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Characterization of bladder acellular matrix hydrogel with inherent bioactive factors

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ABSTRACT

Bladder acellular matrix (BAM) hydrogel may have great potential in tissue engineering due to outstanding biocompatibility and the presence of inherent bioactive factors in BAM. In this study, we prepared the BAM hydrogel by the method of enzymatic solubilization with pepsin and characterize the microrheological properties of the BAM precursor solution. The structures of the BAM hydrogel were characterized by scanning electron microscope (SEM), Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Furthermore, the growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor B (PDGF-BB), keratinocyte growth factor (KGF) were quantified by ELISA. The biological performances of the hydrogels were evaluated by cultivating porcine iliac endothelial cells (PIECs) *in vitro*. Lyophilized BAM showed porous structure with pore diameter ranging from 50 to 100 μm. BAM 4-G hydrogel (4 mg/mL) with a short gelation time of 3.95 ± 0.07 min presents better thermal stability than BAM 6-G hydrogel (6 mg/mL). Growth factors in the BAM hydrogel maintain valuable biological activity even after digestion process. The BAM hydrogel supported the adhesion and growth of PIECs well and has great potential for further tissue engineering.

1. Introduction

Biologic scaffolds based on extracellular matrix (ECM) have been widely used for the repair and reconstruction of variety of tissues [1–3]. The ECM scaffolds are prepared by removing all cells of intact tissues or organs and composed of growth factors, collagen, laminin, fibronectin, glycosaminoglycans, glycoproteins and proteoglycans [4]. An ECM scaffold derived from the porcine urinary bladder refers to bladder acellular matrix (BAM) or bladder acellular matrix graft (BAMG), one of the most representative decellularized tissues. BAM has been demonstrated to preserve bioactive factors well to support tissue regeneration and exhibits good biocompatibility [5,6]. Several studies have indicated that BAM is a suitable scaffold for the repair and reconstruction of the urinary system [7–10]. Although urothelial cells grew well on a simple BAM sheet implanted in bladder [7], BAM cannot promote the satisfactory vessel formation due to limited urothelial coverage which leads to bladder fibrosis and eventually affects long-term bladder function [11]. The bilayer scaffold comprising of a porous network (silk fibroin, SF) and an underlying BAMG sheet was developed to fulfill specific requirements, since these dense and smooth BAM cannot promote satisfactory smooth muscle regeneration, vessel formation, particularly for seeding cells into the scaffold [12]. Suspensions prepared from lyophilized powder of BAM were useful for the treatment of urinary incontinence as an injected particulate bioscaffold [13]. Unfortunately, the wide distribution of BAM particle size limited the injection operation, in which a needle with single inner diameter was normally used.

Nowadays, hydrogels are increasingly utilized in the biomedical application because of variable geometry, tunable strength and porous structure [14]. Hydrogels are three-dimentional polymer networks that are formed by crosslinking for drug delivery and tissue engineering [15,16]. It has been shown that the biologic scaffold materials composed of BAM can be partially digested with pepsin, solubilized and polymerized to form hydrogel [17,18]. BAM hydrogel has attracted attention due to its injectability and the ability to fill an irregularly shaped space. The rheological behavior, morphology, and cell response *in vitro* and *in vivo* of BAM hydrogel have been characterized. It is possible that a hydrogel formed from enzymatically degraded and solubilized BAM may maintain some biological factors that found in the intact BAM. However, the biological factors in the BAM hydrogel were not quantitatively confirmed and investigated.

Therefore, the purposes of this study were: (1) to understand the structural transition of BAM hydrogel from its precursor, (2) to examine the efficiency of preserving extracellular bioactive factors, (3) to determine the releasing ability of the BAM hydrogel, and (4) to evaluate the biological performance of the hydrogel by cultivating porcine iliac endothelial cells (PIECs) on the hydrogels. Instead of regular rheological

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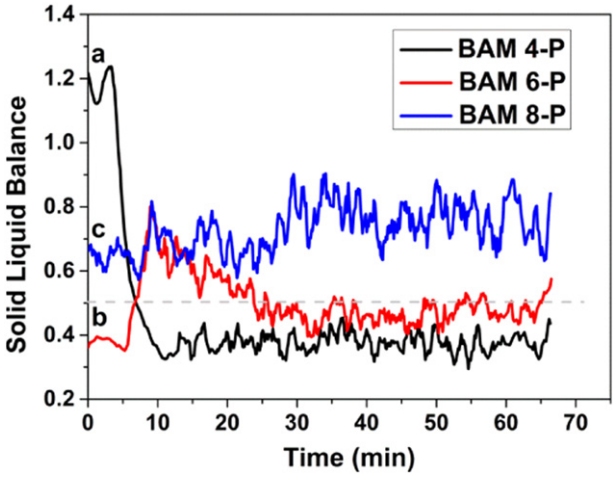

Fig. 1. The value of solid-liquid balance of BAM hydrogel precursors with different concentrations during gelation process: (a) BAM 4-P, (b) BAM 6-P and (c) BAM 8-P.

properties, microrheological behaviors of the hydrogel precursor solution were investigated during the process of gelation to determine the gel points.

