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Review Article

Iron and its alloys for Bone Regeneration Scaffolds – A Review

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Abstract. Permanent implants and bone grafts have been used successfully to repair bone defects for a number of years. However, there are significant limitations, for example patients requiring revision surgery for implant removal, inadequate mechanical properties leading to stress-shielding and osteoporosis, as well as restricted bone development, particularly in paediatric patients. As a result, those implants with a more active involvement in the healing process than the original inert implants, were favoured. Biodegradable scaffolds are porous implants which are incorporated into sizeable bone defects in order to support the damaged area while the bone regenerates. In response to bone healing, the structure is expected to degrade at a controlled rate *in vivo*. Following the promising research published in relation to magnesium-based alloys for cardiovascular stents, iron and its alloys have recently been proposed for this application. *In vivo* evidence show that pure iron exhibited an inadequately slow degradation rate. Therefore, research efforts have been focused on accelerating the corrosion rate by implementing various material design strategies. This review presents an overview of notable research work treating the tailoring of corrosion, mechanical and cytotoxic response as well as promising processing methods for the production of iron-based foam structures. To conclude, based on current research, the clinical potential for these materials will be analysed.

Keywords: iron alloys, bone scaffolds, biodegradable implants, biomaterials, biometals

1 Introduction

Statistics released in the year 2016 showed that, of the 7.9 million fractures that are reported annually, 5–10% result in delayed or impaired healing, of which a significant amount are eventually classified as non-union fractures (Buza & Einhorn, 2016). To encourage bone healing in such cases, surgeons may opt to use bone scaffolds.

In the pursuit of the ideal scaffold, researchers have established a set of optimal characteristics for the final product. Firstly, the implant's porous structure must allow for cell proliferation and vascularisation (the mechanisms that govern the reconstruction process). Moreover, it must be able to mechanically-support the surrounding tissue during reconstruction (Bose, Roy & Bandyopadhyay, 2012). Over the past decades, research into metallic biomaterials have focused on the study of relatively inert materials, including austenitic stainless steel, titanium alloys and cobalt-chrome alloys (Eliaz, 2019). However, the ideal scaffold necessitates the use of a material that has the ability to degrade at a controlled rate in order to match the rate of bone regeneration, while releasing by-products that do not interfere with regular metabolic activity. The use of such materials would eliminate the need of a second surgery to remove the implant after it serves its temporary function, while also contributing to healthcare institutions by lessening the financial burden associated with revision surgeries (Bose et al., 2012; Heiden, Kustas et al., 2015).

The most common bone grafting products available on the market are either made of allograft, that is, human bone taken from cadavers, including demineralised bone matrix (DBM), or ceramic products, like β-tricalcium phosphate (American Association of Surgeons, 2010). However, significant research has also been carried out on biodegradable polymeric materials

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like polylactic acid (PLA), polyglycholic acid (PGA), polylactic co-glycholic acid (PLGA) and polycaprolactone (PCL) (Campana et al., 2014, 10). Despite the fact that results are often positive when using these polymeric and ceramic materials, they generally exhibit inadequate strength, toughness or degradation rates (Heiden, Walker & Stanciu, 2015).

The focus has therefore shifted onto biodegradable metals, which have the potential to offer a more satisfactory mechanical performance, especially for orthopaedic applications. Magnesium and its alloys have been by far the most studied metals. However, their lack of resistance to high amounts of chloride ions results in excessively rapid degradation rates. Apart from this, they produce large quantities of hydrogen gas during degradation, which could result in tissue necrosis (Heiden, Walker & Stanciu, 2015). A more recent development has been centred around the study of zinc alloys. When compared to magnesium alloys, these materials have consistently shown a more adequate corrosion rate *in vitro*, while their low melting point greatly facilitates their processing (Katarivas Levy, Goldman & Aghion, 2017). The main limitation of zinc has been its generally poor mechanical strength, however recent studies have shown that alloying with elements like magnesium and strontium could lead to significant improvements in mechanical strength, coupled with promising biocompatibility in various potential implantation sites, as analysed through acute *in vivo* studies in connective, bone and vascular tissues (Zhu et al., 2019). Despite the positive results, prolonged *in vivo* evaluations are required in order to further evaluate the potential of these alloys.

Over the past couple of decades, iron alloys started being considered for biodegradable orthopaedic applications, as this element is also an essential trace element within the body, although the daily exposure limit is set much lower than that of magnesium (Yuen & Ip, 2010). However, as opposed to the latter, iron does not generate harmful gas during the degradation process, and its mechanical properties are more tailored for load-bearing applications (Cheng, Liu, Wu & Zheng, 2013; Heiden, Walker & Stanciu, 2015). The largest issue with respect to these materials was presented to the scientific community in 2001, when Peuster et al. (2001) indicated that pure Fe stents implanted in New Zealand white rabbits underwent very little degradation after one year, highlighting their slow degradation rate. Consequently, studies have since focused on the acceleration of the degradation rate, while simultaneously treating other issues like cytotoxicity, ferromagnetism, and finding appropriate ways to fabricate implants with the qualities mentioned previously.

2 Degradation of Pure Fe

For researchers in the field to be able to accelerate the corrosion rate of pure Fe, it is essential to first understand the degradation process of pure Fe. This has been discussed in a number of publications (Gorejová, Haverová, Oriňaková, Oriňak & Oriňak, 2019; Hermawan, Purnama, Dube, Couet & Mantovani, 2010; Zheng, Gu & Witte, 2014). The process could essentially be split into four sections, as schematically represented in Fig. 1 (Zheng et al., 2014). In the initial step, the electrochemical processes described in Eqs. (1) and (2), take place at anodic and cathodic areas all over the specimen surface. In pure Fe, a potential difference generally exists between the grains and grain boundaries, as displayed in Fig. 1(a).

$$\mathrm{Fe} \longrightarrow \mathrm{Fe}^{2+} + 2\,\mathrm{e}^- \tag{1}$$

$$\mathrm{O_2} + 2\,\mathrm{H_2O} + 4\,\mathrm{e}^- \longrightarrow 4\,\mathrm{OH}^- \tag{2}$$

In the second stage, the Fe ions (represented as M^{n+}) react with the OH^- ions to form iron (II) hydroxide and consequently, ferric hydroxide, or hydrated iron oxides, as in Eqs. (3) and (4). The products could react to form a magnetite layer in contact with the specimen surface, and haematite on top, as is often reported (Hermawan, Purnama et al., 2010; Gorejová et al., 2019).

$$2\,\mathrm{Fe}^{2+} + 4\,\mathrm{OH}^- \longrightarrow 2\,\mathrm{Fe(OH)_2} \tag{3}$$

$$4\,\mathrm{Fe(OH)_2} + \mathrm{O_2} + 2\,\mathrm{H_2O} \longrightarrow 4\,\mathrm{Fe(OH)_3} \tag{4}$$

As corrosion progresses, weak spots in the hydroxide layer result in the formation of pits on the surface. Such regions are acidified through metal cation hydrolysis, as exemplified in Eq. (5), while gradually being depleted of oxygen, thus limiting the cathodic reaction (Cramer & Covino Jr, 2003). The combined effect of these aspects results in an increasingly aggressive environment that leads to further acceleration of the corrosion process.

$$\mathrm{FeCl_2} + \mathrm{H_2O} \longrightarrow \mathrm{FeOH}^+ + 2\,\mathrm{Cl}^- + \mathrm{H}^+ \tag{5}$$

Fig. 1(c) also shows cell attachment as the surface roughens, as well as the formation of calcium phosphate (or hydroxyapatite-like) compounds, fuelled by the rise in pH as a result of the cathodic reaction. This is particularly positive for bone scaffold applications, as the compounds formed are very similar to the main component of bone itself, encouraging strong attachment. Zhang, Chen and Shen (2010) also describe other reactions that may take place at the metal surface; however, these are not central to the aim of this review.

