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Biomimetic chitosan–nanohydroxyapatite composite scaffolds for bone tissue engineering

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Abstract

We describe a comparative assessment of the structure–property–process relationship of three-dimensional chitosan–nanohydroxyapatite (nHA) and pure chitosan scaffolds in conjunction with their respective biological response with the aim of advancing our insight into aspects that concern bone tissue engineering. High- and medium-molecular-weight (MW) chitosan scaffolds with 0.5, 1 and 2 wt.% fraction of nHA were fabricated by freezing and lyophilization. The nanocomposites were characterized by a highly porous structure and the pore size (~50 to 120 μm) was in a similar range for the scaffolds with different content of nHA. A combination of X-ray diffraction, Fourier transform infrared spectroscopy and electron microscopy indicated that nHA particles were uniformly dispersed in chitosan matrix and there was a chemical interaction between chitosan and nHA. The compression modulus of hydrated chitosan scaffolds was increased on the addition of 1 wt.% nHA from 6.0 to 9.2 kPa in high-MW scaffold. The water uptake ability of composites decreased with an increase in the amount of nHA, while the water retention ability was similar to pure chitosan scaffold. After 28 days in physiological condition, nanocomposites indicated about 10% lower degree of degradation in comparison to chitosan scaffold. The biological response of pre-osteoblasts (MC 3T3-E1) on nanocomposite scaffolds was superior in terms of improved cell attachment, higher proliferation, and well-spread morphology in relation to chitosan scaffold. In composite scaffolds, cell proliferation was about 1.5 times greater than pure chitosan after 7 days of culture and beyond, as implied by qualitative analysis via fluorescence microscopy and quantitative study through MTT assay. The observations related to well-developed structure morphology, physicochemical properties and superior cytocompatibility suggest that chitosan–nHA porous scaffolds are potential candidate materials for bone regeneration although it is necessary to further enhance the mechanical properties of the nanocomposite.

Keywords: Chitosan; Nano-hydroxyapatite; Scaffold; Bone tissue engineering

1. Introduction

In recent years, significant progress has been made in organ transplantation, surgical reconstruction and the use of artificial prostheses to treat the loss or failure of an organ or tissue. However, the drawbacks associated with these treatments have led to the consideration of the tissue engineering approach using cells and scaffolds [1,2]. Attempts are being made to engineer virtually every human tissue. Currently, tissue engineering using cell and biomaterials-based therapy to repair bone, cartilage and intervertebral disk are being explored [3–5]. The approach of tissue engineering is considered promising to repair or regenerate damaged tissue through the substitution of engineered tissue with the aim that it will help restore the functions during regeneration and subsequent integration with the host tissue [2]. In this regard, significant attention is being given to three-dimensional polymer scaffolds [1,6–8]. These scaffolds provide the necessary support as artificial extracellular matrices, allowing cells to proliferate and maintain their differentiated functions. Essentially, they serve as a template to guide the formation of a new tissue. In bone tissue engineering, the biodegradable scaffold is a temporary

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template introduced at the defective site or lost bone to initiate bone tissue regeneration, while it gradually degrades and is replaced by newly formed bone tissue. An ideal scaffold is characterized by excellent biocompatibility, controllable biodegradability, cytocompatibility, suitable microstructure (pore size and porosity) and mechanical properties [7,8]. Additionally, it must be capable of promoting cell adhesion and retaining the metabolic functions of attached cells.

The natural biopolymer chitosan is currently a subject of interest in tissue engineering [3–5]. Chitosan is a linear polysaccharide derived from partial deacetylation of chitin [9]. Chitin consists of 2-acetamido-2-deoxy-β-D-glucose through a β (1 → 4) linkage commonly found in shells of marine crustaceans, insects and cell walls of fungi. Chitosan is a copolymer of (1 → 4)-2-acetamido-2-deoxy-β-D-glucan (*N*-acetyl D-glucosamine) and (1 → 4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) units randomly or block distributed throughout the biopolymer chain depending on the processing method adopted to obtain the biopolymer. Chitosan is considered as an appropriate functional material for biomedical applications because of high biocompatibility, biodegradability, non-antigenicity and adsorption properties [10–13]. Anti-inflammatory or allergic reactions have not been observed in human subjects following topical application, implantation, injection and ingestion [10,11,13].

