OPHTHALMIC ANTIHISTAMINE DELIVERY VIA RECOGNITIVE CONTACT LENSES FOR ALLERGIC RELIEF

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INTRODUCTION

The tenets of biomimesis have been exercised to create novel biomaterials for the controlled release of histamine antagonists on the surface of the eye for the treatment of allergic conjunctivitis. The approach promises to revolutionize the field of ophthalmologic drug delivery for the treatment of conjunctivitis since ocular allergy affects at least one-third of the American population (i.e., roughly 80 million people).

Treatment options for allergic conjunctivitis primarily consist of oral antihistamines and topical treatments. Ocular bioavailability of topical drugs is very poor (typically less than 7% is absorbed by the eye) and there are inherent problems such as patient non-compliance (due to irregularity of recommended dosage administration) **[1]**. Controlling and tailoring the release of antihistamines via novel recognitive contact lenses with significantly enhanced partitioning can solve these problems with increased bioavailability, less irritation to ocular tissue, and reduced ocular and systemic side effects **[2]**. This would remove therapeutic administration from the end-user's hands and circumvent non-compliance issues such as over/underdose. The technology also promises to have a huge impact as conventional contact lenses cannot be loaded with a therapeutic concentration of drug. Also, with conventional topical treatments, the formulation contains significant amounts of drug due to poor bioavailability, which prohibits wear of contact lens during treatment.

Enhanced drug partitioning in hydrogels can be achieved by configurational biomimetic imprinting (CBIP) techniques which involve the formation of a pre-polymerization complex between the template molecule and functional monomers **[3-5]**. Recognitive networks were synthesized on the basis of the non-covalent interactions found in histamine docking sites. Monomer were chosen by providing ligand-binding functionalities similar to those found in histamine-H_1 receptor interactions.

Inspired by Nature, we have successfully synthesized and characterized enhanced loading networks for H_1 antihistamines using the fundamental principles of molecular recognition. The approaches and concepts can be extended to a wider biological spectrum in the design of novel, controlled and modulated delivery devices for biomolecules such as synthetic drugs, nucleic acids, enzymes and hormones. Furthermore, we believe that our science will inspire a wholly new approach, with contact-lens mediated ocular therapies for a number of diseases.

MATERIALS AND METHODS

Synthesis of Recognitive Networks

Acrylic acid (AA), acrylamide (AM), 2-hydroxyethylmethacrylate (HEMA), azobisisobutyronitrile (AIBN) and ketotifen fumarate were purchased from Sigma-Aldrich (Milwaukee, WI). Polyethylene glycol (200) dimethacrylate (PEG200DMA) was purchased from

Polysciences, Inc (Warrington, PA). Lysozyme was purchased was MP Biomedicals. All chemicals were used as received.

Hydrogels of differing compositions 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. Solutions were then allowed to equilibrate in darkness for 12 hours in order to facilitate and ensure non-covalent complexation at the molecular level. The solutions were then transferred to a MBRAUN Labmaster 130 1500/1000 glovebox, where they were 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 0.8 mm thick. The polymerization reaction occurred for ten minutes with light intensity, measured using a radiometer, equal to 40 mW/cm^2 (Dymax UV flood light) at a constant ambient 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. The gels were washed with DI water until ketotifen 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 ketotifen fumarate 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 at 268 nm by drawing 200 µL of solution into a 96-well Corning Costar UV-transparent microplate, and measurements were taken in 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.

Polymerization Reaction Kinetics

Kinetic studies were carried out in a Q-100 modulated differential photocalorimeter (DPC) (TA Instruments, New Castle, DE) at a constant light intensity of 40 mW/cm^2. Pre-polymerization solutions (10 µL) were weighed in aluminum hermetic pans and purged with nitrogen at a flow rate of 40 ml/min, in order to prevent oxidative inhibition. They were then allowed to equilibrate at 35°C for 15 minutes, before opening the shutter of the UV light source (Novecure 2100, Exfo, Mississaugu, Canada) for 12 minutes. Isothermal conditions were maintained throughout the experiment. The DPC records the heat of reaction, from which the rates of polymerization and conversions were calculated using average molecular weight and theoretical enthalpy of acrylate and methacrylate double bonds.

In a typical experiment, pre-polymerization solutions with 0, 0.25, 0.5 and 1 mole percent ketotifen were prepared and allowed to equilibrate for 12 hours. In all studies, the rates of polymerization were determined for five samples and averaged.

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.

