THERAPEUTIC CONTACT LENSES VIA BIOMIMETIC IMPRINTING

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ABSTRACT

A biomimetic approach has been exercised to design and synthesize novel contact lenses, to tackle the unmet need for the controlled loading and release of therapeutics on the surface of the eye. This work will demonstrate the formation and characterization of novel lenses with controlled loading and release of ocular H1-antihistamines and antibiotics. Since ocular bioavailability of topical drugs is very poor (typically less than 7% is absorbed by the eye), a high dosage is needed which typically prohibits contact lens use and warrants multiple dosages a day.

Controlling and tailoring the release of drugs via novel contact lenses with significantly enhanced loading can solve these problems with increased drug bioavailability, less irritation to eye tissue, and reduced eye and body side effects. Conventional soft contact lenses typically do not work due to a lack of sufficient drug loading and poor control of drug release. This new class of recognitive intelligent biomaterials is designed by incorporating motifs with structural and molecular homology to biological receptor docking sites and has a strong potential to work with a wide spectrum of drugs and impact the administration of a number of ocular therapies. Gels of multiple binding points with varying functionalities outperformed gels formed with fewer types of functionality and showed the greatest loading potential (i.e., up to 6 times more than control lenses) with mechanical and optical properties comparable to conventional lenses. Dynamic drug release profiles under in vitro physiological conditions demonstrated that release rates can be tailored via type and amount of functionality and a viable therapeutic concentration of drug can be delivered at a constant rate for extended periods from 16 hours to over a week depending on formulation.

INTRODUCTION

Delivering ocular medications via contact lenses was introduced in 1965 within the first patent/publication in the field from Otto Wichterle [1, 2]. In this work, he stated that "bacteriostatic, bacteriocidal or otherwise medicinally active substances such as antibiotics may be dissolved in the aqueous constituent of the hydrogels to provide medication over an extended period via diffusion." The biggest obstacle to using the fluid within the aqueous portion of a polymer gel is maintaining a significant concentration of drug to have a therapeutically relevant effect, which is ultimately limited by the solubility and partitioning of the drug. Drug soaked contact lenses, which have low drug loading and poor control over release, have not become a clinical or commercial success.

Topically applied drugs in the forms of solutions, suspensions, and ointments account for 90% of ophthalmological formulations on the market today [3]. The caveat with topical delivery is that bioavailability tends to be low (e.g., 1-7% is productively absorbed [4]) due to various factors and ocular protective mechanisms such as lachrymation and tear turnover, nasolachrymal drainage, spillage from the eye, metabolic degradation, and non-productive adsorption/ absorption, etc. Topical solutions and suspensions have remained effective by the administration of very high concentrations of drug multiple times a day, leading to decreased compliance, increased toxicity, burning, itching sensations, and gritty feelings, etc. experienced by the patient. While ointments can lead to slightly increased bioavailabilities over drops, they are difficult to apply, uncomfortable to use, and severely reduce vision. Other methods to improve delivery include viscosity enhancers in topical drops and conjunctival mucin adhesive polymers to increase retention [5], lipophilic modification [4] and

permeation enhancers to increase therapeutic permeability and improve the absorption rate, as well as implantable devices and inserts such as Vitracert®, Ocusert®, and Lacrisert®.

In this work, we explore the potential of therapeutic contact lenses to deliver antihistamines and antibiotics for extended periods with increased bioavailabilty and decreased systemic concentration. Treatment options for seasonal and perennial allergic conjunctivitis primarily consist of oral antihistamines and topical treatments. During allergic periods, the use of contact lenses becomes uncomfortable due to ocular irritation (itch, redness, dryness, and decreased lacrimation). The treatment of ocular allergies is based on the severity of symptoms and the affect on the overall quality of life. Conjunctivitis and seasonal allergic conjunctivitis (SAC) are the predominant forms of ocular immunological hypersensitivity reactions, and the number of people affected by ocular allergies continues to increase (currently 1/3 of US population). In the past ten years, more than ten new products for allergy have been commercialized with sales increasing 20 fold.

