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Development of sodium alginate-xanthan gum based nanocomposite scaffolds reinforced with cellulose nanocrystals and halloysite nanotubes

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ABSTRACT

Sodium alginate (Alg) and xanthan gum (XG) based nanocomposite scaffolds reinforced with various amounts of cellulose nanocrystals (CNCs) and/or halloysite nanotubes (HNTs) were prepared by freeze-casting/drying method. In this study, the structure-property-performance relationship was mainly focused and analysed. Morphological analysis showed high porosity and pore-interconnectivity (pore channels) in all obtained scaffolds. Structural analysis demonstrates the good interfacial interactions and uniform dispersion of the CNCs and HNTs, involving partial orientation within the polymeric network. The water uptake capacity (from 14.73.7 ± 0.46 g/g to 11.34 ± 0.32 g/g) and porosity (from 91.7 ± 0.81% to 88.5 ± 0.64%) were reduced. The compressive strengths (in dry state from 91.1 ± 1.2 kPa to 114.4 ± 0.6 kPa and in wet state from 9.0 ± 0.8 kPa to 10.6 ± 0.8 kPa), thermal stability, cytocompatibility (MC3T3-E1 osteoblastic cells) of the nanocomposite scaffolds improved as compared to Alg and AlgX scaffolds without CNCs and/or HNTs. The obtained scaffolds may be appropriate as scaffolding material in bone tissue engineering.

1. Introduction

Bone tissue engineering needs a scaffold that must be capable of facilitating the formation of new bone tissues under three-dimensional (3D) microenvironment. To achieve this goal, several research studies have extensively been performed and tried to establish appropriate scaffolding systems, but the search of finding of the ideal scaffold for bone tissue regeneration still continues. For this purpose, various natural and synthetic polymers have been applied in their pure and combined forms. Among them, natural polymers such as sodium alginate, xanthan gum, and cellulose have gained special interest in tissue engineering due to their excellent biocompatibility, biodegradability, easy processing, and environment-friendly nature [1–3].

Sodium alginate (Alg) is a natural polysaccharide and produced mainly from brown sea algae. Alg has widely been applied in biomedical applications, especially in tissue engineering due to its biocompatibility, inherent hydrophilicity, easy processing, and gelling properties under mild conditions [1,4,5]. This gelling property comes from the interaction between the carboxylic acid (1,4-linked β-D guluronic acid residues as G units) and bivalent counter ion (e.g. calcium ion) [6] and makes egg-box structure [7]. However, Alg exhibits some limitations in terms of uncontrolled degradation behavior, lack of cell affinity, and low mechanical properties [8]. Further, XG is also gaining considerable attention in tissue engineering applications. XG is anionic polysaccharide with branched chains (produced from *Xanthomonas campestris*). It shows good water-solubility, biocompatibility, and biodegradability. XG also exhibits weak 'gel-like' behavior under an appropriate amount of inorganic salt due to its double-helix conformation as microgel and shear-thinning property under stress. It has broadly been used in several industrial and biomedical applications [2,9–12]. The shear-thinning property of XG is very beneficial in controlling gelling behavior in the targeted site [12].

In addition, Halloysite nanotubes (HNTs) as a dioctahedral 1:1 clay mineral ($Al_2Si_2O_5(OH)_4.nH_2O$) are biocompatible and environmental friendly in nature and show good potential for

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developing new organic-inorganic hybrid hydrogels. HNTs are nanoscaled hollow tubular structures having 0.2–1.5 μm in length and an aspect ratio of 20 as well as inner and outer diameter of the nanotubes 10–20 nm and 40–70 nm, respectively. Chemically, HNTs have innermost aluminium (positive) and outermost silicate (negative) surfaces, respectively [13,14]. Due to their hydrophilicity (Al-OH and Si-OH groups), HNTs show easy dispersion and good interfacial compatibility in water-based processing of polymers for green materials and biomedical applications [15–19]. Also, cellulose-based nanomaterials have attracted a great attention in biomedical applications. In this case, the nanocellulose (e.g., cellulose nanocrystals (CNCs)) has unique properties such as nanoscale size, high aspect ratio, high surface area, low-density, good hydrophilicity, high strength (~7.5 GPa) and modulus (110–220 GPa) [20,21], excellent biocompatibility, and ability to maintain moisture. In recent years, nanocellulose has been used as ideal nano-reinforcement as well as with other nano-reinforcements for the preparation of nanocomposites for tissue engineering applications [1,2,22,23]. Recently, HNTs were incorporated with Alg matrix and the obtained properties of the porous scaffolds were analysed for possible application in the tissue engineering [24]. In another study, HNTs and CNCs were incorporated in the poly (vinyl alcohol) matrix and prepared non-porous nanocomposite films by using solvent-casting method for possible use in food packaging applications [25]. To explore the applicability of the natural polymers and to improve the biological response and mechanical performance of the scaffolds for bone tissue engineering [26], the incorporation of HNTs and CNCs in the Alg/XG matrix may have synergistic effect on the obtained properties of the nanocomposite porous scaffolds.