RESULTS

Enhanced Loading and Performance of Multiple Monomer Pre-Polymerization Mixtures

We hypothesized that gels composed of multiple functional monomers would outperform those composed of single functional monomers. This would better mimic the docking site of histamine at the molecular level providing all the relevant functionality necessary for non-covalent interactions. We have proved that loading properties of gels are improved with multiple monomer mixtures (Fig 1). Gels of multiple complexation points with varying functionalities outperformed the gels formed with lesser functional monomer and showed the highest maximum bound of ketotifen. Equilibrium binding isotherms for Poly(Aam-co-AA-co-HEMA-co-PEG200DMA) networks demonstrate enhanced loading with a factor of 2 times increase in the partitioning of drug compared to conventional networks (i.e., gels prepared without template and comparable to existing contact lenses) depending on polymer formulation and polymerization conditions (Fig 2). Poly(Aam-co-HEMA-co-PEG200DMA) networks demonstrated a factor of 2 or 100% increase in the partitioning of drug compared to control networks with higher bound amounts. While the overall drug bound is approximately 33% higher compared to a similar network with made with acrylic acid, the overall increase in partitioning compared to control is lower. The poly(AA-co-HEMA-co-PEG200DMA) networks show a factor of 6 times increase in the partitioning of ketotifen. ***For all systems, a dramatic increase in the amount of loaded drug has been demonstrated.***

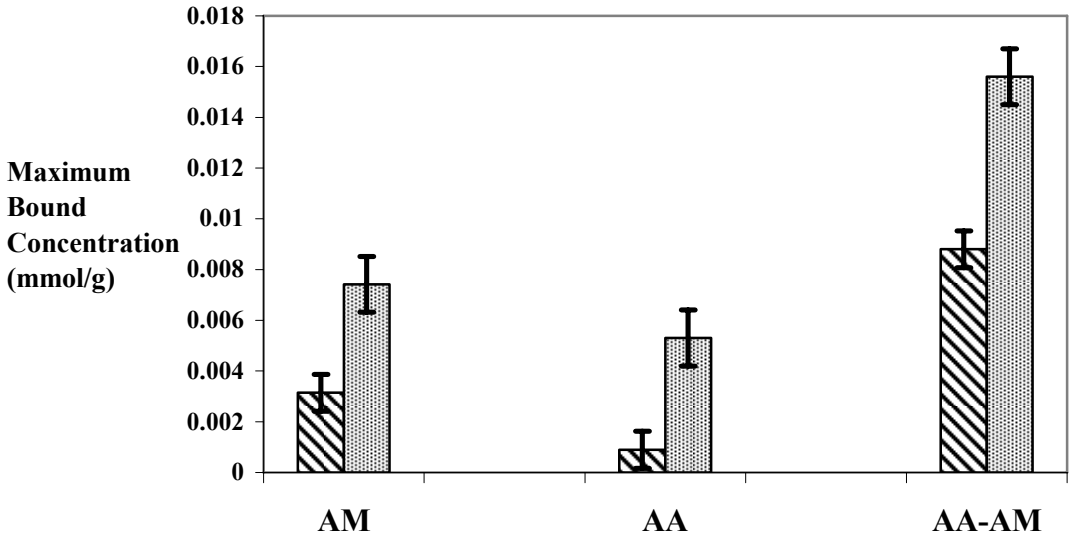

Figure 1: Enhanced Loading of Multiple Monomer Gels for Poly(functional monomer-co-HEMA-co-poly(ethylene glycol)200 dimethacrylate) Networks. Functional monomer is acrylic acid, acrylamide, or an equal mole mixture of both.

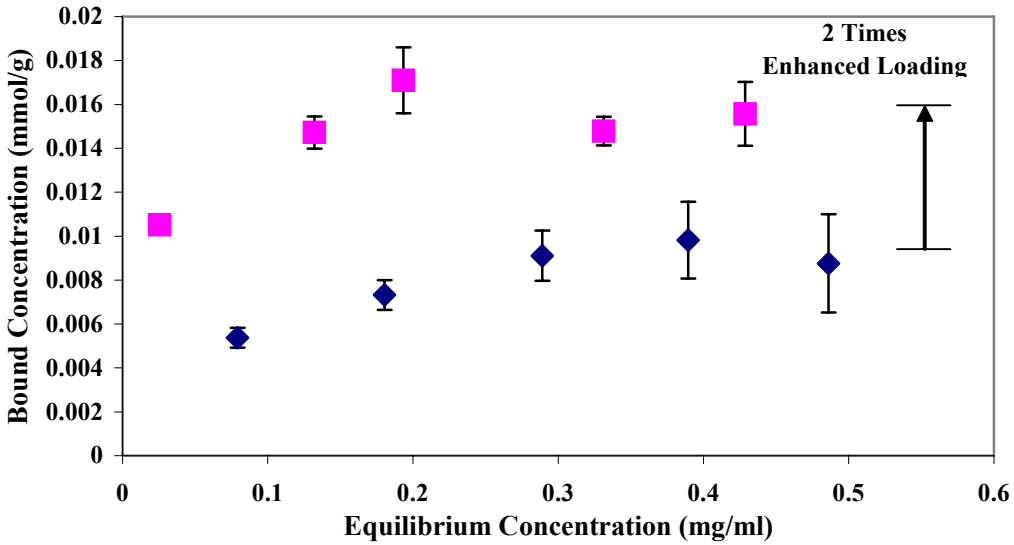

Figure 2: Ketotifen Equilibrium Binding Isotherm in Water for Poly(acrylamide-co-acrylic acid-co-HEMA-co-poly(ethylene glycol)200 dimethacrylate) networks. Recognitive network (■) and Control network (♦)

