Microstructure, mechanical property, biodegradation behavior, and biocompatibility of biodegradable Fe–Fe$_2$O$_3$ composites

J. Cheng, T. Huang, Y. F. Zheng

1Center for Biomedical Materials and Tissue Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, People’s Republic of China
2Department of Materials Science and Engineering, State Key Laboratory for Turbulence and Complex System, College of Engineering, Peking University, Beijing 100871, People’s Republic of China

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Abstract: In this study, the effects of Fe$_2$O$_3$ (addition, 2, 5, 10, and 50 wt %) on the microstructure, mechanical properties, corrosion behaviors, and in vitro biocompatibility of Fe–Fe$_2$O$_3$ composites fabricated by spark plasma sintering were systematically investigated as a novel-structure biodegradable metallic material. The results of X-ray diffraction analysis and optical microscopy indicated that Fe–Fe$_2$O$_3$ composite is composed of α-Fe and FeO instead of Fe$_2$O$_3$. Both electrochemical measurements and immersion test showed a faster degradation rate of Fe–2Fe$_2$O$_3$ and Fe–5Fe$_2$O$_3$ composites than pure iron and Fe–5Fe$_2$O$_3$ exhibited the fastest corrosion rate among these composites. Besides, the effect of Fe$_2$O$_3$ on the corrosion behavior of Fe–Fe$_2$O$_3$ composites was discussed. The extracts of Fe–Fe$_2$O$_3$ composite exhibited no cytotoxicity to both ECV304 and L929 cells, whereas greatly reduced cell viabilities of vascular smooth muscle cells. In addition, good hemocompatibility of all Fe–Fe$_2$O$_3$ composites and pure iron was obtained. To sum up, Fe–5Fe$_2$O$_3$ composite is a promising alternative for biodegradable stent material with elevated corrosion rate, enhanced mechanical properties, as well as excellent biocompatibility.

Key Words: Fe-Fe$_2$O$_3$ composites, biodegradable metals, corrosion, cytotoxicity

INTRODUCTION

Iron (Fe) and its alloys have attracted great attentions as biodegradable implant materials owing to their combination of good mechanical and favorable biological properties. Preliminary in vivo animal tests on pure iron stents show good biocompatibility of iron and validate its feasibility as biodegradable material. However, these results also indicate that a fast degradation rate of pure iron in physiological environment is needed to meet the requirements of clinical applications. On the other hand, from the mechanical point of view, the properties of 316L stainless steel, which is considered as the golden standard for stent material, are aimed at. Thus, improved strength and ductility compared to pure iron are also the goal for the design criterion of iron-based materials.

To date, several efforts have been exerted on iron-based materials including alloying and using new fabrication method, for the sake of increased corrosion rate and superior mechanical properties. Moravej et al. reported that pure iron fabricated by an electroforming technology possessed a high yield strength (YS) (360 MPa) and ultimate tensile strength (423 MPa), and its ductility could be further improved to 18% by annealing. Both static and dynamic in vitro degradation test indicated a faster corrosion rate of electroformed pure iron than that of pure iron produced by casting. Hermawan et al. developed a series of Fe–Mn alloys with Mn content ranging from 20 to 35 wt %. The new Fe–35Mn alloy exhibited a high ultimate strength and YS up to 550 and 228 MPa, respectively, with ductility up to 32%. Moreover, the Fe–Mn alloys showed a corrosion rate two times faster than pure iron. Liu and Zheng comprehensively investigated the effect of eight alloying elements (Mn, Co, Al, W, Sn, B, C, and S) on the biodegradability and in vitro biocompatibility of pure iron in both as-cast and as-rolled state. The results indicated that the addition of alloying elements except for Sn improved the mechanical properties of pure iron after rolling and all the Fe–X alloys showed comparable corrosion rate as that of pure iron.

