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Building towards a standardised approach to biocorrosion studies: a review of factors influencing Mg corrosion *in vitro* pertinent to *in vivo* corrosion

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ABSTRACT The factors that influence magnesium (Mg) corrosion *in vitro* are systematically evaluated from a review of the relevant literature. We analysed the influence of the following factors on Mg biocorrosion *in vitro*: (i) inorganic ions, including both anions and cations, (ii) organic components such as proteins, amino acids and vitamins, and (iii) experimental parameters such as temperature, pH, buffer system and flow rate. Considerations and recommendations towards a standardised approach to *in vitro* biocorrosion testing are given. Several potential simulated body fluids are recommended. Implementing a standardised approach to experimental parameters has the potential to significantly reduce variability between *in vitro* biocorrosion tests, and to help build towards a methodology that accurately and consistently mimics *in vivo* corrosion. However, there are also knowledge gaps with regard to how best to characterise the *in vivo* environment and corrosion mechanism. The assumption that blood plasma is the correct bodily fluid upon which to base *in vitro* methodologies is examined, and factors that influence the corrosion mechanism *in vivo*, such as specimen encapsulation, bear consideration for further studies.

Keywords: corrosion, biocorrosion, magnesium, biodegradable metals, *in vitro*, *in vivo*

INTRODUCTION

Medical magnesium and biocorrosion

Alloys of magnesium (Mg), zinc (Zn) and iron (Fe) are being developed as a new class of metallic biomaterials known collectively as biodegradable metals [1]. These are different to traditional metallic biomaterials, which are dominated by corrosion resistant metals such as titanium, cobalt-chromium alloys and stainless steel [2]. The key application for biodegradable metals is for temporary medical implants and devices [3–7]. Biodegradable metals corrode and disappear after implantation. This eliminates the need for a subsequent, second surgery to remove the implant or device, as well as lowering the risk of infection and foreign body reaction [8–12]. Mg based implants have experienced good clinical successes in recent years [13–17].

In addition to being used effectively in temporary implants, Mg alloys have potential to be developed into multifunction medical materials [1]. Mg encourages osteogenesis [18], which has clear potential for orthopaedic applications [2]. Furthermore, the corrosion products of the Mg implant may be beneficial in certain applications [1]. For example, Mg corrosion releases H_2 gas, as shown by the overall corrosion reaction in Equation (1). This hydrogen may be significant, as rats who inhaled H_2 after brain injury had a lower level of brain tissue infarction [19]. Chen [20] proposed a mesh made from a Mg alloy for cranial repair, as the Mg enhances bone regrowth, and the H_2 that diffuses into the brain tissue may reduce the extent of tissue damage.

$$Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2 \quad (1)$$

However, despite the potential benefits of Mg alloys in medical applications, the key factor that must be addressed is the apparent, relatively-high corrosion rates of Mg alloys [21,22]. The tendency to corrode in the body allows Mg alloys to be used in temporary medical implants or devices. However, these corrosion rates must be

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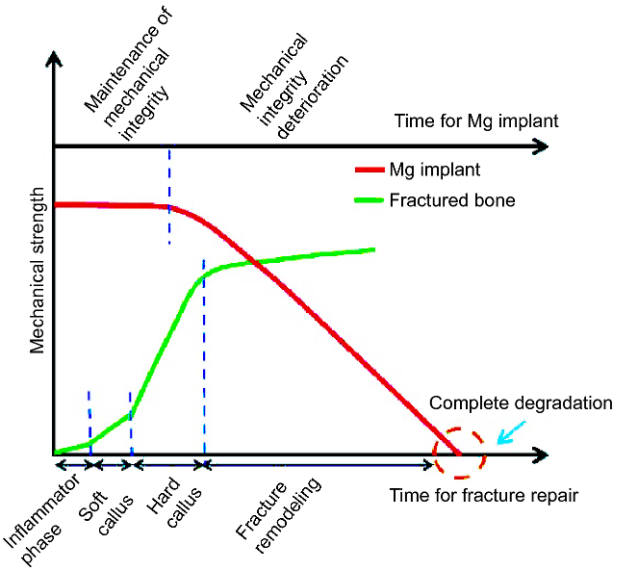

Figure 1 Diagram describing ideal degradation behaviour (and associated change in mechanical integrity) of a degradable orthopaedic implant. Reprinted with permission from Ref. [26], Copyright 2017, Elsevier.

controlled. Too rapid corrosion of the implant can cause issues stemming from (i) rapid ion release, (ii) excessive H_2 gas production forming gas pockets, (iii) local pH increases, and (iv) premature loss of structural integrity of the implant. The creation of hydrogen gas pockets has been of particular concern for biodegradable Mg implants [23,24], and the size of the gas pockets is linked to the samples' corrosion rate [25]. Ideally, the corrosion rate of the implant would match the rate at which the body tissue heals, as depicted in Fig. 1 [26].

In an effort to achieve this goal, a considerable body of research has been conducted to understand and control Mg corrosion in medical environments, which is also referred to as biocorrosion. The majority of this research into Mg biocorrosion has been conducted *via in vitro* immersion tests [27–39].

Variability of *in vitro* testing and the need for standardisation

In vivo and clinical data are undeniably vital to the study and success of Mg biomaterials. However, the high financial and ethical burdens associated with *in vivo* testing should, and does, limit their use. Consequently, *in vitro* testing continues to be the most frequently used method to study Mg biocorrosion.

In vitro testing can be utilised to reduce the ethical burden on *in vivo* studies by pre-screening a large batch of potential alloys to determine the best candidates, significantly reducing the number of animal sacrifices necessary for an *in vivo* assessment [40]. *In vitro* testing can also be used to assess factors that may be impossible, or impractical, to measure *in vivo* or *in situ.* As an example, the authors' pervious work considered the influence of pH on the biocorrosion of several Mg alloys [36]. This study would not been possible *in vivo* as changing an animal's blood pH would have been difficult, dangerous and unlikely to pass ethical scrutiny.

It has long been the goal of biocorrosion research to construct an *in vitro* methodology that accurately compares to *in vivo* behaviour [41]. In order for a laboratory or *in vitro* corrosion test to be able to predict in service corrosion (in this case *in vivo* corrosion), the *in vitro* corrosion testing must adhere to the following principle: the *in vitro* corrosion test must produce the same corrosion mechanism as that operating in service. This typically means considering the essential causative factors for the in-service corrosion, and reproducing these factors in the *in vitro* environment. This can be an iterative process. Success is evident when the *in vitro* corrosion tests and the in service corrosion (*in vivo*) have the same corrosion rates, the same corrosion manifestation, and the same corrosion products.

However, the early testing failed to accurately predict *in vivo* corrosion rates or the corrosion mechanism. Initial testing used immersion conditions in simple NaCl solutions, but these were found to be inappropriate [42]. Subsequently, a wide range of solutions and methodologies have been explored [36,43–46], each with advantages and limitations, although some studies still continue to use NaCl solutions [47]. Table 1 provides a comparison of the constituent elements of several commonly used *in vitro* immersion solution [24,48–50].

This variety in experimental solutions and conditions makes comparison between studies challenging, which has necessitated a number of studies to be repeated. The complex and interconnected factors which influence biocorrosion *in vitro* has split opinions within the field, leading to some distrust of *in vitro* biocorrosion testing [41], which is a significant impediment to progress in the field of biocorrosion and biodegradable metals. A movement towards standardization has been discussed [41], but with such a complex system, lack of complete knowledge, and such varied methodologies, progress towards this end has been challenging.

As the ultimate goal of *in vitro* testing is to accurately predict and mimic the *in vivo* corrosion behaviour [41], it would seem logical to just compare several *in vitro* conditions to the *in vivo* results and determine which is most

Table 1 Comparison of common immersion solutions used in *in vitro* biocorrosion studies compared to blood plasma

	Component	Blood plasma [48,49]	HBSS (H1387) [24,75]	HBSS (14175) [50]	EBSS [48]	MEM [48]
Inorganic ions (mmol L^{-1})	Cl^-	100.0–103.0	145.0	143.3	125.0	125.0
	HCO_3^-	22.0–30.0	4.2	4.2	26.0	26.0
	$H_2PO_4^-$	0.0–0.08	0.4	0.4	–	–
	HPO_4^{2-}	0.0–1.0	0.3	0.3	1.0	0.9
	SO_4^{2-}	0.5	0.8	–	0.4	0.4
	Mg^{2+}	1.0–1.5	0.8	–	0.4	0.4
	Ca^{2+}	2.5	1.26	–	1.8	1.8
	Na^+	140.0–142.0	142.0	142.8	144.0	143.0
	K^+	5.0	5.8	5.8	5.4	5.4
Organic components (g L^{-1})	Protein (e.g., albumin)	35–80	–	–	–	–
	Amino acids	0.25–0.40	–	–	–	0.95
	Vitamins	Variable (in range of μg–mg)	–	–	–	0.008
	Glucose	0.9–1.1	1.0	1.0	1.0	1.0

accurate or representative. However, due to the complex nature of the biocorrosion environment, and the large variations and inconsistencies in the *in vitro* conditions, this direct approach has not been as widely accepted as could be hoped. To illustrate this, a case study is made of two studies, which have directly compared *in vitro* biocorrosion to *in vivo* and effectively came to opposite conclusions. These studies are outlined briefly in Table 2 [24,50].

The studies presented in Table 2 provide valuable insight into the comparison between *in vitro* biocorrosion and *in vivo* biocorrosion conditions. However, their impact is limited by inconsistent experimental conditions. Marco *et al.* [50] compared several Mg alloys in several *in vitro* solutions to samples in Sprague-Dawley rats. This study found a large amount of variation between these conditions, but ultimately concluded that DMEM was most suitable as it more closely reproduced the *in vivo* corrosion layer. However, there are several concerns with the *in vitro* methodology employed. No pH control was employed for the simpler simulated body fluids (SBFs) (PBS and HBSS), while a 5% CO_2 atmosphere was used for DMEM. Additionally, the HBSS used did not contain Ca^{2+} or Mg^{2+}. This omission is sigificant as HBSS usually contain these ions [36,37,51,52], and it is likely the reason that there was poor agreement between the corrosion layers of the HBSS samples compared to *in vivo* in this study.

In contrast, Abidin *et al.* [24] found CO_2 buffered HBSS (containing Ca^{2+} and Mg^{2+}) to be a good model for intramuscular *in vivo* corrosion in Mister rats. It was further proposed that the reason for this good agreement was that the SBF used had a similar ionic content to blood, and a reliable buffer system which was similar to that used in the human respiratory system. However, this was the only *in vitro* environment tested, and this result provides limited scope to the question of the the existance of a more optimum *in vitro* system or solution. It is impossible to determine from these results if this is the best SBF or if additional components, such as protiens, might have further improved the comparision.

These results indicate that there is currently still lacking a "gold standard" methodology which provides optimum comparision to *in vivo* results. However, *in vitro* evaluations continue to be an important component in biocorrosion testing for the foreseeable future. As such, it is important that *in vitro* tests are structured to be as accurate, repeatable, and consistent as possible. This need is reflected in the ASTM standard currently in development which seeks to provide guidance for the *in vitro* assessment of biodegradable/absorable metals [53]. A deep and full understanding of how each aspect of the *in vitro* environment influences the biocorrosion of Mg alloys is an essential part of improving *in vitro* testing, and building towards a standardised biocorrosion methodology.