The ability of chitosan to support cell attachment and proliferation is attributed to its chemical properties. The polysaccharide backbone of chitosan is structurally similar to glycosaminoglycans, the major component of the extracellular matrix of bone and cartilage [4]. Other advantages of chitosan scaffolds for bone tissue engineering include the formation of highly porous scaffolds with interconnected pores, osteoconductivity and ability to enhance bone formation both in vitro and in vivo [3–5].

While chitosan is a biocompatible substrate for cell propagation and is potentially an effective template in the repair of osseous and chondral defects, there is a need to improve the mechanical strength and biological property of chitosan scaffolds in order to make them suitable for bone tissue engineering [14]. The mechanical properties of the scaffold are important for hard tissue like bone in order to transmit mechanical force and form matrix mineralization. Current attempts are focused on improving the mechanical and biological properties of chitosan scaffolds through the incorporation of bioceramics such as hydroxyapatite (HA) [15–20], β-tricalcium phosphate [21,22] and calcium phosphate [14,23–27] biomaterials like gelatin [15,21,28,29], alginate [30] or inorganic material such as wollastonite [31]. Incorporation of calcium phosphate into the chitosan matrix improved biocompatibility and hard tissue integration and assisted in tailoring degradation and resorption kinetics. The demonstration of cytocompatibility of chitosan–HA scaffold suggests their potential use for bone tissue engineering applications using pre-osteoblast or osteoblast cells [18,20,25]. An improved osteogenic differentiation of human mesenchymal stem cells upon osteogenic induction was reported for chitosan–gelatin–HA matrix [15].

Considering that bone is a nanocomposite of minerals and proteins, it is preferred to consider a nanocomposite that mimics natural bone. The extracellular matrix of natural bone is composed of organic and inorganic phases, where the organic component consists of type I collagen, glycosaminoglycans, proteoglycans and glycoprotein and the inorganic constituent is hard and brittle HA. The limitations associated with the independent use of chitosan or bioceramics can be overcome by combining chitosan with hydroxyapatite. In this regard, the development of nanocomposite materials with controllable bioactivity and biodegradability, and suitable mechanical property for bone tissue engineering, is currently being explored [17–20].

In the earlier work by other research groups, methodologies to synthesize chitosan–nanohydroxyapatite (nHA) composites through in situ hybridization by ionic diffusion processes [17], freezing and lyophilization [18], stepwise co-precipitation [19], and mineralization via double diffusion [20] were described. In these studies, structural morphology [17–20], mechanical [17] and physical [17–20] properties, biodegradation [20], and cytocompatibility [18,20] of the composites were examined in the attempt to formulate matrices that closely mimic bone. The use of these approaches led to fabrication of chitosan–nHA composites of defined microstructure and properties. These studies indicated significant improvement in mechanical properties including bending strength (86 MPa) and modulus (3.6 GPa) of the composites [17] and higher cell proliferation and spreading on the chitosan–nHA scaffolds in comparison to pure chitosan scaffolds [18]. The composites fabricated using the stepwise co-precipitation approach had resemblance to biological apatite [19]. Transmission electron microscopy of the mineralized scaffold indicated formation of thin needles of HA entangled in the matrix [20]. The greater cellular response in the mineralized scaffold in relation to non-mineralized scaffold was attributed to the presence of apatite. However, a coherent understanding involving process–structure–functional property relationship and biological response appeared to be inadequately addressed. Thus, our objective here is to describe a focused study that combines the different aspects including microstructure, functional and mechanical properties, biodegradation profile, and biological response to further advance our understanding in the effort to develop three-dimensional cell-nanocomposite constructs.

In order to reach these scopes, we describe here the synthesis of a bone-like organic–inorganic biomimetic nanocomposite consisting of chitosan and nHA for potential use as a bone tissue engineering material. The biomimetic nanocomposite was fabricated using chitosan of different molecular weights (MWs) and various fractions of nHA. The different MW chitosans were used to determine and compare their feasibilities as scaffolding matrices to achieve

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