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Effect of hyaluronan molecular weight on structure and biocompatibility of silk fibroin/hyaluronan scaffolds

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ABSTRACT

The structure of scaffolds is known to play a key role in tissue engineering as it provides structural support and physical environment allowing cells to reside and rebuild the target tissue. In this work we investigated the effects of hyaluronan (HA) molecular weight (M_W: 0.6, 1.6 and 2.6 × 10^6 Da) on the pore structure, secondary structure, and biocompatibility of lyophilized silk fibroin (SF)/HA composite scaffolds. The results showed that HA promoted the pore structure formation and restrained the formation of separate sheet like structures in the SF/HA blend scaffolds, which was dependent on HA M_W. The 3D pore structure maintained the scaffold shape during the process of 75% ethanol annealing. Structural studies indicated that HA did not induce but hinder SF conformation transition from random coil to β-sheet before and after treatment. In addition, SF/HA scaffolds showed an increase in cell proliferation compared to pure SF scaffold. These findings demonstrated the important role of HA M_W in preparing SF/HA blend scaffolds suitable for application in tissue engineering.

1. Introduction

Tissue engineering has been emerged as a promising alternative strategy to treat patients with tissue loss or organ failure, which has the potential to revolutionize the treatment of many diseases [1]. The design of scaffold is important for tissue engineering [2]. Ideally scaffolds should have a proper structure similar to natural extracellular matrices (ECM) which induce regenerative processed by interacting with relevant cell populations.

Many materials have been employed to construct tissue engineering scaffolds, in particular, lots of attention has been focused on naturally derived polymers, such as collagen, silk, chitosan, hyaluronic acid (HA), alginic acid, etc. [3]. Among these native materials, silk has been given much attention due its biocompatibility, slow degradability, remarkable mechanical properties, and excellent processing ability [4]. Silk fibroin has been processed into a variety of shapes including hydrogels, sponges, films and nanofibers, for new biomedical applications [5–7]. Compared to other material formats, porous sponge scaffolds exhibit more important role in providing a versatile 3D porous structure for cell attachment, proliferation, and migration, as well as for nutrient and waste transport [8]. A wide variety of techniques, salt leaching, lyophilization, gas foaming, have been reported to design and fabricate porous scaffolds [4,8]. The lyophilized porous silk sponges from aqueous solution demonstrated excellent interconnectivity between the pores and improved cell attachment compared to the solvent-based porous sponges, likely due to the rougher surfaces and the benefit of not using organic solvents [4,8].

Recently a number of reports demonstrate that the combination of SF and other synthetic or natural polymers, such as poly (vinyl alcohol), collagen, chitosan, polylactic acid, HA, and so forth, has displayed advantages over the use of either material alone for tissue engineering applications [9–11]. SF/HA blend scaffolds could significantly promote cell proliferation, cell infiltration, and tissue formation as compared to plain SF scaffolds, suggesting the important role of HA in manipulating cell behavior [12–14].

Hyaluronic acid (HA), a naturally occurring linear polysaccharide distributed in the ECM of mammalian soft tissues, has been implicated in diverse biological processes such as angiogenesis and migration, tissue development, as well as the proliferation and differentiation of progenitor cells [15]. Scaffolds derived from natural polysaccharides are very promising in tissue engineering applications and regenerative medicine, as they resemble glycosaminoglycans in the ECM [16]. SF and HA have been made into SF/HA blend nanofibers [12], hydrogel [17], film [18], and porous scaffold [19] for application in regeneration medicine. The increased use of these materials will require finely tuned and

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controllable material characteristics, such as HA molecular weight. The function of HA based on the specific interaction with cells and ECM components in the body highly relies on the molecular weight of HA [20]. However, the effect of HA molecular weight on the structure and property of this system (SF/HA blend scaffolds) has not been characterized.

The goal of the present study was to develop an optimum SF/HA blend scaffolds used for tissue engineering. HA with different M_W was blended with SF to prepare SF/HA combined scaffold through freeze-drying. The morphology, structure, and biological properties of these scaffolds were investigated. More importantly, the effect of HA M_W was discussed in detail.

2. Experimental

2.1. Preparation of SF solution

Bombyx mori fibroin solutions were prepared according to our previously published procedures [21]. Cocoons were boiled for 20 min in an aqueous solution of 0.02 M Na_2CO_3 and then rinsed thoroughly with distilled water to extract the sericin proteins. After drying, the extracted silk fibroin was dissolved in 9.3 M LiBr solution at 60 °C for 4 h, yielding a 20% (w/v) solution. This solution was dialyzed against distilled water using Slide-a-Lyzer dialysis cassettes (Pierce, molecular weight cut-off 3500) for 72 h to remove the salt. The solution was optically clear after dialysis and was centrifuged to remove the small amount of silk aggregates that formed during the process. The final concentration of aqueous silk solution was ~7 wt.%, determined by weighing the remaining solid after drying. The silk fibroin solution was then diluted to 6 wt.% with deionized water.