In order to achieve this aim, and to help inform the development of standard testing practice in this area, a systematic analysis is herein conducted on each element or parameter of the *in vitro* biocorrosion system in-

Table 2 Two studies which have directly compared *in vitro* corrosion to *in vivo* corrosion

	Marco *et al.* [50]	Abidin *et al.* [24]
SBF	PBS, HBSS (14175) & DMEM	HBSS (H1387)
Animal model	Sprague-Dawley rat (male), femur	Wistar rat (male), subcutaneous
Solution volume control	30 mL cm^{-2} for PBS & HBSS, 50 mL cm^{-2} for DMEM	0.7 L/sample
pH control & buffer	No control for PBS & HBSS. 5% CO_2 atmosphere for DMEM	Active CO_2
Alloys	Pure-Mg, Mg-10Gd & Mg-2Ag	HP-Mg, WZ21 & AZ91
Number of samples	n=6 *in vitro* n=2 *in vivo* (2 samples per rat)	Minimum of n=4 for various time points
Summary of results	A large amount of variation was found in the corrosion rates of the alloys tested when comparing *in vitro* and *in vivo* results. DMEM was considered the best solution used as it produced a similar corrosion layer to the *in vivo* model.	CO_2 buffered HBSS (H1387) produced similar corrosion rates *in vitro* when compared to *in vivo* for HP-Mg and WZ21. The AZ91 samples had much lower corrosion rates *in vivo*, attributed to these samples being encapsulated in fibrinous tissue.

dividually, in order to create a clearer picture of which aspects are most vital to a standardised approach. Current key knowledge gaps or areas of interest are also identified.

This review focuses on the influence of (i) inorganic ions, (ii) organic components, and (iii) experimental parameters such as temperature, pH and solution flow rate. On this foundation, recommendations are made (i) on the immersion solution and (ii) experimental parameters that should be considered in a standardised biocorrosion methodology.

MAGNESIUM CORROSION ASSESSMENT

Corrosion of Mg in a medical environmental (also known as biocorrosion) converts metallic Mg into the stable Mg^{2+} ion. This occurs in the presence of water, which is simultaneously reduced to form hydrogen gas (H_2). Hydroxide ions (OH^-) are also formed. These readily react with Mg^{2+} ions to form the corrosion product magnesium hydroxide $Mg(OH)_2$, because of the low solubility of $Mg(OH)_2$. The overall reaction is given in Equation (1).

Corrosion of Mg alloys is typically faster than the corrosion of high-purity Mg in simple chloride solutions, because the second phase in the Mg alloy causes micro-galvanic acceleration of the corrosion of the alpha-Mg matrix [22,54–56]. This micro-galvanic corrosion acceleration typically occurs preferentially next to the second phase, and is often wrongly characterised as pitting corrosion. In less aggressive solutions, like sulphate solutions, the corrosion products on the matrix phase can overcome this micro-galvanic acceleration, and the corrosion rate can begin to decrease despite the presence of second phase particles, as for example Mg-Y alloys in the work of Liu *et al.* [57].

The corrosion of Mg is commonly characterised *via* a corrosion rate. This corrosion rate measures the average amount of Mg that has been corroded over a period of time. Typical units are mm y^{-1} or mg cm^{-2} day^{-1}. Both of these units are equally valid, and require a simple conversation factor to compare. For consistency, the corrosion rates discussed herein use mm y^{-1}.

Corrosion rates are measured *via* a number of different techniques, the most common being (i) mass loss, (ii) hydrogen evolution and (iii) Tafel extrapolation of cathodic polarisation curves. A detailed description of each technique can be found in any one of several good reviews [54,55,58]. Each technique has strengths and limitations. Consideration should always be given to using a number of techniques in tandem, in order to mitigate any potential issues or inaccuracies that may occur when only employing a single technique. Corrosion rates are useful for a direct comparison between samples or alloys, however they may not provide a complete picture of the corrosion when considered on their own. Consideration of the corrosion morphology is also recommended, as this provides information on the type of corrosion that has occurred [42,59].

Many studies also conduct an analysis of the corrosion products and the layers these products form on the sample's surface (also known as surface films) [60]. More stable corrosion products or oxide/hydroxide films forming on the surface of the sample reduce the sample's active contact area with the solution, thereby providing some protection from corrosion. This is of particular importance in more complex solutions, such as those

used when measuring biocorrosion. Typical Mg corrosion products, such as $Mg(OH)_2$, are not expected to form a stable corrosion-product film under physiological pH levels, and thus only provide partial protection. However, in more complex solutions, such as those employed in biocorrosion testing, more varied corrosion products can form [21]. These additional corrosion products can form surface films, which provide more substantial protection, and slow the corrosion rate of the sample [21,54].

Nomenclature

To ensure this review provides useful commentary to the widest possible audience, a glossary of terms is presented as Supplementary information to this paper. This glossary defines, explains or otherwise clarifies terms, acronyms, and/or abbreviations that are common in this field of study.

Additionally, it is noted that due to the variable nature of measuring and reporting biocorrosion data, many studies have resisted direct comparison of the biocorrosion rates reported. As such, a series of terms are proposed herein to indicate a percentage range of influence. These are outlined as follows:

Minimal: effectively no change in the corrosion rate;
Slight: ~0–25% change in the corrosion rate;
Moderate: ~25–100% change in the corrosion rate;
Substantial: >100% change in the corrosion rate.

These terms allow the reader to appreciate the magnitude of the influence measured, while avoiding conversations or comparisons which might have appeared disingenuous, and maintaining the integrity of the original study and data set.

INORGANIC IONS

Anions

Human body fluids contain anions such as chlorides (Cl^-), carbonates (HCO_3^-), phosphates ($H_2PO_4^-$ and HPO_4^{2-}), and sulphates (SO_4^{2-}) [61]. The influence of anions, in particularly chlorides, on the corrosion of metals has been well studied [62–64], and such anions have gathered significant interest in the field of Mg biocorrosion.

Chloride ions (Cl^-)

Chloride ions are known to increase corrosion rates of Mg alloys by destabilising partly-protective films in simpler solutions such as 3.5% NaCl [54,65], and this is commonly expected to be the case in more complex solution such as SBFs. The presence of Cl^- destabilises the partially protective $Mg(OH)_2$ corrosion layer to form $MgCl_2$, which is water soluble and therefore does not protect the underlying Mg [66]. This reaction is presented in Equation (2). Increased concentration of Cl^- was found to increase the probability for localised corrosion of AZ91 in a range of modified SBFs [59]. Cl^- were also postulated to have destabilised the corrosion product layer, inducing the dissolution of the mildly protective $Mg(OH)_2$ layer and transforming it into a soluble $MgCl_2$ layer in CP-Mg samples [67].

$$Mg(OH)_2 + 2Cl^- \rightarrow MgCl_2 + 2OH^- \quad (2)$$

These results suggest that the influence of Cl^- may be similar to that in simpler solutions. However, further investigation into the effects of this ion has been pursued as the complexity of SBFs, specifically the wide array of ions present, can require a nuanced approach. Taltavull *et al.* [51] studied the influence of Cl^- on the biocorrosion of HP-Mg, AZ91 and ZE41. Five immersion solutions were tested, and are presented in Table 3 with a comparison to blood plasma [51]. To maintain pH within physiological levels, they used the same buffer system as that by Abidin *et al.* [24] between CO_2 gas and the dissolved $NaHCO_3$. This system is covered in detail in the following section on carbonates, as well as in Section "pH and buffer systems". An increased incidence of localised corrosion was associated with an increase in Cl^- for all three alloys. However, the higher incidence of localised corrosion was not always correlated with an increased corrosion rate.

Fig. 2 presents a comparison between the HP-Mg and ZE41 samples [51], with the AZ91 samples behaving similarly to the ZE41. There was a substantial increase in the corrosion rate between solutions 1 and 2 for AZ91 and ZE41, and a slight increase for HP-Mg. This indicated that the presence of Cl^- caused an increase in corrosion rate, due to the micro-galvanic interaction between the second phase and the Mg matrix for AZ91 and ZE41. The effect was small for HP-Mg because HP-Mg is single phase and there was only a slight increase of corrosion rate attributed to the degradation of the partially protective surface film. Solution 3 was a HBSS, and the additional ions in this solution substantially reduced the corrosion rate of the samples compared with that in solution 2, despite the moderate increase in Cl^- content. The cause of this decreased corrosion rate was attributed to the changed surface film which was in turn caused by the increased phosphate ion concentration, (in combination with Ca^{2+}, Mg^{2+}, and HCO_3^-). These ions all alter or add to the corrosion product layer, which has an influence on the corrosion rate of the sample. For a more

Table 3 Solutions used by Taltavull *et al.* [51] to study the influence of Cl^- on HP-Mg, ZE41 and AZ91 with blood plasma given as a reference. When referring to this study, solutions are referred to sequentially from solution 1 ($[Cl^-] = 0$) to solution 5 ($[Cl^-] = 1$).

Solution	Composition (mmol L^{-1})									
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	$H_2PO_4^-$	HPO_4^{2-}	SO_4^{2-}	OH^-
$[Cl]^- = 0$	4.2	–	–	–	0	4.2	–	–	–	–
$[Cl]^- = 0.1$	104	–	–	–	100	4.2	–	–	–	–
$[Cl]^- = 0.14$ (Hank's)	142	5.8	0.8	2.5	145	4.2	0.4	0.3	0.8	–
$[Cl]^- = 0.3$	302	5.8	0.8	2.5	300	4.2	0.4	0.3	0.8	–
$[Cl]^- = 1$	1002	5.8	0.8	2.5	1000	4.2	0.4	0.3	0.8	–
Blood plasma	142	3.6–5.5	1.0	2.1–2.6	95–107	27.0	–	0.7–1.5	1.0	–

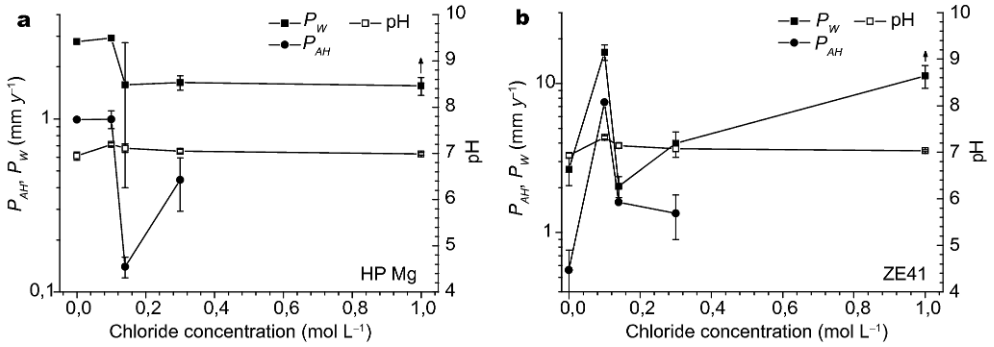

Figure 2 Comparison of (a) HP-Mg and (b) ZE41 in a range of solutions with variable Cl^- content. Reprinted with permission from Ref. [51], Copyright 2013, Springer Science+Business Media New York.

detailed understanding of the effects of these ions, please consult the section of this review dedicated to the ions individually.

In the solutions 4 and 5 (where NaCl was added to the HBSS), there was again a clear distinction between the HP-Mg, and the other alloys. The higher sensitivity to Cl^- continued for the AZ91 and ZE41, again attributed to the micro-galvanic effects between the secondary phases and Mg matrix. The higher Cl^- content, and the associated increased localised corrosion, was associated with a substantially higher corrosion rate for ZE41 and AZ91, whereas there was minimal influence on the corrosion rate of the HP-Mg samples, which had no second phase. This confirms that the influence of Cl^- is dependent on the alloy being tested, however typically an increase in chloride concentration will cause an increase in corrosion rate.

These results support that increasing the Cl^- content tends to increase the biocorrosion rate of Mg alloys by disrupting the partially protective corrosion product layer, and should be maintained within a physiologically relevant range. Additionally, the ability of the Cl^- content to promote localised corrosion is more pronounced in alloys with a greater amount of secondary phase. The micro-galvanic corrosion, which is common to such alloys, leaves them susceptible to this type of localised corrosion attack because the electrochemical potential between the two phases provides an additional driving energy for the corrosion of the Mg matrix. As such, it is recommended that multiple alloys be used when investigating an aspect of biocorrosion, as a single alloy may not tell the entire story.

