Fe–Au and Fe–Ag composites as candidates for biodegradable stent materials

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Abstract: In this study, Fe–Ag and Fe–Au composites were fabricated by powder metallurgy using spark plasma sintering. Their microstructures, mechanical properties, and biocorrosion behavior were investigated by using optical microscopy, X-ray diffraction, environment scanning electronic microscopy, compressive test, electrochemical measurements, and immersion tests. Microstructure characterization indicated that the as-sintered iron-based materials obtained much finer grains than that of as-cast pure iron. Phase analysis showed that the Fe–Ag composites were composed of α-Fe and pure Ag phases, and Fe–Au composites consisted of α-Fe and Au phases. Compressive test showed that the improved mechanical strengths were obtained in as-sintered iron-based materials, among which the Fe-5 wt %Ag exhibited the best mechanical properties. The electrochemical and immersion tests revealed that the addition of Ag and Au could increase the corrosion rate of the iron matrix and change the corrosion mode into more uniform one. Based on the results of cytotoxicity evaluation, it was found that all the experimental material extracts performed no significant toxicity on the L-929 cells and EA.hy-926 cells, whereas a considerable inhibition on the proliferation of vascular smooth muscle cells was observed. The hemocompatibility tests showed that the hemolysis of all the experimental materials was within the range of 5%, which is the criteria value of biomaterials with good hemocompatibility. The amount of platelet adhered on the surface of as-sintered iron-based materials was lower than that of as-cast pure iron, and the morphology of platelets kept smoothly spherical on the surface of all the experimental materials.

Key Words: biodegradable metal, Fe-Ag composite, Fe-Au composite, corrosion, biocompatibility, in vitro

INTRODUCTION

As Sigwart first introduced the use of coronary stents at 1987,1 the coronary stents have been widely applied in the treatment of coronary artery stenosis. At present, clinical use of coronary stents is mainly made from 316 L stainless steel, Co–Cr and TiNi alloys. However, it is generally accepted biodegradable stents as ideal stents as they can effectively avoid the late stent thrombosis. Two material classes have been largely researched for biodegradable stent applications: polymers and metals. Among these two material categories, metals have absolute advantages on their mechanical properties. Iron and magnesium are the most promising candidates for use in cardiovascular intervention and osteosynthesis.2–7 Compared with that of pure magnesium (41 Gpa) and its alloys (about 44 Gpa), the elastic modulus of pure iron (211.4 Gpa) is much larger.8 Besides, pure iron has better plastic deformation ability. With the above two important performances, pure iron can provide higher radial support strength, then lower the stenting and balloon expansion technology requirement, and make the surgery more convenient. From the perspective of biocompatibility, iron is an essential trace element. Iron content in the adult is about 4–5 g, 70% of which is combined with hemoglobin, and the rest combined with myoglobin or...
stored in the body as iron protein and flavin. The earliest research on the pure iron as biodegradable stent material could be derived from 2001. Peuster et al. implanted pure iron stents into 16 New Zealand white rabbits descending aorta, and pure iron’s good biological safety was observed. In their subsequent report, Puster et al. implanted pure iron stents into the descending aortas of swines with 316 L stainless steel (316 L SS) stents as control. At 1-year postoperatively, no significant difference was found between pure iron stents and 316 L SS stents. Thereafter, a series of in vivo tests also proved the feasibility of pure iron as biodegradable material. However, the slow degradation rate of iron was revealed in most of the animal experiments.

To meet the clinical requirements, lots of researches have been done on iron-based biodegradable materials in recent years. The methods to develop new iron-based biomedical materials mainly include alloying treatment and adopting new preparation technologies. For alloying treatment, alloys including Fe–Mn, Fe–X (X=Mn, Co, Al, W, Sn, B, C, S), Fe–Mn–Si, Fe–Mn–C, Fe–Mn–Pd, and Fe–Mn–C–(Pd) were investigated. Hermawan et al. developed four kinds of Fe–Mn alloys with Mn content ranging from 20 to 35 wt %. Fe–20Mn and Fe–25Mn alloys were found containing ε + γ phases, whereas Fe–30Mn and Fe–35Mn alloys were found containing only γ-phase. All those alloys exhibited lower magnetic parameters than 316 L SS owing to the antiferromagnetic property of both ε and γ phases. The results showed that the corrosion rate of Fe–35Mn alloy in Hank’s solution was about three times as that of pure iron. Liu and Zheng systematically investigated in vitro corrosion behavior and biocompatibility of eight kinds of Fe–X alloys (Mn, Co, Al, W, Sn, B, C, and S). Except for Fe–Sn alloy, the other alloys after rolling exhibited better mechanical properties than that of pure iron. But the corrosion rates of all the experimental alloys were similar to that of pure iron. Furthermore, Fe–30Mn–6Si alloy was prepared and the shape memory effect was found. The ultimate tensile strength and ductility of Fe–30Mn–1C alloy were improved to 1010 MPa and 88%, respectively. The corrosion rates of Fe–Mn–Pd and Fe–Mn–C–Pd alloys increased when compared to pure iron. For composites, pure iron enhanced by W, CNT, Fe2O3, Pd, and Pt was prepared through spark plasma sintering. The degradation rates of iron composites were all higher than that of pure iron, especially for the Fe–Pt composite. In addition, to improve biocompatibility of pure iron, Fe–HA, Fe–TCP, and Fe–BCP composites were fabricated. As the results show, these three composites exhibited enhanced corrosion, accompanying with decreased compressive yield strength and ultimate compressive strength. Besides, positive reactions with bone growth were also observed, but decreased the compressive yield strength and ultimate strength. Moravej et al. developed fine-grained pure iron from electroforming. The yield strength and ultimate strength of electroformed pure iron were 360 and 423 MPa, respectively. Chou et al. processed Fe–30Mn alloy into porous bone structure through 3D printing technology, and significantly accelerated corrosion rate was observed.