Ketotifen fumurate is a potent fast acting and highly selective histamine H1 antagonist (competitive and allosteric inhibition) with a sustained duration of action. It inhibits itching, redness, eyelid swelling, tearing, and chemosis induced by conjunctival provocation with allergens and histamine. With topical application in the form of eye drops, absorption is incomplete and bioavailability is low. Thus, the dose is usually administered multiple times daily with a high concentration. Also, due to the high concentration of drug and other constituents of the ophthalmic suspension preparation, patients are advised not to wear soft contact lenses during eye drop treatment.

Bacterial conjunctivitis is typically managed with broad-spectrum antibiotic drops with intensive instillation for the first day (multiple times an hour) until the infection is under control and symptoms and signs reduce. Common organisms are Staphylococcus aureus, Staphylococcus epidermis, Group A Streptococcus and Streptococcus pneumoniae, and to a lesser extent Haemophilus influenzae, seudomonas, and Escherichia coli.

For large or dirty abrasions or lacerations to the cornea, clinicians typically prescribe broad-spectrum antibiotic drops since abrasions have susceptibility for developing microbial keratitis (infectious corneal ulcers) and infections have been shown to delay healing. The healing process, i.e., healthy cells filling the defect, usually takes 24-72 hours for moderate abrasions. More significant abrasions and deep scratches can lead to corneal scarring. Antibiotic drops must be administered every 2 to 3 hours and ointments, which are less comfortable and have shown to retard corneal epithelium healing, need to readministered every 4 to 6 hours.

Inspired by Nature, we adopted a biomimetic strategy to rationally design novel contact lenses capable of delivering drugs to treat allergic and bacterial conjunctivitis. Typical hydrogels with the requisite functionality were synthesized via configurational biomimetic imprinting (CBIP) techniques [6], a variant of the general molecular imprinting technique [7, 8] which involves pre-polymerization complexation between the template molecule and functional monomers with specific chemical structures designed to interact with the template via non-covalent chemistry. The functional monomers were chosen based on a fundamental analysis of biological recognition (i.e., mechanism of action and ligand receptor chemistry). A translational advantage afforded by the CBIP synthesis technique is that it can be easily incorporated into the synthesis scheme of conventional lenses, without several additional steps.

MATERIALS AND METHODS

Synthesis of Recognitive Networks

Hydrogels of differing compositions of acrylic acid (AA), acrylamide (AM), 2-hydroxyethylmethacrylate (HEMA), azobisisobutyronitrile (AIBN) and ketotifen fumarate (antihistamine) or chloramphenicol (antibiotic) were synthesized in a temperature controlled, non-oxidative environment using free-radical UV photopolymerization. Typically, the

reaction solutions (e.g., consisting of monomers, template molecule and initiator) were sonicated to produce a homogeneous mixture. The solutions were then equilibrated in an inert environment and purged with nitrogen until oxygen levels were less than 0.1 ppm. The solutions were pipetted into glass molds (6'' X 6'') separated by a Teflon frame 0.7 or 0.4 mm thick. The polymerization reaction occurred for ten minutes with light intensity of 40 mW/cm^2 (Dymax UV flood light), at a constant temperature of 36 °C. Circular discs of 14 mm were cut with a cork borer. Control gels were prepared without the template molecule, following similar steps.

Equilibrium Binding and Release Studies

Equilibrium binding studies were conducted to examine the enhanced loading potential of the hydrogels for antihistamine (ketotifen fumarate) and antibiotic (chloramphenicol). The gels were washed with DI water until the therapeutic molecule and unreacted monomers could no longer be detected by spectroscopic monitoring. Recognitive and control gels were then dried at room temperature for 24 hours, followed by vacuum drying (T=30 °C, 28 in. Hg vacuum), until no change in dry weight was observed (i.e., less than 0.1 weight percent difference). The gels were placed in concentrated solutions of therapeutic molecule and gently agitated on a Stovall Belly Button Orbital Shaker. After 72 hours, the bound concentration in the gel was determined by mass balances. Dynamic binding studies were conducted to determine the time needed for equilibration.

