

## FERROFERRIC OXIDE/CHITOSAN SCAFFOLDS WITH THREE-DIMENSIONAL ORIENTED STRUCTURE\*

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**Abstract** A facile approach to construct ferroferric oxide/chitosan composite scaffolds with three-dimensional oriented structure has been explored in this research. Chitosan and ferroferric oxide are co-precipitated by using an *in situ* precipitation method, and then lyophilized to get the composite scaffolds. XRD indicated that Fe<sub>3</sub>O<sub>4</sub> was generated during the gel formation process, and increasing the content of magnetic particles could destruct the crystal structure of chitosan. When the content of magnetic particles is lower than 10%, the layer-by-layer structure and wheel spoke structure are coexisting in the scaffolds. Increasing the content of magnetic particles, just layer-by-layer structure could be observed in the scaffolds. Ferroferric oxide particles were uniformly distributed in the matrix, the size of which was about 0.48 μm in diameter, 2 μm in length. Porosity of magnetic chitosan composite scaffolds is about 90%. When the ratio of ferroferric oxide to chitosan is 5/100, the compressive strength of the material is 0.4367 MPa, which is much higher than that of pure chitosan scaffolds, indicating that the layer-by-layer and wheel spokes complex structure is beneficial for the improvement of the mechanical properties of chitosan scaffolds. However, increasing the content of ferroferric oxide, the compressive strength of scaffolds decreased, because of the decreasing of chitosan crystallization and aggregation of magnetic particles as stress centralized body. Another reason is that the layer-by-layer and wheel spokes complex structure makes bigger contributions for the compressive strength than the layer-by-layer structure does. Three-dimensional ferroferric oxide/chitosan scaffolds could be used as hyperthermia generator system, improving the local circulation of blood, promoting the aggradation of calcium salt and stimulating bone tissue regeneration.

**Keywords:** Chitosan; Ferroferric oxide; Scaffold; Three-dimensional oriented structure.

## INTRODUCTION

Bone defect caused by ageing or various traumas is a typical tissue malfunction, which needs three-dimensional scaffolds to repair. The scaffolds should possess hierarchical porous structures to achieve the desired mechanical properties, mass and oxygen permeability, and could guide cell attachment, growth and tissue regeneration<sup>[1–3]</sup>. Many biodegradable scaffolds made of PLA, PLGA, PCL and chitosan have been constructed<sup>[4, 5]</sup>. Among them, chitosan has been widely used for tissue engineering due to its desirable biocompatibility and biodegradability<sup>[6, 7]</sup>. But there is fewer research reported on the three-dimensional oriented structure of scaffolds, so we tried to fabricate three-dimensional oriented chitosan scaffolds to improve the mechanical properties, so as to meet the demand for the bone regeneration. In our previous research, three-dimensional chitosan rod and its composites have been constructed by an *in situ* precipitation method<sup>[8–13]</sup>, which have high

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mechanical properties and could be used for bone fracture internal fixation. The *in situ* precipitation method and lyophilization have been used to fabricate three-dimensional ordered chitosan scaffolds in this research.

Magnetic field could improve local circulation of blood, promote the aggradation of calcium salt and stimulate bone tissue regeneration<sup>[14–16]</sup>. Ferritic stainless steel fibres were used to fabricate porous material, which could stimulate the bone growth under magnetic field<sup>[17]</sup>. Magnetic porous bioceramics made of tricalcium phosphate, bioglass and magnetic material with main crystalline phase of  $\text{ZnFe}_2\text{O}_4$ , have excellent physical and biological properties. Animal experiments approved that the magnetic porous bioceramics could stimulate the formation of new bone and accelerate the repairment of bone defects<sup>[18]</sup>. Magnetic collagen/hydroxyapatite composite material has been prepared by co-precipitation method, which could produce controllable hyperthermia activated by an alternative electromagnetic field after implantation, and could be used for bone cancer treatment. If magnetite content in the composite materials is 1% or 2%, the composites can not produce the necessary hyperthermia. However, the composites with higher content of magnetite (for instance 5%) can be successfully used as hyperthermia generator system<sup>[19]</sup>. Ferroferric oxide is useful for local hyperthermia in bone, and could stimulate bone growth<sup>[20]</sup>, so it has been introduced into chitosan scaffolds to functionalize the bone repair material in this research. The relationship between the structure and properties of ferroferric oxide/chitosan scaffolds is explored in the following sections.

