



## Short Communication

## Biodegradation and compressive strength of phosphorylated chitosan/chitosan/hydroxyapatite bio-composites

Bin Li<sup>a</sup>, Longnan Huang<sup>b</sup>, Xinbo Wang<sup>b,\*</sup>, Jinhuan Ma<sup>b</sup>, Fang Xie<sup>b</sup>

<sup>a</sup>State Key Laboratory of Advanced Welding Production Technology, Harbin Institute of Technology, Harbin 150001, China

<sup>b</sup>School of Materials Science and Engineering, Harbin Institute of Technology at Weihai, Weihai 264209, China

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## ABSTRACT

The research about how to obtain an organic/inorganic bio-composite with excellent comprehensive properties is an active research field. Nowadays, very few of the achievements were applied to the clinical use due to many reasons. In this work, the purpose was to get a three-phase composite with good compressive strength by using phosphorylated chitosan. Phosphorylated modification of chitosan would bring new properties such as metal chelating. Four phosphorylated chitosan/chitosan/hydroxyapatite (PCS/CS/HA) composites with the weight ratios of 40/40/20, 30/30/40, 20/20/60, 10/10/80 were prepared through the coprecipitation method. The maximum value of compressive strength was measured about 70.25 MPa corresponding to the PCS/CS/HA composite rod with a weight ratio of 30/30/40. The composite rod maintained 64% of original compressive strength after soaking in simulated body fluid for 20 days. All the results showed that the PCS/CS/HA composite (30/30/40), had a good compressive strength, was appropriate to be used as a novel bio-composite for bone tissue engineering.

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### 1. Introduction

Remarkable developments can be seen in the field of biomaterials in recent years. One of the most important aspects of this field is the preparation and development of bone substitute materials. A large number of people all over the world suffer from bone disease and require bone implanted materials. More and more bio-composites have been used to effectively enhance implant fixation and bone regeneration [1,2].

There is thus an active research field of developing chitosan (CS) and hydroxyapatite (HA) composites for bone tissue engineering [3]. CS is the fully or partially deacetylated derivative of chitin which is the most abundant natural polymer after cellulose. Fig. 1 shows the chemical conversion of chitin to chitosan [4]. CS is a cationic natural polymer material. In recent years, considerable efforts have been put into the CS due to its specific properties such as biocompatibility, biodegradability and toughness [5]. However, lack of bioactivity limits its use as a bone substitute material. HA [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] is the main inorganic composition of bone tissue, with a high bioactivity, but brittleness and hardness [6–8]. Therefore, there has been growing concern about novel composites of polymer and HA to compensate the weak mechanical properties

of HA. Although there was much progress in research of CS/HA composites, up to now, few of the achievements could accord with clinical use due to many reasons such as the lack of interfacial bonding between organic and inorganic phase [9].

In order to obtain better performance, many researchers have added another polymer material as a reinforcing phase, for example, phosphorylated chitosan (PCS) [10]. Chemical modification of materials is one of the prime methods to generate new functions. The existence of amine groups and hydroxyl groups in chitosan is highly advantageous for conducting modification reaction. Recently, several chemical methods have been employed for the preparation of phosphorylated derivatives of chitosan [11–13] and there has been a steady growth of interest in this subject [14–18]. PCS could possess metal chelating [11] and biocompatible properties [19]. So, if combining the PCS, CS and HA together to take advantage of their beneficial properties and get rid of their shortcomings, it will be hopeful to obtain a good bone substitute material.

In this paper, we proposed to use PCS to obtain a three phase bio-composite which had high compressive strength. PCS powder was prepared by the reaction of orthophosphoric acid, urea and chitosan in N,N-dimethylformamide. PCS/CS/HA composites with different weight ratios were prepared by adjusting the starting content of PCS and CS. The compressive strength of the composite rod was measured by the mechanical testing. Some results of the composite characterization were also carried out by using X-ray diffraction (XRD) and scanning electron microscopy (SEM).

\* Corresponding author. Tel.: +86 631 5687675; fax: +86 631 5687305.

E-mail address: [sdwxb@hitwh.edu.cn](mailto:sdwxb@hitwh.edu.cn) (X. Wang).

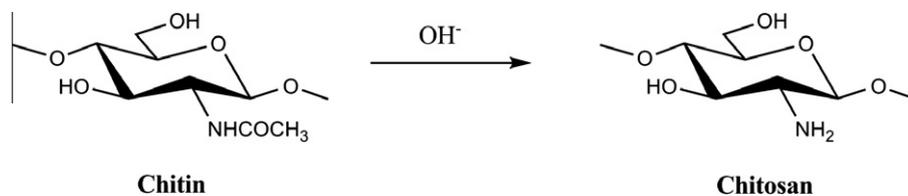


Fig. 1. Synthesis of CS.

