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Corticosteroid-loaded biodegradable nanoparticles for prevention of corneal allograft rejection in rats

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ABSTRACT

Immunologic graft rejection is one of the main causes of short and long-term graft failure in corneal transplantation. Steroids are the most commonly used immunosuppressive agents for postoperative management and prevention of corneal graft rejection. However, steroids delivered in eye drops are rapidly cleared from the surface of the eye, so the required frequency of dosing for corneal graft rejection management can be as high as once every 2 h. Additionally, these eye drops are often prescribed for daily use for 1 year or longer, which can result in poor patient compliance and steroid-related side effects. Here, we report a biodegradable nanoparticle system composed of Generally Regarded as Safe (GRAS) materials that can provide sustained release of corticosteroids to prevent corneal graft rejection following subconjunctival injection provided initially during transplant surgery. Poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles containing dexamethasone sodium phosphate (DSP) exhibited a size of 200 nm, 8 wt.% drug loading, and sustained drug release over 15 days *in vitro* under sink conditions. DSP-loaded nanoparticles provided sustained ocular drug levels for at least 7 days after subconjunctival administration in rats, and prevented corneal allograft rejection over the entire 9-week study when administered weekly. In contrast, control treatment groups that received weekly injections of either placebo nanoparticles, saline, or DSP in solution demonstrated corneal graft rejection accompanied by severe corneal edema, neovascularization and opacity that occurred in ≤4 weeks. Local controlled release of corticosteroids may reduce the rate of corneal graft rejection, perhaps especially in the days immediately following surgery when risk of rejection is highest and when typical steroid eye drop administration requirements are particularly onerous.

1. Introduction

Corneal transplantation is the most common form of solid tissue transplantation [1,2], and is widely used to treat blindness caused by corneal diseases. Approximately 36,000 corneal transplantation surgeries are performed each year in the US alone [1]. The 2-year graft survival rate for avascular and non-inflamed "low-risk" cornea beds is up to 90%, however, the rate for "high-risk" cornea beds, which had either neovascularization, inflammation, or previous graft rejection, is as low as 50% [1,3]. Given the increased risk of future graft failure in patients who have previously rejected a corneal transplant and the limited supply of cornea tissues suitable for transplantation, corneal graft failure is a significant burden on patients, their families and the health care system.

Immunologic corneal rejection is the main cause for graft failure. Immunosuppressive therapies, such as steroids, antimetabolites, and T-cell inhibitors, have been applied to patients after cornea transplantation, either systemically or through eye drops [4–6]. Topical corticosteroids in eye drops are widely used to control rejection rates of both "low-risk" and "high-risk" corneal grafts [7–10]. Drops are generally preferred over systemic steroid administration in order to target the therapy and reduce systemic side effects. However, rapid drug clearance from the ocular surface and low drug penetration into the eye lead to a requirement for frequent administration [9,11,12]. Drop administration requirements can be as often as every 2 h during the first several days after surgery [13,14], a regimen that causes poor patient compliance that increases graft rejection rates [15,16].

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Table 1
Evaluation of clinical parameters after transplantation (score 0–4), modified from [27].

Clinical parameter	Score				
	0	1	2	3	4
Cornea transparency	Clear cornea	Slight opacity	Mild opacity with iris details visible	Moderate opacity, iris details not visible	Severe opacity, white cornea
Edema	None	Slight	Moderate stromal edema	Marked stromal edema	Severe edema
Neovascularization	No observable growth of new vessels	New vessels invading less than 1/3 of the recipient bed	New vessel invading less than 2/3 of the recipient bed	New vessels growing up to the limiting ring of the graft	New vessels invading the graft

In an attempt to address the need for high local steroid levels immediately after surgery, subconjunctival (SC) injection of corticosteroids at the time of surgery is often employed. SC injection of dexamethasone sodium phosphate (DSP) solution has been shown to result in increased concentrations of DSP in the aqueous humor compared to drug concentrations achieved with eye drops [17,18]. However, rapid clearance of small molecules like DSP from the ocular tissue limits the duration of therapeutic drug levels after a single SC injection, and the spike in drug concentration may cause increased ocular side effects.

