PROTEIN REJECTING HYALURONIC ACID-CONTAINING HYDROGEL MATERIALS

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MARK VAN BEEK, B.ENG.

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AUTHOR: Mark Van Beek, B. Eng. (McMaster University)

SUPERVISORS: Professor H. Sheardown and Professor Lyndon Jones

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ABSTRACT

Recently new contact lens materials have been introduced which are said to improve comfort by incorporating wetting agents either in a releasable or nonreleasable form. However, there is little information in the literature to demonstrate whether these claims are indeed true. In the present work, model lens materials based on poly(2-hydroxyethyl methacrylate) (pHEMA) and methacryloxypropyltris (trimethylsiloxy) silane(TRIS) were developed which incorporate releasable or crosslinked and therefore physically entrapped hyaluronic acid (HA) of various molecular weights as a wetting agent.

Studies showed that uncrosslinked high molecular weight HA exhibited burst release kinetics with 80% release in less than 20 hours. While applicable for use in daily wear cycles, releasable wetting agents certainly would have very little effect on extended wear cycles, a common mode of wear. Protein adsorption results suggest however that the wetting agent resulted in no statistical change over the control material.

Crosslinked and therefore physically entrapped HA, despite being only present in very small amounts, showed consistently lower water contact angles over four hours in comparison to controls, indicating that HA is present at the interface and was not being released over time. The presence of HA in the material was further confirmed by increases in the glass transition temperature

measured by differential scanning calorimetry, increases in the stiffness as measured by Instron testing, and slight changes observed in both x-ray photoelectron spectroscopy and Fourier transform infrared spectra. This crosslinking procedure appeared to have no effect on optical transparency using 35 kDa HA whereas small decreases in optical transparency at higher wavelength were noted for the 169 kDa HA crosslinked material as measured by UV spectrophotometry. Most importantly, protein adsorption results indicated that the adsorption of all proteins studied was considerably decreased by the presence of the small amount of crosslinked HA. It is hypothesized that HA acts in a similar manner as PEO protein repulsion, where free HA chains are able to produce an environment which highly rejects protein adsorption. Significant decreases in lysozyme adsorption were also observed on model silicone hydrogel materials. The results suggest that these materials have significant potential for application in contact lens applications.

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1. Introduction

The contact lens industry has seen significant growth in recent years and is expected to continue to grow in the foreseeable future. In a 2003 US report, it was noted that approximately 59% of the population requires some form of vision correction and that 21% of those patients are contact lens wearers. The expected growth will likely be the result of young teens adopting this technology (OptiStock, 2003).

However, despite this rapid growth and the presence of a number of new products on the market, a significant fraction of contact lens wearers discontinue use on a regular basis. In a study examining reasons for discontinuation, discomfort was cited as the principle reason by 51% of the respondents (Glasson, 2002). Discomfort is thought to stem from a variety of sources including dry eye, or protein fouling problems. Protein deposition is of particular interest since the deposition of proteins and lipids from the tear fluid onto contact lenses causes such adverse effects as reduced visibility, reduced wettability, inflammatory complications and reduced comfort. It is estimated that 30% of all after-care visits to optometrists are due to deposits (Brennan, 2000). Material properties such as porosity, water content and surface charge - in addition to protein properties - affect adsorption.

New lens technologies, mainly silicone hydrogels, have allowed for increased wear cycles and have tried removing the need to take out the lens at night. These silicone hydrogels are much more hydrophobic then conventional lenses and require some form of modification to increase hydrophilicity. Many lenses use a form of plasma coating to

increase hydrophilicity but recent products on the market such as Acuvue OASYS incorporate internal wetting agents as a method of increasing the hydrophilicity and comfort. Another brand, Focus DAILIES with AquaRelease, uses releasable wetting agents which are advertised to increase comfort by releasing the wetting agent with every blink. While all these products that incorporate wetting agents are advertised to improve comfort for the wearer, little is currently published on the effect of these wetting agents on material properties such as material strength, surface roughness and protein deposition. Furthermore, there has been no published comparison between releasable wetting agents and internal wetting agents. In previous work, the Sheardown group examined dextran, poly vinyl pyrollidone (PVP) and hyaluronic acid (HA) release from model hydrogel materials. The results suggested that more prolonged release of HA occurred compared with the other materials which were released over a very short (1-2 hour) time period. Furthermore, anecdotal evidence suggested that HA incorporation resulted in more lubricious feeling surfaces.

Therefore, this project aims to directly compare, using HA as a model, releasable and non-releasable internal wetting agents. By use of a novel method of incorporating hyaluronic acid into poly HEMA and model silicone hydrogels, such factors as hydrophilicity, protein deposition, surface roughness, water content and material composition were measured.

2. Literature Review

2.1 Hydrogel Based Soft Contact Lenses

Hydrogels, an important class of contact lens materials, are highly water swollen hydrophilic networks of polymeric chains with potential for biocompatibility. Generally, to take advantage of multiple properties and to generate superior lens materials, most commercially available lenses are comprised of copolymers (Hoffman, 2001). In order to generate chemically stable hydrogels, covalent crosslinking is necessary. In the crosslinked state, the materials are able to reach equilibrium with the aqueous solution. Crosslinking density, estimated by the molecular weight between crosslinks, along with material type, plays an important role in determining the equilibrium water content of the gel. Typically these chemically stable hydrogels are not homogenous in nature, instead consisting of domains of high crosslinking, low swelling or domains of high swelling but low crosslinking. Methods of hydrogel synthesis include crosslinking of polymer solids or solutions, copolymerization of monomer and crosslinker, copolymerization of monomer with multifunctional macromer, polymerization of a monomer within a different solid polymer or through chemical conversion of a hydrophobic polymer (Hoffman, 2001).

The five generations of contact lenses is detailed in Table 2-1 (Nicolson, 2001). Beginning with the use of polymethylmethacrylate (PMMA) as an ophthalmic material, hydrogels have evolved to incorporate softer polymeric components, particularly poly-hydroxyethyl methacrylate (PHEMA). The incorporation of other polymeric components such as poly vinyl pyrollidone (PVP) and poly methacrylic acid (PMAA) has been shown

to result in materials with higher water uptake and hence higher oxygen permeability, although certainly much less than necessary for optimal ocular health. The use of silicone rubber to generate lenses with higher oxygen permeability initially resulted in failure as this highly hydrophobic material interacted strongly with lipids from the tear film and tended to adhere to the corneal epithelial layer, resulting in lens binding and adherence. Rigid gas permeable lenses, with higher oxygen permeability met with some degree of success, although the level of comfort with these lenses was significantly lower than with their softer, less oxygen permeable counterparts. Likely the most significant development in contact lens materials came with the introduction of silicone hydrogel materials, which combine the high oxygen permeability of silicone rubber with the comfort of hydrogels.

Table 2-1: Evolution of contact lens materials detailed by the strengths and weaknesses of each generation of contact lens materials.

Generation	Lens	Strengths	Weaknesses
1	PMMA	High transparency; low cost; ease of manufacture	Low oxygen permeability
2	PHEMA + Copolymer	Moderate oxygen permeability; moderate mechanical strength	High protein adsorption
3	Silicone Rubber	High oxygen permeability	Adhere to corneal surface; high lipid uptake
4	Rigid Gas Permeable	High oxygen permeability	Poor initial comfort
5	Siloxane + copolymer	High oxygen permeability	High cost; need for surface treatment

This evolution of materials has resulted in a variety of modes of wear. Each new mode has resulted in increased convenience for wearers by lengthening the wear time and, in many cases, eliminating the need for lens removal overnight. Daily wear lenses are removed daily and cleaned in a cleaning solution or are disposed of following a single cycle of wear. Extended wear lenses can be worn overnight, for 6 or more days before

disposal. Continuous wear lenses can potentially be worn for periods of up to 30 days without removal.

The FDA has designated contact lenses into four classes based on electrostatic charge and water content as shown in Table 2-2. Materials containing no net charge are designated as nonionic polymers while negatively charged polymers are termed ionic polymers. This grouping is then furthered defined by the water content, where high water content materials contain over 50% water at equilibrium swelling and low water content materials have less then 50%.

Table 2-2: US Food and Drug Administration grading system for contact lens materials.

FDA Grade	Ionic Characterization	Water Content
1	Nonionic Polymers	<50%
2	Nonionic Polymers	>50%
3	Ionic Polymers	<50%
4	Ionic Polymers	>50%

Contact lens developers use trade names for their material compositions such as those listed in Table 2-3 under USAN (United States Adopted Names). The USAN council is governed by the American Medical Association, the United States Pharmacopeial Convention, and the American Pharmacists Association. It aims to globally standardize and unify drug nomenclature. Under their naming conventions, lenses ending with 'filcon' are designated hydrophilic while those ending with 'focon' are hydrophobic. Under their conventions, hydrophilic lenses are those containing more then 10% by weight of water and anything less then 10% is hydrophobic, which is clearly different from the FDA system. Each member of a family of materials is assigned a

nonproprietary name containing the proper suffix and is given a letter (A etc.) to indicate which member it is in that family. Each member of the family is comprised of the same monomers, while the letter indicates which ratios of monomer are used (American Medical Association, 2005).

Table 2-3: Examples of contact lens materials, their USAN, FDA classification, commercial name and manufacturer.

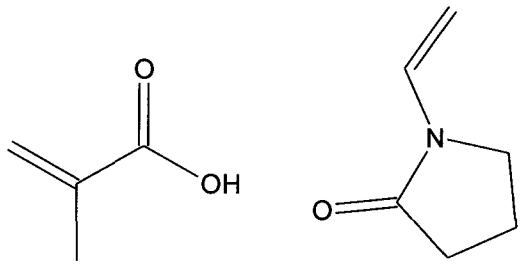
USAN	Monomers	FDA Class	Water Content %	Commercial Name	Manufacturer
Etafilcon A	HEMA/MA	4	58	Acuvue 2	Johnson and Johnson Vision
Alphafilcon A	HEMA/NVP	2	66	SofLens 66	Bausch and Lomb
Polymacon	HEMA	1	38	SofLens 38	Bausch and Lomb
Omafilcon A	HEMA/EDMA	2	62	Proclear Compatibles	CooperVision
Lotrafilcon A	DMA/TRIS/ Siloxane monomer	1	24	Focus Night and Day	CIBA Vision
Galyfilcon A	Silicone Based, Hydraclear	1	47	Acuvue Advance	Johnson and Johnson Vision
Lotrafilcon B	Modification of Lotrafilcon A	1	33	02 Optix	CIBA Vision
Balafilcon A	NVP/NCVE/ TPVC/PBVC	3	36	PureVision	Bausch and Lomb

Abbreviations: MA- Methacrylic acid, NVP- N-vinyl pyrollidone, EDMA- ethylene dimethacrylate, NCVE- *N*-carboxyvinyl ester,TPV- tris-(trimethylsiloxysilyl) propylvinyl carbamate, PBVC- poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]),DMA- *N,N*-dimethylacrylamide)

The major types of hydrogel lenses currently on the market include conventional hydrogels and silicone hydrogels. Conventional hydrogels are comprised primarily of pHEMA or poly vinyl alcohol (PVA) while silicone hydrogel materials incorporate complex silicone chemistry.

2.1.1 Conventional Contact Lens Materials

Conventional contact lenses typically consist of a copolymer mixture with poly HEMA as the base, although other base polymers, particularly PVA, have been used. Increases in oxygen permeation in these lens materials are a result of higher water content because oxygen passes through the water filled channels in the swollen hydrogel materials. PHEMA alone has an equilibrium content of 38%, so typically a second monomer is included to increase the hydrophilicity/water uptake which improves both the wettability and oxygen permeability. Such secondary monomers include nonionic but highly hydrophilic N-vinyl pyrollidone (NVP) or ionic and highly hydrophilic methacrylic acid (MAA) as shown in Figure 2-1. The ionic nature of MAA, while increasing the water content, has been shown to lead to increased protein adsorption, a potentially negative side effect of this modification (Garrett, 2000). While increased oxygen transport through these materials is the primary reason for the addition of the hydrophilic monomer, even the most highly swollen conventional contact lens materials do not possess adequate oxygen permeability for "extended" or overnight wear(Nicolson, 2001). Furthermore, while very high water contents are possible in pHEMA based materials, increased water content is associated with decreased mechanical strength/elasticity.



MAA NVP

Figure 2-1: Chemical structure of methacrylic acid and N-vinyl pyrollidone which are commonly added to conventional contact lenses to improve their water uptake.

2.1.2 Silicone hydrogels

The introduction of silicone hydrogel materials, which can be worn on an extended or continuous wear basis, has revolutionalized the contact lens market. The principle of this technology is very similar to conventional lenses. Conventional lenses add hydrophilic monomers to increase the water content of the material to increase oxygen permeability and wettability whereas silicone hydrogels include monomers with silicon-oxygen bonds into the materials which significantly increase the oxygen permeability. Silicon-oxygen bonds have a significantly higher permeability to oxygen then water. The reported Dk value for pure dimethylsiloxane (DMS) is approximately 600, significantly higher than that required for ocular health (Sweeney, 2004). However, the extremely hydrophobic nature of PDMS results in low water uptake, high protein and lipid affinity and poor wettability. Early silicone lenses with this composition were also found to adhere to the cornea, and were unsuccessful.

The development of silicone monomers which can be copolymerized with hydrophilic monomers to increase the water content, resulted in lenses with increased comfort and high oxygen permeability. The composition of the lens must be carefully

controlled in order to obtain the correct overall composition and ideal properties. While the composition varies from material to material, 60-85% is typically the oxygen-permeable silicone components, with the remainder being chosen for ion permeability, and ability to increase water content, improve wettability and comfort (Sweeney, 2004). The modification of pHEMA to contain Si-O bonds, such as in the monomer TRIS, shown in Figure 2-2, has the advantage of maintaining a reactive hydrophilic tail while adding a hydrophobic, oxygen permeable component. On their own, these materials result in opaque materials not suitable for contact lenses.

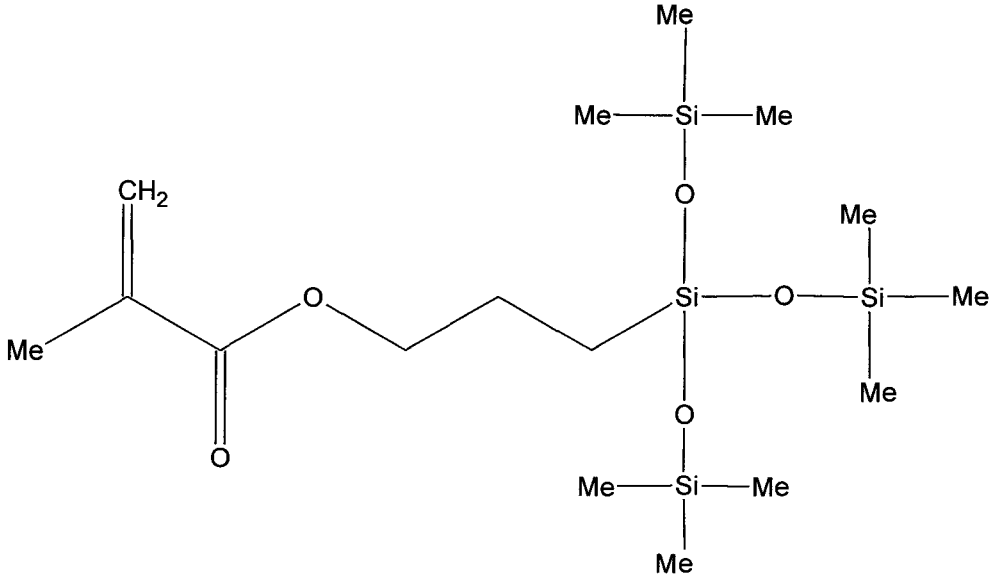


Figure 2-2: Methacryloxypropyltris (trimethylsiloxy) silane(TRIS) with hydrophilic tail attached to a highly oxygen permeable silane groups.

A popular approach for the production of silicone hydrogel contact lenses involves the incorporation of fluorine into the silicone hydrogel (Kunzler, 1996). This modification increases the relative compatibility with hydrophilic monomers, resulting in transparent copolymers. Fluorosilicones also show improved deposit resistance. (Kunzler,

1996) Another approach involves copolymerizing a siloxane monomer (TRIS), hydrophilic monomers (NVP or decyl methacrylate) and fluorinated monomers (Kunzler, 1999).

Silicone based polymers, which are highly surface active, typically require some form of surface modification to improve their wettability. Many of the early silicone hydrogel materials were modified using gas plasma techniques to increase the hydrophilicity (Tighe, 2004). More recently, manufacturers have incorporated wetting agents. Both Acuvue Advance and Acuvue OASYS use PVP as a wetting agent. It is believed that the wetting agent acts as a surface modifying agent, improving the overall comfort of the lens when hydrated. Patent literature (Maiden, 2002) suggests that these wetting agents are added into the monomer mixture as high molecular weight polymers, and are locked into the silicone hydrogel matrix by physical entrapment or weak covalent linking. The wetting agent presumably migrates to the surface under physiologically relevant conditions, improving the in eye performance of the material.

2.1.3 Poly(2-hydroxyethyl methacrylate) (pHEMA) as a Biomaterial

2.1.3.1 pHEMA as a Contact Lens Material

The highly water swollen nature of pHEMA has led to its extensive use as a biomaterial, including its use as a coating for such things as surgical gloves(Chantara, 1999), as the basis of an artificial archilles tendon (Davis, 1992) or in drug delivery applications (Dziubla, 2000). An additional advantage is its ability to co-polymerize with other polymers such as polyurethane or methacrylic acid. This allows for the production

of materials with suitable - and in some cases tunable - properties, such as hydrophilicity/hydrophobicity, or mechanical strength. The most widespread use of pHEMA, either alone or copolymerized, has been as an ophthalmic biomaterial and more specifically in contact lens applications. By increasing the water content through copolymerization, an exponential increase in the oxygen permeability can be achieved, since purely pHEMA based materials cannot achieve the oxygen permeability needed for extended wear applications.

2.1.3.2 pHEMA Chemistry

Among hydrogel materials, poly(2-hydroxyethyl methacrylate) (pHEMA) has received a great deal of interest. 2-hydroxyethyl methacrylate (HEMA), cross-linked using a variety of monomers results in pHEMA materials that are capable of taking up as much as 40% of their weight in water. HEMA monomer solutions are shipped in trace amounts of ethylene glycol dimethacrylate (EGDMA), a common crosslinker of pHEMA, causing pHEMA to always have some permanent crosslinks. Additional crosslinker can be used to further stabilize the gel. The chemical structures of HEMA and pHEMA are shown in Figure 2-3 and Figure 2-4 respectively.

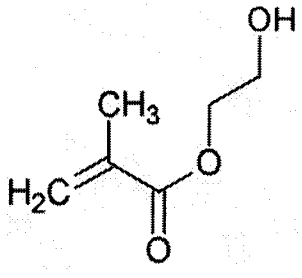


Figure 2-3: Structure of 2-hydroxyethyl methacrylate monomer.

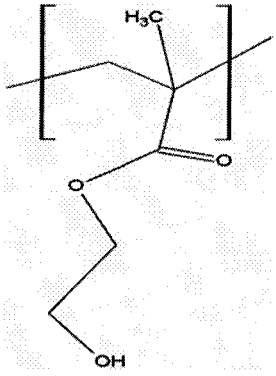


Figure 2-4: Repeat unit structure of poly(2-hydroxyethyl methacrylate).

Many of the beneficial properties of pHEMA, including its ability to form internal physical crosslinks via hydrogen bonding, are a result of the pendant hydroxyl group on the polymer structure. In a dry state, pHEMA is glassy, hard and brittle. However, as a hydrated hydrogel, it is soft and flexible. PHEMA is neutral (non-ionic) and slightly porous in nature. The porosity can be controlled by adjusting the amount of diluents in the monomer mixture, creating micro porous (10 to 100 nm) or macro porous (100nm to 10μm) hydrogel materials. This property is important in that it allows for a high degree of control of the material. For instance, if a biomaterial requires cellular ingrowth and invasion, such as in the case of a porous skirt in an artificial cornea, a 10μm pore size could be used (Chirila, 1996). In terms of contact lenses, protein penetration and absorption is affected by porosity. Larger pores presumably lead to faster and deeper the penetration of proteins which may or may not be beneficial to the properties of the lens (Garrett, 1999).

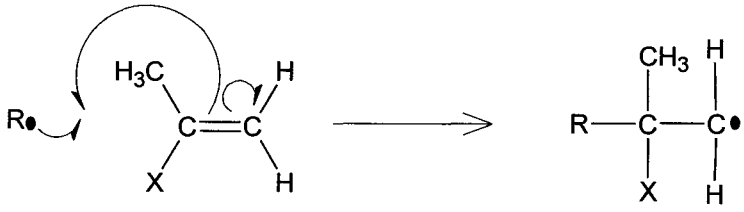
2.1.3.3 pHEMA Curing Methods

pHEMA is most often polymerized by free radical polymerization. Crosslinkers are often used to create permanent hydrogels. However this is not entirely necessary, in part due to the trace amounts of EGDMA that are present in the monomer. While there are several crosslinking agents that can be used, ethylene glycol dimethacrylate (EGDMA) is the most commonly used (Trigo, 1994;Opdahl, 2003). Alternatively, divinyl glycol (DVG) has been shown to result in a material with increased mechanical strength (Clayton, 1997). This is believed to be a result of EGDMA being more prone to phase separation with stiff crosslinks whereas DVG exhibits a relatively low free radical reactivity leading to stronger crosslinks and lower degrees of phase separation.