## EXPERIMENTAL

### Materials

Chitosan (biomedical grade,  $M_n = 5.6 \times 10^5$ , D.D = 85%, Zhejiang Golden-Shell Biochemical Co., Ltd), acetic acid (CP, Yixing Niuji Chemical Reagent Plant), sodium hydroxide (AR, Hangzhou Xiaoshan Chemical Reagent Corporation),  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (AR, Sinopharm Chemical Reagent Co., Ltd).

### Preparation of Ferroferric Oxide/Chitosan Scaffolds

A certain amount of chitosan powder was dissolved in 2 vol% acetic acid solution to get chitosan solution (40, g/L), then ferri ferrous oxide precursor solution was added quickly, in which the molar ratio of ferri ions and ferrous ions was 2/1. The mass ratios between ferri ferrous oxide and chitosan were 5/100, 10/100, 15/100 and 20/100, respectively. Ferroferric oxide/chitosan gel rods were prepared by *in situ* precipitation method. The obtained gel-rods were first rinsed with distilled water, then frozen at  $-60^\circ\text{C}$  for 24 h and lyophilized for 48 h to get the scaffolds.

### X-ray Diffraction Analysis

X-ray diffraction measurements were carried out on a Bruker AXS D8 Advance X-ray Diffractometer (40 kV and 34 mA) with  $\text{Cu K}\alpha$  radiation in the range of  $5^\circ$ – $60^\circ$  at a rate of  $0.1^\circ/\text{s}$ .

### Microstructure Characterization

Scanning electron microscopy (SEM) (HITACHI S-570) was used to observe the microstructure of the three-dimensional ordered ferroferric oxide/chitosan scaffolds after spray coating of gold.

### Measurements of the Porosity of Ferroferric Oxide/Chitosan Scaffolds

The porosity of scaffolds was measured by liquid displacement method<sup>[21–26]</sup>. The materials with certain mass were placed in the ethanol with certain volume ( $V_1$ ), and then treated by cycled vacuum pumping until no bubbles escaping. The total volume of materials and ethanol was marked as  $V_2$ , thus the solid volume of scaffolds can be expressed as  $V_2 - V_1$ . After removing the scaffolds which contained ethanol, the volume of remained ethanol was marked as  $V_3$ . Then the volume of the ethanol ( $V_1 - V_3$ ) contained in the scaffolds can be viewed as the volume occupied by the pores of scaffolds and the total volume of the scaffolds was  $V = (V_2 - V_1) + (V_1 - V_3) = V_2 - V_3$ . So the porosity of ferroferric oxide/chitosan scaffolds can be expressed as  $P = (V_1 - V_3)/(V_2 - V_3)$ .

### Mechanical Properties of Ferroferric Oxide/Chitosan Scaffolds

Compressive strength of samples (15 mm height and 8 mm diameter, three parallel samples each group) was

tested on a Shenzhen Reger Company's universal materials testing machine with the maximum compression ratio 50% and the loading rate 2 mm/min.

## RESULTS AND DISCUSSION

### *Formation Process of Ferroferric Oxide/Chitosan Composites by an in situ Precipitation Method*

Ferroferric oxide precursor solution was mixed with chitosan solution to form a homogeneous brown solution, both of which were acidic, and increasing the concentration of ferroferric oxide could darken the mixture solution. There are a large amount of free amino and hydroxyl groups with lone pair electrons on chitosan macromolecules, while there are empty tracks in ferri ions and ferrous ions, so they can produce strong complexation when the pH value is larger than 7. As in acidic conditions, free amino groups in chitosan macromolecules are protonated, and the polycations are mutually repelled, while ferri ions and ferrous ions are surrounded by the hydrates under acidic condition, and difficult to complex with chitosan, so they can form stable mixture solution.

The protonated chitosan became gel when the pH value was raised to 6.3 because  $\text{OH}^-$  ions permeated into the mixture solution, meanwhile ferri ions and ferrous ions were *in situ* complexed with chitosan molecules. When the pH value was raised to 10, the ferroferric oxide was generated as the following reaction<sup>[27, 28]</sup>:



when  $\text{OH}^-$  diffused into the mixture solution, ferroferric oxide and chitosan were precipitated and formed the first layer. When  $\text{OH}^-$  continued to diffuse into the mixture solution, the second layer was shaped. The precipitation process was repeated until the layer-by-layer structure was finally formed. Meanwhile, the gel rods showed an orderly structure resembling a spoke, which together with the layer-by-layer structure formed the unique three-dimensional oriented gel-rods.

