In vitro study on newly designed biodegradable Fe-X composites (X = W, CNT) prepared by spark plasma sintering

J. Cheng,1 Y.F. Zheng1,2
1Center for Biomedical Materials and Tissue Engineering, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China
2State Key Laboratory for Turbulence and Complex System and Department of Materials Science and Engineering, College of Engineering, Peking University, Beijing 100871, China

Received 11 March 2012; revised 20 June 2012; accepted 7 July 2012
Published online 29 January 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.b.32783

Abstract: Early in vivo animal test on pure iron coronary stent had proved that it is a promising candidate material as biodegradable metal, despite a faster degradation rate and uniform degradation mode is expected. In this work, Fe-X (X = W, CNT) composites were prepared from powders of pure iron and the additive secondary phase X using the spark plasma sintering (SPS) method, aiming to obtain a higher corrosion rate and a more uniform corrosion mode in physiological environment. The microstructures, mechanical properties, corrosion behaviors, and in vitro biocompatibility of these Fe-X composites were investigated. It was found that the additives were uniformly distributed in the iron matrix and relatively high dense Fe-X composite bulk samples were obtained after sintering by SPS. Both the yield strength and ultimate compressive strength increased when compared with that of as-cast pure iron. The corrosion mode of Fe-X composites turned out to be uniform corrosion instead of localized corrosion. Electrochemical measurements and immersion tests indicated that the addition of W and CNT could increase the corrosion rate of the iron matrix. From the results of cytotoxicity evaluation, it was found that all the Fe-X composites extracts induced no obvious cytotoxicity to L929 cells and ECV304 cells whereas significantly decreased cell viabilities of VSMC cells. The hemocompatibility tests showed that all the hemolysis percentage of Fe-X composites were less than 5%, and no sign of thrombogenicity was observed. It might be concluded that Fe composites with suitable second phase can exhibit higher strength, faster degradation rate, and uniform degradation mode than those of pure iron and are promising candidates for future development of new degradable metallic stents. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 101B: 485–497, 2013.

Key Words: biodegradable metal, Fe-X composite, spark plasma sintering, corrosion, biocompatibility


INTRODUCTION

Biodegradable metals have gained widespread attention for making cardiovascular stents because of their numerous advantages over traditional bioinert materials.1,2 Mg-based3–8 and Fe-based9–13 alloys are two typical classes of metallic materials that can be degraded in physiological environment. Biodegradable Mg-based materials have been a research hotspot recently. However, several shortcomings such as poor mechanical properties compared with 316L stainless steel, fast corrosion rate in the body fluid, and hydrogen evolution during degradation may restrict their future application.

Iron is considered to be an alternative candidate for biodegradable metallic stent material. Early results of in vivo animal experiments have validated the feasibility of pure iron as coronary stents. Stents made of pure iron were implanted into the descending aorta of New Zealand white rabbits14 and minipigs15 and coronary arteries of porcines.16 These animal testing results showed that, on the one hand, there were no occurrence of acute neointimal proliferation and no local or systemic iron toxicity, indicating good biocompatibility of iron, however, the pure iron stent strut still stayed within the blood vessel even after 12 months, which means that a fast degradation rate was demanded.14,15

To increase the degradation rate of pure iron, a lot of new researches have been conducted on iron-based materials, such as alloying,10,11 surface modification,17 and new
fabrication method. As proposed by Schinhammer et al., two criteria might be taken into account to achieve increased degradation rate: one is the addition of less noble alloying elements within the solubility limit in Fe to make the Fe matrix more susceptible to corrosion; the other is the addition of noble alloying elements to generate small and finely dispersed intermetallic phases (IMPs) to generate microgalvanic corrosion with Fe matrix. The first criteria has been tried by the present authors, eight different alloying elements (Mn, Co, Al, W, Sn, B, C, and S) were chosen to investigate to effect of alloying elements on biodegradability of pure iron, but the effect was not significant. For the second criteria, the formation and dispersion of IMPs in the iron matrix are difficult to control by traditional casting fabrication technology. Our idea is to fabricate Fe-X (here X represents the second phase that is added in the composite) composite materials, in which the second phase X is functionalized as cathode and the Fe matrix acts as the anode to generate micro-galvanic corrosion. In this way, a fast degradation rate of Fe-based material can be obtained. Besides, if the second phase X is uniformly distributed in the Fe matrix, micro-galvanic corrosion would happen everywhere, taking the place of pitting corrosion of pure iron matrix, and macroscopically uniform corrosion can be achieved. In addition, the mechanical properties of Fe-X composite may be improved in comparison with that of the pure iron, because of the reinforcement of the X phase.