In our recent study, iron-based composites reinforced by W and carbon nanotubes (CNTs) have been reported to show increased corrosion rate and enhanced mechanical properties compared with pure iron.\textsuperscript{13} Fe$_2$O$_3$ is one of the main degradation products of pure iron. As it is reported, the presence of corrosion product layer deposited on the surface decreases the impedance and greatly enhances the anodic dissolution of iron.\textsuperscript{14} Iron oxide (Fe$_2$O$_3$) may have a beneficial effect on the increment of corrosion rate of iron owing to the adsorption of intermediate species such as Fe(I)$_{ad}$ and Fe(II)$_{ad}$ and the resultant self-catalytic effect for corrosion.\textsuperscript{14} Accordingly, the combination of iron matrix and Fe$_2$O$_3$ would be a promising approach to increase the degradation rate of iron matrix, wherein the addition of Fe$_2$O$_3$ may enhance its mechanical properties concurrently. In the view of biocompatibility, Fe$_2$O$_3$ is a well-accepted magnetic material for biomedical applications, such as targeted delivery, cell labeling, and its excellent biocompatibility has been confirmed.\textsuperscript{15} In this study, Fe–Fe$_2$O$_3$ composites reinforced by Fe$_2$O$_3$ particles were fabricated by spark plasma sintering (SPS) and their in vitro degradation behaviors and biocompatibility were systematically investigated by electrochemical measurements, immersion test, cytotoxicity, and hemocompatibility evaluations.

**MATERIALS AND METHODS**

**Material preparation**

Pure iron powder (99.9%, particle size, <10 μm, Alfa) and iron oxide powder (Fe$_2$O$_3$, 99.0%, particle size, <75 μm, SCRC) were used as the starting materials. Different compositions containing 2, 5, 10, and 50 wt % of Fe$_2$O$_3$, respectively, were prepared (Fe–2Fe$_2$O$_3$, Fe–5Fe$_2$O$_3$, Fe–10Fe$_2$O$_3$, and Fe–50Fe$_2$O$_3$ for short). The preparation steps for Fe–Fe$_2$O$_3$ composites were the same as that described in the literature for Fe–W composites.\textsuperscript{15} As-cast pure iron (99.9%) was used as the counterpart.

All the experimental specimens were cut into square pieces (10 × 10 × 2 mm$^3$) for microstructural characterization, corrosion, cytotoxicity, and hemocompatibility tests. Each specimen was mechanically polished to a mirror appearance, and then ultrasonically cleaned in absolute ethanol.

**Microstructural characterization**

X-ray diffraction (XRD) (Rigaku DMAX 2400) using CuKα radiation was employed to identify the constituent phases of Fe–Fe$_2$O$_3$ composites and pure iron controls with a scan range of 10–100° and a scan rate of 8° min$^{-1}$. The specimens were etched with a 4% HNO$_3$/alcohol solution and optical microstructure photographs were obtained by an optical microscopy (Olympus BX51 M). Energy-dispersive spectrometer (EDS, EDAX GENESIS) was used for chemical composition analysis. The densities of Fe–Fe$_2$O$_3$ composites and pure iron were determined by the Archimedes’ method in absolute ethanol.

**Mechanical test**

Compressive tests were adopted to examine the mechanical properties of experimental Fe–Fe$_2$O$_3$ composites and pure iron controls according to ASTM E9–89a,\textsuperscript{16} utilizing an Instron 5969 universal test machine with a strain rate of 2 × 10$^{-4}$ s$^{-1}$. The specimens were in the form of circular cylinder 2 mm in diameter and 5 mm in length. As all the specimens were ductile materials, ultimate compressive strength (UCS) was determined from the stress when the total strain was 40%. At least three measurements were taken for each sample.

**Electrochemical measurements**

A three-electrode cell was used for electrochemical measurements with platinum as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode using an electrochemical workstation (CHI660C, China). The electrochemical measurements were performed in Hank’s solution at 37 ± 0.5°C.\textsuperscript{17} Electrochemical impedance spectroscopy (EIS) was carried out from 100 kHz to 10 mHz at open circuit potential value after 2-h immersion in Hank’s solution. The potentiodynamic polarization curves were measured from −1000 mV (vs. SCE) to 0 mV (vs. SCE) at a scanning rate of 0.33 mV s$^{-1}$. After potentiodynamic tests, corroded surfaces of all the specimens were observed by environmental scanning electron microscopy (ESEM, AMRAY-1910FE).

**Immersion test**

Experimental specimens (10 × 10 × 2 mm$^3$) were immersed in 50 mL Hank’s solution and the temperature was kept at 37 ± 0.5°C by water bath according to ASTM-G31–72.\textsuperscript{18} After 3, 10, and 30 days of immersion, the specimens were removed from the solution, rinsed with distilled water, and dried at room temperature. Changes on the surface morphologies of the specimens after immersion were characterized by SEM and equipped with an EDS attachment (EDAX GENESIS). After the corrosion products were removed into the solution and completely dissolved in HNO$_3$, the inductively coupled plasma atomic emission spectrometry (ICP-AES, Leeman, Profile) was employed to measure the concentrations of released ions in the solution. An average of three measurements was taken for each group.