As suggested in Fig. 1(d), chunks of various sizes from the metal may break off after significant corrosion has taken place, contributing to further material loss.

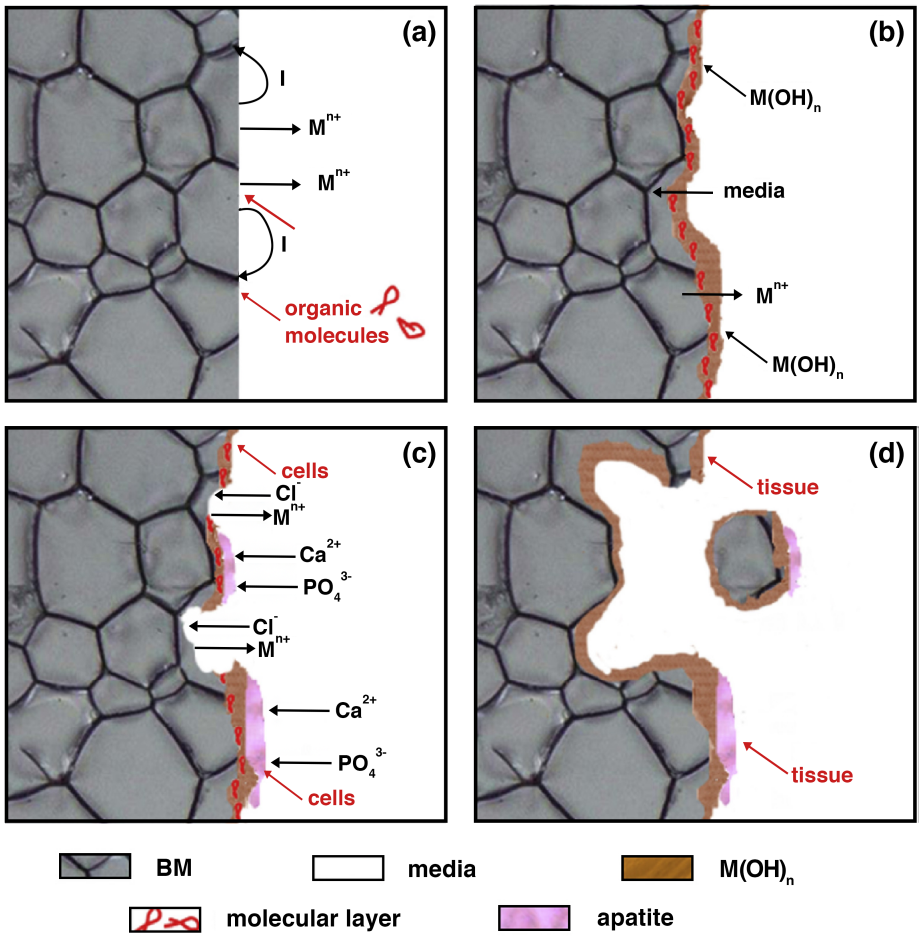

Figure 1: A schematic representation of the degradation process of pure Fe. Reprinted with permission from Elsevier from Zheng, Gu and Witte (2014).

While there have been studies that confirm the described mode of degradation when testing both pure Fe and Fe-alloys *in vitro* (Huang & Zheng, 2016; Huang, Zheng & Han, 2016), both Zhang et al. (2010) and Zhu et al. (2009) have suggested that the dominant degradation mode in pure Fe is uniform corrosion, following analysis of tested samples using Scanning Electron Microscopy (SEM). In fact, Zhu et al. (2009) speculated that a typical vascular stent following the same mean corrosion rate would be completely degraded in 1 month. However, local biological changes and corrosion kinetics *in vivo* are likely to lead to considerably different realities, as discussed in Section 5 of this review.

Another concern that arose when testing pure Fe was related to the corrosion product build-up. When testing pure Fe coupons using static immersion in Hank's solution, Zhang et al. (2010) reported a positive and constant corrosion rate until day 21. The authors attributed the decrease in corrosion rate after day 21 to the accumulation of phosphates on the sample surface. However, that same product did not result in surface passivation when testing the same samples using electrochemical means. On the other hand, in a very similar testing setup, Cheng et al. (2013) reported the build-up of Fe_2O_3 after only 3 days of immersion. It must be noted that this, as well as other Fe corrosion products, are insoluble. Therefore, achieving full degradability without leaving any residuals from the implant is particularly difficult if there are no mechanisms of transport out of the body for the products formed (Cheng et al., 2013). Such products are likely to inhibit further degradation and the remaining implant will stay in the body (Gorejová et al., 2019). However, this should not be an issue, provided that healthy bone is left *in situ* and that the remaining foreign material does not interfere with normal biological activity.

As to the passivating layer, some have also commented on its dependence on the microstructure of the sample. In fact, Obayi et al. (2016) concluded that, in accordance with the study by Ralston, Birbilis and Davies (2010), the corrosion rate actually rises with increase in grain size, contrary to popular belief. As a matter of

fact, the pure Fe samples with the smallest grain size, developed a more dense and stable oxide layer, impeding further release of ions, in the presence of an even slightly alkaline environment (Obayi et al., 2016).

Section 3 will provide an overview of notable works dealing with alloying of pure iron for enhanced degradation rates. Variations in similar corrosion testing procedures make it somewhat difficult to quantitatively compare the performance of different materials, however, general observations could be used to provide an indication of the most promising directions for these innovative materials.