In this study, we fabricated iron-based composites with Ag or Au. Ag has good biocompatibility as well as good antibacterial ability. Au has long been used as dental material. The cytotoxic effect of Au in its ground state is negligible, but the salts of Au are considered to have immunology and cell toxicity. In this study, silver and gold particles were chosen as second phases dispersed in the pure iron matrix. On the one hand, the second phases would hinder the motion of dislocations. On the other hand, the existence of second phases could cause local inhomogeneous deformation, causing dislocation multiplication. Both situations could strengthen the pure iron. Furthermore, the standard electrode potentials of Ag (+0.7996 V) and Au (+1.83 V) are much higher than that of Fe (−0.44 V). Hence, the second phases (Ag or Au) could act as independent cathodes to accelerate the corrosion of pure iron matrix (as anodes).

**MATERIALS AND METHODS**

**Material preparation**

The raw materials, iron (99.99%, <10 μm), silver (99.9%, <44 μm), and gold powders (99.96%, 0.5–0.8 μm) were provided from Alfa Aesar (American). Pure iron powders were uniformly mixed with pure silver powders or pure gold powders in planet grinding machine (XM-4, KELI CERAMICS) under nitrogen atmosphere for 15 h with the rotation speed at 400 r/min. Then, pure iron powders, the mixed Fe–Ag powders, and Fe–Au powders were sintered into Φ 20 × 7 mm2 solids using the SPS-1050 system (Sumitomo Coal Mining) with sintering temperature of 1000 °C, sintering pressure of 40 MPa, and holding time of 5 min. As-cast pure iron (99.9%) was used as control.

Then, the as-cast pure iron and as-sintered specimens were cut into square pieces (10 × 10 × 1.8 mm3) and cylinders (Φ2 × 5 mm3). The cylindrical specimens were used for compressive tests. The square pieces were used into all the remaining tests. Before the tests, all the specimens were mechanically polished to 2000 grit and ultrasonically cleaned in anhydrous ethanol, and then dried in the open air. It should be pointed out that the samples were sterilized under ultraviolet radiation for at least 2 h before cytotoxicity test.

**Microstructure characterization**

First of all, an electronic balance (XS105, METTLER TOL- EDO) attached with density accessories was used to measure the density of all specimens. An average value was taken after measuring three samples for each kind of material. Specimens were further polished by 0.15 μm diamond paste, and then cleaned in acetone. After the specimens were etched with a 4% HNO3/alcohol etching solution, the metallographic structures of specimens were observed by environment scanning electronic microscopy (ESEM, Quanta 200FEG), equipped with an energy-dispersive spectrometer (EDS) attachment. X-ray diffractometer (XRD, Rigaku DMAX 2400) using CuKα radiation was adopted to identify the constituent phases of Fe–Ag and Fe–Au composites. The scanning angle was ranging from 10 to 100° and the scan...
rate was 4/m. EDS was employed for the analysis of chemical composition.

Mechanical test
On account of the limitation of sample size, compressive tests were selected for measuring the mechanical properties of experimental materials. Instron 5969 universal test machine was used for compressive tests according to ASTM E9-89a.40 The specimens were in the form of cylinders, 2 mm in diameter and 5 mm in length. Compressive strain rate was 5 × 10⁻³/min. An average of four measurements was taken for each material.

