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Preparation and evaluation of PLGA nanoparticle-loaded biodegradable light-responsive injectable implants as a promising platform for intravitreal drug delivery

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ABSTRACT

The present study reports on the development of a hybrid system by integrating poly(lactic-*co*-glycolic) acid nanoparticles (PLGA NPs) into light-responsive *in-situ* forming injectable implants (ISFIs) for minimally invasive and safe intravitreal peptide delivery. In the first part of the study, peptide-loaded PLGA based nanoparticles (NPs) were developed using the two-step nanoprecipitation technique. Peptide compatibility with PLGA and polyvinyl alcohol was confirmed via IR spectroscopy. Developed NPs had a size of 149.3–235.4 nm, a polydispersity index between 0.24 and 0.46, a zeta potential of −32.4 to −27.0 mV and an entrapment efficiency of 34.3–55.3%. In the second part of the study, successful synthesis of methacrylated alginate (MA) and its subsequent photocrosslinking upon photoirridiation was confirmed by ^{1}H NMR. Photocrosslinked MA exhibited the required clarity and its gelling time, morphology, syringeability, hardness, rate of swelling and degradation were found suitable for prolonging its residence in the posterior segment. The sulforhodamine B assay on human retinal pigment epithelium cells and the zebrafish embryo toxicity test confirmed the biocompatibility of NPs and ISFIs. Finally, NPs were incorporated into the ISFIs for improved particle retention and to further sustain drug release, suggesting the proposed hybrid system as an innovative and efficient ocular drug delivery platform.

1. Introduction

Effective drug delivery to the posterior segment of the eye is still challenging due to the complex anatomy and physiology of the eye. Ocular barriers (corneal, blood-aqueous and blood-retinal barriers) and elimination mechanisms (tear turnover, nasolacrimal drainage and protein binding) complicate the uptake and further penetration of drug molecules following topical application [1]. Frequent

Abbreviation: AMD, age-related macular degeneration; ARPE-19, human retinal pigment epithelium; ATCC, American Type Culture Collection; AEMA, aminoethyl methacrylate; Cx43MP, connexin43 mimetic peptide; DR, diabetic retinopathy; DSC, differential scanning calorimetry; DMEM, Dulbecco's Modified Eagle's Medium; EMA, European Medicine Agency; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride; FDA, Food and Drug Administration; IVT, intravitreal injection; ISFIs, *in-situ* forming injectable implants; Irgacure-2959, 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone; MA, methacrylated alginate; MES, 2-morpholinoethanesulfonic acid; NHS, N-hydroxysuccinimide; NPs, nanoparticles; PVA, polyvinyl alcohol; PLGA, poly(lactic-*co*-glycolic)acid; PBS, phosphate buffered saline; RMSD, root-mean-square deviation; SA, sodium alginate; SRB, sulphorhodamine B; SEM, scanning electron microscopy; TEM, transmission electron microscopy; UV, ultraviolet; VEGF, vascular endothelial growth factor; ZET, zebrafish embryo toxicity.

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intravitreal (IVT) injections are currently used to overcome the majority of the ocular barriers and provide sufficient drug to the posterior segment of the eye. However, these repetitive injections can be painful and might carry a risk of infection, endophthalmitis, increased intraocular pressure, retinal detachment and cataract formation [2].

To date, only a few site-specific nonbiodegrable ocular implants are commercially available to overcome the need for frequent IVT injections. However, their implantation as well as removal after the release of the payload necessitate surgical procedures [3]. Besides the injection of anti-vascular endothelial growth factor (anti-VEGF) containing solutions, a light-responsive lipid-based formulation, Visudyne® (Verteporfin) is also currently used for the treatment of advanced age-related macular degeneration (AMD). However, this formulation requires frequent systemic administration and is associated with photosensitivity, thus patients have restricted exposure to light and are instructed to wear protective clothing and eye glasses during the therapy [4]. Therefore, strategies that reduce the invasive nature of frequent IVT injections while maintaining safety and therapeutic efficacy of the delivered drug at the site of action are highly sought after.

Poly(lactic-*co*-glycolic)acid (PLGA) has been approved by the US Food and Drug Administration (FDA) for human application and various preclinical studies have shown promising results using PLGA NPs for delivering drug effectively to the posterior eye segment [5–7]. In addition, peptide drugs encapsulated into PLGA NPs may be protected from possible intravitreal degradation and their half-life may be prolonged by slowing down the elimination from the vitreous body [8]. However, PLGA NPs also have major limitations such as the high initial burst release of the drug from the polymer matrix. In addition, after IVT administration, the NPs move freely in the vitreous body and get rapidly eliminated from the posterior eye segment [9]. This results in a decrease of the therapeutic concentration at the target site and thus frequent IVT injections are needed. To overcome these limitations, NPs could be incorporated into a light responsive *in-situ* gelling system where physicochemical properties of the hybrid system may further reduce the limitation associated with each individual system and provide a synergistic effect [8].