Carbonate ions (HCO_3^-)

Another well-studied anion common to most SBFs and biological solutions is HCO_3^-. This is important predominately because of the role it plays in the respiratory buffer system [68]. This system is presented in Equation (3).

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \quad (3)$$

Studying the influence of HCO_3^- on biocorrosion is challenging, as it requires an effort to decouple the influence the ion has on the Mg samples directly from the buffering potential it provides to the solution. For a detailed discuss of the influence of solution pH and various

buffering systems on the biocorrosion of Mg please consult Section "pH and buffer systems". In summary, it has been found that low solution pH promotes the dissolution of Mg, and high solution pH decreases the corrosion rate by stabilising the corrosion layer [36]. Mg corrosion in an unbuffered solution leads to an increase in solution pH. In a simple NaCl solution the pH increases to ~10.3 after several hours due to the low solubility of $Mg(OH)_2$; the physical chemistry by which the pH is controlled by the low solubility of $Mg(OH)_2$ is fully described by Atrens *et al.* [55].

Xin *et al.* [59] considered the influence of a range of ions, including HCO_3^-, on the corrosion of AZ91. The presence of HCO_3^- appeared to initially stimulate corrosion, before aiding passivation due to the precipitation of $MgCO_3$ and $CaCO_3$, which slightly lowered the corrosion rate and completely supressed any incidence of localised corrosion. This result was supported by the results of Ma *et al.* [67] who conducted a primarily electrochemical study of the biocorrosion of CP-Mg and also concluded that the presence of HCO_3^- initially increased the corrosion rate but eventually promoted the deposition of a protective corrosion layer which reduced the corrosion rate. However, these results [59,67] only provide a binary comparison between solutions with and without HCO_3^-. As previously discussed, HCO_3^- are an essential component to the buffer system shown in Equation (3). It is likely that without this buffer, the pH of the solutions without HCO_3^- would have risen quickly due to the corrosion of Mg. These samples would have been more quickly passivated than the samples in buffered solutions containing HCO_3^-. In this way, what may appear as a relative increase in corrosion rate could have resulted from a retardation of the corrosion rates due to increasing solution pH.

In a further study, Xin *et al.* [69] studied a range of HCO_3^- concentrations over a 24 h immersion. A substantially higher concentration of HCO_3^- decreased the corrosion rate substantially in the first few hours; however this effect diminished to a slight or minimal influence by the end of the 24 h test. Additionally, this large initial increase may have been a result of the higher levels of Tris-HCl buffer, which were included in the solutions with less HCO_3^- to better buffer the solution. This was a surprising experimental decision, as the same authors have found that the Tris-HCl buffer substantially increased the corrosion rate of Mg alloys in SBF [70]. In a similar study, but without the Tris-HCl influence, Agha *et al.* [71] considered the biocorrosion of HP-Mg in a range of solutions with varying buffering salt concentrations.

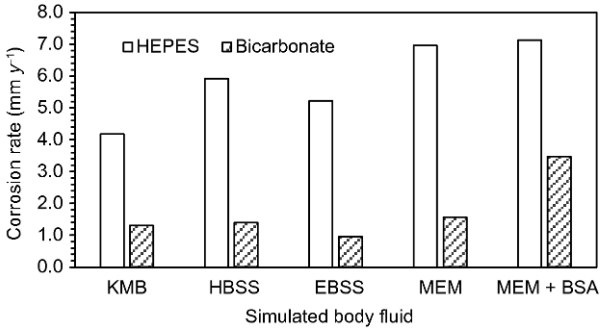

Figure 3 The mass loss corrosion rate of CP-Mg in different SBFs and buffers, at 37°C for 1 week. 40 g L^{-1} of BSA was added to include proteins in the MEM + BSA solution. Note: these corrosion rates were calculated from the % mass loss presented in Kirkland *et al.* [72]. KMB-Kirkland Biocorrosion Medium, a modified SBF.

Increasing concentrations of HCO_3^- (4.4, 22 and 44 mmol L^{-1}) yielded minimal change in the corrosion rate. This result was supported by the study of Kirkland *et al.* [72] where a minimal to slight difference was found between HBSS and EBSS when an active CO_2 buffer was used, as presented in Fig. 3. A comparison between the HBSS and EBSS in Table 1 indicates the primary difference between the balance salt solutions is the higher level of HCO_3^- in EBSS. This result suggests this higher concentration does not significantly influence the corrosion rate, provided the pH is adequately controlled (as is discussed further in Section "pH and buffer systems").

These results suggest that HCO_3^- influences the corrosion through the formation of corrosion products such as $MgCO_3$ and by buffering the solution. However, there is little difference in the corrosion rate in SBFs with HCO_3^- in the range from 4.4, to 44 mmol L^{-1}, once there is a protective surface film.

It should also be noted that increasing HCO_3^- concentration has an influence on the stability of the SBF, and this should be accounted for accordingly. Blood plasma is an inherently unstable solution, which precipitates carbonate based compounds. As previously stated, Table 1 indicates the primary difference between HBSS and EBSS is the higher concentration of HCO_3^- in EBSS. While this does make EBSS more physiologically similar to blood plasma, it comes at the cost of stability, and it is noted that careful preparation, pH control and a 5% CO_2 atmosphere is required to prevent excess precipitation of $CaCO_3$ [49,73]. In contrast, the lower HCO_3^- level in HBSS allows it to be used in normal atmospheric conditions [49]. Adequate buffering and pH controls are nevertheless strongly recommended and can be

achieved by active CO_2 control (i.e., bubbling CO_2 directly into the solution) as discussed in Section "pH and buffer systems".

Phosphate ions ($H_2PO_4^-$ and HPO_4^{2-})

Phosphates are known corrosion inhibitors, and are widely used in protective coatings [74], so that it would be expected that the phosphates in blood plasma would tend to inhibit the corrosion of Mg. The phosphate ions $H_2PO_4^-$ and HPO_4^{2-} are often considered together and discussed simultaneously. In this review, when referring to both species, the term "phosphates" is used.

Xin *et al.* [59] found that HPO_4^{2-} moderately decreased the corrosion rate of AZ91, and delayed the onset of localised corrosion. This result was supported by Ma *et al.* [67] who found that a combination of both phosphate ions slightly retarded the corrosion of CP-Mg and promoted the formation of a magnesium phosphate (Mg-P) precipitation layer on the surface of the samples.

These results help to explain the substantial decrease in corrosion rates shown in Fig. 2 between solutions 2 and 3 [51]. Phosphates are a notable addition to the system in solution 3 (a HBSS), as are sulphates and a number of cations. EDX and XPS analysis of corrosion products in this and other HBSS suggests that phosphates have an influence on the corrosion product layer (usually in combination with Mg^{2+} and/or Ca^{2+}) [75]. This is significant as the (Mg, Ca)-P corrosion layers have been identified in *in vivo* studies [50]. Yamamoto *et al.* [76] considered the solubility limits of phosphate ions in EBSS to determine that Ca-P precipitation should be preferential in this solution at higher pH or when other nucleation opportunities present. These findings were supported by EDX analysis, which found Ca and P incorporated into the corrosion product layer.

These results show that phosphates are an integral component to the biocorrosion system both *in vitro* and *in vivo*. Their incorporation into the corrosion product layers to form (Mg, Ca)-P influences the corrosion mechanism of the Mg alloys and as such, their inclusion in SBFs is essential for an accurate biocorrosion assessment.

Sulphate ions (SO_4^{2-})

Xin *et al.* [59] and Ma *et al.* [67] found that SO_4^{2-} had slight effect in promoting the dissolution of Mg. Moreover, these effects appear to be much less than those of other anions. Agha *et al.* [71] found the addition of $MgSO_4$ did not influence the corrosion rate of HP-Mg samples, although it was claimed that the addition of $MgSO_4$ did have a homogenizing influence on the corrosion product layer [71]. These results indicate that, while sulphates are an important component in most SBFs and should continue to be included, their influence is minimal when compared to other anions present in blood plasma.

Cations

In addition to anions, human bodily fluids also contain a range of cations including magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^+) and potassium (K^+) [61]. The solubility of Mg^{2+} and Ca^{2+} is limited, and it is easy for these to be precipitated from solution. In fact, drinking water is typically deliberately supersaturated in these ions to provide protection for the iron pipes used in the reticulation of drinking water [74]. Thus, it would be expected that these ions would likely become part of the corrosion products and would tend to be mildly protective. In contrast, most salts of K^+ and Na^+ are highly soluble, and these two ions would be expected to have little direct involvement in the corrosion of Mg.

However, unlike anions, the influence of these cations on the biocorrosion of Mg alloys is less well studied. Typically in order to study the influence of an ion on biocorrosion, an ionic salt is added or substituted into the SBF. This results in a change in the concentration of both the anion and the cation. As an example, in the study of Xin *et al.* [59] each increase in anion content was accompanied by an increase in either K^+ or Na^+. As anions and cations are intrinsically linked, it becomes important to have a full understanding of both types of ions in order to decouple the influence that each may be having.

Magnesium ions (Mg^{2+})

The concentration of Mg^{2+} in solution would be expected to influence the precipitation and stability of Mg containing precipitates, due to the common ion effect. Following from this, a decrease in corrosion would be anticipated.

Ning *et al.* [77] considered the influence of four cations (Mg^{2+}, Ca^{2+}, Na^+, K^+) on the corrosion of AZ31B. The influence of the cations in decreasing the corrosion of this alloy was ranked as follows (from most retardation to least):

$$Mg^{2+} > Ca^{2+} > Na^+ > K^+.$$

However, this result was again a binary comparison of presence and absence. Agha *et al.* [71] found that the addition of $MgSO_4$ had a minimal influence on the biocorrosion of HP-Mg in a HBSS. As SO_4^{2-} has been found to slightly increase the dissolution of Mg previously

[59,67], it is reasonable to assume that Mg^{2+} was slightly retarding the corrosion in this case, countering the effect of the addition of SO_4^{2-}. These results suggest that, when a solution is devoid of Mg^{2+}, the addition of these ions has a moderate to substantial influence on decreasing the corrosion rates. However, if a solution already contains a level of Mg^{2+}, which approximates that of blood plasma, then further Mg^{2+} addition to the solution has minimal effect on the corrosion. It is also worth noting that even if Mg^{2+} ions are not present initially, they are always introduced into the system through the corrosion of Mg until the solubility limit of $Mg(OH)_2$ is reached, typically in a matter of hours.

Calcium ions (Ca^{2+})

As previously discussed, the incorporation of (Mg, Ca)-P compounds into the corrosion layer has been found to slow the corrosion rate of Mg alloys [67,75]. These compounds are also essential to forming a more physiologically accurate corrosion layer [50]. Additionally, as with $MgCO_3$, the precipitation of $CaCO_3$ will also inhibit the dissolution and slow the corrosion rate [59]. These results might suggest that addition (or removal) of Ca^{2+} from the *in vitro* system would strongly influence the corrosion rate of Mg alloys.

This hypothesis is supported by the work of Ning *et al.* [77] wherein the presence of Ca^{2+} was found to be secondary only to Mg^{2+} when decreasing the corrosion rate of AZ31B samples. This result was also supported by Agha *et al.* [71] wherein the addition of $CaCl_2$ was associated with a moderate decrease in the corrosion rate, as presented in Fig. 4. This result might be initially somewhat surprising considering the literature strongly suggests that Cl^- promote the corrosion of Mg alloys, as discussed in the section on chloride ions. This result suggests that Ca^{2+} has a greater influence retarding the corrosion of Mg alloys than twice the concentration of Cl^- when the base solution does not originally contain Ca^{2+}. This further highlights the important role that cations play in the corrosion mechanism, and the necessity to include them during analysis.