Electrochemical measurements
Electrochemical measurements were performed with a traditional three-electrode cell using an electrochemical work station (PGSTAT 302 N, Metrohm Autolab). The specimen, a saturated calomel electrode (SCE) and a platinum electrode were acted as the working electrode, reference electrode, and the auxiliary electrode, respectively. All the measurements were maintained at a temperature of 37 ± 0.5 °C in Hank's solution with a pH value of 7.4. The area of working electrode exposed to the solution was 0.3318 cm². The open circuit potential (OCP) measurement was set for 9000 s. Electrochemical impedance spectroscopy (EIS) was measured from 10 kHz to 10 mHz at OCP value. The potentiodynamic polarization curves were carried out from (OCP value, −600) mV (vs. SCE) to (OCP value, +600) mV (vs. SCE) at a scanning rate of 0.33 mV/s. An average of at least three measurements was taken for each group.

Static immersion test
In vitro static immersion test was performed in Hank's solution for 3, 10, and 30 days, 50 mL Hank's solution was used for each sample following ASTM-G31-72 at 37 °C in water bath. After 3, 10, and 30 days, respectively, the samples were removed from the soaking solution, gently rinsed with distilled water, and quickly dried in case of oxidation. Changes on the surface morphologies after immersion were recorded by scanning electron microscopy (SEM). Electrochemical behavior of exposed to the solution, and hence the dissolved oxygen concentration in Hank's solution should be regulated to better simulate the blood environment. In this bench, the wall shear stress (0.68 Pa), temperature (36.0–37.1 °C), pH value (7.35–7.45), and dissolved oxygen (2.8–3.2 mg/L) were well controlled to approach the common values in the human coronary artery. Plate specimens were mounted in paraffin and then placed in a specimen holder. All specimens were exposed to the dynamic fluid flow (Hank's solution) for 30 days (considering the generally bare metallic coronary stents would be covered by endothelial cells after 30 days in vivo implantation and no longer contact with blood directly).18 After the dynamic immersion, the samples were taken out, cleaned by deionized water and ethanol in turn, then rapidly dried by the cool air from a blower. The changes on the surface morphology were characterized by ESEM (Quanta 200FEG). The corrosion rate was calculated based on the weight loss of specimens. Corrosion layers on the surface of samples were peeled off at first. The residual corrosion products were dissolved by 10 mol/L NaOH and rinsed by deionized water and alcohol in turn, and then the samples were weighed after they were dried in the open air. Corrosion rates were calculated by the same formula described in the Static Immersion Test section.

Cytotoxicity test
The cytotoxicity test was performed by an indirect contact method. Murine fibroblast cells (L-929), human umbilical smooth muscle cells (VSMCs), and human umbilical vein endothelial cells (EA.hy-926) were used to evaluate the cytotoxicity of the experimental Fe–Ag and Fe–Au composites. At first, all the cell lines were cultured in the Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO₂. According to ISO 10993-12, extraction medium was prepared using serum-free DMEM with a surface area/extraction medium ratio of 1.25 cm²/mL in a humidified atmosphere with 5% of CO₂ at 37 °C for 72 h. After the extracts were centrifuged, the supernatant fluid was withdrawn and stored at 4 °C before cytotoxicity test. The control groups involved DMEM medium as the negative control and DMEM including 10% dimethyl sulfoxide as the positive control. The concentrations of metallic ions in the extraction medium were measured by inductively coupled plasma atomic emission spectrometry (Leeman, Profile). Cells were incubated in the 96-well plates at the density of approximately 5 × 10³ cells per 100 µL medium in each well and incubated for 24 h to allow attachment. DMEM was then replaced by extraction mediums. Then, 10 µL of serum was added to each well. After 1, 2, and 4 days of incubation in the incubator, respectively, the 96-well plates were observed under an optical microscope. Thereafter, 10 µL of cell counting kit (CCK-8) solution was added to each well. The cells were incubated with CCK-8 for 3 h. Then, the absorbance of each well was tested by using microplate reader (Bio-RAD680) at the wavelength of 545 nm. Viability of cells (X)
was calculated using the following formula according to ISO 19003-544:

\[ X = \frac{OD_1}{OD_2} \times 100\% \]

Here, \( OD_1 \) is the mean absorbance of experimental sample groups and positive control group. \( OD_2 \) is the mean absorbance of negative control group.

