31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Templated Hydrogels for Combination Devices: Therapeutic Contact Lenses

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Abstract—**Molecular imprinting provides a rational design strategy for the development of controlled release drug delivery systems. We demonstrate that imprinting a network results in macromolecular memory for the template molecule, indicated by the two or more times greater partitioning into these networks as compared to non-imprinted networks. Partitioning of drug into networks synthesized from multiple functional monomers was 8 times greater than networks synthesized from single monomers. One-dimensional permeation studies showed that the gel with maximum incorporated chemical functionality had the lowest diffusion coefficient, which was at least an order of magnitude lower than all other gels studied. All imprinted networks had significantly lower diffusion coefficients than non-imprinted networks, in spite of comparable mesh sizes and equilibrium polymer volume fractions in the swollen state. This work also demonstrates molecular imprinting using a "living/controlled" polymerization strategy to enhance template loading/affinity and delay release in weakly crosslinked gels. Recognition studies revealed more than a 50% increase in template loading and dynamic template release studies showed that imprinting via "living" polymerization extends or delays the template release profile by two-fold over that of imprinting via conventional free-radical polymerization techniques and four-fold over the control network. The imprinted gel and imprinted gel prepared via "living/controlled" polymerization release profiles were less Fickian and moved toward zero-order release with profile coefficients of 0.68 and 0.70, respectively.**

I. Introduction

Macromolecular memory within heteropolymer gels is a relatively new method for additional control of therapeutic diffusion within gels [1]-[6] and is especially useful within decreased length scales, as with controlled therapeutic delivery via thin films and coatings. Molecular imprinting techniques introduce "macromolecular memory" within gels and increase the tailorability of the macromolecular structure by producing gel networks with intrinsic affinity and capacity for a template therapeutic. The advantage of tuning co-polymer network functionality is the ability to manipulate the macromolecular structure on the scale of the therapeutic, thus providing better control over the drug release rate [1]-[6]. Increasing the number of therapeutic molecules to polymer chains within a given gel volume is also a significant advantage and significantly increases the therapeutic payload. Recently, these techniques have demonstrated extended therapeutic release and enhanced loading within thin hydrogel films and contact lenses for application within ocular delivery [1]-[6]. Our group was the first to demonstrate enhanced loading and delayed release in hydrogels due to a diversity of monomers [2], and the work of Alvarez-Lorenzo and coworkers was the first to demonstrate significant dependence on the functional monomer/template ratio [6]. A recent review of imprinting within gels highlights the immense potential and status of the field [7].

Two schemes are used to load therapeutics into hydrogels – produce the gel in the presence of drug or synthesize the gel and then load drug into the gel via equilibrium partitioning. Previous work from our laboratory has demonstrated that enhanced loading of imprinted gels results in decreased propagation of polymer chains, probably due to the constraining of monomer configurations and free-radical collisions by the template molecule [2]. However, no work in the field has addressed the feasibility of optimizing drug-functional monomer interactions to influence extended delivery. This work addresses this fundamental issue and presents a novel way to delay therapeutic transport by creating macromolecular memory in the gel, by providing multiple diverse functional groups for extensive non-covalent interactions between the gel and the therapeutic. This could prove to be a valuable tool in the rational design of controlled release hydrogel carriers and significantly increase the applicability of such systems in thin film or 'limited volume' combination devices.

II. Materials and Methods

A. Synthesis of Imprinted Hydrogel Networks

Acrylic acid (AA), acrylamide (AM), 2-hydroxyethylmethacrylate (HEMA), methacrylic acid (MAA), diethylaminoethyl methacrylate (DEAEM), ethylene glycol dimethacrylate (EGDMA), Polyethylene glycol (200) dimethacrylate (PEG200DMA), azobisisobutyronitrile (AIBN), tetraethylthiuram disulfide iniferter (TED), ketotifen fumarate and diclofenac sodium were purchased from Aldrich (Milwaukee, WI) and used as received. Hydrogels of differing compositions were synthesized in a temperature controlled, non-oxidative environment using free-radical UV photopolymerization. Control gels were prepared without the template molecule, following similar steps.