Spark plasma sintering (SPS) is a new rapid sintering method, which was developed recently for the fabrication of ceramics and composites. Using this method, the powders are heated by spark discharge between particles and sintered directly at a higher temperature with a short dwelling time compared with current methods such as hot pressing, resulting in uniform sintering and fine grains of materials.

In this study, typical metal W, as well as a nonmetal carbon nanotube (CNT) were chosen as the adding X phase to fabricate Fe-X composites by SPS to evaluate their effect on the degradation and biocompatibility of pure iron. The standard potential of W (−0.04) and CNT (+0.2) are higher in comparison with that of pure iron (−0.44), so they can act as superior cathode. From the viewpoint of biocompatibility, tungsten coil has been explored as versatile embolic material for different aneurysms and tumor-nourishing vessels, whereas CNTs have been widely used for biomedical applications such as tissue engineering, drug delivery, etc. Thus, both W and CNT are acceptable in the consideration of choosing second phases. The microstructures, mechanical properties, corrosion behavior, cytotoxicity, and hemocompatibility of these composites were investigated, with both as-cast pure iron and pure iron prepared by SPS (SPS pure Fe) as the controls.

**MATERIALS AND METHODS**

**Material preparation**

Pure iron powder (99.9%, particle size <10 μm, Alfa), tungsten powder (99.8%, particle size <75 μm, SCRC), and multi-wall CNT (10–20 nm in diameter and 5 μm in length, CNano) were used as the starting materials. Two compositions which contain 2 wt % and 5 wt % of W were prepared for Fe-W composite, whereas 0.5 wt % and 1 wt % for CNT. The powders of W and iron were mixed with an ARE-310 hybrid mixer (Japan Thinky Co.) at 2000 rpm for 5 min after manually mixed in a mortar, and ball milling was used to mix iron and CNT powders. The mixture powders were put into a 20 mm diameter graphite die and sintered under vacuum by SPS technique using a SPS-1050 system (Sumitomo Coal Mining Company). The sintering process was performed under a pressure of 40 MPa. After the powders were heated to 950°C and kept for 5 min, the pressure was released and the samples were cooled to room temperature. Table I summarizes the composition and SPS conditions of various Fe-X composites. As-cast pure iron (99.9%) and pure iron produced by SPS were used as controls.

The as-sintered Fe-X composite and pure iron disk samples (20 mm in diameter and 8 mm in height) and as-cast pure iron were cut into square pieces (10 × 10 × 2 mm³) for microstructural characterization, corrosion, cytotoxicity, and hemocompatibility tests. Each specimen was mechanically polished to 2000 grit, then ultrasonically cleaned in absolute ethanol and dried in the open air. Before cytotoxicity test, the specimens were further sterilized with ultraviolet radiation for at least 2 h.

**Microstructural characterization and composition analysis**

X-ray diffraction (XRD) (Rigaku DMAX 2400) using Cu Kα radiation was used to identify the constituent phases of Fe-X composites and pure iron controls with a scan range of 10–100° and a scan rate of 8°/min. Optical microscopy (Olympus BX51 M) was used to observe the surface morphology after the specimens were etched with a 4% HNO₃/alkohol solution and energy dispersive spectrometer (EDS) were used for chemical composition analysis. The densities of Fe-X composites and pure iron controls were determined by the Archimedes’ method in absolute ethanol.

