Dexamethasone Drug Eluting Nanowafers Control Inflammation in Alkali-Burned Corneas Associated With Dry Eye

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PURPOSE. To evaluate the efficacy of a controlled release dexamethasone delivery system for suppressing inflammation in an ocular burn + desiccating stress (OB+DS) model.

METHODS. Nanowafers (NW) loaded with Dexamethasone (Dex, 10 μg) or vehicles (2.5% Methylcellulose; MC) were fabricated using hydrogel template strategy. C57BL/6 mice were subjected to unilateral alkali ocular burn with concomitant desiccating stress for 2 or 5 days and topically treated either with 2 μL of 0.1% Dex or vehicle four times per day and compared with mice that had MC-NW or Dex-NW placed on their corneas. Clinical parameters were evaluated daily. Mice were euthanized after 2 or 5 days. Quantitative PCR evaluated the expression of inflammatory cytokines IL-1β and IL-6 and matrix metalloproteinases (MMP) in whole cornea lysates. Myeloperoxidase activity (MPO) was measured using a commercial kit in cornea lysates.

RESULTS. Both Dex drop and Dex-NW groups had significantly lower corneal opacity scores compared with their vehicles. Both Dex drops and Dex-NW significantly decreased expression of IL-1β, IL-6, and MMP-9 mRNA transcripts compared with vehicle drops or wafers 2 and 5 days after the initial lesion. A significant lower number of neutrophils was found in both Dex treatment groups and this was accompanied by decreased MPO activity compared with vehicle controls.

CONCLUSIONS. Dex-NW has efficacy equal to Dex drops in preserving corneal clarity and decreasing expression of MMPs and inflammatory cytokines of the corneas of mice subjected to an OB+DS model.

Keywords: nanowafers, alkali injury, dry eye, neutrophils, MMPs, dexamethasone
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clarity, decreased inflammatory cytokine and MMP expression in the wounded corneas, while reduced neutrophil infiltration. The efficacy of once a day Dex-NW treatment was comparable to four times a day topical Dex eye drops treatment.

**Materials and Methods**

**Materials**

Sodium methylcellulose (MC, molecular weight 90,000) and high performance liquid chromatography (HPLC) solvents (acetonitrile) were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). USP grade dexamethasone sodium phosphate (Dex) was obtained from Spectrum Chemicals (New Brunswick, NJ, USA). Polymerase chain reaction reagents and Oregon Green Dextran (72 KDa) were purchased from Life Technologies (Carlsbad, CA, USA). The drug release study from the Dex-NW was analyzed by HPLC method using a Shimadzu Prominence HPLC system (Kyoto, Japan).

**Animals**

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocols were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee (Houston, TX, USA). Female C57BL/6j mice (6- to 8-weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA).

Two hundred and twenty-two C57BL/6j mice were used in this study. Twenty-two C57BL/6j animals were used per group (vehicle or Dex in the format of drops or NW) and per time point (2 and 5 days): 6 for histology, 12 for real-time PCR, and four for myeloperoxidase assay. Corneal opacity and wound closure rate were evaluated in 12 live mice that were also used for either histology or PCR. Twenty-two naive animals were used for normal controls. For the dose response experiment, a separate group of 24 mice were used (four/group).

**Nanowafer Fabrication**

The NW were fabricated according to a previously published procedure with slight modifications. A clear MC solution (4% wt/vol, 12 mL) was transferred with a pipette onto a polydimethylsiloxane (PDMS) imprint (3500 nm, 500-nm diameter) containing square posts (of 500 × 500 nm, 500-nm high) placed on a flat glass plate and left to dry at room temperature. Thus, formed MC NW was carefully peeled away from the PDMS imprint. The MC NW was filled with Dex by transferring a thick polymer solution (2.5%) to obtain the required thickness of the NW. The MC NW was carefully peeled away from the PDMS imprint. The MC NW was then dried at room temperature. The clear solution was filtered through a 0.2-μm syringe filter and drug concentration was calculated by comparing the peak area of standards and sample detected at 240 nm. The UV–HPLC system was equipped with an autosampler, in line degasser, and column oven set at room temperature.