3 Alloying for Improved Performance

3.1 Fe-Mn Alloys

In a series of studies starting in 2007, Hermawan, Dubé and Mantovani (2007) introduced the idea of iron-manganese alloys as biodegradable metals. The value of −1.18 volt corresponds to the electrode potential of Mn not to the electrode potential of the metal after the addition of Mn. The addition of Mn could have the overall effect of lowering the potential of the bulk material from an initial value of −0.44 V, thus increasing its susceptibility to corrosion (Schinhammer, Hänzi, Löffler & Uggowitzer, 2010). Apart from this, manganese could enhance the strength of the material through the formation of an Fe-Mn solid solution, and also allows for the elimination of the ferromagnetic characteristic of the material, through the formation of the antiferromagnetic γ-austenite or ε-martensite Fe-Mn phases. These two phases are the face-centred cubic (FCC) and hexagonal close packed (HCP) variations of the body-centred cubic (BCC) arrangement of pure Fe at room temperature, brought about by the addition of varying amounts of Mn in conjunction with thermal or mechanical processes, and resulting in specific mechanical, magnetic and electrochemical properties. Originally aimed for application as cardiovascular stents, the authors first investigated an alloy which is produced via the powder metallurgy route, wherein metal particles are uniaxially pressed to form powder compacts. These powder compacts are subsequently subjected to high temperatures below the material melting point, in order to stimulate the particles to bind together. This process is more commonly known as conventional sintering. In the case of Hermawan et al. (2007), the coupons were subjected to various cycles of cold-rolling operations to eliminate the resultant porosity in the specimen from the sintering process. The material could then be laser-cut to produce the typical stent geometry. From their initial *in vitro* degradation studies, the Fe-35Mn coupons tested seemed to exhibit a uniform degradation mechanism, with grain boundaries all over the sample surface acting as anodic sites. No pitting was observed after 144 hours of immersion testing. Moreover, the magnetic susceptibility of the same material was even less than that of AISI 316L stainless steel; a material that has been widely implemented in various biomedical applications. In their second publication on the same material, Hermawan, Alamdari, Mantovani and Dubé (2008) also showed that Fe-35Mn exhibited comparable mechanical properties to AISI 316L, as shown in Table 1, placing the alloy ahead of both pure Fe and most Mg-alloys, in terms of mechanical performance.

Table 1: Mechanical properties of Fe35Mn and SS316L (Hermawan, Alamdari, Mantovani & Dubé, 2008).

Material	UTS[a] (MPa)	YS[b] (MPa)	e_{max}[c] (%)
Fe35Mn	550 ± 8	235 ± 8	31 ± 5
SS316L[d]	580 ± 2	250 ± 2	56 ± 2

[a] UTS: Ultimate Tensile Strength;
[b] YS: Yield Strength;
[c] e_{max}: maximum elongation;
[d] SS316L: annealed foil of 0.5 mm thickness.

Following the positive results obtained using Fe-35Mn, Hermawan, Dube and Mantovani (2010) studied the effect of varying the amount of Mn between 20 and 35wt.%. It must be noted that the harder martensitic phases are more stable at lower concentrations (< 27%) of Mn in Fe (Zhang & Cao, 2015). Apart from this, the same martensitic phases could also be induced through plastic deformation (Sato, Ichinose, Hirotsu & Inoue, 1989). In fact, lower concentrations of Mn (20–25wt.%) led to a bi-phase composition of austenite and ε-martensite, while subsequent plastic deformation resulted in a fully martensitic structure. On the other hand, higher amounts of Mn (30–35wt.%) led to an initially austenitic structure, and a bi-phase composition following plastic deformation. The dual phase compositions enhanced the micro-galvanic degradation effect and resulted in higher corrosion rates, while simultaneously improving the strength, at the expense of ductility. Although the mechanical, corrosion and magnetic performance was improved for all Fe-Mn alloys tested when compared to pure Fe, the authors proposed Fe-30Mn and Fe-35Mn for further investigation (Hermawan, Dube & Mantovani, 2010; Hermawan, Purnama et al., 2010). This research in particular served as the basis for several other studies, as will be noted throughout this review.

The micro-galvanic effect with Fe-Mn alloys was also observed recently by Dehestani, Trumble, Wang, Wang and Stanciu (2017), who studied the effect of varying powder particle size on the degradation rate of Fe-30Mn. Interestingly, the use of a relatively large particle size led to a corrosion rate that was about five times higher than that achieved when using finer particles. The formation

of a homogeneous composition across the sample volume is dependent on the diffusion of Mn into Fe during the heat treatment. The use of larger particles provided diffusion distances that were significantly greater, so as to prevent the complete diffusion of Mn. With respect to the less noble Mn-rich regions, the resultant Fe-rich centre of the particles therefore acted as a cathodic site, accelerating the corrosion rate. Similar outcomes were also observed when studying the microstructure and degradation characteristics of various Fe-Mn alloys (Kupková, Hrubovčáková, Zeleňák et al., 2015; Kupková, Hrubovčáková, Kupka, Oriňáková & Morovska Turonova, 2015). Moreover, when using larger powders, the packing efficiency is naturally poorer, contributing to a higher amount of porosity in the coupon. This translated into a larger surface area exposed to the corrosive medium, which further contributed to an increase in corrosion rate. The latter effect is commonly observed when dealing with powder processed test samples and is a serious consideration when designing the actual bone scaffold (Zhang, Wang, Cao & Gao, 2012; Zhang & Cao, 2015).

Similar to the issues related to corrosion product build-up when testing pure Fe, the agglomeration of oxides on the surface of Fe-Mn alloys could also act as a barrier, preventing further ion exchange between the metallic sample and the corrosive medium. In fact, Hermawan, Purnama et al. (2010) reported the presence of magnetite and other hydrated products on the surface of degradation tested Fe-Mn, which led to this effect on the corrosion rate. Similarly, Heiden, Walker, Nauman and Stanciu (2015) observed no corrosion on Fe-20Mn during the first 50 days of an immersion test in osteogenic cell culture medium, and attributed this lack of weight loss to the formation of the observed oxide layer. However, the corrosion rate drastically increased following the 50 days, as the loosely bound oxide layer fissured and allowed the corrosive fluid to come into contact with the metallic substrate. This effect was not reported elsewhere, however, it is suspected that the residual strains generated during the machining of the coupons for testing, could have stimulated the eventual oxide cracks. In fact, it is generally accepted that the stable, insoluble oxides formed will significantly diminish the degradation rate with time.

Although alloying with Mn had a positive effect on the corrosion rate in the works cited previously, the high Mn compositions used could, if not carefully controlled, result in severe toxicological effects. Therefore, in an effort to reduce the risks of disease, Drynda, Hassel, Bach and Peuster (2015) investigated Fe-(0.5–6.9wt.%)Mn for its cardiovascular application. However, the coupons lost even less weight than pure Fe when immersed for 84 days in 0.9% NaCl. These results were supported by similar poor degradation rates when testing Fe-3Mn, compared to pure Fe (Liu & Zheng, 2011).

3.2 Alloying with Noble Elements

Leading research groups in the field have approached the main issue of pure Fe in either of two primary ways. Corrosion could be accelerated through the addition of a second phase that lowers the overall standard electrode potential of the alloy, and thus makes the material more susceptible to corrosion, which is in essence the target when adding Mn. The alternative is to add a phase that is nobler than pure Fe, in order to trigger micro-galvanic corrosion (Schinhammer et al., 2010). The latter has proven to be the most popular route adopted.

