Design and Fabrication of Disposable, Percutaneous Chemical Sensors

Kuo-Chih Liao[1,2], Thieo Hogen-Esch[3,4], Frances J. Richmond[1], Laura Marcu[2], Gerald E. Loeb[1,2]
[1]Alfred E. Mann Institute for Biomedical Engineering, [2]Dept. of Biomedical Engineering,
[3]Dept. of Chemistry, [4]Loker Hydrocarbon Research Institute,
University of Southern California, CA, USA

Abstract— **Sencils™ (*sen*sory *cil*ia) are chemical sensors that are minimally invasive, disposable and easily readable to make frequent measurements of various analytes *in vivo* over a period of 1-3 months. A percutaneous optical fiber permits precise, reliable photonic measurement of chemical reactions in a nano-engineered polymer matrix attached to the internal end of the fiber. The first Sencils sense interstitial glucose based on measurement of fluorescence resonance energy transfer (FRET) between fluorophors bound to dextran and Concanavalin-A in a polyethylene glycol (PEG) matrix. In vitro experiments demonstrate a rapid and precise relationship between the ratio of the two fluorescent emissions and concentration of glucose in saline over the physiological range of 0-500mg/dl. Chronic implantation in pigs has demonstrated biocompatibility. The Sencil platform can be adapted to detect other analytes in interstitial fluids.**

Keywords— **Optical fiber, chemical sensor, fluorescence resonance energy transfer, affinity-binding assay, glucose, Quantum dots**

1 The Potential of Chemical Sensing in vivo

Although biosensors have been used in clinical application for decades, most of the commercialized products are intended for *in vitro* assays of samples expelled or extracted from body (urine, blood, tissue etc.). These sensors have significant disadvantages for monitoring fluctuating physiological processes because it is impractical to obtain these samples more than once or twice a day.

In order to manage certain diseases and conditions, it is important to make frequent measurements of specific analytes over an extended period of time. These include diabetes, cancer chemotherapy treatment, and hormonal monitoring for fertility and complications of pregnancy. Continuous *in vivo* monitoring could reduce the complications of diabetes from hyperglycemia and hypoglycemia (insulin overdosage). Frequent monitoring could increase the safety and effectiveness of chemotherapy by measuring interstitial drug levels and detecting apoptosis of tumor cells *in situ* without sampling through biopsy.

The technology can also benefit basic research in molecular biology, drug discovery and veterinary medicine. For example, some metabolites and secondary messengers in signaling transduction pathways can only be traced directly through *in vivo* detection (Harper et al.). Tracking such activity without requiring biopsies or sacrificing experimental animals would expedite such research (Wiley et al.). Sencils can be implanted precisely in solid tumors and other tissues to provide highly localized information.

2 Challenges of chemical sensing *in vivo*

Over the past twenty years, there have been many research projects to develop minimally invasive chemical sensors but relatively few products have been approved for clinical use. The requirements discussed below tend to compete with each other, making it difficult to develop and validate strategies that achieve useful tradeoffs among them.

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2.1 Critical sensitivity and selectivity requirement

The chemical sensor involves two steps in the biosensing process: recognition of a specific analyte and transduction into a signal that provides information about the concentration of the analyte over its physiological range. Most analytes of clinical interest are present in low concentrations (micromolar to nanomolar) and are mixed with a huge number of other biochemicals whose concentrations may vary widely and unpredictably. Other physiological variables can affect transduction. For example the catalytic activity of glucose oxidase, the most common transduction molecule in commercial glucose sensors, deteriorates over time and is sensitive to pH, oxygen tension and temperature (Usmani et al.). So the sensor must recognize the analyte efficiently and selectively, and convert the weak interaction between the analyte and the transduction material into a signal with sufficient accuracy and precision to provide clinically useful information about the analyte independent of concurrent pathological conditions (e.g. fever, hypoxia, acidosis, etc.).

One desirable approach is to use transcutaneous optical interrogation of a systemic or locally implanted chemical sensor. Variability of tissue properties over time and between subjects (such as pigment, water content and thickness of skin, or scattering and re-absorption of photons in the eye) make it difficult to achieve reliable and accurate measurement. Absorption spectroscopy has been shown to be useful in monitoring glucose, glutamate, ammonia, lactic acid *in vitro*, and has been explored to detect glucose *in vivo* (Chung et al., McShane and Cote). It detects glucose by measuring IR absorptions due to low energy electronic vibration (700-1000nm) and bond stretching of –OH and –CH (1000-2500nm). The absorption band is sensitive to temperature and hydrogen bond effects. The lack of repeatability of the signal in vivo reflects the signal coupling difference within and between patients. "The primary known drawbacks to taking this technology from an in vitro to an in vivo device include the path length variability when going from a fixed test cell to a pliable tissue; temperature variability when going from a controlled, incubator, environment to peripheral site such as finger or earlobe; other chemical substance (protein, cholesterol, alcohol and urea etc.) that have overlapping spectra and may vary in ways that can not be accommodated by calibration; and the pulsatile nature of blood effects both the path length and the concentration of chemicals."(Cote) Polarimetry measurement of glucose is another technique with complex problems when used in vivo. Glucose can change the polarity of light (rotate light in the right-handed direction with concentration). In addition to the concentration of the chiral material, the amount of rotation of linear vector of the polarized light also depends on the thickness of the layer traversed by the light, the wavelength of light used for measurement, the temperature and pH of the solvent. Because the device must be able to measure millidegree rotations, researchers have focused on detection in the clear fluids of the eye rather than the highly scattering tissues of the skin (McNicols & Cote). Photon energy must be kept low to prevent injury, but that significantly reduces the signal-to-noise ratio. The confounding rotation due to corneal birefringence and the variation in this rotation with eye motion pose significant problems for this technology.

2.2 Perturbation of the analyte by the sensor

Rather than detecting the analyte directly, it may be useful to amplify the strength and selectivity of the signal by means of a specific chemical reaction.. Enzymatic transducers tend to have a high selectivity for their substrates but they consume the substrate irreversibly, creating local concentration gradients that depend on the perfusion of the tissue rather than the systemic concentration of the analyte (McNichols & Cote). The implanted component of the sensor may induce a foreign body reaction that changes the local concentration of the analyte or that blocks diffusion of the analyte to the transduction point.

2.3 Strict biocompatibility and chemical/mechanical integrity requirement for chronic implantation

As with any implanted device, biocompatibility can be divided into effects of the sensor on the tissue and effects of the tissue on the sensor. Because of the intimate contact required between the chemical transducer and body fluids, even reactions that are nominally benign may cause serious degradation of

sensor performance. For example, encapsulation by connective tissue may reduce diffusion of analyte to the sensor surface. Smaller implants tend to provoke lesser foreign body reactions, but their small volume may make them more susceptible to damage by leaching or enzymatic degradation of their critical components (Park & Lakes, and Fraser).

