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Nanostructuring of PEG–fibrinogen polymeric scaffolds ☆

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ABSTRACT

Recent studies have shown that nanostructuring of scaffolds for tissue engineering has a major impact on their interactions with cells. The current investigation focuses on nanostructuring of a biocompatible, biosynthetic polymeric hydrogel scaffold made from crosslinked poly(ethylene glycol)–fibrinogen conjugates. Nanostructuring was achieved by the addition of the block copolymer Pluronic® F127, which self-assembles into nanometric micelles at certain concentrations and temperatures. Cryo-transmission electron microscopy experiments detected F127 micelles, both embedded within PEGylated fibrinogen hydrogels and in solution. The density of the F127 micelles, as well as their ordering, increased with increasing block copolymer concentration. The mechanical properties of the nanostructured hydrogels were investigated using stress-sweep rheological testing. These tests revealed a correlation between the block copolymer concentration and the storage modulus of the composite hydrogels. In vitro cellular assays confirmed that the increased modulus of the hydrogels did not limit the ability of the cells to form extensions and become spindled within the three-dimensional (3-D) hydrogel culture environment. Thus, altering the nanostructure of the hydrogel may be used as a strategy to control cellular behavior in 3-D through changes in mechanical properties of the environment.

1. Introduction

One of the common approaches in tissue engineering is based on growing cells in three-dimensional (3-D) porous extracellular matrix (ECM) analogs, known as scaffolds [1,2]. The ECM replacement should mimic the natural organization of the tissue and provide temporary yet necessary structural support for the initial stages of the tissue regeneration process [3]. Many materials used for preparing scaffolds are polymeric, made from either synthetic or natural building blocks. Typically, scaffolds made from synthetic polymers possess good mechanical properties, but lack cellular interactions due to the absence of natural ligands that enable specific communication with cell surface receptors. On the other hand, natural materials, such as hyaluronate, collagen or fibrin, have good interactions with cells but are unable to form mechanically stable constructs. The preparation of a hybrid biomaterial, composed of a combination of natural and synthetic constituents, is one way to overcome the inherent limitations of scaffolds made from either natural or synthetic polymers alone. As an example, polyethylene glycol (PEG), which generally resists protein and cell adhesion [4], can be chemically conjugated to oligo(peptides) derived from cell-adhesion promoting proteins such as fibronectin, laminin and fibrinogen [5–7] in a reaction termed "PEGylation". Zhang et al. [8] utilized PEGylated fibrinogen as a carrier for isolated mesenchymal stem cells (MSCs) in order to provide a solution in myocardial cell-therapy. Their results suggest that PEGylated fibrin patches increased MSC viability in a rat infarct model and, furthermore, caused phenotypic changes in the MSCs consistent with endothelial cells. Zisch and co-workers [9] developed several synthesis schemes for preparing proteolytically sensitive PEG/peptide hydrogel biomaterials. These include block copolymers of acrylated PEG and oligopeptide domains polymerized by light-induced crosslinking, or end-functionalized branched PEG vinylsulfone chains reacted with bioactive thiol-bearing peptides as structural building blocks. Seliktar and co-workers [3,6,10–14] used PEGylated fibrinogen crosslinked in the presence of cells to form a dense cellularized hydrogel network. The fibrin-like scaffold material maintained its biofunctionality through the fibrinogen component, whereas the PEG component provided malleability over the mechanical properties of the material.

Nanostructuring of scaffolds for tissue engineering has recently been suggested as a way of imparting important structural cues

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into non-native scaffold materials in order to make them more like the natural ECM [15]. The nanostructuring of scaffold materials has been shown to induce major alterations in their interactions with cells. For example, nanostructuring by electrospinning, a technique which provides fibers in a broad size range of 50 nm up to 30 μm, provides a large surface area for improved cell attachment. Cells placed on nanofibers tend to preserve their phenotype and direct their growth along the nanofiber orientation [16]. Nanostructuring can also be achieved using the "phase separation method", which has been traditionally used to create porous membranes and recently adopted for the preparation of 3-D tissue engineering scaffolds [15]. Polymer scaffolds obtained by the phase separation method usually have a porous sponge-like microscale morphology. Another approach for nanoscale design of supported cell matrix is self-assembly, a reversible and cooperative assembly of pre-defined components into an ordered superstructure [15]. Self-assembly can be used to create a variety of structures, such as films, bilayers, membranes, fibers, micelles, multilamellar vesicles and many other structures [17].