Release Profiles

Dynamic release profiles in deionized water (data not shown), artificial lacrimal solution (data not shown), and an artificial lacrimal and lysozyme solution (data not shown) demonstrated extended release of a viable therapeutic concentration of drug. As a representative example, the gels with acrylic acid as co-monomer released over 7 to 8 days. Considering the recommended ocular dosage (0.12 mg/day for an 80 kg. man) and the fact that our gels are slightly larger than conventional contact lenses due to ease of experimental procedure, our gels can deliver a therapeutic dosage. Release profiles were fit for Fickian diffusion in slab geometry. The profiles indicated that template release was purely dependant on diffusion. To investigate the effect of protein on dynamic release, we chose lysozyme as the model since it is the largest protein component in tear fluid. Dynamic release studies in lysozyme and artificial lacrimal solution were also observed to be Fickian. ***The formulations can be tailored to release therapeutic concentrations of drug over longer periods.***

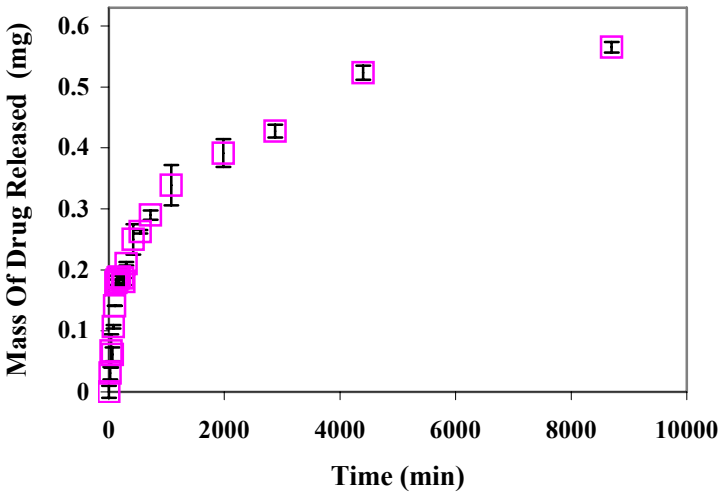

Figure 3: Release Data in Artificial Lacrimal Solution for Poly(acrylamide-co-acrylic acid-co-HEMA-co-poly(ethylene glycol)200 dimethacrylate) Networks. Recognitive Network (▫).

Polymerization Kinetics

The rate of polymerization for a given conversion decreased for increasing mole percentage of template molecule in pre-polymerization monomer solution. The results indicate that the reaction rate for a given conversion decreases as one increases a molecule in the mixture that does not get covalently incorporated into the polymer chains. ***Thus, the formation of polymer chains and the enhanced partitioning due to the configurational biomimetic effect may be related to the propagation of polymer chains.*** The template molecule poses physical constraints to free radical and propagating chain motion and hence effectively lowers the rate of polymerization in the creation of ligand binding pockets. These results also show that CBIP is reflected at the molecular level

Swelling Profiles and Mechanical Properties

Equilibrium swelling studies in DI water and 0.5 mg/ml concentrated ketotifen solution (data not shown) indicated that recognitive and control networks were statistically the same and that 40% of the swollen gels is water, which indicates that the comfort of wearing and oxygen permeability of these gels is in concordance with conventional contact lenses. These studies indicated that CBIP, and not an increased porosity or surface area of the gel, is responsible for the enhanced loading properties. Further studies on the mechanical properties of the gels have shown comparable storage and loss moduli, glass transition temperatures and damping factors to that of conventional contact lenses (data not shown).

DISCUSSION

Ocular inflammation is epitomized by pruritus and rhinoconjuctivitis of ocular tissue. Pharmacological down-regulation of histamine action is possible by the local targeted delivery of antagonistic antihistamines. H_1-antihistamines such as ketotifen fumarate are hydrophobic drugs which downregulate the effects of histamine at the H_1-receptors on endothelial cells and respiratory smooth muscle, resulting in decreased vascular permeability, bronchodilation and decreased exudation of effector cells.

Biomimesis is the application of the fundamental laws of molecular recognition to design systems in order to address pressing problems in biology and medicine **[6, 7]**. Effectively designed configurational biomimetic imprinted polymer networks show enhanced loading by tuning the non-covalent interactions at the molecular level. An inspection of binding motifs in proteins enabled us to design ligand binding pockets based on the positioning and contributions of the amino acids to the non-covalent interactions. The techniques within our work are applicable to a wide range of biomolecules and recognitive networks, in which non-covalent interactions will direct recognition and enhanced loading. In particular, gels of multiple monomers outperformed those of single monomers, highlighting the contributions of each functionality to ligand binding.

Novel network design and synthesis, with a basis in current physiochemical components of commercially available hydrogel contact lenses, has a huge potential to affect the administration of a number of ocular therapies. This new class of recognitive intelligent materials is designed by incorporating motifs with structural and molecular homology to biological receptors and has a strong potential to impact the administration of a number of ocular therapies.

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