Ca^{2+} also forms an important component of a physiologically relevant corrosion product layer. Marco and Myrissa *et al.* [50] compared the biocorrosion of several Mg alloys in a variety of *in vitro* solutions against *in vivo* conditions. The corrosion layer formed in the HBSS (14175) used in this study was found not to match the corrosion layer of samples *in vivo*, largely due to a lack of Ca containing species. This result is surprising as these species have been identified in previous biocorrosion studies which have used HBSS (H1387) [75]. The HBSS (14175) used in this study is compared to another HBSS (H1387) commonly used in biocorrosion studies (both are presented in Table 1). The obvious difference between these solutions is the absence of Mg^{2+} and Ca^{2+} in the HBSS (14175) used in this study [50].

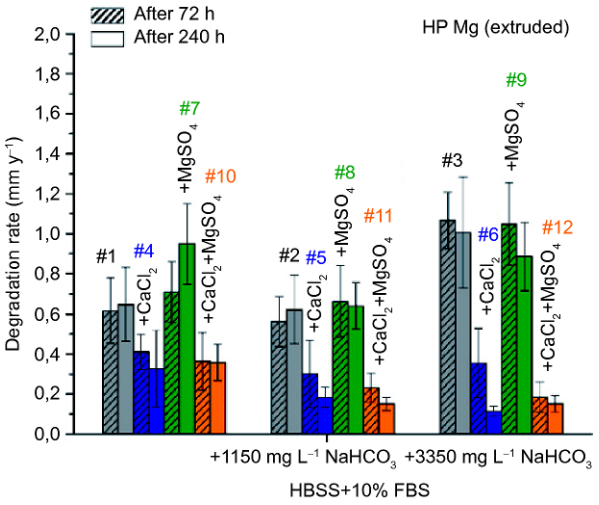

Figure 4 Pure Mg tested in HBSS with a variety of addition elements. Reprinted with permission from Ref. [71], Copyright 2016, Elsevier.

These results suggest that, similar to phosphates, Ca^{2+}, are essential in forming a physiologically accurate corrosion product layer, and therefore may influence the corrosion mechanisms both *in vitro* and *in vivo*.

Sodium (Na^+) and potassium (K^+) ions

Ning *et al.* [77] found that both Na^+ and K^+ have minimal influence on the corrosion of Mg, with K^+ slightly less significant than Na^+. The elemental mapping of the corrosion layer in Marco and Myrissa *et al.* [50] revealed trace amounts of Na^+ and K^+ in the corrosion product layer following *in vitro* immersion with a slightly higher levels of incorporation presenting after *in vivo* corrosion. The incorporation into the corrosion product layer *in vivo* suggests these ions may play a role in the surface chemistry *in vivo*, but to a much lesser role than other elements such as O, Mg, Ca and P. Their inclusion in *in vitro* immersion solutions should not be overlooked, but appears to be less sensitive than other elements studied.

ORGANIC COMPONENTS

In addition to inorganic ions, bodily fluids also contain a number of organic components such as proteins, amino acids, vitamins, macrophages and red blood cells. The addition of these components to the *in vitro* system in-

creases the complexity of solutions that attempt to replicate the *in vivo* corrosion environment. While organic components are undoubtedly an essential component of many bodily fluids, their exact effect on the biocorrosion of Mg alloys is a matter of some debate. Organic components have been applied inconsistently between studies, hindering accurate comparison of corrosion data. These components are largely discussed in relation to blood and blood plasma, as many simulated body fluids are utilised specifically because they have a similar ionic content to that of human blood plasma [24]. Plasma constitutes the majority of human blood by volume, with the remainder coming primarily from red blood cells [78].

Proteins

Proteins, in particular albumin, are an essential competent of human plasma [79] and are the majority component of plasma aside from water, as shown in Table 1. A number of studies have considered the influence proteins have on the biocorrosion of Mg alloys. These studies have often focused on albumin as these are the most abundant protein species in blood plasma [80,81]. In general, proteins are incorporated into an *in vitro* system by the mixing of foetal bovine serum (FBS) or bovine serum albumin (BSA) into a SBF [82,83].

Liu *et al.* [83] conducted an electrochemical study of AZ91 and found the addition of BSA decreased the open circuit potential (OCP) of the sample, but the addition of 1 g L^{-1} protein had minimal effect on the corrosion rate. A further study by Liu *et al.* [81] on Mg-1.5Ca in NaCl solutions containing albumin found that the presence of proteins increased the charge transfer resistance of the corrosion layer on the surface of the Mg sample, and substantially decreased the corrosion rate as measured by hydrogen evolution. Yamamoto *et al.* [76] also found that the addition of protein moderately retarded the corrosion of pure Mg. Zhang *et al.* [84] found the addition of 10% protein to a SBF slightly decreased the corrosion rate of a Mg-Nd-Zn-Zr alloy, while the addition of 1% HUVEC (human umbilical vein endothelial cells) slightly increased it. SEM analysis indicated C and N peaks, and appeared to indicate incorporation of proteins into the corrosion product layer [84]. The proposed mechanism was that the divalent Mg ions interacted with the proteins to form a covering layering, and thereby decreased the corrosion rate [83].

However, this mechanism has not been supported by further studies. Törne *et al.* [85] investigated the corrosion of CP-Mg in a variety of SBFs and buffering systems, as well as citrated whole blood and blood plasma. The corrosion rate in CO_2-buffered SBF was substantially lower than in either blood or in blood plasma (by a factor of 10). This suggests that the inclusions of proteins increased the corrosion rate, rather than decreased it. Additionally, in a previous study by the same authors, biological components were found to be incorporated in the corrosion products of Zn samples [86]. However, this finding was not replicated during the follow up similar study on Mg, and neither EDX nor FTIR found evidence of organic materials incorporated into the corrosion layer on the Mg samples [85].

Further studies have concluded that the addition of proteins to SBFs increased as opposed to decreased the corrosion rates. Gray-Munro and Strong [87] explored the $Mg(OH)_2$-solution interface and found that the presence of proteins (i) encouraged the dissolution of the partially protective $Mg(OH)_2$ corrosion layer and (ii) inhibited the deposition of Ca-P on the sample surface. Both of these surface layers tend to slow the biocorrosion of Mg alloys, and their removal or absence would be assumed to increase the corrosion rate of Mg samples. This analysis has been supported by the findings of Walker *et al.* [48] where a substantial increase in corrosion rate was measured when proteins were incorporated into the SBF for all six Mg alloys tested, as presented in Table 4. However, this increase in corrosion rate did not correspond to a better match between *in vitro* and *in vivo* corrosion rates. In fact, the comparison was actually substantially worse in the solution which contained proteins compared to the simpler SBF used (EBSS). A substantial increase in corrosion was also observed in a study by Kirkland *et al.* [72] when 40 g L^{-1} of BSA was added to MEM, as presented in Fig. 3. The increasing corrosion rate indicates that proteins do influence the biocorrosion of Mg alloys. However, the poor agreement to the *in vivo* results presented in the same study suggests the *in vivo* corrosion environment may not be fully characterised by blood plasma, and additionally factors may play a role which is not captured *in vitro*.

A potential explanation of the disparity between these studies may come from the concentration of proteins tested. Table 5 presents the composition of the solutions used by Yamamoto [76]. In this study, it was claimed that E-MEM+FBS contained approximately half the wt% proteins compared to blood plasma [76]. However, this does not appear to be the case, as the protein concentrations presented in Table 5 suggest that E-MEM +FBS contained less than 10% of the proteins present in blood Plasma. In the initial study by Liu *et al.* [83] proteins were added to a maximum of 1 g L^{-1}, again a small

Table 4 Corrosion rates (mm y^{-1}) measured from mass loss after 7 days. EBSS contains similar ionic content to blood. MEM is similar but also includes amino acids and vitamins. MEM+Protein includes 40 g L^{-1} protein. The *in vivo* model was subcutaneous implantation into male Lewis rats [48].

Alloy	EBSS	MEM	MEM+Protein	*In vivo*
HP-Mg	0.57	0.73	2.19	0.39
AZ31	0.79	1.29	1.94	0.34
Mg-0.8Ca	0.57	0.94	2.81	0.31
Mg-1Zn	0.51	0.96	1.72	0.38
Mg-1Mn	0.76	0.86	2.77	0.30
Mg-1.34Ca-3Zn	1.62	4.72	3.29	0.79

fraction of the levels present in blood plasma. In stark comparison, Walker *et al.* [48] and Kirkland *et al.* [72] incorporated 40 g L^{-1} of proteins, achieving close to physiological levels, and much higher corrosion rates than *in vivo*. Additionally, the pH was controlled in this study, although there is concern over the volume of solution used. Walker *et al.* [48] immersed samples in 30 mL of solution, approximately half of which was changed daily to mimic normal kidney function. This method is discussed in more detail in Section "Ratio of solution volume to sample area", and may be worthy of critical analysis. Kirkland *et al.* [72] used a ratio of 20 mL of solution per 1 cm^2 of sample surface area, which is below the desired level, also discussed in Section "Ratio of solution volume to sample area" and in another work by the same group [58].

Regardless, the results of Walker *et al.* [48], presented in Table 4, suggest the inclusion of proteins (as well as amino acids and vitamins, covered in the next section) do substantially increase the corrosion rate of Mg alloys, but this change does not translate to better predictions of *in vivo* corrosion. This result creates quite a conundrum. The ultimate goal of *in vitro* testing is to accurately and consistently mimic *in vivo* corrosion as closely as possible. As stated previously, the best way to achieve this is to recreate the *in vivo* corrosion mechanisms *in vitro*. From the data presented above it is clear that proteins (as well as other organic components) do interact or influence the Mg corrosion mechanisms. However, their inclusion in the *in vitro* environment produces less comparable corrosion rates compared to *in vivo*. It is clear that this topic requires significant further study to begin answering these questions.

Finally, it is worth noting that the findings of Walker *et al.* [48], as well as those of Gu *et al.* [82], suggest that the influence of proteins on biocorrosion rates may be alloy dependent. The results of Walker *et al.* [48], presented in Table 4, show the corrosion rate increases for all the alloys when comparing MEM and MEM + protein, except for Mg-1.34Ca-3Zn which presented in slower corrosion rate when proteins were included, although these corrosion rates were still greater than those in EBSS. This disparity between alloys was also seen in Gu *et al.* [82] where the corrosion of Mg-Ca, AZ31 and AZ91 was studied *via* electrochemical techniques such as OCP and polarization resistance in HBSS, DMEM and DMEM +10% FBS. Mg-Ca corroded moderately faster in the presence of proteins, whereas AZ91 corroded moderately

Table 5 Composition of solutions used by Yamamato *et al.* [76] compared to blood plasma

	Plasma	NaCl	NaCl+HEPES	NaCl+$NaHCO_3$	Earle (+)	E-MEM	E-MEM+PBS
Na^+ (mmol L^{-1})	142	125	125	151	151	151	151
K^+ (mmol L^{-1})	5	–	–	–	5.37	5.37	5.37
Ca^{2+} (mmol L^{-1})	2.5	–	–	–	1.8	1.8	1.8
Mg^{2+} (mmol L^{-1})	1.5	–	–	–	0.811	0.811	0.81
Cl^- (mmol L^{-1})	103	125	125	125	125	125	125
HCO_3^- (mmol L^{-1})	27	–	–	26.2	26.2	26.2	26.2
HPO_4^{2-} (mmol L^{-1})	1	–	–	–	0.897	0.897	0.9
SO_4^{2-} (mmol L^{-1})	0.5	–	–	–	0.811	0.811	0.81
Amino acids (mg L^{-1})	nd	–	–	–	–	0.86	0.86
Dex/Glu (g L^{-1})	nd	–	–	–	–1	1	1.13
Proteins (g L^{-1})	63–80	–	–	–	–	–	4.3
HEPES (mmol L^{-1})	–	–	10	–	–	–	–
Phenol red (g L^{-1})	–	0.1	0.1	0.1	0.1	0.1	0.1

nd: no data available; Dex: dextran; Glu: glucose.

more slowly following the addition of FBS. AZ31 samples corroded moderately faster in the presence of proteins but only for the first 3 days of the test, whereafter the trend reversed and the solution containing protein corroded moderately more slowly. However, it should be noted that these tests were conducted after 7 days and while the initial pH was correct (~7.4), there is no indication if pH control or a buffer system was employed which suggests that when these measurements were taken the pH was likely far from physiologically accurate. The influence of solution pH on corrosion and corrosion rates is detailed in Section "pH and buffer systems", however it can be simply stated that there exists a negative correlation between solution pH and corrosion rate (i.e. at high pH the corrosion rate decreases). This lack of pH control limits the efficacy of the data collected.