Free radical polymerization is a common polymerization technique which uses carbon-carbon double bonds to form polymer chains. Free radical polymerizations require chemical initiation. The initiators are able to degrade with exposure to heat or ultraviolet radiation to form free radicals which each have one unpaired electron. Initiators include tetramethylethylene diamine (TEMED), ammonium persulphate (APS) (Clayton, 1997), 2-2'-azobis(isobutyronitrile) (AIBN) (Trigo, 1994), and benzoyl peroxide (Li, 2001). These free radicals shown as R* in Figure 8, attack the double-carbon bond forming an unpaired electron on the monomer. This new group then attacks other double-carbon bonds and propagation occurs. A disadvantage of chemically-initiated free-radical polymerization for the preparation of biomaterials is the need to remove residual initiator fragments prior to use. Fortunately these residual fragments can be released by diffusion upon swelling (Hill, 1999).

The general reaction scheme for the polymerization of HEMA to pHEMA is shown in Figure 2-5. The choice of initiator is a matter of preference and thus is represented as R.

Initiation Step



Propagation Step

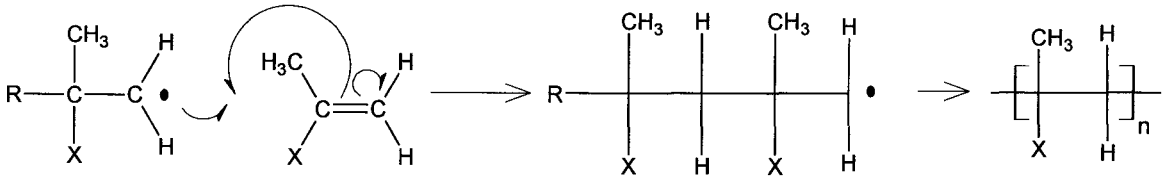


Figure 2-5: Free radical polymerization schemes for 2-hydroxyethyl methacrylate (HEMA) into poly(HEMA). R = free radical initiator

The amount of water present during the reaction affects the transparency of the final product. Warren and Prins (Warren, 1972) reported that <45% water present during polymerization is necessary to produce a transparent material. When greater amounts of diluent are used during the polymerization, the formation of inhomogeneously cross-linked networks occurs with the possibility for phase separation leading to a heterogeneous gel. This reaction can either be conducted by heating or UV

polymerization. Temperatures ranging from 30°C to 80°C (Trigo, 1994;Chirila, 1993) for periods ranging from 12 hours to a week have been reported in the literature.

2.1.4 Silicone Hydrogel Curing Methods

Silicone hydrogel curing processes occur using chemistry that is similar to that which is used for polymerization of pHEMA. Monomers such as TRIS contain the same reactive functional groups that pHEMA contains and can be reacted by the same free radical polymerization technique with an initiator and crosslinker. The only difference is that when reacting HEMA, the addition of water aids in forming larger domains in the material, which are important for increasing water content and transparency. However, in the case of monomers such as TRIS, mixing will not occur and two phases will form during polymerization. The resultant silicone based hydrogel materials have a lower water content then pHEMA.

2.1.5 Contact Lens Oxygen Permeability Requirements

Oxygen permeability, referred to as the Dk [barrer], is a measure of the permeability of the material to oxygen. However, this parameter relates to a material of unspecified thickness. Clinically, an alternative measure termed "oxygen transmissibility" or Dk/t [barrer/mm] is used, which is a measure of the amount of oxygen diffusing through a lens of a particular thickness. The thickness specified is often the centre thickness of the lens (Woods, 2005; Nicolson, 2001). Since most of the oxygen needs of the anterior eye are met via interaction with the tear fluid, the presence of a contact lens provides an additional resistance to oxygen transport, making it more

difficult for the eye to meet its oxygen requirements. The average Dk of conventional contact lenses is 8-20 barrer, while the minimum value required for safely wearing a contact lens overnight has been estimated at 87 Dk/t, implying that conventional polyHEMA-based lenses would not be suitable for extended wear as they will induce chronic hypoxia due to a lack of sufficient oxygen transmissibility (Nicolson, 2001).

2.2 The Eyes and Vision

The need for vision correction affects millions of patients worldwide. It stems from a variety of problems in the visual pathway which consists of the cornea, lens and various internal structures as depicted in **Figure 2-6**. Of particular relevance to the development of new contact lens materials are interactions with the anterior layers of the eye, including the cornea and tear film.

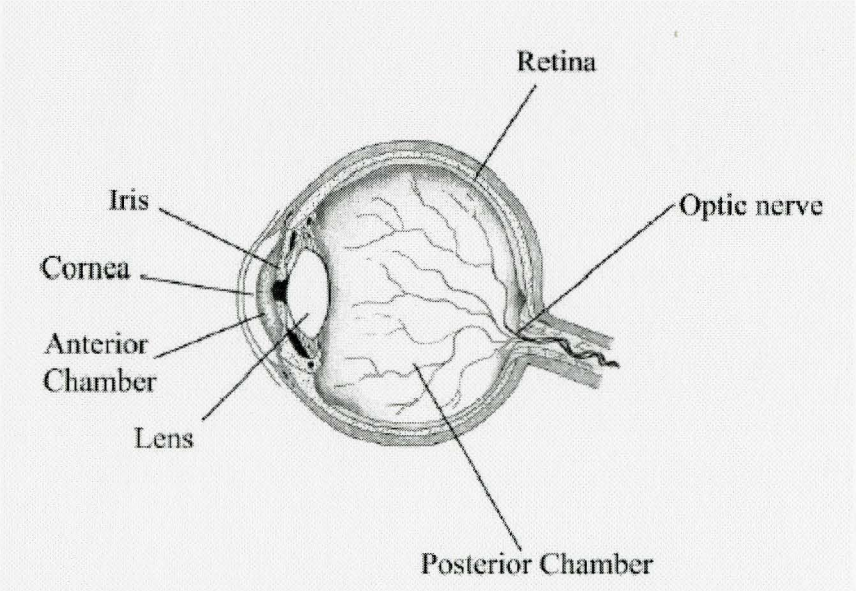


Figure 2-6: Structure of the eye (Lloyd, 2001) Reprinted from *Biomaterials* 22(8) Lloyd, A. W., Faragher, R. G. A., Denyer, S. P. Ocular biomaterials and implants. 769-785. (2001) with permission from Elsevier.

2.2.1 The Cornea

The cornea, as the outer-most tissue in the eye, acts as both the primary refractive element of the eye, providing approximately 70% (Tripathi, 1984) of the optical power, and as a physical barrier, protecting the delicate internal ocular structures from insult from air and tear borne pathogens. The cornea is approximately 0.52 mm thick centrally with peripheral thickness of 0.97 mm and consists of five avascular tissue layers: the epithelium, Bowman's layer, stroma, Descemet's membrane and endothelium (Tripathi, 1984).

The outermost epithelial layer, consisting of approximately 5 layers of epithelial cells, acts as a physical barrier and is responsible for providing a smooth surface wettable by tears. It is a highly innervated tissue (Maurice, 1962), making it highly sensitive to pain, important in preventing injury. Bowman's layer, composed primarily of the cell adhesion peptide laminin, acts as a basement membrane for the epithelial layer, separating it from the stroma. The highly hydrated, high water content stroma comprises the bulk of the corneal thickness (90%) (Maurice, 1962). The structural arrangement of type I collagen fibres of this layer is essential for maintaining stromal transparency and is important for providing the mechanical strength, and elasticity of the cornea. Descemet's membrane, composed of fine type VIII collagenous filaments (van der Rest, 1991), is a basement layer of endothelium. The single cell layered endothelium is a thin layer that acts as a pump for moving excess water from the stroma to the aqueous humor, maintaining the water content of the stroma and hence stromal hydration (O'Neal, 1986).

2.2.2 Tear Film

The tear film layer acts as the interface between the air and the corneal surface and serves many purposes including keeping the eye moist, acting as a barrier to protect the eye from injury and infection, nourishing the eye though the delivery of oxygen as well as providing the optically smooth surface necessary for vision.

The tear film is comprised of at least three distinct regions or layers (Davson, 1990), including the mucus, aqueous and lipid layers. Within these layers, the tear film is known to contain a multitude of proteins and lipids.

The thickness of the tear film has been reported in multiple references (Wang, 2003; King-Smith, 2000; Prydal, 1992), although there is significant disagreement between the reported values. Values range from 3μm, measured by reflection spectra (King-Smith, 2000), to 45 μm, measured by confocal microscopy (Prydal, 1992), although the lower estimates, which were determined using newer techniques, are believed to be more accurate (King-Smith, 2000; Wang, 2003). As shown in Table 2-4, thickness is also dependent on the location of the measurement.

Table 2-4: Human tear film thickness from the literature, adapted from King-Smith, 2004.

Measurement Method	Layer	Thickness
Absorbent disks	PCTF	7 μm
Fluorometric	PCTF	4 μm
ADF	PCTF	<10 -45 μm
Optical pachometry	PCTF	<<40 μm
WDF	PCTF	2.7-11 μm
Theory	PCTF	8-10.4 μm
OCT	PCTF	3.3 μm
Aspiration	Tear film over conjunctiva	3.8 μm
TDF	PLTF	1.5-5.5 μm
WDF	PLTF	2.3-4.9 μm
OCT	PLTF	3.8 μm
Optical pachometry	PolLTF	11.5 μm
WDF	PolLTF	2.3 μm
OCT	PolLTF	4.6 μm
Two wavelength	Lipid	40 nm

Abbreviations: PCTF, pre-corneal tear film; PLTF, pre-lens tear film; PoLTF, post-lens tear film; WDF, wavelength-dependent fringes; TDF, thickness-dependent fringes; OCT, optical coherence tomography.

2.2.2.1 Mucous layer

The innermost mucous layer, secreted by small glands in the conjunctiva called goblet cells, is a highly hydrated, semi solid gel which spreads over the surface of the cornea and conjunctiva. While not strictly part of the tear film, it plays an important role in its stabilization (Holly, 1977). Mucous is hydrophilic, decreasing the surface tension of the tear fluid, allowing for ease of spreading, stability and cohesion (Nichols, 1985).

In a healthy individual, the mucous layer is very thin, a few hundredths of a micron, and has a morphology resembling the microvillous, ridged appearance of the superficial epithelial cell walls (Holly, 1977).

2.2.2.2 Aqueous Layer

The aqueous layer, situated in the middle of tear film, consists of a low viscosity aqueous solution which ensures that the eye is consistently moisturized. While its exact composition varies by individual and time of day, it is composed of inorganic salts, trace ionic elements, glucose, and urea as well as proteins and glycoproteins. It is secreted in the conjunctiva by tiny accessory lacrimal glands. As a result of the presence of bicarbonate ions and proteins, the aqueous layer has some buffering capacity (Holly, 1977). The thickness of this layer is unknown. In a model by Holly, the aqueous layer is depicted as making up the bulk of the tear film; conversely, in a model proposed by Wolff (Wolff, 1946), the mucous layer is much thicker with a relatively thin aqueous layer.

2.2.2.3 Lipid Layer

The outermost lipid layer, produced by the meibomian glands in the eyelids, protects the eye from foreign particles, prevents the evaporation of the aqueous layer and acts as a lubricant during eyelid movement (Tiffany, 1987). In order for this layer to be effective, it must be secreted in appropriate amounts, and must completely cover the hydrophilic aqueous layer (McCulley, 1997). Defects in the lipid layer have been suggested to cause dry spots or even possibly the break up of the tear film (Kaercher,

1994). The measured thickness of this layer using various techniques is summarized in Table 2-5 (Bron, 2004). Its composition, determined from samples collected from human cadavers by column chromatography, is shown in Table 2-6 (Nicolaides, 1981).

Table 2-5: Thickness of the tear film lipid layer adapted from (Bron, 2004)

Method	Tear film lipid layer thickness (nm)
Differential interferometry	100
Photometric reflectometry	32-46
Specular microscopy	13-100

Table 2-6: Composition of lipid layer determined from human cadavers by column chromatography adapted from (Nicolaides, 1981).

Lipid Component	Percent
Hydrocarbons	7.54
Sterol esters	27.28
Diester region	7.74
Triacyl glycerol (TG)	3.70
Post-TG region	2.98
Wax esters	32.32
Free sterols	1.63
Free fatty acids	1.98

2.2.3 Proteins in the Tear Film

Proteins are important components in the tear film, playing important roles in defense, immune response, and wound healing. They are mainly present in the aqueous layer of the tears, and Table 2-7 summarizes the properties of the five primary proteins in the tears.

Table 2-7: Properties of proteins and lipids in tears.

Protein	MW (kDa)	pI	Concentration (mg/ml)
Lysozyme	14.5	10.5	1.9
Albumin	66.3	4.9	0.02-1.24
Secretory IgA	160	4.5-5.5 (Waldo, 1989)	2.76-4.57 (Choy, 2004)
Lactoferrin	80 (Lonnerdal, 1995)	8.7 (Moguilevsky, 1985)	1.9 ± 0.62 (Abe,1999)
Tear Lipocalin	17-18	4.9-5.2	3.32-3.46 (Choy, 2004)

2.2.3.1 Lysozyme

Lysozyme, an abundant, bacteriolytic enzyme found in the tears, is widely reported as the protein found most commonly on worn contact lenses (Moradi, 2004). As such, it has been the most widely studied. It is produced by the acinar and ductal epithelial cells of both the main and accessory lacrimal glands (Janssen, 1983). Lysozyme can also be found in urine, saliva, milk, cervical mucus, leukocytes and kidneys (Berman, 1991).

Lysozyme is a 14.5 kDa compact globular protein comprised of a single chain of 129 amino acids (Osserman, 1974). Due to a large cluster of basic domains, lysozyme has a high isoelectric point of 10.5 and is highly positively charged at physiological conditions. Furthermore, because lysozyme is a "hard" protein, being a small molecule with strong internal cohesion (Garrett, 1999), it tends to present this positive charge at the surface.

Very abundant in the eye, it plays a major role in the resistance of the external eye to infection. Comprising between 20% and 40% of the proteins in the tears, lysozyme catalyses the hydrolysis of N-acetylmuramic acid-N-acetylglocamine β 1-4 linkage of

bacterial cell walls (Saari, 1983). Decreased lysozyme concentrations have been shown in many infections and diseases of the eye including keratitis sicca syndrome (McEwen, 1955), acute and chronic conjunctivitis or keratitis (Thomspon, 1936), and acute herpetic eye disease (Eylan, 1977). Persons suffering from acute trachoma have been reported to show a total absence of lysozyme in tears (Grabner, 1982).

2.2.3.2 Human Serum Albumin

Human Serum Albumin (HSA) is an unglycosylated protein found most abundantly in plasma. With a molecular weight of 66.3 kDa, it consists of 585 amino acids linked in a nine double loop structure, a result of the presence of 17 disulfide bonds. With an isoelectric point of 4.9, HSA is negatively charged at the physiological tear film pH of 7.4. In plasma, HSA is an important contributor to colloid osmotic pressure and acts as a transport vessel for a multitude of substances in the body. Although its role in the tear film is less clear, it can be hypothesized that the role in the tears is likely similar to the role in the plasma. As well, it is known that serum albumin has multiple binding sites for fatty acids and lipids and is important for lipid transport. As lipids are important components of the tear film, a similar role for tear albumin may be assumed. Furthermore, of all the plasma proteins in the body, albumin has the highest buffering capacity, particularly in fluids where hemoglobin is not present (Era, 1995). It is therefore likely that albumin plays a role in maintaining the pH of the tear fluid. It is known that albumin leaks through conjunctival blood vessels into the tear film as a result of stress and inflammation. The concentration of albumin in the tear film has been

reported to range between 0.02 and 1.24 mg/ml, dependent on the tear physiology of the individual and time of day as well as the stress level in the eye (Rebeix, 2000).

2.2.3.3 Immunoglobulin

Immunoglobulins are antibodies found abundantly in blood and tissue fluids. There are five classes of immunoglobulins in our body; IgG, IgA, IgM, IgD, and IgE, of which only IgG and IgA are present in substantial quantities in the tears. IgM is present in very low concentrations in tear solution, a result of transudation from the serum. Both IgG and IgA play a role in defending the ocular mucosal surface from viral and bacterial infections (Bours, 2005). The presence of these proteins in the tears provides evidence of the importance of layer in protecting the internal structures of the eye.

2.2.4 Tear Film and Contact Lenses

Contact lens wear has been shown to significantly affect the nature of the tear film, breaking it up, disrupting its reformation, and increasing tear film evaporation. This has been shown to result in contact lens induced dry eye, the third highest reported problem associated with contact lens wear after lens deposits and patient compliance. Interestingly, it has been demonstrated that the effects of five contact lens materials (Polymacon, Omafilcon A, Phemfilcon A, Balafilcon A and Etafilcon A) on tear thinning time and tear evaporation rates were similar, with no significant differences between materials on tear film physiology; all materials had significant negative effects (Thai, 2004).

2.2.5 Protein Adsorption

Protein fouling of contact lenses is a major problem. Tear film components such as lipids (Bontempo,1994; Jones, 2000; Bontempo, 1997), proteins (Baines, 1990;Bohnert, 1988;Myers,1991) and mucin (Castillo, 1986) have been found to deposit onto the surface of contact lenses. Initially invisible to the wearer, these protein films are capable of buildup to the point where they are visible, resulting in a whitish tint that is composed primarily of denatured proteins (Franklin, 1992). They cause wearer discomfort and, in the extreme case, lead to inflammatory conditions. Protein deposits are believed to induce or aggravate such inflammatory conditions as giant papillary conjunctivitis, one of the most common reasons for discontinuation of lens use (Skotnitsky, 2002), as well as corneal vascularization and infection (Dohlman, 1973). Therefore, successful lenses for use on an extended or continuous wear mode require low protein adsorption.

2.2.5.1 Protein Adsorption Fundamentals

Macromolecular adsorption events such as protein adsorption are largely governed by interfacial forces, including van der Waals (dispersion forces), electrostatic forces as well as hydrophobic and hydration forces in the domain between bulk solution and surface. These forces typically only affect macromolecules on length scales of approximately 10 nm from the surface. Diffusion affects macromolecules at much larger distances, often in the tens of micrometers range at low Reynolds numbers, and at these distances diffusion is the dominant mechanism causing the movement of macromolecules

towards the surface. At further distances in the bulk solution, the macromolecules maintain their native conformations (van Oss, 1994).

Electrostatic effects occur when a surface is placed in an ion containing solution. Fixed charges on the surface cause a disruption in the ion distribution of the aqueous solution resulting in an accumulation of counter-ions at the surface. This establishes a layer of fixed counter ions, known as the Stern layer or an electrical double layer. The adjacent Gouy-Chapman layer is defined by its diffuse ion concentration (van Oss, 1994). The effective length over which this layer affects protein adsorption is described by the ionic strength dependent Debye number. Typical values are less then 1 nm in 0.15 M saline, which is similar to blood and tears (Israelachvili, 1991).

van der Waals dispersion forces arise from fluctuating dipole-induced dipole interactions. While these forces are typically short range, their strength increases as the intervening distance from surface to protein decreases. These forces are the sum of Keesom, Debye and London contributions whose energy decays as the inverse sixth power of separation distance for nonretarded interactions. These forces are typically calculated using a simple approach described by Hamaker (Roth, 1996).

Hydrophobic interactions and hydration forces are related to hydrogen bonding interactions and are attractive and repulsive, respectively. These forces are capable of representing energies that are up to 100 times greater then those of van der Waals and electrostatic forces combined. These forces can extend for tens of nanometers and 1-5 nm, respectively (van Oss, 1994).

It is thought that initially protein adsorption occurs if the balances of forces on the protein to the surface are favored over the protein to solution forces. There is an exchange that occurs each time a protein adsorbs to the surface where for every such reaction, one solvent-surface and one protein-solvent interaction is disrupted. This exchange is governed by Equation 2-1 first introduced by Silberberg (Silberberg, 1968).

$$\chi_s = z'(h_{s1} - h_{s2} + \tfrac{1}{2}h_{22} - \tfrac{1}{2}h_{11})/kT \qquad 2\text{-}1$$

where χ_s is the adsorption enthalpy, h_{11} is the enthalpy of protein-protein, h_{22} is the enthalpy of solvent-solvent, h_{s1} and h_{s2} are enthalpy of the solution at the surface and z' is the total number of possible surface contacts. Proteins can form various orientations on the surface, depending on their affinities for the surface and solvent. In a solution containing many proteins, initially adsorbed proteins can be displaced by higher affinity and lower concentration proteins in a process known as the Vroman effect (Vroman, 1964). Protein adsorption occurs very rapidly with burst adsorption occurring followed by a plateau effect when equilibrium in the system is established.

2.2.5.2 Protein Adsorption Studies on Contact Lens Materials

The most abundant tear film protein, lysozyme, which comprises approximately one third of the proteins in tears, is generally reported as the most abundant protein associated with lenses (Senchyna, 2004). This is thought to be the result of the availability and small size of this protein as well as its positive charge and compact globular nature which gives it a high affinity for negatively charged lens surfaces such as those containing methacrylic acid. Other tear film components are typically of the same

charge as the contact lens material and, while proteins tend to be amphipathic with both positive and negative regions, the net negatively charged proteins would be expected to be repelled to a greater extent than the positively charged proteins (Soltys-Robitaille, 2001). Furthermore, while protein interactions with biomaterials is typically thought to be a surface phenomenon, resulting in the formation of a protein monolayer (Castner, 2002), in the case of high water content gels such as are used in contact lens applications, absorption of the protein is possible and indeed likely, depending on the size of the pores and the nature of the interactions between the gel and the proteins.

