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Fabrication and properties of chitin/hydroxyapatite hybrid hydrogels as scaffold nano-materials

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ABSTRACT

Novel hybrid hydrogels were prepared by introducing nano-hydroxyapatite (nHAp) into chitin solution dissolved in NaOH/urea aqueous solution at low temperature, and then by cross-linking with epichlorohydrin (ECH). Their structure and morphology were characterized by FTIR spectra, wide-angle X-ray diffraction (WAXD), thermo-gravimetric analysis (TGA), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Our findings revealed that hydroxyapatite nano-particles were uniformly dispersed in chitin hydrogel networks. The chitin/nHAP hybrid hydrogel (Gel2) exhibited about 10 times higher mechanical properties (compressive strength: 274 kPa) than that of chitin hydrogel. Moreover, COS-7 cell culture experiment proved that cells could adhere and proliferate well on the chitin/nHAp hydrogels, suggesting good biocompatibility. All these results signified that these biomaterials could be potential candidates as scaffolds for tissue engineering.

1. Introduction

Hydrogels are water-swollen polymeric materials that maintain distinct three-dimensional networks, with the ability to absorb and retain a significant amount of water. Since the discovery of hydrogels as contact lenses (Zohuriaan-Mehr, Omidian, Doroudiani, & Kabiri, 2010), the hydrogel applications are now widespread in the fields of agriculture (Kopecek, 2009), drug delivery (Hoare & Kohane, 2008), sensors (Richter et al., 2008), and so on. Especially, biodegradable hydrogels are currently used as scaffolds in tissue engineering, where the 3D networks may contain cells to repair defective tissue, due to hydrogels having structural similarity to the macromolecular-based components in the body and are considered biocompatible (Lee & Mooney, 2001). There are two major types of hydrogels, synthetic and natural hydrogels, according to their origin. Synthetic hydrogels are made mainly from synthetic polymers, such as poly(acrylic acid), poly(ethylene glycol), poly(vinyl alcohol), polyacrylamide, and polypeptide (Ryan & Nilsson, 2011; Zhu, 2010). Natural hydrogels are from biopolymer-based materials, such as polysaccharides (including hyaluronic acid, chitosan, alginate, and cellulose) and proteins (e.g., elastin, collagen, gelatin, fibrin, and globular proteins) (Jonker, Lowik, & van Hest, 2012; Van Vlierberghe, Dubruel, & Schacht, 2011).

Hydroxyapatite (HAp) exhibits excellent biocompatibility with soft tissue such as skin, muscle and gums, so it has become an ideal candidate for orthopedic and dental implants or components of implants (Zhou & Lee, 2011). However, the intrinsic properties of HAp, such as hardness, fragility, and lack of flexibility, make it difficult to be shaped in the specific form required for bone repair and implantation, which limits its application as a load-bearing implant scaffold material (Sun, Zhou, & Lee, 2011). Therefore, many studies have been reported on composite materials containing HAp in combination with various polymers, such as poly(lactic acid), sodium alginate, cellulose, polycaprolactone, collagen, and chitosan, to overcome the drawbacks of HAp for the application in tissue engineering (Cai et al., 2011; Eosoly, Brabazon, Lohfeld, & Looney, 2010; Grande, Torres, Gomez, & Carmen Bañó, 2009; Jeong et al., 2008; Rossi et al., 2012; Swetha et al., 2010; Tan et al., 2010; Zhang et al., 2010). These polymers have been widely used to develop porous 3D scaffolds. Usually, natural polymer-based composites have been focused with more attention than synthetic polymer composite for bone tissue engineering applications, due to the biocompatible and biodegradable behavior of natural polymers. Chitin is produced by a number of living organisms in lower plant and animal kingdoms, and possesses natural biocompatibility, which is very good candidate of biomaterials. It is noted that nano-sized HAp (nHAp) which has high surface area to volume ratio showed significant improved properties such as bioactivity

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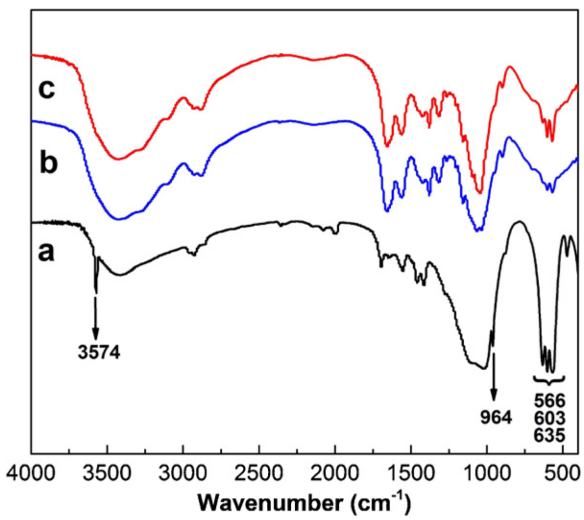

Fig. 1. FTIR spectra of nano-hydroxyapatite (a), Gel1 (b), and Gel2 (c).

