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In situ crosslinking of a biomimetic peptide-PEG hydrogel via thermally triggered activation of factor XIII

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Abstract

There is a medical need for robust, biocompatible hydrogels that can be rapidly crosslinked in situ through the use of gentle and non-toxic triggers, which could be used as a surgical adhesive, a bone-inductive material, or for drug and gene delivery. The complete gelation system described here includes calcium-loaded liposomes, hrFactor XIII, thrombin, and an enzymatic substrate based on a four-armed PEG in which each arm terminates with a 20mer peptide sequence derived from the γ-chain of fibrin. Controlled release of calcium ions for efficient hrFXIII activation was accomplished by thermal triggering of a tailored liposome phase transition at 37°C, which allowed the entire gelation system to be stored in aqueous solution at room temperature without premature gelation. When the system temperature was raised to 37°C (body temperature), the released calcium activates the hrFactor XIII, and gelation was observed to occur within 9 min. Rheological studies performed to quantitatively determine the storage modulus (G') of the gel during oscillatory shear show that it behaves as a robust, elastic solid. Scanning electron microscopy studies revealed the hydrogel to have a very dense morphology overall, however spherical voids are observed in regions where calcium-loaded liposomes were entrapped during gelation.

Keywords: Liposome; Hydrogel; Peptide; Biomimetic; Poly(ethylene glycol); Enzymatic crosslinking

1. Introduction

Biomaterials based upon poly(ethylene glycol) (PEG) are commonly investigated for medical and biotechnological applications such as wound healing, tissue scaffolding, and drug and gene delivery [1]. The intrinsic molecular properties of PEG, including its good water-solubility, resistance to protein adsorption, low immunogenicity, tendency to slow immune system clearance of therapeutics, absence of toxicity, and low cost, make it nearly ideal for use as the basis of an in vivo hydrogel [2–5]. Moreover, covalent incorporation of other synthetic or biological polymers into PEG-based hydrogels can allow for the inclusion of additional desirable physical or biological characteristics [6]. For example, the swelling behavior of an ionic hydrogel system, composed of poly(L-glutamic acid) (PLG) covalently crosslinked to PEG, can be adjusted by the variation of pH, thus altering the ionization of the PLG and resulting in the controlled release of pharmaceuticals [7]. Rathore and Sogah synthesized *N. clavipes* silk-mimetic polymers by selectively replacing amorphous peptide domains with PEG, while retaining the poly (alanine) regions that direct silk self-assembly. These polymers formed β-sheet-rich secondary structures that self-assemble into discrete fibrils, which were shown to have solid-state structures similar to silk proteins and to exhibit good mechanical properties [8].

Biocompatible hydrogels designed for in situ gelation and/or crosslinking may be formed in any desired shape at the site of injection, an advantage that increases their applicability and reduces the need for invasive

Abbreviations: Human recombinant factor XIII; hrFXIII; Scanning electron microscopy; SEM; Calcium-encapsulated interdigitation-fusion vesicles; Ca-IFVs; Poly(ethylene glycol); PEG

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surgery. One approach to in situ gelation is to design polymeric materials that can be crosslinked by photopolymerization. Hubbell and coworkers developed degradable PEG-based diacrylate macromers that were rapidly polymerized in situ using UV and visible light, suitable for tissue repair and prevention of surgical adhesions [9]. Han and Hubbell extended this method to copolymerize acrylated oligolactide with monoacrylated PEG to form gel networks that could serve as tissue engineering scaffolds [9,2]. Photopolymerization has also been used to crosslink another gelation system consisting of a PEG main chain with flanking lactic acid oligomers and tetraacrylate termini, allowing in situ formation of thin hydrogel barriers on the inner surface of injured arteries for reduction of thrombosis and intimal thickening in rats and rabbits [10]. However, photopolymerization strategies often require the use of harmful catalysts and may be exothermic to a degree that can damage neighboring cells and lead to local necrosis. Moreover, the light used to initiate photopolymerization may be limited in its ability to penetrate tissues.