Manuscript received April 7, 2009. This work was supported in part by the National Science Foundation under Grant G00003191, and the US Dept of Education, Grant P200A060184.

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Ketotifen templated gels consisted of compositions of 5 mole% PE200DMA crosslinking monomer and 95 mole% functional monomer (92 mole% backbone functional monomer, HEMA, and the balance 3 mole% as combinations of other functional monomers). Diclofenac sodium imprinted poly(DEAEM-co-HEMA-co-PEG200DMA) gels with a 5% crosslinking percentage were made with 0.336 mL of DEAEM (1.673 mmole), 3.659 mL of HEMA (30.118 mmole), 0.538 mL of PEG200DMA (1.673 mmole), 20 mg of AIBN (0.121 mmole), and 150 mg of diclofenac sodium (0.352 mmole). The solutions were mixed and sonicated until all solids were dissolved. The poly(DEAEM-co-HEMA-co-PEG200DMA) recognitive gel prepared via "living/controlled" polymerization was synthesized with 4.20 mg of TED (0.014 mmole) and 40 mg of AIBN (0.242 mmole). Solutions were transferred to an MBraun Labmaster 130 1500/1000 Glovebox (Stratham, NH), which provided a nitrogen atmosphere for free-radical UV photo-polymerization. Then monomer solutions were pipetted between two 6" x 6" glass plates separated by 0.25 mm Teflon spacers. The solutions were left uncapped and open to the nitrogen atmosphere until the O_2 levels inside reached negligible levels (<1 ppm). The polymerization reaction was carried out for 8 minutes for the poly(DEAEM-co-HEMA-co-PEG200DMA) control and recognitive gels while for the "living/controlled" polymerization prepared polymers the reaction time was 24 minutes. Separate DPC studies revealed exact reaction times. The intensity of light from a UV Flood Curing System (Torrington, CT) was 40 mW/cm^2 at a voltage of 325 V, and the temperature within the glovebox was held constant at 25°C.

After polymerization, the glass plates were soaked in deionized (DI) water and the polymers were quickly peeled off the plates and cut into circular discs using a size 10 cork borer (13.5 mm). The gels were washed in a well-mixed 2 L container of DI water for 7 days with a constant 5 mL per minute flowrate of de-ionized water. Absence of detectable drug released from the polymer gel was verified by removing random gels, placing them in fresh DI water with adequate mixing, and sampling the supernatant via spectroscopic monitoring. The discs were allowed to dry under laboratory conditions at a temperature of 20°C for 24 hours and then transferred to a vacuum oven (27 in Hg, 33-34°C) for 24 hours until the disc weight change was less than 0.1 wt%.

B. Equilibrium Template Recognition, Dynamic In-vitro Release, and Template Transport Studies

In a typical experiment, recognitive and control gels were placed in concentrated solutions of template and gently agitated until equilibrium. Separate dynamic studies were performed to assure equilibrium conditions were reached. After equilibrium was reached over a 7 day period, the solutions were vortexed for 10 seconds, and the equilibrium concentrations were measured. A mass balance was used to determine the bound amount of drug within the polymer gel. All gels were analyzed in triplicate, and all binding values are based upon the dry weight of the gel. Kinetic release studies were conducted in DI water using a Sotax Dissolution Apparatus (Horsham, PA) with 1000 mL of solvent. The solution was stirred at a constant rate of 75 rpm by paddles and kept at a constant temperature of 37°C. At various time points, the absorbance of the solution was measured using a Synergy UV/Vis spectrophotometer (BioTek Instruments, Winooski, VT) at the wavelength of maximum absorbance.

Fractional template release profiles were calculated for the recognitive and control gels by taking the amount of template released at specific times, M_t, divided by the maximum amount of template released during the experiment, M_∞. The fractional template release profile, M_t/M_∞ versus time, was determined for each gel. Template diffusion coefficients were calculated using Fick's law, which describes one-dimensional planar solute release from gels. For polymer geometries with aspect ratios (exposed surface length/thickness) greater than 10, edge effects can be ignored and the problem approached as a one-dimensional process.