**Mechanical test**

The mechanical properties of experimental Fe-X composites and pure iron controls were determined by compressive
test according to ASTM E9-89a. The specimens were in the form of circular cylinder 2 mm in diameter and 5 mm in length. The compressive test was performed at a strain rate of 2 \times 10^{-4}/s with an Instron 5969 universal test machine. As all the specimens were ductile materials, ultimate compressive strength was determined from the stress when the total strain was 40%. An average of at least three measurements was taken for each group.

**Electrochemical measurements**

The electrochemical measurements were performed using an electrochemical workstation (CHI660C, China) at the open circuit potential (OCP) measurement was maintained between the reference electrode, and the auxiliary electrode (SCE) were set as the working electrode, auxiliary electrode, and the reference electrode, respectively. The electrochemical measurements were performed using an electrochemical workstation (CHI660C, China) at the open circuit potential (OCP). A three-electrode cell was used for electrochemical measurements. The specimen, a platinum electrode, and a saturated calomel electrode (SCE) were set as the working electrode, auxiliary electrode, and the reference electrode, respectively. The open circuit potential (OCP) measurement was maintained up to 7200 s. Electrochemical impedance spectroscopy (EIS) was performed from 100 kHz to 10 mHz at OCP 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.

**Immersion test**

The immersion test was performed in Hank’s solution according to ASTM-G31-72. Experimental specimens (10 × 10 × 2 mm³) were immersed in 50 ml solution and the temperature was kept at 37 ± 0.5°C by water bath. After 3, 10, and 30 days 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 environmental scanning electron microscopy (ESEM, AMRAY-1910FE), equipped with an EDS attachment. The surface morphologies of the specimens after immersion were characterized by environmental scanning electron microscopy (ESEM, AMRAY-1910FE), equipped with an EDS attachment.

**Hemolysis test and platelet adhesion**

Healthy human blood containing sodium citrate (3.8 wt %) in the ratio of 9:1 was taken and diluted with normal saline (4:5 by volume). Specimens of Fe-X composites and pure iron controls were soaked in standard tubes containing 10 ml of normal saline that were previously incubated at 37°C for 30 min separately. Then 0.2 ml of diluted blood was added to each tube and the mixture was incubated at 37°C for 60 min. About 0.2 ml of diluted blood added in distilled water was used as a positive control, whereas normal saline solution with diluted blood as a negative control. After this period, all the tubes were centrifuged at 1000 × g for 5 min and the supernatant was transferred to a 96-well plate. The absorbance was measured with a microplate reader (Bio-Rad 680) at 545 nm. Hemolysis percentage was calculated according to the following formula based on the average of three replicates:

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

For platelet adhesion, platelet-rich plasma (PRP) was obtained by centrifuging the whole blood at 1000 rpm/min for 10 min. The specimens were placed in 24-well microplates, and 0.2 ml of PRP was added atop each specimen and incubated at 37°C for 1 h. After that the specimens were gently rinsed with phosphate-buffered saline (PBS) 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%, 60%, 70%, 80%, 90%, and 100%) for 10 min each and finally freeze-dried. The surfaces of platelet attached specimens were observed by ESEM.

**RESULTS**

**Microstructures of Fe-X composites**

Figure 1 shows the XRD patterns of experimental Fe-X composites, with as-cast pure iron and pure iron prepared by SPS (SPS pure Fe) as the controls. It is found that most of
Fe-X composites are composed of two phases, with $\alpha$-Fe (PDF#65-4899) as the dominant phase, whereas for as-cast and SPS pure iron, $\alpha$-Fe is the only phase at room temperature. Fe$_3$C (PDF#35-0772) phase is found in Fe-C composites without any carbon phase, and Fe$_7$W$_6$ (PDF#42-1209) is detected within Fe-5W composites except for $\alpha$-Fe and W (PDF#04-0806). However, a Fe$_7$W$_3$C (PDF#41-1351) phase is found in Fe-2W specimens. The appearance of C in Fe-2W specimen may come from the graphite die used in the sintering process.