Alternatively, temperature can be used to control the gelation of thermo-responsive polymer systems designed for biocompatibility. A thermo-sensitive polymer network composed of copolymerized poly(ethylene oxide) and poly(L-lactic acid) was developed for drug delivery, and was designed to be injected as a fluid solution at 45°C and to form a gel upon cooling to the physiological temperature of 37°C [11]. Vernon et al. synthesized thermoreversible hydrogels composed of high molecular weight poly(*N*-isopropyl acrylamide-co-acrylic acid) for use as a gelation system to entrap pancreas islet cells [12]. Upon heating this system from a solution at room temperature to a gel at 37°C, the total volume was found to not significantly change. Stile et al. exploited the LCST transition exhibited by poly(*N*-isopropylacrylamide) to form porous hydrogels in vitro, which were shown to support the growth of bovine articular chondrocytes and the formation of cartilage-like tissues [13]. Modest temperature changes can also be used to stimulate block copolymer gel formation, as shown by Jeong et al. using PEG grafted with poly(lactic acid-co-glycolic acid), creating a biodegradable material with utility for short-term drug delivery applications [14].

An interesting and biologically compatible approach to hydrogel formation and stabilization is to take advantage of transglutaminase enzymes, whose natural function is to crosslink proteins in vivo. Transglutaminases are generally dependent upon calcium ions as cofactors for their specific activity [15]. One particular transglutaminase, Factor XIII (FXIII), catalyzes the formation of covalent crosslinks between lysine and glutamine residues in the α- and γ-chains of fibrin, stabilizing blood clots in vivo [16,17]. Sperinde et al. took advantage of tissue transglutaminase from guinea pigs to crosslink a biodegradable polymer system composed of a glutaminamide-derivatized PEG and a synthetic, lysine-containing random co-polypeptide. In this case, a calcium chloride solution (6 mM) was added directly to the peptide-polymer-enzyme solution to trigger enzymatic crosslinking, producing a significant viscosity increase within 30 min and gelation within a period of several hours [18]. Systems that utilize enzymes to induce gelation or crosslinking have the advantages of being gentle and biocompatible (if the enzyme is not immunogenic), not requiring a chemical initiator, and not resulting in temperature changes at the site.

With the aim of developing biocompatible hydrogels utilizing triggered enzymatic crosslinking, researchers have also investigated the use of either a high concentration of calcium ions [19] or another blood-clotting enzyme, thrombin [20], to induce high activity in transglutaminase enzymes. A dual activation scheme including both calcium and thrombin, which closely mimics the manner in which FXIII is activated in vivo [15], has been used to crosslink the natural FXIII substrate, fibrin [21], as well as other protein substrates in situ [21,22]. Commercially available, fibrin-based adhesives produce strong, biodegradable gels very quickly (<10 min) and are used as surgical glues, tissue sealants, and drug delivery devices [23]. Through extensive crosslinking of the protein substrate by FXIII, resistance to both mechanical and enzymatic degradation is imparted to the material [21]. Covalent incorporation of bioactive peptides into fibrin gels, again through exploitation of transglutaminase enzymes, can be used to tailor the hydrogel properties for specific functions in vivo. For example, incorporation of neuroactive peptides into fibrin gels by Factor XIII was found to enhance neurite outgrowth and extension into the gel when it was implanted in vivo [22].

We reasoned that stimuli-responsive activation of transglutaminase enzymes (e.g., via changes in pH or temperature) would be useful to accomplish in situ enzymatic crosslinking of a biomimetic substrate and formation of a hydrogel. We have shown previously that stimuli-responsive liposomes can be used to trigger the in situ formation of biomaterials [24], in addition to the more conventional uses of liposomes in drug delivery and other biotechnology applications [25,26]. Recently, Messersmith et al. developed a class of tailored, thermally triggered liposomes, which stably store calcium at ambient temperature [27,28], but rapidly release $>90\%$ of entrapped calcium at 37°C [19]. These thermally triggered liposomes have been successfully utilized for in situ formation of self-assembled peptide hydrogels [29], alginate and fibrinogen-based hydrogels through ionic gelation [19], and the controlled deposition of phosphate mineral for dental applications [27,28].

