Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 3876–3886
Biomaterials
www.elsevier.com/locate/biomaterials

Protein–polymer conjugates for forming photopolymerizable biomimetic hydrogels for tissue engineering

Maya Gonen-Wadmany, Liat Oss-Ronen, Dror Seliktar*

Faculty of Biomedical Engineering , Technion—Israel Institute of Technology, Haifa 32000, Israel

Received 5 January 2007; accepted 5 May 2007
Available online 18 May 2007

Abstract

Collagen, fibrin and albumin are popular proteins for making biological scaffolds for tissue engineering because of their biocompatibility, biodegradability, and availability. A major drawback of biological protein-based biomaterials is the limited control over their physical and biodegradation properties. Our laboratory has been developing new protein-based biomaterials with tunable properties without the use of cytotoxic protein cross-linking techniques. We describe the formation and assembly of photopolymerizable biomimetic hydrogel scaffolds made from protein–polymer conjugates of poly(ethylene glycol) (PEG) and collagen, fibrin or albumin. The conjugation of PEG to these proteins (PEGylation) was verified by SDS-PAGE and the polymerization reaction into a hydrogel network was confirmed by shear rheometry. The differences in rheology and swelling characteristics of the three hydrogel materials underscore the importance of the molecular relationship between the PEG and the protein constituent in this protein–polymer arrangement. The biofunctionality of the PEGylated collagen and fibrinogen hydrogels sustained both cell adhesion and proteolytic degradation that enabled 3-D cell spreading and migration within the hydrogel network. PEG–albumin hydrogels exhibited poor cell spreading and migration by virtue of the fact that the albumin backbone lacks any known cell adhesion sites. Despite differences in the biological and structural composition of the PEGylated fibrinogen and collagen hydrogels, the rate of cellular migration within each material was not significantly different.

Keywords: Albumin; Fibrin; Collagen; Poly(ethylene glycol); Scaffold; Smooth muscle cells

1. Introduction

Natural extracellular matrix (ECM) proteins are widely used to make biomaterial scaffolds for tissue engineering and regenerative medicine. These purified and reconstituted ECM proteins can form hydrogel matrices with unique three-dimensional (3-D) architecture coupled with intrinsic cell signaling that guide remodeling and functional tissue regeneration. Biological scaffolds are easily made from collagen or fibrin, two abundant proteins that have been employed in numerous *in vitro* and *in vivo* applications [1]. Both collagen and fibrin assemble into fibrillar hydrogel networks that are supported by covalent and non-covalent protein interactions [2,3] and can be physically cross-linked with additional factors [3]. The liquid-to-solid transition (gelation) of the soluble protein hydrogel precursors is advantageous for tissue engineering because the matrix can be formed in the presence of cells, soluble growth factors, or living tissues (*in situ* polymerization). The 3-D environment created by protein hydrogels can support cell repopulation and instructive remodeling into hierarchically organized tissues and organs [4]. For this reason, it is important to design the scaffold with pore size, permeability, and material surface properties that favorably affect this remodeling [5,6]. At the same time, the ECM backbone of the material can instruct cells towards important phenotypic transitions [7].

There are some drawbacks to using exogenous or recombinant proteins such as collagen and fibrin as scaffolds for tissue engineering, including a limited control over the physical properties and biodegradation of the polymeric network. For example, collagen gels are made from a network of fibrils that exhibit poor physical strength

*Corresponding author. Tel.: +972 4 829 4805; fax: +972 4 829 4599.
E-mail address: dror@bm.technion.ac.il (D. Seliktar).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.05.005

and super-physiological tissue porosity. The specific conformation of fibrils combined with the open pore structure of the interpenetrating network leaves the protein backbone easily accessible and susceptible to freely diffusing proteases from the surrounding host tissue or cell culture system. This often results in uncontrolled and premature deterioration of the scaffold in the presence of cell-secreted proteases [3,8,9]. The discrepancies in structure and proteolytic susceptibility of reconstituted protein hydrogels compared to natural tissues still leaves much to be desired from the biological scaffold systems in many practical tissue engineering applications.

There are a number of techniques used to improve or modify the physicochemical properties of reconstituted protein hydrogels and to protect them from rapid degradation. Techniques for improving the physical properties based on covalent cross-links include the use of aldehydes, carbodiimides, and *N*-hydroxysuccinimides (NHS) [10,11], to name just a few. Many of the cross-linking procedures offer some improvements over the physical stability and reduced enzymatic susceptibility of the scaffold, but do so by introducing a cytotoxic manufacturing step which requires extensive washes and increases the likelihood that residual toxins in the scaffold will affect cellular activity [3,12]. Collagen and fibrin gels can also be processed by freeze-drying to increase the tensile strength and modulus of the protein network [13–15]. However, freeze-drying necessitates a pre-fabrication freezing step, which eliminates the possibility for gelation of the polymer in the presence of cells and also does away with the benefits of *in situ* fixation.

