Preparation and *In Vitro*/*In Vivo* Evaluation of Cyclosporin A-Loaded Nanodecorated Ocular Implants for Subconjunctival Application

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ABSTRACT: In terms of ocular drug delivery, biodegradable implant systems have several advantages including the ability to provide constant drug concentration at the target site, no necessity for surgical removal, and minimum systemic side effects. Cyclosporin A (CsA) is a neutral, hydrophobic, cyclic peptide of amino acids that frequently used for dry eye disease treatment. The aim of this study was to develop a nanoparticle-loaded implant system for sustained-release CsA delivery following subconjunctival implantation. Poly(lactide-co-glycolide) (85:15) or poly-ε-caprolactone (PCL) were used to prepare two different nanoparticle formulations. These nanoparticles loaded into PCL or poly(lactide-co-caprolactone) implant formulations were prepared by two different methods, which were molding and electrospinning. Size and zeta potential of nanoparticles were determined and the morphology of the formulations were investigated by scanning electron microscopy. CsA-loading efficiencies were calculated and the *in vitro* degradation and *in vitro* release studies were performed. MTT test was also performed using L929 fibroblast cells to evaluate the cytotoxicity of the formulations. PCL–PCL–NP-I formulation was implanted to Swiss Albino mice with induced dry eye syndrome to evaluate the efficacy. *In vitro* release studies showed that the release from the formulations continues between 30 and 60 days, and the cell viability was found to be 77.4%–99.0%. *In vivo* studies showed that healing is significantly faster in the presence of the selected implant formulation. Results indicated that nanodecorated implants are promising ocular carriers for controlled-release CsA application.

Keywords: cyclosporin A; ophtalmic drug delivery; drug delivery; nanoparticle; nano fiber; formulation; ocular implants; controlled release; subconjunctival application

INTRODUCTION

Dry eye syndrome (DES; keratoconjunctivitis sicca) is a common disorder of the tear film caused by decreased tear production, increased tear evaporation, or changes in the tear composition.[1] Tear film has an important role on nutrition of eye tissues and a functional vision. Reduction in tear production causes serious damage and scar formation on the anterior of the eye. Untreated advanced DES may cause an increase in the risk of infection and other visual disorders.[2] DES has some major symptoms, such as photophobia, burning, and stinging, that can have a significant impact on visual function, daily activities, and quality of life.[3,4] Tear film stability and DES might be a function of age, hormonal disorders, menopause, systemic anticholinergic drugs, corneal surgical operations, or systemic autoimmune diseases such as Sjögren's syndrome, rheumatoid arthritis, and progressive systemic sclerosis.[5] As a result of aging population, there is an increase in DES prevalence in recent years. Current lack of understanding makes the diagnosis of DES difficult. Thus, progress in this area is needed for an effective characterization, diagnose, and treatment of DES.[6]

The main purpose of DES treatment is to obtain a smooth ocular surface by re-epithelialization, relieve the symptoms, and prevent the complications.[7] Artificial tears are the mainstay of DES therapy. Occlusion of the lacrimal puncta by plugs is also a common DES therapy that blocks the flow of the tears through the canaliculi that connect eyes to the nose.[8] Although artificial tears and punctual plugs are able to improve DES symptoms, they are not the solution for DES-dependent inflammation; thus, anti-inflammatory therapy may be indicated, such as topical corticosteroids, topical nonsteroidal anti-inflammatory drugs, oral tetracyclines, and cyclosporin A (CsA).[9]

Cyclosporin A is a hydrophobic compound that has been used for its immune suppressor properties in organ transplantation. Numerous reports support that the local immunosuppression caused by CsA is effective for the management of ocular diseases such as corneal graft rejection, autoimmune uveitis, and DES. CsA's mechanism of action is based on its selective inhibitor properties of interleukin-2 release and causes cell-mediated immune response suppression that results in an increase in tear production.[9,10] Topical rather than systemic CsA application has been suggested to avoid systemic side effects,

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in the treatment of ocular diseases. It shows poor biopharmaceutical properties with low water solubility and permeability that makes CsA delivery a challenge for pharmaceutical scientists.[11,12] Topical CsA is the only drug that has been approved by US Food and Drug Administration (FDA) for DES and it has been on the market as an ophthalmic emulsion (0.05%), named Restasis®. Considering topical delivery, liposomes, microparticles, nanoparticles, micelles, emulsions, implants, and CsA prodrugs have been studied; however, none of the described topical systems has really succeeded in to extend the period of time on the corneal surface. Therefore, the administration frequency remains as a problem with these systems.[9] On the contrary, biodegradable ocular implants can have dual drug release profiles that are able to simultaneously deliver a loading and a maintenance dose.[13]

In this study, CsA nanoparticles-loaded extended-release biodegradable implant and fiber formulations were developed and characterized for subconjunctival application of CsA in DES treatment, in order to reduce the administration frequency and improve the patient compliance.

