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Research Paper

Controlled Release of High Molecular Weight Hyaluronic Acid from Molecularly Imprinted Hydrogel Contact Lenses

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Purpose. Current dry eye treatment includes delivering comfort agents to the eye via drops, but low bioavailability and multiple administration continues to be a barrier to effective treatment. There exists a significant unmet need for devices to treat dry eye and for more comfortable contact lenses.
Methods. Using molecular imprinting strategies with an analysis of biology, we have rationally designed and synthesized hydrogel contact lenses that can release hyaluronic acid (HA) at a controlled rate.
Results. Delayed release characteristics were significantly improved through biomimetic imprinting, as multiple functional monomers provided non-covalent complexation points within nelfilcon A gels without altering structural, mechanical, or optical properties. The diffusion coefficient of 1.2 million Dalton HA was controlled by varying the number and variety of functional monomers (increasing the variety lowered the HA diffusion coefficient 1.5 times more than single functional monomers, and 1.6 times more than nelfilcon A alone).
Conclusions. HA can be delivered from a daily disposable lens at a therapeutic rate of approximately 6 μg/h for 24 h. This is the first demonstration of imprinting a large molecular weight polymer within a hydrogel and the effect of imprinting on the reptation of the long chain macromolecule from the structure.

KEY WORDS: biomimetic; comfort contact lenses; controlled drug delivery; dry eye; molecular imprinted hydrogel; therapeutic contact lenses.

INTRODUCTION

Keratoconjunctivits sicca, commonly referred to as "dry eyes", is a condition where the conjunctiva and cornea are not enclosed with a healthy amount or quality of tear fluid (1). Dry eye syndrome affects nearly 50 million people in the US to varying degrees. The disorder occurs when a patient's eyes are not adequately hydrated by the tears they produce, exposing the epithelia of their cornea and conjunctiva to desiccation. While ocular dryness is not immediately threatening to vision, the desiccation of the epithelia triggers a number of uncomfortable symptoms such as itching and burning of the eye, a sensation of grittiness, light sensitivity, excessive watering, blurred vision and inflammation, all of which can significantly affect the quality of a patient's life. Serious symptoms such as bacterial conjunctivitis, corneal ulcers, corneal perforation, and loss of vision have been reported.

The etiology of dry eye can be quite complex. Normal tear film envelops the ocular epithelium in a dynamically stable layer of fluid that continually thins and breaks under evaporation forces while being replenished via the lacrimal glands. Factors promoting premature tear break-up, and hence dry eyes, are hyperosmolarity of the tear fluid (2), environmental factors such as contact lens wear or air quality (3–5), congenital malfunction of tear glands, and autoimmune disorders such as Sjogren's syndrome (6). Factors promoting film stability are the lipid layer at the air-aqueous interface, the hydrophilic epithelial mucus that lowers the film surface tension and decreases evaporation, and the action of blinking, which replenishes and rebuilds the thinning tear film.

Ocular dryness triggered by contact lens-wear is known as contact lens-induced dry eye (CLIDE). The tear film is disrupted by the increased surface tension at the boundary of the lens. It has also been demonstrated that continued contact lens wear leads to decreased blinking rate, so the film is refreshed less often (7). Some studies show that the aqueous layer becomes more stable if the lens gets coated by mucins secreted by goblet cells in the ocular epithelium (8). The mucins make the lens surface more hydrophilic, lowering the aqueous-lens interfacial tension. In one survey, 76.8% of current contact lens users reported ocular dryness, with 26.8% reporting frequent-to-constant symptoms (9).

Most people with dry eyes apply rewetting solutions, lubricants, comfort agents, or artificial tears to their eyes via eye drops to increase the viscosity of the tears in the eye, slowing drainage and evaporation, and to increase the moisture of the ocular surface. But eye drops are inconve-

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nient because they need to be administered multiple times a day, have low bioavailability (as low as 5%), and the application is followed by a time period in which vision is blurred and normal activities must be interrupted. For example, some people need to apply drops over 9 times a day and their quality of life is severely impacted (10). Despite these disadvantages, there is a large market for eye drop formulations to treat dry eye.

Various macromolecules have been used in rewetting drops such as hyaluronic acid (HA), hydroxypropyl methylcellulose, carboxy methylcellulose, polyvinyl alcohol, polyvinyl pyrrolidone, polyethylene glycol, dextran, and polyacrylic acid, etc. With contact lens-induced dry eye, patients significantly decrease or stop contact lens use because of their symptoms (11).

HA has been used as a topically delivered artificial tear solution for over 20 years (12), and it has a proven track record for the treatment of ocular discomfort and dry eye syndrome (13–15). Two HA eye drop formulations that are currently on the market are VISMED® and AQuify™. Hyaluronic acid (HA) is an unbranched non-sulfated glycosaminoglycan composed of units of the saccharides D-glucuronic acid and D-*N*-acetylglucosamine (Fig. 1). It is typically administered in molecular weights of approximately one million Dalton and has a dimer subunit repeating group of 415 Da. The viscoelasticity of HA enhances the tear stability (16), and it slows tear removal (17) by decreasing the flow rate. HA is also mucoadhesive and interacts with ocular mucins when delivered to the surface of the eye. When a contact lens is present in the eye, HA may cover the lens as an "artificial mucin" and counteract the tear film destabilization that occurs in the presence of the lens. Because HA is hygroscopic, it retains water close to the ocular surface and reduces dehydration (18). It also encourages corneal wound healing (19) by promoting epithelial cell migration (20), and thus, HA has therapeutic properties.

A few devices currently on the market can treat dry eye. Lacrisert® (Merck) is a cellulose-based polymer insert placed in the lower eyelid to treat dry eyes (21), administered once-a-day and degradable. Ocular inserts are placed in the eye, deliver drug until depleted, and either degrade or are removed. Phosphorylcholine-coated contacts lenses for extended wear have also been produced that limit drying of the eye and increase biocompatibility by mimicking the tear film

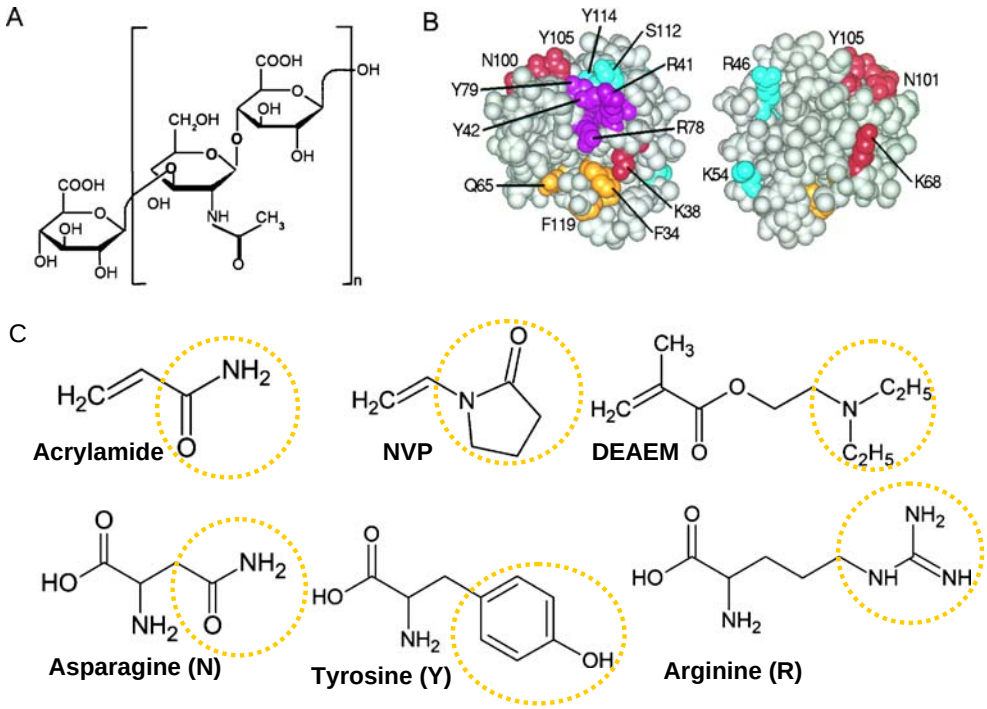

Fig. 1. Hyaluronic acid structure and biomimetic approach: comparison of functional groups on amino acids and monomers. **A** Hyaluronic acid is a long-chain molecule, specifically a polysaccharide consisting of repeating units of glucuronic acid and *N*-acetylglucosamine (dimer molecular weight is 415 Da). The glucuronic acid has a carboxylic group that deprotonates (pKa=4.8) (22) and non-covalently bonds with cationic monomers such as 2-(diethylamino)ethyl methacrylate (pKa=9.5) (23) and amino acids such as lysine and arginine. **B** Hyaluronic acid binding protein CD44, is a naturally found protein with a high affinity for hyaluronic acid. The amino acid residues important for binding are indicated (Fig. 1**B** from (24), used by permission from American Soc. for Biochemistry and Molecular Biology) (blue not important to HA binding, gold important for structural integrity, orange- and pink important and critical for HA binding where mutations significantly decrease or abolish HA binding, respectively. **C** For the biomimetic imprinting of hyaluronic acid, we selected acrylate and methacrylate monomers that bear chemical similarity to the amino acids found on the binding site CD44. Acrylamide and asparagine both have amide moieties, *N*-vinyl pyrrolidone and tyrosine have hydrogen bonding capability while 2-(diethylamino)ethyl methacrylate is positively charged, like arginine and lysine.

