European Polymer Journal 52 (2014) 137–145

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Macromolecular Nanotechnolgy

A novel method for hydrogel nanostructuring

Ortal Yom-Tov [a], Ilya Frisman [b], Dror Seliktar [c,d], Havazelet Bianco-Peled [b,d,*]

[a] Inter-Departmental Program for Biotechnology, Technion-Israel Institute of Technology, Haifa 32000, Israel
[b] Department of Chemical Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel
[c] Department of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel
[d] The Russell Berrie Nanotechnology Institute, Technion-Israel Institute of Technology, Haifa 32000, Israel

ARTICLE INFO

Article history:
Received 25 August 2013
Received in revised form 17 December 2013
Accepted 5 January 2014
Available online 11 January 2014

Keywords:
Hydrogel
Structure
SAXS
Nanostructuring

ABSTRACT

Nanostructured hydrogels tailor-made for specific applications are new grounds for the creation of novel soft materials. The current research presents a new method for hydrogel nanostructuring involving the incorporation of Pluronic® F127 micelles mixed with acrylated blockcopolymer molecules, which enable the attachment of these micelles to the hydrogel matrix through their endgroups. This design impacts the hydrogel nanostructure as well as its swelling and mechanical properties. Small Angle X-ray Scattering (SAXS) and Cryogenic Transmission Electron Microscopy (cryo-TEM) revealed that photochemical crosslinking of the hydrogel caused immobilization of the nanostructured micelles. Mechanical and weight gain experiments demonstrated a significant impact of these nanostructures on the hydrogel's elastic modulus as well as the transient and equilibrium weight gain ability of the material.

1. Introduction

Nanostructuring is becoming increasingly important in the design of precisely defined 3D structures which enable control over the material characteristics. In principle, by optimizing the nanostructure, materials tailor-made for a specific application can be created with structural definition to a sub-cellular level. The development of hydrogel nanostructuring methods are still challenging because the high water content excludes the use of lithographic techniques. One approach for nanostructuring hydrogels utilizes the self-assembly capability of block and graft copolymers driven by hydrophobic interactions between the blocks [1–3]. For example, nanostructured poly(ε-caprolactone)-poly(ethylene oxide)-poly(ε-caprolactone) (PCL-b-PEO-b-PCL) hydrogels were created by PCL removal after synthesis in order to create a nanoporous structure [4,5]. The incorporation of nanometric scaled structures with different morphologies into the hydrogels contributed to the control over the hydrogel nanostructure, mechanical and physical properties [6–8]. Poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogels incorporated with core cross-linked poly(ethylene glycol)-block-poly(ε-caprolactone) (PEG-*b*-PCL) micelles having spherical or rod-like morphologies were likewise prepared and evaluated for use as drug-eluting soft contact lenses [6]. Integration of micelles with crosslinked cores into pHEMA hydrogels led to the formation of different internal nanostructures which were dependent on the amount and morphology of the embedded micelles. Incorporation of cross-linkable micelles was found to reduce the degree of hydrogel weight gain. Combining hexagonal or lamellar lyotropic liquid crystals with poly(ethylene glycol) diacrylate (PEGDA) hydrogels was found to impact their physical properties including network swelling, mechanics, and degradation [7]. Nanostructuring of hydrogels during their formation has recently been reported by our group [8]. This approach utilizes the self-assembly ability of biocompatible, amphiphilic block-copolymers of poly(ethylene oxide)/poly(propylene oxide) (Pluronic®) in order to

* Corresponding author at: Department of Chemical Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel. Tel.: +972 4 8293588; fax: +972 4 8295672.
E-mail address: bianco@tx.technion.ac.il (H. Bianco-Peled).

0014-3057/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.eurpolymj.2014.01.004

impart nanostructured organization in the hydrogels during photopolymerization. Limited stability of these micelles was observed, mostly attributed to their diffusion out of the hydrogels after four days in aqueous buffer. In fact, the gradual escape of Pluronic® molecules from the hydrogel resulted in a significant change to the nanostructure of the polymer network.

The main objective of the current study was to design a novel method for nanostructuring of hydrogels. This method is based on embedding Pluronic® micelles in a hydrogel while anchoring some of their molecules to the surrounding network through their endgroups. Our hypothesis was that in this design, the anchored molecules could provide a means to further crosslink the hydrogel and stabilize the network. In contrast to traditional low molecular weight crosslinkers, the covalently bound molecule can stretch as the unbound Pluronic® diffuse out of the hydrogel and the micellar structures are lost. We postulated that this unique design will allow further manipulation of the gel properties. Herein we describe a comprehensive analysis of hydrogels prepared using this new nanostructuring methodology. Small angle X-ray scattering (SAXS) and transmission electron microscopy at cryogenic temperature (cryo-TEM) were used for structural characterization, whereas mechanical and weight gain experiments were used to explore the impact of nanostructure alterations on these properties.

