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Light-responsive nanoparticle depot to control release of a small molecule angiogenesis inhibitor in the posterior segment of the eye

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ABSTRACT

Therapies for macular degeneration and diabetic retinopathy require intravitreal injections every 4–8 weeks. Injections are uncomfortable, time-consuming, and carry risks of infection and retinal damage. However, drug delivery via noninvasive methods to the posterior segment of the eye has been a major challenge due to the eye's unique anatomy and physiology. Here we present a novel nanoparticle depot platform for on-demand drug delivery using a far ultraviolet (UV) light-degradable polymer, which allows noninvasively triggered drug release using brief, low-power light exposure. Nanoparticles stably retain encapsulated molecules in the vitreous, and can release cargo in response to UV exposure up to 30 weeks post-injection. Light-triggered release of nintedanib (BIBF 1120), a small molecule angiogenesis inhibitor, 10 weeks post-injection suppresses choroidal neovascularization (CNV) in rats. Light-sensitive nanoparticles are biocompatible and cause no adverse effects on the eye as assessed by electroretinograms (ERG), corneal and retinal tomography, and histology.

1. Introduction

Neovascular age-related macular degeneration (AMD) is one of the most common eye disorders that impair vision and is the leading cause of blindness in the elderly [1]. The majority of treatments for AMD require monthly or bimonthly intravitreal injection of anti-angiogenic drugs, such as bevacizumab (Avastin), ranibizumab (Lucentis), and more recently, aflibercept (VEGF-Trap/Eylea) [2]. Though the risk of adverse effects, such as cataracts or retinal detachment, with each injection is rare, it increases with the number of intravitreal injections [3]. Thus, strategies that reduce the frequency of injections while maintaining the therapeutic efficacy of these drugs are highly sought after. An ideal solution would also preserve ophthalmologist control over dosages to allow adjustment for each patient's response, which would maximize the efficacy of each injection.

While several systems have been developed to extend the lifetime of anti-angiogenics following intravitreal injection, including biodegradable implants [4,5], liposomes [6,7], and micro- [8,9] and nanoparticles [10,11], none allow ophthalmologist control over the timing of release. One of the most commonly used materials for this purpose is poly(lactic-*co*-glycolic acid) (PLGA), which can be tuned to release at different rates by varying its composition and molecular weight. This reliance on a very simple and widely-used material does not take full advantage of recently developed technologies in the drug delivery field, which could allow on-demand [12,13] or disease-triggered [14,15] intravitreal release of AMD drugs. Here we propose a nanoparticulate drug delivery depot formulated from a light-degradable polymer for on-demand light-triggered release of drugs post-implantation. This polymer, containing an *o*-nitrobenzyl moiety in each monomer, responds to absorption of UV by degrading into fragments and small molecules through quinone-methide rearrangements [16]. These nanoparticles rapidly release encapsulated small molecules upon exposure to 365 nm light.

To assess the efficacy of this system in vivo, we delivered nintedanib (BIBF 1120), a small molecule inhibitor of the receptors for VEGF, PDGF, and FGF [17], and demonstrate that UV light-triggered release attenuates

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laser-induced choroidal neovascularization (CNV) in rats. To our knowledge, this is the first report of in vivo light-triggered release in the eye.

2. Methods

2.1. Nanoparticle formulation

Light-sensitive polymer was synthesized as previously published (MW 7.6 kDa, PDI 1.3 by gel permeation chromatography) (Fig. S1) [16]. 10 mg polymer was dissolved in 270 μL dichloromethane, and 30 μL dimethyl sulfoxide (DMSO) containing 2 mg payload (fluorescein diacetate (FDA), calcein AM, BIBF1120) was added. The resulting solution was added to 6 mL of sterile-filtered 1% polyvinyl alcohol (PVA) in water, and probe sonicated for 4 min at 9–10 W (S-4000, Misonix Sonicators). Organic solvents were removed by evaporation under light vacuum conditions while stirring at 600 rpm for 3 h. Remaining PVA was removed by concentrated mode tangential flow filtration (Pellicon XL, 500 kDa, Millipore) with 250 mL cell-culture grade water (HyClone) at 45 rpm. The retentate was then freeze-dried with 100 mg trehalose as cryoprotectant. The size and distribution of particles were confirmed by dynamic light scattering (DLS, Zeta Nanosizer, Malvern Instruments), and scanning electron microscopy (SEM, FE-SEM 8500, Agilent). Loading and encapsulation efficiency were measured using UV–vis spectroscopy (UV-3600, Shimadzu) and fluorescence spectrophotometry (Jobin Yvon FL-1000, Horiba).

2.2. In vitro release studies

Raw 264.7 mouse macrophages or retinal progenitor cells were seeded at 20,000 cells/well on a 96-well plate 12 h before the experiment. Cells were washed twice with 100 μL warm Dulbecco's phosphate buffered saline (DPBS) and incubated with 100 μL of 2 mg/mL suspension of FDA-containing nanoparticles in media or media alone as controls for 3 h at 37 °C under 5% CO_2. Cells were again washed twice with 100 μL warm DPBS and replenished with fresh media, and free FDA was added to wells without particles as a positive control. Half of the particle-containing wells were irradiated with 8 mW/cm^2 of 365 nm UV light for 5 min (OmniCure S2000, Lumen Dynamics) to induce particle degradation. Green fluorescence from wells was measured using a plate reader (SpectraMax M5, Molecular Devices), and images were collected using a fluorescence microscope (TS100F, Nikon).

