Journal of Controlled Release 208 (2015) 76–84

Contents lists available at ScienceDirect

Journal of Controlled Release

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

A functionalized, injectable hydrogel for localized drug delivery with tunable thermosensitivity: Synthesis and characterization of physical and toxicological properties

Paul Z. Elias [a], Gary W. Liu [a], Hua Wei [a,1], Michael C. Jensen [c], Philip J. Horner [b,*], Suzie H. Pun [a,*]

[a] Department of Bioengineering and Molecular Engineering and Sciences Institute, University of Washington, Seattle, WA 98195, United States
[b] Department of Neurological Surgery, University of Washington, Seattle, WA 98195, United States
[c] Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, WA 98101, United States

ARTICLE INFO

Article history:
Received 19 November 2014
Received in revised form 20 February 2015
Accepted 1 March 2015
Available online 4 March 2015

Keywords:
Injectable hydrogel
Peptide
Drug delivery
Cell therapy
Immunotherapy
Spinal cord injury

ABSTRACT

Thermosensitive injectable hydrogels have been used for the delivery of pharmacological and cellular therapies in a variety of soft tissue applications. A promising class of synthetic, injectable hydrogels based upon oligo(ethylene glycol) methacrylate (OEGMA) monomers has been previously reported, but these polymers lack reactive groups for covalent attachment of therapeutic molecules. In this work, thermosensitive, amine-reactive and amine-functionalized polymers were developed by incorporation of methacrylic acid N-hydroxysuccinimide ester or 2-aminoethyl methacrylate into OEGMA-based polymers. A model therapeutic peptide, bivalirudin, was conjugated to the amine-reactive hydrogel to investigate effects on the polymer thermosensitivity and gelation properties. The ability to tune the thermosensitivity of the polymer in order to compensate for peptide hydrophilicity and maintain gelation capability below physiological temperature was demonstrated. Cell encapsulation studies using an H9 T-cell line (CD4+) were conducted to evaluate feasibility of the hydrogel as a carrier for cellular therapies. Although this class of polymers is generally considered to be non-toxic, it was found that concentrations required for gelation were incompatible with cell survival. Investigation into the cause of cytotoxicity revealed that a hydrolysis byproduct, diethylene glycol monomethyl ether, is likely a contributing factor. While modifications to structure or composition will be required to enable viable cell encapsulation, the functionalized injectable hydrogel has the potential for controlled delivery of a wide range of drugs.

1. Introduction

Intraparenchymal, organ specific drug delivery is a challenge that could be met through development of engineered biomaterials. Thermosensitive injectable hydrogels are a promising class of biomaterials that are typically characterized by a lower critical solution temperature (LCST) above which they undergo a sol–gel transition. This property allows for injection in a liquid state and subsequent gelation after equilibration to physiological temperature in vivo [1,2]. Injectable hydrogels have utility in a variety of soft tissue applications where minimally invasive implantation and low stiffness can minimize damage to delicate anatomical structures.

Injuries and diseases of the central nervous system (CNS) are particularly difficult to access and challenging to treat, making them logical targets for advanced drug and cellular therapies delivered via hydrogel. One potential application of drug-loaded hydrogels is for treatment of spinal cord injury (SCI). Thermosensitive hydrogels are particularly attractive for spinal cord delivery since the blood–spinal cord barrier (BSCB) restricts the passage of most systemically-administered drugs into the spinal cord [3–5]. Local delivery of a therapeutic hydrogel formulation within spinal cord tissue therefore bypasses the BSCB and creates a local drug depot that increases delivery efficiency and organ specificity, while reducing drug exposure to potentially degradative enzymes in the circulation [4]. This is especially advantageous for delivery of peptide and protein therapeutics, which typically have short half lives in circulation and may be too large or hydrophilic to cross the BSCB. Further, peptides are amenable for covalent attachment to biomaterials since various functionalities for conjugation may be present or incorporated through particular amino acids. Therapeutic peptides have been identified that target various aspects of CNS pathophysiology following injury to the spinal cord, providing a range of potential drugs and associated targets [6]. Conjugation of one such peptide, bivalirudin, is demonstrated in this work. Bivalirudin is a 20 amino acid peptide which acts to directly inhibit thrombin and has been shown to provide beneficial effects in mitigating glial scarring after spinal cord injury [7].