### *Component Determination of the Composite Scaffolds*

Figure 1 shows X-ray diffraction spectra of the composite scaffolds. A characteristic diffraction peak on behalf of chitosan appears at  $2\theta = 20^\circ$ . While the peaks at  $2\theta = 30.2^\circ, 35.7^\circ, 43.4^\circ, 57.2^\circ, 53.6^\circ, 62.8^\circ$  are typical diffraction peaks of  $\text{Fe}_3\text{O}_4$ , which prove that  $\text{Fe}_3\text{O}_4$  are generated in the precipitation process.  $\text{Fe}_3\text{O}_4$  particles prepared by *in situ* precipitation method dispersed well in the chitosan matrix, and showed superparamagnetic properties and no remanence or coercivity. The saturation magnetizations of the prepared  $\text{Fe}_3\text{O}_4$ /chitosan nanocomposites were 49.5 emu/g<sup>[28]</sup>. Magnetic particles strongly interacted with chitosan, so increasing the content of magnetic particles could cause the destruction of chitosan crystal structure, which could be proved by the XRD spectra in Fig. 1.

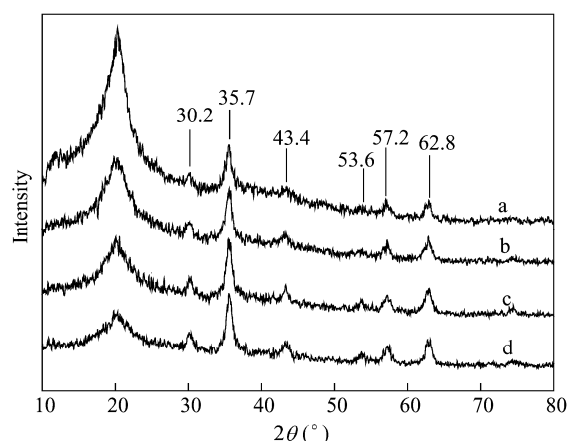


**Fig. 1** X-ray diffraction patterns of composite scaffolds with different ferroferric oxide/chitosan ratios: (a) 5/100, (b) 10/100, (c) 15/100 and (d) 20/100

### Macroscopic Morphology and Microstructure of Magnetic Chitosan Scaffolds

As shown in Fig. 2, there are digital photos of magnetic chitosan scaffolds with different ferroferric oxide/chitosan ratios. In the formation process of gel rods *via in situ* precipitation, layer-by-layer structure and wheel spokes structure are competitive. When chitosan precipitated faster, the layer structure was thinner; when more chitosan gel formed, the penetration rate of  $\text{OH}^-$  became slow, so a significant wheel spoke could be observed. Because of the stronger interactions between chitosan and magnetic particles, magnetic particles within each layer are equivalent to a cross-linking agent, increasing the intermolecular interactions among chitosan macromolecules, the chitosan gel tends to having a layer-by-layer structure. When the content of magnetic particles is lower than 10%, the layer-by-layer structure and wheel spokes structure are coexisting in the composites (Figs. 2a and 2b). Continuing to increase the content of magnetic particles, only layer-by-layer structure could be observed (Figs. 2c and 2d).