structure (25). Daily wear disposable lenses that release or have immobilized comfort agents are also on the market today (e.g., Focus Dailies with AquaRelease release polyvinyl alcohol (CIBA Vision, Inc.) (26) and 1-Day Acuvue Moist have immobilized polyvinyl pyrrolidone (Vistakon) (27). Recently, Van Beek and coworkers reported hyaluronic acid (HA) containing silicone hydrogels (28) and poly(2-hydroxyethyl methacrylate) gels (29) with HA immobilized as a wetting agent. The presence of crosslinked HA within the structure decreased protein adsorption significantly in both studies, which could lead to increased lens wettability and comfort due to less protein deposition. It is important to note that the HA was loaded via equilibrium partitioning and entrapping by crosslinking, and the release of uncrosslinked HA from the gels was also studied. In our work, published in 2007 (30), we were the first to engineer a therapeutic contact lens that releases the comfort molecule hyaluronic acid in a sustained and tailorable fashion with control of immobilization and release via molecular imprinting methods, which exploits multiple non-covalent interactions between the HA and the polymer chains to delay release.

The molecular imprinting technique provides a rational design strategy for the development of controlled release drug delivery systems. Imprinting within hydrogels has received increased attention in the last few years, and we direct the reader to the following reviews highlighting methods to imprint in hydrogels (31–33) and imprinting in drug delivery (34–37). To date, only two groups have successfully imprinted small molecular weight therapeutics in weakly crosslinked structures to produce hydrogel thin films for extended release contact lenses (38–41). While templates and polymers differ, Alvarez-Lorenzo and coworkers have explored optimizing the monomer/template (M/T) ratio (41) using one functional monomer, and Byrne and coworkers have increased the diversity of functional monomers at a fixed M/T ratio (38,39), leading to the highest template loading and delayed template release achieved to date (31). Imprinting is paramount to delaying release in these swollen hydrogels, which cannot use conventional strategies to delay drug transport from the equilibrium swollen polymer network. Imprinting has been shown to delay template transport from the polymer chains, and a tumbling hypothesis was recently proposed by our group analyzing one-dimensional template transport (42). Molecular imprinting creates macromolecular memory for the drug within the network and delays the transport of drug from the matrix via interaction of the drug with numerous functional groups organized within the network (42). The drug's heightened interaction with the memory pockets slows its release from the hydrogel despite comparable free volume within the polymer chains for drug transport (42). We direct the reader to the following articles discussing the impact of such systems in ocular drug delivery (43,44).

The size of the therapeutic and the size of the spaces available for diffusion with the polymer chains also influence drug release. Since HA is a long chain molecule its diffusion from the structure will be reduced compared to a small molecular weight molecule. The hypothesis in this work was that imprinting would also provide a level of control in the release of much larger molecules at a given network mesh size. The contribution of imprinting would become more pronounced as the network mesh size/therapeutic size ratio increased and was much greater than unity. The HA used in this work was approximately 1.2 million Dalton molecular weight.

MATERIALS AND METHODS

Biomimetic Approach: Hyaluronic Acid Binding Moieties

A biomimetic strategy was employed for the choice of functional monomers, an approach recently demonstrated to produce ketotifen fumarate releasing contact lenses (38,39). It is hypothesized that biomimetic imprinting will enhance the affinity of the HA within the hydrogel by introducing memory sites that have similar chemistry to binding sites in the HA-binding protein, a protein found naturally in the body. The increased affinity of the HA for the hydrogel will slow down its diffusion from the gel, leading to a more controlled release rate.

Within the human body, HA binds to various receptors, the most significant is the cell-surface glycoprotein CD44 shown in Fig. 1. Using molecular modeling and site-specific mutagenesis, researchers have identified the amino acid residues most responsible for the binding of CD44 to HA (39). Residues deemed critical for HA binding were tyrosine-42, arginine-78 and tyrosine-79. Residues considered important for HA binding were lysine-38, arginine-41, lysine-68, asparagine-100, asparagine-101 and tyrosine-105.

Based on this analysis, we sought FDA-approved acrylate monomers with functional groups that displayed similarities to the side chains of tyrosine, arginine, lysine, and asparagine. Tyrosine contains a 4-hydroxyphenyl group which features aromatic behavior with some hydrogen bonding capability. Arginine and lysine have amine groups which bear positive charges when protonated. Asparagine possesses an amide group for additional hydrogen bonding.

Acrylate and methacrylate monomers with similar chemical properties are acrylamide (AM), N-vinyl pyrrolidone (NVP) and 2-(diethylamino) ethyl methacrylate (DEAEM). AM has an amide group, like asparagine, and NVP, an aromatic lactam, can be seen as an analog to tyrosine for its aromaticity and hydrogen-binding capability. Finally, DEAEM is a cationic acrylate, and is similar to arginine and lysine because of its positive charge. The structures are shown in Fig. 1.

We hypothesized that these monomers, if incorporated into network, would non-covalently interact with HA and increase the affinity of the molecule for the hydrogel, thereby giving us an additional level of control over the release rate. The DEAEM was expected to form an ionic bond with the carboxylic groups on the glucuronic acid units, and the AM and NVP would hydrogen-bond with varied groups on both glucuronic acid and acetylglucosamine. The increased bonding would improve our ability to tailor the release kinetics of HA, and enable us to design the optimal formulation for an extended release signature.

We hypothesized that when the functional monomers are added to the hydrogel formulation and allowed to equilibrate, the various molecules would spatially arrange relative to one another in a low energy configuration. Such a configuration

favors electrostatic and polar interactions between the monomers and the HA, much like the interactions between amino acids and HA in the CD44 binding site. When the gel is crosslinked the monomers are immobilized in these favorable configurations, flexible sites within the network with stronger affinity for HA are created, as compared to areas with the same chemical composition but a random molecular configuration.

Synthesis of Hydrogel Contact Lenses

Biomimetic, soft contact lenses were produced using functional monomers and a commercial lens formulation known as nelfilcon A (45). Nelfilcon A contact lenses are daily disposable lenses (although such lenses have been tested with wear over for multiple days) (46). Nelfilcon A hydrogel lenses consist of biocompatible polyvinyl alcohol (PVA) macromers crosslinked into a network structure. PVA contains hydroxyl (OH^-) groups attached to a repeating polymeric backbone in the 1,3 position. It is synthesized by the acid hydrolysis of polyvinyl acetate. The 1,3 hydroxyl groups are positioned to undergo cyclic acetal formation upon reaction with aldehydes, and this is a highly useful mechanism for the attachment of moieties necessary for hydrogel formation.

The synthesis of PVA macromers of nelfilcon A lenses was performed by CIBA Vision, Inc. (Duluth, GA) according to a two step procedure (26). First, a diacetal with acrylate functionality, N-acryloylaminoacetaldehyde dimethylacetal (NAAADA), was synthesized by reacting aminoacetaldehyde with acryloyl chloride in a low-temperature alkaline aqueous solution. After neutralization and extraction, the crude product was purified through molecular distillation. In the second step, PVA was transacetylized with NAAADA resulting in PVA chains with a well controlled number of pendant crosslinking acrylamide groups per macromer chain (45), as shown in Fig. 2. A number of reactions were taking place at this acid catalyzed stage: the crosslinker's acetal was hydrolyzed to aldehyde, the aldehyde reacted with the PVA, and some acetate groups remaining on the PVA from its synthesis were converted to hydroxyl groups. Atmospheric oxygen was used as the stabilizer for the acrylamide. The reaction was quenched by neutralizing with alkali. The polymer was then purified by ultrafiltration to the desired purity and concentration. A photoinitator, Irgacure 2959, was then added to the purified polymer.

