Available online at www.sciencedirect.com
SCIENCE DIRECT
Acta Biomaterialia 2 (2006) 241–251
Acta BIOMATERIALIA
www.actamat-journals.com

Physical matrices stabilized by enzymatically sensitive covalent crosslinks

Brandon L. Seal, Alyssa Panitch *

Harrington Department of Bioengineering, Arizona State University, P.O. Box 879709, Tempe, AZ 85287-9709, USA

Received 16 November 2005; received in revised form 14 December 2005; accepted 29 December 2005

Abstract

This work describes a unique system of gel and gel-like materials formed from physical bonds between heparin and heparin binding peptides (dG-PBD) coupled to multivalent poly(ethylene glycol) vinyl sulfone star polymers (PEGVS) and formed from covalent bonds between an enzymatically sensitive crosslinker and PEGVS. Dynamic mechanical testing revealed that the viscoelastic behavior and gelation kinetics of 10% (w/v) gels formed from 2:1 dG-PBD:PEGVS, heparin, and crosslinker (2:1 gels) and from 3:1 dG-PBD:PEGVS, heparin, and crosslinker (3:1 materials) were significantly influenced by the presence of both physical and covalent bonds. Furthermore, the mechanical properties of both compositions were thermally responsive and reversible. At 4 °C, the storage modulus, G', for 2:1 gels (5005.3 ± 592.0 Pa) and 3:1 materials (5512.0 ± 272.7 Pa) were statistically similar; however, at 45 °C, G' of 2:1 gels decreased to 477.9 ± 150.4 Pa, and the viscoelastic behavior of 3:1 materials was dominated by viscous behavior. In addition, the mechanical properties of 2:1 gels and 3:1 materials were sensitive to the frequency of the applied stress at 4 °C, 20 °C, and at 37 °C. Although the covalent bonds within the materials provided mechanical stability, the overall viscoelastic response of this system could be dominated by physical crosslinks under certain conditions. Results from degradation studies indicated that the crosslinker was sensitive to collagenase type I but not to thrombin or heparinase I, and a hemolysis assay suggested that the 2:1 gels and 3:1 materials might be biocompatible. These materials could be useful to study the role of physical interactions within networks that mimic the extracellular matrix.
© 2006 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Keywords: Biomimetic; Hydrogel; Heparin; Enzymatic degradation; Stimuli–responsive

1. Introduction

The self-assembly of biological polymers, such as DNA, proteins, and polysaccharides, into macromolecular structures involves physical associations based on molecular affinity [1–3]. In fact, an increasing body of literature is exploring how physical interactions between proteins and proteins as well as proteins and polysaccharides contribute to the mechanical integrity of various extracellular matrices [4–9]. However, interactions within the extracellular matrix are not limited to physical interactions. The strength of collagen and elastin fibers is enhanced, for example, by crosslinking activities of enzymes such as lysyl oxidase [10]. Also, the physical soft clot formed by polymerizing fibrinogen becomes stabilized by the crosslinking transglutaminase activity of factor XIIIa [11]. Clearly, biology incorporates controlled chemistries as well as diverse assembly mechanisms in order to create macromolecules with specific properties.

New biomaterial designs patterned on the interactions and properties of familiar biological polymers continue to emerge to mimic the complexity and variety of biological molecules. For example, recent work has described advances in stimuli–responsive materials, such as recombinant elastin proteins [12–14], poly(*N*-isopropyl acrylamide) derivatives [15–17], enzymatically crosslinked polymer gels [18–20], enzymatically degradable systems [21,22], cell adhesive polysaccharide matrices [23–26], and

* Corresponding author.
E-mail address: alyssa.panitch@asu.edu (A. Panitch).

1742-7061/$ - see front matter © 2006 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2005.12.008

