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Functionalized, biodegradable hydrogels for control over sustained and localized siRNA delivery to incorporated and surrounding cells

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ABSTRACT

Currently, the most severe limitation to applying RNA interference technology is delivery, including localizing the molecules to a specific site of interest to target a specific cell population and sustaining the presentation of these molecules for a controlled period of time. In this study, we engineered a functionalized, biodegradable system created by covalent incorporation of cationic linear polyethyleneimine (LPEI) into photocrosslinked dextran (DEX) hydrogels through a biodegradable ester linkage. The key innovation of this system is that control over the sustained release of short interference RNA (siRNA) was achieved, as LPEI could electrostatically interact with siRNA to maintain siRNA within the hydrogels and degradation of the covalent ester linkages between the LPEI and the hydrogels led to tunable release of LPEI/siRNA complexes over time. The covalent conjugation of LPEI did not affect the swelling or degradation properties of the hydrogels, and the addition of siRNA and LPEI had minimal effect on their mechanical properties. These hydrogels exhibited low cytotoxicity against human embryonic kidney 293 cells (HEK293). The release profiles could be tailored by varying DEX (8 and 12% w/w) and LPEI (0, 5, 10 μg/100 μl gel) concentrations with nearly 100% cumulative release achieved at day 9 (8% w/w gel) and day 17 (12% w/w gel). The released siRNA exhibited high bioactivity with cells surrounding and inside the hydrogels over an extended time period. This controllable and sustained siRNA delivery hydrogel system that permits tailored siRNA release profiles may be valuable to guide cell fate for regenerative medicine and other therapeutic applications such as cancer treatment.

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1. Introduction

RNA interference is an efficient method to post-transcriptionally turn off the expression of specific proteins and transcription factors using small interfering RNA (siRNA) for cancer therapeutics or tissue engineering applications [1–8]. Naked siRNA bears negative charges which limit its ability to passively diffuse across cell membranes and it is easily degraded by ribonucleases [1,2]. To overcome these limitations, a number of siRNA delivery systems, including liposomes, lipoplexes, nanoparticles and microparticles, have been developed to deliver siRNA to treat a wide range of diseases [1]. Unfortunately, these systems can be easily dispersed in vivo on account of their small size, making it difficult to locally target sites of interest for a prolonged period of time [1,2]. Localized and sustained delivery is a promising strategy for siRNA delivery in vivo, which may enhance its clinical applicability. For example, localized delivery would permit targeted siRNA exposure to non-malignant tumors or sites of tumor resection, which may lower the dose required for efficacy and potentially reduce effects on non-target cells [7]. siRNA delivery at a specific location in the body may also permit regulation of transplanted or host cell gene expression to aid in the regeneration of damaged or diseased tissues [8]. In addition, sustained delivery of siRNA may provide a silencing effect over an extended period of time. There are currently no injectable hydrogel systems that provide temporal control over the local delivery of siRNA to incorporated and surrounding cells.

To address the issues of rapid dispersion and transient delivery of siRNA from the aforementioned nano- or microparticle systems, we previously demonstrated sustained and localized siRNA delivery from macroscale, biodegradable polymer hydrogels [2]. Hydrogels, highly hydrated three-dimensional (3-D) networks of crosslinked hydrophilic polymer chains, have been widely explored for use as bioactive agent delivery vehicles and tissue engineering scaffolds [9–12]. Injectable hydrogels are valuable for these applications because polymer solutions can be easily mixed with bioactive agents and/or cells, administered via a minimally invasive method at desired sites and triggered to form hydrogels in situ [9]. Our previous work using photocrosslinked alginate, calcium crosslinked alginate and collagen hydrogels demonstrated that sustained and localized siRNA release could be achieved, and that the

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release profiles were material dependent [2]. However, siRNA release from those hydrogels could not be easily controlled [2].

The goal of this work was to engineer the first hydrogel system that would permit controlled, localized and sustained delivery of siRNA to cells incorporated within and surrounding the biomaterial. To achieve controlled siRNA release profiles, we designed photocrosslinked dextran (DEX) hydrogels covalently functionalized with cationic LPEI molecules via a biodegradable ester linkage. It was hypothesized that siRNA could be retained within the hydrogels via electrostatic interactions between the negatively charged siRNA and positively charged LPEI, and degradation of the ester linkages would permit tunable, controlled release of siRNA/LPEI complexes. The release profiles could be tailored by regulating the degree of these interactions and controlling the degradation rate of hydrogels. For this purpose, the hydrogels were photocrosslinked from solutions of DEX methacrylate containing various concentrations of methacrylated linear polyethyleneimine (LPEI). We tested whether LPEI modification affects the hydrogel physical properties, such as swelling, degradation profiles and mechanical properties, and the viability of human embryonic kidney 293 cells (HEK293) cultured near and within the hydrogels. Hydrogels containing varying DEX, LPEI and siRNA concentrations were examined to determine the role of these parameters on siRNA release profiles. Bioactivity of released siRNA and its ability to transfect cells inside the hydrogels were also investigated to demonstrate the utility of this system with tunable delivery profiles.