Figure 2 displays the optical microstructures and representative grain size distribution of the experimental specimens. It can be seen that adding phases are uniformly dispersed in the matrix and the majority of the second phase precipitates along the grain boundaries of the Fe matrix without any obvious pores. The average grain size of pure iron specimen prepared by SPS is about 35 $\mu$m, much smaller than that of as-cast pure iron. The addition of both W and CNT largely decrease the grain size of pure iron, with the average grain size of 18 $\mu$m and 20 $\mu$m for Fe-5W and Fe-0.5CNT, respectively. Besides, a significant grain size decrease can be observed as the content of second phase increases and CNTs have a greater impact on the grain size of the material because of their fine profile. The density of all the specimens is listed in Table I, and it reveals that relatively high dense bulk composites are obtained after SPS process.
Compressive properties of Fe-X composites

Figure 3 depicts the compressive properties of Fe-X composites with as-cast and SPS pure iron as the controls at room temperature. Because of the limit of sample size, only compressive test is appropriate for the mechanical properties test. It reveals that both the yield strength (YS) and ultimate compressive strength (UCS) of SPS pure iron increase in comparison with that of as-cast pure iron. This may be because the grain size of SPS pure iron is smaller than that of as-cast pure iron. The UCS of Fe-X composites are significantly increased, especially for Fe-CNT composites. However, the differences of YS between different Fe-X composites and SPS pure iron are not significant and only a slight increase can be observed.

Electrochemical corrosion behavior

The typical potentiodynamic polarization curves and Nyquist plots of Fe-X composites specimens immersed in Hank’s solution, with as-cast and SPS pure iron as the controls are presented in Figure 4(a,b). The average electrochemical parameters and corrosion rate are listed in Table II. It is found that the corrosion potential is greatly decreased after the addition of second phase for all the composite specimens. The addition of second phases in pure iron increase the corrosion current densities compared

![Figure 3](https://example.com/fig3.png)

**Figure 3.** Compressive properties of Fe-X composites with as-cast and SPS pure iron as the control. Ultimate compressive strength was determined from the stress when total strain was 40%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

![Figure 4](https://example.com/fig4.png)

**Figure 4.** (a) Potentiodynamic polarization curves and (b) Nyquist plots of Fe-X composites specimens immersed in Hank’s solution, with as-cast and SPS pure iron as the control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

<table>
<thead>
<tr>
<th>TABLE II. Electrochemical Data and Corrosion Rate Calculated From Different Measurements of Fe-X Composites With As-Cast and SPS Pure Iron as the Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure iron</td>
</tr>
<tr>
<td>As-cast</td>
</tr>
<tr>
<td>SPS</td>
</tr>
<tr>
<td>Fe-W</td>
</tr>
<tr>
<td>2 wt %</td>
</tr>
<tr>
<td>5 wt %</td>
</tr>
<tr>
<td>Fe-CNT</td>
</tr>
<tr>
<td>0.5 wt %</td>
</tr>
</tbody>
</table>

\( V_{corr} \), corrosion potential; \( I_{corr} \), corrosion current density; \( v_{corr} \), corrosion rate in terms of penetration rate; corrosion rate measured by immersion test was calculated by released ion concentration after 30 days immersion in Hank’s solution.
with pure iron. Furthermore, the corrosion current densities increase with the increasing content of additive phases. The Nyquist plots [Figure 4(b)] indicate the same results with the potentiodynamic polarization curves [Figure 4(a)]. The diameters of the semicircle for Fe-W and Fe-CNT composites are smaller than that for as-cast and SPS pure iron groups, revealing their worse corrosion resistance. Because 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.  

