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Investigation of the mechanical properties and degradability of a modified chitosan-based scaffold

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HIGHLIGHTS

- The samples consisting of CS with lesser deacetylation degree had higher bending strength.
- The samples with CS:HA = 6:4 had less bending strength and more fracture strain.
- The samples with CS:HA = 4:6 had higher fracture toughness after immersion test.

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ABSTRACT

The aim of this research is to evaluate the mechanical properties and degradation behavior of the chitosan/hydroxyapatite/ferrite nanocomposites in the ringer solution. The initial materials, including chitosan and hydroxyapatite, were developed from shrimp shells and bovine cortical bone, respectively. Ferrite was also synthesized through wet-chemical method using $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ as precursors. The obtained results showed that the samples containing chitosan with a lesser deacetylation degree (73.5%) possess higher bending strength when compared to the samples with a higher deacetylation degree (82.5%). The samples with higher amounts of chitosan remain healthier and have a lesser weight loss percentage than the samples with more hydroxyapatite. The samples with the ratio of chitosan:hydroxyapatite (6:4) have displayed the smallest amount of degradation. Additionally, the samples with lower ratio of chitosan:hydroxyapatite (4:6) (the sample labeled as B) have demonstrated significantly higher fracture toughness (regarding to compression stress-strain curves) than other samples. Finally, it is concluded that chitosan/hydroxyapatite/ferrite nanocomposite with 6 g chitosan, 4 g hydroxyapatite, 0.5 g $FeCl_2.4H_2O$, and 1 g $FeCl_3.6H_2O$ (the sample labeled as F) is selected as the optimal sample of this research, with regards to both of the mechanical properties and degradation behavior results.

1. Introduction

Several million people per year in the USA alone suffer bone fractures caused by accidents, age-related diseases, and osteoporosis, and this statistic will rapidly rise in the future due to the increased life expectancy. Expenses beyond $1.0 billion per annum were incurred in the national health system in 2004–2005 [1]. Recent commercial bio-metals (e.g., screws and bone plates) have been made of stainless steel, titanium, and cobalt-chromium alloys. However, permanent metal implants have experienced two vital problems—stress shielding phenomenon and surgical interventions. Metallic implants need to be extracted 1–2 years after implantation. For this reason, second surgical operation is required, resulting in personal, medical, social, and economical consequences and costs. To overcome this problem, producing a new generation of degradable biomaterials can have a considerable influence on

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societal and economic factors [1]. Chitosan, as a natural polysaccharide and deacetylated derivative of chitin, is a biopolymer that has the capacity to be involved in different biological activities. It is a linear, cationic polymer consisting of β (1–4)-glucosamine and N-acetyl-D-glucosamine, obtained from N-deacetylation of chitin. Notable properties of this biopolymer are susceptibility to enzymatic degradation, accelerated angiogenesis, slight fibrous encapsulation, capability to deliver growth factors, and improved cell attachment [2–4]. For these reasons, chitosan has been utilized in several biomedical applications, including drug discovery, wound dressing and 3D scaffolding [5–7]. Chitosan has also been found to improve cellular adhesion and to retain and profilerate cells, growth factors and cytokines [6]. As the main component of hard tissue, hydroxyapatite (HA), with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$, is the most commonly used calcium phosphate-based bioceramic with a high bioaffinity, biocompatibility and osseoconductivity [8–21]. Properties of HA have found beneficial application in low-load bearing porous scaffolds [22–25]. HA is an outstanding selection for regenerative medicine due to its bioactivity—it acts as a template for formation and growing of the host bone tissues [26–29]. Ferrite, with the chemical composition of Fe_3O_4, is a ferrimagnetic ceramic material used in magnetic applications. It has been currently used as a medical material with specific applications that require magnetic properties such as biosensors, cancer treatment with magnetic particles known as hyperthermia, magnetic resonance imaging (MRI), immunoassay, and drug-delivery systems (DDSs) [30–33]. Due to the higher surface to volume ratio of nano-scale magnetic particles, they have presented good medical performance, along with a lower internal diffusion resistance [33]. With all these factors in mind, we aim to develop a novel hybrid material composed of chitosan, hydroxyapatite, and ferrite. Another novelty is using inexpensive constituents, including natural HA extracted from the bone and the natural chitosan extracted from shrimp shells. Finally, in this study, the mechanical properties and biodegradation behavior of these nanocomposites are be determined and discussed.

