13

Tear Fluid Photonic Crystal Contact Lens Noninvasive Glucose Sensors

Sanford A. Asher and Justin T. Baca

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, USA

We are developing a photonic crystal contact lens sensor for the noninvasive determination of glucose in tear fluid. Success requires a photonic crystal sensor with the sensitivity to glucose required to differentiate normal levels from hyperglycemic and hypoglycemic levels. In addition, the tear glucose levels need to track those of blood. In this chapter we review our progress in developing a photonic crystal glucose sensor for incorporation into a contact lens. We also review all previous tear fluid glucose studies, which, while suggesting that tear glucose concentrations tracks that of blood, significantly disagree as to the tear glucose concentrations in both normal and diabetic subjects. These studies also disagree as to the relationship between blood and tear fluid glucose concentrations. We also present new measurements of tear glucose concentrations by using a method designed to avoid tear stimulation. We conclude that the various previous tear collection methods biased the measured tear glucose concentrations. We also review recent studies which attempt to monitor tear glucose concentrations *in situ* by using contact lens-based sensing devices. On the basis of these results, we are optimistic about the future of *in vivo* tear glucose sensing.

Key words: glucose, glucose sensing, photonic crystals, photonic crystal sensors,

tear fluid glucose, tear collection, tear production, tear stimulation, conjunctiva, glucose transport, *in situ* tear analysis, contact lens sensors.

13.1 Importance of Glucose Monitoring in Diabetes Management

20.8 million people in the United States and 180 million people in the world are estimated to have diabetes mellitus [1]. The prevalence of this disease is expected to double by 2030 [2]. The cost for treating diabetes related illnesses is estimated to be 10% of all healthcare expenditures in the United States [3].

The Diabetes Control and Complications Trial clearly demonstrated that tight glycemic control is critical to preventing complications such as retinopathy, nephropathy, and neuropathy [4]. Current standards of care require self-monitoring of glucose several times daily, with an increase in frequency for patients receiving insulin [5]. Self-monitoring of blood glucose utilizes finger-stick blood sampling.

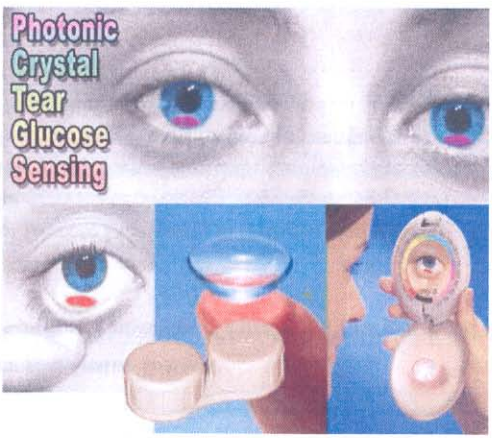

FIGURE 13.1: Photonic crystal glucose sensing material for determining tear fluid glucose concentrations. The sensing material is embedded within a contact lens or ocular insert. The color diffracted changes with the tear fluid glucose concentration. A mirrored compact-like device illuminates the sensor with white light. The sensor color is determined by viewing the reflected (diffracted) light and compared to a color wheel calibrated in blood glucose concentration.

While many noninvasive approaches to blood glucose monitoring have been investigated, none have, as yet, replaced fingerstick direct measurements of glucose in

blood [6]. Recently developed 3-day implantable, continuous glucose sensors show some promise for improving glycemic control [7, 8]. However, the existing approved devices must be calibrated at least twice daily with a direct blood measurement, and must be replaced after 3-7 days. Noninvasive glucose monitoring approaches currently being investigated include IR [9, 10] and Raman spectroscopy [11, 12], birefringence measurements [13], photoacoustic phenomena [14], optical coherence tomography [15], fluorescence [16, 17], surface-plasmon resonance in nanoparticles [18], and electrical impedance measurements [19]. The GlucoWatch (Cygnus, Redwood City, CA) which is a quasi-noninvasive electrochemical measurement device which extracts interstitial fluid through the skin has been approved by the FDA for supplementary blood glucose monitoring. However, it requires frequent calibration against fingerstick blood glucose measurements.

We have been working towards developing a photonic crystal contact lens for the noninvasive monitoring of glucose in tear fluid [20-22,24] (Fig. 13.1). Tear glucose in diabetic patients has been studied for over 80 years [23] and recently at least three independent groups have developed glucose sensors that can be incorporated into contact lenses [24–27]. Obviously, for this approach to be successful it is essential that the blood and tear glucose concentrations correlate. Unfortunately, there is presently significant disagreement as to the actual concentrations of tear glucose in normal and diabetic subjects, as well as to how, or whether, tear glucose concentrations correlate with blood glucose concentrations. There is surprisingly little known about the origin of glucose in tear fluid, as discussed below. In this chapter we will present an abbreviated overview of the present understanding of the correlation of tear and blood glucose concentrations and then discuss our photonic crystal tear fluid glucose sensor approach. We also review the other competing approaches for noninvasive tear glucose contact lens sensing. We refer the interested reader to our more extensive review published elsewhere [28].

13.2 Eye Tear Film

The tear film on the surface of the eye is composed of several layers. Most superficially there is a lipid layer that is less than 100 nm thick which serves several functions including preventing evaporation of the underlying aqueous layer and providing a smooth optical surface over the cornea [29]. This layer is composed of sterol esters, wax esters, and many other minor lipid components [30]. These are secreted from the Meibomian glands located on the margins of the eyelids, just posterior to the eyelashes. Dysfunction of these glands can lead to increased evaporation of tears from the eye, causing an increased tear osmolarity and clinical dry eye [31]. The lipid layer is compromised when contact lenses are worn; this layer may be completely absent over rigid contact lenses [30].

Just below the lipid layer is a predominantly aqueous layer. Measurements of

the thickness of this layer over the cornea varies from 2.7 μm to 46 μm, with the most recently reported value of 3.3 μm [32]. In the presence of contact lenses, the aqueous tear film can be measured both in front of the contact lens (pre lens), and between the lens and the cornea (post lens). Both the pre lens and post lens tear films are approximately 3 μm thick [32]. In both the presence and absence of contact lenses, the tear film is considerably thicker near the margins of the eyelids, where a meniscus forms. The total volume of the aqueous tear film is about 7 μL [33], and its production and elimination are discussed below.

Below the aqueous layer of the tear film is a mucin layer, consisting of glycoproteins which lubricate the eye surfaces. At least 20 different mucins are present. They provide a hydrophilic surface, over which the aqueous fraction rapidly flows [34, 35]. This layer is approximately 30 μm thick [29] and its components are produced by both the cornea and conjunctiva [36]. The mucin layer moves freely over the glycocalyx, which is comprised of membrane-associated mucins bound to the cornea and conjunctiva.

The rate of tear production can vary 100-fold between basal tear production and active tearing [37]. The average rate of tear fluid production ranges between 0.5 and 2.2 μL/min (with an average of 1.2 μL/min) at baseline [33].

While estimates of the rate of baseline tear fluid production have not varied significantly since the 1966 report of Mishima et al. [33], the relative contributions of different sources to the aqueous tear fraction continue to be debated. Aqueous tear fluid was thought to be almost entirely produced by the main lacrimal gland with minor contributions from the accessory lacrimal glands and goblet cells in the conjuctiva [38]. Recent studies, however, have shown that the rate of tear fluid flow across the conjunctiva can be as high as 1–2 μL/min and may account for a large proportion of basal tear production [39]. While estimates of the contribution of conjunctival secretion to basal tear fluid production vary widely, recent models of tear production suggest that 25% of tear fluid is produced by the conjunctiva in the absence of reflex tearing [40]. While the aqueous fraction in stimulated tears derives primarily from the lacrimal glands, unstimulated tears may have significant contributions from conjunctival sources.

13.3 Glucose in Tear Fluid

13.3.1 Tear fluid glucose transport

The source of glucose in tears remains unclear. The studies that used mechanically irritating methods to obtain tear fluid find the highest glucose concentrations and find correlations between blood and tear glucose concentrations [38, 41-43]. Mechanical irritation abrades the conjunctiva and probably causes leakage of glucose from epithelial cells or the interstitial space directly into the tear fluid [38, 44].

Studies that attempt to avoid abrasion of the cornea and stimulation of tearing

measure the lowest glucose concentrations [44–46]. Many studies of chemically stimulated tears also find decreased concentrations of glucose, suggesting that very little, if any, glucose comes from the lacrimal glands [42, 44, 47].

There is only limited evidence for glucose transporters in the tear glands, the conjunctiva, and the cornea. The constitutive glucose transporter, GLUT-1, is present in the apical corneal epithelium [48], but absent in the lacrimal glands and conjunctiva [49]. Expression of GLUT-1 in the corneal epithelium is increased after corneal abrasion, and appears to have a role in corneal wound healing [50]. A sodium/glucose cotransporter, SGLT-1, is present on the apical side of the bulbar and palpebral conjunctiva [51, 52]. This transporter operates in both directions, allowing both secretion and absorption of glucose, depending on sodium and glucose concentrations [53]. While this transporter removes glucose from the tear fluid under physiological conditions [54], it can add glucose to the tear fluid during the hypoosmotic stress that occurs when rinsing the eye with water or swimming [40, 53–55].

Recent models of electrolyte and metabolite transport in the tear fluid suggest that a paracellular transport mechanism is required to fully explain observed electrolyte concentrations [40]. Studies of polyethylene glycol oligomer permeability in the cornea and conjunctiva of rabbits suggest that there are paracellular pores with diameters of ~4 nm and ~2 nm in the conjuctiva and cornea respectively [56]. While glucose should be able to pass through these pores and into the tear fluid, its transit has not been directly measured. While the distribution and regulation of glucose transporters affecting the tear glucose concentration are not yet fully characterized, glucose transport across the conjunctiva appears to be the major determinant of tear glucose concentrations in the absence of reflex tearing.