Despite progress in microminiature technologies, conventional implantable devices (mm to cm) remain much larger than cells and blood capillaries (10-100μm). For example, needle-type glucose sensors are small enough to avoid use of anesthetic for self-implantation but their insertion into the subcutaneous tissue creates locally severe damage to cells and capillaries. The more severe the damage, the more intense is the self –defense:

- Immediately upon contact with extracellular fluid (especially blood), proteins and other chemicals will adsorb onto and diffuse into the sensor, which may block protective or analyte-selective membranes.
- Change in the conformation of native proteins adsorbed onto sensor surfaces may provoke the activation of complement system or elements of the immune response.
- The acute inflammation from the implantation trauma will recruit white blood cells over the next four days, releasing hydrogen peroxide, superoxide radicals, and lytic enzymes that may damage the sensor.
- A fibrous capsule will be formed progressively during postimplant days 4-12, potentially blocking diffusion of the analyte. The capsule may have low or fluctuating concentrations of oxygen, glucose and other metabolites depending on the ratio of metabolic activity of the capsular cells to the circulation provided by the interrupted and reforming capillary bed.

In the worst case, chronic inflammation occurs around the sensor implantation site. It triggers the release of free radical relative materials from the self-defense system and destroys the chronic mechanical integrity of well known biocompatible materials, such as polyurethane (widely used as semi-permeable membrane on implantable device) (Labow et al.).

2.4 Wearable and disposable systems are preferred for chronic patient use.

The major goal of treatment with any medical device is to restore as normal a quality of life as possible. The sensor system should be physically small and unobtrusive but it should also be mechanically robust and require infrequent and simple service or replacement.

Total cost over the lifetime of the patient needs to be minimized, including both replacement costs for disposable and portable components, and professional services for implantation and calibration. We can consider the economics of two competing new technologies for frequent glucose measurements, both based on glucose peroxidase assays. The GlucoWatch costs $872 for the biographer worn on the wrist, $169 for autosensor with a $ 9.38 replaceable chemical patch installed, which can support detection up to 13 hours. The percutaneous C.G.M.S system from Medtonic MiniMed costs $1475 for the external reader and $29.50 for the disposable sensor, with enzyme coated on a hypodermic needle and implanted near abdominal region up to 72 hours. Data analysis software packages are available for the two products and cost in the range from $100 to $300 (www.diabetespromotion.com).

Researchers working on optical spectroscopy methods typically use relatively bulky and expensive bench-top machines such as infrared grating spectroscopy instruments costing $6000-$10000 dollars. For any photonic assay to be used in a practical clinical tool, the size, complexity and cost of the detection instrument will need to be greatly reduced.

3 Sencil platform for *in vivo* sensing

We are developing a platform technology that will support a family of disposable, minimally invasive, *in vivo* sensors that can measure various analytes in a patient over a period of 1-3 months. The key element is a chronically implanted optical fiber that provides a stable and reliable percutaneous port by

which to interrogate the chemical assay. We call this a Sencil, for "*sen*sory *cil*ium". Whenever a measurement is desired, the external end of the fiber will be attached to a portable photonic analyzer by means of a connector that accepts the free end of the fiber. Each Sencil will measure one analyte by fluorescence emission. The biosensing element is a nanoengineered matrix attached to the internal end of the optical fiber (Figure 1). The analyzer sends excitation light through the fiber to reach the biosensor matrix and receives returning fluorescent emissions from the biosensing element through the same fiber.

4 First application: glucose sensing

The first embodiment of our fiber-optic biosensor is a glucose sensor. It is estimated that diabetes mellitus afflicts nearly 16 million people in the United States and over 100 million people worldwide (National Diabetes Clearinghouse). Diabetes is a metabolic disorder in which cells fail to take up glucose either due to a lack of insulin (Type I) or due to insensitivity to insulin (Type II). Insulin is required in order for cells to take up glucose from the blood, and in diabetics, a defect in insulin signaling can give rise to large fluctuations in blood glucose levels. It is now well-established that many of the complications of diabetes that lead to substantial morbidity and mortality are consequences of these fluctuations, including blindness, kidney failure, retinopathy, nephropathy, neuropathy, and heart disease (Davidson).

The incidence of such complications can be reduced substantially by close regulation of blood glucose near the normal range (Diabetes Control and Complications Trial, by The National Institute for Diabetes and Digestive and Kidney Disorders). Such close regulation requires frequent and accurate measurement of glucose levels because hypoglycemia from insulin overdosage can lead to unconsciousness, permanent

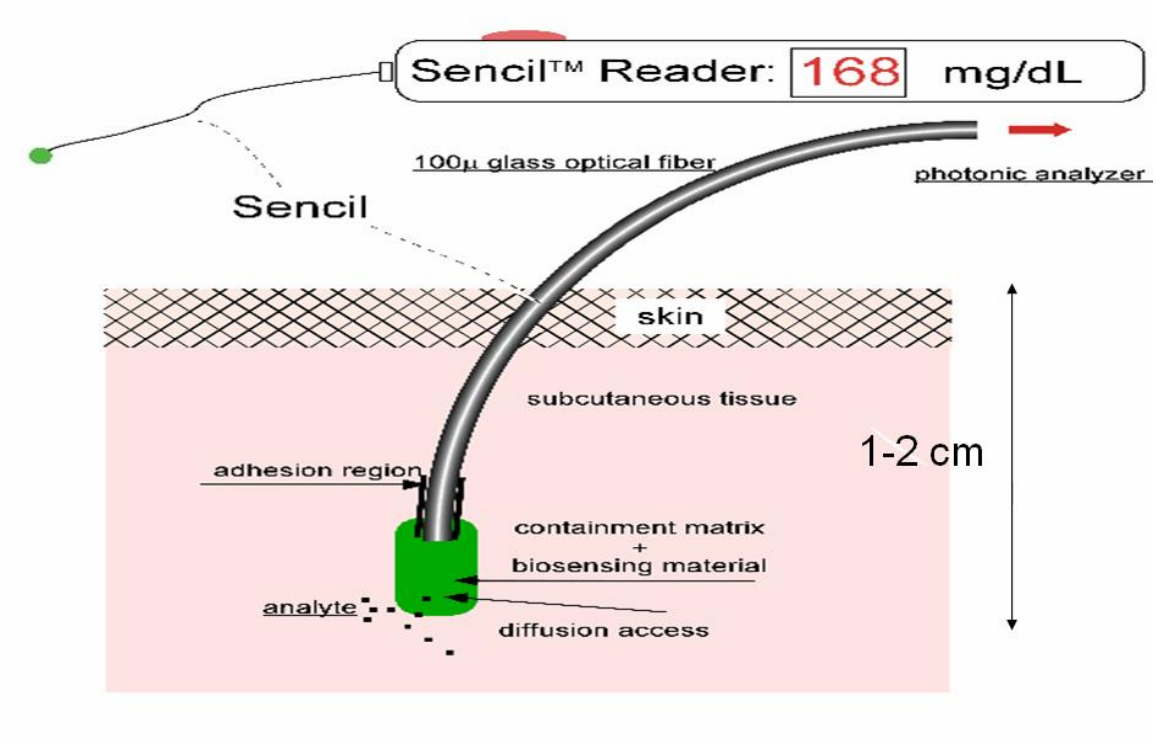

Figure 1: Illustration of biosensor and its implantation position relative to a patient's skin surface.

brain injury and death. Insulin therapy becomes particularly difficult to manage during periods of unusual stress such as pregnancy, trauma and surgery.

Currently, glucose monitoring is usually achieved through invasive means by *in vitro* glucose meters. Typically, glucose measurements require pricking a finger and extracting a drop of blood, which is then applied to a test strip composed of chemicals sensitive to the glucose in the blood sample. An optical meter provides a numerical glucose reading.

