Controlling the Microstructure of Hybrid Scaffolds by Governing Transient Phase Separation in a Polysaccharide-Protein-Organic Solvent System

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Statement of Purpose: The interactions between polysaccharides and proteins have made it possible to create polysaccharide-protein complexes with unique physical and morphological properties for biomedical applications [1]. In a polysaccharide-protein-solvent system, complex coacervation (i.e., associative phase separation) occurs when the interactions between the polysaccharide and the protein are weak attractive and non-specific, giving rise to soluble or insoluble polysaccharide-protein complexes [2]. Evolution takes place in the system as the solvent molecules infiltrate into the complexes over time, leading to the eventual disappearance of the phase separation. As a result, the polysaccharide-protein complex coacervation is transient and reversible. To harness this dynamic process for scaffold fabrication, we have fabricated 3-dimensional (3-D) hybrid porous scaffolds of gelatin (Gtn) and chemically modified photocurable chitosan (Cht) with tunable microstructures and properties for tissue regeneration by governing the polysaccharide and protein interactions in an organic solvent (dimethyl sulfoxide, DMSO) system. These Cht-Gtn scaffolds possess microstructures across the nano, micro, and macro length scales, mechanical properties superior to existing natural biopolymers, and excellent bioactivities. Our approach opens new avenues to achieve hybrid scaffolds of naturally occurring biopolymers for biomedical applications.

Methods: Chemically modified photocurable Cht [3] containing 0.5 wt% Iragure 2959 was added into 5% Gtn solution under stirring to obtain 5% Gtn-5% Cht-DMSO mixture, and 5% Gtn-7.5% Cht-DMSO mixture, respectively. The mixture was then slowly filled into disc molds and set for different lengths of time before exposure to UV light for 2 minutes to crosslink Cht. Another set of the photocured discs were further crosslinked using 4:1 acetone-water (v/v) solution containing 1% (w/v) 1-ethyl-(3-3-dimethylaminopropyl carbodiimide hydrochloride) (EDC) at 4°C overnight to crosslink Gtn, and Gtn with Cht. The scaffold morphologies were examined by SEM. Rheological measurements were performed using an AR-G2 model stress controlled rheometer (T.A. Instruments, U.K.), and the compressive properties were investigated using the Dynamic Mechanical Analyzer Q800 (DMAQ800). Primary bovine osteoblasts were used in cell culture to evaluate the bioactivities of the scaffold discs.

Results: SEM images of the scaffolds (Fig.1) clearly reveal the presences of interconnected pores with varying sizes and morphologies as a function of Gtn-Cht interaction parameters (e.g., the setting time, the Gtn-Cht ratio, and the crosslinking of Gtn). In particular, nanoscale structures in the form of pores, fibers, beads were seen on the skeleton of the scaffolds (Fig.2). All the hybrid scaffolds exhibited elastic-dominant characteristics (Fig 3). The range of the elasticity of the hybrid

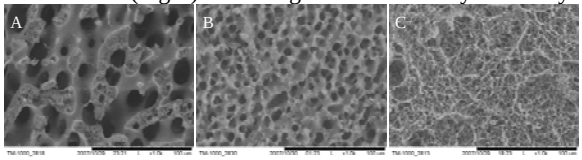

Fig. 1: Representative SEM images of the scaffolds (5% Gtn- 5% Cht) made with different setting time and without crosslinking of gelatin. (A): no setting time; (B): 8 hours setting time; (C): 12 hours setting time.

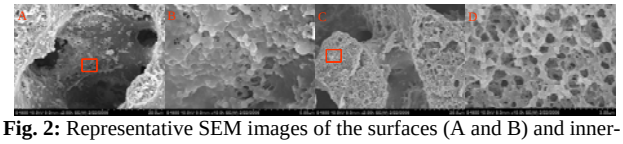

Fig. 2: Representative SEM images of the surfaces (A and B) and inner-structures (C and D) of the scaffolds (5% Gtn- 5% Cht with no setting time) with nanostructures, such as gelatin beads (B) and nanopores (D).

scaffolds has exceeded that in any other natural biopolymers except elastin, indicating the ability of the hybrid scaffolds to expand the mechanical properties of natural biopolymers. The compression tests demonstrate that the hybrid scaffolds are primarily elastic in nature [4] and possess the appropriate strength and toughness to withstand both static and cyclic compressive loadings under simulated physiological conditions (Fig.4). In cell culture, the hybrid scaffolds have supported osteoblast attachment, survival, proliferation, and 3-D organization (Fig.5).

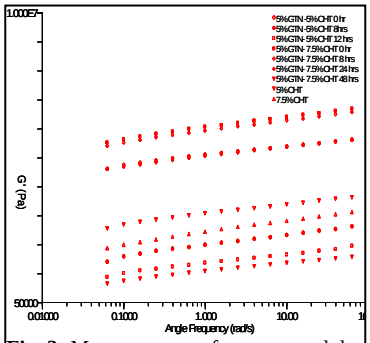

Fig. 3: Measurements of storage modulus of the hybrid scaffolds containing different ratios of gelatin to chitosan and with different lengths of setting time.

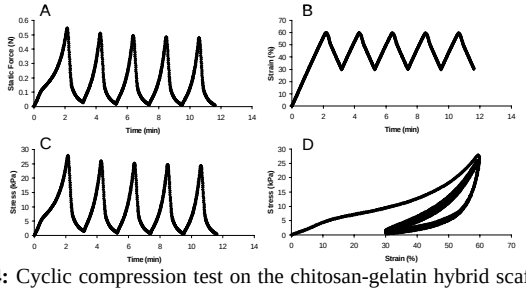

Fig. 4: Cyclic compression test on the chitosan-gelatin hybrid scaffolds (5%-5%, 0 setting time) at a constant strain rate of 1mm/min and a strain range of 30% to 60%. (A) Static force vs. time; (B) strain vs. time; (C) stress vs. time; and (D) stress-strain curve.

Conclusions: 3-D hybrid porous scaffolds of polysaccharide and protein with tunable microstructures across the nano, micro, and macro length scales, superior mechanical properties, and excellent bioactivities can be fabricated by governing a transient phase separation process in a polysaccharide-protein-organic solvent system through the control of interaction parameters.

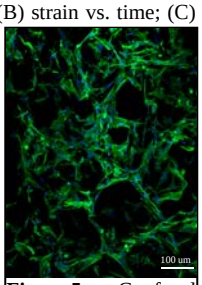

Fig. 5: Confocal image of the osteoblasts cultured in the scaffold of a 5% Gtn-7.5% Cht hybrid scaffold.

References:

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