**Hemolysis test and platelet adhesion**

Healthy human blood (anticoagulant was 3.8 wt % citric acid sodium) extracted from volunteers was diluted by physiological saline according to volume ratio of 4:5. Pure iron, Fe–Ag, and Fe–Au composites were separately installed in centrifugal tubes with 10 mL of physiological saline for 30 min and temperature was kept at 37 \(^\circ\)C. Then, 0.2 mL of diluted blood was added to each tube and incubated at 37 \(^\circ\)C for 60 min, 10 mL of deionized water with 0.2 mL of diluted blood as the positive control, and 10 mL of physiological saline with 0.2 mL of diluted blood as the negative control. After completion of the above operations, samples were removed, and then these tubes were centrifuged at 800g for 5 min. Supernatant was transferred to 96-well multiplates, the absorbance (OD) was determined by a microplate reader (Bio-RAD) at the wavelength of 545 nm. Hemolysis of samples was calculated by the formula:

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

For platelet adhesion, whole blood from healthy human body was centrifuged at 1000 r/min for 10 min. Platelet-rich plasma (PRP) was obtained from the upper fluid. Samples after ultraviolet disinfection were moved to 24-well multiplates and 0.2 mL of PRP was added to each well, and then incubated at 37 \(^\circ\)C for 1 h. After gently rinsed by phosphate-buffered saline (PBS), platelets on samples were fixed with 2.5\% of glutaraldehyde solution at room temperature for 1 h. Then, dehydrated with gradient alcohol solution (50, 60, 70, 80, 90, 95, and 100\%), each concentration for 10 min, and finally freeze-dried for 2 days. The morphologies of platelet adhered on the specimens were observed by ESEM (Quanta 200FEG).
RESULTS
Microstructure of Fe–Ag and Fe–Au composites
Figure 1 shows the ESEM images of metallographs of experimental Fe–Ag and Fe–Au composites, with as-cast pure iron and as-sintered pure iron as control. Figure 2 is the grain size calculation corresponding to the metallographs. It could be seen from these figures that the grain size of as-sintered iron was much smaller than that of as-cast iron, and the addition of gold could further restrict the grain growth. The X-ray diffraction patterns of experimental materials are as shown in Figure 3. The Fe–Ag composites were characterized by \(\alpha\)-Fe and Ag phases, and the Fe–Au composites were composed of \(\alpha\)-Fe and Au phases. Hence, in the metallographs, the second phases were pure Ag and pure Au in the Fe–Ag composites and Fe–Au composites, respectively. According to the binary phase diagrams, there is no solubility of silver in iron, whereas gold can partially dissolve into iron.\(^{45}\) Hence, it can be seen from the metallographs that Ag was totally separated from the iron matrix, there was a clear interface between iron and silver. However, the Au was partially dissolved into the iron, there existed solid solution in the transition region between pure iron and pure gold. It can be seen from the XRD results that the XRD peaks of \(\alpha\)-Fe phase in Fe–Au composites moved a little to the left because of the lattice distortion. These results could be further proved by the detection of EDS (Figure 4). In the Fe–Ag composites, the second-phase Ag (area A) was completely isolated to the iron matrix (area B). On the other hand,
hand, the concentration of Au in the Fe–Au solid solution areas decreased gradually from the iron matrix to the Au center. Table I lists the densities of Fe–Ag and Fe–Au composites with as-cast and as-sintered pure iron as control at room temperature. Relative density of both Fe–Ag and Fe–Au composite was >98%.

**Compresive properties of Fe–Ag and Fe–Au composites**

The compressive properties of Fe–Ag and Fe–Au composites with as-cast and as-sintered pure iron as control at room temperature are shown in Figure 5. Owing to the size limitation of samples, only compressive test was appropriate for the mechanical properties test. As it could be seen from the
diagram, both the compressive yield strength and the compressive strength of as-sintered pure iron were higher than that of as-cast pure iron. When compared to as-sintered pure iron, with the addition of Ag or Au into the iron matrix, only Fe–5 wt % Ag composite among the experimental Fe–Ag composites exhibited better mechanical properties. On the contrary, only Fe–5 wt % Au composite in the experimental Fe–Au composites had a worse mechanical performance.

**Corrosion properties of Fe–Ag and Fe–Au composites**

**Electrochemical corrosion behavior.** Figure 6 shows the electrochemical test results of Fe–Ag and Fe–Au composites as well as as-cast and as-sintered pure iron immersed in Hank’s solution, including potentiodynamic polarization curves [Figure 6(a)], Nyquist plots [Figure 6(b)], and Bode plots [Figure 6(c)]. The average electrochemical parameters are listed in Table II. It was found that the corrosion potentials were largely decreased and the corrosion current densities were increased after the addition of Ag and Au into the iron matrix. The corrosion current density increased continuously with the content of Ag varied from 2 to 10 wt %, whereas decreased as the amount of Au increased. The corrosion rates of all Fe–Ag and Fe–Au composites were increased when compared to as-cast and as-sintered pure iron.