Ketotifen fumarate permeation studies through gels were conducted using Permegear side-by-side diffusion cells (Bethlehem, PA, USA) consisting of donor and receptor reservoirs. Water jackets around the reservoirs provided temperature control at 25°C. Magnetic stirrers (600 rpm) in each reservoir kept the solutions well mixed.

C. Analysis of Network Swelling Behavior and Structural Analysis of Recognition

The equilibrium weight swelling ratio was obtained by taking the ratio of the swollen weight to the dry weight. The equilibrium volume swelling ratio was calculated by dividing the swollen gel volume at equilibrium by the volume of the dry polymer. It is also equal to the reciprocal of the polymer volume fraction in the swollen state. The dry and swollen gels were weighed in air and heptane, a non-solvent, using a microbalance (Sartorius). The volume of the gel in the swollen or dry state was obtained by using Archimedes buoyancy principle with heptane (density of 0.684 g/mL at a temperature of 25°C). Gels in the relaxed state were weighed immediately after polymerization without exposure to solvent.

Dynamic swelling studies were performed by placing dry polymer disks in solvent and weighing at various time intervals by removing the gels from the swelling media and blotting with tissue to remove excess surface solvent.

Hydrogel structural analysis (molecular weight between crosslinking points and network mesh size) was obtained by swelling and tensile experimental studies and by using the theory of rubber elasticity. Static experiments were performed in the equilibrium swollen state. Samples of each gel (1 mm x 5 mm x 15 mm strips) were removed from the solvent and analyzed with a RSA III Dynamic Mechanical Analyzer (DMA), (TA Instruments, New Castle, DE) to obtain stress versus strain.

III. RESULTS AND DISCUSSION

We have proved that loading properties of gels are improved with multiple monomers incorporated into gels **(Fig 1)**. Gels of multiple complexation points with varying functionalities outperformed the gels formed with fewer types of functionality and showed the greatest template loading or partitioning. Transport studies showed that release rates can be tailored via type and amount of functionality. All imprinted networks had lower diffusion coefficients than non-imprinted networks in spite of comparable mesh sizes and equilibrium polymer volume fractions in the swollen state. The diffusion coefficient of the poly(AA-co-AM-co-NVP-co-HEMA-co- PEG200DMA) network was 7.08 x 10^{-10} cm^2/s which was lesser by factors of 15, 76, 49 and 113 than the other imprinted networks. We conclude that the multiplicity and organization of functionality is responsible in binding events and a delay in transport. The imprinted networks of all the gels studied had lower permeability and diffusion coefficients than the control networks, demonstrating that imprinting does delay transport.

Diclofenac sodium binding results for the poly(DEAEM-co-HEMA-co-PEG200DMA) control gel, recognitive gel, and the recognitive gel prepared via "living/controlled" polymerization are presented in Figure 2. The poly(DEAEM-co-HEMA-co-PEG200DMA) recognitive gel had a 140% increase in template loading over that of the control network, with loading capacities of (0.96 ± 0.12 and 2.30 ± 0.20) x 10^{-2} mmole/g, respectively. The recognitive gel prepared via "living/controlled" polymerization had an 54% increase in template loading capacity (3.54 ± 0.16) x 10^{-2} mmole/g) over that of the recognitive gel and a 269% increase over that of the control gel. Both recognitive gels had approximately 1.5 times higher template binding affinity than the control gel which had a template binding affinity of 9.91 ± 0.49 mM^{-1}.

Dynamic fractional template release studies for the diclofenac sodium imprinted poly(DEAEM-co-HEMA-co-PEG200DMA) gels are shown in Figure 3. The recognitive gel showed a two-fold extension in template release time compared to the control gel (i.e., the recognitive gel released 70% of template over 700 minutes compared to 300 minutes for the control). For the gel formed via "living/controlled" polymerization, 70% template was released over a time period of 1400 minutes. Imprinting via "living" polymerization extends or delays the template release profile by two-fold over that of imprinting via conventional free-radical polymerization techniques and four-fold over that of the control network.