**Cytotoxicity test**

The specimens were sterilized with ultraviolet radiation for at least 2 h prior to cytotoxicity test. Murine fibroblast cells (L929), rodent vascular smooth muscle cells (VSMC), and human umbilical vein endothelial cells (ECV304) were adopted to evaluate the cytotoxicity of experimental Fe–Fe$_2$O$_3$ composites with an indirect method. All the cell lines were purchased from American Type Culture Collection (ATCC) with passage number of L929, VSMC, and ECV304 cells maintained at 4, 4, and 5 in this study, respectively. L929, VSMC, and ECV304 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), with 10% fetal bovine serum, 100U·mL$^{-1}$ penicillin and 100 μg·mL$^{-1}$ streptomycin in a humidified atmosphere with 5% CO$_2$ at 37°C. Extracts were prepared using DMEM serum-free medium with a surface area/extraction medium ratio of 1.25 cm$^2$·mL$^{-1}$ in a humidified atmosphere with 5% CO$_2$ at 37°C for 72 h. Then, the extracts were centrifuged, and the
supernatant fluid was withdrawn as the experimental extraction medium. DMEM was set as the negative control and DMEM containing 10% of dimethyl sulfoxide as the positive control. A 100 μL cell suspension at the density of $5 \times 10^3$ cells/100 μL was seeded to each well of 96-well cell-culture plates and incubated for 24 h to allow attachment. Then, the medium was substituted by 100 μL of extraction medium. After 1, 2, and 4 days of incubation, respectively, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide was added to each well and the cells were cultured for 4 h in the incubator. Then, 100 μL of formazan solubilization solution (10% sodium dodecyl sulfate [SDS] in 0.01 M of HCl) was added to each well and incubated in the incubator overnight. The optical density (OD) of the specimens was measured with a microplate reader (Bio-RAD 680) at 570 nm, with a reference wavelength of 630 nm. A blank group of extraction media without cells was used to reduce the disturbance of iron ions on the OD of the solution. In addition, the amount of metal ions in the extraction medium was also measured by ICP-AES. The viability of cells was calculated by the following formula:

$$\text{Cell viability} = \frac{\text{OD(test)} - \text{OD(blank)}}{\text{OD(negative control)} - \text{OD(blank)}} \times 100\%$$

(Hemolysis test and platelet adhesion)

Diluted blood was prepared with normal saline (volume, 4:5) by human blood containing sodium citrate (3.8 wt %) in the ratio of 9:1 from a healthy volunteer (a consensus statement was assigned by the volunteer before the hemolysis and platelet adhesion test were performed). Experimental specimens were immersed in standard tubes containing 10 mL of normal saline preincubated at 37°C for 30 min separately. Then, 0.2 mL diluted blood was added to each tube and the mixture was incubated at 37°C for 60 min. Normal saline solution was used as a negative control and distilled water as a positive control. Then, all the tubes were centrifuged at 1000g for 5 min and the supernatant was moved to a 96-well plate for spectroscopic analysis at 545 nm with a microplate reader (Bio-Rad 680). Hemolysis ratio was calculated by the following formula and averaged by three replicates:

$$\text{Hemolysis} = \frac{\text{OD(test)} - \text{OD(negative control)}}{\text{OD(positive control)} - \text{OD(negative control)}} \times 100\%$$

where OD is the optical density at 545 nm.

For platelet adhesion, platelet-rich plasma (PRP) was prepared by centrifuging the whole blood for 10 min at the rate of 1000 rpm. In brief, 0.2 mL of PRP was added atop each specimen in a 24-well microplate and incubated at 37°C for 1 h. Then, the specimens were rinsed with phosphate-buffered saline to remove the nonadherent platelets and then fixed with 2.5% glutaraldehyde solutions at room temperature for 1 h, followed by dehydration in a gradient ethanol/distilled water mixture (50–100%) for 10 min each. The morphologies of platelet attached specimens were observed by ESEM.

Statistical analysis

All experiments were performed at least three times and the data were expressed in the form of mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance and $p < 0.05$ was considered statistically significant.