**Ocular Burn and Desiccating Stress (OB+DS) Model**

Unilateral alkali burn was created on the right eye of 6- to 8-week-old female C57BL/6j after systemic anesthesia with isoflurane using a vaporizer (SommOVA, Kent Scientific, Torrington, CT, USA), by placing one 2.0-mm diameter filter paper disc presoaked with 1 N NAOH on the central cornea for 10 seconds, followed by extensive rinsing with balanced salt solution (Alcon, Fort Worth, TX, USA), as previously described. Precautions were taken to avoid damage to the peripheral cornea, conjunctiva, and lids. After anesthesia recovery, mice were subjected to desiccating stress (DS) to create an OB+DS model. Desiccating stress was induced by sterile subcutaneous injection of 0.5 mg/mL scopolamine hydrobromide (Sigma-Aldrich Corp.) four times per day (QID) into alternating flanks and exposure to a drafty low humidity (<30% relative humidity) environment for 2 or 5 days as previously described.

**Dosing Regimens**

Mice subjected to OB+DS model were topically treated as described below. Tissues were collected after 2 and 5 days of creating the alkali burn and dry eye.

1. **Dose response experiment:**

   To test the effective dose of Dex-NW, C57BL/6j mice subjected to the OB+DS model received a corneal application of MC NW loaded with 2, 4, or 10 μg Dex once per day (QD) or 10 μg every other day (QOD) and were compared with naive control corneas and 2.5% MC blank nanowafer.

2. **NW drug delivery system:**

   Mice subjected to the OB+DS model for 2 and 5 days received a corneal application of each 10 μg Dex-NW or vehicle (2.5% MC wafer) QD.

3. **Conventional topical anti-inflammatory therapy:**

   Mice subjected to the OB+DS model for 2 and 5 days were topically treated either with 2 μL sodium phosphate Dex (1mg/mL, Spectrum Laboratory, Gardena, CA, USA), or vehicle (2.5% MC) QID and compared with Dex-NW QD–treated corneas. Each drop delivers 2 μg/eye/application, for a total of 8 μg/day/eye.
Opaque, iris and pupils still detectable, grade 3 and photographed by a Nikon fluorescence microscope with no view of the pupils).

pupils hardly detectable, and grade 4.

analyzed.

by two masked observers in a categorical manner (present/

Nikon). Corneal epithelial defects were graded in digital images

graphed with a stereoscopic zoom microscope (SMZ 1500; ocular surface, corneas were rinsed with PBS and photo-

After instilling 1

slightly hazy, iris and pupils easily visible, grade 2

Completely clear, grade 1

Opacity Score. Corneal edema and opacity were graded biomicroscopically by two masked observers in images taken by a color digital camera DS-Fi1 (Nikon, Melville, New York, USA) as previously described.16 Corneal opacity was scored using a scale of 0 to 4 (grade 0 = completely clear, grade 1 = slightly hazy, iris and pupils easily visible, grade 2 = slightly opaque, iris and pupils still detectable, grade 3 = opaque, pupils hardly detectable, and grade 4 = completely opaque with no view of the pupils).

Measurement of Corneal Epithelial Defect. Corneal epithelial healing was assessed daily by fluorescein staining. After instilling 1 μL of 0.1% liquid sodium fluorescein onto the ocular surface, corneas were rinsed with PBS and photographed with a stereoscopic zoom microscope (SMZ 1500; Nikon) under fluorescence excitation at 470 nm (DS-Q1iMc, Nikon). Corneal epithelial defects were graded in digital images by two masked observers in a categorical manner (present/absent) to generate a survival curve. Biological replicate scores were transferred to an excel database where the results were analyzed.

Histology and Immunostaining

A total of six mice per experimental group at 2 and 5 days post injury were euthanized. For immunohistochemistry, eyes and adnexa were excised, embedded in optimal cutting temperature compound (VWR, Suwanee, GA, USA), and flash frozen in liquid nitrogen. Sagittal 80 μm tissue sections were cut with a cryostat (HM 500; Micron, Waldorf, Germany) and placed on glass slides that were stored at −80°C. Immunohistochemistry was performed to detect neutrophils using rat anti-Gr-1 antibody (Ly6G, 1:250, clone 1A8I; BD Pharmingen, San Diego, CA, USA). Cryosections were stained with the primary antibody and appropriate biotinylated secondary antibody (1:100 biotin goat α-rat; BD Pharmingen) using a Vectastain Elite ABC kit and Nova Red reagents (Vector, Burlingame, CA, USA). Sections from each experimental group were examined and photographed with a microscope equipped with a digital camera (Eclipse E400 with a DS–F1 digital camera). Mean fluorescent intensity/area was measured in the corneal epithelium and analyzed using the NIS Elements Software (Nikon).