Kinetic release studies were conducted in DI water, artificial lacrimal fluid (6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, 0.084 g/L $CaCl_2.2\ H_2O$, pH 8), and lysozyme (1 mg/ml) in artificial lacrimal fluid. Gels which had been loaded were placed in 30 ml of DI water, and the solutions were continuously agitated with a Servodyne mixer (Cole Palmer Instrument Co.) at 120 rpm. Release of drug was monitored using a Synergy UV-Vis/Fluorescence/Luminescence Spectrophotometer (Biotek). Absorbances were recorded for three samples, averaged, and corrected by subtracting the relevant controls. Solutions were replaced after each reading.

Dynamic Swelling Profiles

Equilibrium weight swelling studies were conducted under ambient conditions in both DI water and a concentrated ketotifen fumarate solution in DI water (0.5 mg/ml) on recognitive and control networks. Dynamic weight swelling ratios were plotted as a function of time until equilibrium. Weight swelling ratios at time *t*, q, were obtained by the ratio of the swollen weight to the dry weight.

Mechanical and Optical Analysis

The mechanical properties of the hydrogels such as elastic and viscous moduli were tested via dynamic mechanical analysis (Seiko Exstar 6000 DMS-6100). Specifically the storage and loss moduli were calculated in swollen and dry lenses and compared to literature values of conventional lenses. The rheological behavior of the hydrogels when dry and fully swollen were measured in triplicate at 37˚C, applying initial tension of 1 mN and angular frequencies of 0-100 Hz, with corrected offset load. The temperature dependence of the elastic and viscous moduli of hydrogels (G' and G'' respectively) and of damping factor values were recorded for an angular frequency of 5 Hz, by subjecting the gels to a temperature sweep of 20-160˚C. Optical transparency studies were performed via light transmission studies at 600 nm and percentage transmittances were calculated.

RESULTS, DISCUSSION, & CONCLUSIONS

Enhanced Loading of Gels Formed With Multiple Functionality

We hypothesized, based on a biomimetic analysis of structural biology, that multiplicity and type of functional chemistry in a pre-polymerization complex, would prove critical in maximizing loading. This would provide a higher probability of ligand docking or memory sites at the molecular level, with relevant multiple chemical functionality for optimal non-covalent interactions. Monomers were then selected based

on the fact that residues such as aspartic acid, lysine, arginine and tyrosine form the docking site in the H_1-receptor, and by matching the side chain chemistry. Fig 1a shows 2 times improvement in the loading of ketotifen by the poly(AA-co-AM-co-HEMA-co-PEG200DMA) networks over control networks. As hypothesized, with the most biomimetic formulation that included four functional monomers, a significant (6 times) increase in loading was observed over the control network and 3 times increased loading over the networks containing two or three functional monomers (Fig 1b).

