that the white regions contain a large amount of Ca and P. The gray regions contain P too, but no Ca. The P concentrations in the white regions are also much higher than those in the gray regions. Fig. 6 shows the cross-section view of sample in Fig. 5a. The surface layer in the white regions is quite thick (several tens of micrometers), but much thinner in the gray regions, as shown in Fig. 6. Non-uniform corrosion is also observed from other magnesium alloys. As discussed above, the formation of this non-uniform surface layer is suspected to stem from localized corrosion [43], but many other magnesium alloys show a very uniform surface layer after exposure to the simulated physiological environment. For instance, in Hanks’ solution, Mg–Zn–Mn alloy undergoes uniform corrosion from the top, as illustrated in Fig. 5b and c. Substantial buildup of the insoluble corrosion products is observed on Ca–rich Mg alloys [11] and, in 0.9 wt.% NaCl solution, Mg(OH)2 and MgO are the main constituents in this surface layer. Alloying elements may also be incorporated into the corrosion products [41]. Aluminium oxide and hydroxide have been identified on AZ91 after exposure in c-SBF [43].

2.3. Influence of constituents in physiological environment on materials degradation

The physiological environment contains various aggressive components that can attack magnesium alloy. Tables 3 and 4 list...
Apart from these aggressive anions, inorganic cations generally pose little effect on the degradation of magnesium. However, Song and co-workers found that the presence of Ca ions in SBF can slow down corrosion to some extent. This is probably because Ca ions in the solution spur precipitation of calcium-containing corrosion products [13,37].

The influence of organic components on the corrosion behavior of biomedical magnesium alloys has been investigated [42,46,47]. Adsorption of proteins on the materials surface affects the corrosion behavior. For instance, Liu et al. found that bull serum albumin (BSA) adsorption takes place when AZ91 is immersed in SBF with BSA [46]. The positive effects of protein on retarding dissolution of rare-earth containing magnesium are further confirmed [42,47]. Adsorption of albumin induces the formation of a BSA layer which can prevent attack by aggressive ions resulting in reduced cathodic currents and enhanced corrosion potentials. However, this blocking effect is very short lived and weakens dramatically with exposure time. Amino acids are also found to reduce the barrier effects of insoluble salt layers against dissolution of magnesium [42].

It should be pointed out that sample preparation procedures and the test conditions are also important for degradation measurement. The test samples in most publications are mainly mechanically polished with very fine grinding paper. Therefore, the preparation procedures of sample in these publications are suspected not to influence degradation measurement obviously. Another important issue is the test temperature: 37 °C is the most often used ambient temperature, but some published investigations are carried out at room temperature. Although it is difficult to evaluate the exact influence arising from the difference between room temperature and physiological ambient temperature, the temperature is in fact an important factor affecting the degradation performance of alloys, especially for alloys with high degradation rates. Thus it is essential to perform in vitro degradation measurement at ambient temperature ~37 °C.

3. Selection of suitable solutions for in vitro studies

An important concern encountered in in vitro studies of biomedical magnesium alloys is the selection of a suitable test
medium or solution. At present, many types of pseudo-physiological solutions that mimic the composition of body fluids are employed in *in vitro* experiments, and they include 0.9 wt.% NaCl solution, c-SBF, r-SBF, Hanks' solution, DMEM, PBS and so on. Table 5 lists the compositions and ion concentrations of five common solutions and Table 6 shows the components and concentrations of the buffering agents in body fluid and in the five solutions. The ionic composition of the solutions as well as constituents and concentrations of the buffering agents are quite different. The corrosion behavior of magnesium alloys is very sensitive to the aggressive environment and, as discussed in the previous section, HCO$_3^-$ and HPO$_4^{2-}$ alter the degradation behavior of magnesium. HCO$_3^-$ and HPO$_4^{2-}$ result in precipitation of insoluble corrosion products which retard subsequent degradation. Furthermore, magnesium dissolution generates OH$^-$ and consumption of OH$^-$ by the buffering agents in the solutions changes the degradation rate dramatically. Hence, the total concentration of buffering agents will dramatically affect the degradation rate. Thus, conflicting reports concerning the degradation behavior of magnesium alloys are present in Refs. [20,41,42,44]. The degradation behavior in 0.9 wt.% NaCl, 0.9 wt.% NaCl with Hepes buffer, NaCl with HCO$_3^-$ buffer, 0.125 M L$^{-1}$ NaCl in water, Earle's solution containing calcium and magnesium salts, and Eagle's MEM have been studied [42], and the corrosion rates are observed to vary by a factor of 100, depending on the solutions. It should be noted that the concentrations of inorganic ions are similar, but the buffering concentrations in Hanks' solution and c-SBF are different. The results indicate that the degradation rate in c-SBF is about one order of magnitude higher than that in Hanks' solution. The difference is mainly ascribed to the high concentration of Tris–HCl, which can react with OH$^-$, expediting dissolution of magnesium. In the solution containing a high concentration of HCO$_3^-$ (for example r-SBF, which contains ~27 mmol L$^{-1}$), AZ91 is immune to pitting corrosion, whereas in c-SBF (~4.2 mmol L$^{-1}$) or 0.9 wt.% NaCl without HCO$_3^-$ or with a low concentration HCO$_3^-$, AZ91 is very sensitive to pitting corrosion.