Immersion behavior

Figure 5 presents the released ion concentrations of iron in Hank’s solution at different immersion durations. It is found that the ion concentration released increases with a longer immersion time. The released iron concentration of SPS pure iron is higher compared with as-cast pure iron after all three immersion durations, and the difference becomes larger as the immersion time increases. The dissolution of iron concentrations from all the Fe-X composites are almost the same after immersion in Hank’s solution for 3 days, except for Fe-1CNT with a significantly higher ion concentration. Generally, higher content of second phase is related to higher released ion concentration, suggesting faster corrosion rate of the experimental specimens. Addition of W has no significant effect on the increment of long-term corrosion rate, whereas CNTs significantly increase the corrosion rate compared with pure iron, as revealed by the Fe ion concentration released in the solution after 30-day immersion. However, the corrosion rate calculated by released ion concentration after immersion tests are at the same order of magnitude for all the Fe-X composites compared with both as-cast and SPS pure iron controls, except for Fe-0.5CNT, as shown in Table II. In addition, it should be noted that the released Fe ion concentration from Fe-CNT composites are comparable to as-cast pure iron and lower than Fe-W composites after 10 days immersion, which is not consistent with results after 3 and 30 days immersion. This may be ascribed to the partial residual corrosion products adhered on the sample surface, as shown in Figure 6(a).

Figure 6(a) shows the surface morphologies of the Fe-X composites after 3, 10, and 30 days immersion in Hank’s solution, with as-cast and SPS pure iron as the controls. After being immersed in Hank’s solution for 3 days, localized corrosion can be observed with brown corrosion products covered on the surface at the edge of pure iron and Fe-W specimens, whereas for Fe-CNT specimens, corrosion products almost cover the whole surfaces, indicating a relatively uniform corrosion mode. When the immersion duration comes to 10 days, surfaces of all specimens are covered with brown corrosion product, as illustrated in Figure 6(a). However, the corrosion products are easily taken off from the surface of specimens when they are removed from the Hank’s solution due to poor adhesion between the corrosion products and the substrate beneath.

Figure 6(b) presents the XRD patterns of Fe-X composites and two pure iron specimens after 30 days’ immersion in Hank’s solution. For each kind of Fe-X composite, only one pattern is shown in the figure. It is found that the main compositions of corrosion products are iron oxide $(\text{Fe}_2\text{O}_3, \text{PDF} \#52-1449)$, iron hydroxide $(\text{Fe(OH)}_2, \text{PDF} \#38-0032)$, and goethite $(\text{Fe(OH)}_3, \text{PDF} \#29-0713)$ for pure iron, meanwhile XRD spectrums of Fe-W and Fe-CNT show a rather amorphous pattern with a very low intensity, but it approaches the pattern of $\text{Fe}_2\text{O}_3$. Figure 6(c) shows the SEM images of Fe-X composites after 30 days immersion in Hank’s solution with as-cast and SPS pure iron as the controls. It can be seen that (i) The grain boundaries of as-cast pure iron is under obvious and severe corrosion attack inside the grains with an elevated margin can be observed. Lamellar structures showing different corrosion depth are observed with some deep pits visible [marked by arrows in the inset of Figure 6(a)], which indicates the localized corrosion of as-cast pure iron. (ii) Partial corrosion products still can be observed on the surface of SPS pure Fe with no localized attack. The corrosion products are porous, non-compact and composed of Fe, O, Ca, and P, as revealed by EDS results of corroded surface (shown in the inset of SPS pure Fe). (iii) The surfaces of Fe-W and Fe-CNT are full of small tiny holes, with small particles lying at the bottom of holes for Fe-W specimens. EDS analysis indicates that the main composition of these small particles is Fe and W, as displayed in the lower right corner of Figure 6(c). So it is supposed that corrosion initiates around the small particles and then spreads out. Severer corrosion happens when it comes nearer to the particles. SEM image showing cross-sectional profile of Fe-W [Figure 6(c)] after 30 days immersion in Hank’s solution presents almost the same corrosion depth with irregularly notched margin, suggesting uniform corrosion of Fe-W composite.