13.3.2 Tear glucose in diabetic subjects

If tear glucose analysis is to be used to monitor diabetes, we must consider the effects of diabetes on aqueous tear production and glucose transport in tear fluid. While the relative importance of different molecular mechanisms in diabetes pathogenesis is debated, the increased intravascular concentration of glucose that results from diabetes ultimately leads to microvascular and nerve damage [57, 58]. Damage to either the vasculature supplying blood to the eye or the nerves of the lacrimal reflex arc might be expected to alter tear production.

Dry eye is more common in diabetic patients, and correlates with poor glycemic control [59]. Basal tear secretion rates are indistinguishable between normal and diabetic subjects [60, 61]. Reflex tearing, as measured by a Schirmer test, is decreased significantly in diabetic subjects [60, 61], probably due to a decreased sensitivity of the conjunctiva resulting from neuropathy [62]. During episodes of hyperglycemia, increased osmolarity in the extracellular fluid may also impede aqueous tear flux across the conjunctiva or into the lacrimal gland [59].

Increased tear glucose concentrations in diabetic subjects have been repeatedly demonstrated [23, 41–43, 45, 63–66] However, many of these studies used filter paper to collect the tear sample, and the observed high glucose levels may be due to intercellular fluid leaking through the abraded conjunctiva [44]. A recent study of tear

glucose concentration in 50 non-diabetic subjects and 33 diabetic subjects specifically tried to avoid chemical or mechanical stimulation by collecting tear samples with a glass microcapillary [45]. This study observed overlapping ranges of tear glucose concentration in fasting normal and diabetic subjects, but found a statistically significant increase in average tear fluid concentrations in the diabetic subjects (89 μM for normal and 150 μM for diabetic subjects). While tear glucose concentrations are clearly increased in diabetic subjects, the precise mechanism remains unclear. Studies using sampling methods that cause mechanical stimulation of the conjunctiva are likely to be simply measuring analytes in direct equilibrium with intercellular fluid. Studies of non-stimulated tears may measure increased glucose due to paracellular glucose transport in the conjunctiva. It is also possible that these studies did not avoid eye irritation during the required five minute sampling periods [44, 45].

13.4 Previously Reported Tear Fluid Glucose Concentrations

Reported values for tear glucose in normal individuals range from 0 to 3.6 mM (65 mg/dL) [55], while concentrations as high as 84 mg/dL (4.7 mM) were reported for diabetic persons [23]. The reported tear glucose concentrations are generally lower for analytical techniques which require smaller tear volumes. Recently, median glucose concentrations of 89 μM were measured in 5 μL tear samples [45], while we recently measured 28 μM concentrations in 1 μL collected tear samples [46], all from fasting, non-diabetic individuals. Fig. 13.2 shows the variation in the measured tear glucose tear fluid concentrations for all published studies. Older studies generally measured glucose in larger volumes of chemically or mechanically stimulated tears, while more recent studies specifically tried to avoid conjunctival irritation and tear stimulation (Fig. 13.2).

Although the dependence of the tear glucose concentration on the collection method was previously noted [44], there has been little discussion of this sampling bias. As discussed below, we developed a new mass spectral method to determine glucose in 1 μL volumes of tear fluid collected in such a way as to minimally perturb the eye of the subject. We also examined how the tear fluid glucose concentrations track those of blood [28, 46, 67].

13.4.1 Previous measurements of tears in extracted tear fluid

The first quantitative report of glucose in tear fluid in 1930 found tear glucose concentrations of 3.6 mmol/L (65 mg/dL) [68]. The tear volume studied was 0.2 mL, requiring that tearing was induced in order to collect such large volumes. Reported values of tear glucose concentrations have steadily decreased as the analytic methods required less sample volume. At the opposite extreme, LeBlanc et al. recently

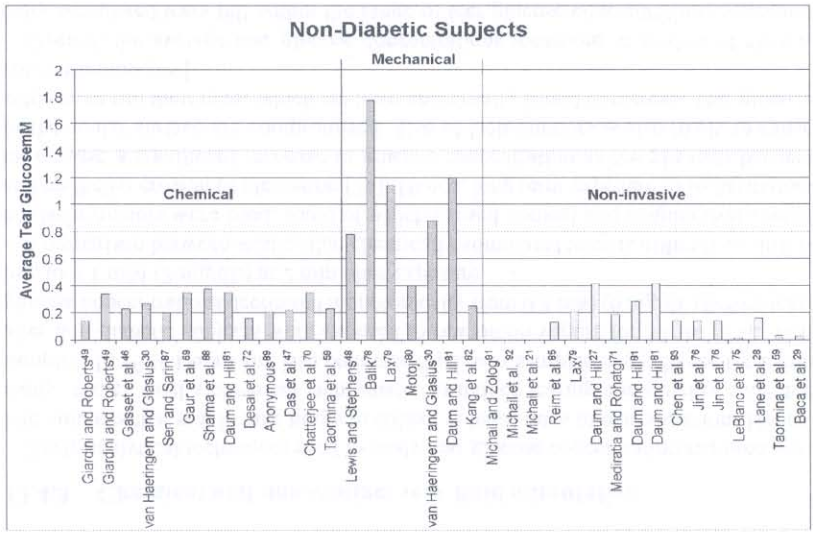

FIGURE 13.2: Summary of average tear glucose concentrations found in non-diabetic subjects from 1930 to present. Studies are grouped by the type of stimulation used to induce tearing (chemical, mechanical, and noninvasive). These are arranged in chronological order from left to right within each section. The details of each study are discussed in the tables and text below. Note that studies employing mechanical stimulation measure the highest tear glucose concentrations.

reported an average tear glucose concentration of 7.25 $\pm$ 5.47 μmol/L in five patients in an intensive care unit [69]. Glucose was measured using HPLC with pulsed amperometric detection. The sample volumes studied were variable and were not individually reported, but were presumably less than 1 μL [70, 71]. The fact that the reported values of tear glucose concentrations varied by more than 1000-fold between all of these studies demonstrates the need for careful consideration and control of experimental parameters such as collection method, analysis method, and selection of the clinical population.

13.4.2 Mechanical tear fluid stimulation

Van Haeringen and Glasius compared glucose concentrations in the chemically and mechanically stimulated tears of normal and diabetic subjects [44]. They first stimulated tearing with 2-chloracetophenon and collected 20 μl tear samples with a capillary tube. They then collected a second tear sample using filter paper strips. Higher tear glucose concentrations for all subjects were measured using filter paper collection. The increase was between 0.1 and 1.5 mM for subjects with blood glucose below 10 mM (180 mg/dL) and as high as $\sim$9 mM for extremely hyper-

glycemic subjects with blood glucose concentrations of ~20 mM (360 mg/dL). A 1969 dissertation by Lax, which is cited by Van Haeringen and Glasius, found glucose concentrations of 0.206 ± 0.027 mM (mean ± SD) using capillary collection and 1.141 ± 0.159 mM using filter paper collection in non-diabetic subjects [44, 72].

Daum and Hill used capillaries to collect 5 μL tear samples at different times from non-diabetic subjects after mechanical stimulation of the conjunctiva with a cotton applicator [73]. They measured an increase in tear glucose concentration from ~0.28 mM (~5 mg/dL) before stimulation to a maximum of ~2.5 mM (~45 mg/dL) 10 min after stimulation. Tear glucose concentrations remained elevated for 30 min, but returned to baseline after 60 min. This indicates that unrecognized conjunctival stimulation early in a time course study of tear glucose could affect many subsequent measurements. A similar increase in tear glucose concentration after corneal or conjunctival irritation occurs in rabbits [74, 75].

Daum and Hill also observed an increase in tear glucose after hypoosmotic stress induced by immersion of the eye in distilled water for 60 sec. Studies of mechanically stimulated tears generally find a correlation between the tear and blood glucose. This is not surprising as the glucose measured in these studies likely comes directly from the interstitial space in the conjunctiva. Rabbit studies suggest that short term contact lens use increases tear glucose concentrations in a manner similar to mechanical stimulation [37, 75]. However, it should be noted that our recent study found no statistically significant evidence of increased tear glucose concentrations in fasting subjects wearing contact lenses [46].

13.4.3 Chemical and non-contact tear fluid stimulation

Early analytical techniques used to study tear glucose concentration required analyte volumes that would take hours to collect at basal rates of tear secretion. Hence, many studies used a chemical lachrymator to induce tearing and quickly collected samples. In 1984 Daum and Hill collected 5 μL tear samples every 20 sec for 2 min after non-diabetic subjects were exposed to raw onion vapors for 30 sec [73]. Tear glucose concentrations decreased monotonically from 0.3 mM (6 mg/dL) before tearing, to 0.1 mM (2 mg/dL) at 2 min after exposure.

Comparison between studies that chemically stimulated tears is difficult, as different lachrymators were used, some of which caused corneal and conjunctival edema and epithelial erosion or ulceration [76]. Hence, long term exposure to lachrymators may cause a significant *increase* in glucose concentration as the physical barriers of the ocular surface are compromised. Use of lachrymators is also likely to cause subjects to rub their eyes, which we have anecdotally found to increase tear glucose concentrations [46].

Overall, the average tear glucose concentrations measured in studies of chemically stimulated tears fall within the range of tear glucose concentrations measured in non-stimulated tears. Some studies of chemically stimulated tears do not find a correlation between tear and blood glucose for all subjects [47]. However, these studies were often able to broadly classify subjects as diabetic or non-diabetic through tear glucose measurements, especially when postprandial samples were considered

[42, 64].