* Corresponding authors.
E-mail addresses: phorner@uw.edu (P.J. Horner), spun@uw.edu (S.H. Pun).
[1] Current affiliation: Department of Chemistry, Lanzhou University, Lanzhou 730000, China.

http://dx.doi.org/10.1016/j.jconrel.2015.03.003
0168-3659/

Drug and cell-loaded hydrogels are also useful in oncology immunotherapies. In particular, injectable hydrogels could potentially be used following brain tumor resection to fill the resulting cavity and deliver drugs and/or anti-cancer T lymphocytes for targeting residual tumor cells [8]. For example, immunomodulatory peptides or proteins may be included in the hydrogel to enhance T-cell therapies [9]. However, cells must be able to survive and migrate within the hydrogel. This work therefore aims to develop an injectable hydrogel system potentially suitable for both controlled drug-delivery and cellular encapsulation.

While a number of natural and synthetic thermosensitive materials have been used for drug encapsulation [10], control of drug release in vivo remains a major challenge. Passive drug loading in hydrogels often results in rapid release due to the inability of many materials to retain lower molecular weight molecules [11]. While drug release kinetics can be delayed by further encapsulation within various microparticle systems, the resulting formulations are more complicated to synthesize and may not fully address burst release [12–14]. Covalent attachment of drugs offers greater control over long-term in vivo delivery, as release typically depends upon enzymatic or hydrolytic cleavage of the chemical bond between the drug and biomaterial [15]. Burst release can therefore be reduced [16,17], while appropriate temporal and spatial release profiles can be designed through environmentally responsive drug linkers [18].

Among the more commonly-used thermosensitive hydrogels are those based on poly(ethylene glycol) (PEG) [19,20], poly(N-isopropylacrylamide) (PNIPAM) [21], and hyaluronic acid blended with methylcellulose (HAMC) [22]. For cell encapsulation, materials such as chitosan [23] and collagen [24] have been used in addition to PEG [20], PNIPAM [21], and HAMC [25]. A chief requirement for these hydrogels is their biocompatibility for the particular cell type to be cultured and delivered in vivo. While modification of hydrogels with cell adhesion peptides or growth factors can affect cell attachment [26], proliferation [26], and differentiation [25], it is possible that incorporation of certain molecules could significantly alter the polymer LCST, thereby shifting a polymer's LCST outside of the temperature range useful for physiological applications [1,27].

Thus, there remains a need for tunable thermosensitive materials that can be readily conjugated to biologic drugs, while also serving as vehicles for delivery of cellular therapies. The Lutz group has pioneered the development of thermosensitive hydrogels synthesized by atom transfer radical polymerization (ATRP) of oligo(ethylene glycol) methyl ether methacrylate ($OEGMA_{475}$) and di(ethylene glycol) methyl ether methacrylate (MEO_2MA) from the arms of a 4-arm PEG ATRP macroinitiator [28,29]. These materials have the advantage of a tunable LCST that depends on the ratio of the two thermosensitive monomers. As a result, the monomer proportions can theoretically be adjusted to maintain an LCST near physiological temperature even in the presence of hydrophobic or hydrophilic drugs. Additionally, linear polymers composed mostly of the same monomers have been shown to be non-toxic at concentrations up to 10 mg/mL [30]. Despite these attractive characteristics, controllable drug conjugation is not possible due to the lack of functional groups in these materials.