RESULTS

Microstructures of Fe–Fe$_2$O$_3$ composites

Figure 1 shows the XRD patterns of experimental Fe–Fe$_2$O$_3$ composites, with as-cast pure iron as the control. It was found that the Fe–Fe$_2$O$_3$ composites were composed of two phases with Fe (PDF#65–4899) as the dominant phase and FeO (PDF#06–0615) was detected instead of Fe$_2$O$_3$ (PDF#24–0072, raw material, data not shown in the result) even when the amount of Fe$_2$O$_3$ increased to 50 wt%, whereas α-Fe was the only phase for as-cast pure iron at room temperature. This may be ascribed to that Fe$_2$O$_3$ powder was reduced by pure iron powder at a high temperature. The possible reaction formula was given as follows. As the content of adding phases increased, the diffraction intensity of second phase increased.

$$\text{Fe} + \text{Fe}_2\text{O}_3 \rightarrow 3\text{FeO}$$

Figure 2 shows the representative optical micrographs of the experimental specimens, the EDS analysis results of Fe–2Fe$_2$O$_3$ composite, and grain size distribution of experimental Fe–Fe$_2$O$_3$ composites. Considering the large amount of Fe$_2$O$_3$ in Fe–10Fe$_2$O$_3$ and Fe–50Fe$_2$O$_3$ composites, only optical microstructures of Fe–2Fe$_2$O$_3$ and Fe–5Fe$_2$O$_3$ composites are shown here. It can be seen that adding phase (black phase) was uniformly distributed in the matrix and
the majority of the second phase precipitated along the grain boundaries of the Fe matrix without any obvious pores. The EDS analysis results (taking Fe–2Fe₂O₃ composite in Fig. 2 as an example) showed that the atomic ratio of Fe to O in position A [Fig. 2(d)] was about 1:1, whereas the composition in the matrix, that is position B [Fig. 2(e)] was almost Fe, indicating that the second phase appeared in Fe–2Fe₂O₃ composites was FeO, which was consistent with the results of XRD analysis. The average grain sizes of Fe–2Fe₂O₃ [Fig. 2(f)] and Fe–5Fe₂O₃ [Fig. 2(g)] composite specimens were 25 ± 10 and 34 ± 12 μm, respectively, much smaller than that of pure iron, which was about 100 μm.

**Compressive properties of Fe–Fe₂O₃ composites**

The compressive properties of Fe–Fe₂O₃ composites with as-cast pure iron as the control at room temperature are shown in Figure 3. Owing to the limit of sample size, only compressive test is appropriate for the mechanical properties test. For Fe–5Fe₂O₃ and Fe–10Fe₂O₃ composites, both the YS and UCS were largely increased, whereas the YS of

**FIGURE 2.** Representative optical micrographs of the experimental specimens (a) pure iron, (b) Fe–2Fe₂O₃, and (c) Fe–5Fe₂O₃. EDS analysis results of Fe–5Fe₂O₃ composite (d) second phase and (e) matrix, and grain size distribution of (f) Fe–2Fe₂O₃ and (g) Fe–5Fe₂O₃ composite. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**FIGURE 3.** Compressive properties of Fe–Fe₂O₃ composites with as-cast pure iron as the control at room temperature, UCS was obtained from stress when the strain was 40%. The samples of Fe–50Fe₂O₃ composite fractured before the strain reached to 40%, and hence no actual strength was obtained. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Fe–2Fe₂O₃ composite increased slightly compared with pure Fe. The UCSs of Fe–2Fe₂O₃ and Fe–5Fe₂O₃ composites were almost two times larger than that of pure iron. However, when the content of Fe₂O₃ reached 50 wt %, fracture happened before the deformation reached 40%, and hence no actual strength was obtained for Fe–50Fe₂O₃ composite (question mark, Fig. 3). Obviously, the addition of low amount of Fe₂O₃ greatly enhanced the UCS and YS of iron matrix.

Corrosion properties of Fe–Fe₂O₃ composites

Electrochemical corrosion behavior: Figure 4 shows the potentiodynamic polarization curves [Fig. 4(a)] and Nyquist plots [Fig. 4(b)] of Fe–Fe₂O₃ composites specimens immersed in Hank’s solution, with as-cast pure iron as the control. The average electrochemical parameters and corrosion rate are listed in Table I. It was found that the corrosion potential was decreased, whereas the corrosion current densities increased after the addition of Fe₂O₃ in the Fe matrix. The corrosion current increased first and then decreased when the amount of Fe₂O₃ varied from 2 to 50 wt % and Fe–5Fe₂O₃ composite showed the largest corrosion current. The corrosion current of Fe–5Fe₂O₃ composites was about 10 times of that of pure iron.