In the first study using noble metals, Schinhammer et al. (2010) obtained promising results when including palladium (Pd), a material commonly used in dental alloys (Wataha & Shor, 2010). The Pd-rich intermetallic phase that precipitated in the Fe-10Mn-Pd tested, not only exhibited a higher corrosion rate when compared to the reference Fe-Mn alloy, but also demonstrated higher compressive strength. The use of just 10wt.% Mn led to the formation of martensite. In their later work, in order to avoid the presence of this brittle ferromagnetic phase, Schinhammer, Steiger, Moszner, Loffler and Uggowitzer (2013) prepared an Fe-21Mn-Pd alloy which was tested using Electrochemical Impedance Spectroscopy (EIS). The alloy was shown to exhibit a polarisation resistance that was five times lower than that of the pure Fe sample, indicating its significantly faster corrosion rate. As opposed to some of the previously mentioned work, the layered corrosion product formed, similar to that previously described by Hermawan, Purnama et al. (2010), did not necessarily result in reduced corrosion rates. In fact, EIS tests showed a decrease in polarisation resistance with time. Čapek, Msallamová, Jablonská, Lipov and Vojtéch (2017) also used Pd as the alloying element in the preparation of Fe-2Pd by Spark Plasma Sintering (SPS). Similar to the previous findings, this alloy displayed approximately four times the compressive yield strength of pure Fe, as well as a high corrosion current density in both static immersion tests and electrochemical corrosion tests.

To prepare Fe-5Pt, Huang, Cheng and Zheng (2014) introduced platinum (Pt) with a standard electrode potential of 1.2 V in pure Fe. They also compared this to the performance of pure Fe and an Fe-5Pd alloy. Since both noble metals have limited solubility in Fe, the documented increase in yield strength was mainly due to the secondary phase strengthening effect. Moreover, the corrosion of both alloys was visibly much more severe than that of pure Fe, with the areas surrounding Pd- and Pt-rich areas corroding most severely. In a similar study, based on the same principal, Huang, Cheng, Bian and Zheng (2016) added silver (Ag) and gold (Au) to Fe.

Interestingly, in the case of Fe-Au alloys, the mechanical performance generally improved as a result of secondary phase strengthening and solid-solution strengthening. However, Fe-10Ag demonstrated poorer mechanical properties compared to pure Fe, indicating that excessive additions of the softer metal into the Fe-matrix is detrimental to the mechanical performance. Again, corrosion results were positive, with Fe-5Ag and Fe-5Au demonstrating the fastest corrosion rates in static immersion tests.

With regards to the effectiveness of the Fe-5Ag, conflicting results were described by both Čapek, Stehlíková, Michalcová, Msallamová and Vojtěch (2016) and Wegener et al. (2011). Čapek, Stehlíková et al. (2016) prepared Fe-2Pd, Fe-2Ag and Fe-2C using powder metallurgy. They reported that while both Pd and carbon (C) additions were successful in accelerating the corrosion rate in static immersion, there was a notable decrease in corrosion rate for the Fe-2Ag alloy due to the formation of AgCl. In fact, there seemed to be no preferential attack near Ag-rich sites in post-corrosion SEM micrographs. Potentiodynamic tests supported these observations. Besides this, when testing Fe-5Ag, Wegener et al. (2011) noted only a slight increase in corrosion, which they concluded was primarily down to the higher amount of micro-porosity present in the sample, due to an increase in surface area exposed to the corrosive fluid. However, in a study by Wiesener et al. (2017) that specifically targeted the understanding of Fe-Mn-Ag corrosion, the authors assured that no AgCl should form on such alloys during the degradation process, and that attack of the Fe-Mn matrix close-by should progress considerably in the first stages. Nonetheless, it is inevitable for phosphates and oxides to deposit on the corroding surface due to the local increase in pH, as a result of the cathodic reaction occurring at the Ag-rich areas, as in Eq. (2).

In fact, Safaie, Khakbiz, Sheibani and Bagha (2015) and Sotoudehbagha, Sheibani, Khakbiz, Ebrahimi-Barough and Hermawan (2018) prepared Fe-Mn-(1-3)Ag alloys and noted no formation of Ag-chlorides. The authors noted that with increase in wt.% Ag the amount of micro-porosity reduced. This is a result of the liquid phase sintering mechanism that is activated during the processing of the material, wherein the Ag enters the liquid phase at 962 °C and flows into the pores present in the surrounding area. This increase in density contributed to the rise in hardness and strength measurements with increase in wt.% Ag. It was also revealed, that the presence of micro-porosity may have a more pronounced effect on the corrosion rate than the incorporation of the noble metal, due to the higher surface area exposed to the corrosive medium. This was revealed through the decrease in corrosion rate with increase in wt.% Ag. On the other hand, Liu, He, Xu and Guo (2018) again confirmed the effectiveness of Ag when reporting a 12 µm/year corrosion rate for Fe-30Mn-Ag alloys, as opposed to the 7 µm/year obtained by both Fe and Fe-30Mn. In this case, the samples were prepared via the casting route and therefore contained no or minimal micro-porosity.

Other techniques for incorporating the noble phase have emerged over the years. Huang and Zheng (2016) used the ion implantation technique in order to introduce Ag in the first few nanometres of the coupon. The ion-implanted coupons exhibited higher corrosion rates and a more uniform corrosion than the pure Fe coupon in the immersion test. However, the shallow depth of the Ag-containing layer limited the acceleration effect to the first few days.

Micro-patterning of pure-Fe surface using Au and Pt has also given positive results (Cheng, Huang & Zheng, 2015; Huang & Zheng, 2016). The authors reported more than double the increase of corrosion rate when depositing circular patterns of Pt, and four times the corrosion rate when patterning with Au. Similar to the issue with ion implantation, the limitation of such techniques is the effective depth of patterning. Moreover, the applicability on more complex 3D structures, such as in the cardiovascular stent and bone regeneration scaffold applications, may be somewhat limited.

3.3 Other Material Design Studies

Another approach for enhancing the performance of pure iron is to alloy with other alloying elements that, unlike Ag, Au and Pd, do not form strong microgalvanic couples. Liu and Zheng (2011) studied the effect on the mechanical properties and corrosion rate with the addition of 3wt.% of cobalt (Co), aluminium (Al), tungsten (W), tin (Sn), boron (B), carbon(C) and sulfur (S). All these elements are commonly used to strengthen steel, yet their potential for use in biomedical applications is yet unknown. Co, W, C and S were found to be effective in increasing the potentiodynamic corrosion rate, although the increase was rather low. Co, W, B, C, and S were found to improve the tensile and yield strengths of the material. Despite the fact that the study deemed certain elements unsuitable for biodegradability applications, the additions were relatively small and the effect of higher concentrations may yield different results, as was proven with additions of higher wt.% Mn (Hermawan, Purnama et al., 2010).