13.4.4 Non-stimulated tear fluid

With the development of more sensitive analytical methods there were attempts to determine glucose concentrations in non-stimulated, or basal tears. These non-stimulated tear samples are generally collected with a capillary by gently touching it to the tear film meniscus. One of the first large studies of non-stimulated tears measured an average tear glucose concentration of 0.42 ± 0.356 mM in 875 tear samples from 12 non-diabetic subjects [55]. This average tear glucose concentration was somewhat higher than more recent measurements of chemically stimulated tears. While studies using lachrymators could be measuring artificially low tear glucose concentrations, it is also possible that some mechanical stimulation was involved in the collection of basal tears in this study. The method used in that study required sample volumes of 5 μL, which would require sample collection times over at least 5 min. Even if mechanical stimulation were avoided during sampling, increased evaporation due to the prolonged suppression of blinking could alter the glucose concentration.

One of the largest and most recent studies of tear glucose by Lane et al. [45] monitored tear glucose concentration in 73 non-diabetic subjects and 48 diabetic subjects before and after an oral glucose bolus. These groups were further divided into fasting and non-fasting groups. The 5 μL tear samples collected at each time point were analyzed with liquid chromatography-pulsed amperometric detection. Average tear glucose concentrations found for non-diabetic subjects was 0.16 ± 0.03 mM while average tear glucose concentrations for diabetic subjects was 0.35 ± 0.04 mM (mean ± standard error). Individual glucose determinations, however, varied from below the limit of detection to over 9.1 mM. They were able to show a modest correlation between average tear and blood glucose concentrations at the five time points in the study. Unfortunately, only results averaged over the subject population were given. Thus, there is no direct indication of how well tear and blood glucose concentrations correlate within individual subjects. The collection of 5 μL tear samples may have precluded the study of truly non-stimulated tears for the reasons noted previously.

A few recent studies analyzed μL or subμL tear volumes [46, 67, 69, 70, 77]. The previously mentioned study of critically ill patients attempted to assess the feasibility of monitoring tear glucose instead of blood glucose in an intensive care unit [69]. The investigators obtained 44 simultaneous blood and tear samples from 5 sedated subjects receiving insulin, two of whom had a history of diabetes. This study measured the lowest average tear glucose concentration (7.25 ± 5.47 μmol/L) of any study where glucose was detected in tears. Despite a wide range of blood glucose concentrations, the study did not detect a clinically useful correlation. However, these data obtained from critically ill patients are likely of little use in predicting whether tear fluid can be used to monitor glucose in more healthy people. However, it is clear that tear glucose monitoring with this method in the ICU is not a feasible replacement for blood glucose monitoring.

13.5 Recent Tear Fluid Glucose Determinations

We recently found a median (range) tear glucose concentration of 28 (7–161) μM or 0.50 (0.13–2.90) mg/dL in 25 fasting subjects [46]. The mean $\pm$(standard deviation) tear glucose concentration was 37 $\pm$ 37 μM. We found a highly skewed distribution of tear glucose values; tear glucose concentrations were <42 μmol/L in 80% of subjects. We found no statistically significant difference between contact lens wearers and non-wearers. Linear regression showed a modest correlation between tear and blood glucose concentrations ($R = 0.5$). We compared tear glucose concentrations within subjects over 30 min and did not see any significant trend with time, suggesting that in our study conjunctival irritation was minimized or eliminated. We believe our study to be the most reliable measurements of baseline tear glucose to date because we collected only 1 μL tear samples, studied non-diabetic fasting subjects, and found negative evidence for the occurrence of conjunctival stimulation.

As described in detail above, there is very little reliable information on whether glucose concentrations in unstimulated tears track blood glucose concentrations. While previous studies showed correlations between averaged tear and blood glucose concentrations, there is little direct information about the existence of such a correlation within individual subjects.

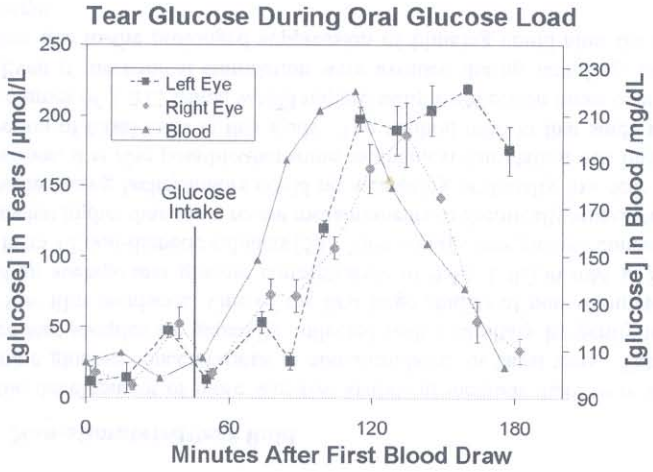

FIGURE 13.3: Tear and blood glucose concentrations in a non-diabetic, male subject. Blood glucose concentration doubles by $\sim$ 70 minutes after glucose ingestion.

We recently used our mass spectrometry method to investigate the relationship between blood and tear glucose concentrations in subjects during a glucose tolerance test (oral administration of 75 grams of glucose) [67]. Fig. 13.3 shows how blood and tear glucose concentrations track over time for a non-diabetic subject. The rise of tear glucose concentrations appear to track that of blood glucose in this subject with a time lag of ~20 min. The left eye tear glucose concentration remains elevated even after the blood glucose concentration decreases. While this may be the true physiological response for this subject, it could also be explained by an unrecognized irritation of the left conjunctiva during the latter half of the experiment (140–160 min). An essential point is that, while the blood glucose concentration increases ~2-fold, the left and right eye tear glucose concentrations both increase ~7-fold. This demonstrates a complex relationship between tear and blood glucose concentrations for this subject.

Fig. 13.4 shows a similar plot of blood and tear glucose concentrations during a glucose tolerance test for a diabetic subject. Blood and tear glucose glucose concentrations are clearly higher for the diabetic subject at all times, even at the beginning of the study, when subjects are fasting. Prior to glucose ingestion, the blood glucose concentration in this subject was about twice that for the non-diabetic subject of Fig. 13.3. However, the basal tear glucose concentration of the diabetic subject is ~10-fold higher than for the non-diabetic subject! This clearly continues to demonstrate a complex relationship between tear and blood glucose concentrations.

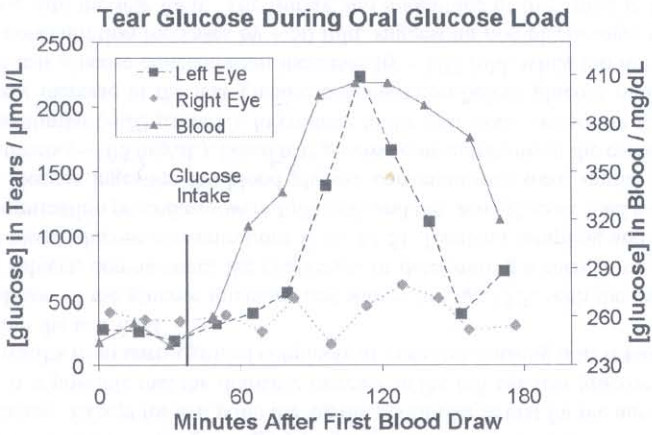

FIGURE 13.4: Tear and blood glucose concentrations in a diabetic, female subject. Blood glucose concentration peaks at ~ 80 minutes after glucose ingestion with a ~ 60% increase.

The extraordinary ~5 fold increase in tear glucose concentration after glucose in-

gestion in the left eye differs completely from the change in the right eye, where (excepting for the single point at ~95 minutes) a ~60% increase in tear glucose concentration occurs which is roughly proportional to the increase in blood glucose concentration. Except for that point the lag time is similar to that for the non-diabetic subject. It is possible that the dramatic increase in the left eye tear glucose concentrations results from unrecognized conjunctival irritation, causing interstitial glucose to leak into the tear fluid.

A replicate of the glucose tolerance test shown in Fig. 13.3, with the same non-diabetic subject, demonstrates the challenges of determining a correlation between tear and blood glucose concentrations (Fig. 13.5). Identical sampling and tear glucose determination procedures were followed, and the same glucose load was given. Prior to glucose ingestion the blood glucose concentrations were similar for both measurements (~105 mg/dL). Basal tear glucose concentrations at the earliest times were also similar (~20 μmol/L). In contrast to the first study, we see an abrupt extraordinary increase in the tear glucose concentration before glucose intake. The right eye tear glucose concentration increases by ~100 fold, while the left eye tear glucose concentration increases by ~50 fold, suggesting non-physiologic transport of glucose into the tear fluid. The timing and magnitude of this spike in tear glucose concentration suggests that the increase may be due to a perturbation during sampling. While we attempted to exclude this possibility, the increased glucose concentration in both eyes might suggest that the subject may have rubbed their eyes after slight conjunctival irritation.

The tear glucose concentrations appear to return to baseline after about 60 min. This timescale agrees with that of Daum and Hill measured for tear glucose to return to baseline after the conjunctiva was stimulated by a cotton applicator [73]. When the results of the replicate study are plotted on the same scale as Fig. 13.3, we see that the tear glucose concentrations at latter times (after 60 min) appear to track changes in the blood glucose concentration. However, the relative tear glucose elevation (~5-fold) in the left eye is much less than observed in the previous study of Fig. 13.3, whereas the relative increase in tear glucose concentration in the right eye is comparable.