2. Experimental

2.1. Materials

DEX from Leuconostoc mesenteroides (average molecular weight of 40,000 g mol^{-1}), 4-(dimethylamino)pyridine (DMAP), glycidyl methacrylate (GMA, 97% pure), 2-hydroxyethyl methacrylate (HEMA), 1,1′-carbonyldiimidazole (CDI), dimethyl sulfoxide (DMSO), chloroform, deuterium oxide (D_2O) and Irgacure D-2959 were purchased from Sigma Aldrich (St Louis, MO, USA). Linear polyethyleneimine (LPEI, 25,000 g mol^{-1}) was purchased from Polysciences Inc. (Warrington, PA). 2-hydroxylethyl methacrylate imidazolylcarbamate (HEMA-IC) was synthesized as previously reported [13]. CellTiter 96 Aqueous One Solution, which contains 3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxy-phenyl]-2-[4-sulfophenyl]-2H-tetrazolium (MTS-tetrazolium), was purchased from Promega Corp. (Madison, WI). Dulbecco's modified Eagle medium with 4.5 g l^{-1} glucose (DMEM-HG) and fetal bovine serum (FBS) were purchased from Hyclone (Logan, UT). Accell eGFP Control siRNA, Accell green cyclophilin B control and Accell Delivery Media (ADM serum-free) were obtained from Thermo Scientific Dharmacon (Lafayette, CO). Accell siRNA can enter cells without the use of a transfection reagent. HEK293 cells stably transfected with destabilized GFP (deGFP) were a generous gift from Piruz Nahreini, PhD (University of Colorado Health Sciences Center). Falcon Transwell inserts were obtained from Becton Dickinson (Franklin Lakes, NJ). Nuclease-free water was purchased from Ambion (Austin, TX). Dialysis membrane (MWCO 3500) was obtained from Spectrum Laboratories Inc. (Rancho Dominguez, CA).

2.2. Synthesis of DEX methacrylate and LPEI methacrylate macromers

DEX methacrylate (DEX-HEMA) was synthesized via the reaction of HEMA-IC to the hydroxyl groups of the DEX main chain as previously described [13]. Briefly, to synthesize DEX with 7% theoretical methacrylation, DEX (10 g) and DMAP (2 g) were dissolved in DMSO (90 ml) in a dry 250 ml round bottom flask. After complete dissolution, HEMA-IC (0.93 g) was added. The reaction occured for 4 days at room temperature, followed by dialysis (MWCO 3500) against ultrapure deionized water (diH_2O) for 3 days and lyophilization. White DEX-HEMA power (9 g) was obtained after lyophilization. LPEI methacrylate (LPEI-GMA) modified at a theoretical degree of 5% was synthesized via the ring opening reaction of epoxy groups of GMA with amine groups of LPEI. LPEI (0.5 g) and GMA (105 μl) were dissolved in chloroform (30 ml) in a 250 ml round-bottom flask for 1 h in a 60 °C silicon oil bath. The chloroform was then completely evaporated under vacuum and the mixture was reconstituted in ultrapure diH_2O (30 ml) at pH 6.0. The LPEI-GMA was purified by dialysis against ultrapure diH_2O at pH 6.0 (MWCO 3500) for 3 days, filtered using a 0.22 μm filter and lyophilized. The final yield was 0.32 g. DEX-HEMA and LPEI-GMA were characterized by proton nuclear magnetic resonance (^{1}H-NMR) in D_2O using a Varian Unity-300 (300 MHz) NMR spectrometer (Varian Inc., Palo Alto, CA). Peaks a and e in Fig. 1b and peak b and the peaks from 2-ethyl-2-oxazoline in LPEI in Fig. 1c were used to determine the actual degree of modification of DEX-HEMA and LPEI-GMA, respectively.

2.3. Photocrosslinking

DEX-HEMA (8 or 12% w/w) was dissolved in phosphate buffered saline (PBS) with 0.05% w/v photoinitiator (Irgacure D-2959) and different LPEI-GMA concentrations (0, 5 and 10 μg/100 μl gel). The polymer solutions (100 μl) were placed into the wells of a 96-well plate and hydrogels were formed by photocrosslinking with 320–500 nm UV light at 3.5 mW cm^{-2} for 85 s using an Omnicure S1000 UV Spot Cure System (Lumen Dynamics Group, Mississauga, Ontario, Canada).

2.4. Swelling and in vitro degradation

To determine the swelling profiles of these photocrosslinked DEX hydrogels, their dry and wet weights were determined at various time points over 17 days. The prepared photocrosslinked DEX hydrogels were lyophilized and their initial dry weights (W_d initial) were measured. Each dried hydrogel sample was immersed in 5 ml PBS at pH 7.4 and incubated at 37 °C. The PBS was changed every three days. At predetermined time points, samples were removed and rinsed with diH_2O, and the weights of the swollen hydrogel samples (W_s) were measured. The swelling ratio (Q) was calculated by $Q = W_s/W_d$ initial.

To determine the degradation profiles, each dried hydrogel sample was immersed in 5 ml of PBS and incubated at 37 °C. The PBS was replaced every three days. At predetermined time points, samples were removed, rinsed with diH_2O and lyophilized. The dry weights of the samples at different time points (W_d) were measured. The percentage mass loss was calculated by $(W_i - W_d)/W_i \times 100$, where W_i is the dry weight of the hydrogel samples at the initial time point. The samples ($N = 3$ for each time point) were prepared and tested at the same time.

2.5. Mechanical properties

Rheological properties of photocrosslinked DEX hydrogels (12% w/w) with various LPEI concentrations with or without encapsulated siRNA (26.6 μg/100 μl hydrogel solution) were measured using a strain-controlled AR-2000ex rheometer (TA Instruments, New Castle, DE) with stainless steel parallel plate geometry (plate diameter of 8 mm, gap of 0.75 mm). Hydrogels were made by pipetting solutions between two glass plates separated by two 0.75 mm spacers and photocrosslinked as previously described. Photocrosslinked hydrogel disks were punched out with an 8 mm biopsy punch to match the diameter of the parallel plates. G′ and G″ of each hydrogel were measured by performing a dynamic