To prepare a pre-polymer mixture, 5 g of the nelfilcon A formulation, containing the modified PVA macromer (CIBA Vision, Inc.), was mixed with 32.5 mg of hyaluronic acid sodium salt (*Streptococcus equi*, Fluka, MW~1.2 million Dalton) in a 15 mL centrifuge tube (the ratio of 6.5 mg HA/g nelfilcon A was the same for all gels studied). The functional monomers acrylamide (Aldrich), N-vinyl pyrrolidone (Polysciences, Warrington, PA) and 2-(diethylamino) ethyl methacrylate (Aldrich) were added to prepare imprinted hydrogels. The mixture was repeatedly stirred, centrifuged (30 min to 1 h at a time, a minimum of four times), and rested overnight to dissolve the HA in the pre-polymer until homogeneous. The mixture was finally centrifuged for 5 to 10 min to remove air bubbles.

Formulations are identified by their total functional monomer content (as a percentage-by-mass of the pre-polymer mixture before addition of HA) and by the relative proportions of each type of functional monomer [AM:NVP:DEAEM]. The formulation 0.125% [0:0:1] contains 0.125% functional monomer by mass, and all of the functional monomer is DEAEM. Similarly, 0.125% [1:1:2] contains 0.0313% by mass AM, 0.0313% by mass NVP, and 0.0624% by mass DEAEM, with the three functional monomers adding up to 0.125% by mass. The formulation 0.125% [1:1:0] consists of 0.0625% AM and 0.0625% NVP. The formulation 0.05% [1:1:2] consists of 0.0125% AM, 0.0125% NVP, and 0.025% DEAEM. Finally, 0.25% [1:1:2] contains 0.0625% AM, 0.0625% NVP and 0.125% DEAEM. However, it should be noted that these are feed compositions and the final polymer composition may vary depending on the reactivity ratios of the corresponding monomers.

Molds for the hydrogels were prepared using glass and Teflon® spacers (Scientific Commodities Inc., Lake Havasu,

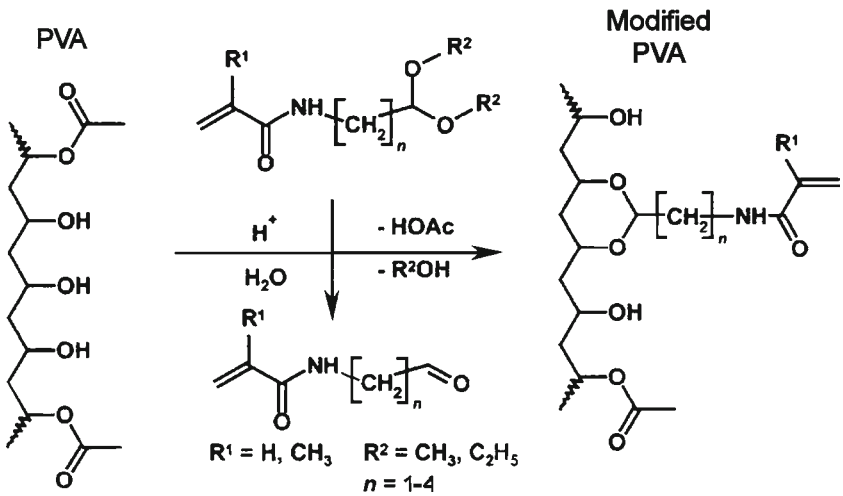

Fig. 2. Synthesis of nelfilcon A macromer from PVA. Nelfilcon A is synthesized using poly (vinyl alcohol) (PVA) as a starting material. *N*-acryloyl-aminoacetaldehyde-dimethylacetal (NAAADA) is reacted with the PVA through transacetylization under acidic aqueous conditions. The product is a PVA macromer with pendant double bonds at well-defined intervals. The product is purified by diafiltration (26).

AZ). Teflon® spacers 127 μm thick were constructed by cutting Teflon® sheets into 2×1.5 in. frames with a 1×1 in. central space. Spacers were affixed to 2×1.5 in. microscope slides. Between 125 and 200 mg of the prepolymer mixture was pipetted into the central space carefully to avoid the introduction of air bubbles, and the mold was closed by placing a second microscope slide on top, sandwiching the prepolymer between the slides and within the spacer, and then clamped. This produced a polymer film of 127 μm in thickness. Hydrogel lenses were also produced with curvature using contact lens molds (CIBA Vision, Inc.).

The mold was exposed to ultra-violet light from a UV light source (Novacure 2100, Exfo, Mississauga, Canada). The intensity of delivered light was 10.5 mW/cm^2 measured by radiometer (International Light IL400A, Peabody, MA). Duration of exposure was 15 s for hydrogels without functional monomers, and 45 s for hydrogels with functional monomers. The exposure times were determined with a Q-100 modulated differential photo calorimeter (TA Instruments, New Castle, DE), measuring the reaction progression.

The mold was opened and the hydrogel was covered by a small volume (2 to 5 mL) of water to soften the material. After 5 min, the hydrogel was peeled from the mold and cut into a disk with a cork borer (size no. 4, diameter 14 mm) for films without curvature.

To prepare hydrogel strips for tensile studies, a Teflon® spacer was cut to an inner space of dimensions 6×3 cm. Crosslinking took place under a UV light source (Dymax UV Flood Light, Torrington, CT) at an intensity of 11 mW/cm^2. The hydrogel was cut with a clean blade to strips 6 to 10 mm wide.

Dynamic HA Release Studies and Analysis of Release

Directly after lens synthesis, dynamic release studies were conducted on the hydrogels to measure and characterize the release of HA. Prepared lenses were placed in 50 mL centrifuge tubes (in triplicate) with 20 mL of artificial lacrimal solution (6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, 0.084 g/L $CaCl_2.2H_2O$, pH 8 (47)) and incubated at 35°C on an orbital shaker (Stovall Life Sciences, Greensboro, NC) at a rotation speed of 30 rpm. After measured time intervals, the lenses were extracted and deposited into fresh lacrimal solution previously incubated at release conditions. The samples were stored at 4°C until assayed with a sandwich HA ELISA assay kit (Corgenix, Denver, CO). The assay kit had a detection range between 20 to 800 ng/mL of HA, and some samples were diluted to prevent signal saturation.

To determine the diffusion coefficient and order of release of HA from this hydrogel, we applied Fick's second law of diffusion by modeling the lens as a slab and assuming one-dimensional diffusion (because aspect ratio of the planar lens was greater than 10 to 1) (48). Analysis shows that during early fractional release ($M_t/M_\infty<0.65$), the relationship between the fractional mass of HA released (M_t/M_∞) and the duration of release (t) can be described by the following equation:

$$\frac{M_t}{M_\infty} = 4\left[\frac{Dt}{\pi L^2}\right]^{\frac{1}{2}} \tag{1}$$

The fractional HA release, M_t/M_∞, was plotted versus $(t/L^2)^{1/2}$ for each release profile and the diffusion coefficient, D, was calculated by setting the slope equal to $4(D/\pi)^{1/2}$. The order of release is $|n\text{-}1|$, and n is equal to the slope of Log $[M_t/M_\infty]$ versus Log $[t]$ (e.g., for planar systems, $n=0.5$ describes Fickian behavior indicating diffusion controlled drug release and $n=1.0$ describes zero-order or case II transport).

Hydrogel Swelling and Structural Studies

After release in lacrimal solution, hydrogel lenses were dried in air for a minimum of 24 h and then dried in a vacuum oven at 30°C and 28 in. Hg until the weight change was less than 0.1% (at least 5 days). The gels were then weighed in air and in heptane, a non-solvent, using a microbalance (Sartorius, Goettingen, Germany). The lenses were equilibrated in DI water and the fully swollen lenses were weighed in air and in heptane. The gels were weighed after removing the gels from the swelling media and blotting with absorbent, lint-free tissue to remove excess surface water.

Swollen lenses without HA were synthesized and weighed after equilibrating in water. Swelling studies were also conducted on lenses containing HA. The equilibrium weight swelling ratio was obtained by taking the ratio of the swollen weight to the dry weight. The equilibrium volume swelling ratio, Q, was calculated as follows:

$$Q = \frac{1}{\upsilon_{2,S}} = \frac{V_{2,S}}{V_{2,d}} \tag{2}$$

where $V_{2,S}$ is the swollen gel volume at equilibrium, $V_{2,d}$ is the volume of the dry polymer, and $\upsilon_{2,s}$ is the polymer volume fraction in the swollen state (49). The volume of the gel in the swollen or dry state was obtained by using Archimedes buoyancy principle. Hydrogels in the relaxed state were synthesized and removed from the mold directly after polymerization without exposure to water, then weighed immediately. These were again weighed after dehydration.