An equivalent circuit model \([R_s(Q_dR_t)]\) (inset, Figure 6(b)) for all the experimental specimens was employed to fit the EIS data. The parameters \(R_s\) and \(R_t\) represented electrolyte resistance and the transfer resistance, \(Q_d\) was a constant phase element, which was deemed as the nonideal capacitive behavior of the electric double layers, respectively. The Nyquist plots [Figure 6(b)] showed the similar results to that of the potentiodynamic polarization curves [Figure 6(a)]. The diameters of the semicircles revealed their worst corrosion resistance. As the diameter of high-frequency capacitive loop can be considered as the charge-

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**TABLE I. Density of Fe–Ag and Fe–Au Composites with As-Cast and As-Sintered Pure Iron as the Control at Room Temperature**

| Materials          | Density \((g/cm^3)\) | Relative density (%)
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>As-cast pure iron</td>
<td>7.831(0.008)a</td>
<td>–</td>
</tr>
<tr>
<td>As-sintered pure iron</td>
<td>7.746(0.005)</td>
<td>98.42</td>
</tr>
<tr>
<td>Fe–2Ag</td>
<td>7.807(0.002)</td>
<td>98.69</td>
</tr>
<tr>
<td>Fe–5Ag</td>
<td>7.870(0.001)</td>
<td>98.73</td>
</tr>
<tr>
<td>Fe–10Ag</td>
<td>7.945(0.004)</td>
<td>98.39</td>
</tr>
<tr>
<td>Fe–2Au</td>
<td>7.841(0.004)</td>
<td>98.45</td>
</tr>
<tr>
<td>Fe–5Au</td>
<td>7.984(0.001)</td>
<td>98.44</td>
</tr>
<tr>
<td>Fe–10Au</td>
<td>8.224(0.006)</td>
<td>98.30</td>
</tr>
</tbody>
</table>

Number in the brackets represents the standard deviation.

**FIGURE 6.** Electrochemical test results of pure iron, Fe–Ag, and Fe–Au composites in Hank’s solution: (a) Potentiodynamic polarization curve, (b) Nyquist plots, and (c) Bode plots.

**FIGURE 5.** Compressive stress–strain curves of pure iron, Fe–Ag, and Fe–Au composites.
TABLE II. Average Electrochemical Parameters of Fe–Ag and Fe–Au Composites (As-Cast and As-Sintered Pure Iron as Control)

<table>
<thead>
<tr>
<th>Materials</th>
<th>$E_{\text{corr}}$ (V)</th>
<th>$I_{\text{corr}}$ (μA/cm²)</th>
<th>$V_{\text{corr}}$ (mm/a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-cast pure iron</td>
<td>−0.7272</td>
<td>3.7416</td>
<td>0.0435</td>
</tr>
<tr>
<td>As-sintered pure iron</td>
<td>−0.8596</td>
<td>6.0179</td>
<td>0.0709</td>
</tr>
<tr>
<td>Fe–2Ag</td>
<td>−0.84118</td>
<td>10.188</td>
<td>0.1196</td>
</tr>
<tr>
<td>Fe–5Ag</td>
<td>−0.85577</td>
<td>12.166</td>
<td>0.1403</td>
</tr>
<tr>
<td>Fe–10Ag</td>
<td>−0.89581</td>
<td>15.189</td>
<td>0.1746</td>
</tr>
<tr>
<td>Fe–2Au</td>
<td>−0.8096</td>
<td>14.967</td>
<td>0.1736</td>
</tr>
<tr>
<td>Fe–5Au</td>
<td>−0.7959</td>
<td>11.498</td>
<td>0.1309</td>
</tr>
<tr>
<td>Fe–10Au</td>
<td>−0.7791</td>
<td>8.833</td>
<td>0.0981</td>
</tr>
</tbody>
</table>

Note: Corrosion potential ($E_{\text{corr}}$) and corrosion current ($I_{\text{corr}}$).

Transfer resistance, smaller charge-transfer resistance corresponds to faster corrosion rate. The Bode plots [Figure 6(c)] showed the same results as that of the Nyquist plots [Figure 6(b)]. There are two characteristic regions for all experimental materials in Bode plots. In the high frequency range ($10^2$–$10^5$ Hz), a flat portion of curves (slope = 0) is observed in the Bode-magnitude plots, whereas the phase angle drops to 0° in the Bode-phase angle plots, which is owing to the response of electrolyte resistance. In the low and middle frequency ranges, Fe–Ag and Fe–Au composites exhibited lower impedance. The impedance of Fe–2Au composite was the lowest. Except for Fe–10Au composite, the phase angle values of Fe–Ag and Fe–Au composites were lower than that of as-cast pure iron (around 60°). This indicated a larger corrosion potential of Fe–Ag and Fe–Au composites.

