Journal of Controlled Release 282 (2018) 156–165

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Accelerated wound healing by injectable star poly(ethylene glycol)-*b*-poly (propylene sulfide) scaffolds loaded with poorly water-soluble drugs

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ARTICLE INFO

Keywords:
Delivery scaffold
Hydrophobic drugs
Tissue repair

ABSTRACT

Injectable hydrogel matrices take the shape of a wound cavity and serve as scaffold for tissue repair and regeneration. Yet these materials are generally hydrophilic, limiting the incorporation of poorly water soluble, hydrophobic drugs. Here we show this shortcoming is circumvented through a *star*-shaped amphiphilic block copolymer comprising poly(ethylene glycol) and poly (propylene sulfide). This *star*-shaped amphiphilic polymer self-assembles in an aqueous medium into a physically stable hydrogel and effectively dissolves hydrophobic molecules delivering them at therapeutic doses. The self assembled hydrogel is a robust three-dimensional scaffold in vivo effectively promoting cellular infiltration, reducing inflammation, and wound clsoure. When combined with a hydrophobic BRAF inhibitor that promotes paradoxical mitogen-activated protein kinase (MAPK) activation in keratinocytes and wound closure, our self assembled scaffold supported dermal wound closure at a reduced drug dosage compared to administering the drug in dimethyl sulfoxide (DMSO) without a polymeric matrix. This family of *star*-shaped amphiphilic polymers delivers poorly water soluble active agents at a fraction of generally required dosage for efficacy and supports three-dimensional cell growth at tissue wounds, showing great promise for novel uses of hydrophobic drugs in tissue repair applications.

1. Introduction

The development of injectable scaffolds to promote tissue repair and regeneration has been primarily driven by the need to match the physical and biochemical parameters of a desired anatomical location, while using a minimally invasive implantation procedure. These scaffolds are generally composed of hydrophilic polymers and are optimized to have bulk properties matching those at the intended target site, such as material stiffness and bioactive signals. However, these approaches are limited by their hydrophilicity narrowing the types of active agents that can be incorporated to hydrophilic macromolecules such as peptides and growth factors.

Many small-molecule drugs or drug candidates are hydrophobic compounds, targeting signaling pathways or directly interfering with protein-protein interactions [1]. The use of small-molecule compounds as modulators of tissue repair and regeneration is currently an underutilized resource. A complication of using hydrophobic drugs is that the functional groups imparting specificity in targeting drug-protein interactions [2] are often nonpolar and hydrophobic, thus disfavored in aqueous solubility. For example, Zhang et al. utilized poly(ethylene oxide)-poly(propylene oxide) based hydrogel to entrap microcrystals of drug agents to assess tissue regeneration in adult mice [3]. However, insufficiently solubilized drug candidates may undergo crystalization and cause acute toxicity [4]. To circumvent this problem, previous

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https://doi.org/10.1016/j.jconrel.2018.05.006
Received 9 January 2018; Received in revised form 3 May 2018; Accepted 7 May 2018
Available online 09 May 2018
0168-3659/

studies have developed carriers such as micelles [5–7], micro − /nanospheres [8], emulsion gels/creams [9,10] and film [11]/patches. In the former two strategies, amphiphlic polymers permits the dissolution of hydrophobic drugs in an injectable vehicle for tissue surface distribution of therapeutics [6,12], but the form of particulates lacks a macroscopic structure to support cell infiltration and tissue remodeling in a tissue cavity. Emulsion gels from ABA block copolymers of PLGA-PEG-PLGA result in thermal gelation polmers that solidify at body temperature and can be used to solublize hydrophobes. However, the resulting gels are not suitable as scaffolding materials since they contain a high amount of surfactants [13]. Other, reverse thermal gelation block *co*-polymers such as poloxamers or their equivalents incorporate hydrophobes through micellar structure in aqueous media, but linear structures and short-range molecular interactions often require excessive polymer concentrations (e.g., 25 w/v% or higher) to increase stability in vivo, but the incorporated drugs easily elute off in a matter of hours [14]. Alternatively, hydrophobic scaffolds support extended drug release via simple diffusion and polymer degradation [15]. A non-covalently associated polymer-nanoparticle hydrogel supported an improved two-stage drug release profile, but it requires separate preparation of nanoparticles besides gel formation and selective adsorption of certain biopolymers [16]. Other scaffolds such as poly(ether urethane) and cyclodextrin provide for oxidation responsiveness [17,18], but the abilities to solubilize hydrophobes and maintain structural integrity are unclear.

Here we demonstrate an amphiphilic star-shaped block copolymer that self-assembles into a stable hydrogel in vivo for tissue regrowth and solubilizes hydrophobic agents for therapeutic dosing at an reduced amount. A one-step approach combining polymer network assembly and solubilization of hydrophobes allows the formation of injectable hydrogel amenable to mechanical manipulation (e.g., injectability) and alterations in response to biochemical changes (e.g., redox responsiveness). Previously, a linear ABA block copolymer of poly(ethylene glycol)-poly(propylene sulfide)- poly(ethylene glycol) (PEG_{16}-*b*-PPS_{50}-*b*-PEG_{16}) formed oxidation-responsive micelles for drug delivery [19]. Branched, four-arm block copolymer of poly(ethylene glycol-poly-propylene sulfide) (*star*-PEG_{113}-PPS_5) was found to support cell cultivation in vitro and serve as a vehicle for stem cell transplantation to the stroke cavity in vivo [20]. Here, *star*-PEG_{113}-PPS_x, coupled with an integrin-binding, cell adhesive peptide based on an amino acid sequence of RGD, provides a self-assembling injectable hydrogel capable of incorporating hydrophobic agents. In particular, a hydrophobic BRAF inhibitor that promotes paradoxical mitogen-activated protein kinase (MAPK) activation in keratinocytes and wound closure [21] was delivered. Our self assembled scaffold supported dermal wound closure at a reduced drug dosage compared to administering the drug in dimethyl sulfoxide (DMSO) without a polymeric matrix.

