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A gelatin composite scaffold strengthened by drug-loaded halloysite nanotubes

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ABSTRACT

Mechanical properties and anti-infection are two of the most concerned issues for artificial bone grafting materials. Bone regeneration porous scaffolds with sustained drug release were developed by freeze-drying the mixture of nanosized drug-loaded halloysite nanotubes (HNTs) and gelatin. The scaffolds showed porous structure and excellent biocompatibility. The mechanical properties of the obtained composite scaffolds were enhanced significantly by HNTs to >300%, comparing to those of gelatin scaffold, and match to those of natural cancellous bones. The ibuprofen-loaded HNTs incorporated in the scaffolds allowed extended drug release over 100 h, comparing to 8 h when directly mixed the drug into the gelatin scaffold. The biological properties of the composite scaffolds were investigated by culturing MG63 cells on them. The HNTs/gelatin scaffolds with excellent mechanical properties and sustained drug release could be a promising artificial bone grating material.

1. Introduction

The need for bone implants is increasing with more than one million bone-grafting operations performed annually in Europe and the United States [1]. Scaffolds mimicking the natural extracellular matrix (ECM) are designed in order to support cell attachment and guide three-dimensional (3D) tissue formation [2–5]. A scaffold should possess adequate mechanical properties, biocompatibility, and a proper degradation profile which would eliminate the need for removal surgery. Composites of inorganic materials and polymers are expected ideal materials to prepare scaffolds for bone substitutes [6–8]. Some researchers suggest that nanoparticles could benefit cell adhesion and proliferation, and even influence cell differentiation [9].

Gelatin, a product extracted from collagen, is also a good choice to prepare scaffolds for tissue engineering due to its excellent biodegradability, biocompatibility, cell adhesion and proliferation, resistance to immunogenicity, pathogen transmission, etc. [10–17]. As a hydrogel, gelatin foam possesses an elastic modulus of only 0.63 MPa in its wet state [18], which suggests that gelatin foam is very weak when it is used in body fluid. The mechanical properties of gelatin are determined by many factors, such as degree of crosslinking, content of water, plasticizers, molecular weight, origin, and processing [10]. Chemical grafting and physical blending gelatin with functional polymer are also two effective strategies to improve the mechanical and proceeding properties of gelatin composites or contribute gelatin with special functions [11–14]. In the past few years, many scientists focused on tailoring the macroporous structures of chemical grafting or physical blending gelatin composite scaffolds and got some interesting advances. A microribbons-based scaffold which could sustain up to 90% strain and 3 MPa stress without failing was prepared by wet-spinning gelatin solution into microfibers and further photocrosslinking [15]. Nanofibrous gelatin scaffolds containing both macropores of 100–500 μm and 1–2 μm were prepared by a thermally induced phase separation technique. The two degrade porous structures and nanofibrous surface were important for cell adhesion, transfer, proliferation and differentiation [16]. HepG2 cells were mixed with gelatin methacrylamide for 3D printing scaffold. The obtained scaffold indicated high cell viability, suggesting a possibility of preparing cell laden scaffold [17].

Infection happened in tissue trauma and invasive surgery due to bacterial colonization at the wound site could cause patient painful including a severe fever and pain, eventually lead to implant failure [19,20]. The foreign body response to an implant is also a big trouble in clinic. Improving the biocompatibility of an implant is an effective strategy to reduce foreign body response, while bacterial infections can be managed by controlled local delivery of antibacterial drugs [21,22]. Thus, the scaffold with high biocompatibility and local delivery of antibiotics is critical for post surgery treatment. The drug release profile can be adjusted by tailoring proper nanoarchitectures of porous materials.

Halloysite nanotubes (HNTs), a kind of aluminosilicate clay, have been used successfully as a nanosized filler to enhance the mechanical properties of polymers due to their high aspect ratio and tough

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structure [23]. They have attracted great interest from scientists in preparing scaffolds for tissue engineering because their excellent biocompatibility has been assessed suitable for both cell culture and animal tissue, and their tubular structure can load various kinds of drugs [24–31]. An electrospun poly(caprolactone)/gelatin microfiber matrix containing 20 wt% HNTs shows interesting anisotropic mechanical properties due to the alignment of HNTs in the microfibers [28]. Addition of HNTs into a chitosan–gelatine–agarose scaffold prepared by freeze-drying can enhance the mechanical properties significantly [32]. These examples implied that HNTs could be an emerging potential candidate for preparing gelatin composite scaffold.

In this study, HNTs was used as a filler to form composite scaffolds with gelatin. Ibuprofen, an analgesic, antipyretic and anti-inflammatory drug extensively used to treat bone diseases like rheumatoid arthritis and osteoarthritis [33], was loaded in HNTs. Ibuprofen can reduce inflammation by the inhibition of the enzyme cyclooxygenase, halting prostaglandin synthesis and therefore relieve the pain and swelling associated with infection [34]. We expected that the tubular structure of halloysite could contribute excellent mechanical properties and a long drug release profile to the composite scaffolds.

2. Materials and methods

2.1. Materials

Gelatin (CP, $M_w = 8000$, Sinopharm Chemical Reagent Co. Ltd.) and genipin (98%, Sigma-Aldrich) were used directly without further purification. HNTs (Yuan Xin Nano Technology Co. Ltd.) were modified by aminopropyltriethoxysilane (APTES (AR, Sigma-Aldrich)) [28]. Deionized water was obtained from a Millipore water purification system.