However, the results of Walker *et al.* [48] and Gu *et al.* [82] do suggest that Mg alloys may react different to the presence of proteins in SBF. This was also supported by the work of Johnston *et al.* [88] where the effect of proteins was tested on CP-Mg and Mg-Y samples in DMEM and DMEM +10% FBS. Samples were tested in two conditions: (i) polished, and (ii) unpolished (i.e., with an oxide layer on the samples surface). The addition of FBS appeared to have a minimal effect on both CP-Mg samples (polished and unpolished). However, FBS was found to substantially slow the corrosion of unpolished Mg-Y samples, while substantially increasing that of the polished Mg-Y. This suggests that the influence of proteins may not only be variable with regards to alloy composition, but may also be dependent on surface condition or the presence (or composition) of an oxide film. However, as with the study of Gu *et al.* [82], there was inadequate pH control in the study of Johnson *et al.* [88] which lead to rapid rise in solution pH. As previously stated, this has a significant, negative influence on the accuracy of the results. Nonetheless, these results suggest that the interaction between Mg alloys and proteins may be more complex than previously thought.

In summary, it is noted that while a small amount of proteins may cause the corrosion rate to decrease, when they are included to a level comparable to that of blood plasma the corrosion rate will likely increase substantially. However, this substantially increase has not been associated with an improved comparison to *in vivo* results. As previously stated, these results create something of a conundrum. The goal of *in vitro* biocorrosion testing is to accurately mimic the *in vivo* corrosion mechanism and therefore the corrosion rate. Proteins clearly influence the corrosion mechanism of Mg alloys, and as they are present *in vivo*, warrant consideration. However, their inclusion produces a poorer correlation between *in vitro* and *in vivo* results, when compared to SBFs such as HBSS and EBSS, which do not contain organic components.

It is also apparent from the data presented that the *in vivo* corrosion environment and associated corrosion mechanism has not been fully characterised. A better understanding of how the samples corrode *in vivo* will improve our ability to mimic this *in vitro*. In the interim, it is left to the experiments discretion whether to include or exclude proteins from the *in vitro* solution. However, if they are to be included it is strongly recommended they be present at levels comparable to blood plasma. Additionally, it is recommended that a reference alloy (preferably HP-Mg) and at least one other alloy be studied.

It is also worth noting that further studies should consider the chemistry of the organic components in the SBF, and the stability of such elements in these solutions.

Amino acids, vitamins and other nutrients

Blood is used to transport nutrients such as amino acids, lipids, sugars, and various vitamins, around the body [89]. Amino acids in particular have gathered interest in biocorrosion studies [76], presumably because of their presence in certain SBFs such as MEM as shown in Table 1. These additional components, such as amino acids, are at much lower concentrations in blood compared to proteins [79,89].

Kirkland *et al.* [72] found a slight increase in the corrosion rate between EBSS and MEM for CP-Mg (presented in Fig. 3), where the effective difference between the SBFs is the inclusion of amino acids and vitamins in MEM (as shown in Table 1). Yamamoto and Hiromoto [76] found a substantial increase in corrosion rate between EBSS and E-MEM, which also indicated that the presence of amino acids accelerated the corrosion of CP-Mg. These results are supported by Walker *et al.* [48] who measured a slight to substantial increase (depending on the alloy) in corrosion rate between samples immersed in EBSS and MEM, as shown in Table 4. As these amino acids and vitamins were added simultaneously, it is difficult to decouple the effect they may have individually on the Mg alloys tested. However, MEM had a higher corrosion rate when compared to EBSS for all 6 alloys tested, across almost all of the time points [48]. This result suggests that amino acids and/or vitamins do indeed accelerate the biocorrosion of Mg alloys *in vitro*. However, this increased corrosion rate did not correspond to a better comparison to *in vivo* data. As can be seen from

Table 4, EBSS was more comparable to the presented *in vivo* corrosion rates than either MEM or MEM with protein for all of the alloys tested [48]. As such, it is difficult to reach a conclusion on the addition of these elements. Nevertheless they are undoubtedly important components in blood plasma, and therefore warrant consideration. This result also suggests that our characterisation of the *in vivo* corrosion environment could be improved, and the factors that influence corrosion *in vivo* could be better understood.

Another nutrient that is commonly included in most SBFs, as shown in Table 1, is glucose. Zeng *et al.* [90] and Cui *et al.* [91] studied the influence of glucose on the biocorrosion of CP-Mg and Mg-1.35Ca respectively in 0.9% NaCl. The formation of gluconic acid was found to moderately increase the corrosion rate of both materials in NaCl solutions. However, Zeng *et al.* [90] also showed a slight decrease in corrosion rate with increasing glucose content in HBSS, which was attributed to the increased Ca-P deposition on the samples surface which, as discussed in Sections "Anions" and "Cations", retarded the samples' corrosion. It should be noted that both of these studies lacked adequate control of the solution pH, which is shown in Section "pH and buffer systems" to have a strong influence on Mg biocorrosion. However, this lack of pH control was of much greater concern in a follow-up study by the same group studying a more complex interaction.

Wang *et al.* [92] studied the influence of amino acids, glucose and a combination of the two components in 0.9% NaCl on CP-Mg samples. Individually, glucose and amino acids were found to have a similar influence, slightly decreasing the corrosion rate of CP-Mg. This result is in contrast to the works previously mentioned which found both constituents increased the corrosion rate. However, perhaps the most interesting result from this study was the combined influence of glucose and amino acids [92]. Each of these constituents individually was found to slightly decrease corrosion by itself, but when combined in the solution they were found to substantially increase the corrosion rate. This increase was attributed to a bonding reaction between the glucose and amino acids which tends to retard the precipitation of the partially protective $Mg(OH)_2$ species. However, it is unclear whether this reaction would take place, or have any effect, in more physiologically accurate conditions as this bonding reactions required alkaline conditions. As stated previously, Section "pH and buffer systems" of this report will detail how vital solution pH and buffering is to accurate biocorrosion studies and this study serves as a further reminder of how important this parameter is.

To the authors' knowledge, at time of writing the influence of other nutrients such as lipids on the biocorrosion of Mg alloys has not been discussed or assessed.

Amino acids and/or vitamins appear to increase the rate of biocorrosion for Mg alloys. However, it is unclear at this time if this increased corrosion rate *in vitro* corresponds to a better correlation to *in vivo* results and in fact current results indicate the opposite. In light of this it is difficult to recommend either their inclusion or exclusion to a standard *in vitro* biocorrosion model. However, if they are to be included it is recommended they be included at a physiologically relevant level. As glucose is already a consistent part of most SBFs, the inclusion of glucose is recommended, again at the physiological relevant level (as is currently the case with most SBFs).

Macrophages

Foreign body reactions are a common complication associated with implanted biomaterials and medical devices [93]. The tendency for these reactions to occur is in fact an argument in favour of the use of degradable implant materials, such as Mg, as opposed to permanent implants, which will be in the body longer and therefore run a greater risk of incurring a negative reaction [93,94]. However, temporary implants can nevertheless illicit a negative immune or inflammatory response from the patient's system. As such, understanding how these reactions alter the biocorrosion of Mg implants is an important point of study. Macrophages are a type of white blood cell that are commonly associated with these reactions [93,94]. Macrophages are found in all tissues [95], and are known to secrete reactive oxygen species (ROS) which have been found to increase the corrosion of other metallic biomaterials such as Ti [96].

Zhang *et al.* [97] studied the influence of macrophages on the biocorrosion of Mg–2.1Nd–0.2Zn–0.5Zr (abbreviated as JDBM). Macrophages were found to substantially increase the corrosion rate of this alloy, owning to the deposition of ROS onto the samples' surface. The influence on the biocorrosion was found to be proportional to the density of the macrophages on the surface of the sample and the incubation time of the cells.

Mg implants must be able to provide consistent and reliable support to the patient in their intended application regardless of host response. As such, understanding the influence of foreign body reaction, implant site infection and other negative host reactions on the biocorrosion of Mg implants is a vital step in safeguarding their success. However, as these organic components are

present in an inconsistent manner *in vivo*, they are not recommended for use in generic biocorrosion studies. Instead, it is suggested that they be specifically added to the standardised *in vitro* conditions when their specific influence is being assessed.

Red blood cells

As previously mentioned, after blood plasma, red blood cells are the most abundant component of human blood, making up almost 50% of the bodily fluid [78]. Despite this abundance in the human system, their inclusion into biocorrosion studies has been rare. However, the works that have included red blood cells have found consistent results. Törne *et al.* [85] found minimal difference in the corrosion current densities, and corresponding corrosion rates, of CP-Mg samples immersed in blood plasma (1.0 ± 0.02 mm y^{-1}) and whole blood (1.10 ± 0.12 mm y^{-1}). As the primary difference between blood plasma and whole blood is the inclusion of red blood cells in the latter, this suggests that these cells had a minimal influence on Mg biocorrosion. This result is supoorted by the work of Geis-Gerstorfer *et al.* [98] who compared the corrosion of a range of Mg alloys in both whole blood and PBS. In this study, red blood cells were found to have minimal adhension on the surface of the Mg alloys.

The current results in the literature suggest that red blood cells have a negligible influence on Mg biocorrosion, and thus there is little evidence to justify their inclusion in SBFs used in *in vitro* immersion testing.

Consideration for other bodily fluids

While blood, and more specifically blood plasma, has been the baseline for many studies developing physiologically accurate biocorrosion environments, consideration should also be given to the intended application of the implant. It is advisable for studies, which consider Mg alloys for applications where the primary bodily fluid in contact with the implant would not be blood, to adjust the composition of their immersion solution accordingly.

The work of Lock *et al.* [99] provides a good example of this during their investigation of an Mg-Y alloy stent for use in the urine tract. In this study, artificial urine (AU) was used as an immersion solution. It was also noted in this study that human urine is much more variable in ionic composition between patients than blood or blood plasma [99]. Another application that could be considered is that of a Mg biliary stent, which has recently been investigated *in vivo* [100]. If future *in vitro* tests prove necessary for this application, a simulated bile solution would seem appropriate for *in vitro* biocorrosion testing.

EXPERIMENTAL PARAMETERS

The variation between *in vitro* studies is not limited to the immersion medium. There is considerable variation in how experimental parameters are set and controlled, which can have a substantial influence on Mg biocorrosion. More consistent management of key experimental parameters will reduce variability between *in vitro* biocorrosion tests, and is an essential component of a standardised biocorrosion methodology.