The goal of this work was therefore (i) to synthesize injectable hydrogels incorporating functional monomers for facile drug conjugation and (ii) to characterize the physical and toxicological properties of the resulting biomaterials. Polymers containing MEO_2MA, $OEGMA_{475}$, and amine-reactive methacrylic acid N-hydroxysuccinimide ester (NHSMA) or amine-containing 2-aminoethyl methacrylate (AEMA) monomers were synthesized. Moreover, activators regenerated by electron transfer (ARGET) ATRP [31,32] was used rather than traditional ATRP because this approach requires less cytotoxic copper catalyst and is insensitive to small amounts of oxygen, allowing for reaction monitoring by NMR. A model peptide, bivalirudin, was then conjugated to the NHSMA-containing polymer; this material was used to demonstrate that polymer thermosensitivity can be iteratively tuned to compensate for changes in hydrophilicity due to conjugation of a peptide. Finally, a study of cellular biocompatibility for potential cell encapsulation applications revealed significant cytotoxicity to cultured mammalian fibroblasts and T-cells. The mechanism of this toxicity was tested, with data suggesting high polymer concentration and release of toxic degradation products as contributing factors, thus precluding the use of these materials for cell encapsulation and delivery. The results suggested, however, that the polymers may retain potential utility as localized drug delivery depots.

2. Experimental section

2.1. Materials and methods

Materials were purchased from Sigma-Aldrich unless otherwise noted.

2.1.1. Macroinitiator synthesis

A 4-arm PEG macroinitiator similar to that reported previously was synthesized [28]. In a typical reaction, 1 g (0.1 mmol) of 4-arm amine-terminated PEG, (MW 10,000, Laysan Bio) was dissolved in 10 mL anhydrous dichloromethane (DCM). The solution was placed under argon and cooled in an ice water bath over a stir plate. 223 μL of triethylamine (1.6 mmol) was subsequently added, and following a 10 minute wait, 202 μL (1.6 mmol) of α-bromoisobutyryl bromide was added. The reaction was stirred for 30 min before removing the vessel from the ice water and allowing it to reach room temperature. The solution was stirred overnight at room temperature. A separatory funnel with 4 mL of aqueous sodium chloride solution (~3.6 g/mL) was used to purify the reaction mixture. The organic phase was collected and dried for several hours with anhydrous magnesium sulfate. The product was then precipitated in ether and dried under vacuum and characterized by NMR. Yields were typically 0.5–0.7 g.

2.1.2. Synthesis of sPEG-b-P(MEO_2MA-co-$OEGMA_{475}$-co-NHSMA) by ARGET ATRP

In a typical reaction, 175.6 mg (0.018 mmol) of 4-arm PEG macroinitiator was added to a 10 mL round bottom flask and dissolved in 5 mL anhydrous anisole. 183.4 μL (0.417 mmol) of $OEGMA_{475}$, 1.944 mL (10.01 mmol) of MEO_2MA, and 97.4 mg (0.521 mmol) of NHSMA (TCI America) were subsequently added. The desired amounts of $CuBr_2$ (0.180 μmol, 0.04 mg) and tris(2-pyridylmethyl)amine (TPMA) (0.9 μmol, 0.267 mg) were so small as to make weighing difficult, so a stock suspension was prepared. 1 mg $CuBr_2$ and 6.6 mg TPMA were added to 1 mL anisole in a 1.5 mL microcentrifuge tube. The suspension was sonicated, vortexed, and vigorously pipetted to evenly disperse the insoluble $CuBr_2$. During rapid pipetting, 40 μL (~0.04 mg $CuBr_2$, 0.267 mg TPMA) was taken from the suspension and added to the 10 mL flask containing the monomers and initiator. A stir bar was added to the flask before it was sealed with a rubber septum and purged with argon for approximately 5 min. 3.1 μL (0.009 mmol) of $Sn(EH)_2$ was dissolved in 1 mL anisole and added to the purged reaction mixture via syringe. The flask was added to an oil bath preheated to 40 °C and the reaction was stirred for 2–3 days. Samples were periodically withdrawn via syringe and analyzed by NMR to determine polymer conversion. When conversion approached 50–60%, the reaction was stopped by removing it from the oil bath and removing the septum. The polymer was precipitated in approximately 140 mL diethyl ether, redissolved in acetone, and transferred into dialysis tubing (Spectropore, #132660, MW cutoff 6–8 kDa). The polymer was dialyzed against acetone for 1–2 days before being concentrated with a rotary evaporator.