The proteolytic degradation of protein scaffolds can also be delayed by protecting the protein backbone of the polymer network using covalent attachment of a shielding polymer such as poly(ethylene glycol) (PEG). The modification of proteins by attachment of one or more PEG chains (PEGylation) has been applied very successfully to increase the plasma half-life of therapeutic peptides or protein drugs [16]. Based on a similar rationale, PEGylation could be a good strategy for protein-based biomaterial design in as much as the PEG chains can slow down the enzymatic biodegradation of the PEGylated protein scaffold [17–19]. At the same time, the PEG chains are non-toxic, non-immunogenic, highly water soluble, and are already approved by the FDA in a number of different clinical indications [20]. Common proteins used in scaffold design may be readily PEGylated using amine group modifications or thiol modifications of the protein backbone to yield a protein–polymer conjugate [21,22]. The PEG shields the protein from enzymes through steric hindrances without blocking all the natural biological function of the structural protein molecule [20].

In the present investigation, we describe the formation of protein–polymer conjugates and their assembly into biomimetic hydrogel scaffolds for tissue engineering. Three biomedical proteins, including collagen, fibrin(ogen), and albumin were PEGylated and formed into hydrogel networks by free-radical photopolymerization. The three biomaterials were characterized and tested with smooth muscle cells (SMCs) in 2-D and 3-D cultures for up to one week. The viability, mobility and cellular remodeling of the cell-seeded materials were documented and summarized.

2. Methods

2.1. PEG-diacrylate synthesis

PEG-diacrylate (PEG-DA) was prepared from linear PEG-OH MW = 10 kDa (Fluka, Buchs, Switzerland) as described elsewhere [30]. In brief, acrylation of PEG-OH was carried out under Argon by reacting a dichloromethane solution of PEG-OH (Aldrich, Sleeze, Germany) with acryloyl chloride (Merk, Darstadt, Germany) and triethylamine (Fluka) at a molar ratio of 1.5:1 relative to –OH groups. The final product was precipitated in ice-cold diethyl ether and dried under vacuum for 48 h. Proton NMR (^{1}H NMR) was used to validate end-group conversion and to verify purity of the final product.

2.2. Fibrinogen and albumin PEGylation

The PEGylation of fibrinogen and albumin was done according to a PEGylation protocol similar to one described in detail by Dikovsky et al. [18]. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP-HCl) (Sigma-Aldrich) was added to a 7 mg/ml solution of bovine fibrinogen (Sigma-Aldrich) or bovine serum albumin (MP Biomedicals, Ohio, USA) in 150 mM phosphate buffered saline (PBS) with 8 M urea (molar ratio 1.5:1 or 2:1 TCEP to fibrinogen or albumin cysteines, respectively). Linear 10-kDa PEG-DA was reacted for 3 h with the protein at a 4:1 or 2:1 molar ratio of PEG to fibrinogen or albumin cysteines, respectively. The PEGylated protein product was precipitated in acetone and redissolved in PBS containing 8 M urea at 7 mg/ml final fibrinogen concentration and 2 mg/ml final albumin concentration. The protein product was dialyzed against PBS at 4 °C for 2 d (Spectrum, 12–14-kDa MW cutoff, California, USA). The PEGylated albumin was concentrated to 8 mg/ml by lyophilization and reconstitution. The PEGylated product was characterized according to published protocols [18].

2.3. Collagen PEGylation

Collagen (type I) was isolated from rat tail tendon according to published protocols [23]. Thiolation of collagen was accomplished using succinimidylacetyl-thioacetate (SATA, Pierce, Illinois, USA) based on the protocols of Chen et al. [24]. Briefly, collagen was dissolved in 150 mM PBS with 8 M urea at a concentration of 5 mg/ml. SATA was reacted for 2 h at RT with agitation at a concentration of 0.075 mg/mg collagen. The resulting acetylated SH groups on the lysine residues were then deprotected for 2 h by reacting 0.5 M hydroxylamine hydrochloride ($NH_2OH \cdot HCL$, Sigma-Aldrich) at a concentration of 0.125 ml/mg collagen. The product was dialyzed overnight against PBS and 8 M urea and PEGylated with TCEP–HCl (molar ratio 2:1 TCEP to the new collagen thiols), 10 kDa PEG-DA (molar ratio 1.5:1 PEG-DA to new collagen thiols) at pH 8 and RT for 3 h. The PEGylated collagen was precipitated in acetone and redissolved in PBS and 8 M urea, and dialyzed in a Slide-A-Lyzer (10,000 MWCO, Pierce, IL, USA) at 37 °C for 2 d to a final concentration of 4 mg/ml collagen. The PEGylated product was characterized the same as PEG–fibrinogen.

2.4. SDS-PAGE

The PEGylation reaction was confirmed by SDS-PAGE whereby PEGylated and unPEGylated proteins were loaded into 8% gels (5–10 μg of protein in each lane). The gels were stained with Coomassie® blue and digitally imaged using a gel documentation workstation.