Preparation and characterization of bionic bone structure chitosan/hydroxyapatite scaffold for bone tissue engineering

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Introduction

As scaffold materials in bone tissue engineering, chitosan(CS) scaffold has been proven to be a potential candidate for bone regeneration because of its good biological and physical properties. Hydroxyapatite (HA) is an excellent candidate for bone repair and regeneration due to its bioactivity and osteoconductivity. However, the mechanical properties of the pure HA are inadequate, which limits its use in bone repair. In order to combine the favorable biocompatibility of chitosan with the osteoconductivity of HA, CS/HA composites with favorable properties have been prepared via in-situ precipitation method.

Materials and methods

The CS/HA scaffolds were prepared by in-situ precipitation and solid-liquid phase separation. The $CaCl_2$ and K_2HPO_4 were dissolved in 2% (v/v) acetic acid solution according to the Ca/P=1.67 (Table1). To produce the CS/HA composite scaffolds, lyophilization and in-situ precipitation were combined.

Table 1. HA precursor content in the CS solution

sample	CS/HA	CS(g)	HA(g)	C_aCl_2(g)	KH_2PO_4(g)
CS/HA5	100/5	10.00	0.50	0.59	0.43
CS/HA10	100/10	10.00	1.00	1.20	0.86
CS/HA15	100/15	10.00	1.50	1.80	1.30

Results and Discussion

Protonated chitosan would reassemble via the electrostatic force between CS-NH_3^+ and OH^- during the process of chitosan multilayer gel-rod formation. In addition, the HA precursors, which distributed homogeneously in the CS organic matrix, would crystallize in situ in the alkaline environment (see Fig.1).

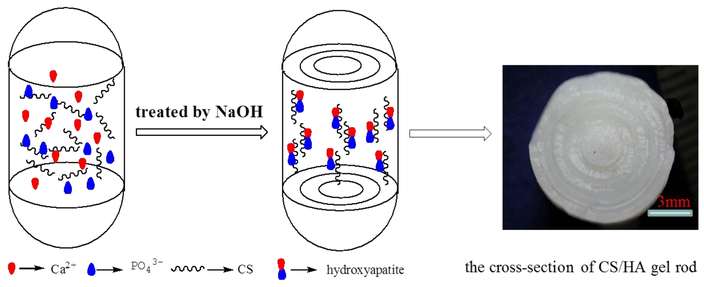

Fig.1. Scheme of the formation mechanism of CS/HA scaffolds

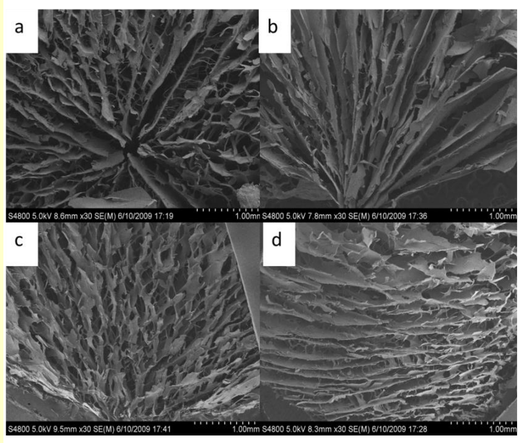

Fig.2 The SEM micrographs of cross-section (a, b, c) and vertical-section (d) of the scaffolds :
(a)CS/HA5;
(b) CS/HA10;
(c) CS/HA15;
(d) CS/HA10

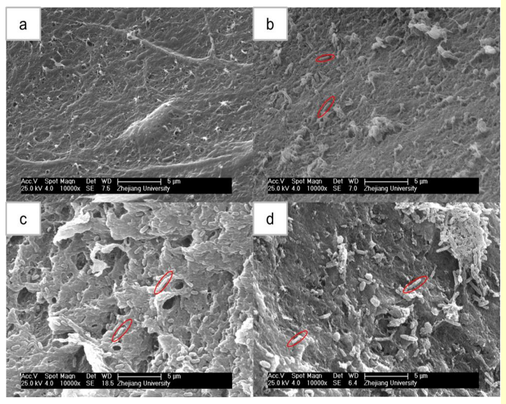

Fig.3 The SEM of CS/HA scaffolds illustrating distribution of HA (marked by the red coil) in the scaffolds:
(a)CS/HA0;
(b) CS/HA5;
(c) CS/HA10;
(d) CS/HA15

The CS/HA scaffolds exhibited a uniform interconnected open pore microstructure and a spoke-like, multilayer and porous structure from Fig.2. The Nano-HA were seen on the scaffold pore walls and were homogeneously dispersed in the matrix form Fig.3. The size of the crystal HA is about 50-500 nm with acicular shape and increased with the content of HA precursor.