## 2. Experimental

### 2.1. Materials

Chitosan with an N-deacetylation degree of 85% was received from Hai Debei bioengineering Co. Ltd., China. The self designed die was made from disposable syringe. Deionized water was used for all samples. Simulated body fluid (SBF) was self-prepared [20]. All other chemicals used in this work were of analytical grade.

### 2.2. Preparation of PCS

PCS was prepared by the following method [11]. In briefly, 10 g of chitosan powder was mixed with 50 g of urea and 100 ml of N,N-dimethylformamide. Then 11.8 g of 85% orthophosphoric acid was added to the chitosan solution. The mixture was reacted at 150 °C for 1 h. After cooling, the reaction mixture was filtered. The obtained precipitate was washed thoroughly with deionized water and then dried at 37 °C for 24 h. The photograph of CS and PCS with the same weight of 2 g was shown in Fig. 2.

### 2.3. Preparation of PCS/CS/HA composite powder

*Step 1:* A PCS/CS aqueous solution of 3.0 wt% was prepared by dissolving the powder of PCS and CS into deionized water containing 1.0 wt% of acetic acid. The solution was stirred for 6 h to allow the chitosan to fully dissolve, and then poured into an 8.5% H<sub>3</sub>PO<sub>4</sub> solution. The PCS/CS/H<sub>3</sub>PO<sub>4</sub> solution was obtained after sufficient stirring.

*Step 2:* The PCS/CS/H<sub>3</sub>PO<sub>4</sub> solution obtained in step 1 was then slowly added into the 5% ethanol solution of calcium hydroxide under vigorous stirring (550 r/min). After that, the pH was adjusted to about 10 with NaOH solution (0.5 M). Then the stirring process was kept for 24 h and the slurry obtained was aged for another 24 h. The reaction temperature was kept at room temperature. Finally, the sample was washed with deionized water several times and the precipitate was dried at 37 °C.

Through the coprecipitation method above [7], four PCS/CS/HA materials with the weight ratios of 40/40/20, 30/30/40, 20/20/60,



Fig. 2. Photograph of CS (left) and PCS (right) with the same weight.

**Table 1**

The amounts of initial reagents used for preparation of PCS/CS/HA composite powder.

PCS/CS/HA composition (weight ratio)	PCS (g)	CS (g)	CH <sub>3</sub> COOH <sup>a</sup> (g)	H <sub>3</sub> PO <sub>4</sub> <sup>b</sup> (g)	Ca(OH) <sub>2</sub> (g)	CH <sub>3</sub> CH <sub>2</sub> OH (g)
40/40/20	1.00	1.00	64.66	3.47	0.37	6.97
30/30/40	1.00	1.00	64.66	9.21	0.99	18.73
20/20/60	0.50	0.50	32.33	10.34	1.11	21.00
10/10/80	0.50	0.50	32.33	27.59	2.95	56.05

<sup>a</sup> CH<sub>3</sub>COOH aqueous solution of 1.0 wt%.

<sup>b</sup> H<sub>3</sub>PO<sub>4</sub> aqueous solution of 8.5 wt%.

10/10/80 were synthesized. Table 1 showed the amount of the initial reagents used.

### 2.4. Preparation of PCS/CS/HA composite rods

Firstly, The PCS/CS/HA powder was ground as fine as possible. The powder obtained was adjusted to paste with 20% citric acid solution quickly and then compressed into the self designed die which was then immersed into 1.5 M sodium hydroxide solution for 6 h. After that, the clava was taken out of the die and immersed in NaOH solution (1.5 M) for another 6 h. The sample obtained was washed with deionized water slowly and dried at room temperature for 5 days. Finally, the sample was cut into a rod with the diameter 3 mm and the length 6 mm for compressive strength test. The photograph of PCS/CS/HA composite rod with weight ratio of 30/30/40 was shown in Fig. 3.

### 2.5. Characterization

The crystal structure of composite powder was examined using XRD (Rigaku D/max-γB, Japan) with Ni-filtered Cu Kα radiation (30 kV, 20 mA) and scanning from 10° to 35° (2θ) at the speed of 8° (2θ)/min. The compressive strength of the composite rod was measured at a loading rate of 1.5 mm min<sup>-1</sup> with an universal testing machine (CSS44300, Changchun Research Institute for Testing Machines, China). The morphology of the synthesized composites was observed with SEM (VEGAI SBH, TESCAN, Czech).

