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Facile method to prepare silk fibroin/hyaluronic acid films for vascular endothelial growth factor release

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ABSTRACT

A facile approach was proposed to prepare silk fibroin (SF) and hyaluronic acid (HA) composite films from aqueous solution without crosslinking or any post treatment. Only by controlling the HA content and film formation temperature during the film casting, the HA/SF films with different composition were prepared. The films were then characterized by structural characteristics, thermal stability, morphology, water stability, water absorption, mechanical properties. After immersing in water for 24 h, all of the films showed good structural integrity. The degradation rate of the HA/SF films in protease XIV can be controlled by changing the film formation temperature and HA content. Decreasing the temperature and adding HA resulted in the rapid release of VEGF (vascular endothelial growth factor) from the HA/SF films. Overall, the 5% HA/SF films formed at 37 °C with more rapid VEGF release exhibited great potential in drug delivery, especially when the rapid vascularization was needed.

1. Introduction

The vascularization of biomaterials, especially timely vascularization, played an important role in tissue survival by transporting metabolic products, oxygen and nutrients to the tissues. This has recently sparked great interest in tissue repair (Novosel, Kleinhans, & Kluger, 2011; Poldervaart et al., 2014). In this context, vascular endothelial growth factor (VEGF) has been added into biomaterials to promote angiogenesis in vivo because it can stimulate the proliferation and migration of endothelial cells that mediate vessel sprouting and neovascularization (Bible et al., 2012; Jay et al., 2010). Therefore, it would be desirable to develop various materials to load VEGF with controllable drug release properties and other essential physicochemical properties to fulfill the specific demands of biomaterials.

Many natural materials with biocompatibility, biodegradability and similarity to macromolecules recognized by the human body such as polysaccharides (chitosan, heparin, chondroitin, hyaluronic acid and alginates) and proteins (collagen, gelatin, silk fibroin, keratin and elastin) were extensively explored for tissue regeneration (Malafaya, Silva, & Reis, 2007; Romero et al., 2015). The natural silk had exceptional properties such as processability, mechanical strength and biocompatibility. After processing into different forms, such as films, nanofibers, microspheres and hydrogels, the regenerated silk fibroin (SF) materials also had properties of biodegradation (Numata, Yamazaki, & Naga, 2012; Wenk, Merkle, & Meinel, 2011; Wenk et al., 2010; Zhou, Cao, & Ma, 2009). Hyaluronic acid (HA) is one of the most ubiquitous glycosaminoglycan found in extracellular tissue in many parts of the body (Collins & Birkinshaw, 2013). HA contains hydrophilic functional groups, including carboxyl and hydroxyl along its backbone which enhances water retention and can be used to introduce functional domains or to interact with other biomacromolecules (Tsai, Liu, Hsu, & Chen, 2006). In spite of these interesting features, HA has some limitations typically related to its inadequate mechanical properties and rapid degradation under physiologically relevant conditions (Jeon et al., 2007; Patterson et al., 2010). Therefore, HA has rarely been used alone. New materials based on binary or ternary blends of several biomacromolecules to avoid the disadvantage of single molecules

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have attracted many researchers (Chi et al., 2012; Garcia-Fuentes, Meinel, Hilbe, Meinel, & Merkle, 2009; Ren, Zhou, Liu, Xu, & Cui, 2009). For instance in the binary blends of HA and SF, SF acted as the main structural conductive component and provided mechanically stable structures that underwent slow biodegradation over an extended period of time while the HA provided a biomimetic surface for cell culture and ingrowth. However, limited information was found about using HA/SF composite materials for VEGF release.

It should be noted that to obtain a more stable structure, most of the insoluble HA/SF composite materials used were fabricated either by using crosslinking agents, such as genipin (Chi et al., 2012) and carbodiimides (Ren et al., 2009) or post treatment with methanol. On the one hand, the crosslinking agents were expensive and the residual crosslinking agent, such as glutaraldehyde, compromised the biocompatibility of the crosslinked structures because the toxic residues and degradation products induce cytotoxicity and calcification (Chan, So, & Chan, 2008; Tam et al., 2015). On the other hand, methanol is toxic, and the post treatment resulted in the complicated process (Garcia-Fuentes, Giger, Meinel, & Merkle, 2008). Hence, there exists an urgent need for exploring a facile method to fabricate the HA/SF materials without using crosslinking agents or toxic organic solvents for VEGF release.

In this study, in order to obtain HA/SF films by a facile method using all-aqueous solution, we hypothesis that the stable HA/SF films will be obtained by regulating the parameters involved in the film formation. To test this hypothesis, we performed a systematic study on how to control HA/SF film properties by modulating the film's HA content and casting temperature. The characteristics of HA/SF composite films including morphology, structure, water stability, water absorption, degradation behavior in protease XIV and mechanical properties were investigated. Furthermore, we prepared VEGF-loaded HA/SF films and studied the VEGF release properties from these films.