Cytotoxicity tests of Fe-X composites

Figure 7 illustrates the cell viability of (a) murine fibroblast cells L-929, (b) rodent VSMC, and (c) human umbilical vein endothelial cells ECV304 expressed as a percentage of the
viability of cells cultured in the negative control after 1, 2, and 4 days incubation in pure iron and Fe-X composites extraction mediums. It can be seen that (i) the L-929 cell viability increases as the incubation time increases. No significant reduction in viability of L-929 cells in all the extracts of pure iron, Fe-W composite and Fe-CNT composite with
appropriately 85% cell viability of negative control can be observed after culturing for 4 days. (ii) For VSMC, the cell viability in all Fe-X composites and pure iron groups increases on day 2 but decreases on day 4. In addition, both pure iron and Fe-X composites extracts lead to decreased cell viabilities in comparison with the negative control. This may be attributed to the inhibitory effect of Fe ions released in the extracts on the proliferation of VSMC cells. (iii) For ECV304 cells, all the Fe-X composites and pure iron groups exhibit higher cell viabilities than that with VSMC and almost no cytotoxicity can be observed. This is in good consistence with results reported by Zhu et al. that low iron concentration (<10 μg/ml) does not induce cytotoxicity on endothelial cells.

Hemocompatibility of Fe-X composites
Figure 8 shows the hemolysis percentage of experimental Fe-X composites and pure iron specimens. The hemolysis ratio of pure iron and Fe-W composites are all less than 3%, whereas the hemolysis ratio of Fe-CNT composites are a little higher than that of as-cast pure iron and reach to about 4% but are still lower than 5%, a judging criterion for excellent blood compatibility. In addition, the content of additive phase has no significant effect on the hemolysis percentage of all the experimental composite specimens.
The morphologies of adhered human platelet on the Fe-X composites and pure iron specimens are shown in Figure 9. There is no significant difference on the shape and number of platelets among a certain kind of Fe-X composite with different contents of X, so only one representative image is shown here as a representative. (i) The number of platelets adhered on the Fe-W composites has no significant difference compared with that on pure iron, whereas for Fe-CNT group, the number is much higher. (ii) Almost all the platelets adhered on the specimens keep the round shape and show no sign of pseudopodia-like structures, implying a negative activation, whereas very few platelets on Fe-CNT specimens have already broken up. (iii) Both the Fe-X composite specimens and pure iron are corroded after immersed samples in PRP for 1 h and the surface are covered with a lot of corrosion products.

DISCUSSION

Previous researches\textsuperscript{14–16} have validated the feasibility of pure iron as biodegradable stent materials, however, a faster degradation rate and better mechanical properties are desirable and localized corrosion should be avoided. The purpose of this study is to investigate the effect of the second phase on the degradation and biocompatibility of pure iron by forming Fe-X composites.

Corrosion mechanism of Fe-X composites

Based on the surface morphologies of experimental Fe-X composite and pure iron specimens after different immersion period in Hank’s solution and other accepted corrosion mechanism,\textsuperscript{11,13} the possible corrosion mechanism of Fe-X composites in comparison with pure iron was discussed in details, with the corresponding schematic illustration shown in Figure 10. It could be divided into three steps:

\textbf{(1) Initial corrosion reaction:} immediately after Fe-X composite was immersed in Hank’s solution, micro-galvanic corrosion resulted from different potentials of second phases and iron matrix occurred, with second phase acting as the cathode and iron matrix as the anode, respectively. As for pure iron, corrosion reaction was initiated from grain boundaries due to potential difference between grains and its boundaries. The iron matrix was oxidized to iron ions (II) following Eq. (1). Electrons generated from the dissolution of iron matrix [Eq. (1)] moved from grains to second phases, where cathodic reaction [Eq. (2)] happened. The accumulated electrons were shown by red cycle in Figure 10(a).