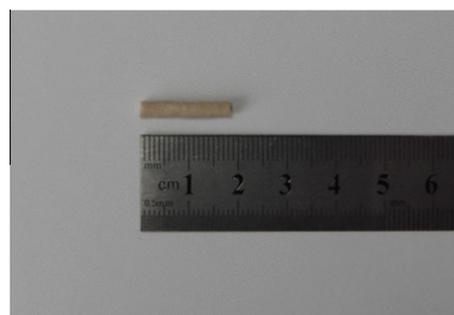


Fig. 3. Rod of PCS/CS/HA composite with weight ratio of 30/30/40.

### 3. Results and discussion

As observed in Fig. 2, the color of the CS changed from the white to light yellow with the phosphorylated modification. Meanwhile, the density of PCS became bigger than that of CS due to the introduced phosphate groups.

Fig. 4 showed the surface morphology of the HA and PCS/CS/HA composite with weight ratio of 30/30/40. SEM observation revealed differences between the HA and the composite (PCS/CS/HA = 30/30/40). Fig. 4a illustrated that the particles of HA were dispersed. In contrast, the composite with a weight ratio of 30/30/40 showed a rough surface morphology and relatively tight structure. According to the SEM, the aspect ratio in the composite was bigger than that of the HA. These results were mostly like to be ascribed to the fact that HA was coated with the PCS and CS. This observation was confirmed by Sailaja et al. in chitosan/polyacrylic acid/hydroxyapatite composites [9].

The XRD spectrum of four composite powder was illustrated in Fig. 5. The XRD spectrum of the PCS/CS/HA composite with weight ratio of 10/10/80 showed strong peaks of HA at  $2\theta = 26^\circ$  and  $32^\circ$  suggesting that HA was formed, but their becoming wide demonstrated weak crystallinity (Fig. 5d). This result was in accordance with that reported by Yamaguchi et al. [3]. The composite (PCS/CS/HA = 40/40/20) exhibited a broad peak around  $2\theta = 20^\circ$  which was the specific diffraction peaks of the PCS and CS (Fig. 5a). This characteristic of PCS and CS was also obtained in previous reports [7,18]. However, the X-ray patterns indicated the peak around  $20^\circ$  ( $2\theta$ ) decreased markedly with increasing the content of HA, meanwhile, the crystallinity of the composite thereupon became higher. New peaks around  $33^\circ$  ( $2\theta$ ) in four composite samples were observed in Fig. 5. These peaks might be the result of the interactions between the composed materials.

Fig. 6 illustrated that the compressive strength increased with the increase of the HA's concentration (less than 40 wt%), and then

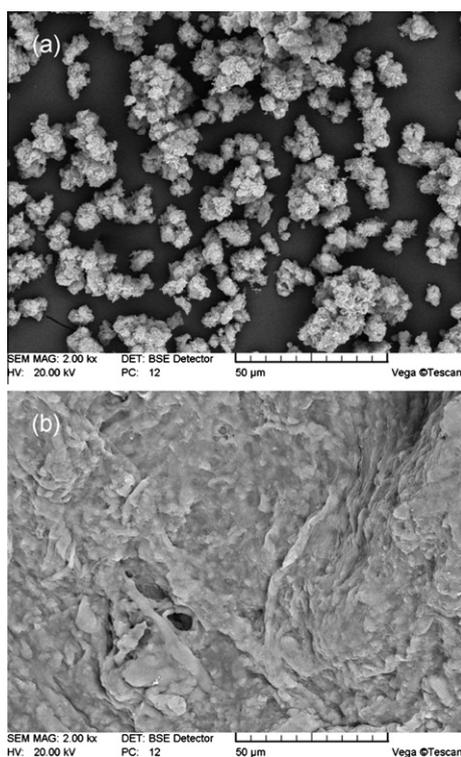


Fig. 4. SEM images of HA (a) and PCS/CS/HA composite with weight ratio of 30/30/40 (b).

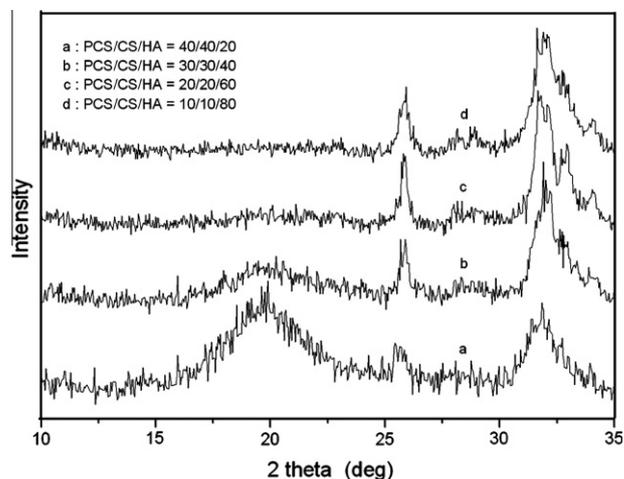


Fig. 5. XRD patterns of four composite powders.

