Colloids and Surfaces B: Biointerfaces 143 (2016) 81–87

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Preparation of collagen/hydroxyapatite/alendronate hybrid hydrogels as potential scaffolds for bone regeneration

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 4 March 2016
Accepted 9 March 2016
Available online 11 March 2016

Keywords:
Hydroxyapatite
Collagen
Alendronate
In situ forming hydrogels
Enzymatically degradable
Bone tissue engineering

ABSTRACT

Development of biomimetic scaffolds represents a promising direction in bone tissue engineering. In this study, we designed a two-step process to prepare a type of biomimetic hybrid hydrogels that were composed of collagen, hydroxyapatite (HAP) and alendronate (ALN), an anti-osteoporosis drug. First, water-soluble ALN-conjugated HAP (HAP-ALN) containing 4.0 wt.% of ALN was synthesized by treating HAP particles with ALN. Hydrogels were then formed from HAP-ALN conjugate and collagen under physiological conditions using genipin (GNP) as the crosslinker. Depending on the ALN/collagen molar ratio and GNP concentration, the gelation time of hydrogels ranged from 5 to 37 min. Notably, these hybrid hydrogels exhibited markedly improved mechanical property (storage modulus G′ = 38–187 kPa), higher gel contents, and lower swelling ratios compared to the hydrogels prepared from collagen alone under similar conditions. Moreover, they showed tunable degradation behaviors against collagenase. The collagen/HAP-ALN hybrid hydrogels supported the adhesion and growth of murine MC3T3-E1 osteoblastic cells well. Such tough yet enzymatically degradable hybrid hydrogels hold potential as scaffolds for bone tissue engineering.

1. Introduction

Repair of large-size bone defects resulted from trauma, resection, or congenital malformations remains challenging. Thus far, a variety of autografts, allografts, and alloplastic materials have been used for the treatment of such defects in the clinical practices of orthopedic and craniofacial settings, each of which have considerable limits [1–4]. Bone tissue engineering, through the use of osteoconductive and osteoinductive scaffolds together with osteogeic cells, represents a promising approach toward bone defect repair and regeneration. As a unique triphasic tissue, bone is composed of cells, a hydrated extracellular organic matrix (mainly Type I collagen), and an extracellular mineral phase (mainly hydroxyapatite) [1,5,6]. In order to support cell adhesion and guide new bone formation in bone tissue engineering, it is critical to develop scaffolds that both chemically and structurally mimic the native extracellular matrix (ECM) of bone [7–11].

Numerous scaffolds have been prepared from natural or synthetic polymers through various techniques such as electrospinning, self assembly, and salt leaching for guiding bone regeneration [12–15]. Among them, hydrogels have gained special attention because they can hold a large amount of water while maintaining the structural integrity [16,17]. More importantly, they allow adequate permeability of water, nutrients, metabolites or pharmaceuticals to promote bone formation [18,19]. However, hydrogels generally do not possess sufficient mechanical strength for regenerating tough tissues such as bone. Improvement of mechanical properties of hydrogels can be achieved by physical, enzymatic, or chemical crosslinking [20–22]. Genipin (GNP), a natural product derived from gardenia fruit extract which has little toxicity, functions as an efficient crosslinker by reacting with the amine groups of amino acids or proteins [23–25]. The biocompatibility of materials or tissues crosslinked by genipin significantly exceeds those crosslinked by glutaraldehyde or epoxy compounds, yet the mechanical strength and enzymatic resistance of them are comparable [26].

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http://dx.doi.org/10.1016/j.colsurfb.2016.03.025
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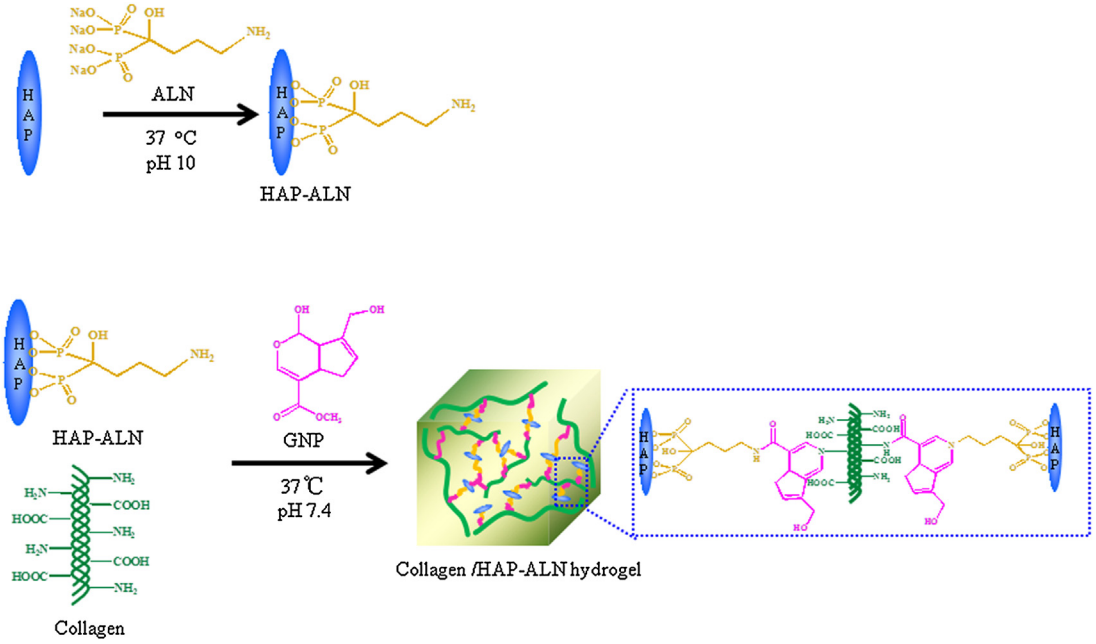