An equivalent circuit model $R_s(QR_t)$ (inset, Fig. 4(b)) for all the experimental specimens was proposed. The Nyquist plots [Fig. 4(b)] indicated the same results with the potentiodynamic polarization curves [Fig. 4(a)]. The diameters of the semicircle for all Fe–Fe₂O₃ composite groups were smaller than that for pure iron group, and Fe–5Fe₂O₃ had the smallest semicircle diameter, revealing its worst corrosion resistance. As the diameter of high-frequency capacitive loop can be considered as the charge-transfer resistance and smaller charge-transfer resistance corresponds to faster corrosion rate. The calculated results summarized in Table I further demonstrated the above results. Fe–5Fe₂O₃ composite showed the smallest transfer resistance $R_t$, followed by Fe–2Fe₂O₃, Fe–50Fe₂O₃, and Fe–10Fe₂O₃ composite and pure Fe possessed the largest transfer resistance.

Immersion corrosion behavior

Figure 5 shows the typical surface morphologies of Fe–Fe₂O₃ composite and pure iron specimens after polarization. For pure Fe, the surface almost kept intact with only very few corrosion pits [Fig. 5(a)], whereas on the surface of experimental Fe–2Fe₂O₃ and Fe–5Fe₂O₃ composites a lot of isolated deeper holes were presented. As for Fe–10Fe₂O₃ and Fe–50Fe₂O₃ composites, only small dimples were observed after the electrochemical measurement in Hank’s solution.

Immersion corrosion behavior

Figure 6 shows the released ion concentrations of iron in Hank’s solution at different immersion durations. It is found...
that the released ion concentration increased with a longer immersion time. The difference between released iron ion concentrations of experimental Fe–2Fe$_2$O$_3$ and Fe–5Fe$_2$O$_3$ composites with that of pure iron was insignificant after 3 and 10 days of immersion in Hank’s solution. However, a little higher Fe ion concentration was detected after 30 days of immersion compared to pure iron, indicating an elevated long-term corrosion rate of Fe–2Fe$_2$O$_3$ and Fe–5Fe$_2$O$_3$ composites. As for Fe–10Fe$_2$O$_3$ and Fe–50Fe$_2$O$_3$ composite, the released iron ion concentrations were much lower than the other two Fe–Fe$_2$O$_3$ composites ($p < 0.05$), which might be ascribed to the low corrosion rate of the materials and partial residual corrosion products on the surface. The corrosion rate of different materials calculated by released ion concentration after immersion tests is summarized in Table I.

Figure 7 shows the representative SEM images of Fe–Fe$_2$O$_3$ composites and pure iron after 30 days of immersion in Hank’s solution with and without corrosion products on the surface. It should be noted that there was no significant difference on the surface morphologies of different Fe–Fe$_2$O$_3$ composites after immersion in Hank’s solution, thus only one typical SEM image was presented. Figure 7(a) shows the surface morphologies of pure iron. The grain boundaries were obviously exposed and relatively uniform corrosion was observed macroscopically although some corrosion pits were visible under high resolution as shown in the inset of Figure 7(a). Additionally, partial corrosion products still could be seen on the surface of Fe–Fe$_2$O$_3$ composite specimens [Fig. 7(b)]. The corrosion products were porous and noncompact with some white particles precipitated on them, which was supposed to be calcium/phosphate compounds. After the corrosion products were removed, the surface of Fe–Fe$_2$O$_3$ composites [Fig. 7(c)] presented the same morphology with pure iron, and severe corrosion attack inside the grains with elevated margins could be observed, indicating similar corrosion mechanism of Fe–Fe$_2$O$_3$ composites and pure iron. Besides, small particle can be observed on the surface of Fe–Fe$_2$O$_3$ composites. It is supposed to be residual second phases after the surrounding Fe matrix corroded.