2. Materials and methods

2.1. Materials

Bombyx mori silk cocoons were kindly supplied by Anhui Xinhai Traditional Chinese Medicine Company (Anhui, China). Sodium carbonate anhydrous, calcium chloride anhydrous, ethanol and cellulose dialysis cassettes (molecular weight cut-off 8000–14,000) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Oligomeric HA with average molecular weight of 6.8 kDa was obtained from Shandong Freda Biopharm Co., Ltd. (Shandong, China). The recombinant human VEGF and VEGF ELISA kits were purchased from Invitrogen trading Co., Ltd. All chemical reagents were of analytical grade.

2.2. Preparation of the SF aqueous solutions

The regenerated SF aqueous solution was prepared following the steps reported previously (Zhou et al., 2009), and the molecular weight of the regenerated SF is about 38.888 kDa (detected by MALDI-TOF and shown in the supporting information). Silkworm cocoons were boiled in 0.005 g/mL Na_2CO_3 aqueous solution for 1 h and then thoroughly rinsed with enough deionized water to extract the glue-like sericin. After drying overnight, the degummed SF was dissolved in a $CaCl_2/H_2O/C_2H_5OH$ ternary solvent (with molar ratio of 1:8:2) at 10% (w/v) for 40 min at 80 °C. The blended solution was put into dialysis cassettes and then dialyzed against deionized water for 3 days to remove the inorganic salt. Then, the SF aqueous solution was filtered to remove the insoluble particulates. Finally, the SF aqueous solution with a preliminary concentration of about 3.5% (w/w) was obtained. The concentration of the SF aqueous solution was determined by weighing the remaining solid content after drying.

2.3. Preparation of the HA/SF Films

The mixed solution of HA and SF was prepared by adding a certain amount of HA (6.8 kDa) to the SF aqueous solution and stirring mildly at room temperature until complete dissolution. The concentrations of HA in the mixed solutions were selected as 0%, 5% and 10% by weight of SF (w/w). Then these aqueous solutions were stirred for 8 h to obtain uniform solutions. To obtain the VEGF loaded SF/HA aqueous solution, 2 μg of VEGF was added into 10 mL of SF/HA aqueous solution. After adding the VEGF the films were prepared per the procedure shown in Fig. 1. To obtain films with similar thicknesses, solutions with the same volume of 8 mL were cast on polystyrene Petri dishes (diameter = 7 cm). The films were designated as SF, 5% HA/SF and 10% HA/SF according to their HA content. The casting temperature was selected at 37 °C and 60 °C, which was efficient in regulating the SF crystalline structure (Hu et al., 2011; Lu et al., 2010a). After 2 days and 1 week, respectively, the SF films were obtained at 37 °C and 60 °C without any further treatment.

2.4. Structural analysis

The structures of the films were analyzed by FTIR (Fourier Transform Infrared Spectroscopy) on a Nicolet 6700 spectrometer with an attenuated total reflectance accessory (ATR). Each spectrum was acquired in transmittance mode on a diamond substrate by accumulating 32 scans with a resolution of 4 cm^{-1} and a spectral range of 4000–500 cm^{-1}.

XRD (X-ray Diffraction) curves were recorded on a Rigaku D/MAXIIA diffractometer using Cu$K\alpha$ radiation (40 kV, 150 mA). The samples were mounted on glass frames and scanned from 5° to 60° (2θ) at a scanning rate of 5°/min.

2.5. Thermal properties

Thermogravimetry analysis (TGA) was tested using a Linseis STA PT1600, with a temperature range of 25–550 °C at a heating rate of 10 °C/min and under a dry nitrogen gas flow of 4 L/h. Differential Scanning Calorimetry (DSC) was investigated with a Linseis DSC PT10 under a dry nitrogen gas flow of 4 L/h and a heating rate of 10 °C/min. A temperature range of 25–305 °C was detected using standard aluminum pans. A sample size of 10 ± 5 mg was used in all the experiments.

2.6. Scanning electron microscopy (SEM)

The surface morphology of the films was observed with a Hitachi S-4800 SEM at an accelerating voltage of 5 kV. All samples were sputter coated with gold for 60 s.

2.7. Water absorption and wet stability

Precisely weighed films were immersed in deionized water at 37 °C and vibrated at 50 rpm in a thermostatic incubation shaker for 24 h. After that excess water on the swollen films was removed with a filter paper and the films were weighed. Samples were then dried in an electro-thermostatic blast-drying oven at 60 °C overnight, and the dry mass of the films was recorded. The experiments were run