Even if the concentration of buffering agents in the solutions are the same, the type of buffering agents affects the degradation behavior of magnesium alloys. The commonly used buffering agents in simulated physiological fluids include Hepes, Tris–HCl, and HCO$_3^-$. Hepes and Tris–HCl are pure buffers which can only consume the generated OH$^-$ during magnesium dissolution. It is well known that HCO$_3^-$ (~27 mmol L$^{-1}$ in body fluids) is the most important buffering agent in body plasma. HCO$_3^-$ is not only capable of consuming OH$^-$, but also induces the formation of insoluble carbonates. This will definitely lead to different degradation behavior in solutions with the same total concentration of buffering agents, but different concentrations of HCO$_3^-$. Xin et al. recently found that in SBF with similar total buffer concentrations but different hydrocarbonate content, the degradation rates of pure magnesium differ greatly [50]. For the same total buffer concentration, the degradation rate of pure magnesium decreases greatly with increased concentrations of HCO$_3^-$. In SBF with HCO$_3^-$ concentrations of 4 mmol L$^{-1}$ and 15 mmol L$^{-1}$, no passivation behavior can be observed from pure magnesium, but a higher concentration of HCO$_3^-$ of 27 mmol L$^{-1}$ can induce fast passivation. It was also found that, in solutions with higher concentrations of HCO$_3^-$ up to 27 mmol L$^{-1}$, a more compact surface layer is formed. The high concentration of HCO$_3^-$ at the corrosion sites leads to precipitation of insoluble carbonates. Fast precipitation of insoluble corrosion products at these corrosion sites is also suspected to be the main reason for the observed passivation behavior. Besides these inorganic ions, organic components influence the degradation characteristics as previously discussed.

According to the above discussions, although Hanks’ solution consists of inorganic ions with concentrations similar to those in body plasma, the lower concentration of buffering agents and much lower concentration of HCO$_3^-$ (~4.0 mmol L$^{-1}$) impedes acquisition of more accurate *in vitro* degradation measurement such as that in the human body. Among these SBF, including c-SBF, modified SBF and r-SBF, r-SBF has the same amounts of inorganic ions, buffering agents and HCO$_3^-$ as body fluids, and thus it is a suitable media for *in vitro* investigations of degradation of Mg alloys. However, r-SBF does not contain proteins, amino acids and glucose, which prevents to some extent its having as accurate a degradation performance as that in a physiological environment. DMEM contains both organic ions and inorganic ions, such as body plasma. However, there are different kinds of DMEM with variable contents, and the DMEM with concentrations of inorganic ions, buffering agents and HCO$_3^-$ equal to those of body plasma is most desirable for *in vitro* degradation studies of biomedical magnesium alloys.

### 4. Conclusion and future trends

There have been many *in vitro* studies of biomedical magnesium alloys, and a wealth of knowledge pertaining to the degradation characteristics and associated mechanisms has been accumulated. In order that *in vitro* studies can be more meaningful and valuable to clinical investigations, future work should focus on the following three aspects. First, investigations on the cytotoxicity of biomedical implants up to now have been preliminary, and studies with more insight into the cytotoxicity of the corrosion products, especially the influence of alloying elements on cell response at gene level (e.g., DNA damage and repair, mutation), should be evaluated systematically. Secondly, much of the inconsistency and controversy pertaining to the degradation characteristics of biomedical magnesium alloys appears to stem from the choice of the test media or solutions. It is also difficult to compare different published results. It is important to establish new standard for *in vitro* studies of magnesium alloy. This standard procedure.
should include the use of a suitable and definite pseudo-physiological solution. The development and application of circulation system that mimics the circulation process in the physiological environment is also of great importance to future research. Last but not least, the effect of protein adsorption and cell attachment on degradation performance must be studied in more detail.

Acknowledgements

This project is jointly supported by Natural Science Foundation Project of CQ CSTC 2010BB4053, Fundamental Research Funds for the Central Universities (Project No. CDJZR11 13 00 01), Hong Kong Research Grants Council General Research Funds (GRF) No. CityU 112307 and City University of Hong Kong Strategic Research Grant (SRG) No. 7008009.

Appendix A. Figure with essential colour discrimination

Certain figures in this article, particularly Fig. 4, is difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.12.004).

References