The underlying hypothesis of the current investigation is that nanostructuring of a poly(ethylene glycol)–fibrinogen (PF) scaffold could potentially provide an additional means to control both the physical properties of the material and the subsequent interaction between the material and the cells. Currently, the range of fabrication techniques by which nanostructured scaffolds can be prepared is very limited. Moreover, few of these methods allow cell entrapment within a hybrid hydrogel during the preparation step. Therefore, studying the properties and cellular compatibility of PF hydrogels with distinct nanostructures requires the development of an appropriate structuring methodology. In the work described herein, the ability to self-assemble biocompatible nanostructures from amphiphilic block copolymers of poly(ethylene oxide)/poly(propylene oxide) (Pluronic®) was utilized for preparing PF hydrogels with distinct ultrastructural characteristics. The aggregation of Pluronic®, which is a function of both concentration and temperature [18,19], requires preparation protocols under controlled conditions. Thus, mixing Pluronic with cold PF hydrogel precursor solution and subsequent crosslinking should enable entrapment of nanometric micelles within PF hydrogels.

The overall objective of this study was to create nanostructures in PF hydrogels and understand the structure–property relationship associated with these nanostructures in cell culture applications. Specifically, we attempted to gain additional control over the material properties of the scaffold using nanostructuring without changing their compatibility for 3-D cell culturing. The initial efforts and preliminary results involved the use of a model system based on the block copolymer $(EO)_{100}$–$(PO)_{65}$–$(EO)_{100}$, available under the registered trademark Pluronic® F127 [20]. The results from this research may have direct implications for any number of hydrogel cell scaffold materials that could benefit from self-assembled nanostructures made from co-polymeric components in their precursor solutions.

2. Materials and methods

2.1. PEGylation of fibrinogen

The PEGylated fibrinogen hydrogel precursor was made according to protocols described elsewhere [3,6]. Briefly, acrylation of linear PEG-OH, mol. wt. 10 kDa (Fluka, Buchs, Switzerland), was carried out under argon by reacting a dichloromethane (Aldrich, Sleeze, Germany) solution of PEG-OH with acryloyl chloride

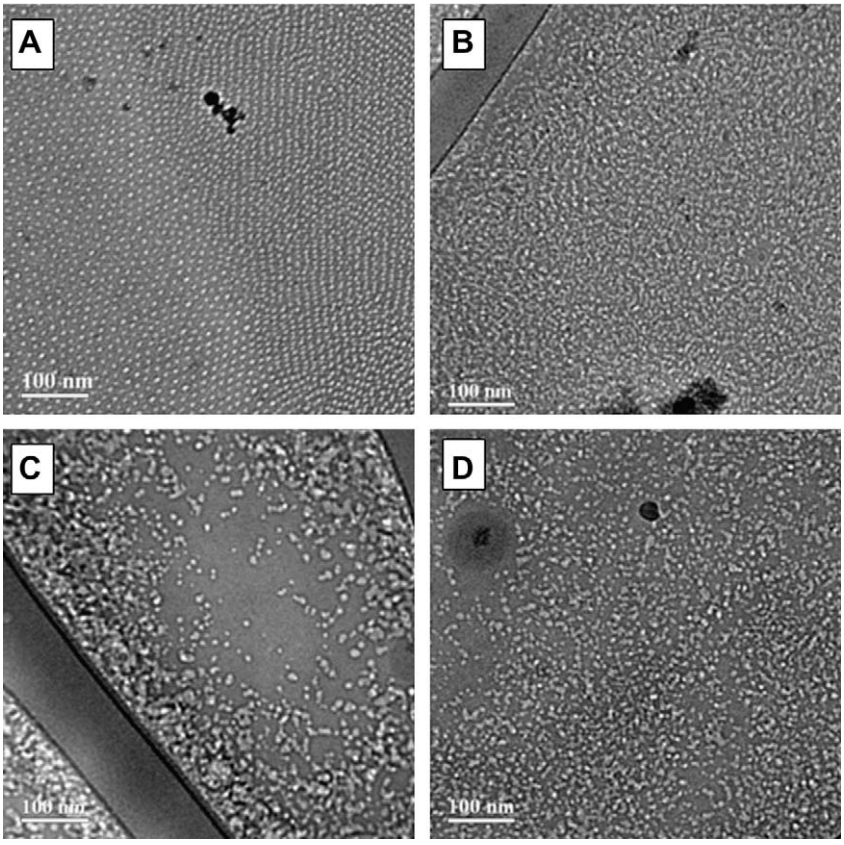

Fig. 1. Cryo-TEM micrographs of (A) 10% w/v Pluronic® F127 solution, (B) PF solution with 10% w/v Pluronic® F127, (C) PF solution with 3% w/v Pluronic® F127 and (D) PF solution with 7% w/v Pluronic® F127.

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