To summarize, considering the large number of studies we have carried out we conclude that tear glucose concentrations generally correlate with an increase in hyperglycemic blood glucose concentrations. Repeat measurements of tear glucose concentrations in the presence of a constant blood glucose concentrations appear to show a constant ratio between the tear and blood glucose concentrations. After glucose ingestion, the blood and tear glucose concentrations generally increase together, with an apparent 20–30 min delay between increases in blood glucose and in tear glucose concentrations.

In our other studies of tear glucose concentrations in subjects undergoing glucose tolerance test, we see only a few instances of the 50–100-fold spikes in tear glucose concentration seen in Fig. 13.5. In general, these abrupt changes do not seem to correlate between the different eyes of the same subject.

Our preliminary results suggest that tear glucose may differ between the left and right eye of a single subject. While we previously showed a general correlation be-

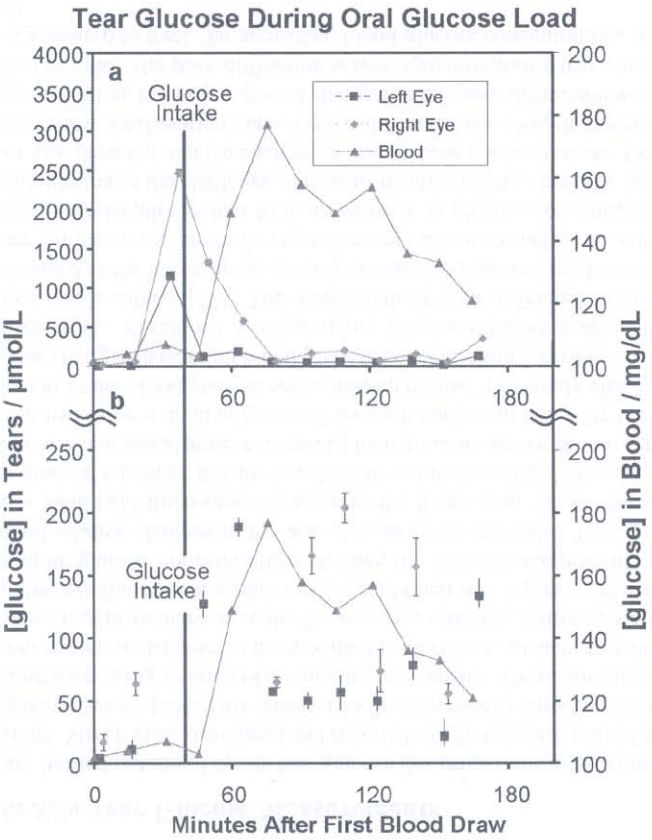

FIGURE 13.5: Tear and blood glucose concentrations in a replicate of the study shown in Fig. 13.2. a) The tear glucose concentration scale must be expanded to show the large and abrupt increase in tear glucose before glucose intake. Blood glucose concentration peaks at ~ 40 min after glucose ingestion with a $\sim 80\%$ increase. b) Plotting the tear glucose on the same scale as in Fig. 13.2 highlights that the tear glucose concentration appears to track the blood glucose concentration except for the early spike in tear glucose concentrations.

tween the tear glucose in the right and left eyes of fasting subjects [46], we occasionally observe significant differences in glucose concentration between eyes. While we do not yet have enough data to specify the precise relationship between tear and blood glucose concentrations over time and the variation of this correlation between subjects, we believe that our tear collection method, which specifically attempts to avoid mechanical stimulation of the conjunctiva, could definitively answer some of the outstanding questions regarding the utility of tear glucose sensing for monitoring

or detecting diabetes, if enough subjects were studied to deconvolve the deviations due to sampling perturbations of the eye.

It is important to note that tear fluid glucose concentration studies represent among the most challenging bioanalytical chemistry sample collection challenges: it is essential to note that reflex tearing can also be elicited for psychological reasons. Nevertheless, the large body of tear fluid studies, including our recent studies, clearly indicate that blood and tear glucose concentrations are correlated for probably most individuals.

13.6 *In Situ* Tear Glucose Measurements

There are limited reports of *in situ* tear glucose determinations using contact lens-based devices. March et al. developed and reported the first clinical trial of a contact lens tear glucose sensor [26]. This sensor uses fluorescence to report on the tear glucose concentration using a competitive binding mechanism. They showed that as the glucose concentration increases in the modified contact lens, quenching groups were displaced resulting in an increase in the fluorescence intensity. Unfortunately, in this study, the absolute fluorescent signal was not calibrated which prevented determination of absolute glucose concentrations. Rather, the fluorescence intensity changes demonstrated relative changes in the tear glucose concentrations. This group also developed a hand held fluorometer to monitor the fluorescent signal. March et al. reported a glucose tolerance test for five diabetic subjects wearing these *in situ* sensors. The fluorescent signal appears to track blood glucose concentrations. However, the response had to be individually scaled for each subject in order for the fluorescence signal to fit the blood glucose concentration profile. This study clearly shows, however, that changes in tear and blood glucose concentration correlate.

Domschke et al. developed a holographic, glucose-sensitive contact lens and tested it in a single subject [27]. The wavelength of light diffracted from the contact lens changed as the holographic spacing changed in response to glucose binding in a manner similar to our photonic crystal sensors discussed below. A red-shift in the diffracted wavelength resulted from an increase in glucose concentration. This sensor motif eliminates the challenge of measuring absolute fluorescence intensities. This sensor also detected relative changes in tear glucose concentrations. Domschke *et al.* did not show a calibration curve for the diffracted wavelength dependence on glucose concentration, but only reported changes in the peak diffraction wavelength as a function of time. The peak diffraction wavelength monitoring tear glucose concentrations appeared to track the increasing blood glucose concentrations with little or no delay.

These important *in situ* results clearly demonstrate correlations between blood and tear glucose concentrations. What is not clear is whether these anecdotal studies are reproducible within individuals over different days and weeks. Also it is still un-

known whether the blood and tear glucose correlations are identical between normal people or between diabetic subjects.

The results to date suggest that the worst case scenario is that a reliable and useful correlations between blood and tear glucose occur for only a subset of the population and that the correlations between tear fluid and blood glucose differ between individuals. Thus, the use of a contact lens glucose sensor in the future may require fitting by a physician who would determine the correlation and sensor sensitivity required for the glucose sensing contact lens.

The value of using a glucose sensing contact lens to achieve a noninvasive method to easily and continuously monitor the glucose concentrations would revolutionize life for people with diabetes mellitus. We believe that there is clearly enough evidence of a correlation between tear and blood glucose to justify continued efforts to develop contact lens glucose sensors such as the contact lens photonic crystal glucose sensor described below.

13.7 Photonic Crystal Glucose Sensors

Our program [20–22, 24] to develop a tear fluid glucose sensing device envisions a photonic crystal sensing disc encased within either a contact lens or an ocular insert, as shown in Fig. 13.1. The ocular insert would be used by subjects who do not tolerate contact lenses well. The ocular insert, which consists of the photonic crystal sensor sandwiched between thin dialysis membranes, would be placed beneath the lower eyelid.

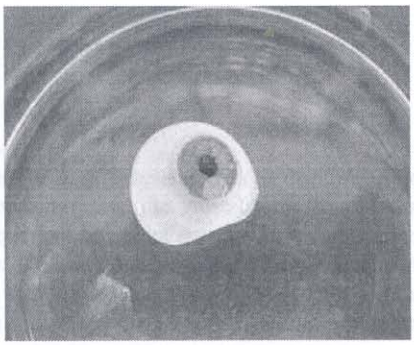

FIGURE 13.6: Prototype of IPCCA photonic crystal glucose sensor in commercial contact lens on glass eye.

Diabetic subjects would use a mirror to examine the color of the photonic crystal sensor in the contact lens or the ocular insert. For the ocular insert, the lower eyelid would be lowered to view the photonic crystal color. The color reflected (diffracted), which will vary with the tear fluid glucose concentration, would be viewed with a mirror and compared to that of a color wheel whose colors are calibrated in terms of the blood glucose concentrations. For those people who find it difficult to do color matching we will instrument this measurement by using a simple spectrometer which utilizes a color camera.

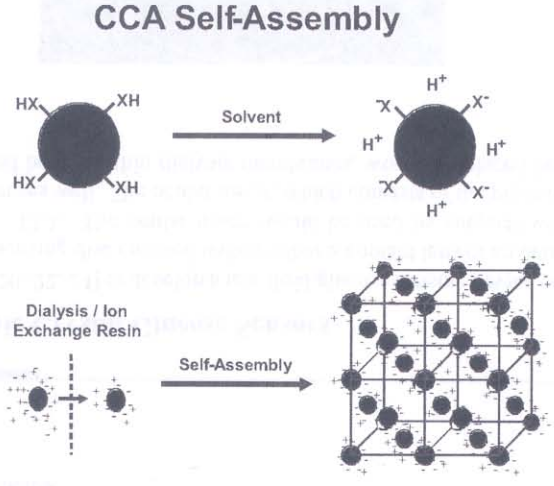

FIGURE 13.7: A dispersion of monodisperse colloidal particles with strong acid groups on their surfaces self-assemble into fcc or bcc crystalline colloidal arrays in low ionic strength solution. Ions can be removed from colloidal dispersions by techniques such as dialysis, for example.

Fig 13.6 shows our first prototype of the photonic crystal sensor embedded within a commercial contact lens placed on a glass eye. The contact lens contours to the eye allowing the embedded photonic crystal sensor to contact the tear fluid. The sensor changes diffraction wavelength in response to changing glucose concentrations.