RNA Isolation and Quantitative PCR

Four whole corneas (including stroma) per group at 2 and 5 days post injury were excised, minced, and total RNA was extracted using a Qiagen MicroPlus RNAeasy isolation Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, quantified by a NanoDrop ND-2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and stored at −80°C. First-strand cDNA was synthesized with random hexamers by M-MulV reverse transcription (Ready-To-Go You-Prime First-Strand Beads; GE Healthcare, Inc., Arlington Heights, NJ, USA), as previously described.17 Real-time PCR was performed with specific Taqman MGB probes (Applied Biosystems, Inc., Foster City, CA, USA) and PCR master mix (Taqman Gene Expression Master Mix), in a commercial thermocycling system (StepOnePlus Real-Time PCR System, Applied Biosystems), according to the manufacturer's recommendations. Quantitative real-time PCR was performed using gene expression assay primers and MGB probes specific for murine targets described in the Table. The beta-2-microglobulin (B2M) gene was used as an endogenous reference for each reaction to correct for differences in the amount of total RNA added. The results of quantitative PCR were analyzed by the comparative C T method where target change = 2−ΔΔC T. The results were normalized by the C T value of B2M and the levels of relative RNA transcripts in the untreated group was used as the calibrator.

Myeloperoxidase Assay

Myeloperoxidase (MPO) activity was measured using a myeloperoxidase colorimetric activity assay kit as described by the manufacturer (Sigma-Aldrich Corp.). Briefly, whole cornea lysates from experimental groups (n = 4/group) were homogenized in MPO assay buffer and the homogenate was centrifuged at 14,000g for 20 minutes at 4°C. Total protein concentration was measured by the BCA protein assay as previously described. A 50 μg/sample was mixed with MPO assay buffer and MPO substrate, incubated at room temperature for 2 hours, and then mixed with tetramethylbenzidine probe. Fluorescence was measured at 412 nm using a Tecan Infinite M200 (Tecan, Inc.) plate reader equipped with Magellan V6.55 software (San Jose, CA, USA). Biologic replicate samples were averaged. Results are presented as mean ± SEM milliunits.

Statistical Analysis

Results are presented as the mean ± SEM. Two-way ANOVA with Bonferroni post hoc testing was used for statistical comparisons of gene expression. P less than or equal to 0.05 was considered statistical significant. These tests were performed using GraphPad Prism 6.0 software (GraphPad Incorporation, San Diego, CA, USA).

Results

Sustained Release of Dexamethasone From Nanowafer

In this study, methylcellulose (MC) polymer was used for NW fabrication because of its water solubility, transparency, and
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Dexamethasone is an anti-inflammatory glucocorticoid commonly used after corneal injury and eye surgery. Topical 0.1% Dex treatment effectively decreased the expression of inflammatory cytokines and MMPs in our OB+DS model. We hypothesized that daily application of a Dex-NW can control inflammation to a similar degree as conventional Dex drops administrated at least four times a day in an OB+DS model. Using this model, we investigated the efficacy of Dex-NW in ameliorating clinical signs and decreasing expression of inflammatory cytokines and MMPs, and limiting neutrophil influx compared with conventional Dex drops.

The in vitro drug release from Dex-NW was analyzed by HPLC method. The in vitro drug release profile of Dex-NW revealed a sustained Dex release for up to 24 hours (Fig. 1A). In the first hour, approximately 15% of the drug was released followed by 5% to 7% release per hour for the next 5 hours. As can be seen from the Figure 1A inset, there was a very small burst release in the first hour followed by a sustained release for up to 24 hours. In practical terms, when a Dex-NW is applied on the injured cornea, a small initial burst release of the drug from the Dex-NW is followed by a prolonged release for sustained anti-inflammatory activity.

To test the maximum effective dose for suppression of inflammation, Dex-NWs loaded with 2, 4, and 10 μg of Dex and tested in our OB+DS model. Four mice per group were subjected to alkali burn and desiccating stress and received daily applications of the different NW (2, 4, 10 μg) for 2 days. After the creation of a unilateral alkali burn, the NW was grasped gently by a jeweler’s forceps and applied to the cornea surface. A 10-μl drop of PBS was applied to hydrate the NW and facilitate its uniform adhesion to the cornea. After hydration, the NW expanded (Fig. 1B). Careful application using this technique provided good adhesion of the NW to the cornea that did not dislodge with blinking.