We are continuing to develop novel biomaterials that mimic the crosslinking strategy of the blood coagulation cascade to produce in situ hydrogels with increasingly tailored properties. In this study, we have created a novel, bioconjugate-based transglutaminase substrate by coupling a 20mer peptide substrate sequence derived from the γ-chain of fibrin [21], which has an N-terminal cysteine and is linked to the termini of a four-armed PEG molecule. Linking of these bioactive peptides to the branched PEGs renders these molecules capable of serving as an enzymatic substrate [21], allowing for the formation of a crosslinked PEG-peptide network. We utilized thermally triggered release of calcium from phospholipid vesicles [19] to activate FXIII to crosslink this polymeric bioconjugate and rapidly form a hydrogel at physiological temperature. We have examined the in vitro rheological and morphological properties of the resulting hydrogel, and find that it has material properties that should be favorable for biomedical use.

2. Materials and methods

2.1. Materials

Amino acids and resin for peptide synthesis were purchased from Novabiochem (San Diego, CA). Remaining peptide synthesis reagents were purchased from Applied Biosystems (Foster City, CA). A four-armed, maleimide-terminated PEG with a weight-average molecular weight of 10,000 g/gmol was purchased from Shearwater Polymers Inc. (Huntsville, AL). 1,2-bis(palmitoyl)-sn-glycero-3-phosphocholine (DPPC, >99%) and 1,2-bis(myristoyl)-sn-glycero-3-phosphocholine (DMPC, >99%) were obtained from Avanti Polar Lipids (Alabaster, AL). Thrombin (lot 48H7611) and fibrinogen (61% protein, 97% clottable, lot 119H7612) from bovine plasma were purchased from Sigma Chemical Company (St. Louis, MO). HEPES, EDTA, NaCl, and arsenazo III (AIII) were purchased from Sigma Chemical Company. All aforementioned chemicals and materials were used as received. Human recombinant Factor XIII (hrFXIII) was generously donated by ZymoGenetics Inc. (Seattle, WA) as a lyophilized powder, which was dissolved at 10 mg/ml in water and stored at –80°C.

2.2. Instrumentation

An Applied Biosystems 433A peptide synthesizer was used to synthesize the 20mer peptide (Foster City, CA). Peptide purity was analyzed using a Waters 2690 analytical reversed-phase high pressure liquid chromatography (RP-HPLC) system equipped with a Vydac C18 column (5 μm beads, 300 Å pore size, column size 2.1 × 250 mm). The peptide was purified on a Varian preparative RP-HPLC system using a Vydac C18 preparative column (15 μm, 300 Å, and 22 × 250 mm). A Bohlin VOR Rheometer (Bohlin Rheologi; Cranbury, NJ) was used to study the rheological properties of this system with a circulating water bath to control temperature.

2.3. Synthesis and purification of the peptide

The 20mer peptide CTIGEGQQHHLGGAKQAGDV [21] derived from the Factor XIII crosslinking site of fibrin, with an additional N-terminal cysteine residue, was synthesized using standard solid phase methods and FastMoc™ chemistry (Applied Biosystems). The peptide was cleaved with a mixture of 95% trifluoroacetic acid (TFA), 2.5% ethanedithiol (EDT), and 2.5% water for 1.5 h at room temperature, and then the mixture was frozen and lyophilized to a powder. The crude peptide was analyzed for purity by RP-HPLC using a gradient of 10–60% B in A (B: acetonitrile with 0.1% TFA, A: water with 0.1% TFA). The N-terminal cysteine residue was reduced for 2 h with the peptide at 1–10 mg/ml in 0.1 M sodium phosphate buffer containing 5 mM EDTA and 10–100 mM dithiothreitol (DTT) with a pH of 6.5–7.0. The reduced peptide was purified by preparative RP-HPLC with a gradient of 10–60% B in A. Peptide molecular weight was confirmed using electrospray ionization (ESI) mass spectroscopy by Northwestern University's Analytical Laboratory.