There has been significant research into the degree to which commercial lenses foul. In most in vitro studies, protein adsorption from artificial tear solutions to commercially available lens materials is measured, although in many cases, this artificial tear solution contains only lysozyme, presumably based on the finding that lysozyme is the most abundant protein on the lens surface.

One study of some relevance explored the uptake of lysozyme on pHEMA copolymers (commercial lenses were not examined (Garrett, 1999)). Radiolabel-tracer techniques were used to quantify protein adsorption. Protein penetration into the lens was assessed by confocal microscopy. It was found, not surprisingly, that in the case of high water content, ionic materials such as HEMA/MA and HEMA/NVP/MA hydrogels, lysozyme adsorption was dominated by a penetration process. The authors postulated that the process was initially driven by electrostatic effects. Furthermore, non-ionic, low water content materials such as pHEMA were found to sorb significantly less lysozyme; this lysozyme was not penetrating the surface.

In a follow up study, (Garrett, 2000) different HEMA containing polymers were prepared which contained various amounts of either methacrylic acid (MAA) or N-vinyl pyrrolidone (NVP) on a pHEMA backbone. Single protein solutions containing either radiolabelled HSA or lysozyme were used. Results of the adsorption experiment are summarized in Table 2-8. It should be noted that, aside from measuring the water content of the modified materials, there was no independent confirmation that the modifications had occurred.

Table 2-8: Summary of results by Garrett, Q. et al. (Q. Garrett et al., 2000)

Surface	***Water Content*** %	***Lysozyme adsorption*** (ng/cm^2)	***Albumin Adsorption*** (ng/cm^2)	***Estimated Porosity***
pHEMA	30.2±1.6	268±11	121±18	13.8
pHEMA, 1% MAA	37.8±2.9	5730±511	103±12	17.3
pHEMA, 3% MAA	59.7±1.8	300000±45000	87±9	31.6
pHEMA, 5% MAA	62.8±2	550000±45000	75±5	34.7
pHEMA, 10% NVP	34.5±1.6	250±11	93±9	16.3
pHEMA, 15%NVP	38.3±2	500±20	110±5	18.6
pHEMA, 25% NVP	43.9±3.1	1900±350	138±11	22.4
pHEMA, 0.5%MAA, 5% NVP	37.2±2.1	1000±150	78±10	

From Table 2-8 it can be seen that increasing amounts of the non-ionic NVP results in increased water content and estimated porosity from theoretical models. These increases also resulted in small increases in lysozyme and albumin sorption. A much more dramatic change was observed with the presence of ionic materials in the HEMA. Increasing amounts of the ionic MAA in the polymer resulted in an expected increase in both the porosity and water content. However, most notable was the exponential increase in lysozyme adsorption with a corresponding linear decrease in albumin absorption. This study demonstrated the effect material composition and surface charge have on lysozyme

adsorption. Albumin adsorption was generally low for all materials. The results suggest that the use of non-ionic materials to increase water uptake has definite advantages over the use of ionic materials. Interestingly, in another study by Garrett and Milthorpe (Garrett, 1996), HSA adsorption to three materials (2 ionic and one non-ionic) was examined. Higher HSA adsorption on one of the ionic materials was found compared with the non-ionic material. This result is opposite to expectations if electrostatic effects play a significant role in HSA adsorption. They also found that HSA did not have high levels of irreversible sorption, but that the amount of irreversibly adsorbed HSA increased with increasing time of exposure.

Other studies have reached similar conclusions (Baines, 1990;Sack, 1987; Bohnert, 1988). Baines (Baines, 1990) used radioiodine tracing techniques to measure protein adsorption and concluded that high water content ionic lenses bound more protein then low water content non-ionic lenses. Sack (Sack,1987) determined that ionic lenses left a thick layer of lysozyme on the surface whereas non-ionic materials left a much thinner film. This study also found that water content itself had little effect on protein adsorption.

Similar conclusions have been reached in clinical studies. Jones et al. (Jones, 2000), examined 22 patients wearing ionic HEMA/MAA or non-ionic HEMA/NVP lenses for periods of up to 28 days and found dramatic differences in total protein deposition. The ionic lens material, for a 28 day study, had a total of 493 ± 101 μg/lens versus only 40 ± 7 μg/lens for the non ionic lenses. This trend of high adsorption for the ionic lens was observed for all time points measured throughout the study. Their total

protein deposition was measured by UV transmission on an ultra violet spectrophotometer.

Literature would seem to indicate charge having a large effect on adsorption and thus, in the design of materials, non-ionic materials are favorable over ionic materials. It is interesting to note that very few studies consider other factors which may affect protein adsorption and only ever consider charge. It is probable that studies were designed around the notion that lysozyme is the major adsorbing protein, and thus focus on methods of preventing only its adsorption. But these studies seldom look at the effect this has on the multitude of other tear proteins present which are often present in equally concentrated amounts by weight.

2.2.5.3 Poly(ethylene oxide) Protein Repulsion

Hydrophilic, water soluble polymers such as poly(ethylene) oxide (PEO) are known to significantly reduce protein adsorption on surfaces. The mechanism by which this occurs is not fully understood. One theory, the steric stabilization/repulsion theory, hypothesizes that inhibition of protein adsorption is a result of end-tethered PEO or other surface bonded, long chain hydrophilic molecules (Napper, 1983). As a protein approaches the PEO layer, they become dehydrated and the compression they cause on the PEO decreases PEO chain conformation leading the system into an unfavorable thermodynamic state. As a result of the regional dehydration that occurs, local osmotic pressures increase causing water to be forced back into the PEO and proteins near the interface. This movement of water is believed to cause the decreased protein-surface interactions (McPherson, 1995).

Others factors that have been shown to affect protein adsorption to PEO modified surfaces are the flexibility of the PEO, how tightly it is anchored, and surface coverage (McPherson, 1995). Chain density of the PEO layer also impacts protein adsorption with each molecular weight having an optimum chain density (Unsworth, 2005). PEO may also mask surface forces such as hydrophobic and van der Waals forces (Malmsten, 2003).

2.3 Hyaluronic Acid

Hyaluronic acid (HA), also referred to as hyaluronan and hyaluronate is a naturally occurring, linear polysaccharide from a group polysaccharides called glycosaminoglycans. Discovered in the vitreous body of the eye in 1934 by Karl Meyer, HA was long assumed to act as an inert filling material between cells (Engstrom-Laurent,1997).

2.3.1 Structure and Properties of Hyaluronic Acid

The structure of HA, shown in Figure 2-7, was first published in 1954 (Weissmann, 1954). It is a polymer consisting of alternating units of glucuronic acid and N-acetylglucosamine. Commercial HA is isolated from animal sources or produced using recombinant technologies. These methods allow for the production and isolation of a range of molecular weights between 1 and 5000 kDa (Brown, 2005). HA is non-immunogenic, biodegradable, and viscoelastic (Leach, 2004). These properties make it particularly of interest for biomaterials research.

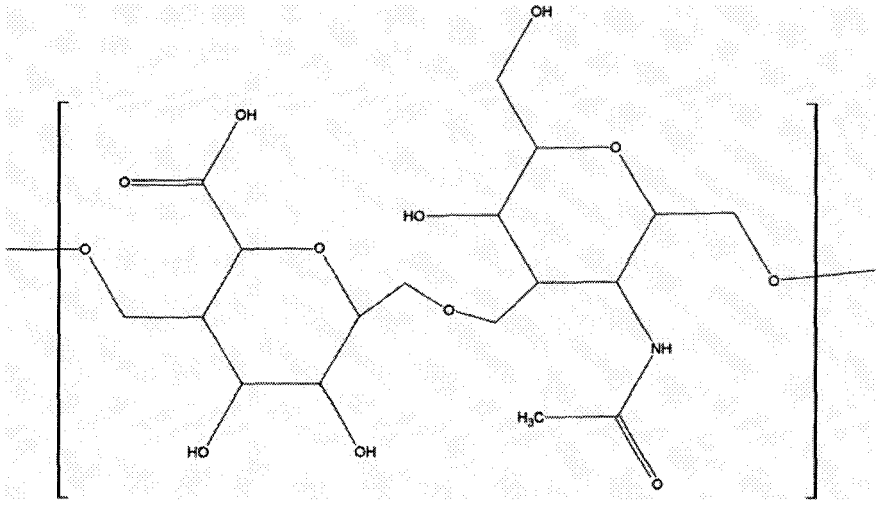


Figure 2-7: Repeat structure of Hyaluronic Acid.

2.3.2 Function of HA

While the exact role of HA in the body may not be fully understood, HA has been shown to play a role in the regulation of the permeability of substances by steric exclusion. It has been implicated in the lubrication of joints (Dougados, 1993) and also in water homeostasis (Laurent, 1996). HA has been to found to bind to proteins in the extracellular matrix, within the cell cytosol, and at the cell surface (Fraser, 1997). The binding indicates that it plays a role in cartilage matrix stabilization, cell motility, growth factor action, embryonic development, morphogenesis, and inflammation (Luo, 2000). HA present in the joints is thought to act as a space filler, lubricant, as well as performing scavenger functions and regulating cellular activities (Laurent, 1996).

2.3.3 Ophthalmic Applications of Hyaluronic Acid

Hyaluronic acid has been reported to be effective in improving dry eye symptoms as well as lessening ocular surface epithelial damage (Polack,1982). It promotes corneal epithelial wound healing by accelerating cell migration, and protecting the corneal epithelium (Nishida, 1991). Studies have found that HA containing drops also improve corneal epithelial barrier function suggesting HA improves the integrity of corneal superficial cells (Yokoi, 1997). The water retentive properties of HA are believed to provide tear fluid stability in dry eyes (Göbbels, 1992). As a result HA is widely used in eye drop solutions for relieving dry eye symptoms, (Sand, 1989;Shinmmura, 1995;Aragona, 2002) other studies have concluded that HA has no effect on dry eye symptoms (Limberg, 1987; Nelson, 1988). Hyaluronic acid has also been used in intraocular lens surgery as a viscoelastic to raise the intraocular pressure (Holzer, 2001). It has also been used to coat IOLs made of PMMA in the hopes of providing more inert surfaces to protein and bacterial adhesion (Cassinelli, 2000).

2.4 Hypothesis and Objectives of the Present Research

New commercial materials use silicone hydrogel chemistry with surface/bulk modifications to produce low fouling surfaces with high oxygen permeability. The use of wetting agents, either releasable or locked into the hydrogel are two new methods of modifying conventional hydrogel lens materials and silicone hydrogels to lead to the development of more hydrophilic interfaces. The objective of this work is therefore to establish a method of incorporating hyaluronic acid into model lens materials and to

compare the effects of HA release versus HA incorporation on the properties of the materials. Surfaces will be characterized using a number of techniques in order to confirm the modification. The thermal properties of these materials will be examined to assess the effect of the presence of HA. Bulk characterization by such techniques as TEM and FTIR will be used in conjunction with surface characterization techniques including XPS. However, of utmost importance, measurement of the adsorption of relevant tear proteins to these materials will be examined. Specifically, it is hypothesized that:

- Extremely rapid HA release will be observed in the absence of any crosslinking step for the HA.
- A more hydrophilic surface interface will result with HA incorporation but, in the case of the uncrosslinked HA, this improvement will be short lived as the HA will be released.
- The presence of carboxylic acid group on HA will be suitable for reactions with amine groups in the presence of EDC to form a crosslinked network, resulting in materials containing physically entrapped hyaluronic acid which cannot be released under simulated physiologic conditions.
- The incorporation of crosslinked HA will not lead to significant changes in the optical transparency of the materials.
- HA modified materials will have protein rejecting properties.

3. Materials and Methods

3.1 Preparation of pHEMA Surfaces

2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA) and benzoyl peroxide were purchased from Sigma Aldrich, Oakville ON. HEMA monomer was purified to remove 4-methoxyphenol (MEHQ), a polymerization inhibitor, by passing the monomer through a column packed with Aldrich inhibitor removers (Sigma-Aldrich, Oakville ON).

HEMA and 1% by weight EGDMA were mixed. To this mixture, an equal amount of water by weight was then added and the mixture stirred. Subsequently, 1% by weight benzoyl peroxide (Sigma-Aldrich, Oakville ON), dissolved in a small amount of tetrahydrofuran (THF) (Caledon, Georgetown ON), in a 1.5 ml vial, was added. This final step required very rapid mixing to prevent the precipitation of the benzoyl peroxide initiator, as any precipitate resulted in the presence of solid white particles in the resulting pHEMA, which were easily visible to the naked eye. The solution was then poured into a custom designed 10 cm diameter Teflon mold depicted in Figure 3-1a/3-1b. The schematic for the molds is shown in Figure 3-2. These molds consisted of sheets of Teflon with a Teflon ring along the edge that provided a tight seal to the sheet. Structural stability was provided by a Plexiglas ring placed on top of the Teflon ring. These rings were sealed to the sheet using 8 screws which could be adjusted to level the mold as needed. The molds were open to air on the top. The mixture was placed in a 400 watt UV chamber (Cure Zone 2 Con-trol-cure, Chicago IL) and cured for 25 minutes. The surfaces were subsequently placed in an oven at 50°C overnight to ensure that all

monomer was fully reacted. Materials were then removed from the molds and placed in water for one to two days to ensure complete swelling prior to cutting as well as to remove any unreacted monomer on the samples. Samples of pHEMA were cut to the desired size, typically ¼ inch disks, and placed in a 48 well plate to be dried at 40°C overnight.

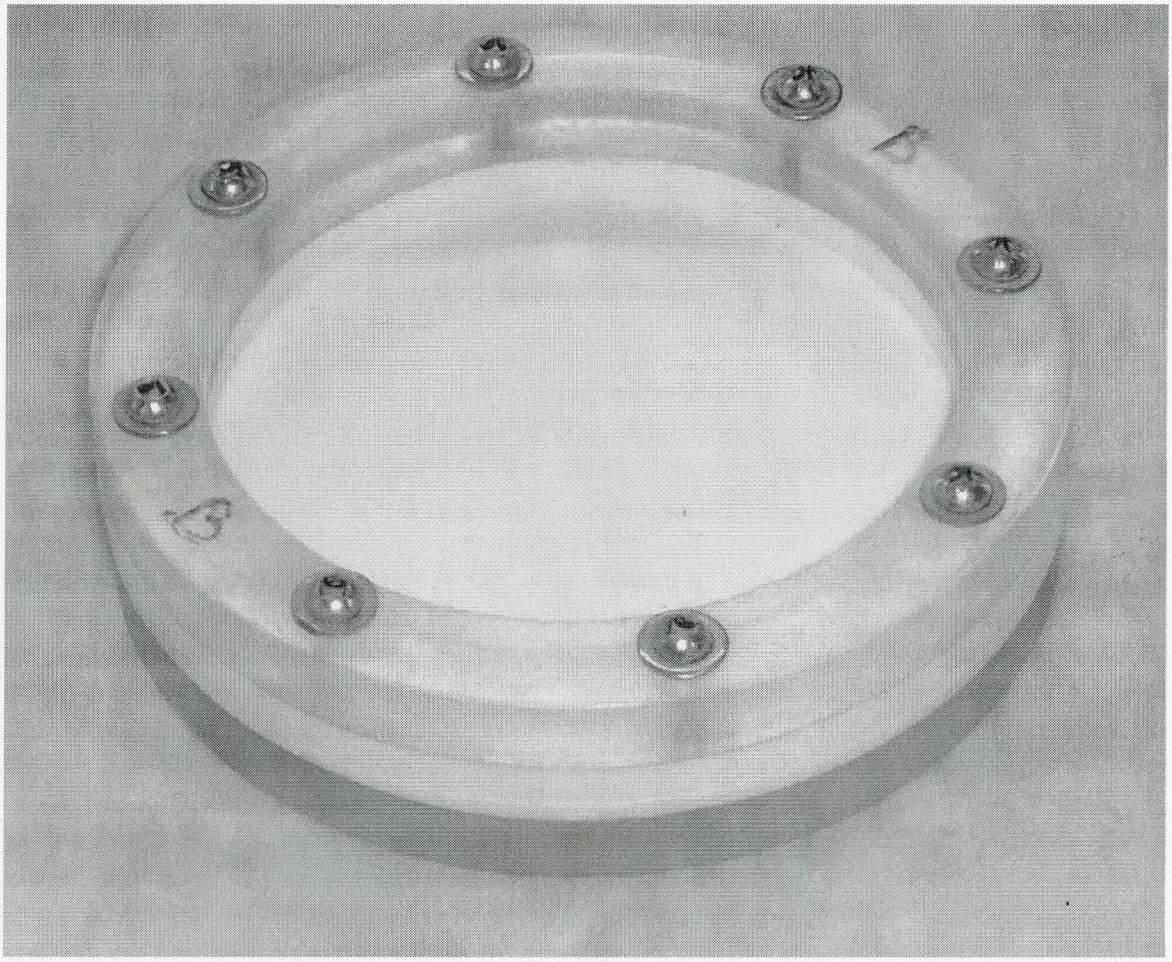


Figure 3-1a: Assembled Teflon mold used for preparation of pHEMA surfaces.

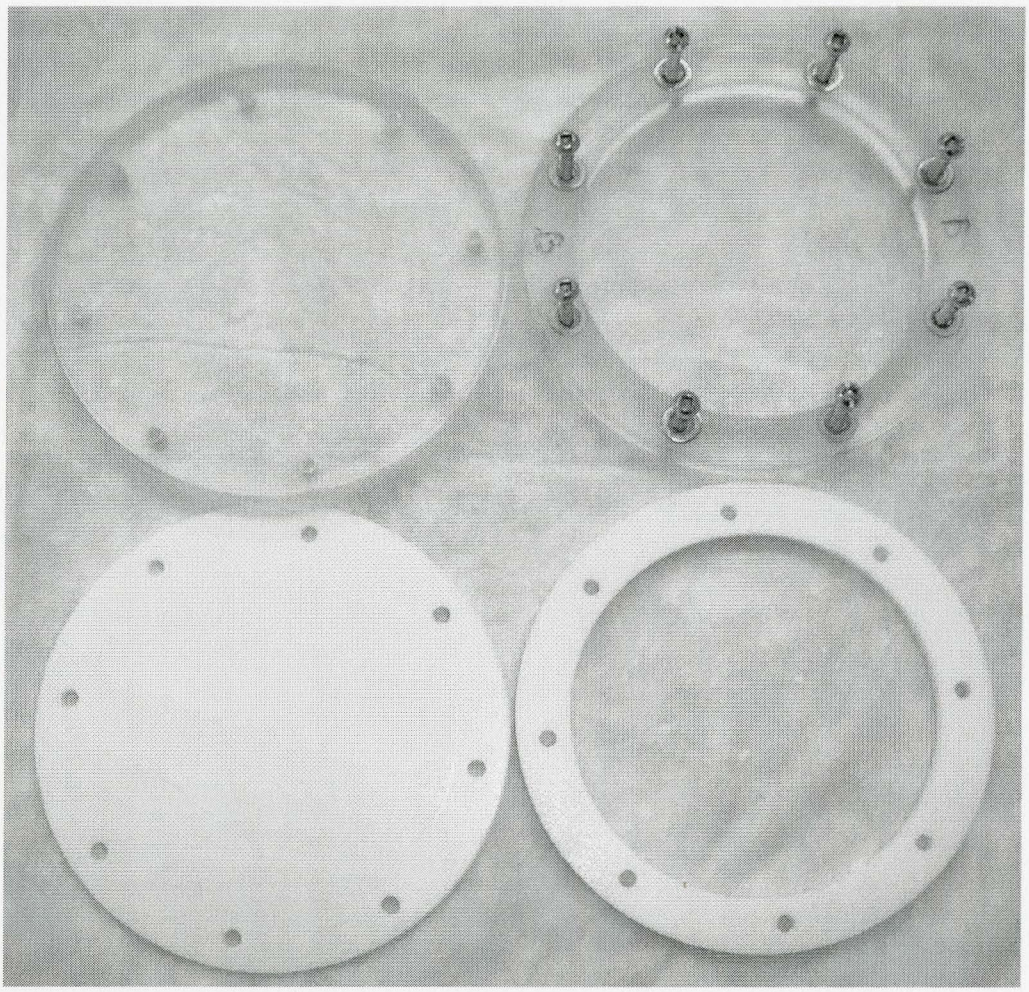


Figure 3-1b: Teflon mold disassembled into pieces.