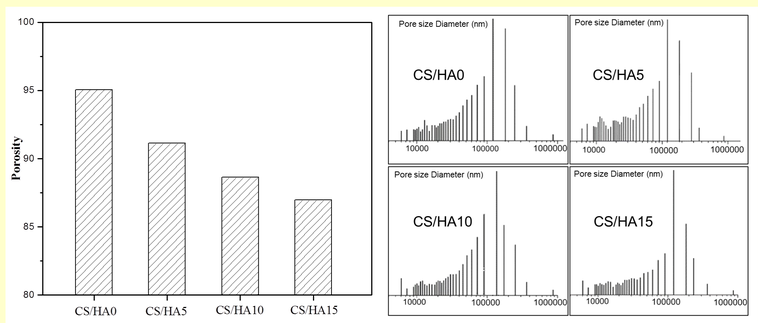

Fig.4 Porosity and Pore size Diameter of CS/HA scaffolds

The porosity of the in-situ scaffolds examined by a mercury porosimeter was above 85% (see Fig.4) and had a decreasing trend with increased content of HA. This trend might be because HA-situ precipitation process affected the formation of the porous structure, which could be proved by the clustering phenomenon in the SEM of Fig.3 d. The main pore diameter was in the range of 100-300 μm from Fig.4, which was more ideal pore size and related to the suitable heat and mass transfer rates.

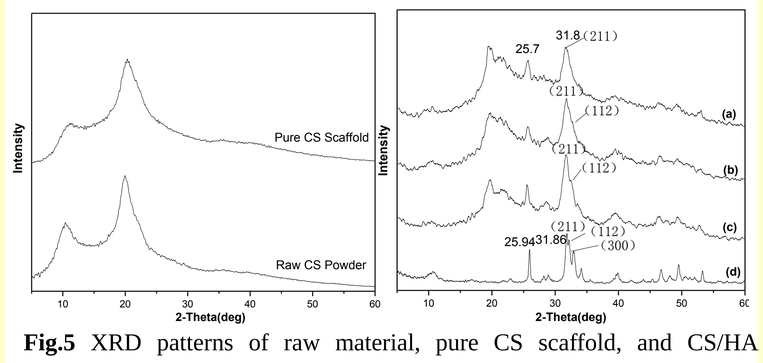

Fig.5 XRD patterns of raw material, pure CS scaffold, and CS/HA composite scaffolds: (a) CS/HA5; (b) CS/HA10; (c) CS/HA15; (d) HA (homemade)

Two broad diffraction peaks of the raw CS powder around 10 º and 20 º were observed, indicating that the forming process of the scaffolds prevented the yield of hydrogen bonds. From the Fig.5 (right), the diffraction characteristic peaks of the composite scaffolds around 31.8 º and 25.7 º corresponded to the peaks of hydroxyapatite (31.86 º, 25.94 º), showing that the hydroxyapatite was exactly formed in the chitosan matrix.

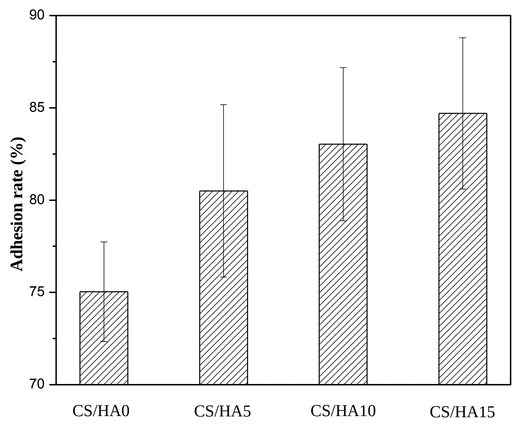

Fig.6 Adhesion of osteoblasts on CS/HA scaffolds

As shown in the Fig.6, the composite scaffolds had the higher ratio of adhesion compared to the pure chitosan scaffold, and the ratio increased with the increase of HA content. This may be due to the change of roughness of the surface with HA content. The larger surface area, another key parameter, also played an important role in the increasing protein adsorption especially adhesive proteins. As shown in Fig.7, the CLSM pictures of the cells that cultured on CS/HA scaffolds for 1 day showed that shutter-like and polygon-shaped cells spread actively on the composite scaffolds and round-like cells grew on the pure chitosan scaffolds.