We fabricate the glucose sensing photonic crystals by using crystalline colloidal self-assembly [78] to form the photonic crystal template (Fig. 13.7). In this approach highly charged monodisperse spherical polystyrene colloidal particles are synthesized, for example, by using emulsion polymerization [79]. These particles are then cleaned by dialysis and ion exchange and then dispersed in pure water.

The synthesized spherical particles are also functionalized with thousands of strong acid groups which ionize in water to result in a high surface charge (Fig. 13.7). Be-

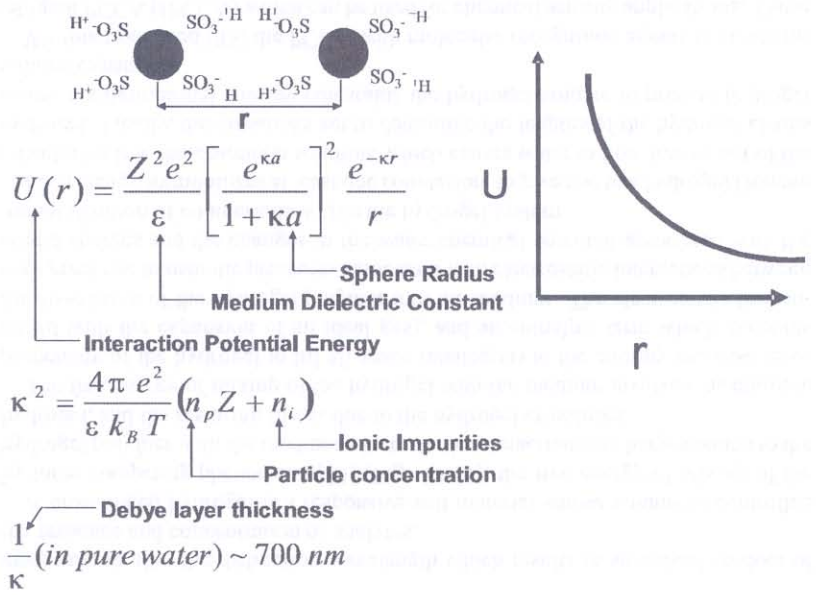

FIGURE 13.8: The potential energy of repulsion between two charged colloidal particles separated by distance r, $U(r)$ is given by the DLVO potential which depends upon the number of charges, Z, the electron charge, e, the dielectric constant, ε, the radius, a, the Boltzmann constant, k_B, the temperature, T, and the particle concentration, n_p. κ is the inverse of the Debye length which depends on the concentration of ionic impurities, n_i. $U(r)$ can be much larger than $k_B T$ resulting in the self-assembly of the colloidal particles into a robust fcc CCA.

cause these highly charged spheres repel each other over macroscopic distances (Fig. 13.8), the system finds a well defined minimum energy state where the particles self-assemble into an fcc lattice [80].

Diffraction from the CCA photonic crystals is extraordinarily efficient. For example, Fig. 13.9 shows transmission measurements through an fcc colloidal crystal [81] made from ~120 nm diameter polystyrene spheres in water which is oriented with the normal to the fcc (111) planes parallel to the incident light propagation direction. The diffraction of ~500 nm light gives rise to a symmetric bandshape as shown in the Fig. 13.9 extinction spectra whose ordinate is scaled as -log of the transmission.

We fabricated more robust photonic crystal materials (Fig. 13.10) by polymerizing acrylamide hydrogels around the CCA lattice of colloidal particles [82]. These polymerized CCA (PCCA) possess the responsive properties of hydrogels. These PCCA can also be chemically functionalized to make them responsive to changes in their chemical environment. This enables the fabrication of novel chemical sensing photonic crystals [83]. The resulting volume changes in response to changes in chemical

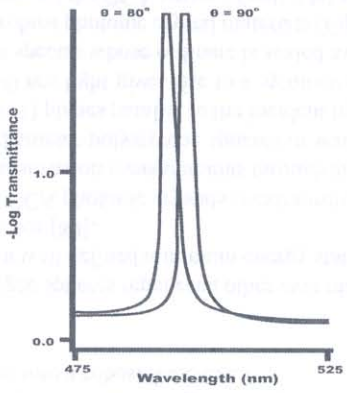

FIGURE 13.9: Extinction spectra of CCA of ~120 nm polystyrene particles. The ~500 nm diffraction band shows a top-hat profile since all incident light within a 5 nm bandwidth is diffracted. The diffraction blue-shifts as the crystal is tilted to 80^o off normal, as expected from Bragg's law.

environment alter the diffraction wavelength which results in an optical readout of the presence and concentration of analytes.

A crosslinked hydrogel is a responsive soft material whose volume is controlled by three competing phenomena [83] (Fig. 13.11), the free energy of mixing of the hydrogel polymer with the medium, electrostatic interactions of charges bound to the hydrogel, and the restoring forces due to the hydrogel crosslinks.

The free energy of mixing of the hydrogel with the medium involves the entropic propensity of the hydrogel to fill all space (analogous to the entropy increase associated with the expansion of an ideal gas), and an enthalpic term which accounts for dissolution of the hydrogel polymer into the medium. The electrostatic free energy gives rise to osmotic pressures associated with electrostatic interactions between bound charges and the changes in the water chemical potential associated with the immobilization of counterions within the hydrogel system.

Each charge immobilizes at least one counterion, to give rise to a hydrogel Donnan potential in low ionic strength solution, which causes water to flow into or out of the hydrogel. Finally, the crosslinks act to determine the lengths of the hydrogel chains whose configurational entropy constrains the hydrogel volume to prevent hydrogel volume expansion.

We functionalized [83] the PCCA with molecular recognition agents to create intelligent PCCA (IPCCA) which can be used for chemical sensing applications. These molecular recognition agents are designed to actuate hydrogel volume changes as they interact with their target analytes. These volume changes alter the embedded CCA lattice constant, which results in diffraction wavelength shifts which report on the analyte concentrations.

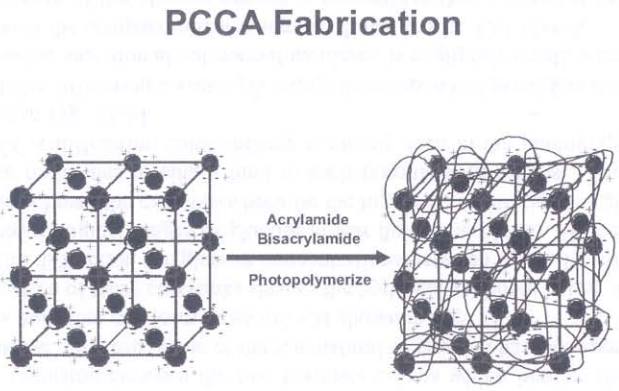

FIGURE 13.10: Synthesis of polymerized colloidal array (PCCA) by adding polymerizable monomers of acrylamide and bisacrylamide and a UV initiator to a CCA. UV polymerization forms a hydrogel network around the CCA (~90 % water). The CCA crystal structure is maintained during polymerization and also upon hydrogel volume changes.

The selectivity of these IPCCA sensing materials are determined by the selectivities of their molecular recognition agents. Our first glucose sensor was highly selective for glucose since we used the enzyme glucose oxidase (GOD) [83]. GOD converts glucose to gluconic acid. During this reaction a flavin is reduced on the enzyme. This anionic flavin acts as an anion immobilized onto the hydrogel, which gives rise to a Donnan potential which swells the PCCA in proportion to the glucose concentration. This GOD PCCA operates as a steady-state glucose sensor since oxygen in solution reoxidizes the flavin. When oxygen is excluded, concentrations of glucose as low as 10^{-12} M are easily determined [83].

We recently developed another glucose IPCCA sensor that also utilizes a Donnan potential by attaching boronic acid recognition groups to the PCCA [20–22, 24]. Boronic acid derivatives are known to bind to the cis diols of carbohydrates such as glucose. The binding of glucose to neutral boronic acid derivatives shifts the boronic acid equilibrium towards the anionic boronate form. This charged boronate attached to the hydrogel results in a Donnnan potential which results in an osmotic pressure which swells the IPCCA. As shown in Fig. 13.12, this IPCCA is an excellent glucose sensor for low ionic strength aqueous solutions [22].

We also developed a glucose sensor for high ionic strength bodily fluids such as in our target tear fluid [20–22, 24]. This sensor motif utilizes changes in the hydrogel

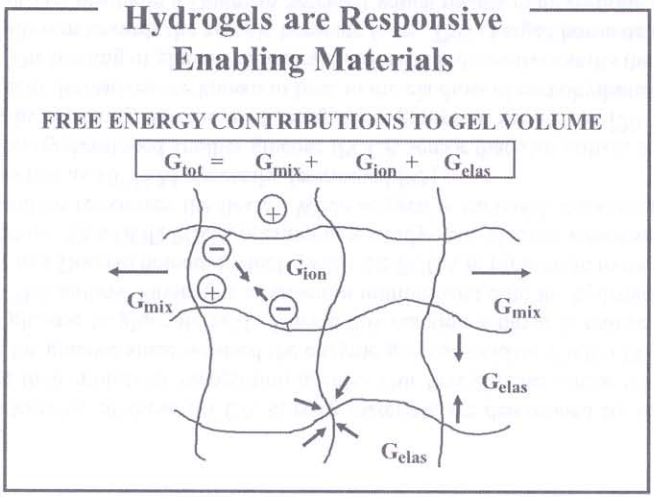

FIGURE 13.11: Dependence of hydrogel volume on the free energy of mixing, the free energy of ionic interactions, and the free energy associated with the elastic constraints of the hydrogel crosslinks. The hydrogel volume in equilibrium with bulk water is determined by the balance of osmotic pressures induced by these phenomena.

crosslinking induced by glucose binding. This sensor, which also utilizes boronic acids, additionally incorporates polyethylene glycols in order to bind Na^+ to screen the charge repulsion between the two boronate anions which bind to the two cis diols of glucose. Glucose is one of the few natural sugars that has two appropriately oriented cis diols that can form cross links as shown in Fig. 13.13.