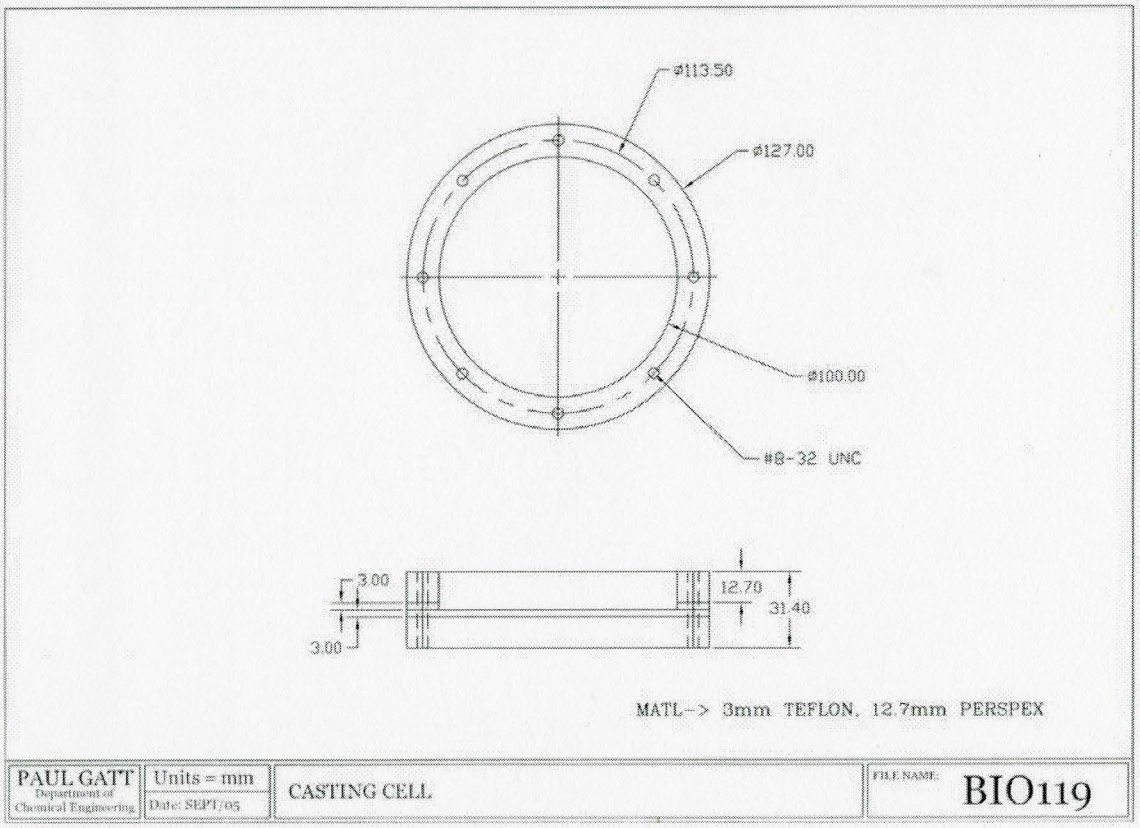


Figure 3-2: Schematic diagram of polymerization molds used in the preparation of pHEMA samples.

3.2 Preparation of pHEMA/TRIS Surfaces

IRGACURE was purchased from Ciba (Mississauga, ON). Methacryloxy propyltris (trimethylsiloxy) silane (TRIS) was purchased from Gelest (Morrisville, PA). TRIS monomer and HEMA monomer were purified to remove the 4-methoxyphenol (MEHQ) polymerization inhibitor as previously described. HEMA, TRIS and 5% by weight EGDMA were mixed. Subsequently, 0.5% by weight IRGACURE was added and mixed thoroughly. The solution was then poured into the custom mold, placed in the UV chamber (Cure Zone 2 Con-trol-cure) and cured for 25 minutes. The surfaces were

subsequently placed in an oven at 50°C overnight to ensure that all monomer was fully reacted. Following polymerization, materials were removed from the molds and placed in water for one to two days to ensure complete swelling and remove any unreacted monomer from the samples. Samples were cut to the desired size, typically ¼ inch disks, and placed in a 48 well plate to be dried at 40°C overnight.

3.3 HA loading of materials

Hyaluronic acid (HA) (LifeCore Biomedical, Chaska, MN) solutions (5 g/L) were prepared in 30% ethanol / 70% water based on the results of the swelling study. Four molecular weights of HA were studied: 6.3, 30, 169 and 900 kDa. Dried hydrogel disks were placed in an excess amount of the HA containing solutions in 48-well plates and sealed using parafilm, for a minimum of four days at 4°C to ensure that a maximum uptake of HA. A temperature of 4°C was selected to ensure that the HA did not degrade. Times of 1, 2, and 4 days were examined in preliminary studies. It was found that there was little difference in the uptake of the HA containing solution as a function of time suggesting that the uptake was relatively rapid. However longer time periods were used for all studies in order to ensure that the maximum amount of HA could be loaded into the materials. Materials generated using this method would presumably have free HA chains which are essential for decreasing protein adsorption. The incorporation of HA into the HEMA backbone by chemical reaction with the HEMA would presumably result in the lower levels of free HA and less control over the crosslinking than the HA incorporated using the current method.

3.3 Hyaluronic Acid Crosslinking

In order to physically entrap the HA into the HEMA structure, crosslinking was effected. Similar to the materials loaded with HA, the materials which contained crosslinked HA were prepared by making a hyaluronic acid and diaminobutane-4 (DAB-4) generation 1 dendrimer (Sigma, Milwaukee WI) in 30% ethanol/70% water. The concentration of each component was 5g/L. The dendrimer was used to amplify the amount of amine groups present and therefore to increase the crosslinking density. Dried samples were loaded in 0.2ml of this solution for a minimum of four days at 4°C to ensure complete uptake. Following loading, a solution containing approximately 1% by weight 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) in water was prepared. The loaded samples were then placed in an excess of the EDC reagent for a period of 24 hours for crosslinking of the loaded HA. The EDC was not added to the initial uptake gelation of the solution surrounding the pHEMA disks occurred. This was followed by release of the unreacted HA and dendrimer by soaking in water for a minimum of two days prior to characterization.

3.4 Bulk Characterization

3.4.1 PHEMA Swelling Studies

Based on prior work within the research group (Princz, 2005), ethanol solutions were used as the swelling solutions. In order to assess the extent of swelling of pHEMA in different solvents, cured pHEMA disks were cut and completely dried at high solution as it was found that the crosslinking of the HA occurred very rapidly and temperatures

(70°C) for 24 hours. Samples were weighed and placed in 48-well plates. To these samples, aqueous solutions containing varying amounts of ethanol ranging from 0% to 100% were added in excess. Three samples were used for each ethanol concentration, with the exception of the 70% and 90% ethanol samples, which an n of two was used. As the amount of ethanol increased, a noticeable decrease in the mechanical properties resulted to damage to one of the samples, making accurate measurement of the mass impossible. The plate was then covered and sealed with parafilm to prevent liquid from evaporating. Samples were incubated at room temperature for 72 hours to ensure maximum uptake, subsequently removed from the ethanol solutions and gently blotted with a Kimwipe to remove any excess solution. The samples were immediately weighed again and the solvent uptake was calculated based on:

$$Solvent\ Uptake = \frac{Wet\ Weight - Dried\ Weight}{Wet\ Weight} \quad [\%] \tag{3-1}$$

These results were then plotted against solvent concentration to determine any relationships that may exist.

3.4.2 Equilibrium Water Uptake Measurement

Water uptake was used as an indirect measurement of HA incorporation and crosslinking of the samples, as the HA would be expected to result in higher water swelling although higher crosslinking tends to result in a lower equilibrium water content in hydrogel polymers (Bryant, 2002; Clayton, 1997). The samples were completely dried, either by heating or by freeze drying. The sample was then weighed, placed in water, and

the weight determined as a function of time. When the weight stopped increasing, it was considered to have reached equilibrium. The water uptake was then determined from:

$$\%\ Water\ Uptake = \frac{Dried\ Weight}{Equilibrium\ Weight} * 100 \quad [\%] \tag{3-2}$$

3.4.3 Tensile Strength Testing

The mechanical properties of the hydrogel materials, were measured at room temperature using an Instron Series XI Automated Material Testing System with a 50 Newton load. Samples with hydrated dimensions of 4 cm by 1.5 cm were prepared. Hydrated samples were loaded and tensile strain and stress were measured using a crosshead speed of 5mm/min. The top and bottom of each sample was wrapped in paper tape to protect the samples from clamping and to measure the properties of the samples. The tensile strain and tensile strength were then determined from Equation 3-3 and 3-4 respectively:

$$Tensile\ Strain(\%) = \frac{Length\ at\ break\ (cm)}{Initial\ Length\ (cm)} * 100\% \tag{3-3}$$

$$Tensile\ Strength\ (Mpa) = \frac{\text{Force at break(N)}}{\text{Crosssectional area}\ (mm^2)} \tag{3-4}$$

3.4.4 Differential Scanning Calorimetry

TA 2910 MDSC Differential Scanning Calorimeter was used to measure the glass transition of the different materials produced. This method was used to discern any changes that may have occurred in the thermal properties of the material as a result of the incorporation and crosslinking of the HA. 2-10mg freeze-dried samples of pHEMA,

pHEMA/35 kDa un/crosslinked HA and pHEMA/169 kDa un/crosslinked HA samples were placed in a hermetically sealed pan and to 300°C at a rate of 15°C/min.

3.4.5 Transmission Electron Microscopy

In order to assess whether visible HA domains were present in the HEMA polymers, transmission electron microscopy, which has been previously used with interpenetrating network polymers (Liu, 2005a) was used. Samples were embedded in a water-miscible melamine Nanoplast resin (Ted Pella Inc., Redding CA). Small sections of sample were placed in the Nanoplast resin for 48 hours at 40°C, during which time, the water in the sample was replaced by the resin. This was followed by 48 hours at a temperature of 60°C to polymerize the resin. After polymerization, the resin could be stored at room temperature. This technique allowed for thinner sections then would be possible using cryo-sectioning techniques. Samples were then analyzed using a JEOL JEM 1200-EX transmission electron microscope (JEOL, Peabody, MA) operated at 80kV.

3.5 Surface Characterization

3.5.1 Water Contact Angle Measurements

Sessile drop advancing and receding contact angles provide relative information about the surface hydrophilicity and hydrophobicity. Samples were placed on glass slides and dried at 37°C overnight. Sessile drop advancing and receding water contact angles were measured using MilliQ water drops with a volume no greater than 3 μL using a Ramé-Hart NRL 100-00 goniometer.

3.5.2 Atomic Force Microscopy (AFM)

Atomic force microscopy is a common technique of analyzing polymer surfaces for properties including surface roughness. This technique provides high resolution images of the material surface capable of showing topography in the nanometer range. 2μm x 2 μm areas were analyzed using a NanoScope IIIa AFM in surface tapping mode. 512 sample lines and 1:1 aspect ratio was used for all measurements. Surfaces were dried prior to analysis.

3.5.3 X-Ray Photoelectron Spectroscopy (XPS)

XPS is a technique widely used to measure the chemical composition of the surface of a material. The surface is bombarded with x-rays; the energy of electrons released from the material is measured and correlated to specific chemical elements in the material. Dried samples were sent to the Surface Interface Ontario Laboratory at the University of Toronto for XPS analysis. XPS spectra were obtained using a Leybold Max 200 X-Ray Photoelectron Spectrometer. Analysis of the data was performed using SPECSlab software and 285 eV was referenced as the C-C peak. A high resolution carbon scan was conducted in the 280 to 290 eV binding energy range. This method produced intensity peaks that are used to quantify the carbon bonds present on the material surface. Low resolution scans for binding energies between 280 and 550 eV were conducted to quantify ratios of carbon, nitrogen and oxygen. These methods could give an indication of whether the reaction between HA and the dendrimer were occurring and if there was HA/dendrimer present on the surface.

3.5.4 Fourier-transfer Infrared Spectroscopy

Fourier-transfer Infrared Spectroscopy (FTIR) was used to determine and to quantify the amount of hyaluronic acid in the crosslinked HEMA materials. Samples were ground into a powder and mixed with KBr at a ratio of 1:5 sample:KBr. They were then molded into a thin disk by compression. Spectra were obtained at room temperature under nitrogen protection on a BIO-RAD FTS-40 machine with Bio-Rad Win-IR software. The parameters used for this study were: Resolution 2, Sensitivity 1, Aperture 2, Scan time 16.

3.6 Protein Adsorption Studies

Lysozyme (chick), human serum albumin (HSA), β-lactoglobulin (Bovine) and immunoglobulin-G (IgG) (Human) were obtained from Sigma-Aldrich (Milwaukee WI). 10 mg/ml solutions of each protein in phosphate buffered saline (PBS) were prepared.

3.6.1 Radiolabelling Protein

Proteins were then radiolabeled with $Na^{125}I$ or $Na^{131}I$ using the iodine monochloride method (ICl) as previously described (McFarlane, 1963). Protein solutions, placed in a 1.5 ml vial (1), were buffered with 0.2 ml glycine buffer (pH 8.8). In a second vial (2), ICl reagent, with amounts being dependent on protein and amount labeled, was added to 0.060ml glycine buffer. To vial 2 was added 5μL of either ^{125}I or ^{131}I and the solution mixed for 1 minute. The contents of vial 1 were then added to vial 2 and mixed for 2 minutes. Unbound ^{125}I and ^{131}I was removed by passing the labeled

(PBS, pH 7.4) samples through a 3ml syringe packed with AG 1-X4 (100-200 dry mesh in chloride form) (Bio-Rad, Hercules, CA). Excess PBS buffer, typically 3mL or more, was passed through the column to ensure all protein was collected.

Excess protein was labeled in each experiment to account for any loss that may occur as a result of the free iodide removal process. As this loss can be in excess of 50%, 2-3 times more protein was labeled than was needed for the experiments. In all cases, 5 μl of isotope was used for labeling each protein, independent of the amount of protein labelled. Approximately 4 mL of solution was collected from the column for each protein. Free iodide was determined by trichloroacetic acid(TCA) precipitation in all cases. Briefly, 100 microliters of radioactive protein solutions was added to 900 microliters PBS buffer. 100 microliters of this solution was added to 0.5ml TCA and 0.9ml 1% bovine serum albumin solution. This was mixed prior to being left for 10 minutes. The TCA precipitates all the protein in the solution. After 10 minutes the vials were centrifuged, and the solution, containing free 125-I was counted and compared with protein containing solution to determine the free iodide content of the protein. The free iodide of labeled of HSA, lysozyme and IgG was in all cases less then 2% of total radioactivity; β-lactoglobulin had a free iodide of 10% on average presumably due to the presence of fewer tyrosine and histidine residues in these samples.

3.6.2 Artificial Tear Solution

A simulated tear solution, the composition of which is shown in Table 3-1 was prepared in PBS. In all studies, two radiolabeled proteins were used, one labeled using

^{125}I and one labeled ^{131}I. Depending on the protein in question, the simulated tear solution contained between 3-10% (w/w) radiolabelled protein.

Table 3-1: Model tear solution and amount of radiolabelled protein used for the study.

Protein	Concentration (mg/ml)	% Radiolabelled Protein
Lysozyme	1.9	8
Albumin	0.2	6
IgG	1.7	3
B-lactoglobulin	1.6	

3.6.3 Sample Preparation

For comparison with the HA containing materials, protein adsorption to commercial contact lenses of various compositions was performed. The lenses were removed from package, wiped dry using a Kimwipe and subsequently placed in 24-well plates. PHEMA and experimental test materials were stored in water prior to testing; the surfaces were blotted dry using a Kimwipe prior to testing in a 96-well plate. The HA containing materials were prepared in a similar manner.

3.6.4 Protein Adsorption

Artificial tear solutions were added to the wells containing the lens materials or the HA containing membranes and incubated for 2 hours at room temperature. This was followed by three 5 minute rinses with fresh PBS buffer to remove any loosely adherent protein. The lenses were counted for radioactivity using a Wizard 3 1480 Automatic

Gamma Counter (Perkin Elmer) and the adsorbed amounts were calculated using background-corrected surface counts relative to the solution count for the individual protein solution.

4. Results & Discussion

4.1 PHEMA and Model Silicone Hydrogel Synthesis

Transparent, water swellable pHEMA membranes were synthesized using the method described. While the exact amount of monomer used was dependent on the mold, typically the lowest amount of monomer that resulted in full mold coverage was used to ensure that a thin polymer sheet was formed. The mixing and incorporation of the initiator was often problematic and slow mixing resulted in the formation of a precipitate which had negative impact on optical transparency. Careful control of curing time was essential as increased curing times resulted in very rough surfaces, seemingly the result of the escape of volatile materials from the forming polymer.

In order to examine the possibility for using this technique to treat silicone hydrogel materials, model silicone hydrogel materials were synthesized. The choice of initiator for this reaction resulted in a slightly yellow material. Decreased transparency and mechanical strength were observed in materials containing over 10% TRIS. This was likely an indication of the formation of sub domains of TRIS in a pHEMA backbone. Compared with pHEMA alone, high amounts of crosslinker were necessary to facilitate polymerization. More than 3% crosslinker was necessary to generate fully polymerized materials. Preliminary studies only were performed using these materials with the intent of observing whether the results obtained with the pure pHEMA could be mimicked with silicone hydrogels.

4.2 Bulk Characterization

4.2.1 PHEMA Swelling Study

In order to determine the optimum solvent for HA loading, the swelling of the pHEMA membranes in a variety of ethanol/water solutions were tested. Completely dry samples were weighed and placed in solvent solutions for 72 hours at which time they were weighed again. Water uptake results, shown in Figure 4-1, demonstrated that maximum solvent uptake was achieved with a solution containing approximately 50% ethanol in water. However, it was observed physically that the mechanical properties of the membranes decreased at concentrations above 30% ethanol, making them difficult to manipulate. Mechanical properties did not appear to be impacted at a concentration of 30% ethanol. High solvent uptake was also noted at 20%, and perhaps 20% could have been more optimal for loading but less specimens were measured for 20% leading to less confidence in the results. However high solvent uptake was achieved at 30% ethanol and this concentration was used to load HA into the matrices.

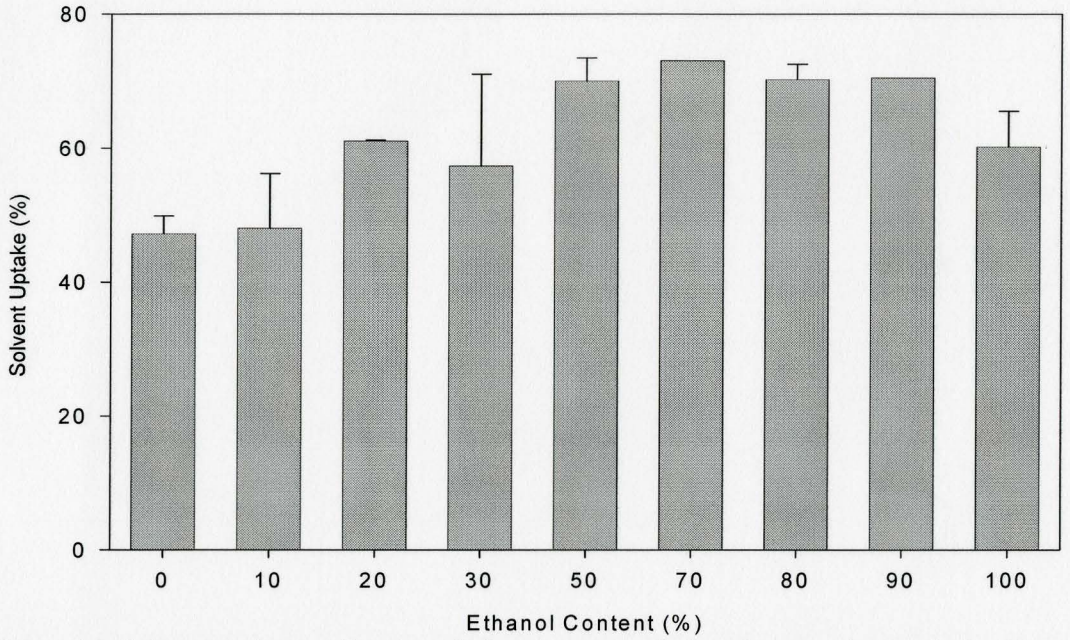


Figure 4-1: Solvent uptake as a function of ethanol content of ethanol water solvent solution. Decreases in mechanical strength were noted in uptakes above 50%, 30% was used for all loadings due to its high solvent uptake and minimal effect on mechanical properties.

4.2.2 Characterization of HA Loading and Release in Non-Crosslinked Materials

In order to determine the rate at which uncrosslinked HA present in the materials could release into a surrounding aqueous fluid, HA release studies were performed. Surfaces loaded with fluorescently labeled HA and released in PBS buffer yielded a release profile shown in Figure 4-2. It was assumed that all HA loaded into the pHEMA was released over time. A high molecular weight of HA was selected for these studies since it was assumed that prolonged release would be observed with higher molecular weight compounds, prolonging any biological effects observed. A commercially available fluorescently labeled HA with a molecular weight of 900 kDa was used for ease of quantification. A typical burst release with a duration of approximately five hours and 90% release after 1 day was followed by gradually decreasing release and exhaustion of

the compound within 60-80 hours. Lower molecular weights compounds, particularly 35kDa HA would be expected to exhibit significantly faster release profile due to the smaller size of the compound relative to the size of the pores of the pHEMA. Given the relatively fast release kinetics of the high molecular weight HA, it can be concluded that HA releasing lenses would not be expected to be effective for periods of more than 1-2 days. Instead, in order to effect any long term change to the biological interactions with the contact lens materials using HA, a method of more permanently incorporating the HA into the matrix must be examined. Given the structure of HA, an obvious method of more permanently incorporating the HA into the material would be to crosslink it. In the current work, this was accomplished using EDC chemistry. In order to produce higher crosslinked structures, dendrimers were used to amplify the terminal amine functional groups in the HA.