MATERIAL AND METHODS

Materials

Cyclosporin A was a kind gift from NOVARTIS (Turkey). Poly(lactide-co-glycolide) (PLGA; 85:15), poly-ε-caprolactone (PCL), poly-L-(lactide-co-caprolactone) (PLCL), high-performance liquid chromatography (HPLC) grade acetonitrile (ACN), and dichloromethane were purchased from Sigma–Aldrich (Germany). Polyvinyl alcohol [PVA; molecular weight (MW): 30,000–70,000 and 80,000 g/mol] was purchased from Merck (Germany) and mannitol was obtained from Riedel de Haën (Germany). Purified water was supplied from Milli-Q Integral Water Purification System (Millipore, Germany). MTT [3-(4–5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] was obtained from Sigma (St. Louis, Missouri, USA), and DMEM (Dulbecco's modified Eagle medium), fetal bovine serum (FBS), trypsin–EDTA, and penicillin–streptomycin were obtained from Biochrom (Germany). L929 (mouse fibroblast) cells were purchased from ATCC (Manassas, VA, USA).

Methods

Preparation of Nanoparticle Formulations

Two different nanoparticle formulations were prepared using PLGA (85:15) or PCL (MW: 30,000–70,000 g/mol). Both blank and CsA-loaded nanoparticles were manufactured by o/w emulsification solvent evaporation technique followed by lyophilization. Briefly, PLGA or PCL was dissolved in dichloromethane and CsA (20% of the polymer amount) was added to dichloromethane phase for CsA-loaded nanoparticles. Then, this organic phase was emulsified in aqueous PVA solution (1% and 0.5%, w/v) by sonication (60 W, 60 s) using an ultrasonic probe. PLGA emulsion was then diluted in PVA stabilizer solution (0.36%, w/v). The organic solvent was allowed to evaporate at room temperature under magnetic stirrer (750 rpm) for 4 h (PLGA) and 2 h (PCL) at room temperature. The nanosuspensions were then centrifuged at 17300 g for 60 min. The resulting nanosuspensions were cooled down to −20°C and lyophilized using 10% mannitol solution as a cryoprotectant.

Characterization of Nanoparticles

A scanning electron microscopy (SEM; FEI-Nova Nanosem 430, FEI; Hillsboro, Oregon, USA) device was used to evaluate surface characteristics and particle sizes of the nanoparticles. Both blank and CsA-loaded nanoparticle formulations were mounted on the metal stubs with conductive silver paint and then coated with a 150-Å thick layer of gold in a Bio-Rad sputter apparatus. SEM images of the samples were obtained at different magnifications. Average diameters of nanofibers were measured by Image J software ($n = 20$).

Mean diameter and polydispersity index values of PLGA and PCL nanoparticles were determined by quasielastic light scattering technique using Malvern NanoZS (Zetasizer NanoSeries ZS; Malvern Instruments, United Kingdom). Surface charge of nanoparticle formulations was also determined by zeta potential measurements using Malvern NanoZS (Zetasizer NanoSeries ZS; Malvern Instruments). Analyses were performed in triplicate at 25°C, before and after lyophilization.

Preparation and loading efficiency of the formulations were calculated. Preparation efficiency was calculated based on the weight of the obtained nanoparticles in comparison with the added amount of the drug and polymer. Nanoparticles were stirred in ACN for 1 h to completely dissolve, and CsA amount in nanoparticles was quantified using a validated HPLC method. The HPLC (Agilent 1200, Germany) separation were made using a reverse-phase C_{18} column and ACN–water (80:20) as mobile phase, with a UV detector at 210 nm. Column temperature was set to 65°C and the flow rate was 1 mL/min.

Preparation of Implant Formulations

Molding Technique

Poly(lactide-co-glycolide) and PCL nanoparticles-loaded implants were prepared by molding method using two different polymers, which were PCL or PLCL.[14,15] Implant polymer (0.5 *g* PCL or PLCL) and CsA (0.5% of the polymer amount) were dissolved in 3 mL dichloromethane, and nanoparticles (20% of the polymer amount) were suspended in 2 mL deionized water. Dichloromethane phase was added into the water phase drop by drop and then mixed with ultrasonic probe for 60 s using 10 W power. Formulations were immediately poured into the molds and cooled down to −80°C and then lyophilized (Fig. 1a).

Electrospinning Technique

Poly(lactide-co-glycolide) or PCL nanoparticles-loaded fiber implants were prepared by two-nozzle electrospinning method. Two-nozzle electrospinning was performed using the simultaneous electrospinning of different solutions from two needle tips.[16,17] In this study, PCL (MW: 80,000 g/mol) was used as the fiber polymer composition. PCL solution (15%, w/v) in dichloromethane–N-N dimethylformamide (1:1) was prepared to form the fiber structure. CsA-loaded nanoparticle suspension was used as the blend solution. Two different solutions were delivered at a constant feed rate of 0.48 mL/h. The solutions were electrospun on the collecting plate at a distance of 20 cm, generating an electric field at 20 kV (Fig. 1b). The collecting plate was moved in a square (50 cm^2), then formed fiber implants were cut into smaller pieces with 1 cm^2 dimensions. CsA concentration was adjusted to obtain 150 μg CsA in 1 cm^2 fiber implant. A list of the prepared implant and fiber formulations with the abbreviations is given in Table 1.

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