Hydrogel structural analysis was obtained by swelling and tensile experimental studies. Using the theory of rubber elasticity (50–52), the average molecular weight between crosslinks ($\overline{M}_c$) can be calculated. Since the imprinted gel was prepared in the presence of solvent, Eq. 3 is used (49),

$$\tau = \left(\frac{RT}{\bar{v}\overline{M}_c}\right)\left(1-\frac{2\overline{M}_C}{\overline{M}_n}\right)\left(\alpha-\frac{1}{\alpha^2}\right)\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} \tag{3}$$

where τ is the normal stress applied, α is the elongation ratio, $\upsilon_{2,r}$ is the polymer volume fraction in the relaxed state directly after polymerization (in this case, $\upsilon_{2,r}/\upsilon_{2,s}\sim1$), R is the ideal gas constant or 8.314472 cm^3 MPa K^{-1} mol^{-1}, T is the temperature of experimental conditions, v is the specific polymer volume (0.909), and $\overline{M}_n$ is the number average molecular weight of uncrosslinked polymers. This model allowed us to measure the relationship between the normal stress applied and the polymer elongation ratio to calculate the average molecular weight between crosslinks.

The slope of τ versus $\alpha-1/\alpha^2$, obtained from the tensile tests, is the shear modulus and enables us to calculate the molecular weight between crosslinks.

Stress-strain data was obtained by performing tensile studies on a dynamic mechanical analyzer (TA Instruments, Wilmington, DE). Hydrogels prepared in strips (in triplicate)

were mounted on a dynamic mechanical analyzer (RSA III, TA Instruments, New Castle, DE) at a gauge length of 30 to 35 mm, and extended at a constant rate of 4 mm/min. The gels were fully hydrated through the experiment, and hydration was maintained with an aerosol diffuser.

Using the molecular weight between crosslinks, we calculated the size of the mesh between crosslinked hydrogel chains. The mesh size or correlation length, ξ, which is defined as the linear distance between two adjacent crosslinks, is related to the molecular weight between crosslinks according to Eq. 4,

$$\xi = \upsilon_{2,s}^{-\frac{1}{3}} \left(\frac{2C_n \overline{M}_c}{M_r} \right)^{\frac{1}{2}} l \tag{4}$$

where C_n is the Flory characteristic ratio, l is the length of the bond along the polymer backbone (which is equal to 1.54 Å for vinyl polymers), and M_r is the molecular weight of repeating units from which the polymer chains are composed.

HA Heat Stability and Optical Transmission Studies

The hydrogels were subjected to simulated sterilization conditions to determine if the release characteristics would be affected. Hydrogels were prepared with 6.5 mg HA/g nelfilcon, no added monomers, and placed in 2 mL microcentrifuge vials with 6.5 mg/mL solution of HA in DI water to prevent partitioning out of the HA. The vials were heated to 120°C for 40 min and then cooled in a room temperature water bath. The lenses were removed from the vials, blotted to remove excess HA from the surface, and studied for their release kinetics.

The effect of heat conditions on HA solution was also assessed. In 2 mL microcentrifuge vials, 1 mL samples of HA solutions of 500 ng/mL and 10 μg/mL were heated to 105°C and 121°C respectively. The 500 ng/mL samples were heated for 0, 5, 30 and 60 min while the 10 μg/mL samples were heated for 15, 30, 45 and 60 min. The sample vials were quenched in a room temperature water bath and assayed with an ELISA assay kit for HA.

Optical transmission studies were conducted by cutting small diameter films and placing in the bottom of a 96-well plate where absorbance valued were measured via spectrophotometric monitoring (Biotek, Winooski, VT). All films were fully hydrated and tested at wavelengths of visible light (380 to 780 nm). The absorbance value of each well in air was calculated and subtracted from the data. Percent transmission values were calculated from the absorbance data.

RESULTS AND DISCUSSION

Non-imprinted hydrogels demonstrate a concentration dependent or Fickian release profile. Fig. 3 shows the cumulative mass of HA released from the hydrogel over a 5 day period. The release rates can be classified into three general zones. Initially HA is released over the first 6–10 h at a rate of around 12 μg/h (A). The intermediate region from 10 to 30 h indicates a nearly linear release profile, delivering HA at a rate of approximately 4 μg/h (B). After approx. 40 h, the release rate gradually decreases until very low amounts are releasing for the last 3 days (C). The calculated diffusion

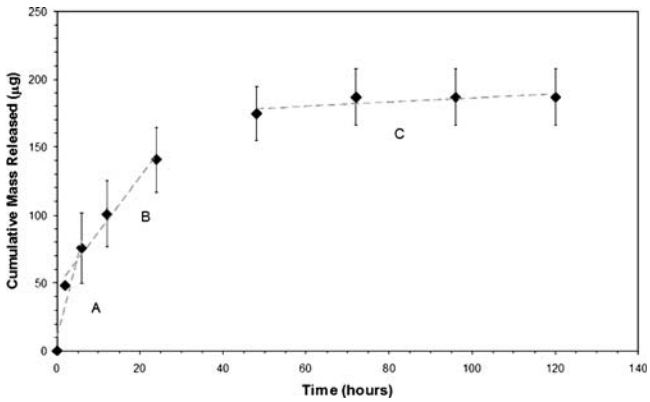

Fig. 3. Cumulative release of HA from Non-imprinted Nelfilcon A hydrogel. The pre-polymer formulation used to make this hydrogel contained only the nelfilcon A macromer and hyaluronic acid (6.5 mg HA/g nelfilcon A). The release profile demonstrates Fickian kinetics over 5 days, with three distinct release rates. The initial rate (**A**) lasts about 6 to 10 h with a release rate of ~12 μg/h. The intermediate region (**B**) demonstrates a nearly linear release profile, delivering ~4 μg/h. After about 2 days (**C**), the release rate significantly tapers off until it is negligible.

coefficient of HA for the release profile shown in Fig. 3 is $(5.69 \pm 0.005) \times 10^{-10}$ cm^2/s, and the order of release is 0.61 ± 0.02. We have produced non-imprinted lenses with varying amounts of HA and analyzed this data in determination of the diffusion coefficient and it appears that the diffusion coefficient is independent of the concentration of HA in the hydrogel, and the total released amount of HA depends on the concentration incorporated into the hydrogel.

To compare the release rate of HA from these hydrogel lenses with the therapeutic regime of HA eye drops, we considered the therapeutic regime of topically delivered HA artificial tear eye drops. In particular, we examined AQuify® Long-Lasting Comfort Drops, a 0.1% solution of HA. According to the package insert, the recommended dose is two drops up to 3–4 times daily. The volume of a typical eye drop is 20 μL (53) so if drops are delivered four times a day, the delivered dose would be 40 μg of HA every 6 h or 6.67 μg/h. As the bioavailability of eyedrops is much less than 100% due to normal removal mechanisms, it appears the hydrogel lens can deliver therapeutic or near therapeutic amounts of HA over the first couple of days. However, by incorporation of functional monomers to the system we may be able to exert an additional level of control over the diffusion rate and tailor the release profile.