5 Requirements and design strategy

Each of the elements of a Sencil (see Figure 1) must be engineered to meet specific requirements for a clinical sensor that is safe and effective.

5.1 Selectivity for glucose (Figure 2)

Our biosensor uses the much-studied affinity-binding assay for polysaccharides based on the jack bean lectin Concanavalin A (Con A) (Meadows & Shultz). Succinylated ConA exists as a dimer (Gunther et al) in neutral environment; each monomer has a potential N-terminal binding site where a fluorophore can be attached and a nearby (2nm away) recognition site for saccharides such as glucose and dextran (or betacyclodextrin) (Hardmen & Ainsworth). Dextran (or betacyclodextrin) binds to Con A but can be displaced by glucose.

Con A is conjugated with fluorescein isothiocyanate (FITC), which fluoresces at 520nm when excited at 470nm. Dextran (or betacyclodextrin) is conjugated with tetramethylrhodamine isothiocyante (TRITC), which fluoresces at 580nm when excited at 520nm (the emission of FITC). The affinity between Con A and dextran (or betacyclodextrin) brings the dye-labeled fluorophores close to each other so that the emission of FITC can be absorbed efficiently by TRITC. The fluorescence emission quenching (of FITC quenched by TRITC) phenomenon is called fluorescence resonance energy transfer, FRET (Figure 2A); the distance between them at which half of the FITC emission is so absorbed is called the Főrster radius. Increasing concentrations of glucose reduces the FRET by displacing the FITC-Dextran and restores the emission intensity of FITC (Figure 2B).

Förster (or fluorescence) resonance energy transfer (FRET) between a fluorescence donor molecule bound to the target and an acceptor attached to a receptor protein has been used widely in sensing studies (Lakowicz, Hermanson). The affinity between receptor and analyte provides the required selectivity to overcome interference from other ambient molecules. The concentration of the analyte is inferred from the ratio of the two fluorescent emissions rather than the absolute intensity of either, making the assay relatively insensitive to variability of the photonic coupling or deterioration of the sensing materials.

5.2 Sensitivity for glucose

Meadows and Schultz coupled Con A (MW 102kD) to TRITC and purchased FITC-dextran (MW 2000kD) from Sigma for use in subcutaneously implantable pellets that were to be interrogated by transcutaneous fluorescence measurements. The combination of the fluorophores in aqueous solution can detect glucose concentrations up to 1600mg/dL (88 mM). When we attempted to replicate these reactions and to adapt the system for the very small volumes and efficient percutaneous photonic coupling of the Sencil matrix, we found it necessary to reengineer the assay method. This effort has given us an armamentarium of materials and methodologies that are more suited to the particular advantages of the Sencil probe geometry for both glucose and other analytes. We identified and addressed successfully the following limiting factors:

High concentrations of the fluorophores are desirable to produce strong fluorescence signals but the concentration of the two fluorophores should be limited below the range where FRET from random-proximity dominates the affinity-triggered FRET.

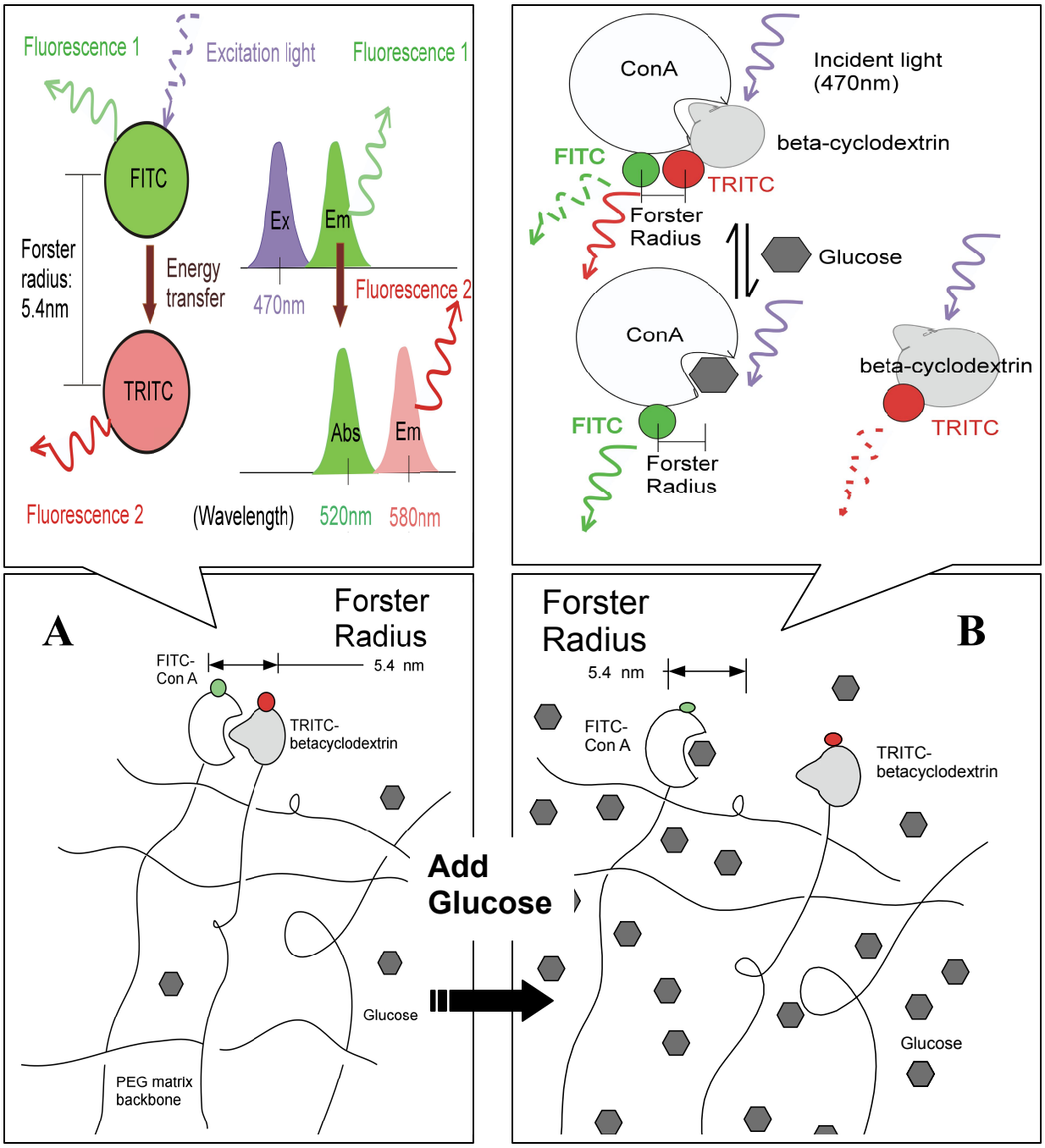

Figure 2: Apply FRET in sensing glucose. Both fluoropjores are covalently immobilized on the backbone of PEG matrix. The backbone of the matrix can wave like the seaweed and preserve the mobility requirement for the FRET phenomenon (A) with low glucose (B) with more glucose to replace betacyclodextrin from the binding site.