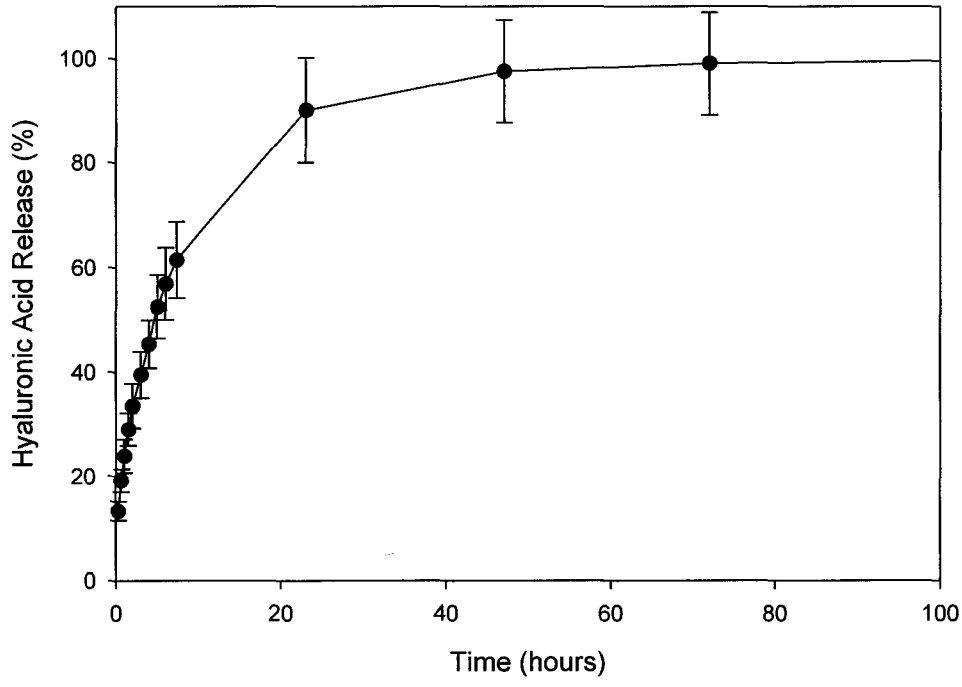


Figure 4-2: 900 kDa hyaluronic acid release from pHEMA membranes. Clearly the bulk of the HA is released at a relatively constant rate over periods that would be much less than a single day of wear, suggesting that long term changes to the interactions between the materials and the surrounding biological fluid would not be released using releasable HA. Error bars: SD (n=3)

4.2.3 Characterization of HA Loading in Crosslinked Materials

Samples containing physically immobilized HA prepared by crosslinking the polymer inside the pHEMA matrices as described in Section 3.1 were analyzed using a variety of different techniques to determine the amount of HA present in the pHEMA matrix. While mass change in the materials was examined, the amount of HA taken up by the materials could not be accurately determined since the accuracy of the balance used to make the measurements of approximately ± 2 mg were similar to the amounts of material taken up by the 10-20 mg pHEMA membranes. However, in all cases a measurable increase in the mass was observed. The studies were repeated using larger

pHEMA membranes. While as much as 30 weight % loading of HA/Dendrimer could be achieved, only a small amount (~2%) of the HA was crosslinked into the material. Therefore, the amount of HA present in the materials is clearly very small.

The loaded and crosslinked surfaces exhibited physical differences from the unmodified controls. They were considerably more lubricious and were often hard to handle. Lubricity has been touted as a desirable property in ophthalmic and particularly contact lens applications (*Rx For Contact Lenses: Mobility + Lubricity = Comfort And Safety.*May 8, 2001) as decreased friction between the lid and lens could be beneficial to the maintenance of the tear film. The lenses would also be expected to show less adhesion to the cornea. Lubricity is difficult to accurately measure; results tend to be very subjective and are often relative only to other materials tested rather than being absolute. One possible method that has been used previously by our group and by others would involve AFM measurements on hydrated hydrogels (Mirmiran, 1999). However, an AFM tip is a poor model of an eye lid and only relative results would be obtained.

4.2.4 Optical Transparency of HEMA/HA materials

Transparency of the HA modified materials and the HA releasing materials was measured by UV spectrophotometry in a wavelength range of 400 nm (blue) to 700 nm (red). Ideally, a material should have a transmittance greater than 90% in order for appropriate light transmission to occur. Figure 4-3 summarizes the change in transparency with modification relative to the unmodified pHEMA controls. Clearly, incorporation of 35 kDa HA did not have an effect on transparency when compared to the control. However, the samples with incorporated 169kDa HA showed slight decreases in

transparency at high wavelengths. This is presumably due to the formation of domains of significant size with this higher molecular weight HA which can potentially block the light. The effect of higher HA loading on the transparency needs to be examined.

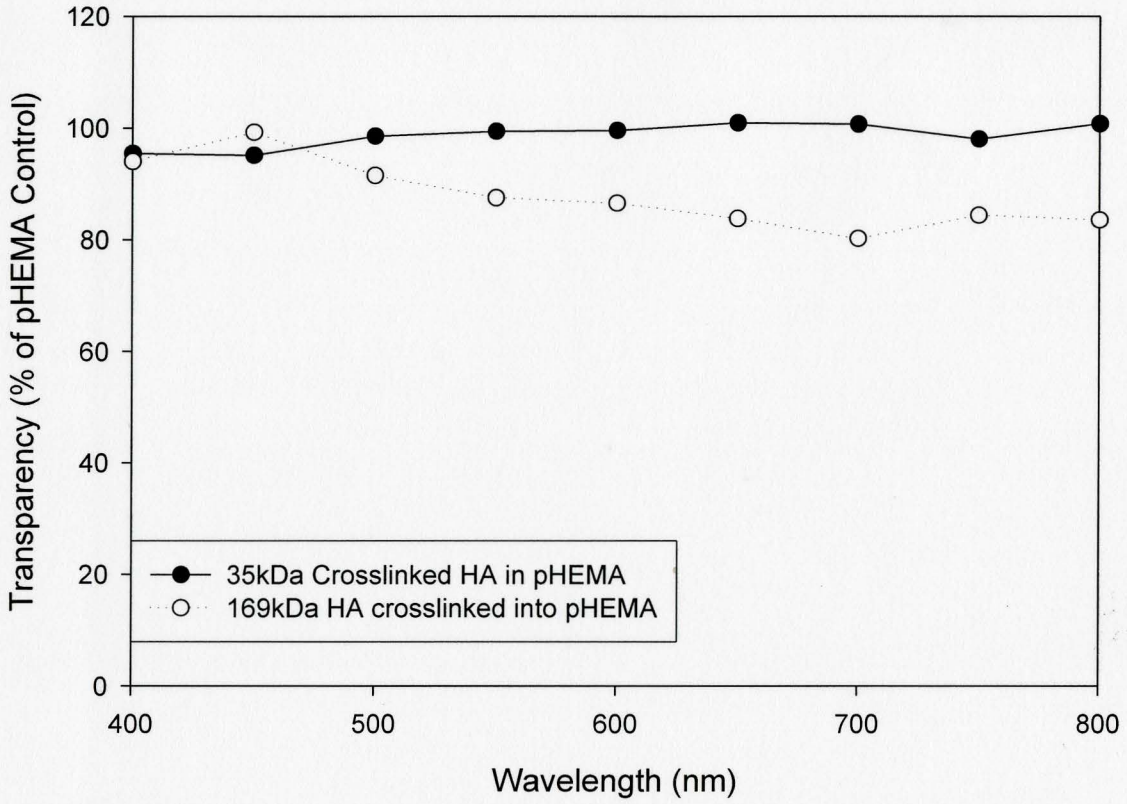


Figure 4-3: Transparency as a percentage of a pHEMA control versus wavelength for pHEMA crosslinked hydrogel networks. Clearly the incorporation of small amounts of low molecular weight HA does not affect the transparency of the pHEMA matrix. However, there is an effect on the transparency when a higher molecular weight of HA is used. The significance of this decrease is not known.

4.2.5 Equilibrium Water Uptake

From Figure 4-4, it can be observed that loading HA into a pHEMA membrane without crosslinking has no effect on equilibrium water uptake. This is thought to be due

to the time required to reach equilibrium, which is in excess of a day, being longer than the time required to release most if not all of the HA from the material. Therefore, the equilibrium water content measurements were likely made on pHEMA materials containing little or no HA to affect the water content.

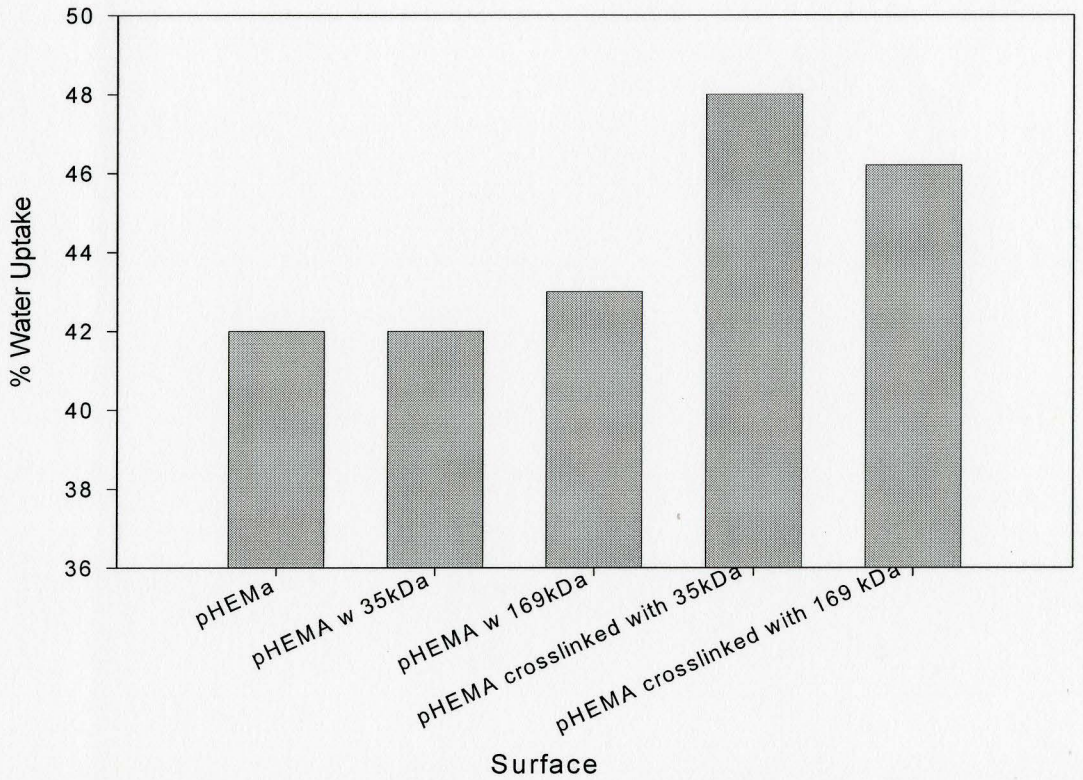


Figure 4-4: Percent water uptake for pHEMA, pHEMA loaded with HA and pHEMA surfaces crosslinked with HA. Crosslinking HA into the material results in a slightly greater water uptake while the uncrosslinked HA materials showed no differences relative to pHEMA controls.

In the case of the crosslinked materials, a small increase in water content was observed suggesting that the crosslinked HA was incorporated into the material. While this increase is small, the actual amount of HA crosslinked into the material is estimated to make up less than 2% of the weight of the materials. Based on these results, an

increase in the amount of HA present in the material would therefore be expected to result in an even further increase in the water content. Furthermore, since most conventional hydrogel lenses contain a hydrophilic component in addition to pHEMA which results in a water uptake of as much as 70%, the incorporation of HA into a conventional hydrogel lens may result in the incorporation of even more water. The water uptake effect of the HA is more important for silicone hydrogel materials which are typically more hydrophobic in nature and show water uptakes in the range of 30-50%. The incorporation of high amounts of crosslinked HA in silicone hydrogels, may lead to significant increases in the wettability and water uptake in addition to imparting other beneficial properties of the material.

4.2.6 Tensile Strength Testing

Mechanical properties of the materials, including maximum tensile strength and modulus of elasticity were determined by Instron testing. Figure 4-5 shows that a slight increase in the modulus of elasticity was observed with HA incorporation and that this was affected by the molecular weight of crosslinked HA. Since modulus is an indicator of the stiffness of a material, the 169kDa HA/pHEMA samples are clearly stiffer than the pHEMA samples. While this is not likely a problem with the HEMA based materials, increased stiffness may be problematic with silicone hydrogel materials as decreased stiffness has been reported as a desired material property with this lens type (Efron, 2005). The formation of high molecular HA domains in the material presumably affects the ability of the pHEMA to resist change in deformation, manifested as increased stiffness.

These results suggest that increases in the HA molecular weight result in larger domains, which manifests itself as increased stiffness and with decreased transparency.

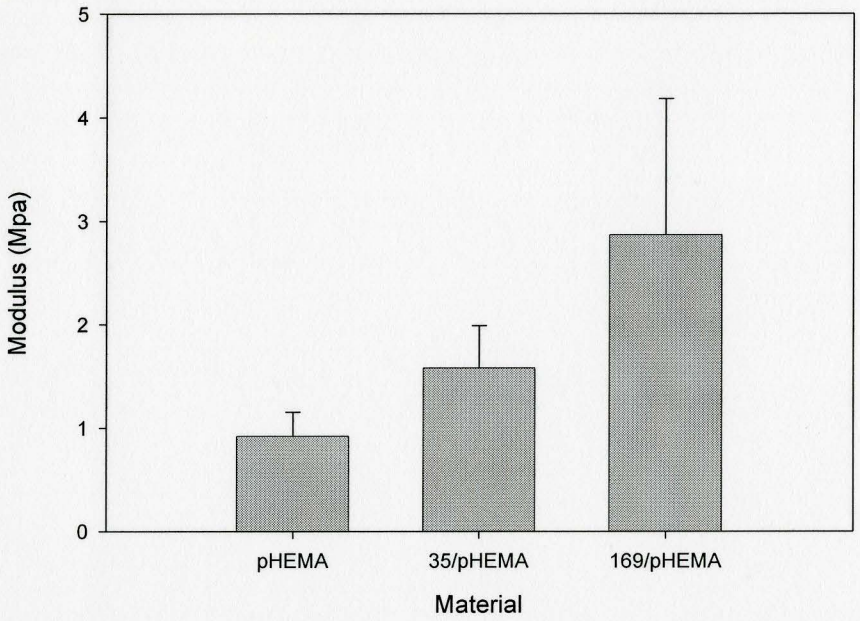


Figure 4-5: Modulus of Elasticity for pHEMA, and the two crosslinked pHEMA/HA materials. Modulus of elasticity is shown to increase with increased hyaluronic acid molecular weight crosslinked into the hydrogel but statistically not significant with F=4.5 being less then F critical=5.14. Error bars: SD (n=3)

Tensile strength results are summarized in Figure 4-6. Statistically, there is no difference between the control pHEMA material and the two crosslinked HA materials as measured by single factor analysis of variance resulting in F=2.5 being less then F critical=5.14. However, it does appear that the 169kDa material is slightly stronger then the control and 35kDa material.

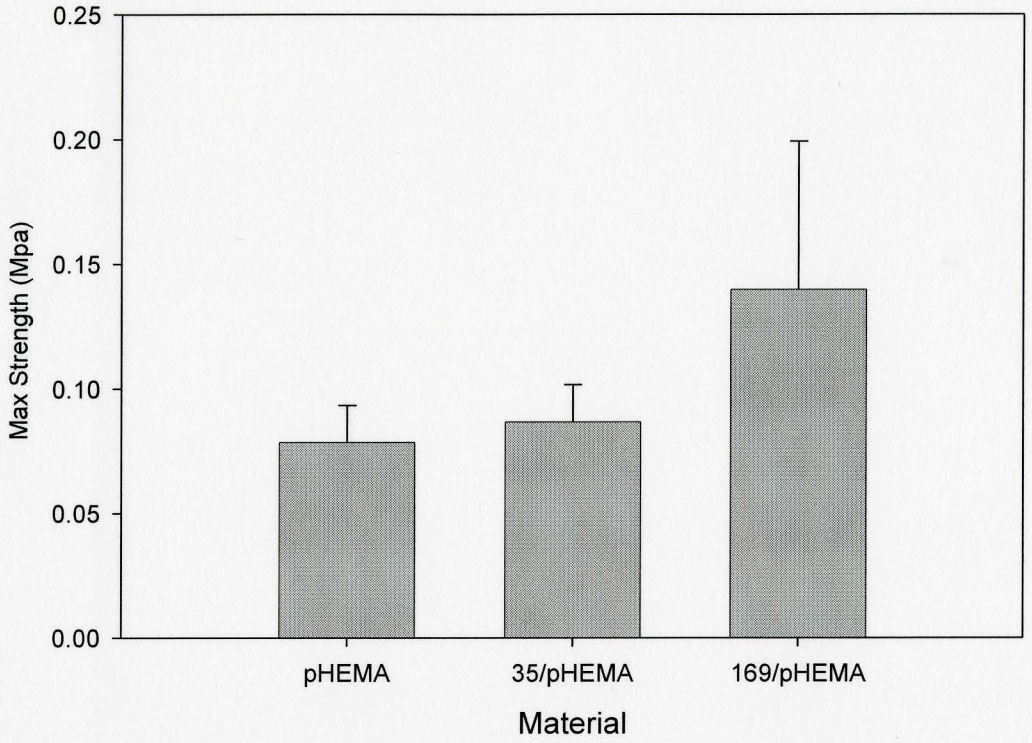


Figure 4-6: Maximum tensile strength of pHEMA, and the two crosslinked pHEMA/HA. There is no statistical difference, as measured by single factor analysis of variance with F=2.5 being less then F critical of 5.14 between maximum mechanical for each material although the 169kDa crosslinked material does appear to be slightly higher. Error bars: SD (n=3)

4.2.7 Differential Scanning Calorimetry

DSC measurements on the samples over a temperatures range of 40-200°C are summarized in Figure 4-7. At temperatures above this, decomposition of the samples was observed. Not surprisingly, given the crosslinked nature of these polymers, no melting points were detected; glass transition temperatures are summarized in Table 4-1. The presence of HA results in an increase in the glass transition temperature by a measurable amount, despite the fact that only a very small amount of HA was present. Crosslinking the samples appeared to have no effect on the glass transition temperature

when compared to samples prepared without crosslinking the HA in the samples. It is expected that the non-crosslinked samples would see decreased glass transition temperatures with exposure to buffer and HA release. In the case of the crosslinked samples, samples were exposed to a buffer solution to release any unbound HA from the sample prior to analysis and the results are due only to the HA which has been physically trapped within the material.

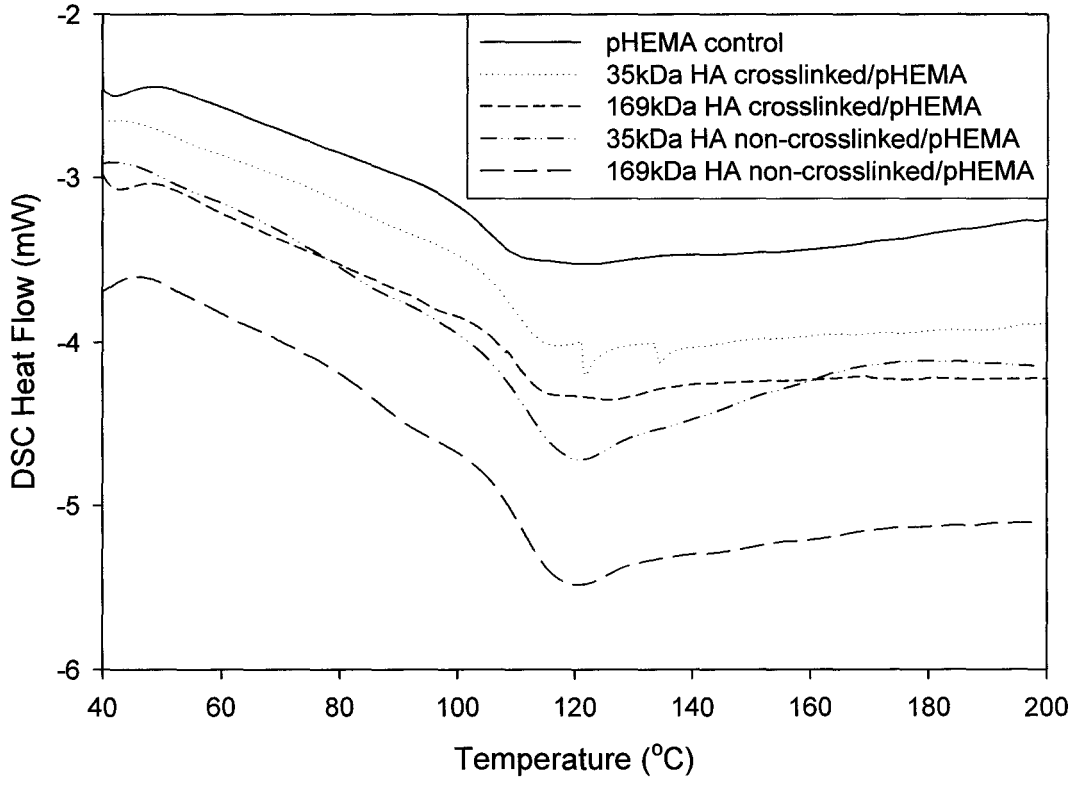


Figure 4-7: Heat flow versus temperature results as measured by DSC for various pHEMA/HA materials.