In a study by Wegener et al. (2011) in which the authors tested compact powder metallurgy processed Fe-0.06B, Fe-0.6P and Fe-1.6P, additions of boron also yielded positive results. The alloys all exhibited a slight increase in corrosion rates when tested in simulated body fluid. This was somewhat unexpected for Fe-P alloys due to the corrosion inhibition effect phosphorus gener-

ally has when added to steels. Moreover, while Wegener et al. (2011) reported only a small increase in corrosion, Oričaková et al. (2016) reported a threefold increase in degradation of Fe-0.5P alloy in Hank's solution. Electrochemical tests carried out on Fe-P foams also indicated an increased corrosion rate, however, changes in the exposed surface area may have had a significant effect (Hrubovčáková, Kupková & Džupon, 2016). Thus, further testing must be carried out to determine the suitability of both Fe-B and Fe-P alloys.

Furthermore, other elemental additions have been made to the promising Fe-Mn alloys. In fact, Hong et al. (2016) tested the electrochemical corrosion rate of Fe-Mn-(1-2)Mg based on the premise that Mg, being a less corrosion resistant material, will induce fast degradation of the scaffold. This was confirmed as Fe-Mn-2Mg exhibited more than nine times the degradation rate of the Fe-Mn matrix in Hank's solution. Encouraging results have also been published with additions of copper to the Fe-Mn matrix (Santanu, Raviteja, Madhuparna, Vamsi & Mangal, 2019). This addition resulted in a highly increased corrosion rate, a pronounced antimicrobial characteristic when tested with *E.coli*, and no problematic cytotoxic effects when tested with MG-63 osteosarcoma. Fe-Mn-Si is an Fe-Mn alloy that has been popularly studied by Xu, Hodgson and Cao (2015). The authors showed that while the forged alloy exhibited a rise in corrosion rate with increase in wt.% silicon (Si), the powder-processed compacts with the same composition demonstrated the opposite effect. In this case, the densification of the compact improved with increase in wt.% Si, and thus the area exposed to the corrosive fluid decreased.

The addition of second phases has also been tested using various other materials. Cheng and Zheng (2013) studied the addition of 0.5wt.% and 1wt.% of carbon nanotubes (CNTs). While CNTs have been used in numerous biomedical applications, they also have a potential of 0.2 V and are thus expected to trigger microgalvanic corrosion. In fact, sintered Fe-CNT composites exhibited significant increases in electrochemical and immersion corrosion rates, potentially due to a finer grain size compared to pure iron, as well as the microgalvanic effect. Fe-CNT also displayed an increase in ultimate compressive stress. The same effect was not observed in the study carried out by Oriňaková et al. (2013), wherein Fe-CNT foams exhibited a lower corrosion rate compared to pure Fe foams in Hank's solution.

Following the conclusion by Liu and Zheng (2011) that the presence of Fe_2O_3 as a corrosion product may lead to enhanced anodic dissolution, Cheng, Huang and Zheng (2014) proposed Fe-Fe_2O_3 for biodegradable implant applications. Results from the study showed that Fe with up to 5wt.% addition of Fe_2O_3 may potentially be applicable for biodegradable orthopaedic implants, as they showed enhanced strength and increase in corrosion rates, resulting from the fine microstructure formed, coupled with the anodic effect of defects present.

Finally, Fe-ceramic composites have also been investigated by various research groups, including Ulum et al. (2014). Additions of 5wt.% of biodegradable materials including hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and biphasic calcium phosphate (BCP), were added to create sintered composites. The materials showed slightly poorer compressive properties, potentially due to the brittle nature of the incorporated ceramic materials. Furthermore, the Fe-ceramic showed increased degradation, however this was mainly attributed to the weight loss of the biodegradable ceramics.

4 Processing and Performance of Fe-based Foams

Apart from finding the ideal scaffold composition, researchers have also been concerned with developing an adequate technique to produce the optimal porous structure. The techniques being researched are heavily based on powder metallurgy, most probably due to the high degree of flexibility that this technology allows. Other methods, including melting and deposition, have been mentioned in select works (Alavi, Trenggono, Champagne & Hermawan, 2017; Drynda et al., 2015; Schinhammer, Steiger et al., 2013; Dehestani et al., 2017).

Quadbeck, Stephani, Kümmel, Adler and Standke (2007) first suggested the use of the replication method for this application. This method involves the use of a polymeric porous template, generally reticulated polyurethane foam, that is coated with a slurry containing metallic powders. The coated foam is then subjected to low-temperature de-binding and template removal, followed by sintering at 1000–1200 °C. The technique has been used successfully in the preparation of Fe-P, Fe-B and Fe-Fe_2O_3, with porosities generally varying between 80 and 90% (Hrubovčáková et al., 2016; Oriňaková et al., 2013; Orinak et al., 2014; Feng et al., 2018). While the low structural density suggests rather poor mechanical properties, Quadbeck et al. (2011) and Wang et al. (2017) have reported the effectiveness of varying the slurry coating thickness in achieving the desired mechanical performance.

Another technique that has been employed is the space-holder method, wherein the metallic powders are uniaxially compacted along with organic space-holding particles. The latter particles are subsequently dissolved or de-binded prior to sintering, leaving behind a porous network. Čapek and Vojtěch (2014) and Čapek, Vojtěch and Oborná (2015) used ammonium bicarbonate (NH_4CO_3) as a space-holder to obtain porous Fe. Resulting structures had up to 82% porosity with

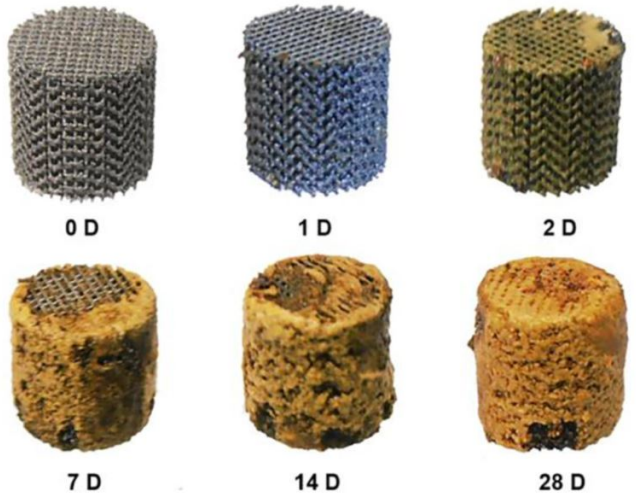

Figure 2: Visual evidence of progressive *in vitro* degradation of iron scaffolds prepared using Direct Metal Printing and tested in revised simulated body fluid for 28 days. Reprinted with permission from Elsevier from (Li et al., 2018).

macro-pore sizes varying between 250–500 µm, as is required for the application (Bose et al., 2012). Moreover, they exhibited mechanical properties comparable to human cancellous bone. Zhang and Cao (2015) used the same space-holder to prepare Fe-35Mn. The authors reported a considerable acceleration in corrosion rate in various physiological media, with increase in percentage porosity, however, the mechanical properties were not deemed suitable for load-bearing applications. Sodium chloride was also used as a space-holder by Feng et al. (2017) to produce porous Fe-Mn-Si-Pd. However, incomplete leaching of this space-holder may lead to premature corrosion of the metallic foam.

