



Silk fibroin/poly(vinyl alcohol) photocrosslinked hydrogels for delivery of macromolecular drugs

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ABSTRACT

Hydrogels are three-dimensional polymer networks widely used in biomedical applications as drug delivery and tissue engineered scaffolds to effectively repair or replace damaged tissue. In this paper we demonstrate a newly synthesized cytocompatible and drug releasing photo-crosslinked hydrogel based on poly(vinyl alcohol) methacrylate and silk fibroin which possesses tailorable structural and biological properties. The initial silk fibroin content was 0%, 10%, 20%, 30%, 40% and 50% with respect to the weight of poly(vinyl alcohol) methacrylate. The prepared hydrogels were characterized with respect to morphology, crystallinity, stability, swelling, mass loss and cytotoxicity. FITC-dextrans of different molecular weights were chosen as model drugs molecules for release studies from the hydrogels. The hydrogels containing different silk fibroin percentages showed differences in pore size and distribution. X-ray diffraction analysis revealed that amorphous silk fibroin in poly(vinyl alcohol) methacrylate is crystallized to β -sheet secondary structure upon gelation. The sol fraction increased with increasing fibroin concentration in the co-polymer gel (from 18% to 45%), although the hydrogel extracts were non-cytotoxic. Similarly, the addition of silk fibroin increased water uptake by the gels (from 7% to 21%). FITC-dextran release from the hydrogels was dependent on the silk fibroin content and the molecular weight of encapsulated molecules. The study outlines a newer type of photo-crosslinked interpenetrating polymer network hydrogel that possess immense potential in drug delivery applications.

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1. Introduction

Hydrogels are insoluble three-dimensional (3-D) networks of crosslinked hydrophilic homopolymers, co-polymers or macromers with a high degree of water uptake in aqueous environments [1]. Hydrogels are widely used in biomedical applications, such as drug delivery vehicles [2], encapsulation materials for immunoisolation-based cell therapeutics, wound dressings and tissue engineered scaffolds [3]. Polymers can be prepared and combined in the form of blends, co-polymers, and interpenetrating polymer networks (IPN). IPN are unique combinations of crosslinked polymers in which at least one component is synthesized and/or crosslinked in the presence of the other [4]. IPN are often created to deliver key attributes of one of the components while maintaining the critical properties of the other polymer. When two polymers form a co-polymer network but only one of them is crosslinked and the other is simply mixed into the network a semi-IPN is formed [5]. IPN formation has been shown by numerous researchers to improve the performance of crosslinked hydrogels. Semi-IPN are usually synthesised by polymerizing a monomer or prepolymer

around existing polymer chains. Alternatively they can be developed by diffusing polymer chains within a preformed polymer network [6].

The chains comprising semi-IPN network hydrogels may be based on natural, synthetic or hybrid combinations of these materials. Hydrogel formation can be achieved when polymer chains interact either physically or chemically into networks [1]. The physical structure and characteristics of hydrogels depend upon the starting monomers and macromers, synthesis and fabrication methods, degradation, etc. Synthetically derived hydrogels have the advantage of controlling the specific gel properties and are tailorable through the use of specific molecular weight, block structure and cross-linking densities [7–9]. Furthermore, hydrogels synthesized from natural macromers generally induce fewer immunogenic reactions as they are produced from basic molecules already used by the body. Biological molecules are incorporated into synthetic hydrogel networks to improve cell attachment and, hence, encapsulation [10,11].

Poly(vinyl alcohol) (PVA) is particularly advantageous, as it offers the possibility of attaching cell signalling molecules or drugs via the numerous hydroxyl groups present on the backbone [12]. PVA can be modified into multifunctional, multivinyl macromers through the plethora of pendant hydroxyl groups, which can be

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substituted by a variety of substituents [8,9,13,14]. Photo-cross-linked hydrogels are gaining importance in biomedical applications because aqueous macromer solutions containing cells and/or bioactive factors can be delivered in a minimally invasive manner and crosslinked under physiological conditions upon exposure to ultraviolet light [15,16]. The cells and bioactive molecules are exposed to limited adverse conditions upon controlling the intensity of the UV exposure and with appropriate selection of the photoinitiator [17].

Biological hydrogels have been developed from agarose, alginate, chitosan, hyaluronan, fibroin and collagen, as well as many other materials [18]. Silk fibroin (SF), a hydrophobic protein obtained from *Bombyx mori* (silkworm), is an abundantly available and reasonable cost resource of natural protein polymer [19]. SF, unlike other natural polymers, has been extensively studied for several biomedical applications due to its extraordinary mechanical strength and toughness, the ease with which it can be chemically modified, its thermal stability, high oxygen permeability, slow in vivo degradability and sterilisability by autoclaving and the ability to control the structure and function [19–21]. SF shows a smaller inflammatory response compared with other polymers such as collagen and poly(L-lactic acid) [19,21]. SF also possesses good processability and thus has been evaluated in various forms, such as fibres, sponges, mats, gels, scaffolds, tubes, etc. [21]. Several methods, like shearing, sonication (which mimics natural spinning), removal of bulk water by osmotic stress, heat treatments and exposure to solvents, are utilized for the preparation of SF gels [22,23]. Regenerated SF can either be blended or chemically cross-linked with other natural or synthetic polymers to form hydrogels with improved properties. Various research groups are working to understand the mechanism involved in the gelation of SF and it has been postulated that the transition to β -sheet structure is one of the main reasons for gelation. SF can self-assemble to form strong and resilient bonds. The dominance of β -sheet forming regimes within the SF structure gives these protein-based materials high mechanical strength and toughness [19,20]. SF composite gels have been prepared with PVA, gelatin, collagen, poloxamer-407, modified polyethylene glycol (PEG), N-isopropylacrylamide (NIPAAm), polyacrylamide, etc. [24–33].