2.2. Preparation of HNTs/gelatin scaffolds

Gelatin solution (10 wt%) was prepared by dissolving gelatin in water at 60 °C for 1 h. The modified HNT dispersion was mixed with gelatin solution under stirring at 60 °C for 2 h. Different amounts of HNTs were added into the gelatin solution to determine the highest tube content that could be doped. Then, 5 ml 2% (g ml^{-1}) genipin solution as crosslinking agent was added to 50 g the gelatin solution and allowed to age for half an hour at 60 °C. The mixtures were casted into polyethylene molds and aged for 48 h, turning into HNTs/gelatin gel and freezing at −50 °C for 6 h. Finally, porous composite scaffolds were obtained by freeze-drying for 48 h. The porous scaffolds of gelatin and HNTs/gelatin were kept in a dryer for further use. The information on the samples was summarized in Table 1.

2.3. Characterization of scaffold structures

2.3.1. Structure and morphology examination of HNTs, gelatin and HNTs/gelatin scaffolds

A transmission electron microscope (TEM) (TECNAI-12, Philips, Holland) was used to characterize the structure of HNTs and a field emission scanning electron microscope (FE-SEM) (S-4800II, Japan) was used to characterize the surface morphology and microstructure of the gelatin and HNTs/gelatin scaffolds. The samples were sputtered with Au before SEM observation.

Table 1
Information of gel and HNT/gel scaffolds.

Sample	NHT(g)	Gelatin(g)
Gelatin	0.00	5.00
NHT 10	0.56	5.00
NHT 20	1.25	5.00
NHT 30	2.14	5.00
NHT 40	3.33	5.00
NHT 50	5.00	5.00

2.3.2. Porosity

Porosity was measured by the anhydrous ethanol substitution method [35]. Firstly, the volume and weight of the scaffolds were measured and noted as V_0 and W_0, respectively. Secondly, the density (ρ) of the corresponding non-porous pure gelatin film and HNTs/gelatin nanocomposite films was determined by measuring their weight and volume. Finally, the porosity of the sample was calculated based on the following formula, $\varepsilon = \frac{V_0 - (\frac{W_0}{\rho})}{V_0} \times 100$.

2.3.3. Compositions of the gelatin and HNTs/gelatin scaffolds

TGA measurement was carried out on a Pyris 1 TGA thermogravimeter. The samples were heated from 30 to 800 °C at a heating rate of 10 °C min^{-1} in an oxygen atmosphere.

FT-IR spectra were performed on a Tensor 27 spectrometer. The scan range was 4000–600 cm^{-1} with a resolution of 2 cm^{-1}. The scaffolds were grounded to a powder by a mortar. KBr tablet method was used to test FT-IR. X-ray diffraction (XRD) studies were conducted on a D8 Advanced X-ray diffractometer equipped with Cu-K α source (40 kV, 40 mA) in the range of 10–70° at a scan rate of 5°/min^{-1}.

2.3.4. Mechanical test

Uniaxial compression tests were performed on a universal test machine with a 5 kN load cell under a cross-head speed of 1 mm·min^{-1} until the scaffolds were compressed to 5%. Samples for mechanical testing were cylinders with a diameter of 30 mm and a height of 15 mm. The elastic modulus (E) was determined from the elastic region of stress-strain curves. Five samples were measured for each composition to check the reproducibility.

2.3.5. Water uptake ability

The water uptake ability (E_A) of a scaffold was studied using the following procedure. Dry scaffolds were weighed (W_1) and immersed in distilled water for 48 h. Then the scaffolds were gently removed from the beaker after 48 h and placed on a wire mesh rack. Excessive water was drained and scaffolds were weighed (W_2) to determine water uptake.

$$E_A = \frac{W_2 - W_1}{W_2} \times 100$$

2.3.6. Hydrophilicity of the scaffolds

Hydrophilicity of the scaffold was evaluated by measuring the contact angles of water droplets on the HNTs/gelatin films with an OCA20-type contact angle analyzer (Data Physics Co, Ltd., Germany). All the samples were dried at 60 °C for 24 h before measurement. Water droplets (5.0 μl) were dropped carefully onto the surface of the films, and the dynamic change of the water was recorded by a video. Five samples were measured for each film.

2.4. The load of IBU and in vitro release tests

A 2 mg/ml IBU methyl alcohol solution was mixed with dry HNT powder and placed in a vacuum chamber. After 30 min, the vacuum was stopped, and air was allowed into the chamber. The process was repeated twice for the most efficient loading. The most efficient loading is around 20 wt%. After loading, the samples were separated from the solution by centrifugation and washed with water to remove any unloaded drug. The drug loaded HNTs were dried in an oven at 50 °C and milled to fine powder. The in vitro release test was conducted using a THZ-1028 table concentrator. Dialysis bags (molecular mass cut-off: 3500 Da) sealed with approximately 0.5 g of IBU-loaded HNTs, gelatin scaffold and HNTs/gelatin scaffold were soaked into 500 ml of phosphate buffers (pH 7.4) at 37 °C with a stirring speed of 170 rpm. At suitable intervals, 5 ml of the medium was withdrawn for testing,