2. Experimental section

2.1. Synthesis of PEG-PPSx

The synthesis of PEG-PPS follows a three-step reaction, primarily described in previous publication with slight modifications [22]. Briefly in the first step, 10 g four-arm poly(ethylene glycol) (PEG) (MW 20,000, 2 mmol arms; A starting material of 20,000 Da, four-arm PEG was used, each arm having approximately 113 repeating units of ethylene glycol)) was dissolved in 120 mL dried tetrahydrofuran (THF) (pretreated with activated molecular sieves for overnight) and refluxed under argon gas at 90 °C for 4 h. After the flask was cooled down, 0.6 g sodium hydride (8× excess over arms = 16 mmol) was slowly added to the dissolved PEG and stirred for 15 min under argon. Subsequently 1.6 mL allyl bromide (10× excess over arms = 20 mmol) was injected into the mixture and the reaction was stirred under argon for overnight. To purify the reaction product of PEG-allyl ether, the reaction mixture was filtered under vacuum and the filtrate was dried to remove excess solvent. The viscous sample was redissolved in a small amount of dichloromethane and precipitated out in 200 mL ice-cold ethyl ether for two times. The precipitant was collected and dried under vacuum for overnight and subsequently stored in argon at − 20 °C. NMR was used to characterize the final sample for modification [23]. ^{1}H NMR (400 MHz, $CDCl_3$): 3.39–3.89 (broad, PEG chain protons), 5.85–5.98 (m, 1H, $–CH_2OCH_2C\mathbf{H}=CH_2$), 5.15–5.30 (m, 2H, $–CH_2OCH_2CH=C\mathbf{H}_2$).

Second, PEG-allyl ether (3.78 g, 0.73 mmol arms) was dissolved in 130 mL anhydrous toluene with stirring and warming below 45 °C in a schlenk tube. The solution subsequently underwent freeze-pump-thaw degassing cycles until no bubbles were seen in the thawing step. The radical initiator 2,2′-Azobis(2-methylpropionitrile) (AIBN) (1.5 g, 9 mmol) was freshly activated via recrystallization in methanol. Recrystalized AIBN and 2 mL thioacetic acid (26 mmol) dissolved in 20 mL anhydrous toluene were added to PEG-allyl ether solution in five aliquots over one day. The reaction was carried out at 80 °C for 72 h in argon with aliquots of AIBN/thioacetic acid added at an interval of 2–3 h. The reaction product of PEG-thioacetate was dried and precipitated in ice-cold ethyl ether. NMR was used to characterize the final sample for modification. ^{1}H NMR (400 MHz, $CDCl_3$): 1.81–1.9 (q, 2H, $-OCH_2C\mathbf{H}_2CH_2S$-), 2.35 (s, 3H, $-SCOC\mathbf{H}_3$), 2.92–2.97 (t, 2H, $-OCH_2CH_2C\mathbf{H}_2S$-), 3.39–3.89 (broad, PEG chain protons).

Third, PEG-thiolacetate (0.78 g, 0.153 mmol arms) was dissolved in freshly distilled THF. Sodium methoxide (83 mg, 10× excess over arms = 1.53 mmol) was added to PEG-thiolacetate/THF under argon and stirred for 30 min at room temperature. Subsequently specific amounts of propylene sulfide (2.5×, 5× and 16× molar equiv. of PEG arms) was added under argon and the reaction mixture was stirred for one hour. The end-capping reagent 2,2′-dithioldipyridine (168 mg, 5× excess over arms = 0.77 mmol) was later added and the reaction mixture was stirred under argon for overnight. The sample of PEG-PPSx was later dried via rotary evaporator and dialyzed extensively against water. Lastly, the sample was lyophilized and stored under argon at − 20 °C. NMR was used to characterize the final sample for modification. ^{1}H NMR (in $CDCl_3$): 1.35–1.45 (d, CH3 in PPS chain), 1.81–1.9 (broad q, 2H, $-OC\mathbf{H}_2CH_2CH_2S$), 3.6–3.7 (broad PEG chain protons).

2.2. Rheometry

PEG-PPS hydrogels were allowed to self-assemble overnight before transferred to an 8 mm plate-to-plate rheometer (Physica MCR 301, Anton Paar, Ashland, VA). An evaporation blocker system was used during measurements. For frequency sweep, the data were collected for the modulus with a frequency range of 0.1–100 rad/s under a 1% constrain at 37 °C. For amplitude sweep, the data were collected for the modulus with a frequency of 20 rad/s under a constrain range of 0.1–100% at 37 °C.

2.3. Water content measurements

Hydrated self-assembled PEG-PPS hydrogels were weighed for the wet mass (W_{wet}). Subsequently they were stored in a vacuum oven for two days until the mass did not change. The dried polymers were weighed (W_{dry}). Water content (%) was calculated as $(W_{wet} - W_{dry})/W_{wet} * 100\%$.

2.4. Oxidation of PEG-PPS

The reduction modification on PEG-PPS was performed in two steps. The first step was to use Ellman's assay (following manufacturer's instructions) to determine all the available thiol groups on a certain percentage of aqueous solution of PEG-PPS. The second step was to use different amounts of TCEP to treat PEG-PPS, resulting in various degrees of disulfide-crosslinked PEG-PPS (confirmed by Ellman's assay).