The formation of these crosslinks shrinks the hydrogel as shown in Fig. 13.14 and blue shift the diffraction for glucose concentrations between 0 and 10 mM (which spans the physiological ranges of glucose in tear fluids and blood). Higher glucose concentrations break the crosslinks because the higher concentrations of glucose allow glucose molecules to singly bind to each boronic acid groups. The visually evident IPCCA diffraction color shifting is clearly seen in the photographs of the IPCCA seen in Fig. 13.14.

Although the diffraction wavelength is angularly dependent as evident from Bragg's law, the possible variation about normal incidence is negligibly small, when viewed by reflection in the compact mirror assembly shown in Fig. 13.1 above.

The sensitivity of the glucose sensors is presently within a factor of four of that needed to function as a hyperglycemic sensor in tear fluid. We also now understand the equilibrium sensing mechanism in sufficient detail such that we can fully model this sensor response using our fundamental understanding of the hydrogel volume phase transitions.

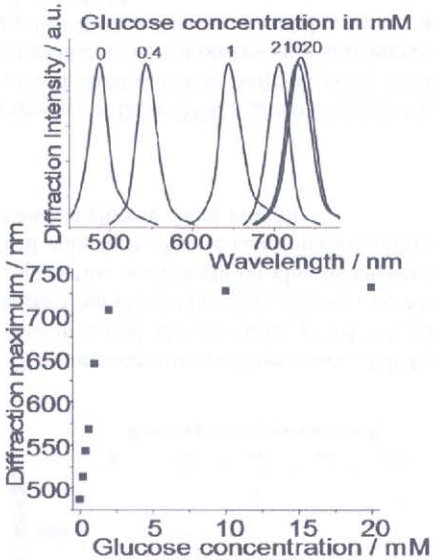

FIGURE 13.12: Response to glucose of boronic acid IPCCA. The IPCCA in pure water redshifts as the glucose concentration increases until ~ 3 mM glucose concentration where the response saturates.

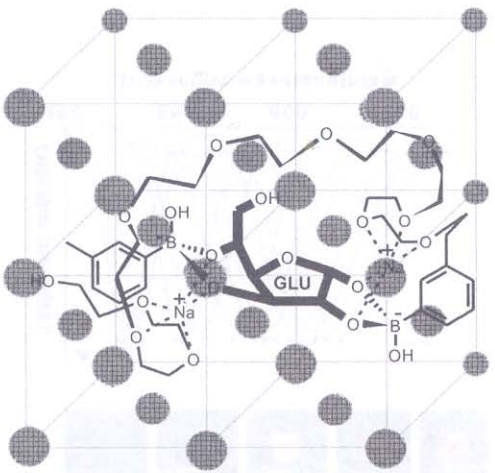

FIGURE 13.13: Model of crosslinks formed by a glucose molecule across two boronic acid groups within the IPCCA. The polyethylene glycol serves to localize two Na^+ cations next to the boronates to reduce the electrostatic repulsion between them.

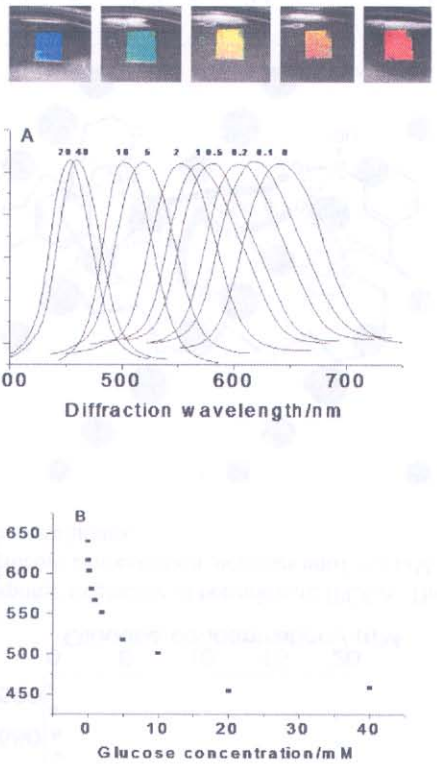

FIGURE 13.14: Glucose concentration dependence of diffraction of the AFBA-AA-PEG PCCA sensor in 2 mM gly-gly buffer at pH 7.4, 150 mM NaCl. Top: diffraction color changes from red to blue upon glucose concentration increases. A) The dependence of diffraction wavelength on glucose concentration. The diffraction peaks are labelled with their glucose concentration (mM). B) Dependence of diffraction peak maxima on glucose concentration.

We find similar responses of these IPCCA glucose sensors to glucose in synthetic tear fluid solutions as compared to that in buffered saline (2 mM gly-gly, pH=7.4, 150 mM NaCl). The fact that we do not observe any interference from the additional species present in the tear fluid indicates the viability of our sensing approach for determining glucose in tear fluid.

The acrylamide IPCCA glucose sensors originally responded rather slowly to changes in glucose concentrations (~30 min). We worked to modify the hydrogel composition to increase the response rate such that it could sense changes in glucose concentrations at rates comparable to the expected rates of change of glucose

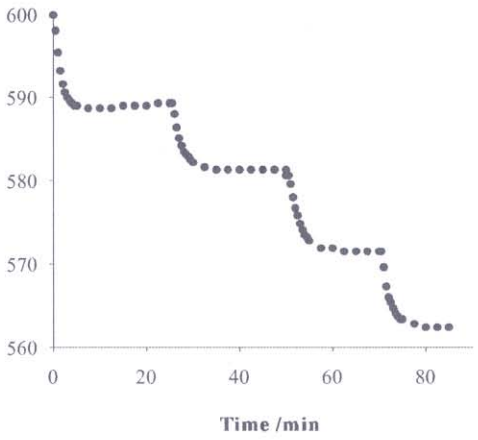

FIGURE 13.15: Response kinetics of hexylacrylate IPCCA glucose sensors upon exposure to multiple additions of a freshly prepared 0.2 mM D-glucose solution in 5 mM gly-gly buffer, 150 mM NaCl, pH=7.4 at 37°C. A repeatable rapid blue shift of diffraction is observed which saturates within ~5 min.

concentrations in blood.

After examining diffusion constants of molecules in the IPCCA hydrogel we hypothesized that the response speed limit was determined by the friction experienced by the water and hydrogel polymer that limit the water and hydrogel polymer terminal flow velocity in response to the osmotic pressure. We recently incorporated hydrophobic monomers into the hydrogel to decrease the extent of hydrogen bonding of water to the hydrogel polymer. This successfully increased the response rate, making it adequate for physiological sensing [21] (Fig. 13.15).

We are still working to increase the sensor glucose sensitivity to make it useful for the visual determination of the normal ~30 μM tear fluid glucose concentrations. The remaining challenges are to demonstrate that these sensors successfully determine glucose *in situ* and that variations in the basal tear glucose concentration are not large enough to confound correlations between tear fluid and blood glucose concentrations. We are presently investigating these issues.

13.8 Summary

It is clear that the glucose concentrations in tear fluid track that of blood for a significant number of individuals. This indicates that a glucose sensor within a contact lens has the potential to revolutionize glycemic determination and glycemic control

in subjects with diabetes mellitus. We have developed a new photonic crystal glucose sensor that we are incorporating into a contact lens. While there is significant risk in this endeavor, it also has the possibility to dramatically aid human health.

Acknowledgements

This research was supported by the National Institutes of Health Fellowship 1 F31 EB004181-01A1 to Justin T. Baca, and Grant 2 R01 EB004132 to Sanford A. Asher.

Financial Disclosures

Sanford A. Asher is the scientific founder of Glucose Sensing Technologies LLC, a company developing PCCA sensors for glucose sensing.

References

[1] National Institute of Diabetes and Digestive and Kidney Diseases. National Diabetes Statistics fact sheet: general information and national estimates on diabetes in the United States. http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm#7 (Accessed 6/14/2007 2007).

[2] World Health Organization. Fact sheet N° 312. http://www.who.int/mediacentre/factsheets/fs312/en/ (Accessed 6/14/2007 2007).

[3] American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2002, *Diabetes Care*, vol. 26, 2003, pp. 917–932.

[4] H. Shamoon, H. Duffy, N. Fleischer, S. Engel, P. Saenger, M. Strelzyn, et al., "The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes-mellitus," *N. Engl. J. Med.*, vol. 329, 1974, pp. 977–86.

[5] American Diabetes Association (ADA). Standards of Medical Care in Diabetes, *Diabetes Care.*, vol. 27 (Suppl. 1), 2004, pp. 15–35.

[6] O.S. Khalil, "Non-invasive glucose measurement technologies: an update from 1999 to the dawn of the new millennium," *Diabetes Technol. Ther.*, vol. 6, 2004, pp. 660–697.

[7] S. Garg and L. Jovanovic, "Relationship of fasting and hourly blood glucose levels to HbA1c values: safety, accuracy, and improvements in glucose profiles obtained using a 7-day continuous glucose sensor," *Diabetes Care,* vol. 29, 2006, pp. 2644–2649.