2. Experimental procedures

In this research, chitin was synthesized from the shrimp shells according to the previous work from Bazargan et al. [34]. Natural hydroxyapatite powder was also extracted from the bovine cortical bone (Bahrololoom et al. [35]). To prepare the chitosan/hydroxyapatite/ferrite nanocomposite powder, an acetic acid solution (2% (v/v)) was prepared; $FeCl_2.4H_2O$:$FeCl_3.6H_2O$, with the weight ratio of 1:2, was added to that solution and blended for about 30 min. Hydroxyapatite powder was then poured into the prepared solution and mixed for 1 h to produce a yellow solution. Chitosan was also added to the previous solution and mixed for 1 h. All mixing processes were carried out on a magnetic stirrer at room temperature. A model was designed, in which the viscous solution was casted inside. The model was placed in a sodium hydroxide solution (5% (w/v)) for 12 h, and a gelation layer was formed on the internal surfaces of the model. The produced gel was placed in an oven at 60 °C for 24 h. The original amount of the components for the preparation of chitosan/hydroxyapatite/ferrite nanocomposites is presented in Table 1. As observed in this table, the samples with different compositions have been labeled *A* to *I*. Mechanical properties were evaluated by compression and bending tests using a universal testing machine (Zwick/Roell Z020). Cylindrical samples with the diameter of 10 mm and length of 10 mm were fabricated for compression test and rectangular samples with the dimensions of 75, 10, and 3.3 mm for bending test. Compression test was conducted according to ISO 5833 standard, at a loading rate of 20 mm/min and load versus displacement were curved at the frequency of 100 Hz. Bending test was performed by three-point mode with the span length of 40 mm and the loading rate of 1 mm/min. Compression strength was calculated considering compressive strength = F/A, whereas *F* is failure load (N) and *A* is the sample cross-sectional area A (mm^2). Bending strength was also determined by the equation of bending strength = $3FL/2bh^2$, where *F* is failure load (N), *L* is upper span (mm), *b* is width (mm), and *h* is thickness (mm). Each experiment was repeated 5 times and the mean value was reported. In order to observe the degradation behavior of the samples, they were immersed in the ringer solution for 1, 2, 3, 4, 5, 6, 7, 8 and 9 weeks and the amount of weight loss was measured. For this purpose, the dried samples were weighed before immersion and then were immersed in the ringer solution (30 mL) at room temperature. The samples were extracted from the solution after the aforementioned times and weighed again. The weight loss percentage (W_L) was finally calculated, according to the equation $W_L = (W_0 - W_1)/W_0 \times 100\%$, where W_0 is the weight of the sample before immersion and W_1 is the weight of the sample after immersion.

Table 1
The original amount of the components for the preparation of chitosan:hydroxyapatite:ferrite nanocomposites. CS_1: Natural Chitosan with 75% degree of deacetylation. CS_2: Natural Chitosan with 82% degree of deacetylation.

Samples	CS_1 (g)	CS_2 (g)	HA (g)	$FeCl_2.4H_2O$ (g)	$FeCl_3.6H_2O$ (g)
A	4	0	4	0.25	0.5
B	4	0	6	0.25	0.5
C	6	0	4	0.25	0.5
D	4	0	4	0.5	1
E	4	0	6	0.5	1
F	6	0	4	0.5	1
G	0	4	4	0.25	0.5
H	0	4	6	0.25	0.5
I	0	6	4	0.25	0.5

Cell proliferation assays on different samples was assessed using PrestoBlue Cell Viability Reagent. A standard graph was obtained by plotting number of cells [Mesenchymal Stem Cells (MSC)] versus fluorescence. The samples immersed in media without cells were utilized as the control group (Ctrl). The percentage of cell viabilities for certain days (7 days) were calculated using the equation: (mean optical density (OD) of sample at 7 days incubation/OD of Ctrl × 100%.

3. Results

Fig. 1 shows the bending stress-strain curves of the prepared samples. According to this figure, samples C, F, and I, with the ratio of chitosan:hydroxyapatite = 6:4, have indicated lesser bending strength and more fracture strain compared to the samples with the ratios of chitosan:hydroxyapatite of 4:6 and 4:4. In fact, the samples with a high level of hydroxyapatite were more brittle, had more strength and lesser fracture strain. As observed in Fig. 1, the samples consisting of the chitosan with lesser deacetylation degree (73.5%) have shown higher bending strength when compared to the samples with a higher deacetylation degree (82.5%). This can be explained by the side groups of acetyl forming a link to the main chains and being replaced to the existing hydrogen in amino groups in chitosan with a higher deacetylation degree. A shrinkage in the side chains show a decrease in mechanical properties of the polymeric chains [36]. Moreover, the samples with more ferrite have presented higher bending strength and lower fracture strain compared to the sample with less ferrite. The surface area under the stress-strain curves was calculated and considered as the fracture toughness of the samples. The results of fracture toughness after the bending test have been presented in Table 2. According to