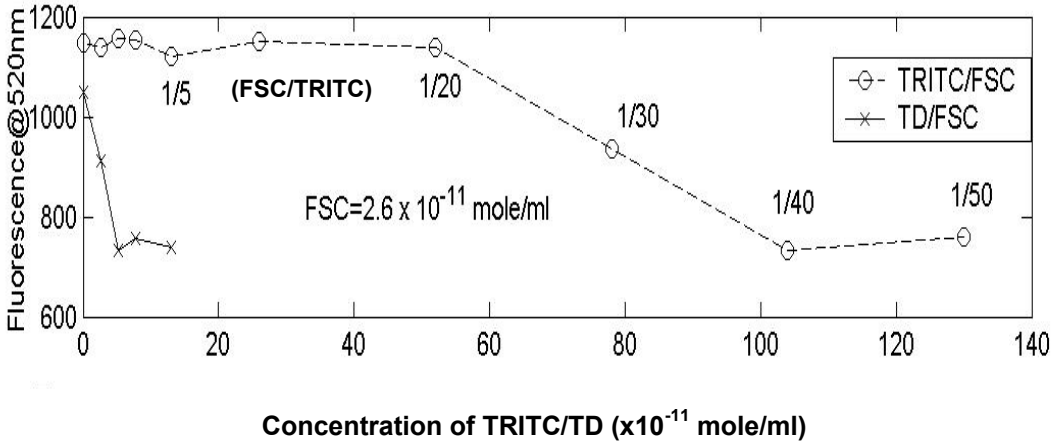

Figure 3: Comparison of fluorescence quenching effect from TRITC/FSC (random proximity) and TD/FSC (affinity-binding), when [FSC] = 2.6 x 10^{-11} mole/ml.

The concentration of fluorophores has a major influence on the average distance between the two fluorophores in aqueous solution. In binding-affinity model, FRET quenching should be triggered solely by affinity, which condition obtains only at concentrations of both labeled receptor and labeled ligand that are low enough to minimize random proximity.

To test this hypothesis, we obtained the following commercially available materials:

- FITC-succinyl-ConA with 2:1 molar ratio (L-9385, Sigma Chemicals)
- TRITC-dextran (155kD, ~861 glucose monomers) with 2:1 molar ratio (T-1287, Sigma Chemicals)

- TRITC (T-5654, Sigma Chemicals)

In the preliminary result (figure 3), the TRITC/FSC curve demonstrates the FSC emission quenching from the random proximity (with free TRITC dye and FSC in aqueous solution). It indicates that when the molar ratio of TRITC:FITC is larger >20, the average distance between the two random and uniformly distributed fluorophores is smaller than the distance required to trigger efficient FRET quenching, resulting in detectable emission reduction. The TD/FSC curve demonstrates the FRET quenching resulted from affinity between dextran and Con A. So the molar ratio of TD: FSC should be <20 to avoid interference from random proximity (when the concentration of FSC is fixed at 2.6x10^{-11} mole/ml but see below).

A large dextran molecule can bind to ConA in a spatial orientation for which the distance between the fluorophores precludes FRET, if the dye-labeled ratio is not high enough.

The fluorophore is randomly conjugated on the dextran (the other fluorophore is conjugated on the N-terminal of Con A, which is 2nm away from the saccharide recognition site), and Con A recognizes dextran through an essential oligosaccharide sequence (core), which favors the formation of hydrogen bond or van der Waal force with the surrounding functional region on the peptide backbone of Con A binding site (Bryce et al.). For dextran of a given size, the higher the labeling ratio (mole of fluorophore per mole of dextran), the better is the FRET efficiency. Similarly, most of the fluorophores attached to a large dextran molecule would be distant from an attached ConA and could not contribute to FRET efficiently.

To test this hypothesis, we obtained the following commercially available materials:

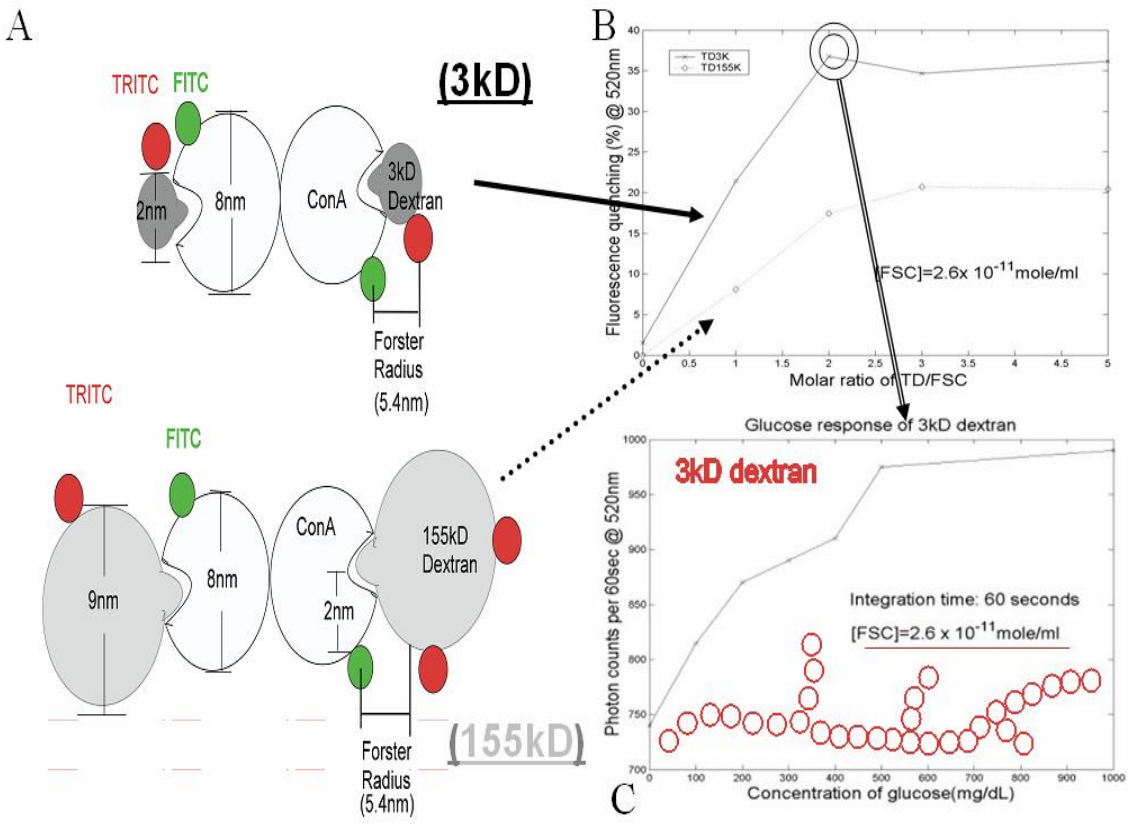

Figure 4: Effects of molecule size on the FRET quenching efficiency. The quenching (%) is define as the (fluorescence of FSC-fluorescence of FSC quenched by TD) / fluorescence of FSC.

- FITC-succinyl-ConA with 2:1 molar ratio (L-9385, Sigma Chemicals)
- TRITC-dextran (155kD, ~861 glucose monomers) with 2:1 molar ratio (T-1287, Sigma Chemicals)
- TRITC-dextran (3kD, ~22 glucose monomers) with 1:1 molar ratio (D-3307, Molecular Probes)

When adding more labeled dextran (TD 3kD or 155kD) to labeled ConA (FSC), the fluorescence quenching (of FITC emission) increases until the quenching effect saturates (see figure 4B). As predicted, the amount of quenching achievable for the large molecular weight dextran (155kD) is less than for the smaller dextran (3kD) (figure 4A and 4B) (20% vs. 35%). In order to maximize the sensitivity of the quenching to displacement by glucose, we picked the molar ratio 2:1 (double-headed arrow in figure 4B). We then added increasing concentrations of glucose across the physiological range (figure 4C below). As predicted, the quenching effect is extinguished by displacement of the dextran from the Con A, restoring the fluorescence intensity of the FITC, which is bound to the Con A.