Temperature

Initially, the corrosion tests were conducted at room temperature [42] in simple chloride solutions and were found to not give a good indication of *in vivo* corrosion. One aspect contributing to this mismatch was that the simple nature of these solutions was not appropriate as already discussed. Another aspect was that the temperature was below the human body temperature of 37°C [101]. This is significant as an increase in temperature is associated with increased chemical activity, due to the increase in kinetic energy that is afforded from the increased temperature. This increased activity would be expected to be correlated with a higher corrosion rate, and experimental results have found that is indeed the case with biocorrosion testing.

Kirkland claimed that a change of 17°C would increase the corrosion rate moderately to substantially, depending on the alloy being investigated [102]. This claim was supported in a following publication [103] that indicates a substantial increase in the corrosion rate of CP-Mg, Mg-0.8Ca and Mg-1Zn as the immersion temperature was increased from 20°C to 37°C in buffered HBSS. As such, biocorrosion testing at the physiological accurate temperature of ~37°C is seen to be both reasonable and necessary.

pH and buffer systems

Another consideration for Mg biocorrosion testing is solution pH and the consequent requirement for a buffer system. As shown in Equation (1), Mg corrodes to produce $Mg(OH)_2$ and in NaCl solutions the pH typically rises to ~10.3 within hours of immersion due to the low solubility of $Mg(OH)_2$ [55]. While unbuffered SBFs can reach this high pH value [104], they also have a tendency to plateau at a somewhat lower pH depending on experimental conditions [90,105]. Regardless, this plateau is always much higher than the physiological pH of 7.35–

7.45 and therefore will strongly influence the corrosion and corrosion rates.

Increased solution pH has been found to cause a moderate to substantial decrease in the biocorrosion of Mg alloys [36,106]. The solubility of the corrosion layer decreases with increasing pH, and at elevated pH values $Mg(OH)_2$ is able to provide increasing protectiveness to the samples' surface, lowering the rate of biocorrosion. Therefore, it is essential that solutions be maintained at a physiological pH level of ~7.35–7.45 [107,108].

To maintain the pH at the appropriate value, a buffer system is usually employed. A number of buffer systems have been used including CO_2-HCO_3^- (often called the bicarbonate buffer), HEPES and Tris-HCl [72]. The bicarbonate buffer is easily used because $NaHCO_3$ is an integral part of SBFs such as HBSS, so the addition of CO_2 into the system is all that is required to complete the buffer. This buffer is the same as that in the human respiratory and has been found to be effective in a range of SBFs [51,109]. HEPES has been found to be effective at controlling the pH of solutions, however it also substantially increases the corrosion rate of Mg alloys as presented in Fig. 3, as well as alters the composition of the corrosion layer [72,85]. The Tris-HCl buffer has also been found to substantially increase the biocorrosion rate of CP-Mg, particularly in the first 24 h of immersion [70,110]. Consequently, the bicarbonate buffer is strongly recommended, as it is both the most effective and most physiologically comparable buffer system.

However, the bicarbonate buffer system requires active CO_2 input in order to be effective [109]. When utilising balanced salt solutions as cell cultures, pH is commonly regulated *via* a 5% CO_2 atmosphere. Several studies have attempted to utilise this method in biocorrosion testing, but it has proven ineffective. Witecka *et al.* [49] considered the corrosion of ZM21 in two different conditions (as cast and ECAPed) in three SBFs (HBSS, EBSS and E-MEM+10%FBS) while controlling the pH with a 5% CO_2 atmosphere. The pH of the solutions throughout the first 24 h of the tests is presented in Fig. 5. It was noted that the pH of the HBSS and EBSS quickly rose above physiological pH in this test, while the E-MEM+10%FBS was maintained at approximately the ideal pH. From this result it is clear that passive pH control (e.g., a 5% CO_2 atmosphere) is not sufficient for HBSS or EBSS. This result was used as a rationale for using E-MEM+10%FBS instead of HBSS or EBSS in future studies. However active control, where CO_2 is fed as needed into the solution directly, has shown to be effective in balanced salt solutions such as HBSS and EBSS [24,36,37,51,109]. A specific

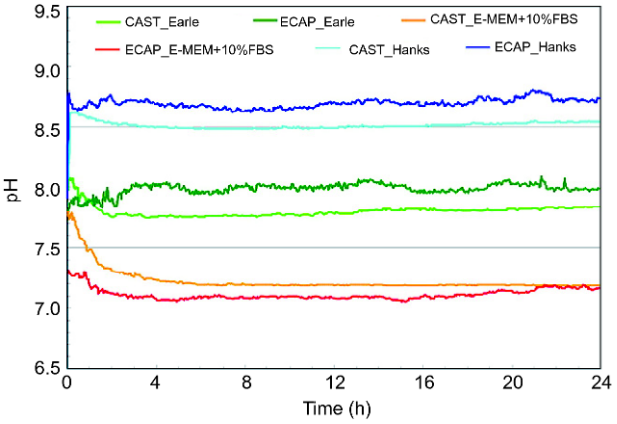

Figure 5 pH of solutions (HBSS, EBSS and E-MEM+10%FBS) over the first 24 h of testing in a 5% CO_2 atmosphere. Reprinted with permission from Ref. [49], Copyright 2016, Elsevier.

partial pressure of CO_2 has been discussed [75], however a simpler method utilising a pH controller to regulate the CO_2 release has also proven effective [36,111].

The conclusions drawn from Witecka *et al.* [49] are a good example of how seemingly small changes in the experimental procedure can significantly impact the results and conclusions generated. The assertion that E-MEM+10%FBS is the most suitable solution for future studies was the conclusion drawn from the data gathered. However, it is impossible to determine if E-MEM+10% FBS is actually a better solution than HBSS or EBSS, or if it is simply a factor of more favourable pH control. If the pH of the other SBFs was controlled more actively, and maintained within the physiological range, a very different conclusion may have been drawn.

In summary, utilising active pH control with a CO_2-bicarbonate buffer system (where CO_2 is bubbled directly into the solution) is recommended to maintain the solution pH within a physiological range of 7.35–7.45 and is essential to accurate biocorrosion assessment *in vitro*.

Ratio of solution volume to sample area

Equation (1) indicates that the corrosion of Mg alloys is associated with an increase in $Mg(OH)_2$, which is partially dissociated into Mg^{2+} and OH^-. As discussed in Sections "Cations" and "pH and buffer systems" respectively, Mg^{2+} and OH^- are expected to influence the corrosion of Mg alloys during *in vitro* biocorrosion testing. A basic principle of corrosion testing is to use a sufficiently large volume of solution so that the composition of the solution does not change significantly during the corrosion test, or to change the solution periodically as the solution becomes contaminated.

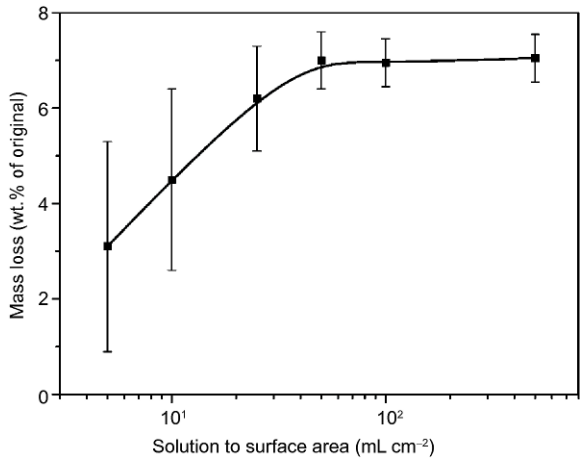

Figure 6 Influence of the ratio of solution volume to sample surface area on the corrosion of pure Mg in Hanks' solution. Reprinted with permission from Ref. [58], Copyright 2012, Elsevier.

In light of this, a number of techniques have been considered in order to minimise the impact the samples' corrosion has on the immersion solution. The most conventional of which is ensuring a sufficiently large ratio of solution volume to sample surface area. The influence of this ratio was considered by Kirkland *et al.* [58], as well as Yang and Zhang [104] on the biocorrosion of Mg. Both studies found that at low levels, this ratio was very impactful on the corrosion of the samples, and this influence tapers out at large ratios, as depicted in Fig. 6. By ensuring a sufficiently large solution volume, when compared to the size of the sample, pressure is taken off the buffer system, as the corrosion of the sample will have a smaller net influence on the pH of the solution.

A different approach to determine the desired volume of solution has been to scale the experimental solution volume to an amount of body plasma, and then daily replace an equivalent amount of solution as could reasonably be processed by the kidneys [48,76]. In these studies the amount of blood plasma in a body is taken to be within 2.75–3.0 L [112,113], and the amount of urine excreted to be 1.4–1.5 L [113,114]. Therefore samples are submerged in 2.75–3.0 L, and 1.4–1.5 L is removed and replaced daily with fresh solution [48,76]. However, this method fails to scale the size of the sample, and therein the amount of corrosion occurring. As shown in Fig. 5, when such a small volume of solution is used, the system can quickly become overwhelmed.

Replenishing part of the solution each day to avoid (i) build-up of Mg^{2+}, (ii) deletion of other important ions, and (iii) increase of solution pH, is another potential approach. This technique may prove particularly effective for long term testing (> 7 d), or for samples with high corrosion rates. However, this approach involves the necessity to disturb the corrosion experiment daily. Moreover, relying solely on this technique for pH control when using a small body of fluid is not recommended, as solution pH can change quite rapidly, as shown in Fig. 5 where only 110 mL of solution was used [49]. Therefore it is recommended that a reasonable ratio of solution volume to sample area (equal to or greater than 1 cm^2: 50 mL) is employed, with solution replacement used in tandem for long term studies. We have typically used ~500 mL of solution per specimen.

Solution flow rate

The majority of *in vitro* testing is conducted in static immersion conditions. However, several studies have considered the influence of solution flow rate on the corrosion rate and morphology of Mg samples. The solution flow rate can affect corrosion behaviour in a number of ways such as (i) enhancing mass transfer on the samples' surface, (ii) stirring the solution and preventing build-up of ions or localised pH increases in stagnant solution, (iii) flow induced shear stress (FISS) on the surface of the sample or (iv) solution flow removing the corrosion products from the samples' surface, exposing the surface to the corrosive medium.

Soya *et al.* [115] found the corrosion of AZ31 plates increased by 13% when the flow rate was doubled from 85 to 170 mm s^{-1}. The effects of flow in this test appeared gradual, and as the test was only conducted over a 24 h period, it is possible the difference would have increased or decreased over time.

The authors have previously considered the influence the hydrogen capture apparatus may have on the corrosion of samples, in terms of stagnating solution flow and causing localised changes in the immersion solution [36]. This study compared specimens suspended under hydrogen capture burettes to specimens in mild flow conditions. The mild flow conditions were created with a pump and drain which cycled solution between two containers at a rate of ~85 mL s^{-1}. These may appear like high flow conditions, but it is worth noting that the relatively large pump and drain diameter (~10 mm) resulted in mild flow conditions spread across six samples. These samples in the mild flow conditions had a slightly higher corrosion rate on average than those in stagnant conditions beneath the hydrogen capture burettes.

However, the slightly higher corrosion rate was associated with a lower incidence of localised corrosion. The

change in corrosion morphology was attributed to the mild flow conditions removing some of the corrosion products from the samples surface. Build-up of corrosion products on the surface of samples is known to retard corrosion, but may also create conditions necessary for localised corrosion (such as crevice corrosion) to occur. Thus it is possible that the increased flow rate removed corrosion products from the samples surface, exposing the Mg beneath and thus simultaneously increasing the corrosion rate and inhibiting the environment necessary for certain types of localised corrosion.

Maybe the most detailed analysis of solution flow on Mg corrosion was conducted by Wang *et al.* [60]. In this study Mg-5Zn-0.3Ca plates were studied under a number of flow conditions, and AZ31 stents in stagnant conditions were compared to those under solution flow. One of the novelties of this study was to calculate the FISS created on the sample from the solution flow. These flow rates and associated shear stresses, as well as the average corrosion rate of the Mg-5Zn-0.3Ca plate samples, are presented in Table 6 [60]. It is worth noting that the spread of data was relatively large for all the samples bar in stagnant conditions.