In this paper we demonstrate the fabrication of a newer type of drug releasing photo-crosslinked hydrogel based on a semi-interpenetrating network of poly(vinyl alcohol) methacrylate and SF, which possess tailorable structural and release properties. We also examined the morphology, crystallinity, stability, swelling properties, mass loss and cytotoxicity of the fabricated hydrogel. FITC-dextrans of different molecular weights were chosen as model drugs to evaluate the release behaviour of macromolecules from these hydrogels.

2. Materials and methods

2.1. Materials

Bombyx mori silkworm cocoons were obtained from Local Sericulture Farm Debra, West Midnapore, India. Poly(vinyl alcohol) (PVA) (13–23 kDa, 98% hydrolysed), 2-isocyanatoethyl methacrylate (2-ICEMA) (Aldrich), 2,6-di-tert-butyl-4-methylphenol, deuterium oxide (Sigma–Aldrich), 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (the photoinitiator I-2959, Ciba Speciality Chemicals Ltd.), sodium bicarbonate, lithium bromide (Himedia Chemical Laboratories, India), thiazolyl blue (MTT), cellulose dialysis membrane (12 kDa exclusion limit, Sigma), tissue culture grade polystyrene-coated plastic flasks and plates (Tarsons, India), FITC-dextran (FD4, M_w 4 kDa; FD20, M_w 20 kDa; FD40, M_w 40 kDa) were obtained from Sigma (St Louis, MO). Cell culture grade chemicals like DMEM-F12 medium, foetal calf serum, penicillin and streptomycin (Gibco BRL, Grand Island, NY) and 0.22 μ m millex syringe filters (Millipore, Billerica, MA) were used in the experiments as received.

2.2. Synthesis of the PVA macromer

PVA was reacted with 2-ICEMA to provide polymerizable pendant groups using a method adapted from Bryant et al. [12]. 10 g of PVA was dissolved in 90 ml of dimethyl sulphoxide (DMSO) at 80 °C. As the solution was cooled to 60 °C one small grain of 2,6-di-tert-butyl-4-methylphenol was added with stirring. The solution was purged with nitrogen gas for 30 min to remove traces of moisture within the vessel. 2-ICEMA was added dropwise to the PVA solution in a stoichiometric amount (reaction scheme shown in Fig. 1). The reaction was maintained at 60 °C in a N_2 atmosphere. After 5 h purging with nitrogen gas was stopped and the solution was precipitated with an excess of toluene. The precipitate was air dried and then redissolved in deionized water and filtered through a 0.22 μ m syringe filter. The filtered PVA methacrylate is freeze dried to obtain the final product.

2.3. Macromer characterization

The PVA methacrylate macromers were prepared by the reaction of polymer hydroxyl groups with methacrylate moieties. The success of methacrylate substitution was verified by the presence of the vinyl resonances at 5.6 and 6.0 p.p.m. Per cent methacrylation of PVA was calculated by comparing the area of the methacrylate vinyl proton peaks with the area of the protons in the PVA backbone (δ_H 3.7–4.1 and 1.4–1.8 p.p.m.). The number of

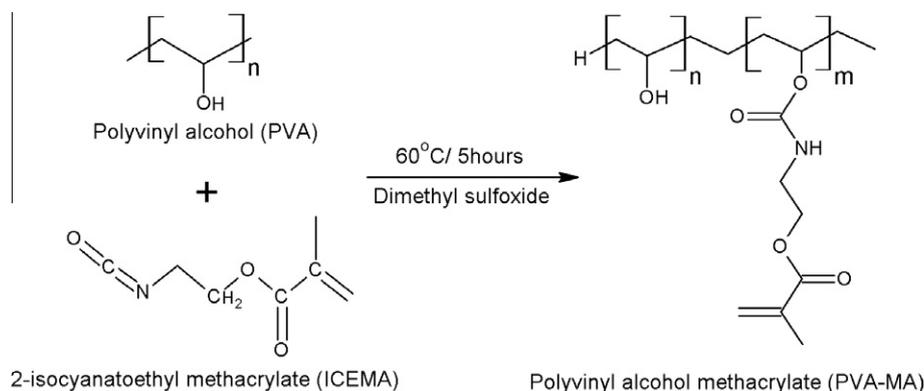


Fig. 1. Schematic representation of the reaction conditions for the synthesis of poly(vinyl alcohol)–2-isocyanatoethylmethacrylate macromer (PVA-ICEMA macromer) in a DMSO solvent system.

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