In the present study, we describe the research study on rheological, microstructural, mechanical, and thermal properties of the Alg/XG based solutions, and Alg/XG based porous nanocomposite scaffolds. To the best of our knowledge, this the first report on the combined effect of CNCs and HNTs as nano-reinforcement in the sodium alginate-xanthan gum polymeric system. First, Alg and XG were mixed homogeneously and further mixed with CNCs and/or HNTs to form homogenous organic-inorganic nanocomposite solutions, which were subsequently freeze-dried for 3D porous nanocomposite scaffolds. The obtained nanocomposite solutions were characterized by rheological analysis and scaffolds were characterized by field emission scanning electron microscopy, fourier transform infrared spectroscopy, X-ray diffraction analysis, dynamic mechanical analysis (under compression), thermal stability, and cytocompatibility. In addition, water uptake capacity and porosity of the nanocomposite scaffolds were determined.

2. Materials and methods

2.1. Materials

Sodium alginate (Alg; 300–400 cps) was purchased from Wako Pure Chemical Ltd., Japan and xanthan gum (XG; 800–1200 cps) and halloysite nanotubes (HNTs; average size (diameter x L): 30–70 nm x 1–3 μm, surface area: 64 m^2/g, pore size: 1.26–1.34 ml/g) were purchased from Sigma-Aldrich (St Louis, MO, USA) (TEM image is shown in Fig. 1). The aqueous suspension of cellulose nanocrystals (CNCs; rod-like nanocrystals with diameter: 5–35 nm and length: 40–290 nm) was prepared as described in our previously reported work [1,8], as shown in Fig. 1. All used chemicals were of analytical grade.

2.2. Preparation of Alg/XG based nanocomposites

For Alg/XG (AlgX) composite solutions, Alg (1.35 g) and XG (0.15 g) were dissolved respectively in 50 ml of distilled water at room temperature for overnight under mechanical stirring. The AlgX based nanocomposite scaffolds reinforced with HNTs and CNCs were prepared by freeze-casting/drying method. For the preparation of AlgX-HNTs and AlgX-CNCs nanocomposite scaffolds, homogenous aqueous suspensions of CNCs (5 and 10 wt% of AlgX content) and HNTs (2.5 and 5.0 wt% of AlgX content) were prepared separately in 50 ml distilled water. AlgX powder mixtures (3.0 wt% in total) were dissolved gradually in these prepared aqueous suspensions with continuous stirring for overnight at room temperature and then for 30 min at 25 °C under sonication, respectively. In addition, for the preparation of AlgX-CNCs-HNTs nanocomposite scaffolds, CNCs aqueous suspensions with specific amounts (5 and 10 wt% of AlgX content) were prepared and then HNTs (2.5 and 5.0 wt% of AlgX content) were added and dispersed in CNCs aqueous suspensions (5.0 and 10.0 wt%), respectively in order to obtain CNCs-HNTs suspensions. Alg/XG (2.7/0.3 wt%) were subsequently added to these CNCs-HNTs aqueous suspensions followed by constant mechanical stirring for complete dissolution at room temperature for overnight in order to obtain AlgX-CNCs-HNTs nanocomposite solutions. The resulting nanocomposite solutions were sonicated for 30 min at 25 °C before being poured into the plastic mold (24 well plates) for freeze-casting/drying at −40 °C to obtain the porous AlgX-CNCs-HNTs nanocomposite scaffolds. For the comparison, Alg scaffold forming solution (3.0 wt%) was also prepared by dissolving 1.5 g of Alg in 50 ml of distilled water at room temperature for overnight under mechanical stirring to obtain Alg porous scaffold. The samples codes were developed and presented as Alg, AlgX, AlgX-5.0CNCs, AlgX-10.0CNCs, AlgX-2.5HNTs, AlgX-5.0HNTs, AlgX-5.0CNCs-2.5HNTs, AlgX-5.0CNCs-5.0HNTs, AlgX-10.0CNCs-2.5HNTs, and AlgX-10.0CNCs-5.0HNTs, respectively for the final scaffolding materials. The various compositions of the scaffolds are given in Table 1.

2.3. Characterization

2.3.1. Transmission electron microscopy (TEM)

The morphology of HNTs was examined by using transmission electron microscopy (TEM; FEI Tecnai G2 F20) with 100–120 kV as an accelerating voltage. A drop of a dilute aqueous suspension of HNTs was deposited on the surface of a copper grid coated with a carbon film. Before analysis, sample was dried at room temperature.

2.3.2. Rheological analysis

The rheological behavior of the pure and nanocomposite solutions was performed using a modern Rheometer (Physica MCR301; Anton Paar, Graz, Austria) at 25 °C. The Rheometer was setup with a parallel plate geometry using 25 mm diameter plates. A circulating water bath (TC10, Dagan Corporation, Minneapolis, MN, USA) was used to control the temperature and UDS 200 software provided by manufacturer was used to determine the rheological parameters. The measurements were evaluated at frequency range of 1.0–100 rad/s.

2.3.3. Field emission scanning electron microscopy (FESEM)

The morphology of the HNTs and nanocomposite scaffolds was analysed by field emission scanning electron microscope (FESEM, Hitachi S-4800) at 8 mm working distance, 15 kV and 10 μA. Before analysis, HNTs powder was sprinkled on stab, while nanocomposite hydrogels were freeze-dried (−40 °C) and coated with a fine platinum layer at a low deposition rate using an ion sputter coater.

2.3.4. Fourier transform infrared (FTIR) spectroscopy

For characteristic the functional groups of polymers and their