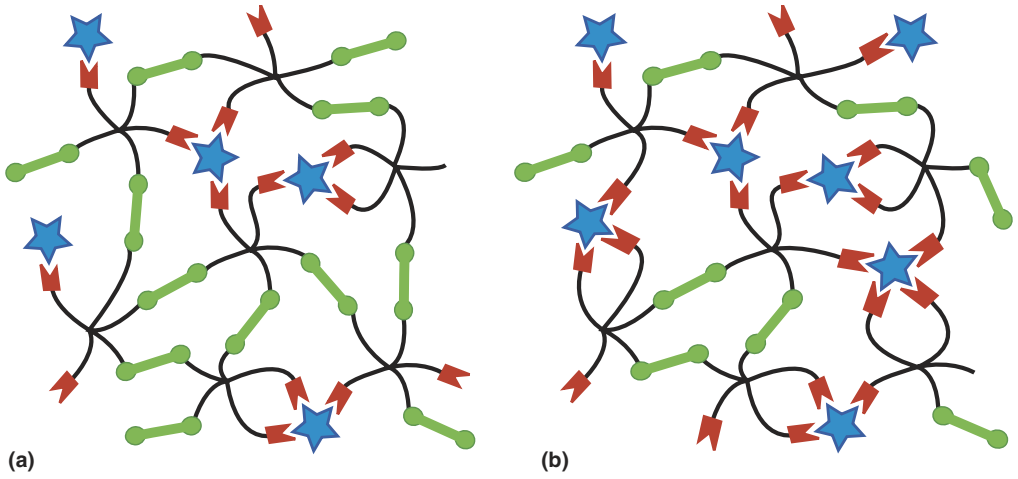

Fig. 1. A schematic of the described material systems. In each panel, the red pentagons represent dG-PBD (dansyl-GKAFAKLAARLYRKAGC), the blue stars represent heparin, the green dumbbells indicate crosslinker peptide (GCRGDSGPQGIAGQGC), and the black lines represent multiarm PEG vinyl sulfone. This schematic represents the structure of gels formed when 2:1 dG-PBD:PEG is combined with heparin and crosslinker (a) as well as the structure of gel-like materials formed when 3:1 dG-PBD:PEG is combined with heparin and crosslinker (b). (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

self-assembling artificial proteins [27,28]. Most of these materials have mechanical properties representative of either covalent or physical networks. However, a growing number of researchers are designing unique systems that incorporate both physical and covalent gel properties often to create materials sensitive to local pH, ionic strength, and/or temperature [13,14,17,29–33].

Recently, we have characterized a physical gel-like matrix composed entirely of interactions between heparin and heparin binding peptides [34]. Although this system had interesting viscoelastic properties at physiologically relevant pH and ionic strength, the material was fairly weak at physiologically relevant temperatures. In an attempt to improve the mechanical properties at higher temperatures, we have designed two variations of this system chemically linked through both physical and covalent bonds. As seen in Fig. 1, both variations are based on a hydrophilic and biocompatible multiarm poly(ethylene glycol) (PEG) backbone polymer, depend on the association of heparin and heparin binding peptides, derived from antithrombin III [35], to coordinate physical bonds, and contain enzymatically sensitive crosslinks introduced by a difunctional crosslinker peptide. Throughout this study, the difunctional crosslinker peptide contained the substrate GPQGIAGQ, which has been shown to be readily degraded by the matrix metalloproteinase collagenase I [36].

Fig. 1a represents a schematic of the variant consisting of, on average, three covalent crosslinks and two physical crosslinks per PEG molecule. It is hypothesized that this material will form a covalent network sensitive to collagenase I and that these covalent interactions will dominate viscoelastic behavior at elevated temperatures. Furthermore, the presence of physical crosslinks will contribute to the overall viscoelastic response of the materials at temperatures for which interactions between heparin and heparin binding peptides exist. The system variant shown in Fig. 1b depicts a material based primarily on physical crosslinks since each PEG molecule has three potential sites for physical crosslinks and only two sites for covalent bonds. It is not expected that the system will form a true covalent network. However, since the physical associations between heparin and heparin binding peptides have been shown to form immediately upon mixing, we hypothesize that the presence of physical crosslinks will initiate the rapid formation of a three-dimensional structure and that this matrix will be stabilized over time by the relatively slower chemical reaction between the crosslinker peptide and PEG.

2. Materials and methods

2.1. Materials

Eight-arm poly(ethylene glycol) (PEG) (avg. MW 20,000 g/mol) was purchased from Nektar Therapeutics (Huntsville, Al). All of the reagents used herein were purchased from commercial sources and used without further purification. For PEG modification reactions, analytical grade chemicals were obtained from Sigma–Aldrich (St. Louis, MO) and EM Science (Gibbstown, NJ). Synthesis grade amino acids and solid phase peptide synthesis chemicals were purchased from Advanced ChemTech (Louisville, KY), Mallinckrodt (Phillipsburg, NJ), Burdick and Jackson (Muskegon, MI), Alfa Aesar (Ward Hill, MA), EM Science, and Sigma–Aldrich. Heparin from porcine intestinal mucosa (avg. MW 18,000 g/mol) was obtained from Sigma–Aldrich, and Dulbecco's phosphate-buffered saline, pH 7.4 (PBS) was purchased from Invitrogen, Inc. (Carlsbad, CA). All of the water used throughout the study

ID	Title	Pages
2179	Physical matrices stabilized by enzymatically sensitive covalent crosslinks	11

Download Full-Text Now

http://fulltext.study/article/2179

fullText.study

Categorized Journals
Thousands of scientific journals broken down into different categories to simplify your search

Full-Text Access
The full-text version of all the articles are available for you to purchase at the lowest price

Free Downloadable Articles
In each journal some of the articles are available to download for free

Free PDF Preview
A preview of the first 2 pages of each article is available for you to download for free

http://FullText.Study