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Effects of halloysite nanotubes on physical properties and cytocompatibility of alginate composite hydrogels

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ABSTRACT

Sodium alginate (SA)/halloysite nanotubes (HNTs) composite hydrogels were successfully prepared by solution blending and cross-linking with calcium ions. HNTs can improve the physical properties and cytocompatibility of composite hydrogels. The static and shear viscosity of SA/HNTs solution increase by the addition of HNTs. FTIR suggests the presence of hydrogen bond interactions between HNTs and SA. The crystal structure of HNTs is retained in the composites as showed by the X-ray diffraction result. A porous structure with pore size of 100–250 μm is found in the hydrogels, which can provide a space for cell growth and migration. The compressive mechanical properties of composite hydrogels significantly increase compared to the pure SA hydrogel. The SA/HNTs composite hydrogels with 80% HNTs loading exhibit the compressive stress at 80% strain of 2.99 MPa, while the stress at 80% strain of pure SA hydrogel is only 0.8 MPa. The dynamic storage modulus of composite hydrogels also markedly increases with HNTs concentration. The differential scanning calorimetry endothermic peak area and swelling ratios in NaCl solution of the composite hydrogels decrease by the addition of HNTs. Preosteoblast (MC3T3-E1) culture results reveal that the SA/HNTs composites especially at relatively low HNTs loading show a significant increase in cells adhesion and proliferation compared to the pure SA hydrogel. All the results demonstrate that the SA/HNTs composite hydrogels show a promising application in bone tissue engineering.

1. Introduction

Tissue engineering is considered as a potentially valuable method to repair the tissue defects by autologous cells/tissue transplantation technology [1]. Porous degradable scaffolds provide a specific environment for cells/tissue growth in vivo and vitro. Many types of materials, including polymers, inorganics, and metals, have been applied to fabricate tissue engineering scaffolds [2–4]. Recently, composite scaffolds which are a combination of the different advantage materials have been designed to satisfy the rigorous need for tissue growth. For ideal tissue engineering scaffolds, they should have sufficient porosity, interconnection channels employed to transport nutrients, well biocompatibility and biodegradability, as well as with a high mechanical strength [5]. Among the materials used for tissue engineering scaffolds, synthetic biodegradable polymers represent an important type since their properties can be readily adjusted via the control of the polymerization reaction [6]. The main drawback of the synthetic biodegradable polymer scaffolds is lack of the bioactivity. Biomacromolecules (such as protein, nucleic acid or polysaccharide) derived from natural resource is another type of scaffold materials. They have many advantages in the application of biomedical areas, such as fine biodegradable, biocompatible, antithrombotic (prevents blood clots) and hemostatic properties, remarkable healing activity, water retention capacity, antibacterial, immunological and anti-tumor properties, and low cost [7–9].

Alginic acid is a natural polysaccharide copolymer consisting of β-D-mannuronic acid (M units) and α-L-guluronic acid (G units) [10], which has widespread application in biomedical areas. Sodium alginate (SA) can form a gel rapidly in the presence of divalent metal ions by the complexation. The SA hydrogels have been extensively applied in drug/gene delivery, tissue engineering, wound healing, cell encapsulation, and so on [11,12]. SA hydrogel as scaffold for tissue engineering has unique advantages, for example, they can be mixed with the cells in liquid form into the body to fill the damaged tissue, the three-dimensional network structure is similar to skeleton to provide a three-dimensional growing space for cells growth [13,14]. Owing to the high rate of degradation in cell culture and the weak mechanical properties, a single component SA hydrogel limits the practical application. To solve this issue, SA has been combined with various components to obtain different composite scaffolds. The nanosilica, α/β-tricalcium phosphate, graphene oxide (GO), halloysite nanotubes (HNTs), and chitin whisker, have been incorporated into alginate matrix which is aiming to improve the adsorption efficient, mechanical strength, and other physical properties [15–18]. For example, the addition of GO can greatly increase the thermal stability and mechanical properties of the SA composite [19,20]. Chitin whiskers can also markedly promote the cell adhesion and proliferation of

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osteoblast cells into the SA nanocomposite hydrogels [15]. Our previous work has revealed that HNTs can effectively improve the compressive mechanical properties and cytocompatibility of SA scaffold [21].

Halloysite is a natural mineral nanotube with high aspect ratio and an empty lumen. HNTs have many virtues as polymer nanofiller such as good dispersion properties, numerous active groups on their surfaces, low toxicity, good biocompatibility, and inexpensive [22–26]. They can easily be dispersed in different polymers and shows a good reinforcing ability for the composites [27]. In recent years, HNTs have attracted wide attentions as novel biomaterials. Firstly, HNTs can be used as drug delivery carriers attributed to their unique nanostructure. Anticancer drugs such as curcumin and doxorubicin (DOX), DNA, protein, antibacterial agent have been included in the tubes for slow releasing [28–31]. HNTs-chitosan sponges can also be found with high hemostatic performance and accelerate wound healing process [23]. Recently, HNTs patterned nanosurfaces have been used to capture circulating tumor cells, which can be used as cancer early diagnose [32,33]. HNTs have good interfacial interactions with SA via hydrogen bonding, which can be employed to prepare composite hydrogel beads or porous scaffolds. Wang et al. prepared diclofenac sodium-loaded SA/hydroxyapatite/HNTs nanocomposite hydrogel beads by the method of dropping the solution into a calcium chloride solution. The tubular structure of the HNTs can restrict movability of the SA polymer chains, which is the main reason for the improved drug loading and release behavior [34]. Liu et al. also prepared SA/HNTs beads for removal of dye from aqueous solution, and they found that not only the adsorption capacity of SA/HNT hybrid beads was improved but also the stability in the solution was enhanced significantly [35]. The effect of HNTs on the physical–chemical properties of SA hydrogel beads has also been studied [36]. However, adopting the dropping-precipitation method is notoriously difficult to prepare large size and size-controllable SA hydrogel. The hydrogel beads cannot satisfy the requirement of tissue engineering scaffold.