2.4. Conjugation of the peptide to the four-armed PEG

For the conjugation reaction, purified peptide was added in 5-molar excess to a solution of the maleimide-functionalized PEG in a 0.1 M sodium phosphate buffer with 0.15 M sodium chloride at pH 7.2. This mixture was allowed to react at room temperature for 24 h, after which the polymeric bioconjugate was dialyzed against water using a Slide-A-Lyzer™ Dialysis Cassette from Pierce Chemical Company (Rockford, IL) with a molecular weight cut off (MWCO) of 2000. The correct molecular weight of the peptide-PEG bioconjugate was confirmed by matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry at the University of Illinois at Urbana-Champaign.

2.5. Liposome preparation

An interdigitation-fusion approach to liposome preparation was adapted to produce thermally triggerable, calcium-loaded phospholipid vesicles, as previously reported by Messersmith et al. [21,18]. A thin film of phospholipid (90 mol% dipalmitoylphosphatidylcholine (DPPC) and 10 mol% dimyristoylphosphatidylcholine (DMPC)) in a round-bottom flask was hydrated with aqueous $CaCl_2$ (0.2 M, 520 mmol/kg) for 15 min at 50°C.

The vesicle suspension was sonicated at 50°C for ~20 min until optically clear, using a probe-type ultrasonicator, to form small, unilamellar vesicles (SUVs). The SUV suspension was allowed to cool to room temperature and was then centrifuged to remove any metal particles released from the probe tip. Absolute ethanol was added to form an interdigitated phase, with a final ethanol concentration of 4 M. The flask was stoppered, vortexed vigorously, and incubated for 15 min at room temperature. The flask was then transferred to a 50°C water bath and incubated for 7.5 min stoppered and 7.5 min unstoppered. Nitrogen was used for 45–60 min to purge the ethanol, yielding calcium-encapsulated interdigitation-fusion vesicles (Ca-IFVs). The liposome solution was cooled to room temperature for 20–30 min, after which unentrapped calcium was removed from the vesicle solution by washing with iso-osmotic buffered saline, centrifuging at $20{,}000 \times g$, and decanting the supernatant. This washing process was repeated a minimum of five times or until calcium could not be detected in the supernatant with a calcium sensitive dye arsenazo III (AIII).

2.6. Hydrogel formation

The gel precursor fluid consisted of 25 mg of polymeric bioconjugate dissolved in 40 μl buffer containing 0.3 M NaCl, 10 mM HEPES, and 1 mM EDTA. Then, 80 μl of hrFXIII at 10 mg/ml was added to the solution, along with 40 μl at 2 units/μl of bovine thrombin and 17 μl of calcium liposomes. The concentration of calcium in the precursor solution was 12 mM. The solution temperature was maintained at 37°C for the duration of the enzyme-crosslinking experiment. The gel was photographed with a Nikon Coolpix 990 digital camera (Tokyo, Japan) after formation in a glass test tube.

2.7. Rheometry

Rheological measurements of the gelation process were made with a Bohlin VOR Rheometer using a stainless steel cone and plate geometry with a 15 mm diameter and 5.0° cone angle. Measurements were taken at both 20°C and 37°C in the oscillatory mode at 0.1 Hz and 1% applied strain. 110 μl of liquid precursor solution (22 mg of polymeric bioconjugate in 30.75 μl of buffer with 0.3 M NaCl, 10 mM HEPES, and 1 mM EDTA, 33.9 μl of thrombin (2 units/μl), 67.8 μl of hrFXIII (10 mg/ml in water), and 17.6 μl of calcium liposomes) was transferred to the thermostatted plate prior to positioning of the cone. The precursor solution contained a calcium concentration of 12 mM. Approximately 2 min elapsed between the application of the sample to the plate and the initiation of data collection. Data were collected every 10 s over 135 min. Mineral oil was applied to the edges of the cone to prevent dehydration during the rheological experiments. After the oscillation experiment, a frequency sweep was performed with the frequency varying from 0.01 to 10 Hz and with 1% applied strain. Finally, a strain sweep was performed with a frequency of 0.1 Hz.