Polymeric nano- and microparticles can be administered by injection and provide controlled release of drugs to target tissues at effective levels, which can increase treatment efficacy and decrease associated side effects. For this reason, polymer particle based therapies are being evaluated to deliver therapeutic agents to the eye by various routes, including intravitreal injection, topical administration and SC injection [19–23]. Here, we describe the development of a nanoparticle formulation for SC injection that can provide sustained release of DSP (DSP-NP) both *in vitro* and following SC injection in rats, and we demonstrate that it is effective in preventing corneal graft rejection in a corneal transplant rat model *in vivo*.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-*co*-glycolic acid; 50:50, MW ~3.2 kDa, acid terminated) (PLGA) was purchased from Lakeshore Biomaterials (Evonik, Birmingham, AL). Dexamethasone sodium phosphate salt (DSP) was purchased from MP Biomedicals (Santa Ana, CA). [^{3}H]-labeled DSP was purchased from American Radiolabeled Chemicals (St Louis, MO). Pluronic F127 (a polyethylene oxide-polypropylene oxide-polyethylene oxide triblock copolymer, or PEO-PPO-PEO), triethanolamine (TEOA), ethylenediaminetetraacetic acid (EDTA) solution (0.5 M), zinc acetate dihydrate and all other organic solvents were purchased from Sigma-Aldrich (St. Louis, MO). Methoxy-poly(ethylene glycol)-amine (MeO-PEG-NH_2) was purchased from Creative PEGWorks (Winston Salem, NC).

2.2. Preparation of model nanoparticles

Red fluorescent carboxyl-modified PS particles of 100, 200, and 500 nm (Molecular Probes®, Life Technologies, Co., Frederick, MD) in size were covalently modified with methoxy-PEG-amine by carboxyl acid-amine reaction, as described previously [24]. PEGylated PS particles (PS-PEG) were thoroughly washed, re-suspended in water, characterized and stored at 4 °C prior to use.

Table 2
Physicochemical properties of nanoparticles.

Formulation	Diameter (nm)	PDI	ζ-potential (mV)	Drug loading (wt.%)
DSP-NP	200 ± 8	0.12	−8 ± 1.4	8
Placebo NP	186 ± 13	0.086	−15 ± 1	n/a
PS-PEG 100 nm	100 ± 1	0.01	−5 ± 2	n/a
PS-PEG 200 nm	260 ± 12	0.08	−4 ± 1	n/a
PS-PEG 500 nm	510 ± 3	0.05	−6 ± 1	n/a

2.3. Preparation of placebo nanoparticles

Placebo PLGA nanoparticles (placebo NP) were prepared by a solvent diffusion method. Briefly, 20 mg of the polymer was dissolved in 1 mL of tetrahydrofuran (THF), and added dropwise to 40 mL of 5% F127 aqueous solution under magnetic stirring at 700 rpm. After stirring for about 1 h, the solution was rotoevaporated for 30 min to remove the residual THF. PLGA nanoparticles were washed with 5% F127 by centrifugation at 10,000 *g* for 25 min, and re-suspended in 0.2 mL of ultrapure water.

2.4. Preparation of DSP-loaded PLGA nanoparticles

Dexamethasone sodium phosphate (DSP) was encapsulated into PLGA nanoparticles following a modified solvent diffusion method, [25,26]. Briefly, a DSP-zinc complex was formed by adding 1 mL of

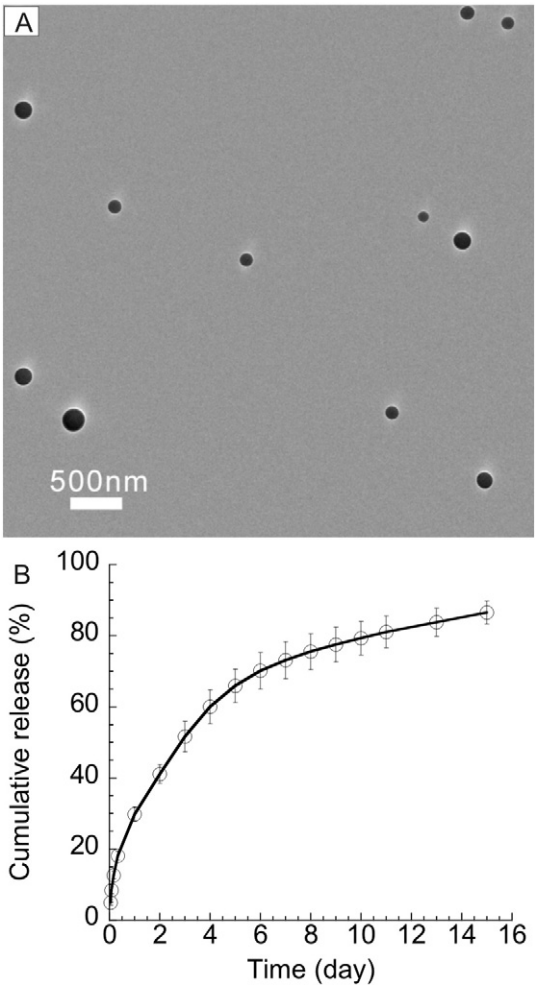

Fig. 1. (A) TEM image and (B) *in vitro* DSP release profile from DSP-NP.