2.2. Preparation of SF–HA scaffolds

Pure 0.6 wt.% HA solutions with molecular weight (M_W) of 0.6, 1.6, and 2.6×10^6 Da were prepared by dissolving HA powder in deionized water. The above 6 wt.% SF solution was mixed with HA solutions of different M_W at room temperature for 2 h. Finally, mixed solution containing 3 wt.% SF and 0.3 wt.% HA with different M_W was prepared.

The prepared SF and SF–HA suspensions were then frozen at −20 °C for about 24 h to freeze and then lyophilized for about 72 h. Lyophilized SF and SF–HA scaffolds were placed on a removable platform under which 75% ethanol was filled in a desiccator with a 25 in. Hg vacuum for 2 h to produce water-insoluble scaffold.

2.3. Cell seeding and culture

Primary cultures of OECs were prepared from 1-month male Sprague-Dawley rats (Experimental Animal Center of Soochow University) as reported previously [22]. All experiment procedures were carried out in accordance with the regulations for the administration of affairs concerning experimental animals of Soochow University. The complete culture medium for OECs consists of DMEM/F-12 (Gibco, Grand Island, NY, USA), with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 1% glutamine (Sigma, St. Louis, MA, USA), and 2% penicillin–streptomycin (Hayao, Haerbin, China). After 10 days in culture, OECs were used for biocompatibility evaluation with SF/HA blend scaffolds.

Scaffolds were cut into disks with diameter 8 mm and thickness 2 mm, transferred to 96-well plates and then sterilized by γ radiation. The scaffolds were incubated with the culture medium overnight, then seeded with OECs at a density of 1.0×10^5 OECs. The cells were allowed to adhere to the scaffolds for 3 h and then the cell-scaffold complexes were covered with 150 μl of culture medium. The culture medium was changed every 3 days up to the indicated time points.

2.4. Scanning electron microscopy

The cross-sections of the silk-based scaffolds were platinum-coated and examined using scanning electron microscope (SEM, Hitach S-4800, Tokyo, Japan). Cross-sections were prepared by cutting the dried silk-based scaffolds with a razor blade in liquid nitrogen.

2.5. Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra were obtained using a Magna spectrometer (NicoLET5700, America) in the spectral region of 400–4000 cm^{-1}, the powdered SF and SF–HA scaffolds were pressed into potassium bromide (KBr) pellets prior to data collection.

2.6. X-ray diffraction

X-ray diffraction (X'PERT PRO MPD, PANalytical Company, Holland) was operated at 40 kV tube voltage and 40 mA tube current, CuKα radiation was used with diffraction angle $2\theta = 2°–45°$, the scanning rate is 2°/min with powdered SF and SF–HA scaffolds.

2.7. Thermogravimetry

Thermogravimetry analysis was performed in a TG-DTA, PE-SII (America) in the temperature range of 40–600 °C with a ramp rate of 10 °C/min, and nitrogen flux of 50 ml/min.

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed in TA instruments Q100 DSC (TA Instruments, New Castle DE) scanning from 40 to 300 °C. The sample was weighed and placed into aluminum pans. The pans were heated at a constant rate of 2 °C/min and a nitrogen gas flow rate of 50 ml/min.

2.9. Cell morphology

The cell morphology on the scaffolds was observed by SEM. OECs were cultured for 10 days on the scaffolds, then fixed with 2.5% glutaraldehyde for 3 h at room temperature, rinsed three times with PBS and dehydrated in a gradient of alcohol (50%, 70%, 80%, 90%, 100%, 100%). Samples were then lyophilized, coated with gold and observed by SEM (Hitach S-4800, Tokyo, Japan).

2.10. DNA content

To study cell proliferation on the scaffolds, samples were harvested at the indicated time point (from day 1 to day 15), and digested with proteinase K buffer solution for 16 h at 56 °C. The DNA content was determined using the Quant-iT™ PicoGreen dsDNA assay, following the protocols of the manufacturer (Invitrogen, Carlsbad, CA). Samples ($n = 3$) were measured at an excitation wavelength of 480 nm and emission wavelength of 530 nm, using a BioTec Synergy 4 spectrofluorometer (BioTec, Winooski, UK). The amount of DNA was calculated by interpolation from a standard curve prepared with lambda DNA in 10×10^{-3} M Tris–HCl (pH 7.4), 5×10^{-3} M NaCl, 0.1×10^{-3} M EDTA over a range of concentrations.