Table 4-1: Glass transition temperatures as measured for pHEMA and various pHEMA/HA materials.

Material	Glass Transition Temperature (°C)
pHEMA	105.29
35kDa HA Crosslinked into pHEMA	110
169kDa HA Crosslinked into pHEMA	109.54
35kDa HA non-Crosslinked in pHEMA	111.07
169Da HA non-Crosslinked in pHEMA	112.08

4.2.8 Transmission Electron Microscopy

TEM results are shown in Figure 4-8 and Figure 4-9. While TEM has been previously used to examine the formation of domains in interpenetrating network polymers (Liu, 2005b), in this case it was not possible to make any distinction between the HEMA and the HA materials using this technique. In the images, the polymer material (pHEMA) appears lighter in color then the resin. However, the addition of HA did not lead to the formation of separate visible domains, presumably due to the small amounts of HA which are incorporated or due to the similarities in the properties of the two materials.

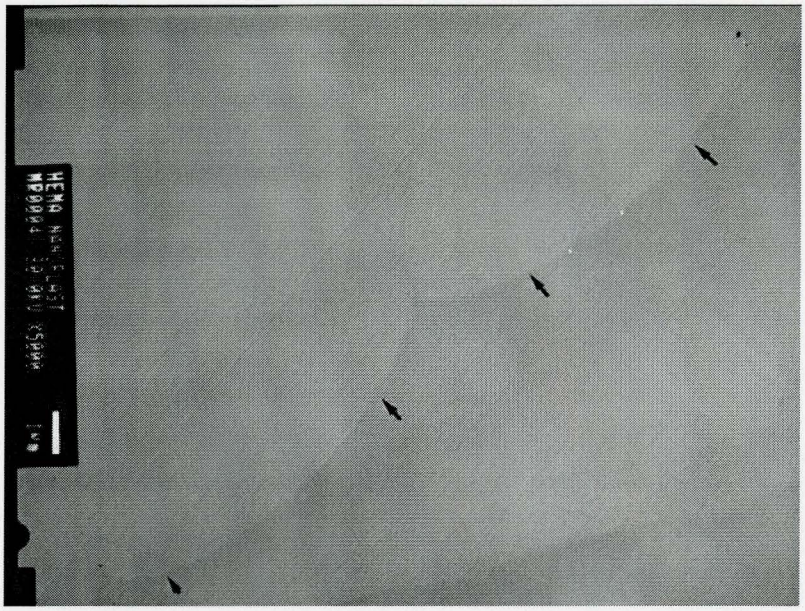


Figure 4-8: pHEMA TEM arrows point to the pHEMA material interface.

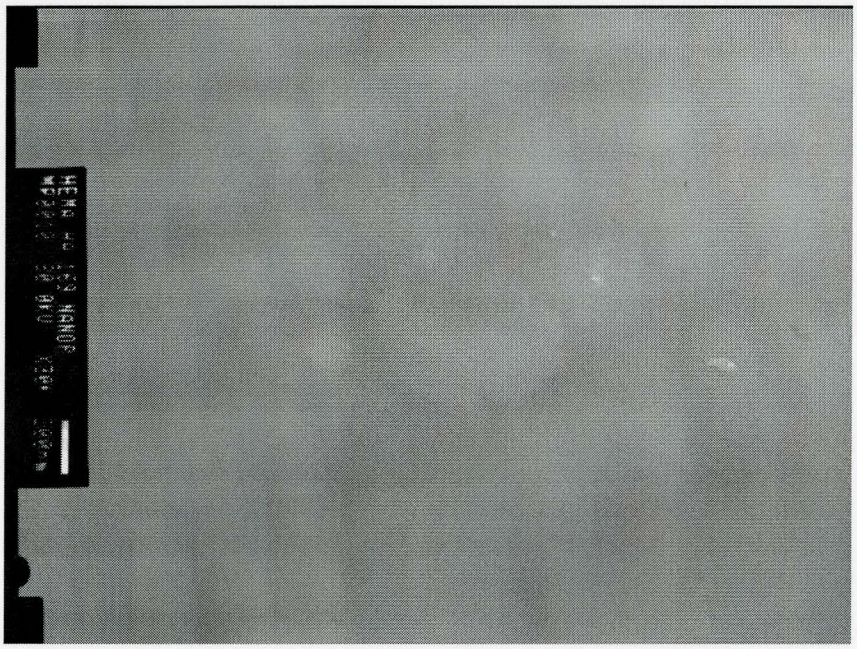


Figure 4-9: pHEMA/ 169 kDA HA surface as scanned by TEM. Only two phases were noted on the materials, that of the nanoplast resin and the pHEMA.

4.3 Surface Characterization

4.3.1 Water Contact Angles

4.3.1.1 Commercial Materials

Water contact angles were measured on 9 brands of commercial contact lenses in order to compare with the results obtained on the novel HA containing materials. Measurements were taken on dry contact lenses. While this is not likely the best model of hydrophilicity for these hydrogels, consistent results can be obtained using this method compared with measurements on wet lenses. As expected, shown in Figure 4-10, the silicone hydrogel materials were generally more hydrophobic in nature then the conventional lens materials under these measurement conditions. It is not possible to determine the effect of the wetting agents or any surface modification in the case of these materials since unmodified controls were not available. Oddly, although both PureVision and O_2Optix silicone hydrogels use plasma surface modifications and have similar water contents, their contact angles are very different. The PureVision lenses have the highest contact angles seen for all materials studied, perhaps indicating that the plasma treatment does not have full surface coverage of the material leaving some of the bulk material present at the surface. As plasma treatments are done to add a hydrophilic layer to the material surface, the O_2Optix results are much more in line with what is expected. Furthermore, this layer is clearly stable and reorientation of the silicone in a dry environment does not seem to occur. The Acuvue brands of silicone hydrogels with incorporated wetting agents (OASYS and Advance) had high contact angles, likely due to

reorientation and resulting in high amounts of silicone and very little of the wetting agent being present at the interface. Contact angles were measured on dried samples which may impact the ability of the wetting agent to impart its hydrophilic capabilities. Receding contact angles, which may provide a more relevant understanding of wetting, are for example, more consistent on the silicone hydrogels.

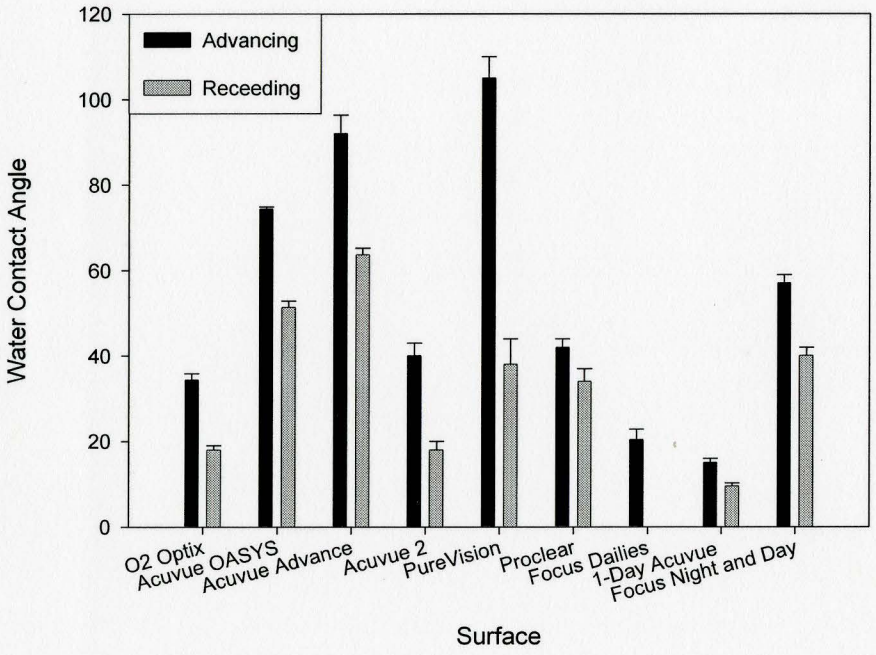


Figure 4-10: Advancing and receding water contact angles for 9 commercially available contact lens materials. Receding contact angles were too low and p8not measurable for Focus Dailies brand of materials. Error bars: SD (n=3)

4.3.1.2 HA Containing Hydrogel Materials

Materials containing uncrosslinked HA showed decreased contact angles relative to pHEMA controls immediately following exposure to the liquid at a time which would correspond to zero release time as shown in Figure 4-11. Following exposure to PBS buffer, the contact angles for these samples increased. This correlates with the release of

HA from the pHEMA over a relatively short period of time. For the 35kDa sample, the HA appeared to be released within thirty minutes whereas the contact angles for the 169kDa samples remained slightly lower then the pHEMA controls throughout the entire four hours of the experiment. The crosslinked samples showed significantly lower contact angles then the pHEMA controls; these lowered contact angles remained consistent for the entire 4 hour study. While analysis of contact angles for periods closer to wear cycles should have been performed, little or no change would be expected over this period of time as there were no changes over the four hour period of the study and little or no measurable HA release was observed from these materials. Due to the high hydrophilicity of HA, a decrease in the contact angles was not unexpected with the incorporation of this molecule. Clearly a small amount of crosslinked HA at the pHEMA surface results in the formation of a more hydrophilic surface. It is somewhat surprising however, that such a small amount of HA crosslinked into the pHEMA results in water contact angles that are similar to those observed with pHEMA materials containing significant amounts of the hydrophilic components methacrylic acid or n-vinyl pyrollidone. Since all contact angles were measured on completely dried samples, it is probable that even lower contact angles would be observed if the measurements were made a humid environment.

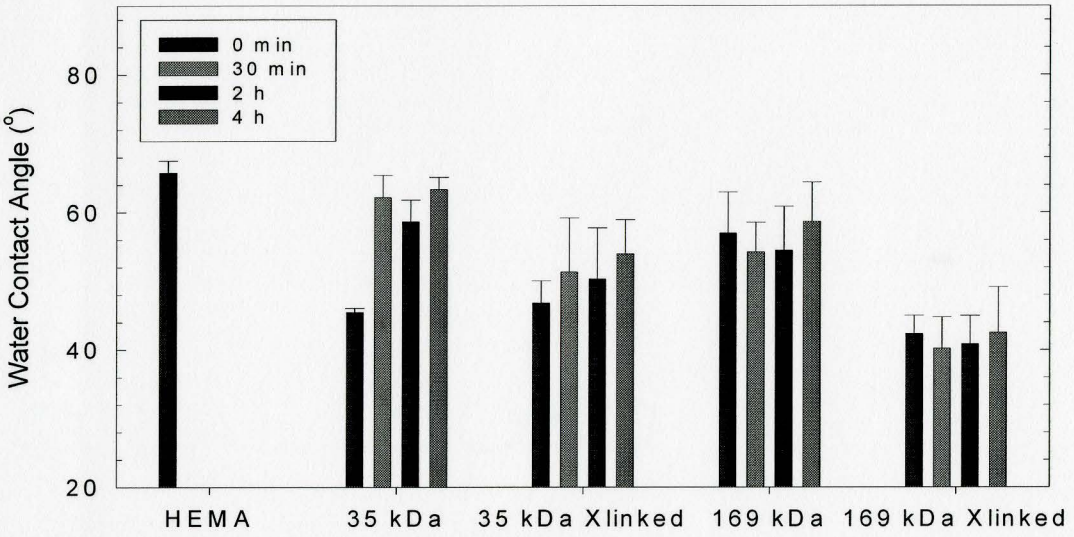


Figure 4-11: Water contact angle for pHEMA and pHEMA HA containing materials either crosslinked or loaded. Error bars: SD (n=3)

4.3.2 Atomic Force Microscopy

Atomic force microscopy was used to determine whether the incorporation of the relatively small amounts of HA into the pHEMA membranes resulted in changes to the surface roughness of the materials. Since the curing process was performed using Teflon sheets with the other side of the materials being open to the air, not surprisingly, significant differences in the roughness of the two sides were observed. Therefore, roughness values, presented in Table 4-2, summarize changes to the material on the Teflon side as there was considerably less variability than on the air side. Clearly, the incorporation of the crosslinked HA resulted in increases in the surface roughness. This is the expected result and is consistent with observations of others for materials modified, for example, with PEO (Chen, 2005). The hydrophilic HA would be expected to present

itself at the interface, assuming that there are free chains of HA, which would presumably result in the formation of a rougher surface. This effect would be expected to be enhanced if the hydrogels were analyzed under hydrated conditions, such that any free HA chains would be extended into the aqueous solution. Topography images for pHEMA, 35kDa and 169kDa HA crosslinked into pHEMA on the Teflon side are shown in Figure 4-12, Figure 4-13 and Figure 4-14 respectively. Artifacts from the machined Teflon molds are noticeable on the images. It may also be of interest to perform surface profilometry measurements of these materials which will provide information about roughness on a more macroscopic scale.

Table 4-2: Root mean square roughness and mean roughness results for all materials for both the Teflon and air side.

Material	Teflon Side	Air Side
pHEMA		
Root mean square roughness (nm)	1.09	4.401
Mean roughness(nm)	0.832	3.423
35kDa HA Crosslinked into pHEMA		
Root mean square roughness (nm)	1.481	4.519
Mean roughness (nm)	1.118	3.26
169kDa HA Crosslinked into pHEMA		
Root mean square roughness (nm)	2.02	5.104
Mean roughness (nm)	1.728	3.882

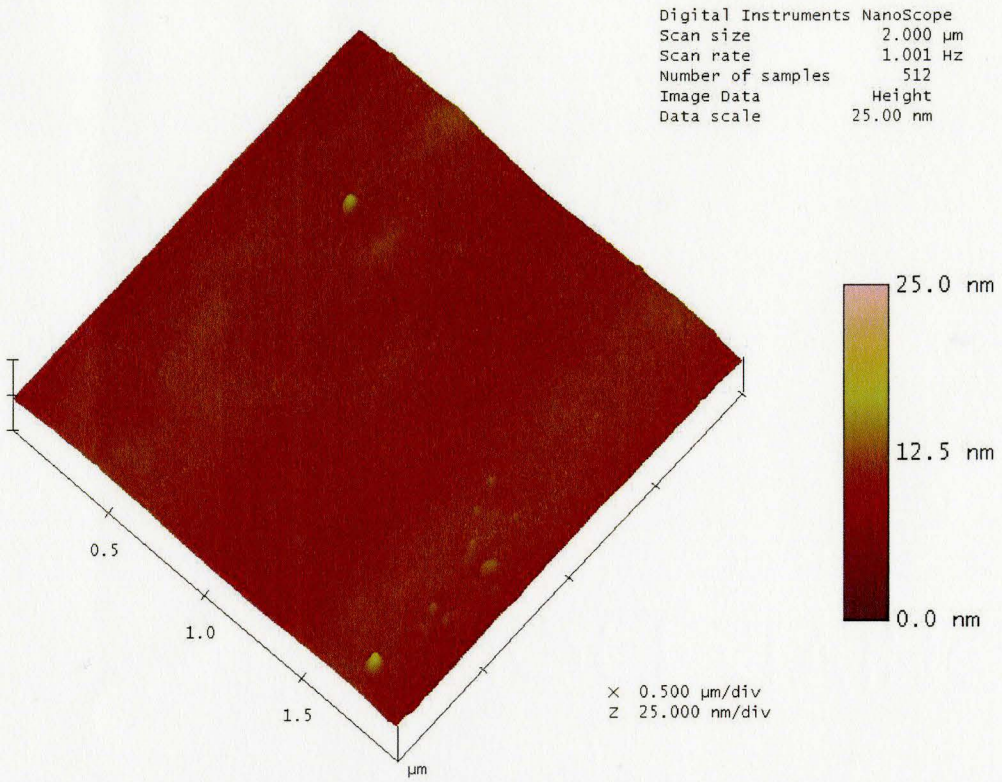


Figure 4-12: AFM surface plot of pHEMA on the Teflon side of the material. The surface appears very smooth but has the presence of a few artifacts or possible surface contamination.

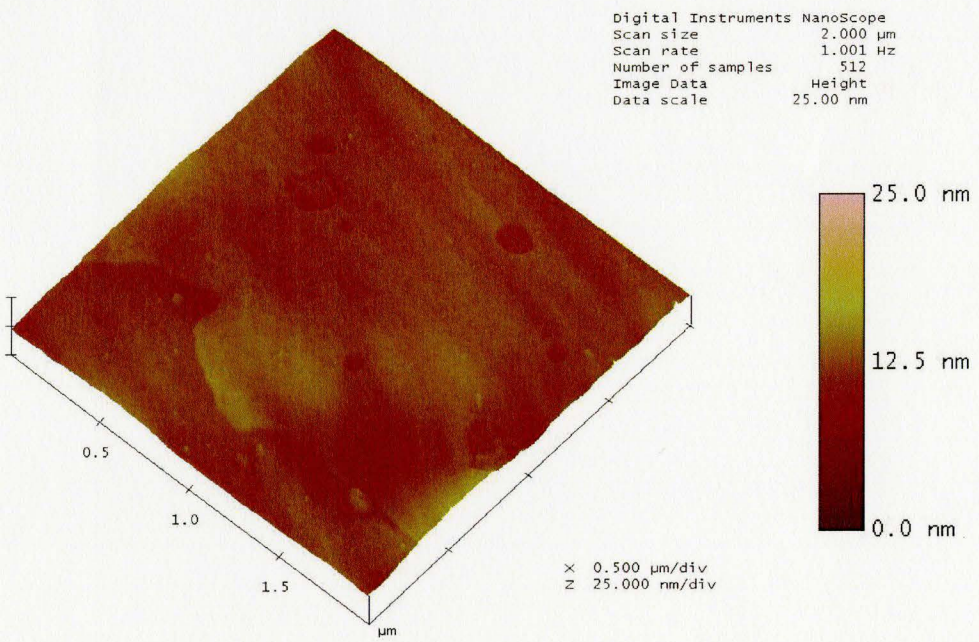


Figure 4-13: AFM surface plot of 35 kDa HA crosslinked into pHEMA on the Teflon side of the material.

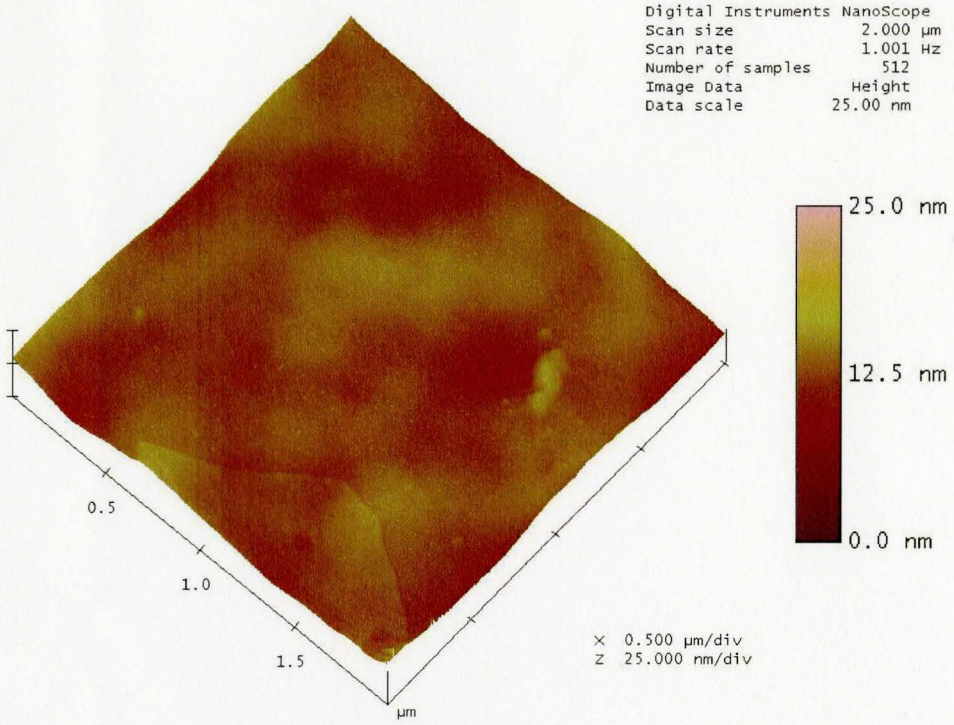


Figure 4-14: AFM surface plot of 169 kDa HA crosslinked into pHEMA on the Teflon side of the material.

4.3.3 X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy was used to assess the chemical composition of the surface. However, a limitation of this technique is that it is performed under high vacuum conditions meaning that the composition of the surface may be quite different than it would be if the surface was examined under aqueous conditions. A sample high resolution carbon 1s scan and a low resolution scan for pHEMA are shown in Figure 4-15 and Figure 4-16 respectively. Summaries for all high resolution carbon 1s scans are presented in Table 4-3 while summaries for low resolution scans are shown in Table 4-4.