A more recent approach has been the use of 3D inkjet printing; Chou et al. (2013) were the first to develop Fe-30Mn foams using this technique. The process involves the printing of powders mixed with a water-based organic material which bind in discrete layers, approximately 100 µm thick, followed by a thermal de-binding step to remove the organic material, and sintering. Due to the absence of compacting pressure during the procedure, the initial powders are generally mechanically milled in order to facilitate the alloying of Fe and Mn. Miniature human femurs with pore sizes of 500 µm and 1 mm were successfully developed using this technique. The process was further developed by Hong et al. (2016) to produce Fe-35Mn and Fe-34Mn-Ca alloys with up to 53% porosity. A similar technique was used by Li et al. (2018) to print scaffolds with an approximate pore size of 749 µm. These scaffolds showed almost twelve times the corrosion rate of cold-rolled iron due to the increase in surface area and the reduced grain sizes, resulting from the Direct Metal Printing (DMP) technique employed. The mechanical properties of these scaffolds remained within the acceptable range for the application after 28 days of immersion in revised simulated body fluid. The results of the DMP technique and consequent corrosion studies can be better appreciated in Fig. 2. It is interesting to note that in both the study by Chou et al. (2013) and the study by Li et al. (2018), the authors noted very similar corrosion product morphology through SEM analysis. Li et al. (2018) indicated that the products were mainly iron hydroxides, phosphates and carbonates, as analysed through XRD and Fourier-transform Infrared Spectroscopy (FTIR). EDS analysis reported in the study by Chou et al. (2013) indicate very similar compositions, although Mn-based products were also present in this case. These results also concur with those obtained when performing corrosion tests on representative coupons, wherein the typical corrosion products observed are generally iron and other metal oxides, calcium and metal phosphates and metal carbonates (Hermawan, Purnama et al., 2010; Schinhammer, Steiger et al., 2013; Wiesener et al., 2017; Zhang et al., 2012; Čapek, Stehlíková et al., 2016). However, with the most common method of analysis being EDS during SEM imaging, few studies pinpoint the exact composition of the degradation products.

Sharma and Pandey (2018a) proposed an alternative method for creating topologically ordered porous iron foams (TOPIF) that incurred fewer capital costs. The technique includes the development of a polymeric 3D printed template with a specific pore architecture. The template is used to create a phosphate-based investment mould, from which the template is removed thermally in a tube furnace. The mould is ultrasonically filled with Fe powder, which is subsequently sintered in a microwave heating furnace. The mould is removed, and the structure is post-processed. In a later study, the authors proved the flexibility of this technique in the fabrication of foams with various unit cell shapes, aimed at improving compressive performance. In fact, these structures showed a mechanical performance similar to that of human bone, however, further studies are required to analyse the degradation performance and to optimise the control over the final geometry, through the initial CAD design (Sharma & Pandey, 2018b)

On the subject of processing porous structures, an interesting development has seen the preparation of nanoporous topologies on the surface of Fe-based implants, aimed at improving tissue/implant interaction through the increase of surface area. The technique used by Heiden, Johnson and Stanciu (2016) in their studies is known as selective dealloying, wherein the more anodic component of the alloy composition is chemically removed in a four-step process. In their initial study, the

authors chose to prepare an Fe-Mn-Zn alloy, in which the more anodic components were the Mn and Zn. The first step in the creation of the nanoporous surface is to immerse the Fe-30Mn substrate in a molten Zn bath, allowing Zn to diffuse into the first few micrometers of the coupon. An etching step then removes most of the Zn from the surface. In the next annealing heat treatment, the remaining zinc moves via bulk diffusion to the surface and is consequently etched to leave behind nanometric pores. The drawback of this process was that stable oxides were formed on the surface during the annealing process, possibly limiting the biodegradability of the alloy despite the potential better osseointegration. A possible improvement was reported in their second study, when Heiden, Huang, Nauman, Johnson and Stanciu (2016) demonstrated that the use of citric acid as the final etchant led to the removal of the passivating oxide layer. This subsequently exposed the underlying metal and successfully accelerated the potentiodynamic corrosion rate, when compared to polished Fe-30Mn, while simultaneously increasing the attachment and proliferation of mouse bone marrow stromal cells (D1-UVA). The large number of optimisable parameters in this technique leaves much to be studied, however this method holds promise for the enhanced performance of biodegradable implants.

5 Cytotoxicity and *In Vivo* Corrosion of Fe Alloys

Biocompatibility is an important factor to consider whenever foreign material is to be implanted *in vivo*. In the case of corroding materials, the corrosion products must be accepted by the body while causing as little cytotoxicity as possible. For this reason, implants are often based on materials which form part of the body's normal biochemical milieu, many elemental metals falling within this bracket (Wolber, Beck, Conlon & Kruger, 2013). Fe is an essential element needed for the normal physiology of the body and, as explained elsewhere in this paper, it possesses other characteristics which render it an even more attractive option. However, in spite of this, too great an amount of Fe within the body may cause accumulation of Fe deposits within different organs, such as endocrine organs, the liver, kidneys and heart, ultimately leading to negative effects, secondary to cytotoxicity. This is often seen in diseases such as thalassaemia (Ballas, 2001). For this reason, corrosion products which are harmful at low concentrations, especially when forming part of a rapidly corroding material, may cause significant cytotoxicity and the ultimate failure of the implant.

Initially it was pure Fe that was studied as a potential material for such corroding implants, these materials having been studied both *in vivo* and *in vitro*. The first *in vivo* studies included leading work by Peuster et al. (2001), where $> 99.8\%$ pure Fe was used to construct an arterial stent. This stent was then implanted into the descending aorta of 16 New Zealand White Rabbits over a study period of up to 18 months. The animals were eventually sacrificed in order to retrieve the implant, along with organs such as the heart, lungs and kidneys. This study reported no events of thrombosis within the stent in any of the studied animals. Histological examination was performed and revealed that there were only mild inflammatory reactions surrounding the stent. However, it must be noted that there were only very small amounts of corrosion products seen, suggesting only minimal corrosion of the stent, providing a potential reason for the lack of cytotoxicity. Examination of the other retrieved organs revealed no signs of Fe overload or Fe-related toxicity.

Other *in vivo* studies tend to mirror these results, with both short and long-term studies on pigs and minipigs showing good biocompatibility, some results in fact comparable to those of the widely-accepted cobalt chromium coronary stents (Waksman et al., 2008; Peuster et al., 2006; Wu et al., 2012). In studies where internal organs such as the kidneys, spleen and heart were harvested, cytotoxicity due to iron accumulation was not observed. In one study, Fe deposits were observed in the spleens of minipigs after a one-year period, but once again no signs of toxicity were noted (Peuster et al., 2006). *In vitro* results agree with those obtained from *in vivo* tests. Fe appears to have minimal cytotoxic effects on red blood cells and may in fact produce a lesser inflammatory response than stainless steel AISI 316L (Walker, Nauman, Allain & Stanciu, 2015).