**Static immersion corrosion behavior.** Figure 7 shows the corrosion rates that were calculated from the weight loss after samples immersed in Hank’s solution for 3, 10, and 30 days, respectively. As shown in Figure 7, the corrosion rates of all experimental materials increased gradually with the immersion tests proceeding. The as-sintered pure iron corroded faster than as-cast pure iron, and the corrosion rates were further speeded up with the addition of Ag or Au. At the first 10 days, the corrosion would be accelerated with the content increasing amount of second phases. However, the iron-based composites with 5 wt % of Ag or Au became the materials with the fastest corrosion rate after 30 days of immersion.

Figures 8–10 show the surface morphologies of specimens after 3, 10, and 30 days of immersion in Hank’s solution, respectively. After 3 days of immersion, on the surface of as-cast pure iron, most of the area kept intact and only several separated etch pits were found. On the surface of as-sintered pure iron, some etch pits were also found, but unlike the as-cast pure iron, there were a lot of cracks on the surface of the as-sintered pure iron. When it came to the Fe–Ag composites, there were a large number of small corrosion pits existing on the specimens’ surface. The corrosion became more serious as the content of Ag increased. For the Fe–Au composites, the iron matrix between the Fe–Au solid solution phases was corroded severely and the surface of Fe–Au solid solution phases kept intact. After 10 days of immersion, most areas of the surface of as-cast pure iron still kept intact. However, the grain boundary could be observed clearly on the surface of the as-sintered materials. Furthermore, the area which corresponded to the Fe–Au solid solution phase kept intact but narrowed. As shown in Figure 10, grain boundary could be seen on the as-cast pure iron after 30 days of immersion. The intracristalline corrosion rate was much faster than that at grain boundary in all experimental materials, and the intracristalline corrosion in the as-sintered pure iron and Fe–Ag composites exhibited ladder-like corrosion.

**Dynamic immersion corrosion behavior.** Figure 11 shows the corrosion rates of experimental materials in the dynamic immersion test. The comparison between corrosion rates of materials was in good agreement with the result of the static immersion test, only the corrosion rates in the dynamic immersion test were over two times than the static ones. Figure 12 shows the surface morphologies of experimental materials after 30 days of dynamic immersion. The corrosion severity was much serious than the static ones. In addition, the surface morphology of as-sintered pure iron was different from the static one. Only rough morphology was observed, rather than morphology of grain boundaries.

**Cytotoxicity of Fe–Ag and Fe–Au composites**

Figure 13 shows the ion concentrations of extractions (a) and the cell viabilities of (b) murine fibroblast cells L-929, (c) human umbilical vein endothelial cells EA. hy-926, and (d) rodent VSMC expressed as a percentage of the viability of cells cultured in the negative control after 1, 2, and 4 days of incubation in experimental materials in extraction medium. It could be seen from these figures that: (1) The order of iron ion concentration in extraction medium matched well with the corrosion results of the 3 days of static immersion test in Hank’s solution; (2) L-929 cell viabilities were basically maintained around 100% through the whole test period; (3) The viabilities of EA. hy-926
increased after 2 days of incubation, followed by a drop on the fourth day; (4) The viabilities of VSMC decreased as the incubation time increased, and after 4 days of incubation, the VSMC cell viabilities of all the experimental composites decreased to lower than 70%. This might be attributed to the inhibitory effect of iron ions released into the extracts on the proliferation of VSMC cells.48

Hemocompatibility of Fe–Ag and Fe–Au composites

Hemolysis. Figure 14 shows the hemolysis percentage of Fe–Ag and Fe–Au composites, as-cast, and as-sintered pure iron as control. The hemolysis of all the experimental materials was <3%, lower than the judging criterion for biomaterials (5%).49 The additive phases Ag and Au exhibited no significant effect on the hemolysis percentage of all the experimental composites.

Platelet adhesion. The morphologies of adhered human platelet on the Fe–Ag and Fe–Au composites and pure iron specimens are shown in Figure 15. It could be seen from this figure that: (1) the as-sintered pure iron as well as composites corroded more seriously than as-cast pure iron after immersed samples in PRP for 1 h and the surfaces were covered with a lot of corrosion products. This result matched well with the electrochemical and immersion tests; (2) The number of platelets adhered on the experimental as-sintered materials was lower than that on as-cast pure iron; (3) Almost all the platelets adhered on the specimens kept the round shape and showed no sign of pseudopod-like structures, implying a negative activation; (4) There was no significant difference in the shape and number of platelets among a certain kind of Fe–Ag and Fe–Au composite with different contents as well as as-sintered pure iron.