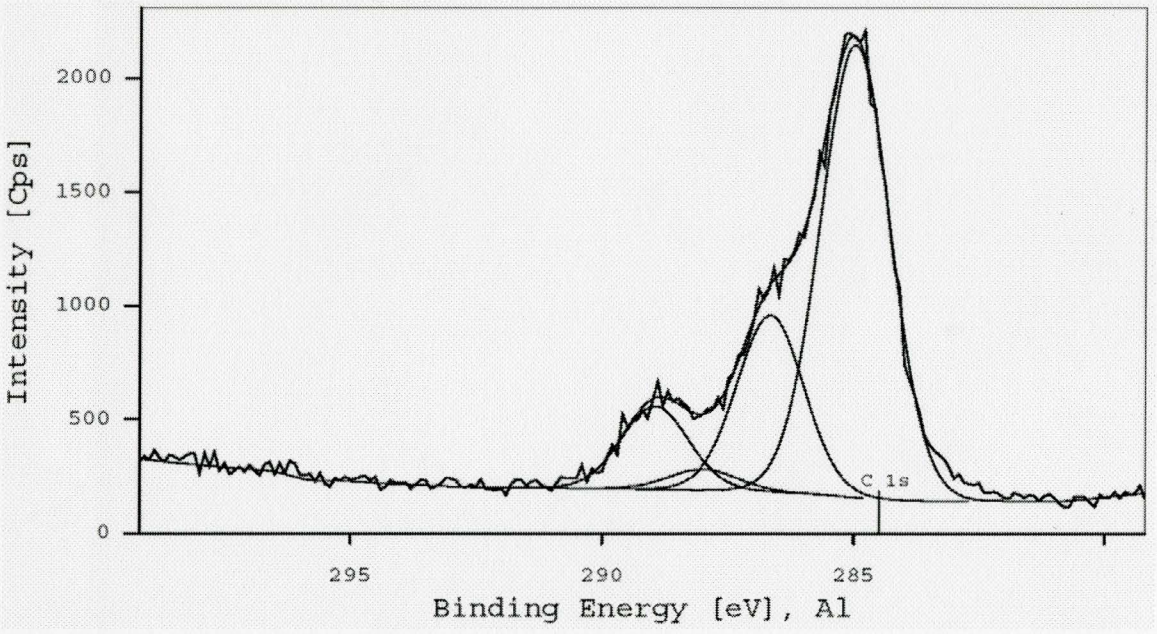
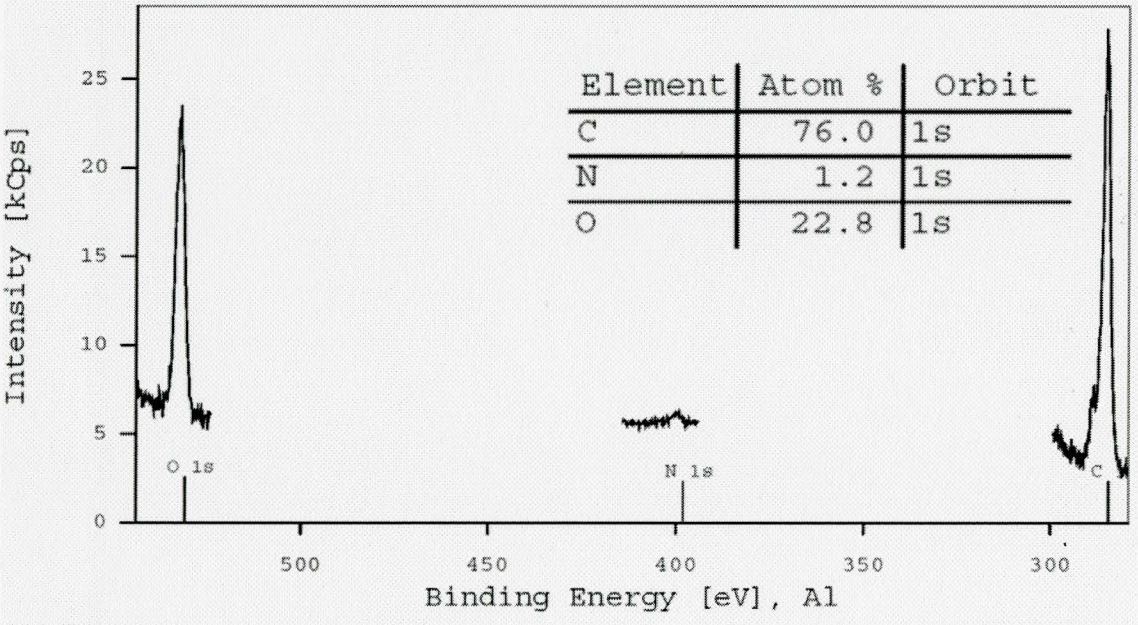


Figure 4-15: High resolution carbon 1s scan from XPS analysis for pHEMA material. Possible surface contamination noted with the presence of a C-N peak.



Element	Atom %	Orbit
C	76.0	1s
N	1.2	1s
O	22.8	1s

Figure 4-16: Low resolution surface scan of pHEMA measuring overall amounts of carbon, oxygen and nitrogen on the material surface by XPS.

Table 4-3: High resolution carbon 1s scans from XPS for materials of interest. A slight increase is noted in the C=N for the crosslinked materials, indicating the presence of HA at the surface. The numbers represent their respective areas on the curve.

Binding Energy [eV]	pHEMA Control	35kDa HA Crosslinked into pHEMA	169 kDa HA crosslinked into pHEMA
285	62.6	65.6	55.1
286.66	23.5	17.4	26.8
289	11.1	9.7	13.7
288	2.8	7.3	4.4

Table 4-4: Low resolution scan by XPS of carbon, oxygen and nitrogen for the materials of interest.

Element	pHEMA Control	35kDa HA Crosslinked into pHEMA	169 kDa HA crosslinked into pHEMA
C	76	78.3	73
O	22.8	20.9	26
N	1.2	0.8	1

While there are differences from the expected results, particularly for the pHEMA control, these are likely due to the high vacuum conditions under which the samples were examined. For example, an unexpected N1s peak was observed on the pHEMA surface, presumably due to surface contamination. Furthermore, the carbon to oxygen ratio, expected to be approximately 2 to 1, was found to be closer to 3 to 1. Again, this is presumably due to the high vacuum environment of the XPS which would be expected to

result in the enrichment of the more hydrophobic carbon under the hydrophobic XPS conditions.

There were however clear differences between the modified materials and the unmodified control, particularly in the high resolution spectra. The N1s peak was also present and expected on the HA crosslinked materials although of a slightly lower magnitude than on the pHEMA only. The presence of higher amounts of nitrogen on the surface of the HA containing materials is expected due to the presence of nitrogen in the HA and in the amine terminated dendrimer is used to facilitate crosslinking. However, as the dendrimer amount is relatively small, its contribution would be expected to be relatively minimal. It can be observed that there were changes in the high resolution spectra, particularly an increase in the peak at 288 eV. This peak is typically C=O or O-C-O. HA contains C=O but no O-C-O bonds. The material containing 169 kDa HA also showed increases in the peaks at 286.66 and 289 eV which are typical peaks for C-O/C-N and O-C=O/Aromatic rings respectively. HA contains significantly more C-O bonds then pHEMA so these increases are likely due to the presence of the HA at the material surface. These increases were not seen on the 35 kDa material. The results in general seem to suggest that there is more HA present when the high molecular weight compound was used.

While there are some indications of HA incorporation, overall, the XPS results were inconclusive in determining the presence of HA/dendrimer in the crosslinked materials. The presence of nitrogen in the pHEMA samples coupled with the lack of an

increase in the N1s peak suggests that the changes in this element as a result of crosslinking are negligible.

4.3.4 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectra are presented in Figure 4-17-Figure 4-19. Since pHEMA and HA have numerous functional groups in common, it is difficult to clearly identify the small amount of HA that is thought to be incorporated into the matrix. The primary difference between pHEMA and the crosslinked materials would be expected to be in the COOR peak, at a wavelength of approximately 1734.4 cm^{-1}. The presence of HA, with its COOH peaks, would be expected to cause a shift in the COOR peak to a slightly lower wavelength. Such a shift is observed in Figure 4-19 with a decrease in this peak to 1733.67 cm^{-1}. It is expected that the addition of more crosslinked HA will result in more definite changes to the peak. Consistent with other results, the FTIR results clearly demonstrate that only a very small amount of HA is incorporated into the pHEMA structure.

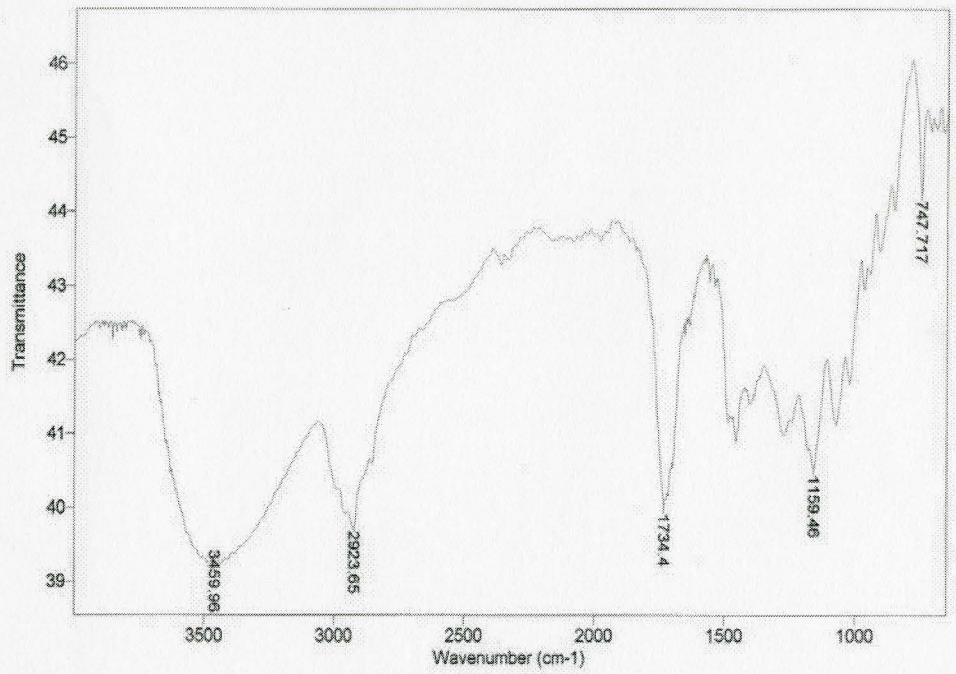


Figure 4-17: FTIR analysis of pHEMA with characteristic COOR peak found at 1734.4.

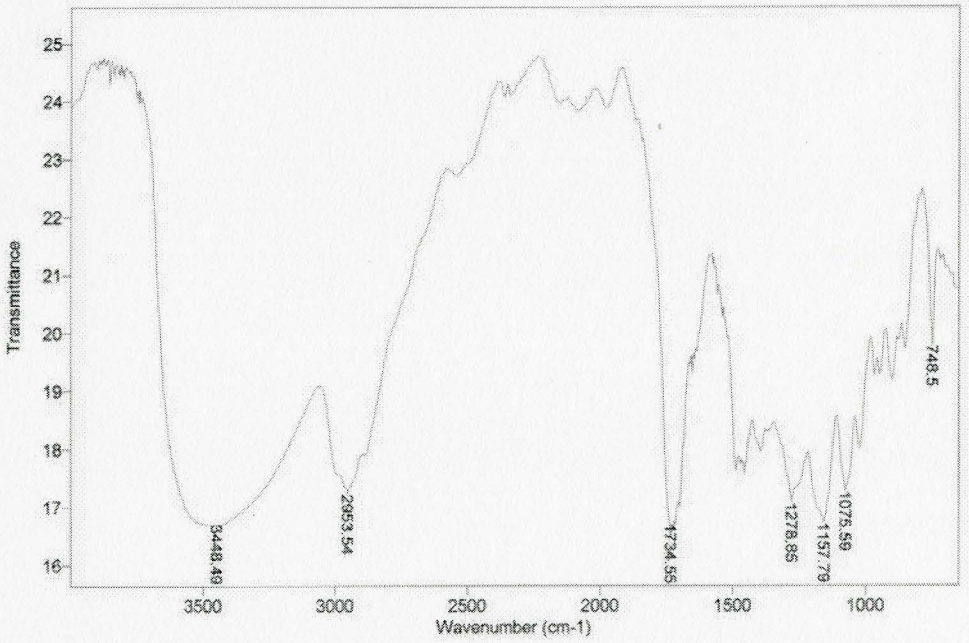


Figure 4-18: FTIR analysis of 35 kDa HA crosslinked into pHEMA with no real noticeable differences when compared to the pHEMA control.

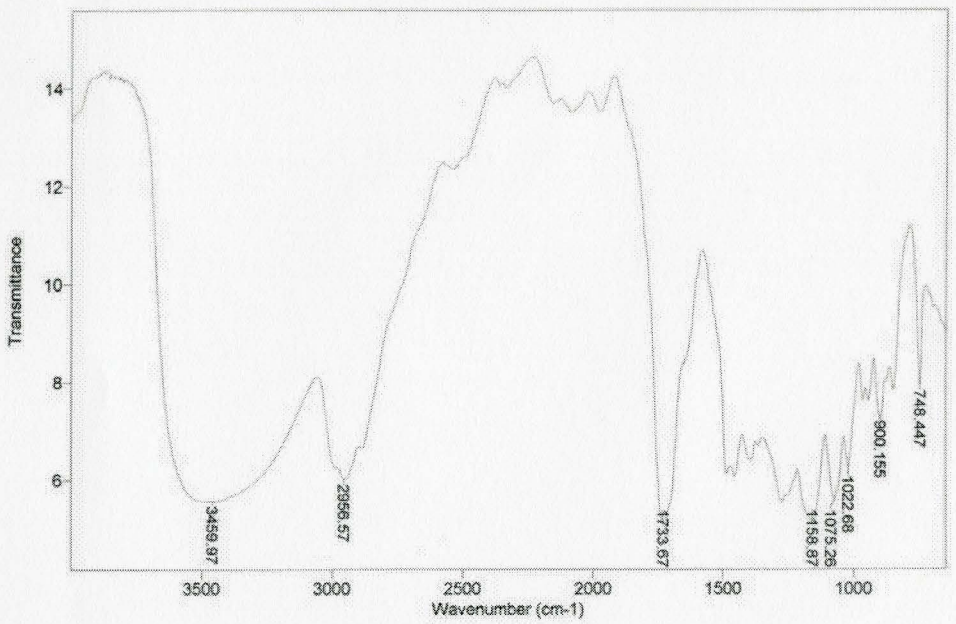


Figure 4-19: FTIR analysis of 169 kDa HA crosslinked into pHEMA.

4.4 Protein adsorption studies

4.4.1 Protein adsorption to Commercially Available Contact Lenses

In order to compare the protein adsorption results obtained with the HA containing materials, a study of tear protein adsorption to a variety of commercially available contact lenses was conducted. An artificial tear solution containing the four most abundant proteins was used. The results are shown in Figure 4-20. Theoretical monolayer values for lysozyme and HSA are 207-310 ng/cm^2 (Q. Garrett, 1999) and 400-460 ng/cm^2 (He, 1992) respectively.

Both the Acuvue 2 and 1-Day Acuvue, comprised of pHEMA/MA showed significantly higher lysozyme sorption then any of the other materials. This measured sorption is likely due to the absorption of the protein as the amounts taken up are

approximately three orders of magnitude greater than the value of a theoretical monolayer of lysozyme. Research has shown that materials such as these, which have a net opposite charge to a protein such as lysozyme tend to have higher absorption than neutral polymers (Garrett, 2000). These materials showed very similar albumin and IgG adsorption values as the other conventional materials, presumably a charge related phenomenon but also possible the result of the size difference between lysozyme and these proteins. The other conventional materials studied, Proclear and Focus Dailies, had significantly less lysozyme adsorption then the 1-Day Acuvue and Acuvue 2 but still had significantly more lysozyme adsorption then most of the silicone hydrogels. However, these materials had lower overall albumin and IgG adsorption then the silicone hydrogels.

In contrast, the silicone hydrogel materials had the lowest protein adsorption of the materials studied, in most cases adsorbing less than a monolayer of protein. These materials can be broken up into two categories; plasma treated silicone hydrogels and wetting agent containing materials. The plasma treated silicone hydrogels had considerable higher albumin and IgG adsorption then the conventional hydrogels but two of the three brands had considerably lower lysozyme adsorption. Those two brands, Focus Night and Day and O_2Optix have complete surface coverage of plasma whereas the PureVision, which had considerably higher lysozyme adsorption, was treated to have islands of silicate. The presence of wetting agents, mainly PVP based, generally resulted in a decrease in the levels of adsorption. There were particularly low levels of lysozyme present on the Acuvue OASYS lenses for example. These materials also had higher albumin and IgG adsorption, comparable to the other silicone hydrogel materials

indicating that silicone hydrogels have a tendency to adsorb more albumin and IgG then conventional hydrogel chemistry. The relevance of this remains to be determined.

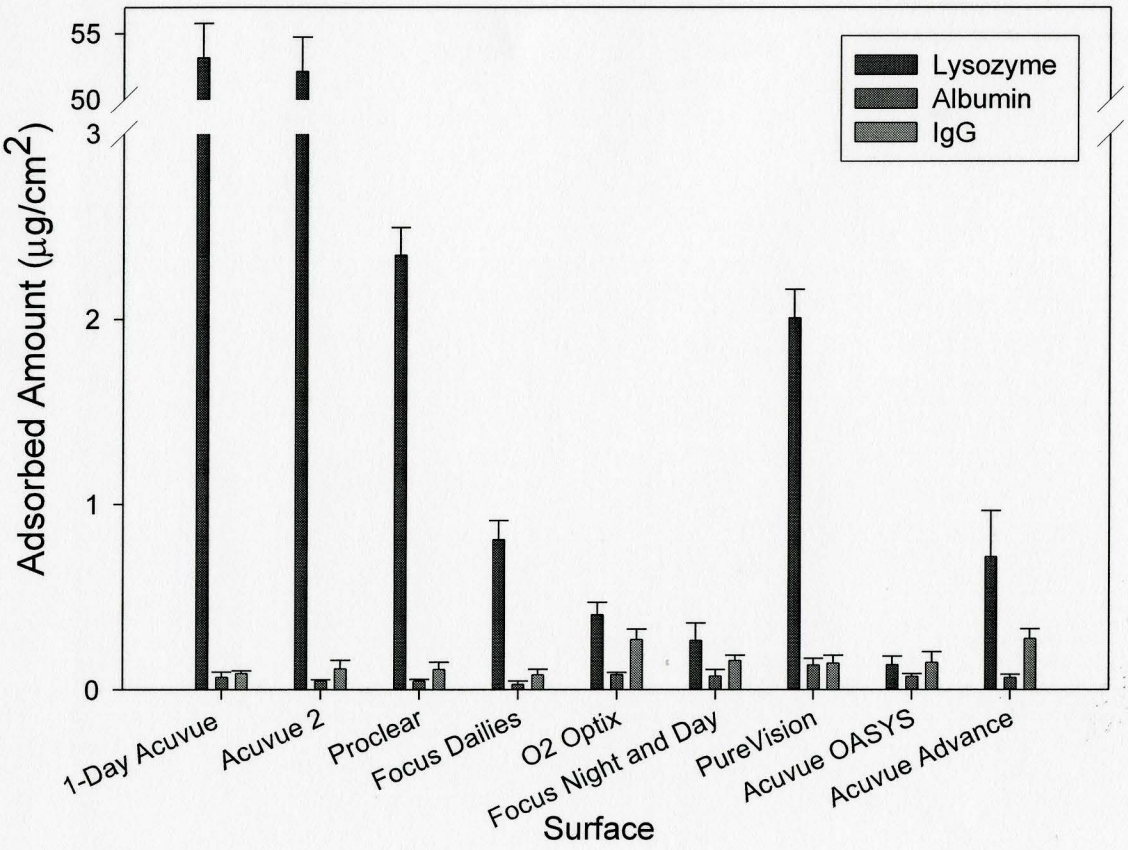


Figure 4-20: Protein sorption of radiolabelled lysozyme, albumin and IgG to ten brands of commercial contact lenses. Error bars: SD (n=6)

4.4.2 Protein adsorption to HEMA and HA Hydrogel Materials

The impact of the addition of HA to the hydrogel materials on adsorption of tear film proteins was examined using these lens results as a basis. HA, being hydrophilic, was hypothesized to decrease protein adsorption possibly by the same mechanisms as PEO (Napper, 1983). The aim of the current work was to determine whether the

incorporation of crosslinked HA would result in the formation of a protein repellent wettable layer. Furthermore, it was of interest to determine whether similar effects were observed with HA which released from the material over the wear period. While it was expected that uncrosslinked HA containing materials would release HA for periods of only two to four hours, the effect of this release on protein adsorption was determined using a two hour protein adsorption from a simulated tear solution. Although the crosslinked materials were shown by XPS and FTIR to contain very little HA, it was hypothesized that under aqueous conditions this HA would be present at the surface and would significantly decrease the levels of protein associated with the materials.