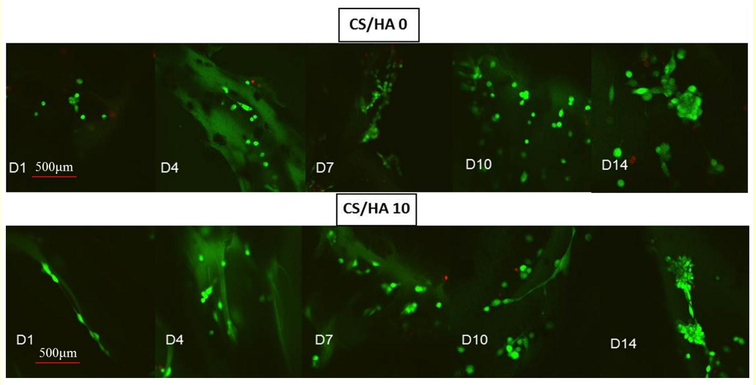

Fig.7 Confocal micrographs illustrating proliferation of osteoblasts on the CS/HA scaffolds. The green points in pictures were living osteoblasts. The red points were dead osteoblasts. All the micrographs had the same scale bars.

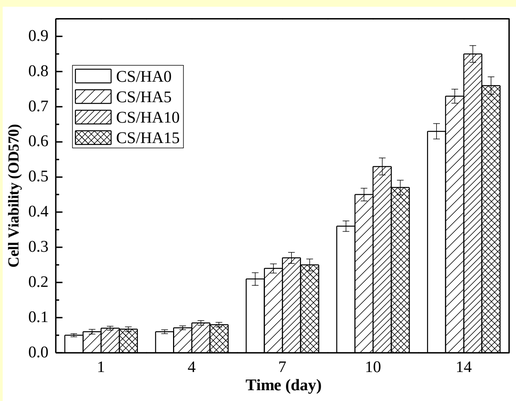

Fig.8 Viability of osteoblasts on CS/HA composite scaffolds as a function of time measured by MTT assay

A higher degree of proliferation of MC3T3-E1 was observed on the CS/HA10 scaffolds compared with the other scaffolds from Fig.8. Throughout the culture process, the state of cell growth on composite scaffolds kept always good, showing good cell compatibility.

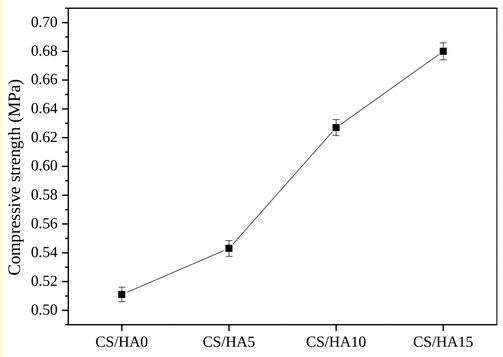

Fig.9 The compressive strengths of CS/HA scaffolds

From Fig.9, the compressive strength of the scaffolds was improved to 0.680 MPa from 0.511 MPa with the increase of the content of HA. Although the chitosan molecules and calcium ions had complexation reaction which resulted in the decrease of chitosan crystallinity and affected the strength of the scaffold, a large number of hydroxyl groups were brought into in the composite scaffold because of the formation of HA, which generated a large number of hydrogen bonds that made chitosan and HA more closely integrated. Moreover, HA, as commonly used as inorganic ceramic materials, especially in the form of human bone with acicular shape crystals of HA, had a high strength. So compared with the pure CS scaffold, the CS/HA composite scaffold had a higher strength.

Conclusions

Three-dimensional oriented chitosan/hydroxyapatite(HA) scaffolds were prepared via in-situ precipitation method. SEM images indicated that the scaffolds with acicular nano-HA had the spoke-like, multilayer and porous structure. The results of XRD and FTIR showed that the mineral particles deposited in the scaffold had phase structure similar to natural bone and confirmed that particles were exactly HA. In vitro biocompatibility evaluation, the composite scaffolds showed a higher degree of proliferation of MC3T3-E1 cell compared with the pure chitosan scaffolds and the CS/HA10 scaffold was the highest one. The CS/HA scaffold also had the higher ratio of adhesion of osteoblasts compared to the pure chitosan scaffold, and the ratio increased with the increase of HA content. The results suggested that the composite scaffolds possessed good biocompatibility. The compressive strength of CS/HA15 increased by 33.07% compared with the pure CS scaffold. This novel porous scaffold with three-dimensional oriented structure might have potential application in bone tissue engineering.

Acknowledgement

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References

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