2.8. Scanning electron microscopy

Polymeric bioconjugate gels were prepared for electron microscopy by fixing the sample in a 2.5% glutaraldehyde solution at 4°C, followed by dehydration in ethanol solutions of increasing concentration at room temperature (20%, 40%, 60%, 80%, 95%, and 100% ethanol). It was observed that the hydrogel volume was reduced by ~75% during the dehydration process. The samples were critical point dried, sputter-coated with 9 nm of gold/palladium, and imaged using a Hitachi S-4500 scanning electron microscope (Tokyo, Japan).

3. Results and discussion

A 20-residue peptide derived from the γ-chain of fibrin with the sequence CTIGEGQQHHLGGAKQAGDV [21], which includes residues 374–392 of the fibrin γ-chain plus an additional N-terminal cysteine, was synthesized. This substrate contains the two residues, glutamine 379 and lysine 387, which have been shown to be the preferred sites for FXIII crosslinking of fibrin [21,30–32]. In particular, the N-terminal glutamine is preferentially crosslinked to the ε-amino group of the lysine residue, releasing ammonia [32]. The molecular weight of the purified peptide was confirmed with electrospray mass spectrometry (expected: 2005.2, observed: 2005.8). The peptide-polymer bioconjugate was synthesized by reacting the purified peptide via its N-terminal cysteine to the maleimide groups of the four-armed PEG (Fig. 1). Successful conjugation in good yield was confirmed using MALDI-TOF mass spectrometry (expected: ~19,000, observed: ~18,800).

Thermal activation of gelation was accomplished by employing calcium-loaded liposomes, whose properties and performance for similar applications have been characterized extensively by Messersmith et al. [19,27,28]. Studies of these liposomes have shown that storage of Ca-IFVs at room temperature in buffered saline does not appreciably change vesicle size nor lead to significant leakage of entrapped calcium. Additionally, heating of calcium-loaded liposomes from room temperature to 45°C in the presence of AIII was shown to cause a sharp release of nearly 90% of the entrapped calcium from the vesicles, at temperatures between 36°C and 38°C [19]. Therefore, these thermally triggered, calcium-loaded liposomes may be considered an efficient method for activation of transglutaminase enzymes

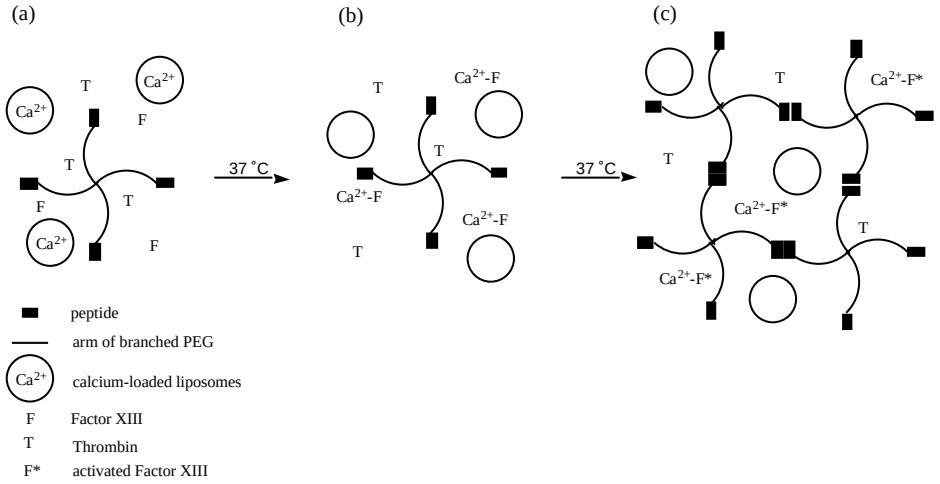

Fig. 1. Schematic illustration of the components of the hydrogel system and the process by which they gel: (a) components are mixed together as a liquid solution containing the peptide-PEG bioconjugate, calcium-loaded liposomes, Factor XIII, and thrombin; (b) the temperature of the solution is raised to 37°C, causing the calcium to be released from the liposomes, activating the thrombin and thereby activating Factor XIII; and (c) the activated Factor XIII crosslinks the peptide-PEG bioconjugate, resulting in hydrogel formation.