[8] D. Deis, J. Bolinder, J.-P. Riveline, T. Battelino, E. Bosi, N. Tubiana-Rufi, et al., "Improved glycemic control in poorly controlled patients with type 1 diabetes using real-time continuous glucose monitoring," *Diabetes Care,* vol. 29, 2006, pp. 2730–2732.

[9] S.F. Malin, T.L. Ruchti, T.B. Blank, S.N. Thennadil, and S.L. Monfre, "Noninvasive prediction of glucose by near-infrared diffuse reflectance spectroscopy," *Clin. Chem.*, vol. 45, 1999, pp. 1651–1658.

[10] I. Gabriely, R. Wozniak, M. Mevorach, J. Kaplan, Y. Aharon, and H. Shamoon, "Transcutaneous glucose measurement using near-infrared spectroscopy during hypoglycemia," *Diabetes Care*, vol. 22, 1999, pp. 2026–2032.

[11] X.M. Dou, Y. Yamaguchi, H. Yamamoto, H. Uenoyama, and Y. Ozaki, "Biological applications of anti-Stokes Raman spectroscopy: quantitative analysis

of glucose in plasma and serum by a highly sensitive multichannel Raman spectrometer," *Appl. Spectr.,* vol. 50, 1996, pp. 1301–1306.

[12] K. Shafer-Peltier, C. Haynes, M. Glucksberg, and R. Van Duyne, "Toward a glucose biosensor based on surface-enhanced Raman scattering," *J. Am. Chem. Soc.,* vol. 125, 2003, pp. 588–593.

[13] W.F. March, B. Rabinovitch, and R.L. Adams, "Non-invasive glucose monitoring of the aqueous-humor of the eye. Part II. Animal studies and the scleral lens," *Diabetes Care,* vol. 5, 1982, pp. 259–265.

[14] H. MacKenzie, H. Ashton, S. Spiers, Y. Shen, S. Freeborn, J. Hannigan, *et al.*, "Advances in photoacoustic noninvasive glucose testing," *Clin. Chem.,* vol. 45, 1999, pp. 1587–1595.

[15] K. Larin, M. Eledrisi, M. Motamedi, and R. Esenaliev, "Noninvasive blood glucose monitoring with optical coherence tomography - A pilot study in human subjects," *Diabetes Care,* vol. 25, 2002, pp. 2263–2267.

[16] R. Russell, M. Pishko, C. Gefrides, M. McShane, and G. Cotè, "A fluorescence-based glucose biosensor using Concanavalin A and Dextran encapsulated in a poly(ethylene glycol) hydrogel," *Anal. Chem.,* vol. 71, 1999, pp. 3126–3132.

[17] R. Badugu, J.R. Lakowicz, and C.D. Geddes, "Boronic acid fluorescent sensors for monosaccharide signaling based on the 6-methoxyquinolinium heterocyclic nucleus: progress toward noninvasive and continuous glucose monitoring," *Bioorgan. Med. Chem.,* vol. 13, 2005, pp. 113–119.

[18] K. Aslan, J.R. Lakowicz, and C.D. Geddes, "Nanogold-plasmon-resonance-based glucose sensing," *Anal. Biochem.,* vol. 330, 2004, pp. 145–155.

[19] A. Caduff, E. Hirt, Y. Feldman, Z. Ali, and L. Heinemann, "First human experiments with a novel non-invasive, non-optical continuous glucose monitoring system," *Biosens. Bioelectron.*, vol. 19, 2003, pp. 209–217.

[20] V.L. Alexeev, A.C. Sharma, A.V. Goponenko, S. Das, I.K. Lednev, C.S. Wilcox, D.N. Finegold, and S.A. Asher, "High ionic strength glucose sensing photonic crystal," *Anal. Chem.,* vol. 75, 2003, pp. 2316–2323.

[21] M. Ben-Moshe, V.L. Alexeev, and S.A. Asher, "Fast responsive crystalline colloidal array photonic crystal glucose sensors," *Anal. Chem.*, vol. 78, 2006, pp. 5149–5157.

[22] V.L. Alexeev, A.V. Goponenko, A. Sharma, I.K. Lednev, C.S. Wilcox, D.N. Finegold, and S.A. Asher, "Photonic crystal carbohydrate sensors: low ionic strength sugar sensing," *J. Am. Chem. Soc.*, vol. 125, 2003, pp. 3322–3329.

[23] D. Michail, P. Vancea, and N. Zolog, "Lacrimal elimination of glucose in diabetic patients," *C.R. Soc. Biol.Paris*, vol. 125, 1937, pp. 194–195.

[24] V.L. Alexeev, S. Das, D.N. Finegold, and S.A. Asher, "Photonic crystal glucose-sensing material for noninvasive monitoring of glucose in tear fluid," *Clin. Chem.*, vol. 50, 2004, pp. 2353–2360.

[25] R. Badugu, J.R. Lakowicz, and C.D. Geddes, "Ophthalmic glucose monitoring using disposable contact lenses," *J. Fluores.,* vol.14, 2004, pp. 617–633.

[26] W.F. March, A. Mueller, and P. Herbrechtsmeier, "Clinical trial of a noninvasive contact lens glucose sensor," *Diabetes Technol. Ther.,* vol. 6, 2004, pp. 782–789.

[27] A. Domschke, W.F. March, S. Kabilan, and C. Lowe, "Initial clinical testing of a holographic non-invasive contact lens glucose sensor," *Diabetes Technol. Ther.,* vol. 8, 2006, pp. 89–93.

[28] J.T. Baca, D.N. Finegold, and S.A. Asher, "Tear glucose analysis for the noninvasive detection and monitoring of diabetes." *Ocul. Surf.*, vol. 5, 2007, pp. 280–293.

[29] A.J. Bron, J.M. Tiffany, S.M. Gouveia, N. Yokoi, and L.W. Voon, "Functional aspects of the tear film lipid layer," *Exp. Eye Res.,* vol. 78, 2004, pp. 347–360.

[30] P.J. Driver and M.A. Lemp, "Meibomian gland dysfunction," *Surv. Ophthalmol.,* vol. 40, 1996, pp. 343–367.

[31] J.P. Gilbard, "The scientific context and basis of the pharmacologic management of dry eyes," *Ophthalmol. Clin. N. Am.,* vol.18, 2005, pp. 475–484.

[32] P.E. King-Smith, B.A. Fink, R.M. Hill, K.W. Koelling, and J.M. Tiffany, "The thickness of the tear film," *Curr. Eye Res.,* vol. 29, 2004, pp. 357–368.

[33] S. Mishima, A.Gasset, and S. Klyce, "Determination of tear volume and tear flow," *Invest. Ophthalmol.,* vol. 5, 1966, pp. 264–276.

[34] Y. Ohashi, M. Dogru, and K. Tsubota, "Laboratory findings in tear fluid analysis," *Clin. Chim. Acta.*, vol. 369, 2006, pp. 17–28.

[35] P. Argueso and I.K. Gipson, "Epithelial mucins of the ocular surface: structure, biosynthesis and function," *Exp. Eye Res.,* vol. 73, 2001, pp. 281–289.

[36] D.A. Dartt, "Regulation of mucin and fluid secretion by conjunctival epithelial cells," *Prog. Retin. Eye Res.,* vol. 21, 2002, pp. 555–576.

[37] R.L. Farris, "Tear analysis incontact lens wearers," *Trans. Am. Ophthalmol. Soc.,* vol. 83, 1985, pp. 501–545.

[38] N.J. van Haeringen, "Clinical biochemistry of tears," *Surv. Ophthalmol.*, vol. 26, 1981, pp. 84–96.

[39] Y. Li, K. Kuang, B.Yerxa, Q. Wen, H. Rosskothen, and J. Fischbarg, "Rabbit conjunctival eithelium transports fluid, and P2Y22 receptor agonists stimulate Cl{-} and fluid secretion," *Am. J. Physiol. Cell Physiol.*, vol. 281, 2001, pp. C595–C602.

[40] H. Zhu and A. Chauhan, "Tear dynamics model," *Curr. Eye Res.*, vol. 32, 2007, pp. 177 – 197.

[41] A.R. Gasset, L.E. Braverman, M.C. Fleming, R.A. Arky, and B.R.Alter, "Tear glucose detection of hyperglycemia," *Am. J. Ophthalmol.*, vol. 65, 1968, pp. 414–420.

[42] B.N. Das, S. Sengupta, B.K. Das, and N.R. Goswami, "Tear glucose estimation — an alternative to blood glucose estimation," *J. Indian Med. Assoc.*, vol. 93, 1995, pp. 127–128.

[43] J.G. Lewis and P.J. Stephens, "Tear glucose in diabetes," *Br. J. Ophthalmol.*, vol. 42, 1958, pp. 754–758.

[44] N.J. van Haeringen and E. Glasius, "Collection method-dependent concentrations of some metabolites in human tear fluid, with special reference to glucose in hyperglycemic conditions," *Albrecht V Graefes Arch. Klin. Exp. Ophthal.*, vol. 202, 1977, pp. 1–7.

[45] J.D. Lane, D.M. Krumholz, R.A. Sack, and C. Morris, "Tear glucose dynamics in *diabetes mellitus*," *Curr. Eye Res.*, vol. 31, 2006, pp. 895–901.

[46] J.T. Baca, C.R. Taormina, E. Feingold, D.N. Finegold, J.J. Grabowski, and S.A. Asher, "Mass spectral determination of fasting tear glucose concentrations in nondiabetic volunteers," *Clin. Chem.*, vol. 53, 2007, pp. 1370–1372.

[47] A. Giardini and J.R.E. Roberts, "Concentration of glucose and total chloride in tears," *Br. J. Ophthalmol.*, vol. 34, 1950, pp. 737–743.