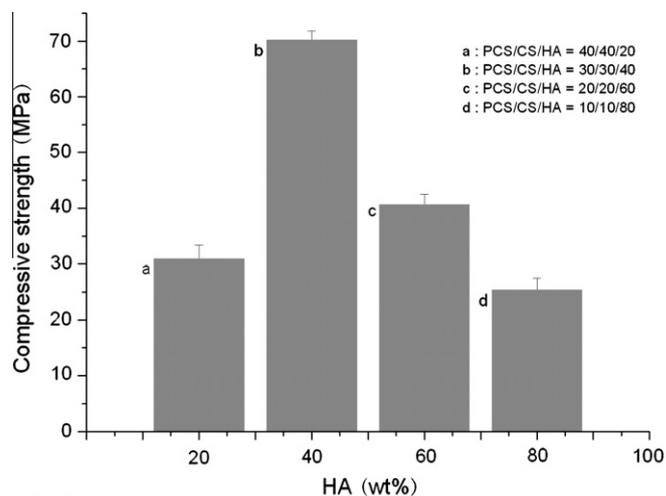


Fig. 6. Changes in the compressive strength of the composite rods.

decreased above 40 wt%. The reason might be that when the content of HA was less than 40 wt%, too much PCS and CS could not provide high strength due to their own properties of the polymer. In contrast, when it was above 40 wt%, the organic phase might be insufficient to bind so many particles of HA. This observation was similar to the result reported by other researchers [9]. The value of the compressive strength of the PCS/CS/HA composite rod with weight ratio of 30/30/40 was measured about 70.25 MPa which was the highest value in this study.

The morphology of the sections of four composite rods was examined by SEM (Fig. 7). The SEM pictures showed that the composite rod (PCS/CS/HA = 30/30/40) had a tightest section in all of the samples. The surface morphology studies of the sections were also in accord with the law of compressive strength appeared in Fig. 6. The results suggested that in these composites, there were different degree of interactions between three parent phases.

Fig. 8 showed that, as the soaking time was prolonged in SBF, the compressive strength of the PCS/CS/HA composite rod with weight ratio of 30/30/40 decreased continuously. In the first 10 days, the reduction rate of its compressive strength was slow and increased gradually. The main reason was that the water swelling was unobvious due to tight composite rod at the initial stage of soaking. At this time, the biodegradation mainly occurred