All three studies were in agreement that increasing flow rate, and thereby shear stress, is associated with an increased corrosion rate. However, in addition to an increase in general corrosion, Wang *et al.* [60] also discovered a higher incidence of localised corrosion at high flow rates. This result is in conflict with the authors' previous work, however it is believed this disparity in an artefact of flow conditions [36]. The samples in Wang *et al.* [60] were studied as stent materials and as such were placed directly in the pipe, in order to better mimic services conditions. The solution flow in the authors' previous study [36] was spread across the 6 samples in a large body of solution (~3 L). This indirect solution flow would likely be associated with a much lower shear stress.

This comparison highlights an important consideration for solution flow in *in vitro* tests: expected application. The expected services conditions of the implant are strongly dependant on the implant site and intended application of the device. These factors determine what level of solution flow the implant is subjected to *in vivo*. As an example, a cardiovascular stent must be substantially more resilient to solution flow conditions when compared to an orthopaedic implant.

For generic biocorrosion assessment, where an intended application is not yet determined, a low flow rate is recommended, in order to maintain homogeneity within the solution in terms of pH and composition of the elements discussed in Sections "INORGANIC IONS" and "ORGANIC COMPONENTS". Examples of apparatus, which make use of slight solution flow can be found in the literature [36,111]. For a large body of solution, a flow rate of <100 mm s^{-1} is recommended, and this should be coupled with a large pipe diameter (>8 mm) to minimise the shear stress induced on the samples. Samples should also not be placed in close proximity the pump inlet or pipe outlet, again in order to minimise the FISS influence on the samples corrosion. For an apparatus where a smaller body of solution is used, a lower flow rate (<50 mm s^{-1}) is recommended. This style of testing may also be applicable where the intended application has a low expected flow rate (i.e., orthopaedics).

If the intended application has a high expected flow rate (i.e., a cardiovascular stent) the expected service shear stress should be estimated and the test adjusted to best mimic this. Wang *et al.* [60] tabulated a range of mean wall shear stresses associated with various cardiovascular sites and these are presented in Table 7 [60]. Values such as these should be combined with a shear stress (τ) calculation such as Doriot's equation [116], presented in Equation (4), to better approximate *in vivo* service conditions. This equation factors in variables such as solution shear viscosity (η), solution laminar flow rate (Q), and pipe diameter (D). These shear stress values are of greater

Table 6 Flow rate, associated FISS and approximate average corrosion rate for Mg-5Zn-0.3Ca plates and AZ31 stents in DMEM. Pipe diameters are 3.2 mm and 6.3 mm, respectively [60].

Sample	Flow rate (mm s^{-1})	FISS (Pa)	Corrosion rate (mm y^{-1})
Mg-5Zn-0.3Ca plates	0.0	0.00	~1.2
	10.4	0.07	~1.6
	20.8	0.15	~1.9
	41.6	0.31	~2.2
	83.2	0.62	~2.4
AZ31 Stent	0.0	0.00	~0.4
	57.7	0.05	~1.2

Table 7 Mean wall shear stress in various cardiovascular environments [60]

Cardiovascular site	Shear stress (Pa)
Coronary artery	0.68 ± 0.03
Femoral artery	0.36 ± 0.16
Supraceliac aorta	0.35 ± 0.08
Infraenal aorta	0.13 ± 0.06

concern than the solution flow rate because they better capture the complex fluid dynamics of the system that is being approximated.

$$\tau = \frac{32\eta Q}{\pi D^3} \quad (4)$$

Finally, it should be noted that while most tests, which approximate body fluid flow, have a consistent flow rate, body systems tend to pulsate. This is especially true of the cardiovascular system, where the patient's heart rate will frequently change the rate of solution flow across the device. Saad *et al.* [117] have developed an apparatus which is claimed to mimic the dynamic environment surrounding an orthopaedic implant. This system is yet to be verified with *in vivo* results, but still is a promising step towards models and apparatus which can correctly characterise the influence of physiologically accurate fluid flow on biocorrosion. Presumably a similar apparatus, also employing a peristaltic pump system, could be developed to mimic the cardiovascular system as well.

CONSIDERATION FOR SELECTING AN APPROPRIATE *IN VIVO* MODEL

This review, and indeed most biocorrosion studies, are predicated on matching *in vitro* corrosion and corrosion rates to the service condition. This service condition is usually approximated with an *in vivo* animal model. However, there are factors that should be considered when selecting an appropriate *in vivo* model, and before attempting such a comparison.

If there is an intended application or final product already in mind, this decision is narrowed considerably. The two most commonly considered applications for Mg medical devices are in orthopaedics and cardiovascular surgery [1,16,17]. The selection of appropriate animal models which reflect or match the part of the targeted part of the human system most effectively is a topic which gathers much discussion and research in biomedical materials [118,119]. For orthopaedic applications small animal studies are generally conducted in rodents such as rats or rabbits [18,27,120,121]. For larger animal studies sheep [122] and pigs [123] have been used previously, with dogs and goats also established in the field [119]. In cardiovascular applications rats are also used as a small animal model [124–126], with pigs generally favoured once large animal trials are required [127].

However, for many biocorrosion studies the end product is not yet determined, or perhaps there are multiple end products being considered. This creates a problem because it would be expected that the different environments within the body would change the corrosion behaviour of the samples. For example the likelihood of flow shear stress related corrosion, as discussed in Section "Solution flow rate", would be expected to be quite different for a sample implanted in an artery compared to an intramedullary nail implanted into bone.

Table 8 Comparison of the corrosion rate of RS66 samples, as measured from volume loss, after implantation into various tissues of female New Zealand white rabbits [128]

	1 week	2 weeks	3 weeks	4 weeks	8 weeks
Bone (mm y^{-1})	1.65	2.65	2.45	3.82	5.45
Subcutaneous (mm y^{-1})	5.70	6.58	6.30	5.29	3.46
Intramuscular (mm y^{-1})	5.34	3.89	5.67	4.18	3.04

Willbold *et al.* [128] explored the influence of implant site on the corrosion of RS66 samples in New Zealand white rabbits. Samples were implanted (i) into the medial femur (bone), (ii) under the skin of the back (subcutaneous) and (iii) into the lumbar musculature (intramuscular). The corrosion rates for various time points are presented in Table 8 [128]. After 1 week, the corrosion rates of the subcutaneous and intramuscular samples were quite similar (5.70 and 5.34 mm y^{-1}, respectively), and both substantially higher than the samples implanted into bone (1.65 mm y^{-1}). However, there were moderate-substantial changes in these corrosion rates for most of the time points measured, culminating in the final time point of 8 weeks where this trend was reversed with bone having the highest corrosion rate (5.45 mm y^{-1}), and subcutaneous and intramuscular were again quite similar (3.46 and 3.04 mm y^{-1}, respectively).

These results suggest the corrosion rate may not be intrinsically linked to the implant site, and indeed this was the conclusion of Sanchez *et al.* [32]. During a comparative review of *in vitro* and *in vivo* corrosion rates, it was concluded that "when comparing the corrosion rates for one specific material, the rates are not very different, or at least the differences cannot be attributed to the implant location" [32]. Verifying this is challenging, as there are similar obstacles present when comparing *in vivo* data as with *in vitro* in terms of variable experimental methodologies. Factors such as (i) the animal model, (ii) alloy and process history, (iii) sample geometry and (iv) time points the corrosion was evaluated, can all influence the corrosion rate measured and inhibit direct comparisons. However, there are some few studies which have enough similarities to justify a direct comparison. Two such studies are in Table 9 [126,129]. These studies considered CP-Mg wire in both an orthopaedics and cardiovascular location, and while the time

Table 9 The corrosion of CP-Mg measured *in vivo*, comparing the corrosion rate of an orthopaedic and cardiovascular model

Intended application	Animal model	Implant site	Sample description	Time points	Corrosion rate (mm y^{-1})	Ref.
Orthopaedics	Male Wister rats	Left femora	0.4 mm 99.9% Mg wire	42 days	~0.6	[129]
Cardiovascular	Male Sprague-Dawley rats	Abdominal aorta	0.25 mm 99.9% Mg wire	5–30 days	~0.3–0.6	[126]

points of analysis were not identical, they were comparable. This data indicates that the difference in corrosion rate between these two different applications may not be as great as was initially thought. This is a single point of comparison and likely does not complete the picture but it does suggest that further direct comparisons and studies are warranted.

A potential explanation for the unexpected similarity between the orthopaedic and cardiovascular corrosion rates in this case may be tissue encapsulation. In the study of Abidin *et al.* [24] the Mg samples were all completely encapsulated in fibrous tissue following 1 or 2 months of intramuscular implantation in male Wistar rats. This encapsulation was suggested as the reason the corrosion rate of the fast corroding AZ91 samples was substantially decreased (by a factor of 10) *in vivo*. Fibrous encapsulation was also present during the subcutaneous implantation of fine WE43B wires in adult female mice [130], and was also suggested as the reason for the slower *in vivo* corrosion rates. This type of soft tissue reaction is also present in the exposed parts of orthopaedic implants. Erdmann *et al.* [131] compared orthopaedic screws made from Mg-0.8Ca and 316L stainless steel implanted into the tibiae of New Zealand white rabbits. Both implants were tolerated well, and had similar inflammatory reactions. There was also some degree of tissue encapsulation on the head of the screws, which was in contact with the cranial tibial muscle. A similar result was observed by Willbold *et al.* [122] following implantation of AZ31 screws into the hip bone of mature female sheep. Myrissa *et al.* [132] implanted Mg pins transcortically into the femoral bone of male Sprague-Dawley rats. No tissue reaction was recorded for the pure Mg or Mg-2Ag pins. However, fibrous and bone tissue encapsulation was reported in some of the Mg-10Gd pins, which corroded substantially faster than the other alloys. It should be noted that while an excessive amount of fibrous encapsulation may indicate a negative host response, the formation of some amount of fibrous tissue may be considered normal. Well integrated, biocompatible implants can still induce a fibrous tissue reaction [133].

Additionally, a similar phenomenon has also been reported for stents where a layer of endothelium coated the struts of a Mg stent implanted into the abdominal aorta of New Zealand white rabbits [134]. Tissue encapsulation appears to be an expected result of contact with blood [98,135]. As suggested by Abidin *et al.* [24], this encapsulation may be slowing the corrosion rate *in vivo*, helping to explain why *in vitro* corrosion rates often overestimate those seen *in vivo* [32]. This would also help to explain the good agreement between the different applications presented in Table 9.

A question that is raised by this hypothesis is the nature of SBFs and what bodily fluid should be the basis for *in vitro* biocorrosion tests. Currently blood plasma has been used as it was assumed most applications would have the implant in contact with this fluid. However, as discussed in Section "Consideration for other bodily fluids", if the expected service condition encounters a different bodily fluid it is recommended that this fluid be used as the basis for the SBFs during corrosion assessment. To the author's knowledge, the nature of the extracellular fluid within the encapsulated tissues has not been considered by any biocorrosion studies. There is limited data available on this fluid, however it is known that the protein content has been measured as only 50%–60% of that of blood plasma [136]. This may help to explain the poor agreement found by Walker *et al.* [48] when proteins, amino acids and vitamins were added to their SBF. A full exploration of the nature of the tissue encapsulation and how it affects the corrosion mechanism and associated corrosion rates would undoubtedly improve our ability to mimic *in vivo* corrosion *in vitro*.