As implied earlier in this review, these trials led to the conclusion that Fe on its own was not producing a sufficiently fast corrosion rate *in vivo*, thus spurring the studies on various other alloys, with Fe-Mn alloys quickly becoming the focus of many research groups. Mn is another elemental metal that is essential to normal human physiology, most frequently implicated as a co-enzyme. It is therefore beneficial in small amounts for normal body processes, but in excess it is known to cause disease, including one similar to Parkinson's disease (Horning, Caito, Tipps, Bowman & Aschner, 2015). As previously discussed, Fe-Mn alloys have been found to possess favourable mechanical and corrosion properties, but questions arose as to the alloys' potential cytotoxicity. Mn is found at much lower concentrations than Fe in the human body and therefore, it followed that Mn could have cytotoxic effects at lower concentrations than Fe. Once again however, this would depend on the rate of corrosion of the alloy.

Studies have shown that pure Mn is significantly more cytotoxic than pure Fe or AISI 316L (Cheng & Zheng,

2013; Hermawan, Purnama et al., 2010). Metabolic rates of 3T3 mouse fibroblast cell lines decrease rapidly when exposed to growing concentrations of pure Mn, especially when compared to similar concentrations of pure Fe and AISI 316L. Pure Fe and AISI 316L only have minimal effects on metabolic activities of cells, this being comparable to control cell lines cultured in standard culture medium. However, when considering the Fe-Mn alloy, its effect on metabolic rates lies somewhere in between those of pure Fe and pure Mn. Cell lines are seemingly able to tolerate significantly higher concentrations of Fe-Mn alloy than pure Mn (Hermawan, Purnama et al., 2010). However, it must also be kept in mind that Fe-Mn has a different corrosion rate to that of pure Fe and therefore, this also plays a part in the cytotoxicity profile of this alloy. With regards to haemocompatibility and thrombogenicity, *in vitro* testing appears to have positive results. Fe-Mn alloys do not seem to cause haemolysis, but instead produce results similar to those exhibited by pure Fe. Thrombogenicity is also not thought to be an issue with such implants, with *in vitro* test results being even more promising than those given for AISI 316L (Liu & Zheng, 2011; Walker et al., 2015).

In vitro cytocompatability studies confirm that Fe-Mn does have an effect on cultured cells, decreasing their metabolic rate. However, this effect does not appear to be too dissimilar from the effect of pure Fe on similarly cultured cell lines. In fact, when taking standards such as ISO 10993-5 into account, Fe-Mn still appears to have less of an effect on the metabolic rate of L929 cell lines than is accepted by this standard (Čapek, Kubásek et al., 2016). A study by Liu and Zheng (2011) documents a decrease in metabolic rate of L929 (mouse fibroblast), VSMC (rodent vascular smooth muscle cells) and ECV304 (human umbilical vein endothelial cells) after 2-day exposure to Fe-Mn corrosion products, but an increase of their metabolic functions after a further 2-day exposure to these same corrosion products (Liu & Zheng, 2011). There are other *in vitro* studies published in the literature on L929 cell lines which corroborate these works and tend towards a good biocompatibility of Fe-Mn alloys (Xu, Hodgson & Cao, 2016). Certain works include the seeding of cells such as MC3T3 (mouse osteoblast) onto alloys such as Fe-30Mn, in order to study cell growth on the alloy itself. These studies also document encouraging results, with only a few dead cells being seen on the scaffolds *in vitro* (Chou et al., 2013).

It is worth mentioning one particular study performed by Caligari Conti et al. (2018), where the effects of the corrosion products of Fe-13Mn-1.3C on hFOB 1.19 human osteoblast cell lines were studied. This study group used this material in potentiostatic tests and gathered the elute for cytocompatibility testing. The obtained solutions were split into 'Complete' and 'Supernatant' groups, the former containing both ionic and particulate products from the corrosion tests. The latter on the other hand contained only ionic products, this group allowing the cell lines to proliferate well, similar to control solutions with no corrosion product. In contrast, the 'Complete' solutions containing particulate corrosion products showed a significantly reduced rate of cell growth, inferring the important effect of particulate material on the rate of cell proliferation and growth.

Thus far, there is little in the way of *in vivo* testing of Fe-Mn alloys, but available results appear to be positive. Wires of Fe-30Mn and AISI 316L have been implanted into the femora of rats for comparison purposes. This study performed by Traverson et al. (2018) explains that once retrieved from the euthanised rats six months after implantation, Fe-30Mn showed very little cytotoxicity. In fact, Fe-30Mn produced a slightly more fibrotic tissue reaction than AISI 316L, leading to it being classified as a mild local irritant. In spite of this, the well-being of the rats did not seem to be affected during the study period. Of note is the differing corrosion results of these two materials. Stainless steel showed no signs of corrosion, while Fe-30Mn showed minimal signs of corrosion with the formation of iron oxide crystals on the surface of the implant (Traverson et al., 2018).

Another study by Drynda et al. (2015) took Fe-Mn alloys with a Mn weight below 10wt.% and implanted discs of these alloys into a subcutaneous pouch created above the gluteal muscles of mice. These animals were studied up to a maximum period of nine months, within which no ill effect was observed. However, none of the alloys exhibited significant signs of corrosion. Of note is that this study was performed with alloys with a relatively low wt.% Mn and with samples of a low surface area to volume ratio. Another reason this study gives for the poor corrosion rate is the formation of passivation layers, including layers of metal hydroxides and phosphates. These form protective layers effectively blocking the corrosion of the alloy *in vivo*, as described earlier in this review (Drynda et al., 2015).

From the discussed results, *in vivo* alloy performance is evidently different from *in vitro* performance. Considerable effort has been made on several fronts to make *in vitro* testing reflect *in vivo* conditions more. Hermawan, Purnama et al. (2010) often used a dynamic test bench for corrosion testing. This allowed for fresh medium to deliver a fresh supply of oxygen to the material surface, while also removing lingering corrosion product. In fact, researchers who made use of such setups, including Huang, Cheng, Bian and Zheng (2016), generally reported higher corrosion rates than those obtained using static setups. Apart from this, Schinhammer, Steiger et al. (2013) also revealed that using gaseous CO_2 to