(whose activity is calcium-dependent) for quick, controlled gelation.

Optimal gelation conditions were determined by visual observation of the gelation time required for different substrate/enzyme mixtures. The polymeric bioconjugate was dissolved at room temperature in aqueous buffer along with hrFXIII, Ca-IFVs, and the enzyme thrombin. This precursor solution was translucent and somewhat viscous and remained stable at room temperature with no visible settling of liposomes (Fig. 2a). The reaction mixture had a notably low viscosity at 20°C, as the calcium ions remained sequestered within the liposomes, leaving the enzyme inactive. Upon heating of the precursor solution to 37°C, a solid, enzymatically crosslinked hydrogel was observed to form within 9 min, as shown in Fig. 2b. Even after cooling to room temperature, the material remained in a gelled state, indicating that the apparent solidification did not arise from physical association nor from precipitation of PEG at increased temperatures.

To characterize the viscoelastic properties of the hydrogel as it was being formed at 37°C, we conducted quantitative measurements using oscillatory rheometry with a low-amplitude strain, to minimize disruption of the gel network during the formation process [33]. Peptide-polymer bioconjugate was dissolved in aqueous buffer along with thrombin, hrFXIII, and Ca-IFVs. This precursor solution was transferred to the rheometer plate, which was thermostatted at 20°C, and the cone was lowered into position (note that the cone was not thermostatted and was therefore initially at room temperature). During an initial 30 min of data collection at 20°C, oscillatory rheometry showed that the storage modulus (G', characterizing the elastic behavior of a

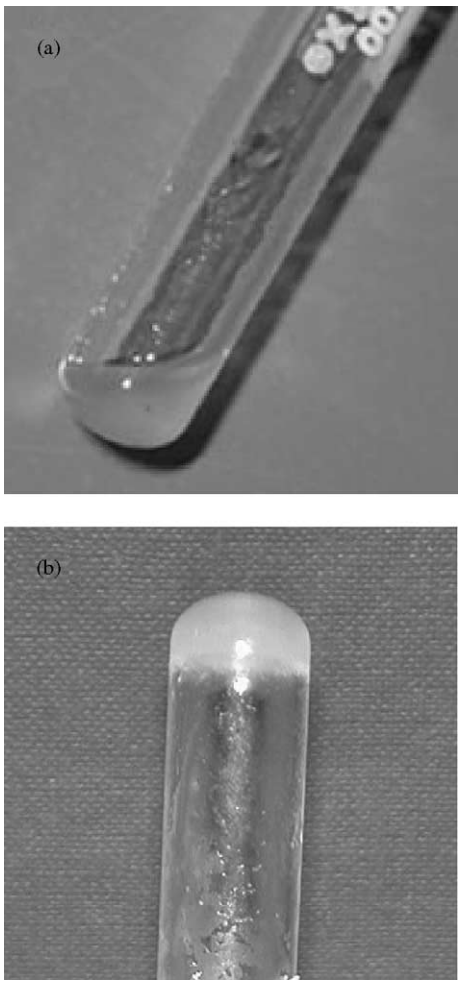

Fig. 2. Photograph of the precursor solution (a) before and (b) after enzymatic crosslinking forms a hydrogel.

material) remained very low (<20 Pa) because the solution maintains fluid characteristics at 20°C. At 30 min, the temperature was raised to 37°C, and gelation occurred rapidly due to calcium release from the liposomes and subsequent activation of hrFXIII, resulting in crosslinking of the peptide-polymer bioconjugate and producing an increase in G' until at 135 min, $G' \sim 13$ kPa (Fig. 3). Apparently, a substantial degree of crosslinking had occurred during the 10–15 min required to complete the temperature step, which corresponds well with our visual observation of the gelation process. After the solution had gelled completely, the phase angle δ was almost zero ($\sim 0.15^\circ$), the loss modulus (G'', denoting the viscous character of the material) was low (~ 30 Pa), and G' was approximately equal to the complex modulus (G^*), indicating that the hydrogel behaved as an elastic solid.