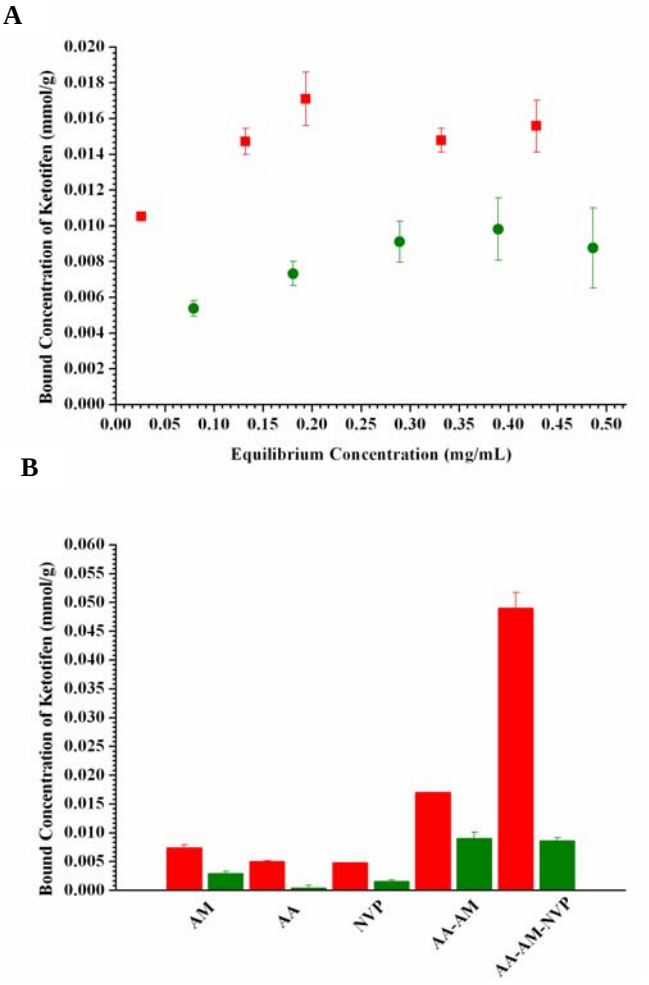

Figure 1- (A) Ketotifen equilibrium binding isotherm in water for poly(AM-co-AA-co-HEMA-co- PEG200DMA) networks with a crosslinking percentage of 5 mole %. N=3, and T=25˚C. Recognitive network (■) and Control network (●). (B) Enhanced loading of ketotifen for multiple monomer gels for poly(n-co-HEMA-co-PEG200DMA) networks at 0.4 mg/mL loading concentration. Functional monomer (n) is AA, AM, NVP, AM-AA, or AM-AA-NVP. Recognitive network (■, left) and Control network (■, right).

In order to verify that CBIP, and not increased porosity or surface area of the gel is responsible for the enhanced loading properties, we conducted dynamic penetrant studies in deionized water and 0.5 mg/ml concentrated ketotifen solution. Recognitive and control networks, for each formulation presented were statistically the same and 40-45% of the swollen gels at equilibrium was water. This indicates that the comfort of wearing and oxygen permeability of these gels is in agreement with conventional lenses synthesized from these monomers, as there is a direct correlation between water volume fraction and oxygen permeability. Dynamic mechanical analysis of the hydrogels showed comparable storage and loss moduli, and damping factors to that of conventional contact lenses . The gels were optically clear and had comparable transmittance values to those of conventional lenses.

Extended Release of Therapeutic

Dynamic release profiles, performed in artificial lachrymal solution to mimic ocular physiological conditions, demonstrated extended release of a viable therapeutic concentration of ketotifen. Release conditions were performed under infinite sink conditions in order to maintain a maximum concentration driving force. We confirmed that release rates can be tailored via type and amount of functionality (Fig 2a). The most structurally biomimetic network, poly(AA-co-AM-co-NVP-co-HEMA-PEG200DMA), exhibited an extended release profile for a duration of 5 days (80% of drug was released in approximately 4 days). To investigate the effect of protein on dynamic release, we chose lysozyme as a model protein since it is the largest protein component in tear fluid. Figure 2b highlights the poly(AA-co-AM-co-HEMA-co-PEG200DMA) network release profile in artificial lachrymal solution with lysozyme, which leads to a factor of 5 increase in the duration of release. Release studies were also conducted with gels loaded with chloramphenicol, which showed controlled release for 10 hours (Figure 2c).