2.3. UV calibration using lens explants

Adult Sprague–Dawley rats were euthanized by CO_2 asphyxiation followed by exsanguination, and their eyes were enucleated and stored at 4 °C in Belzer UW cold preservation media (Bridge to Life), supplemented with 100 μg/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen), and 200 μg/mL D-glutamine (Invitrogen) following published protocols [26,27]. Lenses were then carefully dissected from the globes and incubated in 4 mL Media 199 (Invitrogen) supplemented with 1% w/v of penicillin (100 μg/mL) and streptomycin (100 μg/mL) pre-incubated in 5% CO_2 atmosphere at 37 °C for 24 h. Non-cloudy lenses were either exposed to 10 min of UV light (365 nm, 12 mW/cm^2) or left unirradiated. The cloudiness was assessed visually and quantified through histograms in Adobe Photoshop CS2.

2.4. In vivo release of fluorescent dye

Sprague–Dawley rats (male, 4–8 weeks old) were anesthetized using 100 mg/kg ketamine and 10 mg/kg xylazine administered intraperitoneally. Both eyes were injected with 3 μL of a 200 mg/mL suspension of dry nanoparticle powder in DPBS by first piercing the inferotemporal quadrant with a 31 G insulin needle (BD Products), then inserting a 33 G syringe (Hamilton) through the puncture directly into the vitreous cavity. Both eyes were lubricated using a lubricant eye gel (GenTeal Severe, Novartis). The irradiation protocol to induce drug release from light-sensitive nanoparticles was as follows: one eye was dilated by corneal application of 0.5% proparacaine hydrochloride (ophthalmic solution, Bausch & Lomb), followed by a drop of 0.5% tropicamide (ophthalmic solution, Bausch & Lomb). After 5 min, the rat was anesthetized with ketamine/xylazine intraperitoneally, set on one side, and covered with 2 layers of nitrile gloves to protect the body from UV light. The dilated eye was irradiated for 5 min with 365 nm UV light at 8 mW/cm^2 (OmniCure S2000, Lumen Dynamics). After 45 min, rats were euthanized, and eyes were enucleated and fixed in 4% paraformaldehyde for 45 min. Retinas were then extracted, flat-mounted and imaged under a fluorescence microscope (Biorevo BZ-9000, Keyence).

2.5. In vitro cytotoxicity assay

Ultraviolet-sensitive polymer (UVSP) (5 mg) was dissolved in sterile DMSO (10 μL), and the solution was added to clear Dulbecco's Modified Eagle's Medium (DMEM) (990 μL). The resulting suspension was sonicated until uniform and further diluted to appropriate concentrations in DMEM/fetal bovine serum (FBS). Lyophilized particles containing FDA (5 mg) were resuspended in sterile media (1 mL) and half of the volume was irradiated for 5 min with UV light (10 mW/cm^2, λ_{ex} = 365 nm, OmniCure S2000 Curing System). Solutions were then diluted to appropriate concentrations in cell culture media. Raw 264.7 cells, seeded 24 h prior to incubation on a tissue culture treated 96-well plate (Corning) at 20,000 cells/well in DMEM, were washed twice with PBS at 37 °C, incubated with polymer/particle suspensions in triplicate for 24 h at 37 °C in 5% CO_2, then washed twice again with PBS. Mitochondrial activity was then measured according to MTT assay kit instructions (Sigma-Aldrich). Triton-X (1% w/v, Sigma-Aldrich) was used as a positive apoptosis control. Absorbance at 570 nm normalized to background absorbance at 690 nm was measured using a plate reader (SpectraMax M5, Molecular Devices).

2.6. In vivo biocompatibility of materials

Sprague–Dawley rats (4–8 weeks, male) were anesthetized with ketamine/xylazine and injected intravitreously with 3 μL of a 200 mg/mL suspension of empty UVSP or PLGA nanoparticles in DPBS or 3 μL DPBS. Uninjected rats served as controls. Intraocular pressure (IOP) was measured in non-anesthetized animals at the same time of day using a veterinary tonometer (TONOVET, Icare) at 1, 5, and 7 days post-injection. Electroretinograms (ERGs) were also performed at 1 and 8 days post-injection following a previously reported protocol [28]. Briefly, rats were dark-adapted for 12 h, anesthetized, and given pupil-dilating solutions as described in the irradiation protocol above. Rats were examined within a Ganzfeld bowl (Diagnosys LLC), and electrodes were placed on each cornea, with a subcutaneously placed ground needle electrode in the tail. For scotopic ERG, the retina was stimulated with a xenon lamp at 0.01 and 0.3 cd·s/m^2. For photopic ERG, rats were adapted to a background light of 10 cd·s/m^2, and light stimulation was set at 30 cd·s/m^2. Recordings were processed in Matlab and Excel.

2.7. mRNA extraction and reverse transcription

Sprague–Dawley rats (4–8 weeks, male) injected with UVSP, PLGA nanoparticles or DPBS, as well as controls (no injection) were euthanized 7 days post-injection, and their eyes were enucleated and retinas extracted. Tissue was homogenized by trituration in lysis buffer (QIAGEN), followed by centrifugation through QIAshredder homogenization columns (QIAGEN). mRNA was then extracted using an RNEasy Kit (QIAGEN). RNA concentration was determined by spectrophotometric optical density ratio ($OD_{260\ nm}/OD_{280\ nm}$, NanoDrop 2000, Thermo Scientific). Reverse transcription was carried out using Superscript III