\begin{align*}
\text{Fe} & \rightarrow \text{Fe}^{2+} + 2e^- \quad \text{(anodic reaction)} \\
2\text{H}_2\text{O} + \text{O}_2 + 4e^- & \rightarrow 4\text{OH}^- \quad \text{(cathodic reaction)}
\end{align*}
Formation of hydroxide layer [Figure 10(b)]: because of the significant alkalization near the second phases [Eq. (2)], iron hydroxide was expected to form around them preferentially according to Eq. (3). Because the iron(II) hydroxide was not stable, it was easily oxidized to iron(III) hydroxide by dissolved oxygen following Eq. (4).

\[
\begin{align*}
\text{Fe}^{2+} + 2\text{OH}^- & \rightarrow \text{Fe(OH)}_2 \quad \text{or} \quad \text{FeO} \cdot \text{H}_2\text{O} \quad \text{(3)} \\
4\text{Fe(OH)}_2 + \text{O}_2 + 2\text{H}_2\text{O} & \rightarrow 4\text{Fe(OH)}_3 \quad \text{or} \quad 2\text{Fe}_2\text{O}_3 \cdot 6\text{H}_2\text{O} \quad \text{(4)}
\end{align*}
\]

Generally, pits were easily formed in the corrosion of pure iron due to localized acidification beneath the hydroxide layer, where the surface was loose with small micro-pores. For Fe-X composites, as second phases uniformly distributed in the iron matrix, widespread galvanic corrosion took place with multiple tiny pits formed and hydroxide products uniformly covered the surfaces, resulting in general corrosion of the material macroscopically.

Formation of Ca/P compounds [Figure 10(c)]: Ca/P compounds precipitated on the surface of hydroxide layer from Hank’s solution as the corrosion proceeded. The process could be confirmed by EDS results of sample surface after immersion test, which revealed significant amount of calcium and phosphorus, as shown in the inset of SPS pure Fe in Figure 6(c). Figure 10(d) showed enlarged schematic image of surfaces of the Fe-W composites after corrosion products were removed from the surface. Second phases lay at the bottom of pits because the iron matrix corroded more severely when it came nearer to second phases.

Comparing with as-cast pure iron, it was found that SPS pure Fe degraded faster in Hank’s solution from both electrochemical and immersion tests. This may be attributed to the fine grain size with more grain boundaries of SPS pure iron in comparison to that of as-cast pure iron. The same results have been reported by Moravej et al., which electroformed iron with fine grain size showed faster corrosion rate than that of as-cast pure iron.

However, as discussed above, galvanic corrosion between second phases and iron matrix played the leading role instead of that between iron matrix and the grain boundaries, though it still happened in the corrosion of Fe-X composites. As it is commonly accepted that micro-galvanic corrosion between iron matrix and noble IMPs had a favorable effect on the improvement of corrosion rate of Fe matrix, an elevated degradation rate of Fe matrix after these two phases were added in the composites could be easily understood.

Biocompatibility of Fe-X composites

From the mechanical point of view, the properties of 316L stainless steel, which is considered as the golden standard for making stents, are at least aimed at. For pure iron, its yield strength and ultimate strength are a little lower than...
316L SS. In this study, the ultimate compressive strength of Fe-X composites is significantly enhanced compared with pure iron. This would be favorable to the patient because superior strength contributes to thinner strut of the stent and simultaneously the amount of material released in the body would be smaller while offering the same support to the tissue.