It is important to note that the diffusion coefficient of HA through the swollen hydrogel is predominantly influenced by two factors. First, the hydrogel network presents a steric barrier to the Brownian movement of particles through the solvent. For a particle to diffuse through a hydrogel, it needs to pass through the open space or mesh between crosslinked polymer chains. The smaller the mesh size is, the slower the diffusion of the particle. Second, a long-chain molecule diffuses differently than a simple particle, behaving like a series of particles joined together. The chain-like nature of HA restricts the path that each constituent "particle" can pass through to diffuse through the chain—each must follow the one in front of it. The motion of a long-chain molecule through a hydrogel mesh can be described by a reptation

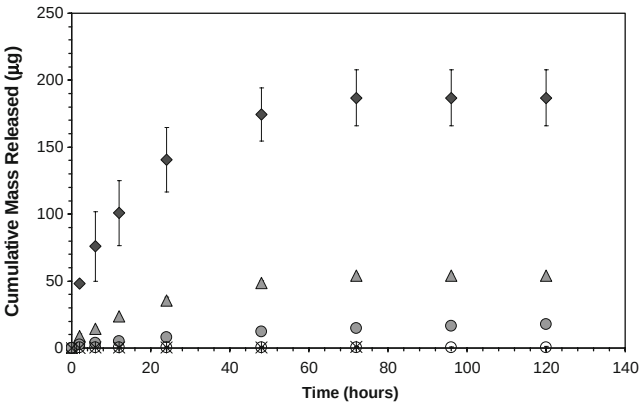

Fig. 4. Tailorable release of HA from imprinted nelfilcon hydrogels with varying percent functional monomers. Cumulative dynamic release studies were conducted on a series of hydrogels containing different %-by-mass of functional monomers: 0.125% [1:1:2] (▲), 0.25% [1:1:2] (●), 1% [1:1:2] (×) and 5% [1:1:2] (○). A hydrogel made with pre-polymer containing no functional monomers is also shown (♦). Increasing the %-by-mass of functional monomers in the hydrogel decreases the release rate and the cumulative released mass of HA. The release profile for a gel prepared with 0.05% functional monomer was statistically similar to the gel prepared without functional monomer.

model (49, 54). The HA chain does not slide through the mesh in one smooth motion. Rather the "tail" end of the polymer moves slightly forward to form a loop, and it is the loop that travels along the length of the chain, with none of the individual units traveling a large distance. By the time the loop reaches the "head" of the chain, the entire chain has undergone a small displacement through the hydrogel.

Using 5% of functional monomers as a starting point, we modified the nelfilcon A macromer sol by adding acrylamide (AM), *N*-vinyl pyrrolidone (NVP) and 2-(diethylamino) ethyl methacrylate (DEAEM) as functional monomers and formed crosslinked gels in the presence of HA (6.5 mg HA/nelfilcon A). The monomers AM, NVP and DEAEM were added in a ratio of 1:1:2 by moles, and together comprised 5% by mass of the pre-polymer solution before addition of HA.

Dynamic release studies revealed negligible amount of HA released in a 24 h period. Theorizing that the functional monomer content was too high restricting HA mobility, we reduced the monomer content to 1% by mass of the pre-polymer. Again, negligible HA was released.

To elucidate the mechanism by which the functional monomers were immobilizing the HA, we produced hydrogels with 1% by mass functional monomers and placed them in lacrimal solutions of varying pH values. We found that the 1% imprinted hydrogels released negligible HA in pH 8 solution but released significantly increased amounts in pH 12 solution. The excessive OH^- ions in the alkaline solution deprotonated the DEAEM, which reduced the electrostatic interactions with HA (in hyaluronate form with negative charges) and allowed the HA to be released from the hydrogel. However, as we are designing our lenses for ocular drug delivery, they need to release HA at ocular, physiological pH.

With evidence indicating that electrostatic (and potentially other non-covalent) interactions were responsible for the immobilization of HA in the hydrogel, we further reduced the functional monomer content of the hydrogel. We hypothesized that if each HA chain interacted with fewer functional monomers, it would undergo faster reptation and diffusion. Our hypothesis can be illustrated by making an analogy with fabric hook-and-loop fasteners such as Velcro™. Velcro™ consists of two fabric surfaces, one with minute loops and the other with hooks. If one isolated hook is connected to an isolated loop, the link between them can be severed with the application of a very small force. But if large fabric surfaces are brought together, the combined links between thousands of hooks and loops require much more applied force to dissociate. Similarly, if the HA is in contact with fewer affinity sites in the hydrogel, it encounters less resistance while diffusing through the network.

As we tailored down the functional monomer content of the prepolymer, we were successful in attaining HA release at ocular physiological pH from a hydrogel containing 0.25% functional monomers by mass. Release studies were also carried out on hydrogels with 0.125% monomers by mass. The cumulative release data is presented in Fig. 4. We see a clear trend that increasing the amount of functional monomer in the gel reduces the cumulative mass of HA released. Thus, HA release can be tailored by the amount of functional monomer in the network. The diffusion coefficients and orders of release for these profiles are summarized in Table I.

The decrease in mass of HA released indicates that as more functional monomers are incorporated into the hydrogel, less HA is able to diffuse out. That is, a larger fraction of the HA is immobilized inside the hydrogel. The fraction of HA that does diffuse from the hydrogel has diffusion

Table I. Diffusion and Release Order of HA from Imprinted Nelfilcon A Hydrogels Produced with Varying Functional Monomer Amounts and Monomer Proportions

	Diffusion coefficient cm^2/s ($x10^{10}$)	R^2	Order of release	R^2
Varying hydrogel functional monomer content at [1:1:2] ratio [AM:NVP:DEAEM]				
Nelfilcon A	5.689±0.005	0.99	0.61±0.02	0.99
0.05% Mass Functional Monomers	4.923±0.007	0.98	0.55±0.05	0.96
0.125% Mass Functional Monomers	3.553±0.004	0.99	0.47±0.02	0.99
0.25% Mass Functional Monomers	1.797±0.001	0.99	0.50±0.02	0.99
Varying hydrogel functional monomer diversity at 0.125% mass functional monomers				
Nelfilcon A [0:0:0]	5.689±0.005	0.99	0.61±0.02	0.99
[AM:NVP:DEAEM] [0:0:1]	5.306±0.015	0.96	0.66±0.01	0.99
[AM:NVP:DEAEM] [1:1:0]	4.824±0.010	0.99	0.57±0.03	0.97
[AM:NVP:DEAEM] [1:1:2]	3.553±0.004	0.99	0.47±0.02	0.99

coefficients dependent on the percent of functional monomer content in the hydrogel. The monomer content and diffusion coefficient are strongly correlated, as shown in Fig. 5.

Fig. 5 indicates that hydrogels with monomer content less than 0.36% are likely to allow HA chains to diffuse through the mesh, while diffusive release is not expected from hydrogels with monomer content much higher than 0.36%. This agrees with our data from the 1% and 5% monomer content hydrogels, which released negligible amounts of HA.

The above analysis demonstrates that the presence of the functional monomers tailors the diffusion coefficient of HA within the hydrogel. It is unclear at this point in the analysis whether the HA interacts with the hydrogel solely because of the electrostatic interaction between HA carboxylate groups and the protonated DEAEM, or whether the AM and NVP contribute to the interaction. To explore this, we produced a series of hydrogels all containing 0.125% functional monomers by mass in the pre-polymer mixtures, but containing varying proportions of AM, NVP, and DEAEM. The compositions are described in the methods section. Release studies were conducted on these hydrogels as described above, and the cumulative release profiles are presented in Fig. 6. The cumulative mass released tends to decrease as the proportion of DEAEM is increased. This suggests that DEAEM has a very strong affinity for HA and immobilizes a large fraction within the hydrogel.

However, if we compare the fractional release of HA from these hydrogels, another phenomenon with a distinct trend is revealed, as shown in Fig. 7. During the first 0.6 fraction of the cumulative release, the diffusion of HA from both 0.125% [1:1:0] and 0.125% [0:0:1] hydrogels is faster than diffusion from the 0.125% [1:1:2] hydrogel. In other words, even if the mass of functional monomers in the gel is kept constant, increasing the *variety* of functional monomers reduces the diffusion coefficient of the HA. The results indicate that an overall functional monomer percentage greater than 0.125% is needed to significantly alter the shape of the release curve. It may be highly possible to push this towards zero order release moving closer to 0.33% functional monomer percentage composition.

For comparison, we also juxtapose the cumulative release profiles of 0.25% [1:1:2] and 0.125% [0:0:1] in Fig. 8. They both contain the same amount of DEAEM, but the former contains an additional 0.0625%-by-mass each of AM and NVP. The cumulative released mass from both hydrogels is the same because the DEAEM immobilizes the same amount, but the diffusion coefficients vary because the two hydrogels have different diversity of functional monomers.