High concentrations of the fluorescent reactants produce strong signals but they may require unphysiologically high concentrations of glucose to displace the dextran from the Con A. This can be overcome by selecting a dextran with a lower affinity for Con A.

Rather than using the limited set of commercially available fluorophore-labeled reagents, we selected betacyclodextrin (instead of dextran). Its rigid circular structure reduces the affinity between this saccharide and ConA, permitting higher concentrations of the fluorescent analytes to be used while preserving sensitivity to physiological concentrations of glucose. We used the isothiocyanate reaction to conjugate TRITC to betacyclodextrin. Betacyclodextrin is dissolved in methyl sulphoxide containing a few drops of pyridine. TRITC is added, followed by dibutyltin dilaurate, and the mixture is heated for 2 hr at 95C. The TRITC-betacyclodextrin is dried by reduced pressure evaporation to remove solvent (De Belder). The residue is dissolved in PBS (phosphate buffer saline). Dialysis and fluorescence measurements on the dialysate are used to separate unbound fluorophor and quantify the stoichiometry of the bound fluorophor.

By using betacyclodextrin, the fluorescence signal is increased about 60 fold (900 photon counts/60s for 3kD dextran vs. 1950 photon counts/2s for betacyclodextrin, when concentration of glucose is 300mg/dL).

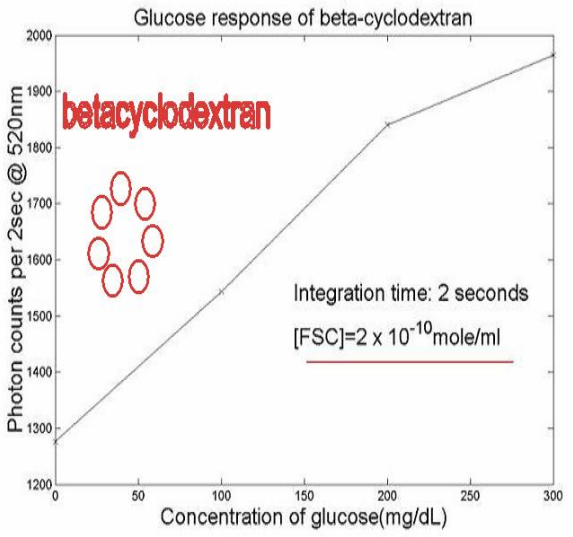

Figure 5: Performance of TRITC-betacyclodextrin/FSC.

5.3 Materials and fabrication for the Sencil implants

As mentioned in section 3, the fiber-optic biosensor resembles the configuration of chronically implanted artificial hair used for cosmetic purposes. Such hairs consist of filaments of synthetic polymer that can be injected into the scalp, where they form an epithelial interface that is stable for months at least (Yamada). Likewise, our biosensor is injectable underneath the skin into a well-vascularized subcutaneous space such as the scalp. A single optical fiber makes up the "shaft" of the hair; the sensing matrix, based on polyethylene glycol (PEG) hydrogel, serves as the "follicle".

Mechanical Properties

Photonic technology is still undergoing rapid evolution, particularly with regard to miniaturization and mass production, which are critical for biomedical applications such as the one proposed herein. Optical fibers are small, thin, lightweight, chemically stable, generally biocompatible, immune to electromagnetic interference and inherently electrically isolated, all desirable properties for medical devices. A multimode silica fiber with 100µm core size and 110µm cladding was selected for this application. The small diameter (about twice that of a human hair) offers the potential for use as a continuous monitoring device by its efficient photocoupling ability, easy insertion and minimal irritation to the surrounding tissue. The silica fibers can bear more than 500g tensile force, the point at which our clamps of the force gauge tended to slip. The 100µ fibers could be bent repeatedly to a 5mm radius with 36±6g peak force detected by force gauge.

Instead of attaching a hollow tube sealed with a membrane onto the end of the optical fiber (Meadows & Schultz), a roughly droplet-shaped hydrogel made of polyethylene glycol (PEG) is polymerized onto the end of the fiber (Fig. 6). The follicle-shaped droplet is formed by shining UV light through the fiber into the precursor solution, which photopolymerizes into the desired shape according to the absorption and dispersion of the light from the end of the fiber. The polymer serves to increase the surface area, raising

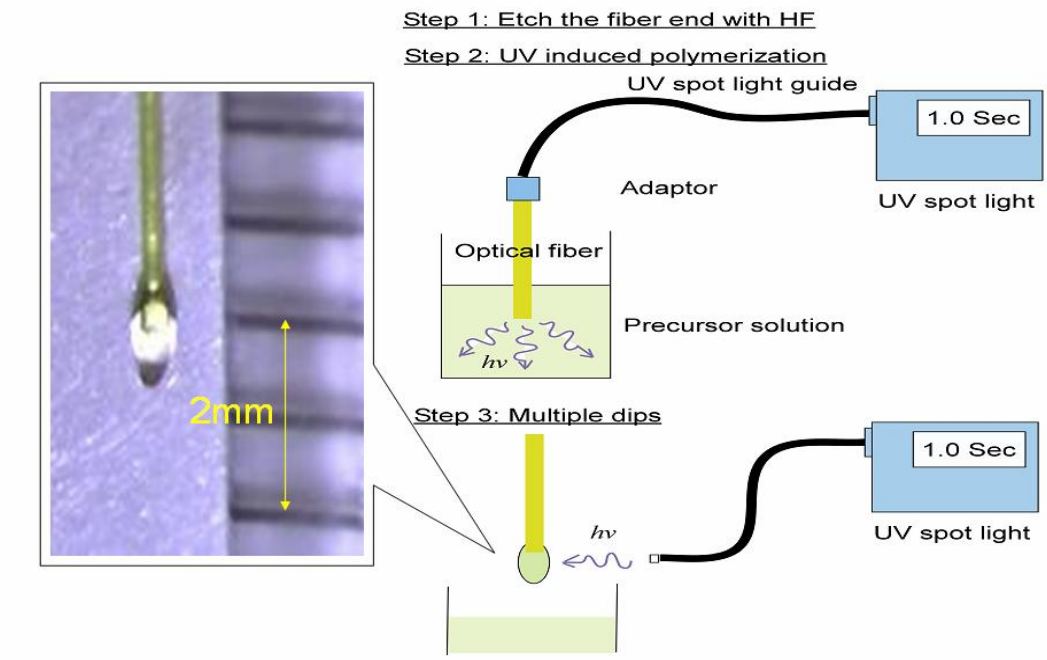

Figure 6: Manufacture method with adhesion enhancement. Step 1: Increase surface roughness by HF etching. Step 2: Dip etched fiber into precursor solution and guide UV light through fiber to induce polymerization. Step 3: Multiple dips and UV polymerization to increase interface area.

the intensity of fluorescence emission, but adds little to the size of the device (the follicle is about 200µm diameter by 500µm length). The pore size and hydrophicility of the matrix can be modified by using larger molecular weight PEG monomer and mixing different composition of copolymer to facilitate the diffusion of the analyte. A similar configuration was used to improve the response time of a pH sensor by using a polymer instead of a membrane barrier to control the leaching of sensing materials (Munkholm & Walt).

