

Evaluation of the mechanical properties, in vitro biodegradability and cytocompatibility of natural chitosan/hydroxyapatite/nano-Fe₃O₄ composite

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ABSTRACT

The main goal of this research was the preparation and evaluation of the mechanical properties, in vitro biodegradability and cytocompatibility, of natural chitosan/hydroxyapatite/nano magnetite (nano-Fe₃O₄) composite. Different ratios of these components were investigated, including chitosan/hydroxyapatite: 4/4 (S1), chitosan/hydroxyapatite: 4/6 (S2), and chitosan/hydroxyapatite: 6/4 (S3). Mechanical properties of fabricated composites were examined using bending and compression tests before immersion, and after 2 and 9 weeks of immersion in the Ringer's solution. Scanning electron microscope (SEM) was employed for observing the bending fracture surface and analyzing the degradation morphology. Human mesenchymal stem cells (hMSC) were also cultured on the samples in order to assess the cytocompatibility. The obtained results revealed that S1 had the highest bending strength before immersion, while S3 had the highest bending strength after 9 weeks immersion. Compressive strength of S2 was greater than that of S1 and S3 not only before immersion, but also after 9 weeks immersion. Although the bio-minerals were deposited on the surface of all samples during the immersion in Ringer's solution, S2 appeared to have the highest quantity of bio-minerals. According to the weight loss percentage ($\Delta W(\%)$), the biodegradation resistance of S1 was the lowest. Finally, the cytocompatibility of S1 was greater than that of S2 and S3.

1. Introduction

Metallic implants have been widely used for bone treatments [1–6]. However, some specific drawbacks of metallic implants include the stress shielding phenomenon leading to the bone loss and weakening, chronic inflammation due to degradation and ion release, and also wear, fatigue and loosening of the implant [7]. As a result, a second surgery is often required to remove the metallic implant after bone healing, which is very inconvenient and costly for patients. On the other hand, biodegradable polymers remove the necessity for a second surgery and are able to avoid the troubles related to the stress shielding. Moreover, they can simultaneously be used to deliver drugs or growth factors in order to accelerate bone growth [8]. Metallic implants have

higher strength than these polymeric implants, which improve bone growth. Consequently, the healing process is accelerated because of polymer degradation in the body, allowing replacement of remaining porosities with osteogenic cells [9].

Immersion of the implants in the Ringer's solution is necessary because the solution simulates an environment similar to that of the body, allowing investigation of the implant's mechanical properties in this comparable environment. Researchers have shown interest in developing scaffold materials for bone tissue engineering [10–20]. Many studies have focused on double hydroxyapatite (HA)/chitosan (CS) composites and its triple composites by using one of the following compounds: gelatin [21], poly lactic acid [22], collagen [23], Ag-nanoparticles [24], carbon nanotube (CNT) [25] or

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carboxymethylcellulose [9].

Magnetite is a material which is used in biodegradable composites with biomedical applications. Magnetic resonance imaging (MRI), hyperthermia, and drug-delivery systems are some examples of this material's application [26]. Nano-sized magnetite particles have higher specific surface area and thus show better performance compared to larger size magnetite particles [27]. If nanocomposites are in the form of porous scaffolds, they provide sites for tissue development and give temporary mechanical support. Nanocomposites may also be suitable for enhancement of osteoblast proliferation and osteogenesis [28]. The biomaterials produced for regenerative purposes need to have mechanical properties close to that of natural bone. CS/HA could not provide bending strength (100–150 MPa) and modulus (7–25 GPa) of human cortical bone [29]. It seems that numerous factors, including particle size and distribution of HA into the CS, the mechanical behavior of CS matrix and the interfacial bonding between the CS and HA, may contribute to the observed mechanical response of these composites. Hu et al. [8] have reported that bending strength of CS/HA: 100/5, was 68 MPa produced with blending technique, Pu et al. [29] have shown that this amount has reached to 100 MPa for CS/HA:20/4 by co-precipitation technique, and Spence et al. [30] have determined the bending strength 19 MPa for CS/HA:70/30. Compressive strength of human bone is 2–10 MPa [31], while in the scaffolds, it varies with different proportions of CS/HA. The compressive strength of CS/HA:3/7 by traditional co-precipitation technique obtained 23.3 MPa according to a published report by Zhao et al. [32], and Nikpour et al. [33] have reported 14.47 MPa for CS/HA composite with 4 g CS. Adding a third constituent, such as polylactic acid (PLLA) [34] and genipin [29], to the CS/HA composite can alter the mechanical properties. The novelty of the present investigation is the fact that natural HA, extracted from bone, and also natural CS, prepared from shrimp shells, have been used. These natural sources are certainly more economic than synthetic HA and CS. Another point of interest in the present investigation is that the magnetic nanoparticles, Fe_3O_4 , were precipitated by in situ precipitation in the CS/HA matrix. In addition, it is also important to evaluate the mechanical properties of the dry nanocomposites after soaking them in the Ringer's solution. Therefore, in this study, mechanical properties (in vitro biodegradability and cytocompatibility) of these nanocomposites were examined.