[48] A.K. Kumagai, B.J. Glasgow, and W.M. Pardridge, "GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye," *Invest. Ophthalmol. Vis. Sci.*, vol. 35, 1994, pp. 2887–2894.

[49] S.I. Harik, R.N. Kalaria, P.M. Whitney, L. Andersson, P. Lundahl, S.R. Ledbetter, and G. Perry, "Glucose transporters are andant in cells with "occluding" junctions at the blood-eye barriers," *Proc. Nat. Acad. Sci.*, vol. 87, 1990, pp. 4261–4364.

[50] L. Lu, P.S. Reinach, and W.W.Y. Kao, "Corneal epithelial wound healing," *Exp. Bio. Med.*, vol. 226, 2001, pp. 653–664.

[51] H.C. Turner, L.J. Alvarez, V.N. Bildin, and O.A. Candia, "Immunolocalization of Na-K-ATPase, Na-K-Cl and Na-glucose cotransporters in the conjunctival epithelium," *Curr. Eye Res.*, vol. 21, 2000, pp. 843–850.

[52] K.-I. Hosoya, V.H.L. Lee, and K.-J. Kim, "Roles of the conjunctiva in ocular drug delivery: a review of conjunctival transport mechanisms and their regulation," *Eur. J. Pharm. Biopharm.*, vol. 60, 2005, pp. 227–240.

[53] S. Eskandari. E.M. Wright, and D.D.F. Loo, "Kinetics of the reverse mode of the Na+/glucose cotransporter," *J. Membrane. Biol.*, vol. 204, 2005, pp. 23–32.

[54] O.A. Candia, "Electrolyte and fluid transport across corneal, conjunctival and lens epithelia," *Exp. Eye Res.*, vol. 78, 2004, pp. 527–535.

[55] K.M. Daum and R.M. Hill, "Human tear glucose," *Invest. Ophthalmol. Vis. Sci.*, vol. 22, 1982, pp. 509–514.

[56] K.M. Hamalainen, K. Kananen, S. Auriola, K. Kontturi, and A. Urtti, "Characterization of paracellular and aqueous penetration routes in cornea, conjunctiva, and sclera," *Invest Ophthalmol. Vis. Sci.,* vol. 38, 1997, pp. 627–634.

[57] The Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. "Effect of intensive therapy on the microvascular complications of type 1 *diabetes mellitus*," *J. Am. Med. Assoc.,* vol. 287, 2002, pp. 2563–2569.

[58] G.L. King and M. Brownlee, "The cellular and molecular mechanisms of diabetic complications," *Endocrinol. Metab. Clin. North Am.*, vol. 5, 1996, pp. 255–270.

[59] I. Kaiserman, N. Kaiserman, S. Nakar, and S. Vinker, "Dry eye in diabetic patients," *Am. J. Ophthalmol.*, vol. 139, 2005, pp. 498–503.

[60] M. Goebbels, "Tear secretion and tear film function in insulin dependent diabetics," *Br. J. Ophthalmol.*, vol. 84, 2000, pp. 19–21.

[61] T.R. Stolwijk, J.A. van Best, H.H.P.J. Lemkes, R.J.W. de Keizerl, and J.A. Oosterhuis, "Determination of basal tear turnover in insulin-dependent *diabetes mellitus* patients by fluorophotometry," *Int. Ophthalmol.,* vol. 15, 1991, pp. 337–382.

[62] M. Dogru, C. Katakami, and M. Inoue, "Tear function and ocular surface changes in noninsulin-dependent *diabetes mellitus*," *Ophthalmol.*, vol. 108, 2001, pp. 586–592.

[63] M.S. Gaur, G.K. Sharma, S.G. Kabra, P.K. Sharma, and L.K. Nepalia, "Tear glucose in uncontrolled and chemical diabetics," *Indian J. Ophthalmol.*, vol. 30, 1982, pp. 367–369.

[64] P.R. Chatterjee, S. De, H. Datta, S. Chatterjee, M.C. Biswas, K. Sarkar, and L.K. Mandal, "Estimation of tear glucose level and its role as a prompt indicator of blood sugar level," *J. Indian Med. Assoc.*, vol. 101, 2003, pp. 481–483.

[65] R.K. Mediratia and J.N. Rohatgi, "Glucose-estimation in tear fluid–its diagnostic significance–a preliminary study," *Indian J. Ophthalmol.*, vol. 31, 1983, pp. 635–638.

[66] B.M. Desa, M.T. Desai, and B.C. Lavingia, "Tear and blood glucose levels in diabetes mellitus," *J. Assoc. Physicians India.*, vol. 35, 1987, pp. 576–577.

[67] C.R. Taormina, J.T. Baca, S.A. Asher, J.J. Grabowski, and D.N. Finegold, "Analysis of tear glucose concentration with electrospray ionization mass spectrometry," *J. Am. Soc. Mass Spectrom.*, vol. 18, 2007, pp. 332–336.

[68] F. Ridley, "The intraocular pressure and drainage of the aqueous humor," *Exp. Path.*, vol. 11, 1930, pp. 217–240.

[69] J.M. LeBlanc, C.E. Haas, G. Vicente, and L.A. Colon, "Evaluation of lacrimal fluid as an alternative for monitoring glucose in critically ill patients," *Intensive Care Med.*, vol. 31, 2005, pp. 1442–1445.

[70] Z. Jin, R. Chen, and L.A. Colon, "Determination of glucose in submicroliter samples by CE-LIF using precolumn or on-column enzymatic reactions," *Anal. Chem.*, vol. 69, 1997, pp. 1326–1331.

[71] Y.C. Lee, "Carbohydrate analyses with high-performance anion-exchange chromatography," *J. Chromatogr. A.*, vol. 720, 1996, pp. 137–149.

[72] F. Lax, "Uber die Spiegel einiger Metaboliten in der Tranenflussigkeit," Inaugural- Dissertation, Marburg, 1969.

[73] K.M. Daum and R.M. Hill, "Human tears: glucose instabilities," *Acta. Ophthalmol.*, vol. 62, 1984, pp. 530–536.

[74] M. Reim, F. Lax, H. Lichte, and R. Turss, "Steady state levels of glucose in the different layers of the cornea, aqueous humor, blood and tears *in vivo*," *Ophthalmologica*, vol. 154, 1967, pp. 39–50.

[75] H. Kilp and B. Heisig, "Glucose and lactate concentration in tears of rabbits following mechanical stress and wearing of contact lenses," *Albrecht V Graefes Arch. Klin. Exp. Ophthal.*, vol. 193, 1975, pp. 259–267.

[76] E.J. Olajos and H. Salem, "Riot C," *J. Appl. Toxicol.*, vol. 21, 2001, pp. 355–391.

[77] S.A. Perezand and L.A. Colon, "Determination of carbohydrates as their dansylhydrazine derivatives by capillary electrophoresis with laser-induced fluorescence detection," *Electrophoresis,* vol. 17, 1996, pp. 352–358.

[78] R.J. Carlson and S.A. Asher, "Characterization of optical diffraction and crystal structure in monodisperse polystyrene colloids," *Appl. Spectrosc.*, vol. 38, 1984, pp. 297–304; P.L. Flaugh, S.E. O'Donnell, and S.A. Asher, "Development of a new optical wavelength rejection filter: demonstration of its utility in Raman spectroscopy," *Appl. Spectrosc.*, vol. 38, 1984, pp. 847–850; S.A. Asher, U.S. Patent # 4,627,689, #4,632,517 (1986); S.A. Asher, P.L. Flaugh, and G. Washinger, "Crystalline colloidal Bragg diffraction devices: the basis for a new generation of Raman instrumentation," *Spectroscopy*, vol. 1, 1986, pp. 26–31.

[79] C. Reese, C. Guerro, J. Weissman, K. Lee, and S.A. Asher, "Synthesis of highly charged, monodisperse polystyrene colloidal particles for the fabrication of photonic crystals," *J. Colloid and Interface Sci.*., vol. 232, 2000, pp. 76–80; C. Reese and S.A. Asher, "Emulsifier-free emulsion polymerization produces highly charged, monodisperse particles for near infrared photonic crystals," *J. Colloid Interface Sci.*, vol. 248, 2002, pp. 41–46.

[80] J.C. Zahorchak, R. Kesavamoorthy, and R.D. Coalson, "Melting of colloidal crystals: a Monte Carlo study," *J. Chem. Phys.*, vol. 96, 1992, pp. 6873–6879.

[81] P.L. Flaugh, S.E. O'Donnell, and S.A. Asher, "Development of a new optical wavelength rejection filter: demonstration of its utility in Raman spectroscopy," *Appl. Spectr.*, vol. 38, 1984, pp. 847–850.

[82] S.A. Asher, J. Holtz, L. Liu, and Z. Wu, "Self assembly motif for creating submicron periodic materials. Polymerized crystalline colloidal arrays," *J. Am. Chem. Soc.*, vol. 116, 1994, pp. 4997–4998; S.A. Asher and S. Jagannathan, U.S. Patent # 5,281,370 (1994); G. Haacke, H. P. Panzer, L.G. Magliocco, and S.A. Asher, U.S. Patents # 5,266,238 (1993), #5,368,781 (1994).

[83] J.H. Holtz and S.A. Asher, "Polymerized colloidal crystal hydrogel films as intelligent chemical sensing materials," *Nature,* vol. 389, 1997, pp. 829–832; J.H. Holtz, J.S.W. Holtz, C.H. Munro, and S.A. Asher, "Intelligent polymerized crystalline colloidal arrays: novel chemical sensor materials," *Anal. Chem.*, vol. 70, 1998, pp. 780–791.