Another group of four mice received the 10 μg NW QOD. Whole corneas were harvested and gene expression investigated by real-time PCR. We observed that the 2 μg Dex-NW failed to reduce the expression levels of IL-1β, IL-6, and MMP-3 in the wounded corneas (Fig. 1C), while the 10 μg Dex QD had the lowest expression of IL-1β, IL-6, MMPs-3, and -9 in the wounded tissue compared with 4 or 10 μg Dex-NW QOD. Therefore, due to its greater anti-inflammatory potency, 10 μg Dex-NW was chosen as the drug concentration for the remainder of the experiments.

Dexamethasone Loaded-Nanowafer has Equal Efficacy to Conventional Dexamethasone Drops

Dex has been used to treat eye inflammation caused by injury, surgery, or other conditions. Our previous studies showed that Dex decreased corneal opacity scores and inflammatory cytokine and MMP expression in eyes subjected to our OB+DS model. Following unilateral alkali burn, mice were placed in a desiccating environment and treatment was initiated (either topical eyedrops or NW placement on the central cornea over the lesion). Clinical parameters (wound healing and corneal opacity) were evaluated daily for the duration of the experiments. We found that both Dex-NW and Dex drops treatments resulted in lower corneal opacity scores compared with their respective vehicle (Fig. 2A). Five days post injury, Dex-NW and Dex drops treatment yielded similarly lower corneal opacity scores, indicating that once daily Dex-NW had equal efficacy to Dex drops applied QID in preserving corneal clarity. Dex treatment, either by conventional drops or by NW, had similar effects on wound closure rate compared with their vehicle (Fig. 2B).

We have shown that expression of inflammatory cytokines and MMPs in the OB+DS model is greater than either of the individual treatment. Next, we compared the expression of IL-1β, IL-6, MMPs-1, -2, -3, -9, and -13 in the Dex-NW-treated corneas subjected to the OB+DS model by PCR to corneas treated with conventional Dex drops. Dex topical treatment significantly decreased IL-1β, IL-6, MMPs-1, -9, and -13 transcripts at 2 days post injury and significantly decreased IL-1β, IL-6, and MMP-9 at 5 days. In contrast, Dex-NW QD-treated corneas had lower expression of IL-1β, IL-6, and MMP-9 at both 2 and 5 days while it showed a significant decrease in MMPs-1 and -13 at 5 days post initial burn (Figs. 3A, 3B). Interestingly, significantly lower IL-6 and MMP-3 at 2 days and lower IL-1β, IL-6, MMPs-1, and -13 transcripts were measured in the Dex-NW compared with Dex drops at 5 days post initial burn, indicating that sustained Dex release produced greater anti-inflammatory and anti-MMP activity especially in the late stage of disease. There was no change in MMP-2 expression with any of the treatment groups. These data support our hypothesis that this sustained release formulation promotes diffusion of drug molecules directly into ocular tissues to produce greater anti-inflammatory effects in the late stage of the injury.

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Dexamethasone Nanowafer Decreases Neutrophil Infiltration in Alkali-Burned Corneas Associated With Dry Eye

Neutrophils play an important role in the host defense against infection and neutrophil infiltration is the hallmark of acute inflammation. Dex has been reported to inhibit neutrophil migration to a site of inflammation. To test if Dex-NW can inhibit the influx of neutrophils, histologic sections of corneas were analyzed at different time points using neutrophil marker Gr-1 antibody (Fig. 4A). A significantly influx of Gr-1 positive (+) cells into the wound site was observed in both vehicle wafer and vehicle drops groups while both Dex-NW and Dex drops significantly decreased neutrophil infiltration (Fig. 4B).

Myeloperoxidase (MPO), an enzyme mostly abundantly expressed in neutrophil granulocytes, has been used as a marker of neutrophil infiltration into tissue. As shown in Figure 4C, increased MPO levels were noted in the control groups (MC eyedrops and MC wafers) at 5 days. Consistent with the Gr-1+ staining results, both Dex-NW and Dex drops treatment significantly decreased MPO activity at 5 days post injury.