DISCUSSION

A lot of research has been carried out on increasing corrosion rate of iron as a biodegradable stent material to meet the clinical requirement. The previously developed iron-based materials, however, are not satisfactory. New iron-based materials with higher degradation rate and better mechanical properties are needed. In this study, noble metallic elements Ag and Au were added into iron matrix to form a large number of galvanic corrosion sites, then accelerated the corrosion rate of iron matrix. The purpose of this
study was to investigate the effect of the second-phase Ag and Au on the mechanical properties, corrosion behavior, and biocompatibility of pure iron.

**Microstructure and mechanical properties**
The grains of as-sintered pure iron were much finer than as-cast pure iron. It should be mainly ascribed to the process of spark plasma sintering. For spark plasma sintering, the grain growth was significantly limited at lower temperature and shorter holding time.\(^{50}\) To some extent, the second-phase particles such as pure Ag and Fe–Au solid solution could also restrict grain growth. As there is no solubility of silver in iron,\(^{45}\) the silver exists in the Fe–Ag composites as pure elementary substance. The pure silver has highest electrical conductivity and thermal conductivity among metallic elements,\(^{51}\) it is helpful to the heat generation and transmission. Grains in the Fe–Ag composites could effectively get energy to grow, and this effect might partly offset the restriction effect of second phase on grain growth. Therefore, the grain size of Fe–Ag composites was similar to that of the as-sintered pure iron. The decrease of grain size could contribute to the improvement of mechanical properties. The second-phase strengthening effect should also be considered as one reason for the enhanced strength. However, as the strength of silver is lower than pure iron,\(^{52}\) the strength of Fe–Ag composites decreased when the content of Ag is too high. To Fe–Au composites, Au could be partly dissolved into iron, and hence when the Au content increased, the amount of solid solution increased. Therefore, besides the refined crystalline strengthening and second-phase strengthening, the improved strength of Fe–Au composites could also be attributed to the solid solution strengthening. According to the data of grain size (Figure 2), the grain sizes of Fe–5Au and Fe–10Au were larger than that of Fe–2Au, the grain-boundary strengthening became weaker, and hence the strength of Fe–5Au decreased. On the other hand, the compensation caused by the second-phase strengthening and solid solution strengthening was more intensive in Fe–10Au composite, which exhibited a relatively high strength.

**Effect of Ag and Au on the corrosion behavior of Fe–Ag and Fe–Au composites**
Based on the surface morphologies after corrosion, it was found that Fe–Ag and Fe–Au composites corroded much
seriously than pure iron. The corrosion initiated at the iron matrix surrounded pure Ag phase or Fe–Au solid solution phase. This phenomenon should be attributed to the galvanic corrosion. Pure Ag or Fe–Au solid solution with relatively high corrosion potential acted as the cathodes, and the iron substrate acted as the anodes. Then, a large number of galvanic cells were formed. In this way, corrosion of iron was accelerated. The corrosion mechanism and processes are shown in Figure 16. It can be seen from this figure that the electrochemical corrosion was the main corrosion mechanism occurred on the Fe–Ag and Fe–Au composites in the physical environment. As shown in Figure 16(a), iron matrix, acting as the anode, would be oxidized into iron ions [Eq. (1)]. Electrons generated from the dissolving iron matrix would transfer to the second phases which set as cathodes. Then, they would be consumed by dissolved oxygen [Eq. (2)].

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

Owing to the solution alkalization near the Ag and Fe–Au solid solution phases [Eq. (3)], iron hydroxide formed around them preferentially according to Eq. (4). Owing to the instability of ferrous hydroxide, it was easily oxidized to

![FIGURE 10. SEM images of samples' surface morphology after statically immersed in Hank's solution for 30 days.](image)

![FIGURE 11. Corrosion rates calculated from the weight loss of samples after dynamic immersion in Hank's solution for 30 days.](image)
ferric hydroxide by dissolved oxygen [Figure 16(b)], the reaction is shown in Eq. (4):

$$
\text{Fe}^2+ + 2\text{OH}^- \rightarrow \text{Fe(OH)}_2
$$

$$
4\text{Fe(OH)}_2 + \text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{Fe(OH)}_3
$$

Corrosion gradually spread into the area beneath the Ag or Fe–Au solid solution phases [Figure 16 (c)] via the penetration of the solution. Widely distributed microgalvanic corrosion couples resulted in an uniform corrosion at macroscopic level.