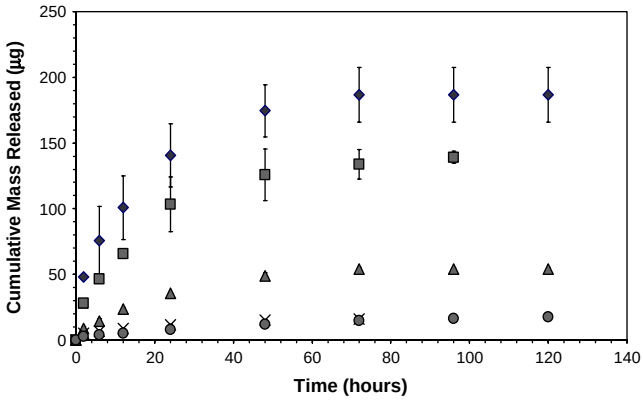

Fig. 6. Cumulative release of HA from imprinted nelfilcon hydrogels with different proportions of functional monomers. Dynamic release studies were conducted on a series of hydrogels containing different proportions of functional monomers [AM:NVP:DEAEM], all at 0.125%-by-mass of pre-polymer: [1:1:0] (■), [1:1:2] (▲), and [0:0:1] (×). For comparison we also plot the release profiles of hydrogels with 0.25% [1:1:2] (●) and hydrogels with no functional monomers (♦). Cumulative mass released decreases as the proportion (and hence total amount) of DEAEM increases.

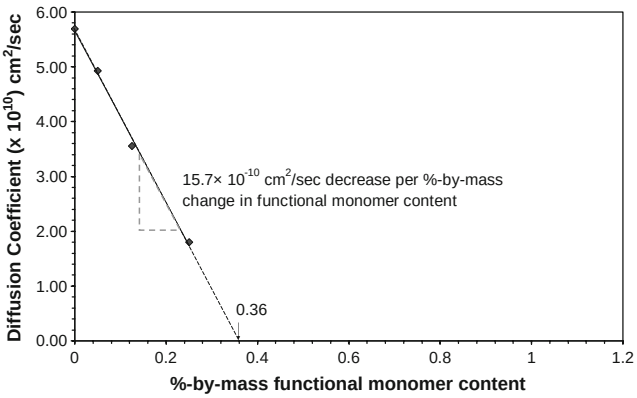

Fig. 5. HA Diffusion Coefficient versus Percent Functional Monomer Content. There is a strong inverse correlation between the diffusion coefficients of HA from hydrogels against the %-by-mass functional monomer content in the gel. The diffusion coefficient is expected to decrease to minute levels as the functional monomer content approaches 0.36%. This extrapolation agrees with our data that shows negligible release of HA from hydrogels with 1% [1:1:2] and 5% [1:1:2] functional monomer content. The slope indicates there is a 15.7×10^{-10} cm^2/s decrease per %-by-mass change in functional monomer content.

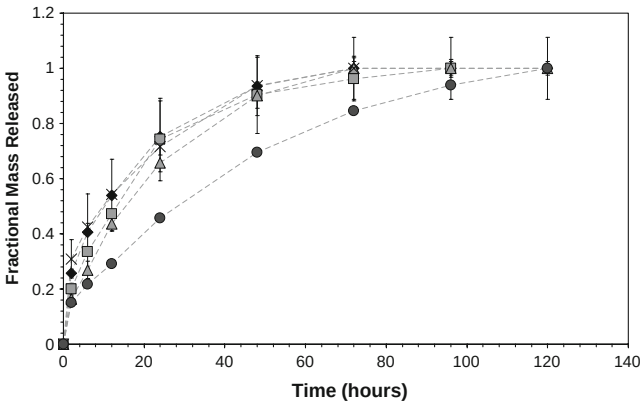

Fig. 7. Fractional release of HA from imprinted nelfilcon hydrogels with different proportions of functional monomers. Dynamic release profiles were normalized with the total amount of HA released by hydrogels prepared from different proportions of functional monomers [AM:NVP:DEAEM], all at 0.125%-by-mass of prepolymer: [1:1:0] (■), [1:1:2] (♦) and [0:0:1] (×). For comparison, we also plot the fractional release profiles of hydrogels with 0.25% [1:1:2] (●) and hydrogels with no functional monomers (♦). The diffusion coefficient decreases with greater diversity of functional monomers, and the release profile is pushed closer to constant or zero order release.

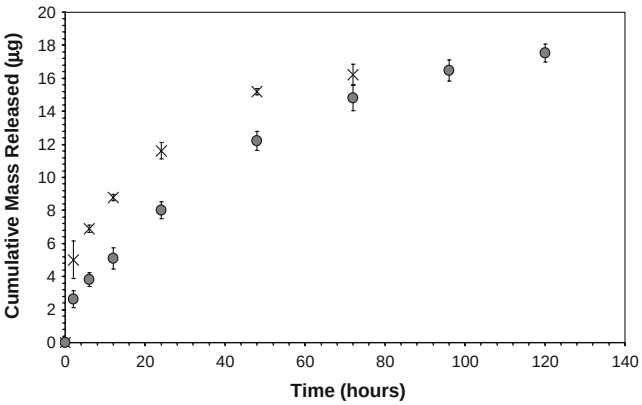

Fig. 8. Cumulative Release of HA from Imprinted Nelfilcon Hydrogels with the same %-by-mass of DEAEM. The hydrogels of composition 0.125% [0:0:1] (×) and 0.25% [1:1:2] (●) contain the same amount of DEAEM. They release similar cumulative masses of HA, but their diffusion coefficients are different. The composition with greater diversity of functional monomers has the lower diffusion coefficient.

We can conclude from this that the release rates of HA can be controlled in two distinct ways—we can vary the cumulative mass released by varying the total amount of functional monomers added, and we can vary the diffusion coefficient by varying the diversity of incorporated monomers. The two trends are made more apparent in Figs. 9 and 10.

The cumulative mass released in 24 h is compared for all 0.125% functional monomer compositions in Fig. 9. The composition with no DEAEM, 0.125% [1:1:0], releases a high cumulative mass of HA, almost as much as is released by the nelfilcon hydrogel without any added monomers. As the proportion of DEAEM is increased, the release amount decreases to the level released by 0.25% [1:1:2], which contains all functional monomers.

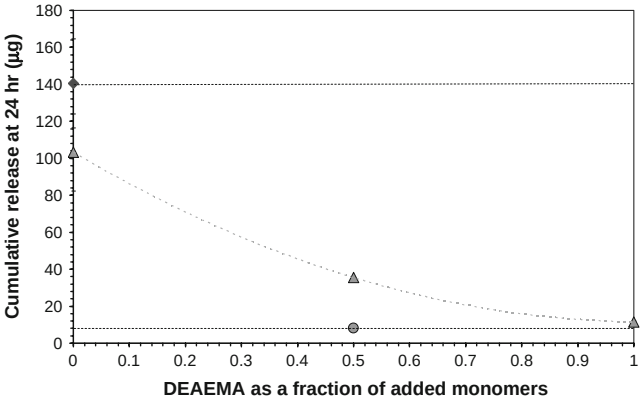

Fig. 9. 24 h Release of HA from Imprinted Nelfilcon Gels Versus Proportion of DEAEM. The amounts of HA released over 24 h by various hydrogels containing %-by-mass of functional monomers 0.125% (▲) and 0.25% (●) were compared, along with non-imprinted hydrogels with no functional monomers (◆). The three data points for 0.125% correspond to different proportions of DEAEM. The 0 on the x-axis indicates [AM:NVP:DEAEM] of [1:1:0], the 0.5 refers to [1:1:2] and the 1 refers to [0:0:1]. It is clear that increasing the amount of DEAEM in the hydrogel decreases the cumulative mass of HA released. Furthermore, the 0.125% [0:0:1] and 0.25% [1:1:2] hydrogels contain the same amount DEAEM, and release the same cumulative mass of HA.

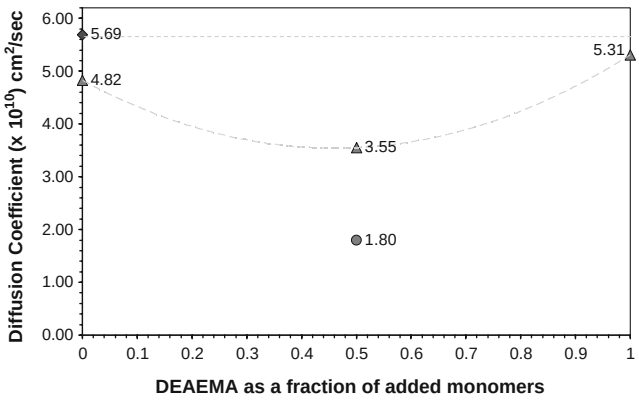

Fig. 10. Diffusion coefficients versus proportion of DEAEM in imprinted nelfilcon gels. The diffusion coefficients of HA through various hydrogels containing %-by-mass of functional monomers 0.125% (▲) and 0.25% (●) were compared, along with hydrogels with no functional monomers (◆). The three data points for 0.125% correspond to different proportions of DEAEM. The 0 on the x-axis indicates [AM:NVP:DEAEM] of [1:1:0], the 0.5 refers to [1:1:2], and the 1 refers to [0:0:1]. We can see that increasing the diversity of the functional monomers, by incorporating all functional monomers AM, NVP and DEAEM in the hydrogel, lowers the diffusion coefficient.