Immediately after completion of the oscillatory shear experiments, the effects of frequency and strain on the storage modulus of the hydrogel were examined. A frequency sweep from 0.01 to 10 Hz was flat with respect to G', confirming that this material behaves as an elastic solid (Fig. 4a). Also, three successive strain sweeps (to a maximum strain of 11.1%) demonstrated that this hydrogel is robust, as it did not tear under these conditions, indicated by a similar linearity of the storage modulus with respect to strain for all three sets of data (Fig. 4b).

Scanning electron microscopy (SEM) experiments were performed to study the morphology of this synthetic, biomimetic hydrogel (Fig. 5). SEM micrographs of critical point dried gels show a dense structure with spherical voids that apparently represent regions where calcium-loaded liposomes were located during gelation. The lipids comprising the membrane of the liposomes were dissolved during the critical point drying, so only the liposome remnants are expected to be visible with this approach. The dimensions of the

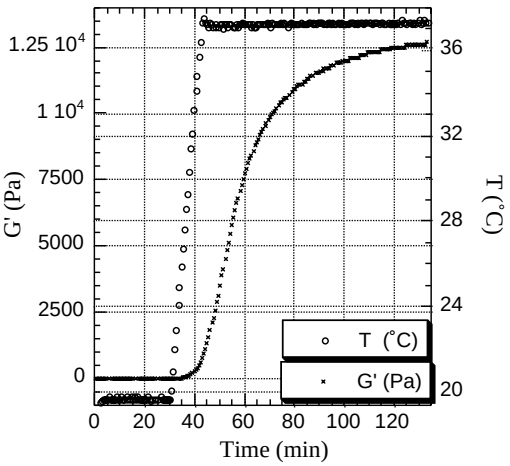

Fig. 3. Plot of rheological data collected during conjugate gelation. The storage modulus and temperature are plotted vs. time.

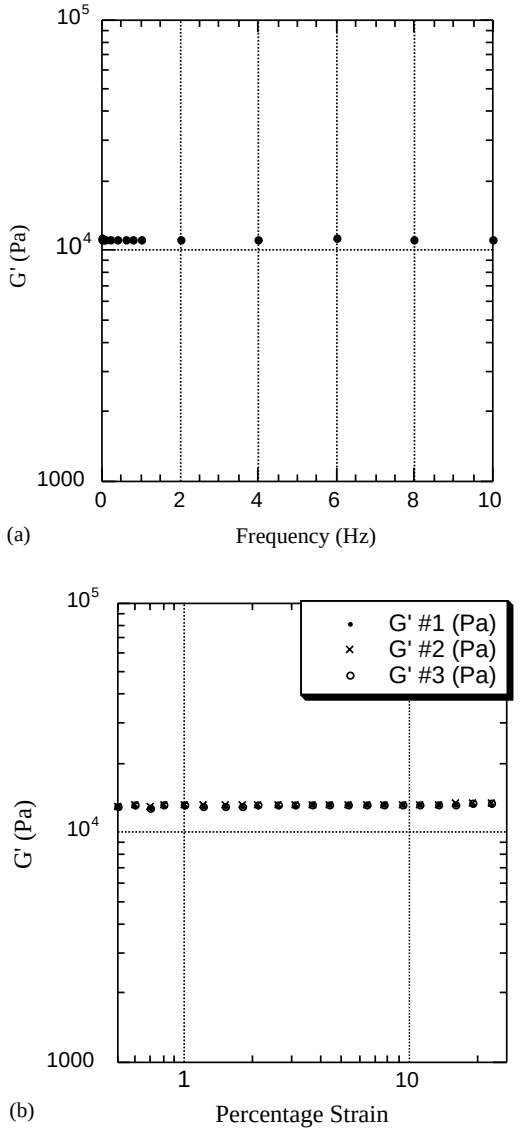

Fig. 4. Plots of rheological data collected after conjugate gelation: (a) storage modulus vs. frequency of oscillation and (b) storage modulus vs. percentage strain, where 1–3 describe successive repeats of the strain sweep.