Template diffusion coefficients were determined from the release data and quantitatively highlight these differences. The template 'effective' diffusion coefficients for the control gel, recognitive gel and the recognitive gel prepared via "living/controlled" polymerization were 6.37 ± 1.02 x 10^{-9} cm^2/s cm^2/s, 3.20 ± 0.48 x 10^{-9} cm^2/s, and 1.49 ± 0.33 x 10^{-9} cm^2/s, respectively.

The recognitive gel and recognitive gel prepared via "living/controlled" polymerization release profiles are less Fickian and moving toward zero-order release with profile coefficients of 0.68 and 0.70, respectively.

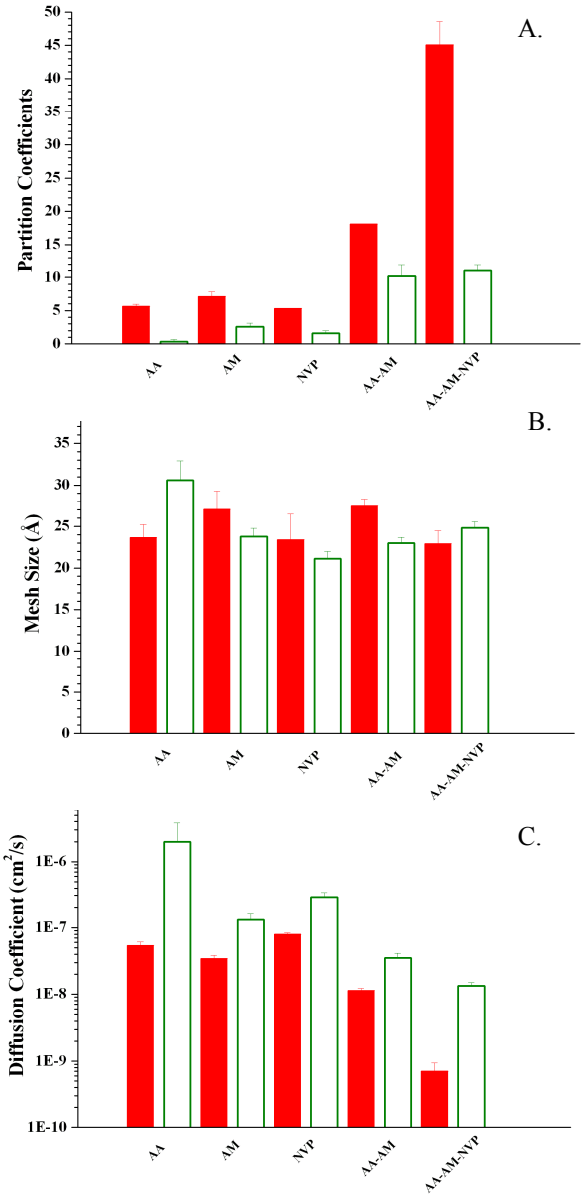

Fig. 1. Ketotifen Fumarate Imprinted Gels (a) Partition coefficients, **(b)** Mesh sizes obtained *via* tensile studies, **(c)** Diffusion coefficients obtained *via* permeation studies, for poly(n-co-HEMA-co-PEG200DMA) networks with a crosslinking percentage of 5 mole %. N=3, and T=25˚C, where n is AA, AM, NVP, AA-co-AM, and AA-co-AM-co-NVP. Imprinted network (■) and Control network ( ).

The control gel conforms very well to a Fickian release profile with n = 0.48. Thus, molecular imprinting shifted the release profiles toward zero-order template release, or constant release that is not dependent upon time or template concentration. Exploiting "living/controlled" polymerization methods contributes further control upon the template release characteristics of the hydrogel. The slower release profile for imprinted gels is hypothesized to be due to the