The diffusion coefficients of HA from all 0.125% monomer compositions are compared in Fig. 10. The compositions with no DEAEM (0.125% [1:1:0]) and with only DEAEM (0.125% [0:0:1]) have diffusion coefficients close to that of nelfilcon gels without added monomers. The composition containing all monomers (0.125% [1:1:2]) has a significantly lower diffusion coefficient: 1.5 times lower than the 0.125% [0:0:1], and 1.6 times lower than the nelfilcon gels without monomers. Also, compare the diffusion coefficients of 0.125% [0:0:1] and 0.25% [1:1:2], which contain the same amount of DEAEM. Although they release equivalent cumulative amounts of HA, their diffusion coefficients differ by a factor of 3. The diffusion coefficients and orders of release of compositions in relation to the monomer diversity are summarized in Table I.

We can explain the changes in diffusion coefficients in the above experiments by referring to the biomimetic imprinting process. In the prepolymer mixture containing the modified PVA macromer (nelfilcon A formulation), functional monomers, and HA; the functional monomers configure themselves around the HA so that the interactions between the functional monomers and HA moieties decreases the free energy of the system. When the mixture is crosslinked, the functional monomers are incorporated into the hydrogel in these favorable configurations through the pendant acrylate groups on the PVA chains. In this manner, the hydrogel is synthesized with memory sites that have an affinity for HA. While interactions do occur between the HA and each functional monomer individually, the presence of all three monomers allows multiple moieties on the HA to interact with the hydrogel at one site. The multiple functional interactions are believed to increase the similarity of the interaction sites with the binding sites on HA-binding protein CD44, leading to enhanced affinity and lower diffusion coefficients.

The possibility remains that the addition of monomers resulted in a hydrogel network with a tighter mesh structure,

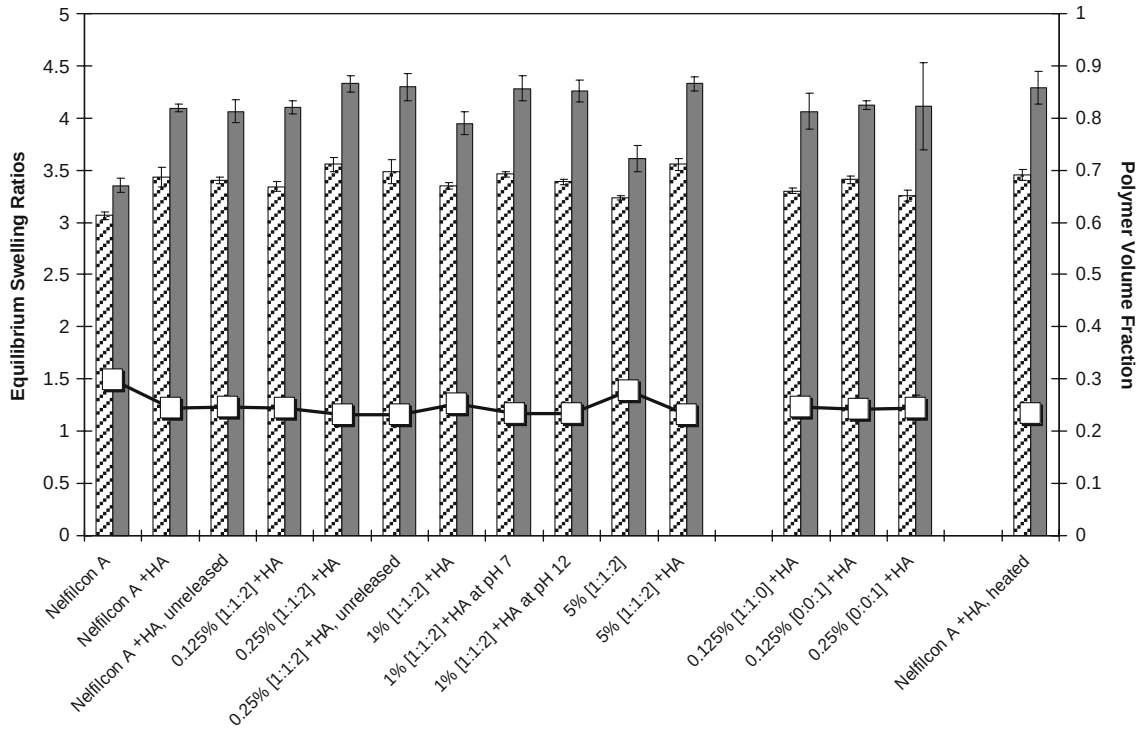

Fig. 11. Equilibrium weight and volume swelling ratios. The equilibrium weight swelling ratios and volume swelling ratios are compared for all hydrogel formulations. The *hatch-marked bars* plot the equilibrium weight swelling ratio, and the *solid gray bars* plot the equilibrium volume swelling ratio (left axis). The □ symbols compare the polymer volume fractions of the hydrogels (right axis). Nomenclature:*+HA* indicates gels synthesized with HA in pre-polymer mixture. Apart from the formulations marked *unreleased*, all *+HA* gels were assessed after release studies were performed. *Heated* indicates lenses that had been tested under heat sterilization conditions.

and the diffusion coefficient decreased because reptation takes longer as steric hindrance increases. To explore this possibility fully, we performed hydrogel swelling studies and mechanical analysis to obtain information about the network mesh size.

The equilibrium weight and volume swelling ratios and the polymer volume fraction in the swollen state were calculated for all hydrogels and are summarized in Fig. 11. The hydrogels synthesized with HA generally have slightly higher weight and volume swelling ratios than the hydrogels synthesized without HA. The former also have slightly lower polymer volume fractions in the swollen state indicating higher water content. Two factors influence this difference. First, the presence of HA in the prepolymer mixture can influence the formation of polymer chains and associated crosslinking points, making the polymer chains more mobile and increasing the hydrogels' capacity to hold water. Second, the residual HA in the hydrogels is highly hydrophilic and increases the hydrogels' capacity to hold water.

In general, all the hydrogels had swollen polymer volume fractions falling within the range of 0.23 and 0.29 suggesting that the mesh size is similar for all the gels synthesized. In particular, we note that the swelling parameters of gels with 1%-by-mass functional monomers remain the same at pH 7 and 12, indicating that the pH dependent increase in HA release above did not arise from change in polymer structure. Furthermore, the swelling parameters do not change for nelfilcon gels synthesized with HA despite heat sterilization. Finally, addition of functional monomers and HA does not have an impact on the optical transmission, with percent transmission of all lenses in the range 94±1%.

In Fig. 12, we illustrate the relationship between the diffusion coefficient and polymer volume fraction in gels with

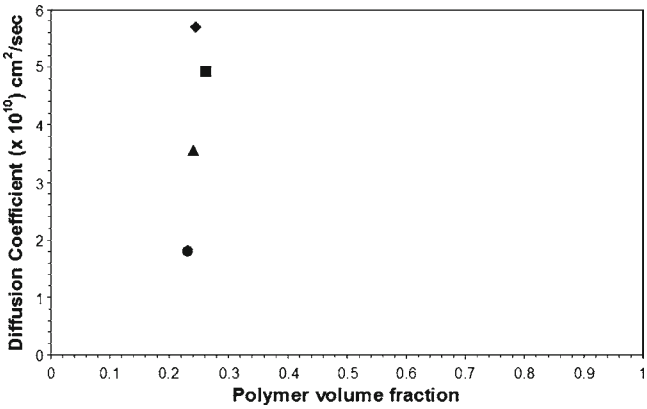

Fig. 12. Diffusion Coefficients versus Polymer Volume Fraction for Imprinted Nelfilcon Hydrogels with Varying Percent of Functional Monomers. The diffusion coefficients of hydrogels with different %-by-mass of functional monomers are plotted against their polymer volume fractions: 0.05% [1:1:2] (■), 0.125% [1:1:2] (▲), 0.25% [1:1:2] (●), and no added functional monomers (♦). The diffusion coefficients vary significantly while the polymer volume fractions are limited to a narrow range. This along with the data in Table II indicate that structural changes such as mesh size are not responsible for the changes in diffusion coefficients.