In the view of biodegradation, the toxicity of a metallic implant material mainly relies on the metal ion itself against cell metabolic activities, metal ion concentrations released in the living body, and toxicity of the degradation products, especially for biodegradable metallic materials. In a physiological environment, pure iron will be degraded into ferric and ferrous ions, iron oxide and iron hydroxide, as demonstrated by previous works and present work. Iron is an essential element for human body with a total content of 4–5 grams in adults and people are clear about the uptake, transport, and excretion of iron in the intact organism. Extracellular iron exclusively binds to transferrin after being oxidized to its ferric form (Fe³⁺) and then iron-loaded transferrin is delivered to the cell surface, where endocytosis happens and iron is absorbed. However, with the addition of W and CNTs into iron matrix, an accelerated degradation rate of iron is obtained in comparison to pure iron. However, the released Fe ion concentration of Fe-X composites in the extract media varies from 5.22 μg/ml to 9.25 μg/ml, as shown in Figure 7(d), much lower than the half-maximal inhibitory concentration (IC₅₀) of Fe. According to the literature, iron ions almost have no inhibitory effect on the metabolic activities of endothelial cells when the concentration is less than 50 μg/ml. Therefore, taken the low amount of iron in a single stent and slow degradation rate of the stent into consideration, the biocompatibility of Fe-X composites is acceptable. In this study, the cytotoxicity test results showed no significant decreased cell viabilities to endothelial cells but reduced cell viabilities to smooth muscle cells for all Fe-X composites, which might be prominent advantages for the application as coronary stents because the former result was favorable to the fast endothelialization of stents, reducing the possibility of inflammatory reactions and thrombosis formation, and the latter might be beneficial to the control of neointima proliferation.

Considering both the second phases are nondegradable except for tungsten, which degrades very slowly, they will be left after iron matrix degrades. So it is necessary to consider the biocompatibility of these second phases. (1) Tungsten is a kind of metallic material that has been used as coils for the occlusion of cerebral arterial aneurysms. Degradation of tungsten coils is not associated with local or systemic toxicity while leads to a steady increase in serum tungsten levels, demonstrating excellent biocompatibility of tungsten. In the present work, the released tungsten ion concentration was much lower than the critical concentration (>50 μg/ml) needed to produce local cytopathological effects on human endothelial and smooth muscle cells and human dermal fibroblasts. (2) CNTs have been widely investigated for a variety of biomedical applications, such as tissue engineering, drug delivery, biosensor, etc. However, the biocompatibility of CNTs is complicated and different results on the biocompatibility of CNTs have been reported considering a lot of variables, such as different dispersed concentration, purification, the form of composites etc. Flahaut et al. have reported that CNTs synthesized by catalytic chemical vapor deposition (CCVD) show no cytotoxicity to human umbilical vein endothelial cells.

In order to further investigate the biocompatibility of second phases, we performed the cytotoxicity evaluation of powders of W, CNTs as well as pure iron powders at the concentration of 10 μg/ml by MTT assays. It was found that for all these powders, no significant decreased cell viabilities was observed for both vascular smooth cells and endothelial cells after 4 days’ direct contact incubation, as shown in Figure 11(a) and (b).

CONCLUSIONS
A series of Fe-X composites with typical metal W and nonmetal CNT as the reinforcement phase, respectively, were fabricated by SPS to obtain a faster degradation rate of
iron-based materials by micro-galvanic corrosion mechanism, and their in vitro degradation and biocompatibility were investigated systematically. The addition of second phases was found to significantly increase the ultimate compressive strength of pure iron, especially for CNT, while the yield strength increased slightly for all the phases. Electrochemical measurements results indicated an increased corrosion rate of Fe-X composites compared with pure iron, whereas immersion tests results revealed that corrosion rate of Fe-X composites and SPS pure iron were at the same order of magnitude except for Fe-CNT with a faster corrosion rate. The corrosion rate of Fe-X composites increased as the amount of X increased. More uniform corrosion mode took place instead of localized pitting corrosion for Fe-X composites. The extracts of Fe-W composites showed no significant cytotoxicity to L-929 cells and ECV304 cells, but all Fe-X composite extracts showed mildly cytotoxicity to VSMC cells. The hemolysis percentage of all Fe-X composites and pure iron were all less than 5% and platelets adhered on these specimens were presented in inactive state with a round shape and shown a comparable number with pure iron except for Fe-CNT. To sum up, iron-based composites are promising candidates for biodegradable coronary stent material with better mechanical properties and faster degradation rate than pure iron.

REFERENCES