2. Experimental

2.1. Fabrication of hydrogel

BAM sheets provided by Shanghai Jiao Tong University Affiliated Sixth People's Hospital were rinsed with deionized water and then frozen at −80 °C. BAM powder was obtained by lyophilizing the sheets using a cryogenic mill (FREEZER/MILL 6770, SPEX SamplePrep). Pepsin (Sigma) was further mixed with the BAM powder in 0.01 M HCl with the ratio of 1:10:1 (w:w:v) and kept at a constant shake of 125 rpm for 48 h at 25 °C. The resultant viscous solution of the digested BAM had a pH ranging from 3.0 to 4.0. The pH of the solution was adjusted to 7.4 by adding 0.1 M NaOH (1/10 of the volume of digested solution), which led to the irreversible inactivation of pepsin. The BAM gels were formed when 10 × PBS (1/9 of the volume of digested solution) was added and stored at 37 °C for 1 h. The desired concentration of the hydrogels can be achieved using a cold (4 °C) 1 × PBS. The hydrogels were frozen at −80 °C and lyophilized to prepare dry samples for future characterization. The hydrogels with different BAM concentrations (4 mg/mL, 6 mg/mL) was assigned as BAM 4-G and BAM 6-G, respectively.

2.2. Rheological measurements during gelation

Rheolaser Master (Formulaction, France) was used to analyze the microrheological behavior of the hydrogel precursor solutions with different BAM concentrations of 4, 6, 8 mg/mL, which were assigned as BAM 4-P, BAM 6-P, and BAM 8-P, respectively. The viscoelastic characteristic of the materials was analyzed according to the displacement of micro-size particles resulted from thermodynamic energy. Brownian movement of the particles can be monitored by dynamic light scattering based on Multi Speckle Diffusing Wave Spectroscopy [19,20]. The light is multiply-scattered by these particles, which leads to interfering backscattering waves [21,22]. It is a non-contact measurement in a static condition by loading the samples of 2 mL into the measurement cell of 4 mL. The temperature of the cuvette was set to 37 °C to induce the gelation of the precursors. The gel points of the samples were obtained based on Time Cure Superposition method.

2.3. Hydrogel structural characterization

2.3.1. Morphology assessment

Surface morphology of the hydrogels was performed using a scanning electron microscope (SEM) (JEO JSM-5600LV, Japan) at a voltage of 10 kV. Dry samples were quenched in liquid nitrogen and cut into small pieces to prepare cross sections for imaging. Then the samples were sputter-coated with a thin layer of gold before observation.

2.3.2. Differential scanning calorimetry (DSC)

Thermal properties of dry specimens of the BAM hydrogels were acquired using a differential scanning calorimeter (Modulated DSC 2910, TA Co., USA) with N_2 gas flow at a heating rate of 5 °C/min from 0 to 300 °C.

2.3.3. Fourier-transform infrared spectroscopy (FTIR) analysis

Structural characteristics of the BAM hydrogels were investigated and compared to those of the corresponding precursors using Fourier Transform Infrared Spectrophotometer (Nicolet Nexus 670). All specimens were lyophilized for 48 h before characterization. FTIR spectra were collected as a mean of 37 acquisitions in a spectral region between 4000 cm^{-1} and 600 cm^{-1} at a 4 cm^{-1} resolution.

2.4. In vitro release investigation

The main growth factors in BAM, vascular endothelial growth factor (VEGF), platelet-derived growth factor B (PDGF-BB), and keratinocyte growth factor (KGF), are closely related to the growth and proliferation of cells and tissue regeneration. VEGF promotes angiogenesis and

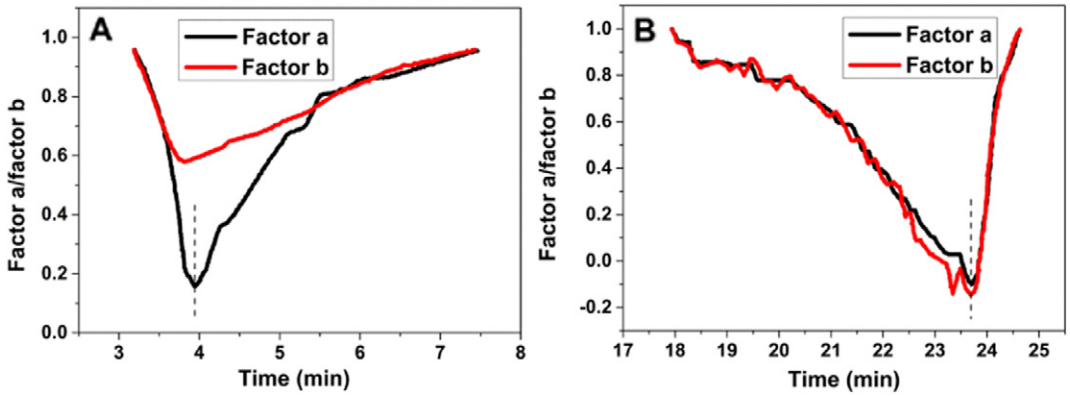

Fig. 2. The gel points of BAM hydrogel precursors with different concentrations: (A) BAM 4-P and (B) BAM 6-P.