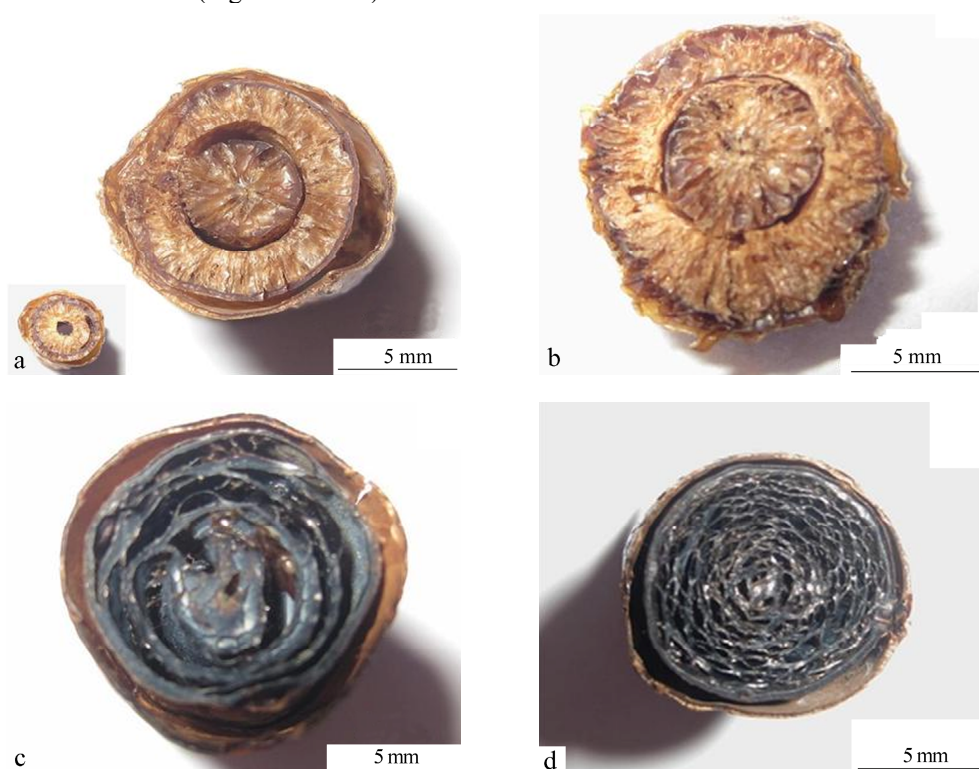


**Fig. 2** Digital photographs of ferroferric oxide/chitosan composite scaffolds with different ratios: (a) 5/100, (b) 10/100, (c) 15/100 and (d) 20/100

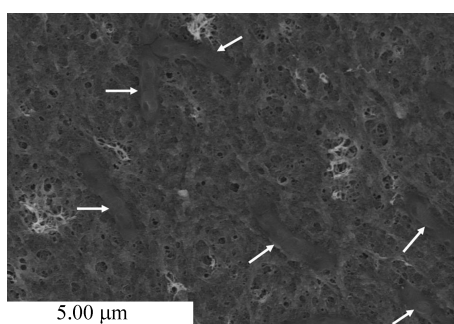


**Fig. 3** SEM of ferroferric oxide/chitosan composite scaffold (20/100)

As shown in Fig. 3, microstructure of ferroferric oxide/chitosan (20/100) composite scaffolds was observed by scanning electron microscopy (SEM). Columnar crystals of  $\text{Fe}_3\text{O}_4$  were uniformly distributed in chitosan matrix (shown by white arrows), the size of which was about  $0.48\ \mu\text{m}$  in diameter,  $2\ \mu\text{m}$  in length. The size is slightly larger than conventional magnetic nano-particles, because the freeze-drying process caused the phase separation between chitosan and water, reducing the fixation of  $\text{Fe}_3\text{O}_4$  particles, so the magnetic particles became aggregated to form larger size particles.

#### **Porosity and Mechanical Properties of Magnetic Chitosan Scaffolds**

Chitosan as a natural biopolymer has been widely investigated for biomedical applications due to its excellent biocompatibility and biodegradability. But the mechanical property of porous scaffolds made of chitosan is low. The electrochemistry assisted deposition method has been used to deposit hydroxyapatite on the porous chitosan scaffolds to improve the strength and osteo-conductivity of the biomaterial. The compressive strength of the treated scaffold is increased to  $0.0998\ \text{MPa}$ , comparing with  $0.0550\ \text{MPa}$  of pure chitosan scaffold<sup>[29]</sup>. In this research, layer-by-layer and wheel spokes complex structure of chitosan scaffolds was designed, which not only improved the mechanical properties of the scaffolds, but also could supply appropriate connected holes to transport nutritive material and oxygen for the cell growth and proliferation. Figure 4 shows the porosity of magnetic chitosan composite scaffolds, which is about 90% in different ferroferric oxide/chitosan ratios.