**Cytotoxicity tests of Fe–Fe$_2$O$_3$ composites**

Figure 8 shows the cell viability of (a) murine fibroblast cells L929, (b) rodent vascular smooth muscle cells VSMC, and (c) human umbilical vein endothelial cells ECV304.
expressed as a percentage of the viability of cells cultured in the negative control after incubation in pure iron and Fe–Fe₂O₃ composites extraction mediums for 1, 2, and 4 days, respectively. It can be seen that (i) the viabilities of L929 cells increased as the incubation time increases. An appropriate cell viability of 80–90% in extracts of Fe–Fe₂O₃ composites could be observed compared with the negative control after culturing for 4 days. (ii) For VSMC, the cell viabilities in Fe–Fe₂O₃ composites and pure iron groups increased on day 2 but decreased on day 4. The addition of 2 and 5 wt % Fe₂O₃ led to decreased cell viabilities after 1 and 2 days of incubation in comparison with the other specimens \( p < 0.05 \). In addition, all the sample extracts led to decreased cell viabilities compared to the negative control (cell viability was considered as 100%) \( p < 0.05 \), which was in consistence with the early result reported by Liu and Zheng \(^ {12} \) on various Fe–X binary alloys fabricated by casting and hot deformation and our previous study \(^ {12} \) of (i), the number of platelets adhered on the Fe–Fe₂O₃ composites. Immediately after the specimens were immersed in Hank’s solution, corrosion reaction occurred resulted from galvanic corrosion between grain/phase boundaries and grains. Formula \( 4 \) and \( 5 \) depict the anode and cathode reaction of pure iron in Hank’s solution, respectively. The only difference from pure Fe was that the addition of Fe₂O₃ largely increased the amount of phase boundaries, the active sites, as shown in Figure 11(a). Electrons generated from the dissolution of iron (anode reaction) moved from iron matrix to grain/phase boundaries.

\[
\begin{align*}
Fe + 2OH^- - 2e^- & \rightarrow Fe(OH)_2 \quad \text{(anode reaction)} \\
O_2 + 2H_2O + 4e^- & \rightarrow 4OH^- \quad \text{(cathode reaction)}
\end{align*}
\]

As the corrosion proceeded, iron hydroxide formed [Fig. 11 (b)] and Ca/P compounds precipitated on the surface of samples [Fig. 11(c)]. This process was similar to that of Fe–X (X = W, CNTs) composites prepared by SPS composite which has been discussed in detail in our previous study. \(^ {13} \) However, for Fe–Fe₂O₃ composites, the difference is that second-phase Fe₂O₃ is a kind of semiconductor\(^ {23} \) whereas W and CNT are conductive. Electrons cannot move through Fe₂O₃ and gather at the grain/phase boundaries. As the corrosion spreads from the grain/phase boundaries to inner interior of the grains, much more active sites and relatively fine grains led to general corrosion of the Fe–Fe₂O₃ composites. Besides, owing to the fact that corrosion happened inside the grains, protrudent edges could be observed when corrosion products were removed away [Fig. 11(d)] the enlarged schematic image of sample surface after the corrosion products was moved away, whereas for Fe–W composites, the effect of grain boundaries on the corrosion behaviors was limited as galvanic corrosion between iron.

**Hemocompatibility of Fe–Fe₂O₃ composites**

The hemolysis percentage of experimental Fe–Fe₂O₃ composites and pure iron specimens is shown in Figure 9. The hemolysis ratios of pure iron, Fe–2Fe₂O₃, Fe–5Fe₂O₃, Fe–10Fe₂O₃, and Fe–50Fe₂O₃ composite were about 2.4 ± 0.3, 2 ± 0.07, 2.1 ± 0.3, 2.66 ± 0.16, and 2.74 ± 0.27%, respectively, and were all <5%, the judging criterion for excellent blood compatibility in ASTM F756-08. \(^ {19} \)

The morphologies of adhered human platelet on the Fe–Fe₂O₃ composites and pure iron specimens are shown in Figure 10. It can be seen that (i) the Fe–Fe₂O₃ composite specimens and pure iron had already corroded after immersed in PRP for 1 h and large amount of corrosion products precipitated on the sample surface. (ii) As a result of (i), the number of platelets adhered on the Fe–Fe₂O₃ composite and pure iron was difficult to count. However, based on the observed platelets, there was no significant difference among the number of adhered platelets on pure iron and Fe–Fe₂O₃ composites \( p > 0.05 \) except for Fe–50Fe₂O₃, on which platelets were difficult to be found. (iii) Almost all the platelets adhered on Fe–Fe₂O₃ composites and pure iron kept the round shape and showed no sign of pseudopodia-like structures, implying a negative activation, as indicated by morphologies of platelets at high magnification.

**DISCUSSION**

**Effect of Fe₂O₃ on the corrosion behaviors of Fe–Fe₂O₃ composites**

Based on the surface morphologies of Fe–Fe₂O₃ composites and pure iron after immersed in Hank’s solution, it is supposed that the corrosion mechanism of Fe–Fe₂O₃ composites is similar to pure iron. Figure 11 schematically illustrates the corrosion mechanism of Fe–Fe₂O₃ composites. Immediately after the specimens were immersed in Hank’s solution, corrosion reaction occurred resulted from galvanic corrosion between grain/phase boundaries and grains. Formula \( 4 \) and \( 5 \) depict the anode and cathode reaction of pure iron in Hank’s solution, respectively. The only difference from pure Fe was that the addition of Fe₂O₃ largely increased the amount of phase boundaries, the active sites, as shown in Figure 11(a). Electrons generated from the dissolution of iron (anode reaction) moved from iron matrix to grain/phase boundaries.
matrix and W played the leading role on the corrosion process.