Figure 4-21 details the lysozyme adsorption to the various materials synthesized. The presence of the releasable HA did not significantly affect protein adsorption. Despite the fact that the release of the protein was found to occur over a period of at least four hours and protein adsorption experiments were carried out over a period of only 2 hours, there was little or no effect of the released agent. These results suggest that commercial brands such as CIBA Visions Focus DAILIES with AquaRelease, which are reported to contain a releasable PVA polymer would not be expected to take up significantly less protein as a result of the release of a wetting agent. Furthermore, while these agents are PVA based and would therefore be expected to show increased interaction with the lens material and therefore slower release, it is likely that, based on the pore size of PVA materials and the size of the polymers, controlled release throughout the wear period is unlikely. The incorporation of the HA into the pHEMA and crosslinking however resulted in a material which showed dramatic decreases in protein adsorption for all of

the proteins examined. The adsorbed amounts were below a monolayer of protein in all cases, which is surprising since pHEMA would be expected to absorb significant amounts of protein. These crosslinked materials had comparable protein adsorption to the commercial lens which showed consistently the lowest amounts of adsorbed protein, Acuvue OASYS. The Acuvue OASYS lenses also contain an internal wetting agent, (PVP), which is not released over time.

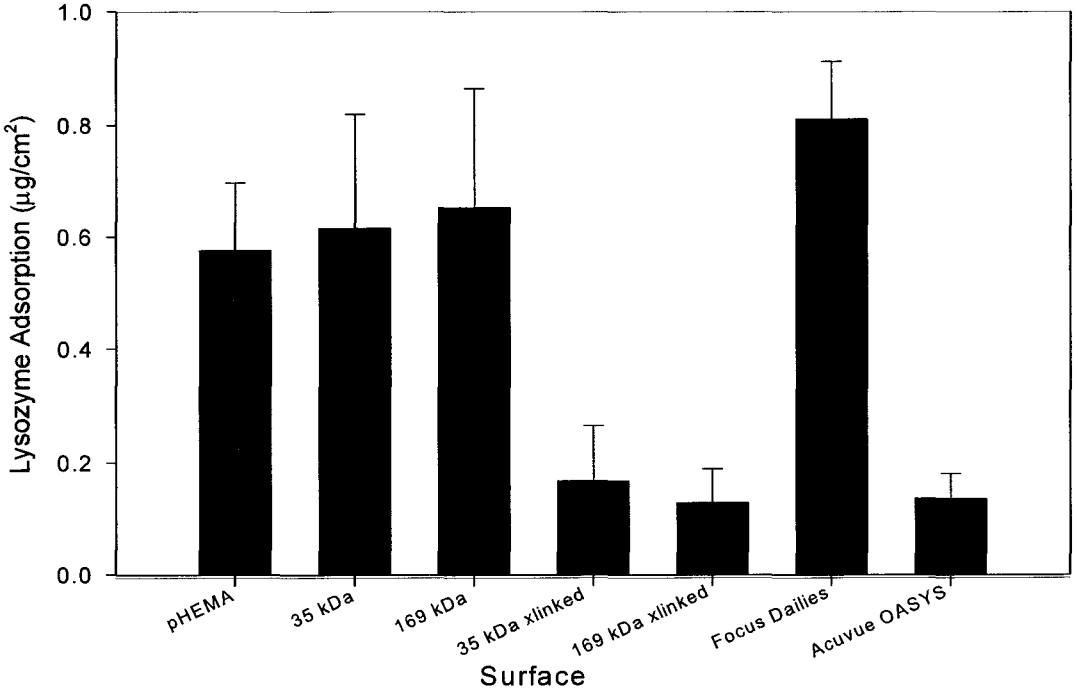


Figure 4-21: Lysozyme sorption as measured by radiotracer technique on PHEMA, PHEMA + releasable HA, PHEMA/crosslinked HA and two leading brands of contact lens for comparison. Error bars: SD (n=6)

Similar to lysozyme results, surfaces loaded with HA but not crosslinked had albumin adsorption values, shown in Figure 4-22, that appear to be the same as pHEMA. The crosslinked materials once again yielded much lower values which were roughly

10% of those observed with pHEMA, and which were much lower then any commercial lens on the market.

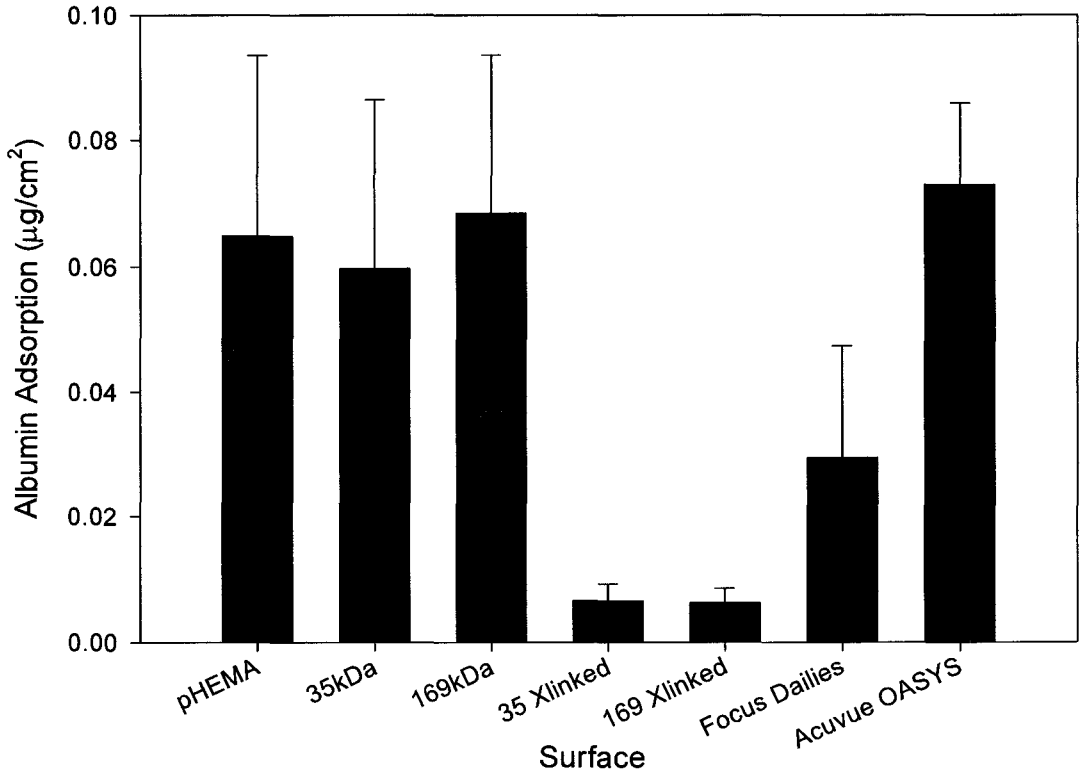


Figure 4-22: Albumin sorption as measured by radiotracer technique on PHEMA, PHEMA + releasable HA, PHEMA/crosslinked HA and two leading brands of contact lens for comparison. Error bars: SD (n=6)

Somewhat different results were observed for IgG. Surfaces containing unbound HA showed decreased IgG adsorption over the control. This suggests that there is still some HA at the liquid surface interface, and while in small amounts, it is capable of inhibiting the adsorption of high molecular weight proteins such as IgG. When the HA is crosslinked in the material, IgG adsorption is further decreased indicating that a higher amount of HA is present at the interface, resulting in increase levels of protein repulsion.

All values, including the pHEMA control were considerably lower than observed with commercially available lenses.

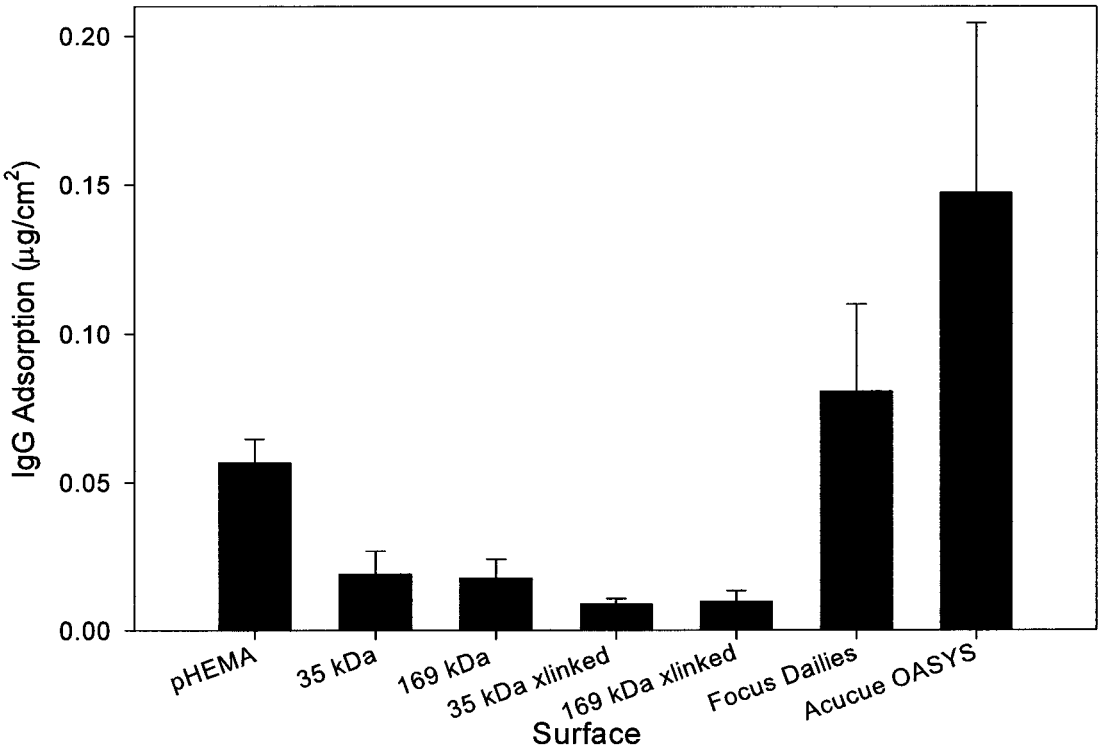


Figure 4-23: IgG sorption as measured by radiotracer technique on PHEMA, PHEMA + releasable (labeled by only the molecular weight of the HA) HA, PHEMA/crosslinked HA and two leading brands of contact lens for comparison. Error bars: SD (n=6)

One limitation of the protein studies is that this method cannot differentiate between adsorbed and absorbed protein. While some values indicate multilayer of protein on the surface, it is probable that the materials are absorbing significant amounts of protein. Significant absorption would be expected for lysozyme given its low molecular weight. The effect of charge in these experiments is expected to be relatively small given that pHEMA and HA are non-ionic, although it is likely significant with methacrylic acid containing lens materials. Therefore, the observed differences in protein

adsorption are the result of molecular weight, protein concentration, protein affinity, and protein conformation. This study was also unable to determine the state of the protein which is also important and which has been shown to be different depending on the composition of the lens and is a parameter that should be examine prior to further development of these materials.

In the current work, two molecular weights of hyaluronic acid were examined. Protein adsorption results indicate that molecular weight of HA incorporated into the material has no impact on the protein adsorption. However, the molecular weight of HA was shown to play an important role on loading. Increased loading of the lower molecular weight HA would be expected due to its higher solubility and due to its smaller size. In comparison, the 169 kDa HA is barely soluble at 10g/L. However, as a result of its relatively small size, it would also tend to be released faster during the EDC uptake/crosslinking step and this may result ultimately in a decrease in the amount of HA present in the pHEMA membrane.

Beta lactoglobulin, also included in the tear solution, was used as a substitute for tear lipocalin, the appropriate tear film component. Both proteins have similar molecular weight and isoelectric points but beta lactoglobulin is easily commercially available. However the ^{125}I binding affinity of this protein resulted in low specific radioactivity and high free iodide. Therefore, extensive adsorption studies were not carried out with this protein.

Protein sorption studies were able to demonstrate that the incorporation of very small amounts of crosslinked hyaluronic acid into a pHEMA backbone results in a

decrease in the sorption of tear proteins. While studies were only conducted on three tear proteins, given the vast differences in the properties of the proteins examined it is reasonable to conclude that decreased protein adsorption would be observed with a much broader range of proteins than were examined in the current study. However the effect of increased amounts of HA crosslinked into the surface should be further explored. The materials synthesized in the current work showed lower protein adsorption then any of the commercial lenses examined.

It would appear that the HA is capable of providing an environment that suppresses protein adsorption. The mechanism by which this occurs is unclear, although given the increased water content, and higher hydrophilicity, it is probable that HA behaves in a manner similar to PEO on a biomaterial surface, in effect blocking protein adsorption. PEO has been widely examined for its ability to decrease the adsorption of proteins to biomaterials surfaces.

While the exact mechanisms are largely unknown, it is possible that even small amounts of HA at the interface bind water in such a manner that any protein approaching the solid interface causes compression of the free HA chains which results in unfavorable thermodynamics for protein adsorption. It is also possible that the decrease in protein sorption is a result of significant decreases in protein absorption. Lysozyme penetration studies for Acuvue OASYS showed that nearly all protein was at the surface, perhaps indicating that wetting layer suppressed absorption of lysozyme (Zhang, 2006). Regardless, HA crosslinked materials seem to result in considerably less protein fouling

than other modifications. The translation of this result into other desirable factors such as lubricity for example remains to be determined and are beyond the scope of this study.

4.4.3 Silicone Hydrogel Lysozyme Adsorption

Preliminary protein adsorption studies were performed using model silicone hydrogel materials prepared using ratios of TRIS and pHEMA in order to determine whether similar levels of protein repulsion were possible with these materials. In this case, lysozyme adsorption from a simulated tear solution was used to examine the potential of this modification for reducing protein fouling on these materials. Any unreacted monomer or free HA was extracted by soaking in water for a period of 4 days. Figure 4-24 summarizes adsorption results for the low and high TRIS content hydrogels. Samples labeled 35kDa or 169kDa indicate the presence of crosslinked HA in the hydrogel. Control represents materials which had no modification. Ratios shown are indicative of the TRIS:pHEMA ratio. Consistent with the results obtained for the pHEMA only materials, the presence of crosslinked HA was shown to result in significant decreases in lysozyme adsorption when compared to unmodified controls. A slight trend was noted in the 10%-2% TRIS to pHEMA range showing increased TRIS ratios resulted in decreased lysozyme adsorption. However a similar trend was not observed for the high TRIS content materials. This difference may be the result of fundamental differences in the mechanisms of protein adsorption with these materials – absorption would be expected for the high HEMA content materials, while the addition of

higher amounts of TRIS would be expected to shift the interactions to one which is much more one of adsorption.

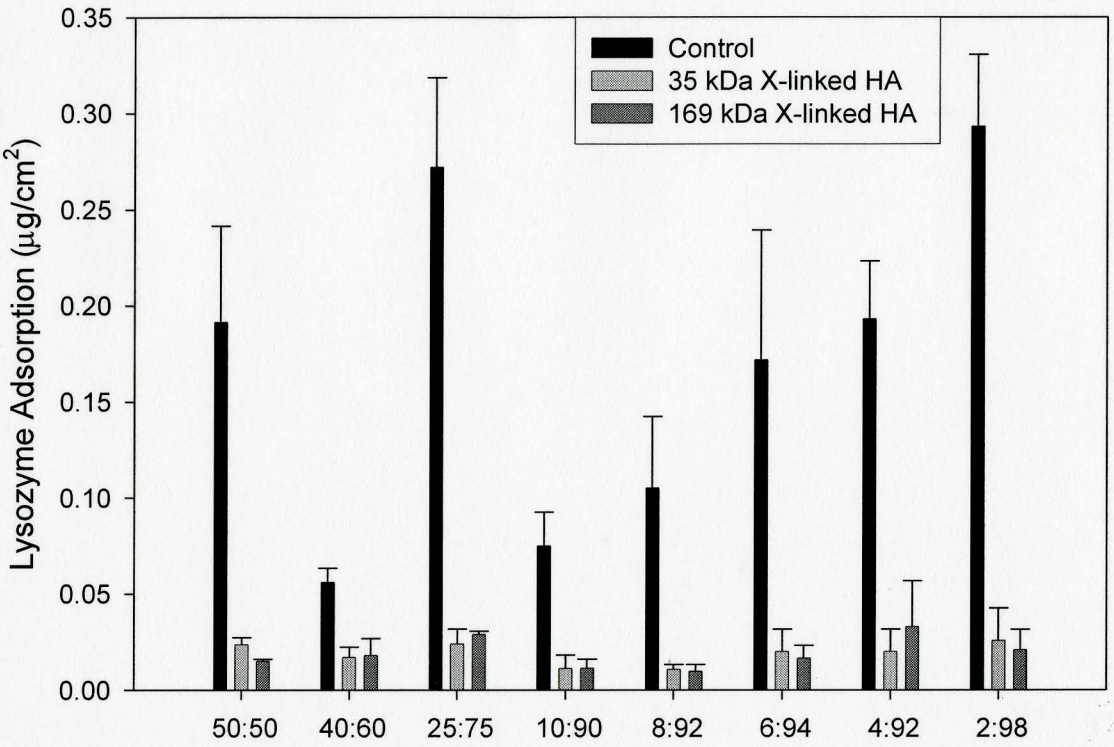


Figure 4-24: Lysozyme adsorption to TRIS containing silicone hydrogels. Numbers along x-axis represents the TRIS:pHEMA ratio used to prepare the material. Molecular weights indicate the presence of crosslinked HA in the sample while Control represents unmodified materials. The presence of crosslinked HA significantly decreases lysozyme adsorption for all materials. Error bars: SD (n=3-6)

5. Conclusions

5.1 Conclusions

In this work, pHEMA hydrogels and silicone hydrogels were modified to incorporate hyaluronic acid as a wetting agent, either releasable or crosslinked. In all cases, the loading of HA in the disks used was too small to quantify using a balance. Releasable high molecular weight HA was shown to have fast release kinetics. Approximately 80% of the HA was released in the first 20 hours in solution as measured by fluorometric analysis. While this release profile is acceptable for one day disposables, it is unacceptable for extended wear lenses.

Surface analysis by water contact angles demonstrated that the crosslinking modification resulted in more hydrophilic surfaces which remained constant throughout the four hour study. This suggested that the HA was physically entrapped in the hydrogel membrane by the crosslinking technique. Chemical analysis of crosslinked materials was conducted by FTIR and XPS. Only slight, inconclusive changes were observed which, taken together with the water uptake and contact angle measurements, indicated that the amounts of HA entrapped are relatively small. The presence of HA in materials was confirmed by DSC with increases in glass transition temperatures for HA containing materials. Optical transparency was not impacted by the presence of 35 kDa crosslinked HA but slight decreases in optical transparency were observed by UV spectroscopy for materials containing crosslinked 169 kDa HA, particularly at higher wavelengths.

Extensive studies measuring the competitive adsorption of lysozyme, albumin and IgG were conducted on the modified materials as well as on many commercial contact lenses as a method of comparison. Releasable HA showed no effect on protein adsorption as compared to a pHEMA controls, with the exception the adsorption of IgG onto releasable 169kDa HA which showed moderate decreases over control. This was thought to be the result of the presence of small amount of HA at the surface being capable of blocking the large molecular weight protein. Conversely, the crosslinked HA materials showed considerable decreases in protein adsorption for all proteins studied. These decreases resulted in levels of associated protein which were well below the values for all the conventional contact lens materials studied. It was hypothesized that crosslinked HA presents a similar interface to surface repelling modifications such as PEO. In terms of protein adsorption, even small amounts of crosslinked HA in the material showed drastic decreases in protein adsorption.

Preliminary work on model silicone hydrogel materials containing crosslinked wetting agents demonstrated that, similar to the pHEMA system, the presence of crosslinked HA resulted in significant decreases in lysozyme adsorption. This further demonstrates the potential of physically entrapped HA for use as a low fouling material in biomaterials applications and in particular as a contact lens material.

5.2 Recommendation for Future Work

A number of additional studies are suggested to more fully examine the potential of these materials as protein repelling surfaces.

- Future work in quantifying the HA loading on the material by casting the pHEMA system on a crystal in order to perform high accuracy mass measurements by quartz crystal microbalance are suggested. This method will further allow for quantification of the amount of crosslinking that is occurring.
- Protein and lipid penetration studies by confocal microscopy analysis should be conducted to understand the localization of proteins and lipids are in the material. This analysis may help to better understand the mechanisms of protein repulsion by these materials.
- Increasing the amount of HA loading and crosslinking should also be explored. In the current study, two molecular weights were used at the same loading concentrations due to the solubility limit of the higher molecular weight HA. In future studies, lower molecular weight HA could be used as it has considerably higher solubility. By increasing the initial concentration of the HA loading solution, it may be possible to increase the loading. Alternatively, increased loading may be facilitated by performing many repeats of HA loading and crosslinking on the same samples. While less economic for commercial applications, this method has the potential to result in highly crosslinked HA networks and will allow for the full potential of these materials to be examined. Increased loading would be expected to result in conclusive HA detection by

XPS and FTIR analysis. Additional studies should be conducted to measure the effect increased loading has on optical transparency, contact angles, mechanical properties and protein adsorption.

- Additionally, AFM studies should be performed using a hydrated system rather then the dry system utilized. This method would produce much more applicable results to real world systems and perhaps give an indication of the lubricity of the materials.
- Further studies should be conducted on silicone hydrogel materials, including, if possible, studies incorporating HA into commercially available lenses. Also, a more commercially relevant silicone hydrogel model should be studied rather then the simple TRIS/pHEMA model used for this study.
- Ultimately, it would be interesting to explore this modification in other areas where protein repelling surfaces are required, including perhaps modifying commonly used blood contacting biomaterials such as poly urethane to determine its effect on protein adsorption.

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