**Biocompatibility of Fe–Ag and Fe–Au composites**

The cytotoxicity of biodegradable metallic materials is mainly attributed to the released metallic ions\(^{16}\) and degradation particles\(^{53}\) which can promote or inhibit cell metabolic activities and proliferation. Iron has been proved as a good candidate with excellent biocompatibility for vascular stent applications.\(^{9,12,54}\) As reported by Zhu et al.,\(^{55}\) there was almost no effect on the metabolic activity of endothelial cells when the concentration of iron ion was lower than 50 \(\mu\text{g/mL}\), and much lower iron ion concentration (<10 \(\mu\text{g/mL}\)) might even have a beneficial effect on the metabolic activities of endothelial cells. The results in this study are in good agreement with the above statement. Iron ion concentrations in the pure iron, Fe–Ag, and Fe–Au composite extracts were all lower than 25 \(\mu\text{g/mL}\) despite the viability of EA. Hy-926 cells decreased slightly as the iron concentration increased; there was no significant toxicity to the EA. hy-926 cells when compared to the negative control. However, the viabilities of VSMC cells increased obviously after incubation in the extracts for 4 days. Mueller et al.\(^{48}\) found that ferrous irons could exert adverse effect on the proliferation of VSMC cells in the sight of
gene expression, and the effective regulation of iron ion on cellular response might benefit the control of neointima coverage. According to the report of Schaffer et al., Fe$^{2+}$ and Fe$^{3+}$ ions could repress the migration of smooth muscle cells at the concentration of 1 mM, whereas good endothelial average was found on iron wires. As for L-929 cell line, the viabilities of L-929 cells cultured in different extraction mediums maintained around 100%. There was almost no impact on the proliferation of L-929 cells from the released metallic ions and corrosion particulate products.

Hemolysis is employed to detect the destructive effect of medical materials to the erythrocyte. Iron is an essential component of hemoglobin and various enzymes. Therefore, it is suggested that iron will not perform destructiveness to erythrocyte. Gu et al. found that the high hemolysis ratio of magnesium alloys might be attributed to the significant increase in pH value resulting from the corrosion. For the iron-based composites, no significant increase of pH value was found after corrosion, which could also be an important reason to explain the low hemolysis ratio of iron-based materials. Platelet adhesion can be considered as a good indicator to detect the probability of thrombogenesis when implants are in contact with blood. Generally, the excellent hemocompatibility of 316 L SS is used as a golden standard for stent materials. Hulander et al. investigated

![Figure 13](image1.png)

**Figure 13.** Ion concentration in experimental materials’ extraction mediums (a) and cell viability after cultured in extraction mediums and positive control for 1, 2, and 4 days: L-929 (b), EA. Hy-926 (c), and VSMC (d).

![Figure 14](image2.png)

**Figure 14.** Hemolysis rates of pure iron, Fe-Ag, and Fe-Au composites.
the blood interactions of noble metals including Ag, Au, Pd, and Ti which are used as control. The results showed that the Ag and Au exhibited relatively worse hemocompatibility than that of Pd and Ti. Nonetheless, the shape of platelets adhered on Fe–Ag and Fe–Au composites kept its integrative shape and was better than 316 L SS as reported by Zhu et al. There was nearly no difference on the number of adhered platelets among different Ag or Au contents of

FIGURE 15. Morphology of platelets adhered on the surface of pure iron, Fe–Ag, and Fe–Au composites.

FIGURE 16. Illustration of the corrosion mechanism for Fe–Ag or Fe–Au composites: (a) initial corrosion reaction; (b) and (c) the formation procedure of hydroxide layer.
experimental composites as well as as-sintered pure iron, whereas the number of adhered platelets adhered on the as-sintered materials was less than as-cast pure iron. Taking the low hemolysis ratio and good antiplatelet adhesion properties into consideration, it is concluded that Fe–Ag and Fe–Au composites possess excellent hemocompatibility.

CONCLUSIONS
A series of Fe–Ag and Fe–Au composites with different contents using Ag or Au were fabricated by powder metallurgy using spark plasma sintering. The microstructure, mechanical properties, in vitro corrosion behavior, cytotoxicity, and hemocompatibility were investigated systematically. The grain sizes of as-sintered pure iron as well as Fe–Ag and Fe–Au composites were much smaller than as-cast pure iron. The Fe–5 wt % Ag, Fe–2 wt % Au, and Fe–10 wt % Au composites exhibited enhanced mechanical properties when compared to as-cast pure iron. Based on the electrochemical and immersion results, higher corrosion rates than both as-cast pure iron and as-sintered pure iron were obtained by the addition of Ag or Au. The extracts of Fe–Ag and Fe–Au composites showed almost no cytotoxicity to EA.hy-926 and L-929 cells, whereas significantly suppressed cell viabilities of VSMC cells. The hemolysis percentages of all Fe–Ag and Fe–Au composites and pure iron were lower than 5% and platelets adhered on these specimens were round with almost the same amount. In summary, iron-based composites reinforced by 5 wt % Ag or Au are promising alternatives for biodegradable materials with good combination of mechanical and biocompatible performance as well as a proper degradation rate.

REFERENCES


