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Characterization of sequential collagen-poly(ethylene glycol) diacrylate interpenetrating networks and initial assessment of their potential for vascular tissue engineering

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ABSTRACT

Collagen hydrogels have been widely investigated as scaffolds for vascular tissue engineering due in part to the capacity of collagen to promote robust cell adhesion and elongation. However, collagen hydrogels display relatively low stiffness and strength, are thrombogenic, and are highly susceptible to cell-mediated contraction. In the current work, we develop and characterize a sequentially-formed interpenetrating network (IPN) that retains the benefits of collagen, but which displays enhanced mechanical stiffness and strength, improved thromboresistance, high physical stability and resistance to contraction. In this strategy, we first form a collagen hydrogel, infuse this hydrogel with poly(ethylene glycol) diacrylate (PEGDA), and subsequently crosslink the PEGDA by exposure to longwave UV light. These collagen-PEGDA IPNs allow for cell encapsulation during the fabrication process with greater than 90% cell viability via inclusion of cells within the collagen hydrogel precursor solution. Furthermore, the degree of cell spreading within the IPNs can be tuned from rounded to fully elongated by varying the time delay between the formation of the cell-laden collagen hydrogel and the formation of the PEGDA network. We also demonstrate that these collagen-PEGDA IPNs are able to support the initial stages of smooth muscle cell lineage progression by elongated human mesenchymal stems cells.

1. Introduction

Collagen hydrogels have been widely investigated as scaffolds for vascular tissue engineering due in part to the abundance of collagen in the vessel wall and due to the capacity of a range of cell types to elongate and spread within collagen networks [1–4]. Yet, collagen hydrogels also have critical shortcomings which limit their broader utility in vascular graft applications. For instance, L'Hereux et al. employed collagen hydrogels to form the medial layer of their engineered vascular grafts [2]. However, the encapsulated smooth muscle cells contracted the collagen gels by up to 70% within 4 days of culture. Although adult mesenchymal stem cells (MSCs) are increasingly used as a source of smooth muscle cells for tissue engineered vascular grafts [5–9], MSC-laden collagen hydrogels are also prone to cell-mediated compaction [10]. In addition, Weinberg and Bell noted that vascular grafts based on tubular collagen hydrogels were so highly distensible that they ruptured at very low pressures (<10 mmHg) and that increasing the concentration of collagen had limited effect on hydrogel strength [4]. This restricted capacity to manipulate collagen hydrogel strength is also reflected in the relatively limited range of stiffnesses achievable with pure collagen hydrogels [11,12]. Specifically, the elastic modulus of collagen hydrogels ranges from 1 to 100 Pa [13,14], which is significantly lower than the modulus of small-diameter vascular tissue (40–900 kPa) [15,16]. Furthermore, collagen hydrogels have a tendency to undergo rapid cell-mediated degradation, which can be challenging to control and predict, and the thrombogenicity of collagen requires graft pre-endothelialization prior to deployment [4,17].

To reduce the thrombogenicity of collagen, researchers have linked thromboresistant molecules, such as heparin and poly(-ethylene glycol), to the collagen network with promising results [18,19]. Similarly, several strategies have been employed to enhance collagen hydrogel stiffness, strength, and resistance to degradation and cell-mediated compaction. For instance,

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Girton et al. demonstrated that glycation can be used to stiffen and strengthen collagen networks [20]. Further studies have since demonstrated that glycation reduces collagen susceptibility to matrix metalloproteinase degradation [21]. In addition, glutaraldehyde, hexamethylene diisocyanate, cyanamide, and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) have each been examined in terms of their capacity to increase the mechanical properties and slow the degradation rate of collagen hydrogels [22]. However, these chemical treatments can also have unwanted side-effects. For instance, glutaraldehyde-treated tissues are prone to calcification, a situation which is undesirable for vascular graft applications [23].

To address the limitations of pure collagen hydrogels while avoiding the drawbacks of chemical crosslinking treatments, we propose to combine collagen hydrogels with a poly(ethylene glycol) diacrylate (PEGDA) hydrogel to form an interpenetrating network (IPN). IPNs comprised of two distinct polymer networks have recently been shown to result in increased hydrogel stability, stiffness, and strength relative to either single component network [24–27]. Importantly, the component networks often contribute to IPN properties in a synergistic, rather than simply an additive, manner [24,25]. For instance, recent polyacrylamide-alginate IPNs [26,27] demonstrate an elastic modulus, tensile strength, and strain at failure that exceed the sum of the corresponding properties of the individual networks.

In the present work, we combine a covalently-crosslinked PEGDA network with a physically-crosslinked collagen network. PEGDA was selected as the second component of this collagen-based IPN due to the established biocompatibility, low thrombogenicity, and resistance to cell-mediated compaction characteristic of PEGDA hydrogels [28–30]. In addition, the degradation rate and mechanical properties of PEGDA hydrogels can be systematically tailored. Specifically, although pure PEGDA hydrogels degrade relatively slowly, their degradation rate can be modified by introduction of hydrolytically or enzymatically-degradable segments within the PEG network [31–35]. Similarly, the mechanical performance of PEGDA hydrogels can be tuned by varying the molecular weight (MW) and concentration of PEGDA in the hydrogel precursor solution [36,37]. Given these properties, it is reasonable to assume that a collagen-based IPN which includes PEGDA as the second network may display improved thromboresistance, higher resistance to cell-mediated compaction, and an increased range of mechanical properties relative to pure collagen.