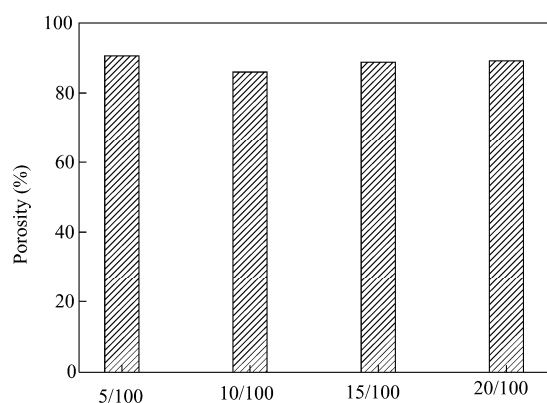


**Fig. 4** Porosity of ferroferric oxide/chitosan composite scaffolds with different ratios: 5/100, 10/100, 15/100 and 20/100

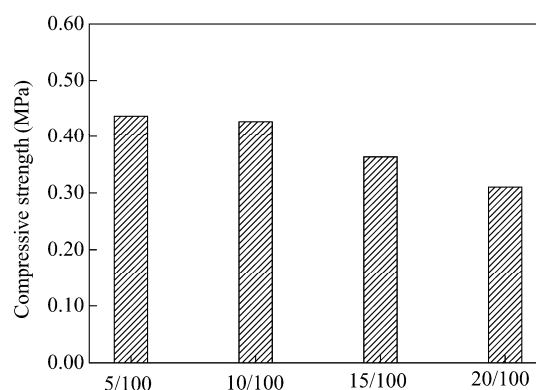


**Fig. 5** Compressive strength of ferroferric oxide/chitosan composite scaffolds with different ratios: 5/100, 10/100, 15/100 and 20/100

Figure 5 shows the compressive strength of magnetic chitosan composite scaffolds with different ferroferric oxide/chitosan ratios. When the ratio of ferroferric oxide to chitosan is 5/100, the compressive strength of the material is  $0.4367\ \text{MPa}$ , which is much higher than that of pure chitosan scaffolds and hydroxyapatite/chitosan composite scaffolds<sup>[29]</sup>, indicating that the layer-by-layer and wheel spokes complex structure is beneficial for the improvement of the mechanical properties of chitosan scaffolds. However, with increasing the content of  $\text{Fe}_3\text{O}_4$  the compressive strength of composite scaffolds decreased, because of the decreasing of crystallization of chitosan and aggregation of magnetic particles as stress centralized body. Another reason is that: when ferroferric oxide/chitosan ratios are 5/100 and 10/100, the composite scaffolds have layer-by-layer and wheel spokes complex structure; while ferroferric oxide/chitosan ratios are 15/100 and 20/100, the composite scaffolds just have layer-by-layer structure. The layer-by-layer and wheel spokes complex structure makes bigger contributions for the compressive strength than layer-by-layer structure.

## **CONCLUSIONS**

Three-dimensional ferroferric oxide/chitosan composite scaffolds with layer-by-layer and wheel spokes complex structure have been fabricated by using *in situ* precipitation and lyophilization methods. XRD proved that  $\text{Fe}_3\text{O}_4$

was generated during the precipitation process, and increasing the content of magnetic particles could cause the destruction of chitosan crystal structure. When the content of magnetic particles is lower than 10%, the layer-by-layer structure and the wheel spokes structure are coexisting in the composites. Increasing the content of magnetic particles, just layer-by-layer structure could be observed.  $\text{Fe}_3\text{O}_4$  particles were uniformly distributed in the chitosan matrix, the size of which was about 0.48  $\mu\text{m}$  in diameter, 2  $\mu\text{m}$  in length. Porosity of magnetic chitosan composite scaffolds was about 90%. When the ratio of ferroferric oxide to chitosan is 5/100, the compressive strength of the material is 0.4367 MPa, which is much higher than that of pure chitosan scaffolds, indicating that the layer-by-layer and wheel spokes complex structure is beneficial for the improvement of the mechanical properties of chitosan scaffolds. With increasing the content of  $\text{Fe}_3\text{O}_4$ , the compressive strength of composite scaffolds decreased, because of the decreasing of crystallization of chitosan and aggregation of magnetic particles as stress centralized body. Another reason is that the layer-by-layer and wheel spokes complex structure makes bigger contributions for the compressive strength than the layer-by-layer structure.

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