Safety of the PEGs has been studied and reported for over fifty years: protein absorption studies have shown that fibrinogen absorption was significantly reduced after coating polymers either linear or branched PEG (Bergström et al.); photo-crosslinked PEG copolymers induced a much less fibrous encapsulation after subcutaneous implantation in rat (Quinn et al.). The lack of toxicity is reflected in the fact that PEG is one of the few synthetic polymers approved for internal use by the FDA, appearing in food, cosmetics, personal care products and pharmaceuticals.

It is likely that very small quantities of PEG (approximately 10µg) and concanavalin A ($<10^{-12}$g/sencil; the lethal dose in mice is 50mg/Kg by intravenous injection) will be left behind subcutaneously each time a percutaneous fiber is removed. We believe and intend to demonstrate that this PEG will gradually biodegrade without producing adverse or cumulative effects.

Our first preclinical biocompatibility testing (section 6), indicated that adhesion between the containment matrix and the optical fiber is required to prevent these two components from physically separating prematurely due to small amounts of relative motion expected between the percutaneous fiber and the subcutaneous tissues. Much of the fixation in the body may arise from encapsulation of the follicle portion rather than the smooth glass shank, so it would be desirable for the adhesion between the matrix and the fiber to be similar to the withdrawal force for a hair. In order to investigate the adhesion strength between the sensing matrix and fiber end, a peak-force meter was clamped to the free end of the Sencil.

The sensing matrix bulb was pulled until it separated from the fiber. We measured a peak tension force of only 2-10g for untreated 100 μm fibers. Etching the silica fiber in hydrofluoric acid improved the adhesion of the PEG matrix without altering its optical characteristic, depending on time and concentration. The limiting factor of the adhesion appears to be the surface area of the fiber actually in contact with the matrix, which tends to be small for the 100μ fibers. Repeat dipping in the PEG matrix precursor followed by UV-polymerization builds up a thicker coat in the meniscus between fiber and matrix, particularly when the fiber surface has been etched with HF. Combining 10 min etch in 25% HF with 10 dip-and-cure cycles in PEG produced 77g adhesion to a 100μ fiber; this represents the high end of the range of extraction force for a human scalp hair.

Matrix Chemistry

The cohesive strength and biodegradability of the containment matrix are both related to the molecular weight and cross-linking of the PEG. We used the method described in Russel et al. – UV-induced polymerization of unhydrated liquid polyethylene glycol- diacrylate (PEG-DA 575, Sigma) followed by hydration in phosphate buffer saline (PBS). In order to immobilize Con A, a trace amount of α-acryloyl, ω-N-hydroxysuccinimidyl ester of poly (ethylene glycol)-propionic acid, MW 3400 (PEG-NHS 3400, Shearwater Polymer Inc.) was added into the precursor solution (1/200 mass ratio to PEG-DA) and incubated with Con A for 30 minutes. The NHS ester of PEG-NHS reacts with lysine residue on the surface of Con A to form a covalent bond. Another acrylate end-group of PEG-NHS crosslinks with PEG-DA to form the hydrogel matrix during UV-induced polymerization. Dextran was physically immobilized inside the crosslinked hydrogel by using short chain PEG-DA oligomers (MW 575) for polymerization.

The precursor solution of the polymer matrix was prepared with 1 mL PEG-DA, 5 mg PEG-NHS, and 0.5 mL of fluorophore solution in PBS at maximum solubility concentrations of 5 mg/mL FITC-Succinyl Con A (MW 56K Dalton, Sigma Aldrich), and 9 mg/mL TRITC-betacyclodextrin (MW 1K Dalton, Sigma Aldrich). After vortexing for 30 minutes, 100 μL of trimethylolpropane triacrylate (TPT) and 10 mg of 2,2-dimethoxy-2-phenylacetophenone (DMPA) were added to the solution, and vortexed for another 30 minutes. This is the precursor solution that is dip-coated and photopolymerized onto the end of the optical fiber.

5.4 Design considerations for a portable reader

In order to deploy the Sencil system, the bulky and mechanically sensitive laboratory instrumentation must be miniaturized into a robust and simple portable device that can be operated easily by patients. The prototype Sencils that we are testing now have two multimode glass fibers embedded in a single sensing matrix, providing separate paths for the excitation light from the source and for the fluorescence to the photometer. Clinical Sencils could be built in this manner, but this would complicate the manufacturing process and user handling of the proximal ends, where the two optical fibers would need to be inserted into the reader. We are now experimenting with a fiber-fiber fused tapped coupler (OZ Optics LTD to mate the excitation source and detection paths with a single Sencil fiber. This can be mated efficiently with a single multimode fiber of the Sencil via a tapered entry port equipped with a flushing port to expel dust that may interfere with coupling.

The major challenge of the Sencil photonic analyzer design is to filter out the scattering of the excitation source (now 470nm), which is 100dB higher in intensity (at its source) than the two fluorescence emissions to be detected (520nm, 575nm). We are examining various fixed-wavelength photonic filter technologies.

Acoustic-optical tunable technology (Fabry-Perot filter; Birk) is attractive because a single reader could be used with multiple Sencil types. Micron Optics Inc., has advised us that they can provide such a filter with narrow bandwidth (~2nm) whose passband is steered by a voltage-controlled oscillator over the 400-600nm visible spectrum. The filter uses a single-mode fiber (8μ core/125μ o.d.), so a lens coupler

(designed by RPC Photonics Inc.) is required to transfer efficiently the light energy to the filter from the multiple mode fiber of the Sencil and the tapped coupler. The detector at the output of the filter will use a MEMS integrated miniature connector and photodiode, signal processing and power system available as a chipset from Xponent Photonics Inc., called a Fiber-Ready Optical Assembly (FROA). Fabry-Perot filters are not particularly accurate in their ability to specify absolute filter wavelength, so a closed-loop control circuit will allow the filter to be fine-tuned successively to each of the two fluorescence peaks.