2. Experimental

2.1. Synthesis of PEG diacrylate (PEG-DA) and F127 diacrylate (F127-DA)

F127-DA and PEG-DA were prepared from Pluronic® F127 (BASF™) and poly(ethylene glycol) (PEG, molecular weight 10 kDa), respectively, as described elsewhere [9]. Briefly, acrylation was carried out under Argon by reacting the polymers with Acryloyl-chloride (Merck, Darmstadt, Germany) and triethylamine (TEA) (Fluka) at a molar ratio of 150% relative to the hydroxyl groups. The resulting product was precipitated and dried under vacuum for 48 h.

Preparation of PEG-fibrinogen (PF) precursor solution is described elsewhere [8]. Briefly, bovine plasma fibrinogen (Sigma–Aldrich) was dissolved in phosphate buffer saline (PBS) containing 8 M Urea. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (Sigma–Aldrich) was added to completely dissolve the protein dissolution, the pH was adjusted to 8, and PEG-DA (10 kDa) in PBS solution was added while maintaining a ratio of 4:1 PEG-DA to protein cysteines. After the reaction was completed, the product was diluted, precipitated, redissolved, homogenized and dialyzed against 150 mM PBS at 4 °C for 24 h with two changes of PBS (Spectrum, 12–14-kDa MW cutoff, California, USA). Protein concentration was determined by Bis-Cinchoninic Acid (BCA) protein assay.

2.2. Nanostructured PF hydrogels

Mixtures of Pluronic® F127 and F127-DA at different ratios were prepared with PF precursor solution (protein concentration 8.5 ± 0.5 mg/ml) at 4 °C until complete dissolution of the block-coloymers was achieved. Total concentration of the block-copolymer (Pluronic® F127 plus F127-DA) was kept constant at 10% (w/v). The solution was mixed with 0.1% (v/v) photoinitiator stock solution containing 10% (w/v) Irgacure™2959 in 70% ethanol and 30% deionized water. The hydrogel precursor solution was heated to 37 °C for 10 min in order to induce micelle formation, followed by irradiation with UV light (365 nm, 4–5 mW/m^2) for 5 min in order to achieve a chemically crosslinked hydrogel.

Small Angle X-ray Scattering (SAXS) experiment were performed using a small-angle diffractometer (Molecular Metrology SAXS system) with Cu Kα radiation from a sealed microfocus tube (MicroMax-002+S), two Göbel mirrors, and three-pinhole slits (generator powered at 45 kV and 0.9 mA). The scattering patterns were recorded by a 20 × 20 cm two-dimensional position sensitive wire detector (gas filled proportional type of Gabriel design with 200 μm resolution) that was positioned 150 cm behind the sample. The resolution of the SAXS system was approximately 2–3 nm^{-1}. The scattered intensity $I(q)$ was recorded in the interval $0.07 < q < 2.7$ nm^{-1}, where q is the scattering vector defined as $q = (4\pi/\lambda)\sin(\theta)$, 2θ is the scattering angle, and λ is the radiation wavelength (0.1542 nm). The sample under study was sealed in a thin-walled glass capillary of about 2 mm diameter and 0.01 mm wall thickness, and measured under vacuum at constant temperature. The $I(q)$ was normalized to the following parameters: time, solid angle, primary beam intensity, capillary diameter, transmission, and the Thompson factor. Scattering from the solvent, empty capillary and electronic noise were subtracted. SAXS curves were measured at q vs. I, where I has the units of $(1/\text{nm}^3)$.

Cryogenic Transmission Electron Microscopy (cryo-TEM) micrographs were obtained from ultra-fast cooled vitrified cryo-TEM specimen prepared under controlled conditions 37 °C and 100% relative humidity as described elsewhere [10]. Specimens were examined in a Philips CM120 cryo-TEM operating at 120 kV, using an Oxford CT3500 cooling-holder system that kept the specimens at about −180 °C. Low electron-dose imaging was performed with a Gatan Multiscan 791 CCD camera, using the Gatan Digital Micrograph 3.1 software package.

Samples for *water weight gain experiments* were prepared by using round Teflon molds with diameter of 14 mm, whereby PF hydrogels with the addition of Pluronic® F127/F127-DA mixtures at different ratios were tested. The control groups included PF hydrogels with the addition of PEG-DA at different percentages. Each sample was made by transferring precursor solution (0.5 ml) into the Teflon mold, heating the solution to 37 °C for 10 min and then crosslinking the hydrogel with UV light for 5 min as described before. The hydrogels were subsequently submerged in 150 mM PBS containing 0.2% sodium azide in Petri dishes. The dishes were incubated at 37 °C and the water weight gain ratio was determined gravimetrically as follows:

$$\%\text{Weight gain} = \frac{(m_W - m_D)}{m_W} \times 100 \quad (1)$$