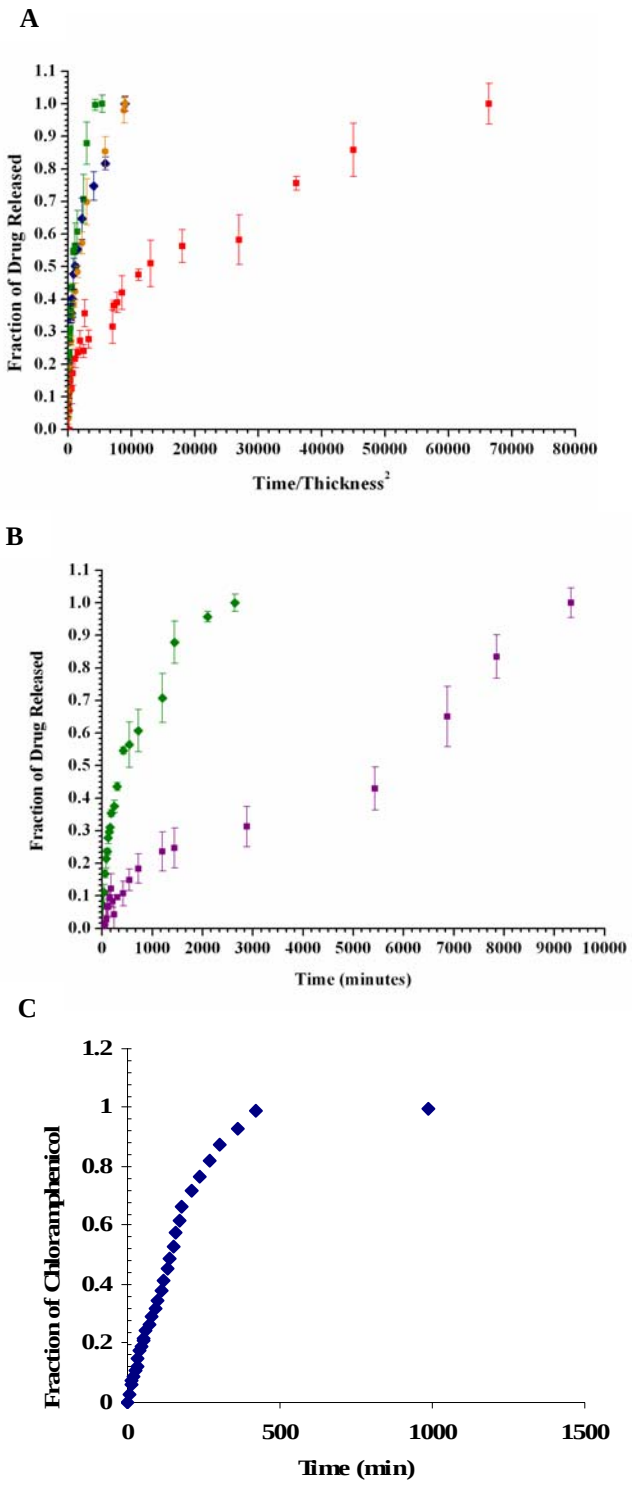

Figure 2 - (A) Tailored release profiles of therapeutic contact lenses for poly(n-co-HEMA-co-PEG200DMA) networks in artificial lachrymal fluid at 25˚C, where n is AM (●), AA (♦), AA-AM(■), and NVP-AA-AM (■) recognitive networks respectively. Fraction of drug released is the ratio of mass released at an instantaneous time t and that at infinite time. The abscissa is time normalized to the square of thickness, (due to slab geometry) as the thicknesses of the gels differed; 700 µm (●,♦,■) and 400 µm (■). (B) Introduction of lysozyme (1 mg/ml) to an artificial lachrymal solution (■) versus artificial lachrymal solutions (♦) to observe effects of proteins on release profiles of a poly(AA-AM-co-HEMA-co-PEG200DMA) network (C) Chloramphenicol kinetic release profile of contact lenses for poly(AM-co-HEMA-co-PEG200DMA) (♦)

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