Taken together, these results demonstrated that a single application of Dex-NW had similar or greater effects to conventional Dex eyedrops applied QID, suggesting that slow
FIGURE 1. (A) In vitro drug release of Dex from NW. The in vitro drug release profile of Dex-NW revealed a sustained Dex release for up to 24 hours. In the first hour, approximately 15% (inset) of the drug was released followed by 5% to 7% release per hour for the next 5 hours for up to 24 hours. (B) Nanowafer placement. Representative bright field digital images of burned eyes immediately after placement of NW in the central cornea up to 3 minutes. The NW was grasped gently by a jeweler’s forceps and then applied to the cornea. A 10-μl drop of BSS was applied to hydrate the NW and facilitate its uniform adhesion to the cornea. After hydration, the NW expanded. Careful application using this technique provided good adhesion of the NW to the cornea that did not dislodge with blinking. T, time. (C) Dose response of the MC Dex-NW on expression of inflammatory cytokine and enzymes.

MC Blank MW  MC 2μg Dex-MW  MC 10μg Dex-MW QD
MC 4μg Dex-MW  MC 10μg Dex-MW QOD

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MMP genes. Corneal expression of inflammatory cytokines (IL-1β and IL-6) and matrix metalloproteinase (MMP-3 and -9) are significantly increased in the combined model of alkali burn and dry eye. Ten micrograms of Dex-NW applied every day at 2 days post injury has greater anti-inflammatory potency to suppress the expression of inflammatory cytokines (IL-1β and IL-6) and matrix metalloproteinase (MMP-3 and -9) compared with vehicle controls. n = 4 right corneas/group. ****P < 0.0001: NS versus MC blank NW; ^^^^P < 0.001, ^^^P < 0.0001: 2 μg Dex-NW NW versus 4 μg Dex-NW, 10 μg Dex-NW. NS, nonstressed.

release of drug content from the wafer improves biological outcomes and would facilitate compliance.

**DISCUSSION**

Eye injuries due to chemical spills cause corneal epithelial damage, opacification, and corneal scarring, which often result in loss of vision and eventual blindness. After chemical injury, the therapeutic goal is to suppress ocular inflammation, to promote corneal epithelial healing and to restore corneal clarity by using anti-inflammatory drugs during the acute stage.1 Currently, eye injuries are treated with topical eyedrops of ophthalmic solutions. Although topical eye drop treatment is a simple and noninvasive mode of drug delivery, because of rapid blinking and tear turnover, most of the drug will be cleared from the ocular surface within a few seconds resulting in unsatisfactory treatment outcomes. In this study, we have presented the fabrication of Dex-NW and its therapeutic potential evaluated in an OB-iDS murine model representing a severe ocular burn condition.2

Previous studies have shown that either corneal alkali burn21–23 or dry eye stress24–26 induce the production of inflammatory cytokines and increase MMPs activity in the ocular surface. In clinic, some patients with ocular burns also undergo dry eye stress: (1) there are many patients with ocular burns who also have damaged conjunctival goblet cells and lacrimal gland dysfunction, leading to decreased tear production, (2) patients with extensive facial burns have eyelids defects which can lead to corneal exposure, (3) some patients stay in environmentally controlled intensive care units (ICU) that have low humidity drafty environments. Therefore, we have recently established a mouse model that combines corneal alkali burns and desiccating stress that represents more severe ocular injury.2

Delayed or impaired wound healing has been an issue for Dex application to open wounds by blocking the fibrogenic effect of TGF-β.27 Conflicting data were reported on corneal wound healing following topical Dex treatment,28 with corneal epithelial healing retarded, but the basement membrane was well maintained. In our previous study, no wound healing was observed in the mice that receiving topical Dex treatment during OB-iDS.11 Because the Dex-NW has longer drug retention time and high tissue drug absorbance, we asked whether Dex-NW affects corneal wound healing. After monitoring corneal epithelial healing on a daily basis, Dex-NW and Dex drops showed the same wound healing rate at 5 days post injuries, indicating that Dex-NW did not impair corneal wound healing. Another important clinical parameter, corneal clarity, is essential for vision and has also been evaluated after treatment with Dex-NW. As expected, administration of Dex-NW QD preserved corneal clarity at the same level as four times a day–administered Dex drops.

The NW drug delivery is novel in the sense that it can be fabricated with mucoadhesive polymers, such as polyvinyl alcohol, carboxymethyl cellulose, hydroxypropyl cellulose, and polyvinyl pyrrolidone, in addition to methylcellulose.12,13 Because of the mucoadhesive nature of the NW, it readily adheres to the ocular surface and releases the drug in a tightly controlled fashion. The NW increases the drug residence time on the cornea, which was found to increase diffusion of drug into the cornea stroma.12,13 On the contrary, drugs delivered as topical eye drop formulations are rapidly cleared from the ocular surface through tear production and drainage, thus limiting therapeutic effect.28 In addition to small molecules such as Dex, the NW can deliver hydrophilic and hydrophobic drugs, and also macromolecules such as antibodies, growth factors, and siRNA. The NW after application on the cornea will remain for up to 3 hours before it completely dissolves as described in one of our previous studies.12,13 Because the NW is fabricated with a mucoadhesive polymer, it will tightly adhere to the corneal surface and withstand blinking while the mouse is awake.