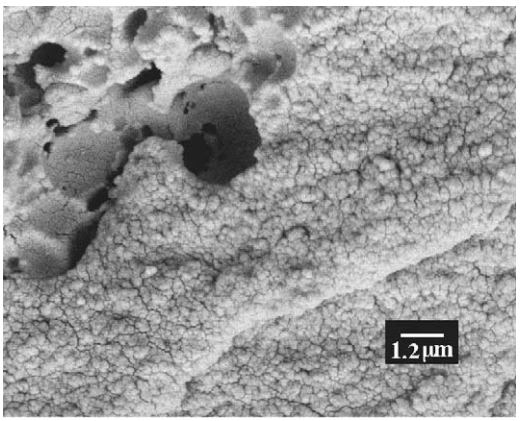

Fig. 5. SEM micrograph of the conjugate gel. The spherical voids represent regions, where the Ca-IFVs were entrapped during gelation.

liposomes used in this study [28] and the spherical features shown in Fig. 5 are consistent with this interpretation. The overall dense structure of the hydrogel matrix observed in the SEM micrograph is consistent with the insolubility of PEG in ethanol during critical point drying, the lack of a self-assembled structure such as that which arises in fibrin clots [23], and the high concentration of substrate that was used ($\sim$150 mg/ml). In contrast, SEM micrographs of commercial fibrin gels, which gel by physical association prior to enzymatic crosslinking, generally show a highly organized and more open pore network [23]. Given that the SEM experiments were performed on a dehydrated sample that exhibited significant reduction in volume from the hydrated state, we cannot draw direct conclusions about the morphology of the hydrogel from the SEM images. However, it is likely that the water-hydrated peptide-PEG hydrogel adopts a dense structure with regular incorporation of polypeptide throughout the gel. Thus, we expect that it would be proteolytically degraded from its outside surface via enzymatic erosion. Biodegradation and in vivo implantation studies are ongoing in our laboratories.

4. Summary

We have successfully coupled a 20-residue peptide from the γ-chain of fibrin to a four-armed branched PEG to create a novel polymeric bioconjugate. This bioconjugate was mixed with hrFXIII in the presence of thermally triggerable, calcium-loaded liposomes and thrombin to create a stable liquid precursor solution. Upon increasing the temperature of the solution from 20°C to 37°C, the solution formed a semi-solid hydrogel within 9 min, as indicated by both visual observations and oscillatory rheometry ($G' \sim 13$ kPa). Rheological experiments suggest that the gel behaves as an elastic solid, as indicated by the near-zero values for G'', the phase angle δ in the oscillatory experiment, and a lack of dependence of G' on frequency in the frequency sweep experiment. During the rheological strain sweep experiment, the gel was found to be robust and not to tear under applied oscillatory shear. Morphological studies using SEM show that the matrix of this peptide-PEG hydrogel is extremely dense, most likely due to the high concentration of substrate used ($\sim$150 mg/ml) and the lack of a self-assembled structure such as that observed in fibrin clots. SEM micrographs also show large, spherical voids within the hydrogel matrix left by the liposomes that were used for controlled enzyme activation.

These materials could be useful for drug and gene delivery and as tissue adhesives. For application as tissue adhesives, the design of the peptide-PEG bioconjugate should facilitate good adhesion to native tissues. In addition to coupling with each other to crosslink the hydrogel, the peptide-PEG bioconjugate can function as both amine donor and amine acceptor in transglutaminase-catalyzed reactions with extracellular matrix proteins of adjacent tissues. Such reactions will lead to covalent coupling and enhanced adhesion between the hydrogel matrix and adjacent tissue. The replacement of fibrinogen taken from pooled-blood sources with a synthetic, biomimetic substrate such as the one we have created could reduce material cost and eliminate the risk of disease transmission, while allowing for consistent formulations to yield a surgical adhesive or tissue scaffold with reproducible properties. The use of peptide substrates as hydrogel crosslinks will be useful for short-term in vivo applications, as protease degradation could efficiently remove the hydrogel from the body within a matter of a few weeks, obviating the need for surgical removal.

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