6 In vivo stability and biocompatibility (without adhesion enhancement)

Initial chronic experiments in rats were unsuccessful because the animals removed the implants when grooming. We implanted 7 pigs (2-week survival) with Sencils (2 fibers/animal), and 10 pigs (4-week survival) with Sencils (4 fibers/animal), all in the region above the shoulder blades. The 2-week animal

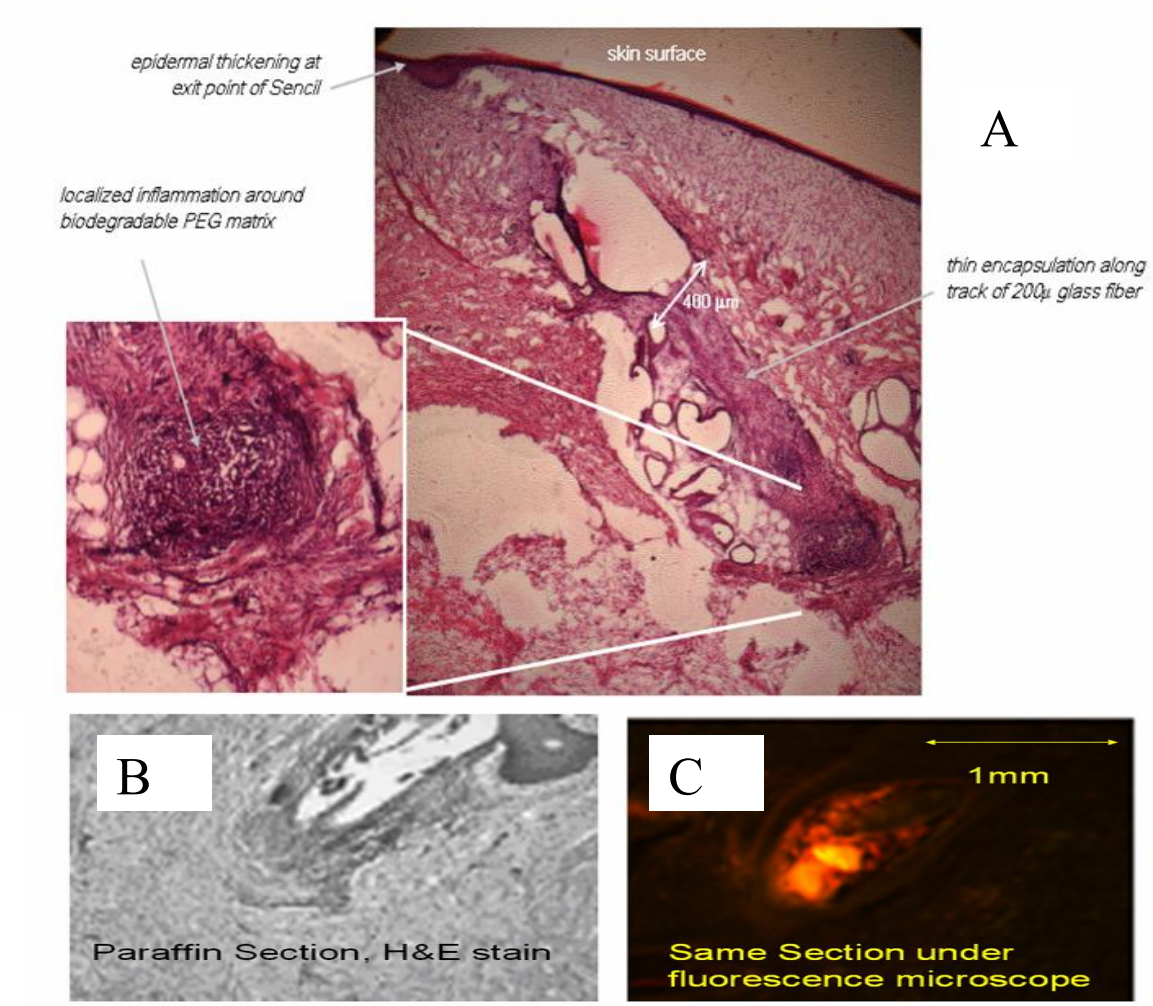

Figure 7: Histology of the track of a Sencil (A) frozen section, H&E stain at 2 weeks (B) paraffin section, H&E stain at 4 weeks (C) same section in B under fluorescence microscope.

group had only 4 sensors remaining at euthanasia; the 4-week animal group had 15 sensors remaining at euthanasia. The few surviving fibers were useful to assess biocompatibility, but extraction forces were lower than required for chronic use in freely behaving animals (8-12g).

To investigate biocompatibility, each animal was sacrificed and the tissue around the remaining Sencils was harvested for conventional histology (hematoxylin & eosin staining of frozen sections and paraffin sections). There was a thin tissue encapsulation along the fiber track with epidermal thickening at the exit point. Minimal localized inflammation around the biodegradable PEG matrix was also observed. The thickness of fibrous capsulation does not have a significant increase from week 2 to week 4 (Figure 7).

7 In vitro performance of the glucose sensor

The in vitro performance of the prototype sensor has been evaluated by measurements in glucose solutions with different concentrations. Figure 8 presents the corresponding spectra of glucose solutions. The fluorescence peak for the conjugated FITC-Con A-PEG shifts to 530nm, and the truncated peak to the left is from the back scattering of the shorter wavelength excitation light. Normally, the Sencil output would be the ratio of the two fluorescence peak, but they are affected by the skirt of the back-scatter. So the results presented below show only the absolute photon-count of the FITC fluorescence at 530nm.

Fluorescence response time (Figure 9) of the device to an increase in glucose is about 5-15 minutes. (Compared to 10-12 minutes response time in Russel's research, with 2mm diameter PEG bead.). Our sensing matrix is about 4 times smaller in diameter (500μm) and should respond more rapidly. We suspect that the covalent immobilization restricts the mobility of Con A and dextran, so the response time is longer than expected. Fluorescence response time to a decrease in glucose is about 15-25 minutes. The reason is that outward diffusion of glucose is further slowed by binding to Con A.

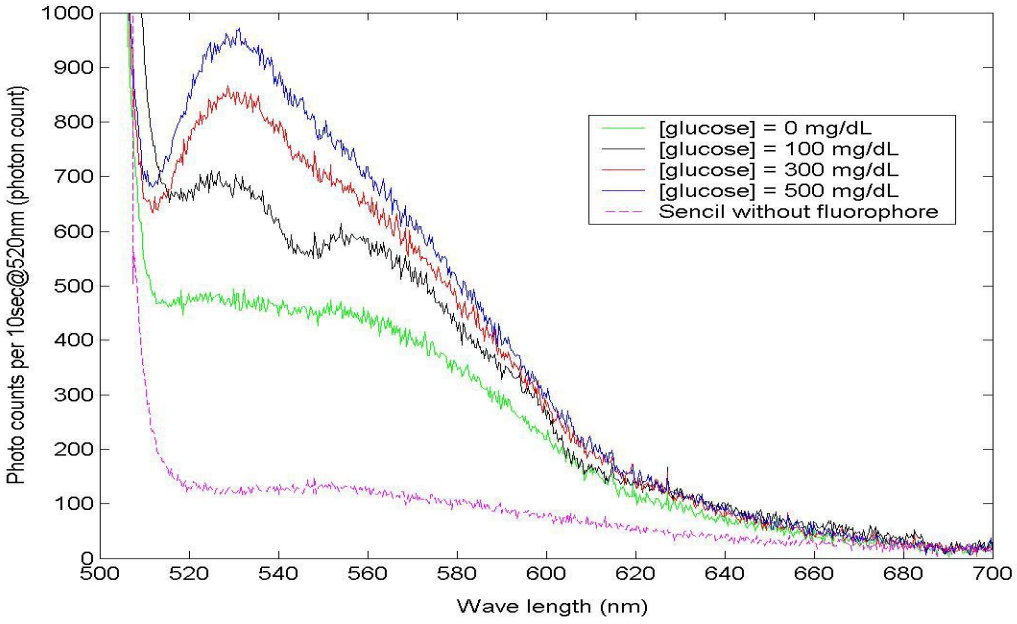

Figure 8: Spectrums of Sencil emission under different glucose concentrations. The pink dash curve is the spectrum of control group (Sencil without fluorophores).