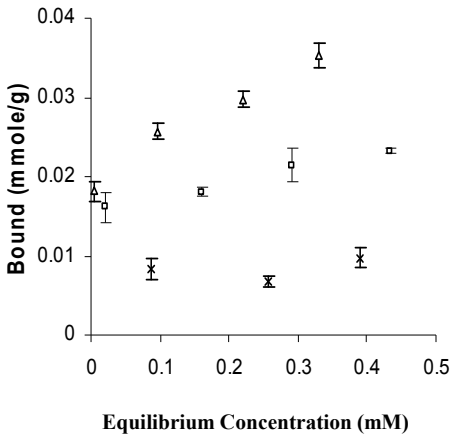

Fig 2. Poly(DEAEM-co-HEMA-co-PEG200DMA) Gel Binding Isotherms for Diclofenac Sodium in Water. The imprinted gel via "living/controlled" polymerization techniques (Δ) shows a 54% increase in loading capacity over that of conventional imprinted gel (□). Both imprinted gels bound more diclofenac sodium compared to the control gel (x), and the imprinted gel had a 140% increase in template loading over that of the control network. The imprinted gel prepared via "living/controlled" polymerization had a 269% increase over that of the control gel (N=3).

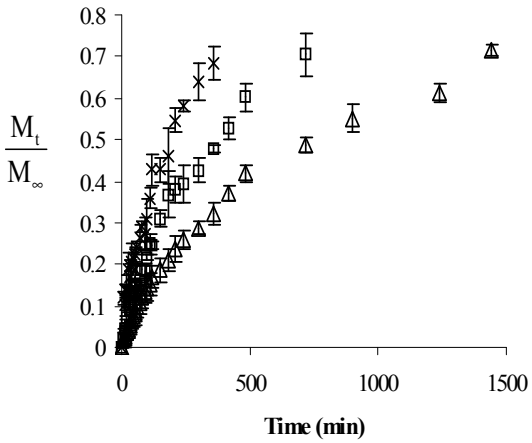

Fig. 3. Fractional Release of Diclofenac Sodium Template for Poly(DEAEM-co-HEMA-co-PEG200DMA) Gels. The imprinted gel prepared via "living/controlled" polymerization (Δ) had extended release in water when compared with the imprinted gel (□) and the control gel (x). The error bars represent the standard error with N=3.

intrinsic concentration. Exploiting "living/controlled" polymerization methods contributes further control upon the template release characteristics of the hydrogel. The slower release profile for imprinted gels is hypothesized to be due to the intrinsic template binding characteristics of the polymer network, as the template molecule diffuses through the network it binds and unbinds a number of times to multiple binding sites as it diffuses through the network.

Binding analysis on a control gel prepared via "living polymerization" was conducted and confirmed that these results were not due to incorporation of more functional monomer within the network. Also double bond conversion data conclusively ruled out differences due to increased double bond conversion. There were no statistical differences between the poly(DEAEM-co-HEMA-co-PEG200DMA) recognitive gel and the gel prepared via living/controlled polymerization, which had double bond conversions of 80 ± 5.1 and 83 ± 4.7%, respectively.

At equivalent double-bond conversions, diclofenac imprinted poly(DEAEM-co-HEMA-co-PEG200DMA) recognitive gels prepared with conventional polymerization and "living/controlled" polymerization methods had average mesh sizes of 30.3 ± 1.7 and 19.7 ± 2.1 Angstrom, respectively. "Living/controlled" polymerization created smaller mesh sizes, which may originate from smaller and more monodisperse kinetic chain lengths within the copolymer network which contributes to the overall homogeneity of the network structure.

Template diffusion through an imprinted polymer network can be influenced by three main variables, average mesh size, template size, and template–polymer chain interactions (i.e., the imprinting effect). Manipulation of one or more of these variables can alter the template diffusion coefficient. An increase in polymer mesh size holding template size and template-polymer chain interactions constant would correspond to an increase in template diffusion. An increase in template size holding mesh size and template–polymer chain interactions constant would correspond to a decrease in the template diffusion coefficient. An increase in template–polymer chain interactions holding template size and mesh size constant would correspond to a decrease in the template diffusion coefficient. It is clear that imprinting plays an important role in the transport of the template from the network. For the recognitive gels prepared via "living/controlled" polymerization, there is a 6.6 fold increase in template affinity with a 4.9 fold decrease in template transport with much less difference in the polymer mesh size on the associated template release.

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