Table II. Structural Parameters of Imprinted Nelfilcon A Hydrogels

Tensile parameters					Normalized average molecular weight between crosslinks and mesh sizes			
Hydrogel	Young's Modulus (MPa)	SD	Shear Modulus (MPa)	SD	Normalized M_c (g/mol)	SD	Normalized ξ (Å)	SD
Nelfilcon A	0.557	0.023	0.201	0.011	1.006	0.037	1.000	0.023
Nelfilcon with HA	0.423	0.060	0.153	0.022	1.201	0.100	1.165	0.053
Nelfilcon with 0.25% [1:1:2]	0.535	0.032	0.195	0.015				
Nelfilcon with 0.25% [1:1:2] and HA	0.550	0.015	0.203	0.009				
Nelfilcon with 0.25% [1:1:2] without HA					1.028	0.050	1.053	0.030
Nelfilcon with 0.25% [1:1:2] with HA					1.000	0.037	1.083	0.023

various percent functional monomers. The figure clearly reveals the narrow range of polymer volume fraction of the hydrogels. In contrast, the diffusion constants of HA through these networks varies dramatically. The highest diffusion coefficient (from the gel without functional monomers) is nearly four times higher than the lowest diffusion coefficient (from the gel with 0.25% functional monomers). This is strong evidence that the diffusion coefficients do not vary because of structural parameters of the network.

To determine mechanical properties, molecular weight between crosslinks, and mesh size, further structural analysis was done through tensile testing for four types of hydrogel samples: Gels consisting of the nelfilcon formulation without added functional monomers or HA, nelfilcon formulation with HA, nelfilcon formulation with 0.25% by mass functional monomers, and nelfilcon formulation with HA and 0.25% by mass functional monomers. Structural parameters for the hydrogels are summarized in Table II. Of the four gels, three have very similar structural parameters. Nelfilcon formulation with HA (no functional monomers) has moduli that are lower than the other hydrogels. This suggests that the presence of HA in the gel without added functional monomers results in a network with longer chains between crosslinks.

This confirms that the mesh of gels consisting of the nelfilcon formulation crosslinked with only HA has a more open network than the other hydrogels. The presence of HA along with modified PVA macromer in the prepolymer in the absence of functional monomers appears to produce a hydrogel with a greater molecular weight between crosslinks than the PVA macromer does alone. The HA seems to influence the formation of polymer chains and associated crosslink points in the hydrogel resulting in a slightly larger average mesh size. This agrees with the results of the swelling studies, in which gels produced with the nelfilcon formulation alone have a greater polymer volume fraction than gels produced with nelfilcon formulation and HA. However, the addition of functional monomers leads to a decrease in the molecular weight between crosslinks, indicating that the presence of the functional monomers decreases the mesh size to an extent comparable with nelfilcon gels prepared with no added HA or functional monomers. Thus, this confirms that

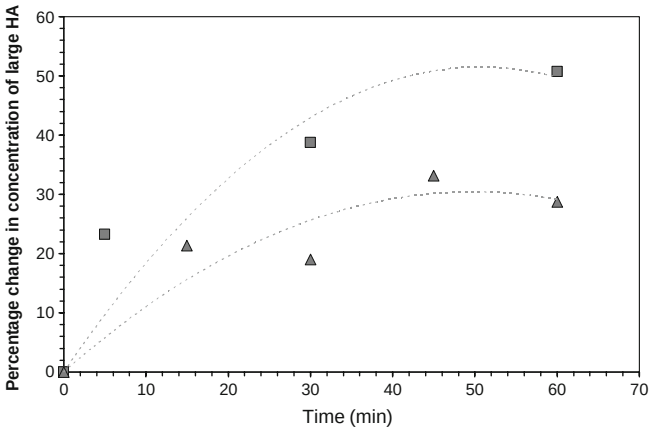

Fig. 13. Comparison of Stability of HA Solutions Under Heat Sterilization Conditions. When solutions of HA in water are heated to above 100°C, they undergo some heat degradation. In the less concentrated solution (500 ng/mL, ■) nearly 50% of the large HA degrades to shorter HA over 60 min, whereas in the higher concentration solution (10 μg/mL, ▲), the degradation is only 30% in the same time. This suggests that higher concentrations have a protective effect on the large HA molecule.

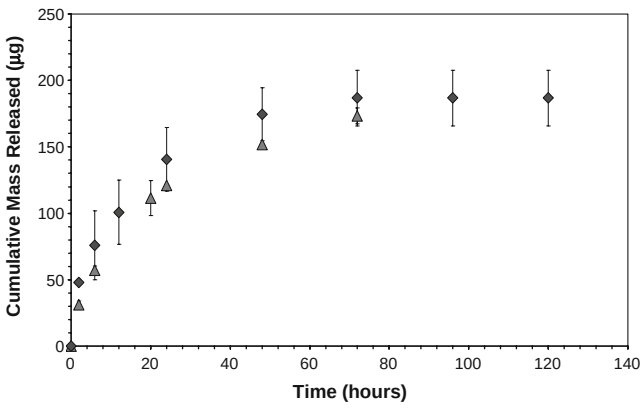

Fig. 14. Cumulative Release of HA from Nelfilcon Hydrogels Before and After Heat-Sterilization. When hydrogels containing HA with nelfilcon were heated to 121°C, the dynamic release profile of HA for such lenses (▲) was similar to the release profile from hydrogels that did not undergo heat treatment (◆).

the diffusion coefficients do not vary because of different mesh sizes or structural parameters of the network.

As HA is known to undergo denaturation when heated to high temperatures, we needed to assess the effect of the high temperature sterilization procedure on the HA incorporated in the lenses. We heated aqueous solutions of HA (500 ng/mL and 10 μg/mL) to temperatures of 121°C for various time intervals. When the samples were assayed, the concentration of one million Dalton HA chains was lower for samples that had been heated longer.

Interestingly, the percentage change in concentration over time was lesser for the more concentrated solution (10 μg/mL) than the more dilute solution (500 ng/mL). The change in concentration in the 10 μg/mL solution was 30% over 60 min while the change in concentration in the 500 ng/mL solution was 50% over 60 min. The percentage change in concentration versus time of heating is shown in Fig. 13. This suggests that higher concentrations of HA may have protective effects on the stability of the long-chain HA molecule. We conducted a dynamic release study to assess the heat effects on the nelfilcon-HA hydrogel lenses. Comparing the heat-treated lenses with the control untreated lenses we measured similar release profiles, suggesting that heat-treatment does not denature the HA within the hydrogel. The release profiles are shown in Fig. 14.

CONCLUSIONS

Hydrogel films and contact lenses composed of nelfilcon A, acrylamide (AM), *N*-vinyl pyrrolidone (NVP), and 2-(diethylamino) ethyl methacrylate (DEAEM) were biomimetically imprinted in the presence of hyaluronic acid (HA), for the controlled release of HA over 24 h. The lenses were designed for the therapeutic delivery of HA to the eye surface, to improve the wettability of lenses and to treat symptoms of dry eye. We demonstrated that by changing the mass content and relative proportions of the monomers AM, NVP and DEAEM within the hydrogel, we can dramatically vary the diffusion coefficients and release profiles of the HA. The variations arise through the biomimetic imprinting process and not through structural changes in the hydrogel. This is the first demonstration in the literature of imprinting a large molecular weight polymer within a hydrogel and delayed reptation.

Increasing the total mass content of monomers in the hydrogel decreases the amount and diffusion rate of HA. Increasing the proportion of DEAEM immobilizes more HA within the hydrogel. Increasing the diversity of monomers lowers the diffusion coefficient. Structurally the mesh size of the hydrogels is fairly uniform. The HA in the lenses also demonstrates tolerance to sterilization conditions. At a limiting amount of functional monomer that led to a crucial number of complexation points between HA and the network, HA release was turned off and did not occur until the ionic bonds between HA and DEAEM were disrupted by altering the pH. This on-demand release can be applied to a number of imprinted hydrogel systems to create novel release mechanisms with 'imprinting control'.

A range of feasible monomer compositions in nelfilcon A has been determined, and can be tailored to produce desired release kinetics. The nelfilcon-based biomimetically imprinted hydrogels for release of HA can deliver comfort molecule to the eye in therapeutic amounts, and may lead to a dramatic shift in the treatment of dry eye symptoms and more comfortable contact lenses. However, the hydrogel lenses produced in this work could serve dual roles as a comfort and therapeutic contact lens, since HA has been shown to have therapeutic properties in corneal wound healing and epithelial cell migration. With lenses of decreased mesh size, release can be tailored further to achieve release for days to months depending on a multiple-day daily or 30-day, extended wear platform.

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