The effect of Fe$_2$O$_3$ on the corrosion rate of Fe–Fe$_2$O$_3$ composites could be expressed in two aspects. On the one hand, the addition of Fe$_2$O$_3$ in the iron matrix decreased the grain size of the material and increased structural defects. Moreover, the SPS technique contributes to the fine grain size of the composites$^{24,25}$ All these might be favorable to the increment of corrosion rate, as Moravej et al.$^7$ had reported that electroformed pure iron possessing a fine grain size exhibited faster degradation rate than as-cast pure iron. On the other hand, Fe$_2$O$_3$ was semi-conductive$^{23}$ and micro-galvanic corrosion between these phases and iron matrix was difficult to happen. However, the uniformly dispersion and coexistence of second phase in the boundary region might serve as a barrier to electron transfer from iron matrix to the surrounding grains, thus decreased the corrosion rate. This may give an explanation for the elevated corrosion rate for Fe–2Fe$_2$O$_3$ and Fe–5Fe$_2$O$_3$ composites but a decreased for Fe–10Fe$_2$O$_3$ and Fe–50Fe$_2$O$_3$ composites. From this point of view, Fe–5Fe$_2$O$_3$ composite may be the most suitable for biodegradable stent material with the fastest corrosion rate among those composites and a corrosion rate comparable to Fe–W composites.

In general, there are two methods to calculate the corrosion rate of iron-based materials in \textit{in vitro} immersion test, released iron ion concentrations in the solution based on CR $= \frac{cV}{St^2}$ and weight loss measurement based on CR $= 8.76 \times 10^{-4} W A t^{-1.6,8,12}$ Large amount of corrosion products precipitate on the surface of iron specimens after long-term immersion in simulated body fluid and the corrosion products layer is loose. The amount of Fe in the corrosion products should be taken into account for both the two methods. For the first method, only mechanical cleaning method is appropriate for the removal of corrosion products. For weight loss measurement, both mechanical and chemical cleaning methods are suitable. However, after corrosion, corrosion products will partially remain on the surface of specimens after mechanical cleaning inevitably, which leads to great error on the evaluation of corrosion rate of the material. On the other hand, in the view of stent material, fast endothelialization happens after the implantation of an iron stent and the corrosion environment has changed. Pierson et al.$^{28}$ has validated that pure iron encapsulation within the arterial wall extracellular matrix degrades faster than that exposed to blood in the arterial lumen using a novel experimental model by implanting iron wire into rat artery wall and artery lumen, respectively. Therefore, it seems that the \textit{in vitro} corrosion test is unnecessary and unsuitable for mimicking the \textit{in vivo} biocorrosion in the long run.

**Biocompatibility of Fe–Fe$_2$O$_3$ composites**

Generally, the cytotoxicity of degradable metallic materials mainly relies on the released metal ions against cell metabolic activities$^{10}$ and toxicity of the degradation products.$^{29}$ Iron has been validated as a biocompatible element for human body. Zhu et al.$^{30}$ pointed out that the critical concentration of Fe ion to induce cytotoxicity of endothelial cells is 50 $\mu$g mL$^{-1}$, and much lower Fe concentration ($<10 \mu$g mL$^{-1}$) may even have a favorable effect on the metabolic activities of ECs. Our results are in good agreement with the above statement. Fe ion concentrations in the pure Fe, Fe–2Fe$_2$O$_3$, Fe–5Fe$_2$O$_3$, Fe–10Fe$_2$O$_3$, and

