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In situ assembly of the collagen–polyacrylamide interpenetrating network hydrogel: Enabling decoupled control of stiffness and degree of swelling

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ABSTRACT

In the past several decades, hydrogels have been extensively studied for use in various biomedical and industrial applications. Successful use of hydrogels in these applications greatly relies on the ability to control mechanical and transport properties in a refined manner; however, current hydrogel design often encounters an undesirable inverse dependency between the degree of swelling and elastic modulus. To resolve this challenge, we hypothesized that an interpenetrating network (IPN) gel in which collagen fibers are chemically linked to a polymeric gel would allow us to control the elastic modulus over a broad range while reducing the change in the degree of swelling. We examined this hypothesis by preparing a collagen–polyacrylamide IPN gel via *in situ* assembly, so that two disparate networks are chemically linked via Michael reaction between collagen and polyacrylamide. The resulting IPN gel successfully demonstrated the independency between the degree of swelling and the elastic modulus. We suggest that the results of this study will be broadly useful in controlling hydrogel properties in an elaborate manner, in turn advancing various molecular and cell therapies.

1. Introduction

Hydrogels are formed from the chemical or physical cross-linking of polymers dissolved in aqueous media, and are increasingly used for various applications including sensing, actuation, controlled molecule delivery, and *in vitro* cell culture [1–6]. One of the important factors required for successful use of a hydrogel in these applications is the ability to control its mechanical stiffness over a broad range [7]. In particular, controlling gel stiffness helps maintain hydrogel structural integrity and regulates adhesion and phenotypic activities of adhered cells [8]. It is common to control stiffness by simply altering the number of cross-links in the gel; however, this approach often results in a significant change in gel pore diameter and permeability. Thus, increasing gel rigidity simultaneously decreases gel permeability and swelling ratio [9].

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To resolve this challenge, efforts were made to control mechanical properties of the gel with a minimal change in permeability. To this end, rigid nanofibers or nanoparticles were introduced into a hydrogel by mixing them with pre-gelled solution followed by activation of cross-linking reaction; however, few successful gels were reported, to our knowledge. Rather, these reinforcing components decreased the gel stiffness or ultimately inhibited gel formation [10]. These results were attributed to uncontrollable aggregation between nano-sized materials in a gel matrix as well as poor interfacial bonds with the gel-forming polymer [11].

In this study, we hypothesized that the *in situ* assembly of an interpenetrating polymeric network (IPN) gel consisting of polyacrylamide (PAAm) and collagen fibers would allow us to control gel stiffness over a broad range while reducing the change in gel permeability. Collagen fibers, a major extracellular component of various tissues in living organisms, present an elastic modulus and ultimate strength around 5 and 0.4 GPa, respectively [12,13]. In particular, the *in situ* assembly process would facilitate a Michael reaction between the amine groups of collagen and the vinyl groups of acrylamide. Thus, the resulting collagen fibers are chemically connected to the PAAm gel without interfering with the cross-linking reaction between PAAm. We examined this hypothesis by systematically studying the effects of the concentration of collagen and the molar ratio between cross-linker and polyacrylamide on elastic modulus, swelling ratio, and the number of cross-links of the gel composite. In addition, we evaluated whether the resulting collagen–hydrogel composite systems would regulate cellular adhesion behavior with their stiffness. We believe that the results of this study will greatly serve to improve the controllability of structure–property–functionality of various hydrogel systems.

2. Experiments

2.1. Materials

Acrylamide, *N*,*N*′-methylenebisacrylamide (bis-acrylamide), ammonium persulfate (APS), tetramethylethylenediamine (TEMED), sodium hydroxide (NaOH), formaldehyde solution, trinitrobenzenesulfonic acid (TNBS, 5% (w/w)), and 1 N hydrochloric acid (HCl) were purchased from Sigma–Aldrich. Bovine collagen solution (3 or 6 mg/mL) was purchased from Advanced Biomatrix.

Cell culture medium and its supplements, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Invitrogen. NIH 3T3 fibroblasts were purchased from American Type Culture Collection (ATCC). Phalloidin and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen.

2.2. In situ assembly and step fabrication of PAAm–collagen IPN gel

A 20% (w/w) acrylamide aqueous solution was prepared with different bis-acrylamide concentrations, including 0.03%, 0.1%, 0.3%, and 0.5% (w/w). The acrylamide/bis-acrylamide solution then was mixed with chilled collagen solution, either at 3 mg/mL or 6 mg/mL. The volumetric ratio between the two solutions was kept constant at 1:1. The final concentration of the acrylamide was kept constant at 10% (w/w) while the bis-acrylamide was varied from 0.015% to 0.25% (w/w). Then, 1% (v/v) of APS and 0.1% (v/v) of TEMED were added to the gel solution to activate cross-linking. These gels were known as the "IPN" gels or the "*in situ* fabrication" gels. For control experiments, collagen–free polyacrylamide gels were prepared with the same masses of acrylamide and bis-acrylamide. To form a gel, the pre-gelled solution was placed between two glass plates with a 1-mm thick spacer. Afterwards, hydrogel disks with 10 cm-diameter and 1 mm-thickness were punched out using a biopsy punch with a 10 cm-diameter.

Separately, a pure collagen gel was prepared by changing pH of the collagen stock solution to 7.0–7.4 by adding 1 M NaOH. Then, the mixture was incubated at 37 °C for 3 h to form a collagen gel. The pre-formed collagen gel was mixed with the acrylamide/bis-acrylamide solution by a vortex mixer. This gel was termed the "step fabrication" or "step" gel. The final concentration of the acrylamide was kept constant at 10% (w/w) while the bis-acrylamide was varied from 0.015% to 0.25% (w/w). Finally, the mixture was mixed with APS and TEMED to activate gel formation.

2.3. Measurements of elastic moduli and swelling ratios of hydrogels

Following incubation of hydrogel disks in DI water for at least 24 h at room temperature, the disks with 10 cm-diameter and 1 mm-thickness were uniaxially compressed at a rate of 1 mm/min using a mechanical testing system (MTS Systems Insight). The tester measured the force required to keep the deformation rate of the gel constant. Then, the force was automatically divided by cross-sectional area of the gel, in order to calculate the stress. In parallel, strain was calculated by dividing the magnitude of gel thickness change by the original thickness of the gel. Finally, elastic modulus was calculated from the slope of a stress versus strain curve at the first 10% strain. This parameter is termed "compressive elastic modulus" or simply "elastic modulus".

In parallel, the swelling ratio was characterized by measuring the mass of water in each gel. First, the mass of the hydrated gel was measured after incubation in DI water over 24 h at room temperature. Then, the gel was lyophilized to measure the dried solid mass. Finally, the degree of swelling (Q) was calculated from a mass ratio of the hydrated gel to the dried solid, Q_m, using Eq. (1):