Most IPNs, including those previously formed from PEG and collagen [38], are fabricated by mixing the two component polymers followed by their simultaneous crosslinking into interwoven networks [24,26,27,38]. Unfortunately, in the case of collagen-PEG IPNs, this fabrication approach forces cells encapsulated within the IPN to take on a rounded cell phenotype, despite the presence of collagen. This is due to the nanoscale mesh structure and slow degradation rate of pure PEGDA hydrogels [39]. We propose to circumvent this limitation by first forming the collagen hydrogel and then infusing this hydrogel with PEGDA. Subsequent exposure of the infused network to longwave UV light will result in the formation of a PEGDA network interlaced with the pre-formed collagen network. As shown schematically in Fig. 1, the degree of cell spreading in the IPN network can be controlled by varying the time delay between collagen hydrogel formation and PEGDA infusion and polymerization. In the current work, we demonstrate that this hybrid natural-synthetic IPN retains the benefits of collagen in terms of enabling robust cell elongation while improving scaffold stiffness and strength as well as scaffold resistance to compaction and platelet adhesion. Furthermore, we show the ability of these collagen-PEGDA IPNs to support the initial stages of MSC progression toward a smooth muscle cell lineage.

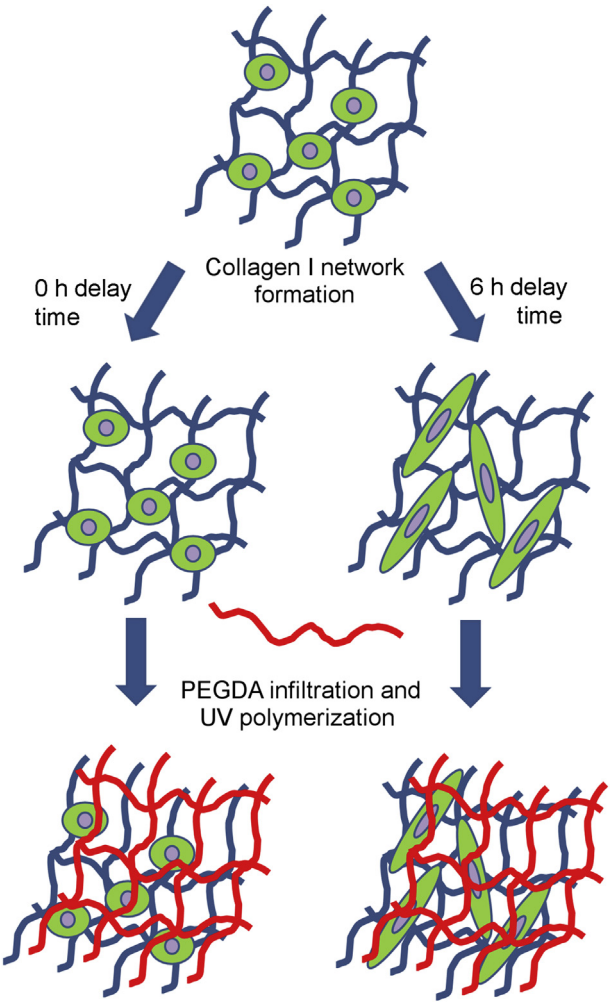

Fig. 1. Schematic representation of the sequential collagen-PEGDA IPN fabrication process.

2. Materials and methods

2.1. Polymer synthesis and characterization

PEGDA was prepared as previously described [40] by combining 0.1 mmol/mL dry PEG (3.4, 6.0, or 10.0 kDa; Fluka), 0.4 mmol/mL acryloyl chloride, and 0.2 mmol/mL triethylamine in anhydrous dichloromethane and stirring under argon overnight. The resulting solution was washed with 2 M K_2CO_3 and separated into aqueous and dichloromethane phases to remove HCl. The organic phase was subsequently dried with anhydrous $MgSO_4$, and PEGDA was precipitated in diethyl ether, filtered, and dried under vacuum. Acrylation of the PEG end hydroxyl groups was characterized by ^{1}H NMR to be ≈ 95%.

2.2. IPN fabrication process

Collagen-PEGDA IPNs were fabricated under sterile conditions via a three-step process outlined schematically in Fig. 1: 1) the physical crosslinking of a pure collagen network, 2) followed by the infiltration of the collagen hydrogel with a 3.4 kDa, 6.0 kDa or 10.0 kDa PEGDA solution, and 3) UV polymerization of the infiltrating PEGDA solution. In brief, ice-cold high-concentration rat tail collagen I (BD Biosciences) was diluted and neutralized with 1 M NaOH, 10× PBS and dIH_2O to achieve the desired final collagen concentration (1.5 mg/mL, 3 mg/mL, or 5 mg/mL) in 1× PBS. Three hundred microliters of the neutralized ice-cold solution was then pipetted into BD Falcon culture inserts (12 mm diameter, 0.8 μm pore size) followed by polymerization via 30 min incubation at 37 °C and 5% CO_2. The resulting hydrogels were then immersed for 30 min in serum-free medium (SFM) composed of phenol-red free, high glucose DMEM (Gibco, Life Technologies) supplemented with 1% sodium pyruvate (Gibco, Life Technologies) and 1% Glutamax (Gibco, Life Technologies).

The second polymer network was created as follows: the SFM solution surrounding the previously cured collagen constructs was carefully removed and