Dex, an anti-inflammatory glucocorticoid, is commonly used after corneal injury and eye surgery. In an outpatient clinical setting, current preferred treatment regimens for ocular burn include multiple topical administrations, including Dex every 6 hours. Hamill and colleagues4 proposed hourly administration of corticosteroids after corneal burn, which lead to improved visual acuity in patients with corneal alkali burns.1 Frequency of eyedrop administration is a main factor influencing patient compliance. Most children are uncooperative with eyedrops,30 older patients have even lower compliance due to motor disabilities and reduced visual acuity31 and critically ill patients depend on caregivers to properly administer drops. However, the outcomes of many eye diseases largely depend on the compliance of the patient to follow the treatment regimens. Herrmann and colleagues5 have used electronic compliance monitoring of topical treatment for 28 patients after ophthalmic surgery, and a mean dose compliance of 50.2% was observed. Moreover, dose compliance was below 25% in approximately one of five patients. The observed mean dosage interval for each patient ranged from 4.6 to 19.7 hours with 30% of dosage intervals exceeding 12 hours. These results implied that the necessity to improve compliance with topical ophthalmic treatment regimens. Therefore, a controlled sustained release drug delivery system for administration of anti-inflammatory agents would be a major advance in the management of the blinding eye injuries and infections.32–34

**Figure 2.** Dex drops or Dex-NW treatment decreases corneal opacity score (A). Corneal opacity ([A] mean ± SEM) and wound closure rate (B) categorical) in corneas subjected to ocular burn with concomitant desiccating stress and treated with Dex drops or Dex-NW and compared with its controls. Both Dex-NW and Dex drops treatments resulted in lower corneal opacity scores compared with their respective vehicle. n = 12 animals/group. *P < 0.05. Dexamethasone Nanowafer Treats Ocular Inflammation

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**Figure 3.** Methylcellulose NW loaded with 10 µg Dex decrease inflammatory cytokines and MMPs in corneas of the combined model of alkali burn and dry eye. (A, B) Mean ± SEM of results of gene expression analysis of inflammatory cytokines (IL-1β and IL-6) and MMPs-1, -2, -3, -9, and -13 RNA transcripts in whole corneas from animals subjected to ocular burn + desiccating stress for 2 (A) or 5 (B) days and topically treated with Dex drops or Dex-NW and compared with its vehicle controls. The horizontal line at each figure represents the level of the mRNA expression for untreated group, which was used as the calibrator and normalized as 1. n = 4–5 right corneas/group. (C, D) Representative merged pictures of MMP-1 (C) and -9 (D) immunofluorescent staining shown in green of central cornea cryosections from animals subjected to a combined model of alkali burn and dry eye topically treated with Dex drops or Dex-NW and compared with its vehicle controls. Counterstaining was PI = red; n = 6 right corneas/group. (E, F) Quantification of mean fluorescence intensity (MFI) values from MMP-1 (E) and MMP-9 (F) staining are displayed. n = 6 right corneas/group. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001: MC blank wafers versus MC Dex-NW, MC blank drops versus MC Dex drops. MC blank-NW versus MC Dex-NW; NS = nonstressed; NTC, negative control; MFI, mean fluorescence intensity.
In conclusion, our data show that NW provided sustained release of Dex, improved clinically graded corneal clarity while decreasing neutrophil infiltration and MMP expression in the cornea. Treatment with Dex-NW QD is as efficacious as four times a day Dex drops. The sustained drug release provided with Dex-NW may improve patient outcomes by improving efficacy of the drug as well as compliance.

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Baylor College of Medicine has filed for intellectual property rights for the use of nanowafers in the treatment of ocular diseases. S.C. Pflugfelder, G. Acharya, and C.S. De Paiva are co-inventors of a patent application.

Disclosure: F. Bian, None; C.S. Shin, None; C. Wang, None; S.C. Pflugfelder, P; G. Acharya, P; C.S. De Paiva, P

References