Scheme 1. Schematic illustration of the synthesis of collagen/HAP-ALN hydrogels.

In this study, we aim to develop a type of biomimetic hydrogels for the applications as bone tissue engineering scaffolds. These hydrogels were designed as a hybrid system which contains hydroxyapatite (HAP), collagen, and alendronate (ALN) and was reinforced by crosslinking using genipin. Among them, HAP is one of the frequently used bioceramics for bone and dental tissue reconstitution. It has excellent biocompatibility and osteoconductivity despite its slow degradation, low mechanical strength and poor osteoinductive potential [27,28]. Collagen is the major ECM of bone tissue and can promote bone regeneration [29]. When associated with HAP particles to form a biocomposite, it results in an easily molded biomaterial and can prevent HAP dispersion in implants [30]. Alendronate (ALN), a potent nitrogen-containing bisphosphate for the treatment of osteoporosis, affects the activity of osteoclasts by inhibiting farnesyl diphosphate synthase (FDPS) in the mevalonate pathway and results in apoptosis of osteoclasts [31–33]. Meanwhile, it may also promote the activities of osteoblasts toward bone formation [34,35]. Therefore, we have prepared a series of GNP-crosslinked collagen/HAP-ALN hydrogels with various GNP concentrations and ALN/NH_2 molar ratios and studied their properties and biocompatibility.

2. Materials and methods

2.1. Materials

Hydroxyapatite nanocrystals (HAP, ca. 20 × 150 nm) and alendronate (ALN, 99%) were purchased from Nanjing Emperor Nano Material Co., Ltd. and Beijing JHYB Pharmaceutical Technology Co., Ltd, respectively. Collagen was purchased from Sichuan Mingrang Bio-Tech Co., Ltd. Genipin (GNP, 98%) was purchased from Linchuan letter Biological Technology Co., Ltd.

2.2. Synthesis of HAP-ALN

HAP (1.0 g) was dispersed through brief sonication in 150 mL aqueous NaOH (5 mM) and ALN (0.2 g) was dissolved in 50 mL aqueous NaOH (5 mM). After adjusting pH to 10 using 20 mM NaOH, the ALN solution was added to the HAP dispersion. The mixture was stirred for 3 days at 37 °C. The crude product was isolated through extensive dialysis (MWCO 1000) against water for 24 h at room temperature to remove free ALN and then freeze-dried.

2.3. Characterizations of HAP-ALN

^{1}H NMR spectra were recorded on a Unity Inova 400 spectrometer operating at 400 MHz. The chemical shifts were calibrated against residual solvent (D_2O) signal. Fourier transform infrared spectrometer (FT-IR) was performed on a Thermo Scientific NICOLET 6700 spectrophotometer. Powder X-ray diffraction (XRD) was performed on an X'Pert-Pro MPD X-ray diffractometer with a Cu tube anode. Thermo gravimetric analysis (TGA) was carried out on a Pyris 1 TGA (Perkin Elmer) under a nitrogen atmosphere at a heating rate of 10 °C/min. HAP and HAP-ALN nanocrystals were heated from 30 to 800 °C.

2.4. Preparation of collagen/HAP-ALN hydrogels

The synthetic route of the hydrogels is shown in Scheme 1. In brief, collagen and GNP were separately dissolved in phosphate buffered saline (PBS, pH 7.4, 10 mM) at concentrations of 2 wt.% and 1 wt.%, respectively, at 37 °C. The collagen/HAP-ALN hydrogels were prepared from HAP-ALN and collagen at molar ratios of ALN/NH_2 from 2.7/1 to 10.8/1 to ensure that collagen and HAP-ALN uniformly dispersed in the solution. After that, the obtained mixtures were crosslinked by GNP at concentrations ranging from 0.04 wt.% to 0.4 wt.% at 37 °C.

2.5. Characterizations of hydrogels

2.5.1. Rheological analysis

Rheological measurements of the hydrous collagen/HAP-ALN hydrogels were carried out using HAAKE RheoStress 6000 (Thermo Fisher, Germany). The sample was placed between parallel plates of 20 mm diameter and with a gap of 0.5 mm, a frequency of 1 Hz and a strain of 1% were applied to maintain the linear viscoelastic