![FIGURE 8. Cell viability of (a) L929, (b) VSMC, and (c) ECV304 after 1, 2, and 4 days of incubation in the experimental Fe–Fe$_2$O$_3$ composites extraction media, with pure iron as the control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]](image)
Fe–50Fe₂O₃ composite DMEM extraction medium for cytotoxicity test are 5.21 ± 0.28, 8.63 ± 0.76, 10.52 ± 0.42, 6.42 ± 0.35, and 7.22 ± 0.25 mg mL⁻¹, respectively, and more than 90% cell viabilities of ECV304 were observed for all experimental materials compared with negative control. However, for VSMCs, significant decreased cell viabilities can be found after incubation in the extracts for 4 days. As reported by Mueller et al., ferrous iron ions could reduce the VSMC proliferation rate in the sight of gene expression, and the regulation of iron on cellular response may play a beneficial role in the control of neointima proliferation. Schaffer et al. also confirmed that Fe²⁺ and Fe³⁺ ions repressed the migration of SMCs at concentration of 1 mM and good EC coverage and endothelial proliferation were found on iron wires. As for L929 cell line, decreased cell viability compared with negative control might be ascribed to iron ions in the extracts. On the other hand, the cell viability of L929 increased as the incubation time increased. This may be owing to the function of iron transport protein transferrin and the iron storage protein ferritin. As incubation time increases, partial iron ions may combine with transferrin and ferritin, and thus the amount of iron in the extract media decreases, resulting in increased cell viability of L929.

Considering the adding second phase, Fe₂O₃ is a well-accepted magnetic material for various biomedical applications such as targeted delivery, cell labeling, and magnetic hyperthermia. Both in vivo and in vitro studies on Fe₂O₃ nanoparticles indicated that Fe₂O₃ is well tolerated and shows excellent biocompatibility. In this study, Fe₂O₃ might be partially or completely reduced to FeO, as indicated by the XRD results shown in Figure 1. However, there are very few studies on the biocompatibility of FeO. Zhu et al. reported that FeO film synthesized by plasma immersion ion implantation and deposition exhibited excellent hemocompatibility and HUVECs shown good adhesion and proliferation behavior on FeO films. When the iron matrix degrades, FeO/Fe₂O₃ particles will be left in the surrounding vessel tissues, together with partial corrosion products of pure iron. As reported in the animal test of pure iron stent, after 18-month implantation, iron laden macrophages and multinucleated giant cells were found to accumulate within the adjacent media or fibrous adventitia of the aorta. Thus, as the main composition of degradation products of iron, it is supposed that these FeO or Fe₂O₃ particles might be phagocytized by macrophages and the
biocompatibility of Fe₂O₃ should not be a problem, as indicated by the exciting results of early animal tests. However, comprehensive interaction between corrosion products and macrophages still needs to be further investigated.

Hemolysis ratio is employed to determine the destructive effect of medical materials to the erythrocyte. Iron is a component of hemoglobin and a variety of enzymes; therefore it is suggested that iron will not lead to disruptive effect on erythrocyte. As reported by Gu et al., high hemolysis percentage of magnesium alloys may be ascribed to the significant increase in pH value as a result of the corrosion of Mg alloys. However, no significant increase of pH value after the corrosion of iron-based composites was found, which also explains the low hemolysis ratio of iron-based materials. Platelet adhesion test can be used to detect the likelihood of formation of thrombus when medical devices are in contact with blood. 316L stainless steel is widely used as stent material owing to its excellent blood compatibility. The shape of platelets adhered on Fe–Fe₂O₃ composites kept its integrative shape and was better than 316L SS as reported by Zhu et al. There was not much difference on the number of adhered platelets among different Fe–Fe₂O₃ composites and pure iron in this study, whereas the number of adhered platelets adhered on Fe–Fe₂O₃ composites was larger than 316L SS as reported by Liu and Zheng. As revealed by the SEM image of platelets in the literature, the low hemolysis ratio and good antiplatelet adhesion properties into consideration, it is concluded that Fe–Fe₂O₃ composites possess excellent hemocompatibility.

CONCLUSIONS
A series of Fe–Fe₂O₃ composites with different contents of Fe₂O₃ powders were fabricated by SPS. FeO was detected as a new phase in the Fe–Fe₂O₃ composites instead of Fe₂O₃. The Fe–2Fe₂O₃ and Fe–5Fe₂O₃ composite exhibited increased UCS and enhanced corrosion rate than pure iron, whereas with the increase of Fe₂O₃ to 10 and 50 wt %, the corrosion rate decreased. The extracts of Fe–Fe₂O₃ composites showed no cytotoxicity to ECV304 and L929 cells, whereas significantly suppressed cell viabilities of VSMC cells. The hemolysis percentages of all Fe–Fe₂O₃ composites and pure iron were all <5% and platelets adhered on these specimens were round with almost the same amount. In summary, Fe-based composite reinforced by 5 wt % Fe₂O₃ is a promising alternative for biodegradable biomaterial with good combination of mechanical and biocompatible performance as well as a proper degradation rate.

REFERENCES