Light-responsive *in-situ* forming injectable implants (ISFIs) are basically free flowing liquids that undergo sol-gel transition once in contact with light to form a gel or semi-solid depot [10]. Their structural similarities to body tissues, such as high water content, soft nature, flexibility and porous structure make them an ideal biomaterial for wide applications including drug delivery [11,12]. Moreover, the transparent nature of cornea and lens render light-responsive systems ideal for ocular applications where light of a certain wavelength can easily reach the posterior segment in a non-invasive manner [13]. The advantage of light-responsive systems over other stimuli-responsive systems receptive to a change in pH, temperature and ions is their rapid sol-gel transformation resulting in better mechanical properties with a reduced initial drug burst [14]. Nevertheless, despite this rapid sol-gel transformation, there is still some drug burst which could be further reduced by incorporating drug-loaded NPs into the light-responsive ISFIs.

Within the light of these facts, we propose a combined system where peptide-loaded PLGA NPs are incorporated into light-responsive ISFIs. We hypothesize that this system will prevent the rapid elimination of peptide-loaded NPs from the posterior eye segment and increase their residence time in the vitreous body. In addition, the incorporation of NPs into the ISFIs will reduce the possible high initial burst release of the drug from NPs and ISFIs alone, and thus allow more control over the drug release. Connexin43 mimetic peptide (Cx43MP) was used as a model peptide drug as it has shown significant therapeutic effects in the treatment of various retinal disorders [15,16]. The main focus of this study was to develop and investigate the physicochemical properties of the NPs, the light-responsive ISFIs and the combined system to ensure their acceptability as a promising platform for minimally invasive and safe drug delivery to the posterior eye segment. In addition, the biocompatibility of these systems with ocular tissues was also evaluated ensure safe clinical application. Overall, the biodegradable nature of the combined system would avoid the need for surgical implantation and removal after the release of the loaded drug.

2. Materials and methods

2.1. Materials

Connexin43 mimetic peptide (Cx43MP; MW 1396 g/mol, purity >98% by HPLC) was purchased from China Peptides Co. Ltd. (China). Fluorescein isothiocyanate labelled Connexin43 mimetic peptide (FITC-Cx43MP, MW 1856 g/mol; purity >40% by HPLC) was purchased from Auspep (Australia). Poly (lactic-*co*-glycolic acid) (PLGA; 50:50, MW 30,000–60,000), polyvinyl alcohol (PVA; MW 85,000–124,000 g/mol, >99% hydrolyzed), Sodium alginate (SA; low viscosity, MW 12,000–40,000 g/mol), 2-morpholinoethanesulfonic acid (MES), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), aminoethyl methacrylate (AEMA), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure-2959), formic acid (purity >98%, LC-MS grade), phosphate buffered saline (PBS), sulphorhodamine B (SRB) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma-Aldrich (New Zealand). Human retinal pigment epithelial (ARPE-19) cells were sourced from American Type Culture Collection (ATCC; USA). 4′,6-diamidino-2-phenylindole (DAPI) and glycine were purchased from Invitrogen (USA). Paraformaldehyde was purchased from Ajax Finechem (New Zealand). Zebrafish embryos were provided by the Zebrafish Facility, University of Auckland, and used according to institutional animal ethics approval. Fresh bovine eyes were procured from the local abattoir (Auckland Meat Processor Ltd., New Zealand). Ultrapure water was prepared via a Milli-Q system (Millipore, USA). All other reagents and solvents were of analytical grade.

2.2. Development and characterization of Cx43MP-loaded NPs

2.2.1. Fabrication of peptide-loaded NPs

NPs were prepared using the two-step nanoprecipitation technique with some modification [17]. Cx43MP (10 mg/ml) was solvent precipitated from 1 ml of aqueous solution by adding it dropwise to acetone at a 1:3 volume ratio with 5 min of sonication followed by continuous stirring for 20–30 min to form the peptide suspension. Separately, PLGA (20, 30 and 50 mg/ml) was completely dissolved in acetone and the resulting PLGA solution was added dropwise to the Cx43MP suspension with 5 min of sonication followed by continuous stirring for 15–20 min. The resulting mixture was then added directly into a 5% w/v PVA solution under continuous stirring. PLGA NPs formed were immediately centrifuged for 30 min at 13,000 rpm and the pellet re-suspended in distilled water. This washing step was repeated thrice before freeze-drying the NPs at a condenser temperature of −45 °C for 48 h. Three nanoformulations (NP1-NP3) using different polymer compositions (20–50 mg/ml) were prepared (Table 1).

2.2.2. Drug-excipient compatibility studies

FTIR spectra of the physical mixtures (1:1) containing Cx43MP, PLGA or PVA were recorded in the range of 4000–600 cm^{-1} using a