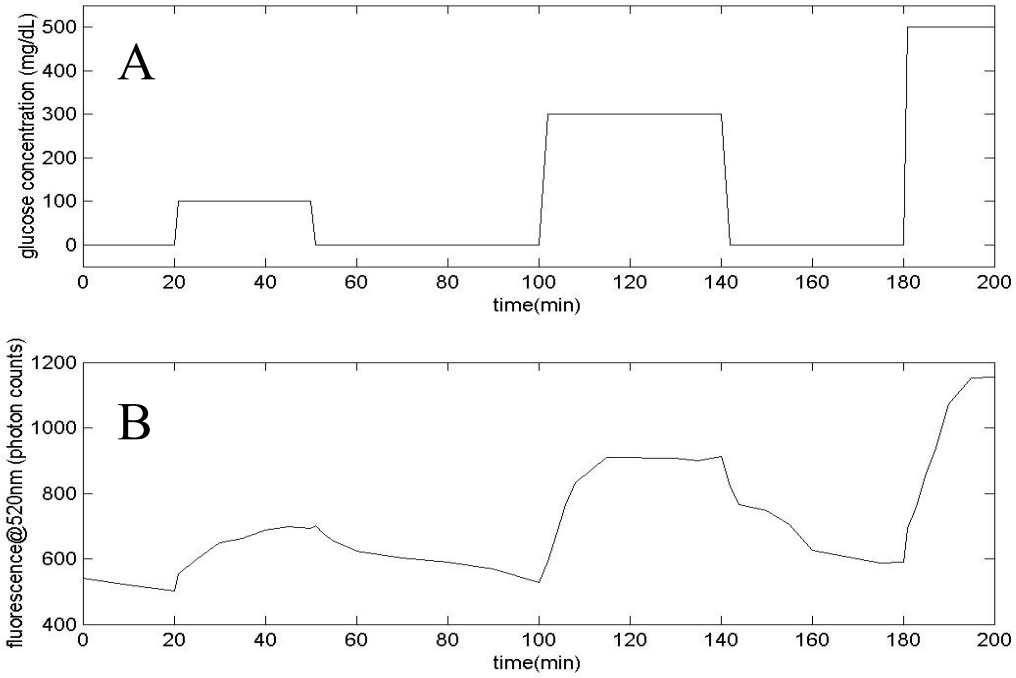

Figure 9: Dynamic of Sencil. (A) The curve indicates the duration and concentration of glucose concentration where the Sencil is placed. In the beginning, Sencil was placed in PBS, and then it was placed in 100mg/dL glucose solution from 20th min to 50th min, in PBS again from 50th min to 100 min, in 300mg/dL glucose solution from 100th to 142nd min and so on. (B) The curve demonstrates the response of the sensor by using fluorescence intensity at 520nm as index.

The PEG polymer derivative from our formula is rather rigid instead of the jelly-like appearance of many hydrogels; the dry material swells in saline much less than 10%. This suggests a relatively low hydrophilicity . We improved this by mixing the precursor with poly(acrylic acid), which is used as a water absorbent in hygiene products such as diapers. The abundant carboxylic groups on the polymer chain increase its hydrophilicity (Elliott et al.). Its swelling ratio is around 10-15, depending on the temperature, pH, and ionic strength in solution. The synthesis of poly(acrylic acid) resembles the radical polymerization of PEG, which is initiated by UV light. The mixture of PEG and poly(acrylic acid) will form a copolymer. When we mixed the hydrogel with 30% poly(acrylic acid), the response time of the Sencil was reduced by 50%.

In order to test the stability of the device, the Sencils were separated into three groups and stored in de-ionized water, PBS and 100mg/dL glucose solution (dissolved in PBS). The spectrum pattern and fluorescence intensity were stable (similar to figure 8 and 9) for the first 8 weeks, no matter which method was used to preserve the Sencil. The fluorescence decreased (about 10-20% at 530nm) at the 9th week for all three aqueous storage conditions. The correlation is shown in figure 10. Both of them still represent almost linearly positive correlation. The response time is stable no matter the decrease of the fluorescence emission at the 9th week.

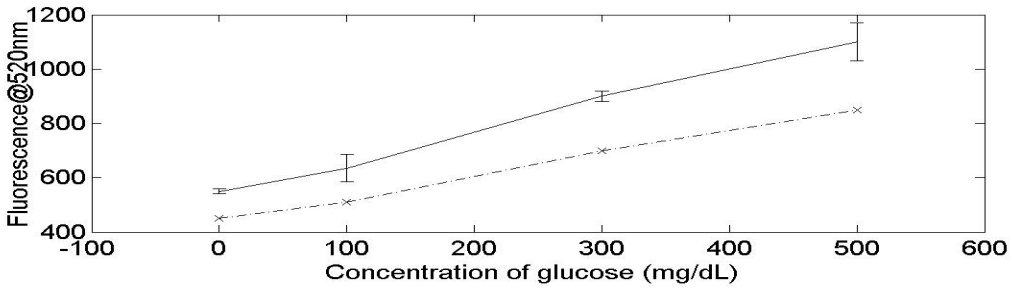

Figure 10: Correlation between (A) emission of FSC and glucose concentration (B) emission ratio of FSC/TD and glucose concentration. The solid curve represents the average values detected every other week for the fist 7 weeks. The dash line represents the detected value at 9th week.

8 Discussion

The materials and fabrication methods to produce Sencil implants appear to be robust and inexpensive. The physical and chemical properties of the polymer matrix can be modified to alter its hydrophilicity, crosslinking density, and pore size for specific application in detecting analytes other than glucose. Systematic nano-engineering of reactant binding affinities, fluorophor attachment, reactant entrapment and matrix structure provides the key to a wide range of clinical applications. We now have a variety of chemical tools with which to optimize systematically the nanostructure of the sensing matrix. Methods to improve adhesion between the matrix and the optical fiber are promising but remain to be demonstrated *in vivo* The photonic integrity and chemical stability of the sensing matrix also need to be determined by serial photonic measurements over the design requirement of 1-3 months *in vivo*.

We have demonstrated the necessary capabilities to test Sencils in the laboratory with the dual fiber Sencil, but the real design challenge for the detection instrument is to separate the two relatively weak fluorescence signals from the strong excitation in a single fiber Sencil. It should be possible to produce stronger fluorescence and more readily detectable FRET in this and other assays by using Quantum Dot® (Butkus, 2004) technology for one (but not both) of the fluorophores. Because the Qdot can be excited by much shorter wavelengths than traditional fluorescence photon donors, this will simplify the optical splitter and filter requirements.

We plan to conjugate biotinylated Con A with the Qdot 525 Streptavidin through the covalent bonding between Streptavidina and biotin. The Qdot labeled Con A will be paired with the nano-engineered TRITC-betacyclodextrin. A broadband UV light source around 400nm can be used as the excitation light source, providing a 120nm difference between the excitation and the emission of the Qdot photon donor (compared to 50nm difference between FITC absorption and emission wavelengths).

The Qdot-based glucose sensor is now being tested for stability of FRET over the physiological range of glucose concentration *in vitro.* The next stages of research will involve chronically implanting single fiber Sencils in animals to demonstrate mechanical stability and allow comparison of measured interstitial glucose concentrations with simultaneous blood glucose measurements with laboratory equipment. If the technology appears promising for glucose, we will explore the performance limitations of photonic instruments suitable for miniaturization into a handheld, battery-powered detector.

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