Ultimately, an *in vivo* model should match the expected in service condition as closely as practicable. However, it may well be the case that there is not yet a planned service condition, or the alloy(s) are being investigated for multiple applications. In this case, the current state of the literature appears to support simpler *in vivo* models such as subcutaneous or intramuscular implantation. More complex *in vivo* models (such as implantation into the bones or vascular system) require greater expertise and therefore have a higher cost. Additionally, the ethical burden for this kind of surgery is likely to be higher. As

Table 10 Recommended components (and concentrations) that SBFs should contain in future *in vitro* biocorrosion studies

Component	Recommended concentration	Rationale	Refs.
Cl^-	Required (100–150 mmol L^{-1})	Cl^- change the corrosion morphology of the specimen by facilitating micro-galvanic corrosion (and therein localised corrosion), as well as influencing the solubility of the corrosion product layer. However, their influence is lessened in complex solutions. Therefore the use of SBFs which have slightly higher Cl^- content (between 20–50 mg greater than that of blood plasma) are still believed to be applicable.	[51,59,67]
HCO_3^-	Required (4–30 mmol L^{-1})	HCO_3^- play an important role in the corrosion product layer and are essential in maintaining a physiologically accurate buffer system and pH level. However, their inclusion to physiological levels can prove challenging *in vitro* as the solution may easily become supersaturated and thus excess precipitation may occur. As the corrosion rate and pH levels are both maintained in solutions with lower levels of HCO_3^-, it is recommended that they be included in the solution but it is not mandatory to be present in concentrations similar to blood.	[51,59,71]
$H_2PO_4^-$ and HPO_4^{2-}	Required (0.5–1.0 mmol L^{-1})	Phosphates are key components in a physiologically accurate corrosion product layer as they allow the precipitations (Mg, Ca)-P species.	[24,50,59]
SO_4^{2-}	Recommended (0.4–0.8 mmol L^{-1})	SO_4^{2-} were found to have a minimal effect on Mg corrosion compared to the other anions.	[59,67,71]
Mg^{2+}	Recommended (0.5-1.5 mmol L^{-1})	Mg^{2+} play an important role in the precipitation of most species in the corrosion layer (as would be expected). Their inclusion is recommended. However, unlike most other ions analysed they will be incorporated into the SBF naturally during the corrosion process. As such, any ill effects from their exclusion will likely be corrected quickly as the sample/s corrode.	[71,77]
Ca^{2+}	Required (1.25–2.5 mmol L^{-1})	Ca^{2+} are integral to a physiologically accurate corrosion product layer. Their inclusion (or exclusion) has a significant effect on the corrosion mechanism.	[50,77]
Na^+	Recommended (140–150 mmol L^{-1})	Found to have minimal influence on biocorrosion.	[77]
K^+	Recommended (5–6 mmol L^{-1})	Found to have minimal influence on biocorrosion.	[77]
Proteins	May be included at experimenter's discretion (35–80 g L^{-1})	The inconsistencies within the approaches and results in this area make a recommendation challenging. Some studies report a decrease in corrosion rate when proteins are included (although it should be noted they were present at levels much lower than in the body). In contrast, several studies indicate that, when included to a physiologically relevant level, proteins substantially increase the corrosion rate. However, this increase does not correspond to a better comparison to *in vivo* results and in fact weakens such a comparison. This result creates a conundrum. It is clear that proteins do influence or interact with Mg corrosion and are therefore worthy of study. However, the ultimate goal of *in vitro* tests is accurate prediction of *in vivo* corrosion and the addition of proteins to the *in vitro* system appears to harm this goal. As such, it is difficult to categorically recommend either their addition or exclusion from *in vitro* biocorrosion studies and it is left to the experimenter's discretion. If they are included they should be present in physiologically relevant quantities.	[48,76,81–83,85,87]
Amino acids, vitamins and other nutrients	May be included at experimenter's discretion (quantity will vary depending on nutrient)	The inclusion of amino acids and vitamins appears to increase the corrosion rate, however this was also found to create a less accurate correlation to *in vivo* results than an SBF without these elements. As such, it is difficult to determine if they should be included in future. Therefore, it is left to the experimenter's determination whether to include these elements. However, if they are included, they should be present in physiologically relevant quantities. As glucose is common to many solutions, and at similar concentrations, its inclusion is recommended in the physiologically accurate range.	[48,76]
Macrophages	Only to be included in specialised studies	Found to increase the corrosion rate by depositing reactive oxygen species onto the samples surface. However, as macrophages are associated with an immune response their inclusion is not recommended in a general biocorrosion testing protocol. Instead, they should be added when trying to determine specifically how the alloy/s perform when a negative immune reaction occurs.	[97]
Red blood cells	Not necessary to include	While red blood cells make up almost 50% of blood by volume, current literature suggests they do not influence Mg biocorrosion. As such it is not necessary to include them in biocorrosion testing.	[85,98]

Table 11 Recommended experimental parameters for future *in vitro* biocorrosion studies

Parameter	Recommendation	Rationale	Refs.
Temperature	~37°C	Physiological temperature is essential for accurate characterisation of biocorrosion.	[103]
pH	~7.35–7.45	Physiological pH level, essential for accurate characterisation of biocorrosion.	[36,106]
Buffer	CO_2-bicarbonate	A buffer system is essential to maintain a stable pH level throughout testing. The bicarbonate buffer system has been shown to be more accurate than zwitterion-based buffers. Active control of pH (where CO_2 gas is bubbled directly into the solution) is preferred over passive application (i.e., 5% CO_2 atmosphere) as active control is more effective at maintain pH levels.	[49,51,72,75]
Sample area to solution volume ratio	~50 mL cm^{-2} (minimum)	To minimise the influence of ions released during the immersion test (e.g., Mg^{2+} and OH^-), a high ratio of solution volume to active sample surface area is recommended. This will also ease pressure on the buffer as any change in pH will be smaller in a larger body of solution. Additionally, replenishing solution at a rate similar to that at which the kidneys process blood *in vivo* can be employed. This method is particularly suggested for long term studies to avoid saturation of Mg^{2+} ions.	[58,76]
Flow rate	Application dependant. For generic studies: A large body of solution (>5 L) with a low flow rate (<100 mm s^{-1}) and a large pipe diameter (>8 mm)	In general a slight or mild flow rate is recommended to maintain solution homogeneity. For large apparatus with sufficient solution volume (>5 L) a flow rate of less than 100 mm s^{-1} is recommended with a large pipe diameter (>8 mm) to minimise the shear stress influence on the biocorrosion. This style may also be applicable for low flow rate applications (e.g., orthopaedic). For higher flow rate applications (e.g., cardiovascular stents) the expected service shear stress should be estimated and conditions recreated to best mimic this. Factors that will influence this *in vitro* (as well as *in vivo*) include: solution viscosity, flow rate, and pipe geometry.	[36,60, 116]

ISO and ASTM standards both recommended either subcutaneous or intramuscular implantation for short-term assessment of biomaterials [137–139], and the data gathered from these simpler tests appear as valid as more complex surgeries by and large, it seems this is a reasonable place to begin testing.

CONCLUDING DISCUSSION AND RECOMMENDATIONS

Immersion solution

The variability between immersion solutions has been a point of contention when discussing biocorrosion testing for a number of years. At this point in time, the authors do not believe that the selection of a single "appropriate" solution is possible. However, when each component is analysed carefully and systematically, it is possible to clarify which components are necessary, and at what concentrations. Blood plasma is the most common bodily fluid and thus is an appropriate baseline for *in vitro* biocorrosion testing. However, as discussed in Section "Consideration for other bodily fluids", if an application would involve contact with another fluid primarily, consideration should be given to incorporating the characteristics of this fluid into the testing.

The inclusion and concentrations of components (i.e., inorganic ions and organic elements) can have a varied influence on the biocorrosion of Mg alloys. Certain components can have a large influence on corrosion rate and mechanism, while others have a comparatively small effect. As such, the importance of including each component (and at what concentration) has been summarised in Table 10.

Experimental parameters

Unlike the immersion solution, where there is still work to be done to select an ideal or "gold standard" solution, the selection of appropriate experimental parameters for *in vitro* biocorrosion testing is a more straight forward task. The recommended experimental parameters for use in future studies are summarised in Table 11, with a rationale given for each and a reference to the relevant literature. If these parameters are followed, it is strongly believed that the variability between *in vitro* biocorrosion tests will be greatly reduced. Examples of experimental apparatus, which incorporate many of the parameters outlined in Table 11 can be found in the literature [36,111], and are a good starting point when considering future *in vitro* biocorrosion testing.

Additionally, consideration should be given to a minimum number of samples tested. A minimum of 3 samples per condition is recommended to account for variability

within the alloy or preparation. It is also recommended to characterise not only the corrosion rate but also the corrosion products and morphology. It is recommended that HP-Mg samples be included as a reference. However, including additional alloys is also recommended, as there is evidence that different alloys may vary considerably during biocorrosion testing [50].

Final thoughts and recommendations

The recommendations presented herein are not intended to be prescriptive, and likely will not result in a perfect *in vitro* environment, which mimics *in vivo* conditions. Instead, this review is meant to be a guide for future studies so we can begin to build a standardised approach to biocorrosion testing. Currently progress has been slow as there is much variability between *in vitro* methodologies, which makes comparison between studies increasingly difficult. A standard approach will allow researchers and readers to see exactly what the effect of a certain element or parameter is on the *in vitro* corrosion, free from the concern that the method in question did not consider a certain factor (i.e., didn't control pH). These comparisons are the basis for iterative improvements, and once a standard approach to *in vitro* biocorrosion testing is established, we can begin in earnest the iterative approach to improving it. A selection of studies which are predominately consistent with the recommendations in Tables 10 and 11 are presented in the Supplementary information.

Additionally, as the ultimate goal of *in vitro* testing is to better approximate or mimic the in service corrosion, it would behove us to improve our understanding of the corrosion environment *in vivo*. A full and deep understanding of the *in vivo* corrosion environment is essential to accurate characterisation of biocorrosion, and should include an understanding of the influence of factors such as (i) proteins, (ii) amino acids, (iii) vitamins, and (iv) tissue encapsulation.

Once we have a full and accurate understanding of the *in vivo* corrosion environment we can begin to better mimic it *in vitro*. In the interim, our progress will continue to be slow and studies will continue to be suspect or require repetition until a standard approach to *in vitro* biocorrosion can be established. Following this point, this standard can be iteratively improved to better match *in vivo* corrosion once factors influencing the corrosion mechanisms become better understood.

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Author contributions Johnston S conceived the review topic, reviewed and analysed the relevant literature, and prepared the manuscript. Dargusch M and Atrens A reviewed and revised the manuscript, and made suggestions for additional sections, points of discussion and/or areas of interest which were subsequently included in the final edition.

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Supplementary information Supplementary details are available in the online version of the paper.

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建立一个生物腐蚀研究的标准化方法: 与体内腐蚀相关的体外镁腐蚀影响因素综述

Sean Johnston[1], Matthew Dargusch[2], Andrej Atrens[1*]

摘要 本文通过综述相关文献, 系统地评价了影响体外镁(Mg)腐蚀的因素. 分析了以下因素对镁体外生物腐蚀的影响: (i) 无机离子, 包括阴离子和阳离子, (ii) 有机成分, 如蛋白质、氨基酸和维生素, (iii) 实验参数, 如温度、pH值、缓冲体系和流速. 通过这些归纳分析, 为建立一个体外生物腐蚀测试的标准化方法提供了思考和建议, 并推荐了几种有潜力的模拟体液. 实施实验参数的标准化方法具有显著减少体外生物腐蚀试验差异的潜能, 并有助于建立准确一致模拟体内腐蚀的方法. 然而, 在如何更好地表征体内环境和腐蚀机理上尚存在着知识上的空白. 本文审查了血浆是进行体外腐蚀测试的合适体液这一假设, 并提出在今后研究中需进一步考虑影响体内腐蚀机理的因素, 如样品封埋等.