2. Experimental procedure

2.1. Materials

2.1.1. Chitin extraction from shrimp shell

In this research chitin was synthesized from shrimp shells. Method of extraction was followed according to the report by Bazargan et al. [35]. In this method, diluted HCl solution was used for demineralization. Shrimp shell powder (100 g) was added to 1000 ml of 7% (w/w) hydrochloric acid at ambient temperature (25 °C) for 24 h. After filtration with a filter paper, the residue was washed with distilled water to neutral. Then, the residue was immersed in 1 L of 10% (w/w) sodium hydroxide at 25 °C for 24 h for deprotonation. The chitin was removed by filtration, and distilled water was used to wash the residue to neutral. Lastly, ethanol was utilized to eliminate ethanol-soluble materials from the crude chitin and to dehydrate it. An air oven was used to dry the chitin at 50 °C overnight [35].

2.1.2. Preparation of chitosan

The chitin was put into 50% NaOH at 110 °C for 4 h in order to prepare crude chitosan. After filtration, the residue was washed three times with hot distilled water at 60 °C. The crude chitosan was obtained by drying in an air oven at 50 °C overnight. The degree of deacetylation of chitosan was determined to be 82.3%. It is important to note that this determination was according to the method reported by Sabnis et al. [36].

Table 1

The original amounts of the components for the fabrication of HA/CS/nano-magnetite composites.

Samples	HA (g)	CS (g)	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (g)	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (g)
S1	4	4	0.5	1
S2	6	4	0.5	1
S3	4	6	0.5	1

Table 2

Composition of Ringer's solution, with distilled water basis.

Substance	Composition (g/l)
NaCl	9.0
KCl	0.42
CaCl_2	0.24
NaHCO_3	0.2

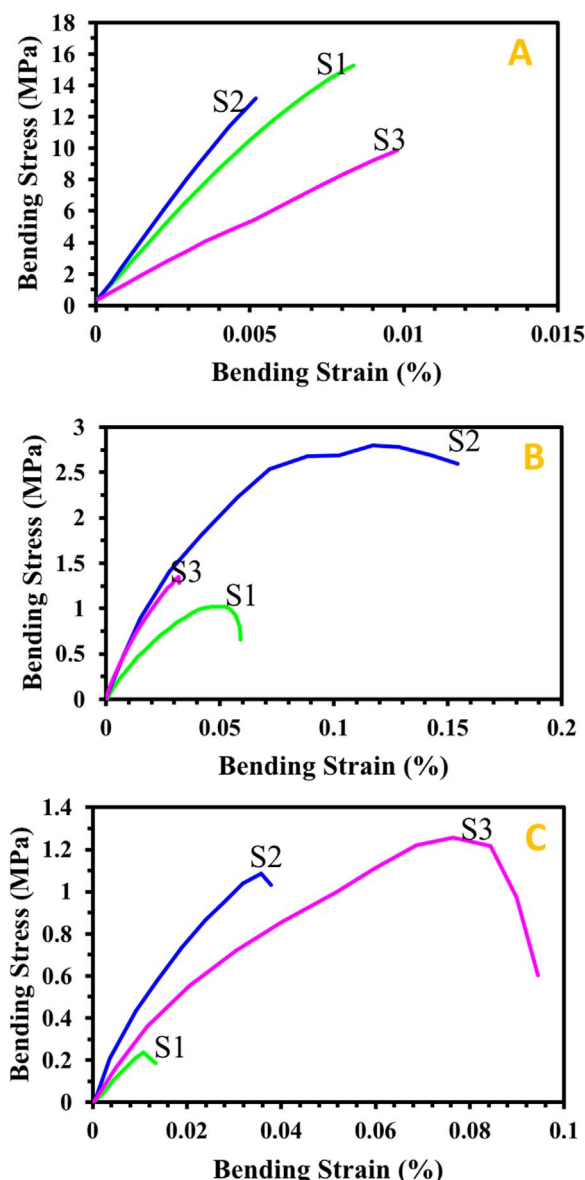


Fig. 1. Bending stress-strain curves, a) before immersion, b) after 2 weeks immersion and c) after 9 weeks immersion in the Ringer's solution.

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