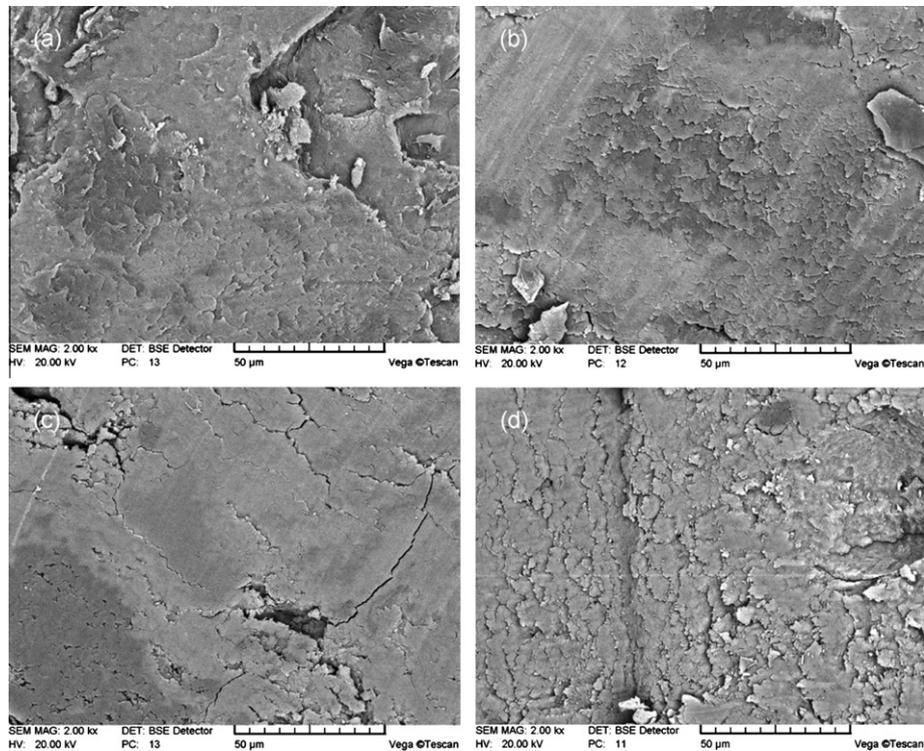


Fig. 7. SEM images of the sections of PCS/CS/HA material rods with the different weight ratios: 40/40/20 (a), 30/30/40 (b), 20/20/60 (c), and 10/10/80 (d).

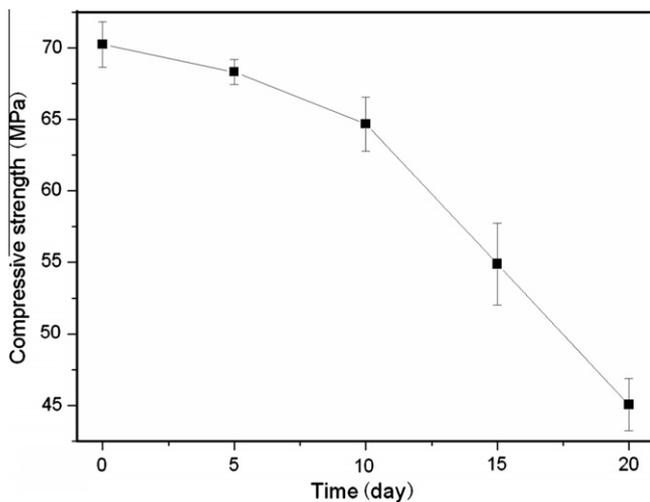


Fig. 8. Changes in the compressive strength of the PCS/CS/HA composite rods (with weight ratio of 30/30/40) after soaking in SBF.

at the surface of rod. After soaking in SBF for 10 days, the reduction rate of compressive strength became rapid because the water swelling was more obvious. Meanwhile, the reduction rate tended to be stable owing to existence of biomineralization which hindered partial degradation. Furthermore, many studies of biomineralization in SBF were indicated by Varma et al. [14] and Yokogawa et al. [19]. The compressive strength of rod changed into 45.07 MPa which was 64% of the original after soaking in SBF for 20 days.

As shown in Fig. 9, compared with the section of original rod, the cracks of section became more and more with the soaking time increasing. At the same time, the structures of section became looser due to degradation. They accorded with the law of changes in compressive strength appeared in Fig. 8.

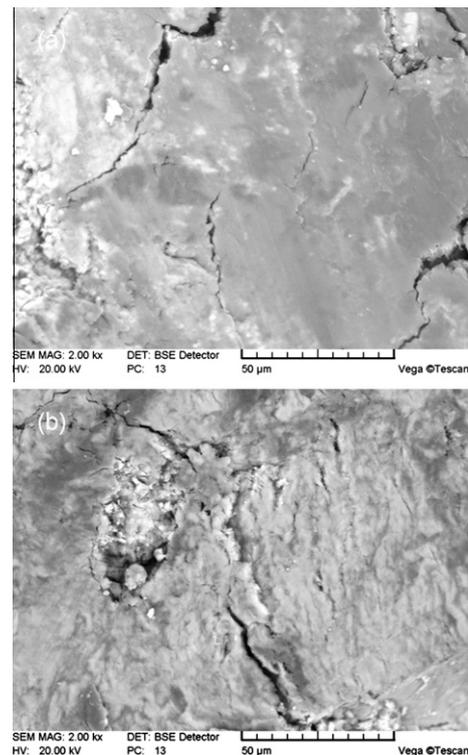


Fig. 9. SEM images of the sections of PCS/CS/HA material rods (with weight ratio of 30/30/40) after soaking in SBF for 10 days (a) and 20 days (b).

#### 4. Conclusions

In this study, a novel bio-composite was synthesized through the coprecipitation method. The composite was expected to have high biocompatibility and bioactivity simultaneously due to that

it was composed of the PCS, CS and HA. The PCS/CS/HA composite rod with weight ratio of 30/30/40 had a good compressive strength (70.25 MPa) and kept 64% of original compressive strength after soaking in SBF for 20 days. Although further studies for the composite are needed, the obtained results have an enormous potential in artificial bone repairing area. The related research including bio-activity experiment via soaking in SBF is ongoing.

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