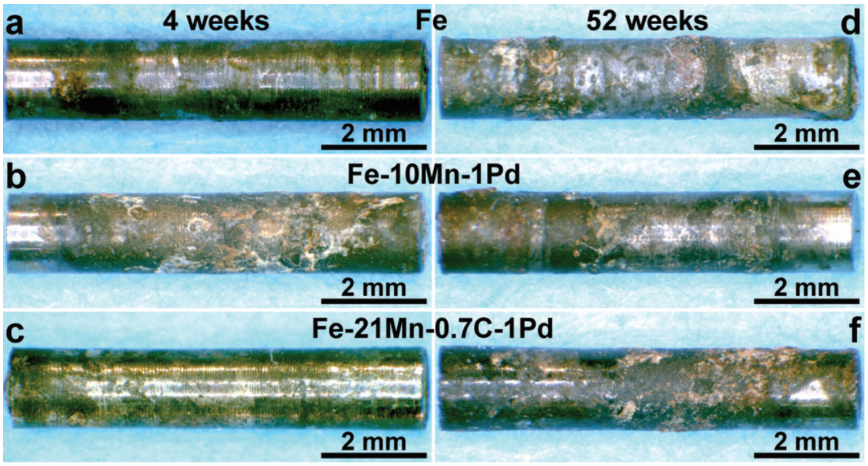

Figure 3: Optical micrographs of pins implanted in the femora of Sprague-Dawley rat, after implantation for (a–c) 4 weeks and (d–f) 52 weeks. Reprinted with permission from Elsevier from (Kraus et al., 2014).

control the pH of the physiological medium produced more realistic results, than when using pH buffers. Another interesting result was obtained by Caligari Conti et al. (2018) when adding protein (bovine serum albumin) to Hank's solution during static immersion testing of Fe-13Mn-1.2C. The corrosion rates calculated for coupons exposed to protein additions were significantly lower than those tested in only Hank's solution. The difference in corrosion rates was attributed to the formation of a biofilm on the specimen surface that limited the dissolution of metal ions. However, the same effect was not observed in potentiodynamic tests with the same solutions. In this case, the anodic potential at the surface interfered with the formation of a stable biofilm, rendering the addition of protein to the solution insignificant, when it came to altering the corrosion rate.

A review by Martinez Sanchez, Luthringer, Feyerabend and Willumeit (2015) attempts to outline the reasons for which *in vitro* and *in vivo* corrosion testing give dissimilar results when testing magnesium-based alloys. The same points seem to be valid when discussing the discrepancies noted when testing iron-based alloys, indicating that there could be a similar trend that applies to the field of biodegradable metals. In this study, the authors proposed multiple reasons for these *in vitro* and *in vivo* discrepancies. For example, the use of several different media during *in vitro* studies which differ from the *in vivo* environment in various ways, including higher concentrations of chloride, the absence of certain buffering mechanisms, or the difference in biochemical milieu, such as in the proteins present. In response to these difficulties, more physiological solutions, such as simulated body fluid (SBF), which mimic the *in vivo* environment to which such scaffolds will be exposed, now exist. These solutions appear to give more similar results *in vitro* to what is observed *in vivo*, but they are still imperfect. Another issue raised, is that within the available literature dealing with corrosion rates *in vitro*, different testing methods have been utilized, including for example potentiodynamic testing and immersion testing. These different methods inherently give varying results, in particular, potentiodynamic testing tending to overestimate *in vivo* corrosion rates compared with other methods.

Changes in methodology have also been proposed for cytotoxic testing. Wegener et al. (2011) used a perfusion chamber to better simulate the flow of medium through cancellous bone. While cytotoxicity tests using monolayer cell culture of hFIB fibroblasts indicated strong cytotoxic effects, the dynamic setup led to a gradual increase of fibroblast levels over the 4-day testing period. However, in spite of these advances, *in vitro* testing still leaves a lot to be desired, with results often being poorly transferable to *in vivo* testing.

While Fe-Mn promises a lot in the field of bone graft substitutes, other elements have also been added to Fe-Mn to contribute to an improved property profile. Two such elements include C and Pd. It seems that Fe-Mn-C-Pd alloys offer good mechanical properties, but these too must be balanced against their cytotoxic performance (Schinhammer, Gerber, Hänzi & Uggowitzer, 2013). Both Fe-Mn-Pd and Fe-Mn-C-Pd alloys were observed in this *in vivo* study by Kraus et al. (2014). Cylindrical pins formed of these alloys were implanted into the femora of Sprage-Dawley rats, after which the test animals were observed for a period of 52 weeks. While the rats showed no signs of ill health, very limited corrosion was observed on the pin surfaces, despite the positive results obtained by Schinhammer et al. (2010) *in vitro*, as described in Section 3.2. The authors attributed this effect, displayed in Fig. 3, to the lack of oxygen present near the implantation site. The lack of

oxygen did not seem to present considerable issues when Fe stents were implanted in the coronary and illiac arteries of juvenile domestic pigs by Waksman et al. (2008) and Feng et al. (2013), respectively. Both groups reported the presence of brown-ish corrosion product after 28 days and after 12 months, indicative of the presence of Fe_2O_3. No further characterisation of the corrosion product was done in these studies, however, Fântânariu et al. (2015) published EDS results of the surface of FeMnSi coupons implanted in the tibia crest of Wistar rats and indicated the presence of a high percentage of oxides along with large quantities of calcium, phosphorus and sulphur compounds. Even in *in vivo* studies, the presence of calcium and phosphorus is a very positive outcome, indicating the promise of implant bioactivity following implantation.

Another popular addition to Fe-Mn is Ag. While the effect of Ag on the corrosion rate of the alloys has varied across various studies, Ag also imparts a separate, yet very important characteristic. Increasing the Ag proportion tends to increase the alloy's antibacterial characteristic. Sotoudehbagha et al. (2018) have observed a decrease in the number of colonies of both *E. coli* and *S. areus* after exposure to Fe-Mn alloy. This effect is even more pronounced after exposure to Fe-Mn alloys with increasing amounts of Ag. Such a material response is invaluable, and may in fact help to decrease graft infection rates in practice.

6 Conclusions

Research on the application of iron alloys for bone regeneration scaffolds has clearly made significant leaps in the past decade. With the largely positive outcomes of most studies on structure development, corrosion and cytocompatibility, the potential of these materials in matching the popularity of Mg-alloys in the field of biodegradable metal research has become even more probable. This review has mainly covered advances in the development of iron alloyed with other metals, along with manufacturing methods for these same materials. However, a recent review by Gorejová et al. (2019) highlights the interesting work being done on the application of ceramic and polymer based coatings, in order to deal with the issues of slow degradation and on occasion, cytotoxicity and osseointegration.

With a considerable basis of knowledge, it is now crucial for the focus to be shifted to the study of more realistic test samples and the development of testing procedures that better reflect the conditions *in vivo*. Several alloys have been proven to significantly accelerate the corrosion rate of pure Fe, while an increase in porosity has generally led to the same effect. Combining the two aspects of scaffold design to gain control over the degradation and mechanical performance could pave the way for targeted scaffold design, wherein custom products could be developed for optimal healing dynamics, depending on specific clinical cases. Naturally, with *in vivo* Fe alloy trials being rather limited, clinical application still seems to be a goal for the rather distant future. However, with the available *in vivo* results, as well as consistent *in vitro* mechanical performance, these scaffolds show great promise for application in orthopaedic and trauma surgery. More specifically, they promise an alternative to both autografts, that is, bone removed from another site from the same patient, and allografts, especially when managing injuries involving bone loss from load-bearing bones such as the tibia.

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