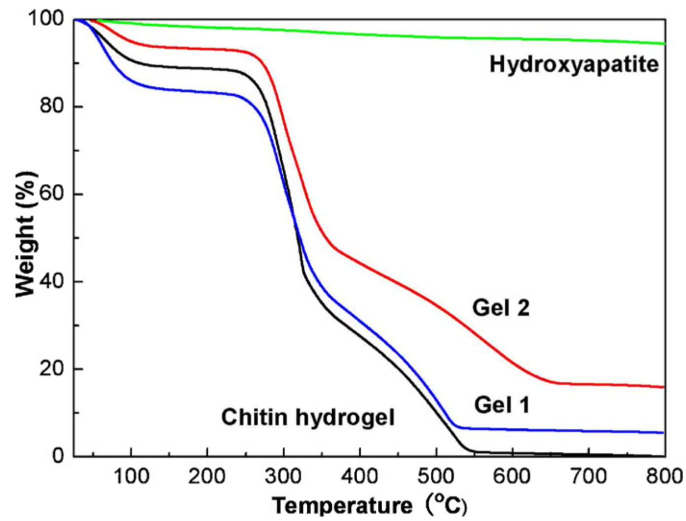

Fig. 2. TGA curves of nano-hydroxyapatite, chitin hydrogel, Gel1, and Gel2.

including osteoblast adhesion, osteoconductivity, and osseointegration, protein adsorption, and mechanical strength (Kumar et al., 2011; Wei & Ma, 2004). Therefore, the inclusion of nHAp into polymer matrix can not only improve the mechanical properties but also incorporate the nanotopographic features that mimic the nanostructure of natural bone.

In our previous work, chitin hydrogels were fabricated by dissolving chitin in NaOH/urea aqueous solution at low temperature. These hydrogels were non-toxic and had good biocompatibility (Chang, Chen, & Zhang, 2011). However, they are not bioactive to induce desired bone regeneration. Herein, we attempted to construct chitin/nHAp hydrogels as scaffolds for tissue engineering, where chitin hydrogels were used as matrix for nHAp. The structure and morphology of the hydrogels were characterized by wide-angle X-ray diffraction (WAXD) and scanning electron microscopy (SEM). The content and distribution of nHAp in chitin hydrogels were determined by thermo-gravimetric analysis (TGA) and transmission electron microscopy (TEM). Moreover, the mechanical properties and biocompatibility of hydrogels were also evaluated.

2. Experimental

2.1. Materials

Chitin powder was purchased from Jinke Chitin Co. Ltd. (Zhejiang, China). The degree of acetylation (DA) was determined by elemental analysis to be 0.98. The weight-average molecular weight (M_w) and radius of gyration ($\langle S^2\rangle^{1/2}$) of chitin, measured by dynamic light scattering (DLS, ALV/CGS-8F, ALV, Germany) in 5% LiCl/DMAc (w/w), were 5.0×10^5 and 68 nm, respectively. $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2HPO_4$ (Sinopharm Chemical Reagent Co. Ltd.), NaOH, $NH_3 \cdot H_2O$, and urea (Shanghai Chemical Reagent Co. Ltd., China) were used as received. Epichlorohydrin (ECH, Chemical Agents, Ltd. Co., Shanghai, China) (density, 1.18 g/mL) was analytical-grade and used without further purification. COS-7 cells were obtained from China Typical Culture Center (Wuhan University) and cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 4 mM L-glutamine, 10% fetal bovine serum (FBS), antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) at 37 °C in a humidified air atmosphere containing 5% CO_2.

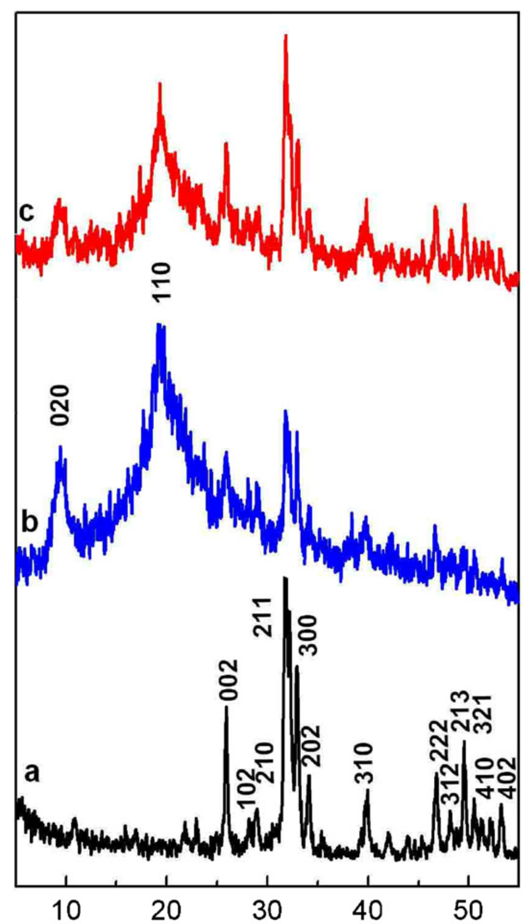

Fig. 3. WAXD patterns of hydroxyapatite (a), Gel1 (b), and Gel2 (c).

2.2. Preparation of chitin/nano-hydroxyapatite hydrogels

Nano-hydroxyapatite (nHAp) was prepared according to our previous work as follows (He, Chang, Peng, & Zhang, 2012). $Ca(NO_3)_2$ solution was added into $(NH_4)_2HPO_4$ solution with stirring, and the pH of solution was adjusted by $NH_3 \cdot H_2O$ to be 10. The mixture was stirred for 2 h and the nHAp precursor was