In this work, columnar composite hydrogel composed with HNTs and SA solution was prepared by casting the mixture solution in a mold and then cross-linking with $CaCl_2$ solution method. The influences of the addition of HNTs on the solution viscosity, dimensional stability, mechanical properties, pore structure, and cell attachment of SA are investigated. The results show that the compressive strength, structural stability, and biocompatibility of SA/HNTs composite hydrogel improved, which contribute to the potential application of SA/HNTs composite hydrogel in bone tissue engineering.

2. Experimental

2.1. Materials

Alginic acid sodium salt from brown algae (SA) (medium viscosity, ≥2000 cP, 2%(25 °C)) was purchased from Sigma-Aldrich (Shanghai, China). Halloysite nanotubes (HNTs), with molecular formula of $Al_2Si_2O_5(OH)_4 \cdot 2H_2O$, were purchased from Guangzhou Runwo Materials Technology Co., Ltd., China. Ultrapure water from a Milli-Q water system was used in all experiments. Acridine orange (AO), RPMI 1640 medium, fluorescein isothiocyanate isomer I (FITC), and dimethyl sulfoxide (DMSO) were purchased from Nanjing Keygen Biotech Co., Ltd., DAPI (4′,6-diamidino-2-phenylindole) and Phalloidin–Tetramethylrhodamine B isothiocyanate (phalloidin-TRITC) were purchased from Guangzhou Jetway Biotech Co., Ltd. Calcium chloride ($CaCl_2$) and other chemicals reagents were purchased from Aladdin (Shanghai, China) were analytically pure without further purification.

2.2. Preparation of SA/HNTs composite hydrogels

The SA/HNTs composite hydrogels were prepared by the method of solution blending and subsequent cross-linking in calcium ions. The typical procedure was as follows. 1.5, 3, 6, and 12 g HNTs were dispersed separately in ultrapure water (100 mL) by magnetic stirring for 30 mins and ultrasonic at 700 W for 30 min. Subsequently, 3 g of SA powder was added into the solution above, continuously stirred overnight. 2 mL mixed solution was cast in 24-well plastic culture plates with syringe, then the mixed solutions were cross-linked with 5 wt.% $CaCl_2$ solution after 24 h. Afterwards, the hydrogels were removed from the plastic culture plates and kept in ultrapure water at 4 °C. According to the HNTs concentration from low to high, the hydrogels were decoded in turn as SA (prepared with pure SA solution), SA2N1, SA1N1, SA1N2, and SA1N4 (the weight ratio of SA and HNTs was separately 2:1, 1:1, 1:2, 1:4; For example, the 'SA2N1' means the weight ratio of SA and HNTs in the hydrogel was 2:1). Thin hydrogel films for storage modulus test and cell experiment were prepared by casting and paving 1 mL solution on the glass slide immersed in 5% $CaCl_2$ solution. The thickness of the films is about 1 mm.

2.3. Characterization

The Brookfield viscometer (DV2TRVTJ0, Brookfield) was used to determine static viscosity of pure SA and SA/HNTs mixed solutions at a speed of 100 RPM for 1 min at 25 °C. A rotational rheometer (Kinexus pro+, Malvern Instruments, Malvern, UK.) was used to measure the dynamic viscosity of the solutions at room temperature at the shear rate of 1–100 s^{-1}. Compression testing of the wet hydrogels was performed using the Zwick/Roell Z005 machine under 25 °C at a speed of 2 mm/min. The section morphology of the composite hydrogels was analyzed by means of SEM (S-4800 FE, Hitachi) at voltage of 2 kV. Before observation, the wet hydrogels were freeze–dried, sectioned, and sputtered with gold. The pore structure was further visualized by fluorescent microscope (XDY-2, Yuexian optical instruments, Guangzhou, China). Before observation, the wet hydrogel samples were stained by 1 mg/mL FITC aqueous solution and then freeze–dried. X-ray diffraction (XRD) profiles for milled and lyophilized samples were obtained using X-ray diffractometer (D8, Bruker) at room temperature. The scanning angle was from 5° to 60° and a scanning speed of 10°/min with 40 kV voltages and 15 mA current. The powder samples were also analyzed in a FTIR spectrometer (VERTEX 70, Bruker) at room temperature, the

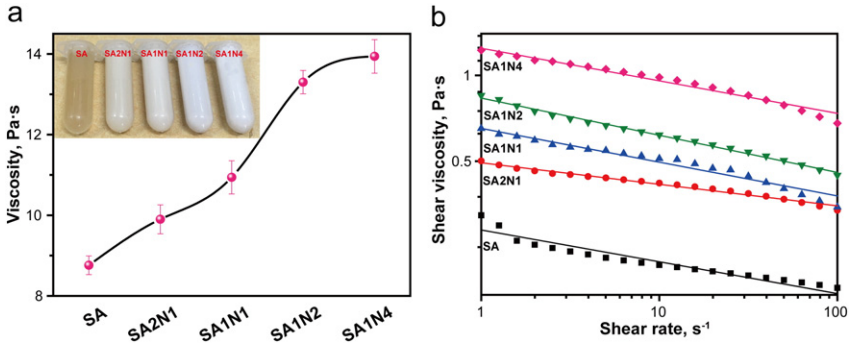

Fig. 1. (a) Static viscosity of pure SA and SA/HNTs solutions; (b) dynamic viscosity of pure SA and SA/HNTs solutions (the solid lines is fitting curve according to power-law equation).