

Single Walled Carbon Nanotubes as Reporters for the Optical Detection of Glucose

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Abstract

This article reviews current efforts to make glucose sensors based on the inherent optical properties of single walled carbon nanotubes. The advantages of single walled carbon nanotubes over traditional organic and nanoparticle fluorophores for *in vivo*-sensing applications are discussed. Two recent glucose sensors made by our group are described, with the first being an enzyme-based glucose sensor that couples a reaction mediator, which quenches nanotube fluorescence, on the surface of the nanotube with the reaction of the enzyme. The second sensor is based on competitive equilibrium binding between dextran-coated nanotubes and concanavalin A. The biocompatibility of a model sensor is examined using the chicken embryo chorioallantoic membrane as a tissue model. The advantages of measuring glucose concentration directly, like most optical sensors, versus measuring the flux in glucose concentration, like most electrochemical sensors, is discussed.

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Introduction

For the 194 million people afflicted with diabetes worldwide,¹ the key issue is meeting blood glucose and glycated hemoglobin targets.²⁻⁴ Meeting targets in blood glucose is largely dependent on sensor technology. A reliable and simple-to-use sensor allowing continuous monitoring would impact both patient behavior and glucose control. Next-generation sensor technology is focused on continuous, *in vivo* analyte detection.⁵⁻⁷ Current glucose sensors that are Food and Drug Administration approved for use in humans are electrochemical in nature⁸ and take the form of devices that work via a transdermal electrode that require frequent replacement every few

days to models in development utilizing telemetry, which require some form of surgical implantation.⁹ Sensors based on optical signal transmission instead can either be minimally invasive, implanted fluorescence-based devices or involve completely noninvasive spectroscopic detection techniques. Fluorescence-based devices often utilize fluorescence resonance energy transfer between fluorophores¹⁰⁻¹³ or some type of competitive binding.^{14,15} While noninvasive spectroscopic techniques would be the most desirable, they currently lack analyte specificity and dynamic range,⁷ as detailed in reviews by Moschou *et al.*,⁵ Wickramasinghe *et al.*,⁶ and Wilson *et al.*⁸

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Abbreviations: (CAM) chorioallantoic membrane, (Con A) concanavalin A, (GOx) glucose oxidase, (MW) molecular weight, (NIR) near-infrared, (PEG) polyethylene glycol, (SWNT) single walled carbon nanotubes

Keywords: carbon nanotube, glucose sensor, fluorescence

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Several photonics applications have been proposed for glucose sensing.^{16,17} The required optical components for Raman-based schemes are extremely expensive.^{16,17} A low-resolution fluorescence method would be more desirable because current instrumentation is already affordable and amenable to microfabrication technologies.¹⁸ Additionally, a small fluorescence-based device could readily be implanted beneath the skin without requiring surgical procedures. **Figure 1** shows a schematic of a sensor based on nanotube fluorescence and the detection of nanotube fluorescence through the tissue of a rat.

Why Single Walled Carbon Nanotubes (SWNT)

Single walled carbon nanotubes are cylindrical tubes of graphite with diameters on the order of 1 nm and lengths up to several millimeters.^{19,20} Their nanometer scale dimensions give rise to a host of interesting phenomena. Depending on how the graphite sheet is rolled, SWNT can be either semiconducting or metallic.^{19,21} While SWNT have been used in a wide number of applications, from composites to transistors, they have received a large amount of interest in regards to sensing. Because of their one-dimensional nature and the fact that all atoms are surface atoms, SWNT are especially sensitive to surface adsorption events. Metallic SWNT have been used to enhance the performance of electrochemical sensors for the detection of a number of analytes ranging from DNA²² to glucose.²³ Semiconducting SWNT have been used as field effect transistors for the detection of a wide variety of biomolecules; however, there is still some controversy about the ability of nanotubes to transduce specific biomolecular events. The discovery of SWNT near-infrared (nIR) fluorescence in 2002²⁴ opened a whole new avenue for sensing. Changing how the graphite sheet making up the nanotubes is rolled affects the optical properties. Smaller diameter nanotubes will have larger band gaps and thus higher energy absorption and fluorescence than those of tubes with larger diameters, with nanotube fluorescence ranging from 900 to 1600 nm.^{24,25}

In the case of optical sensors, the maximum emitted signal detected from an implanted sensor is dependent on the fluorophore quantum yield^{26–29} and the absorption of both excitation and emission light within the tissue.³⁰ Both of these factors together limit the fluorescent probe that can be used for this purpose. **Figure 2A** plots the absorbance of deoxygenated and oxygenated human whole blood, the dominant absorber in the human body, as a function of wavelength. Furthermore, photobleaching, which is a property of all organic³¹ and nanoparticle

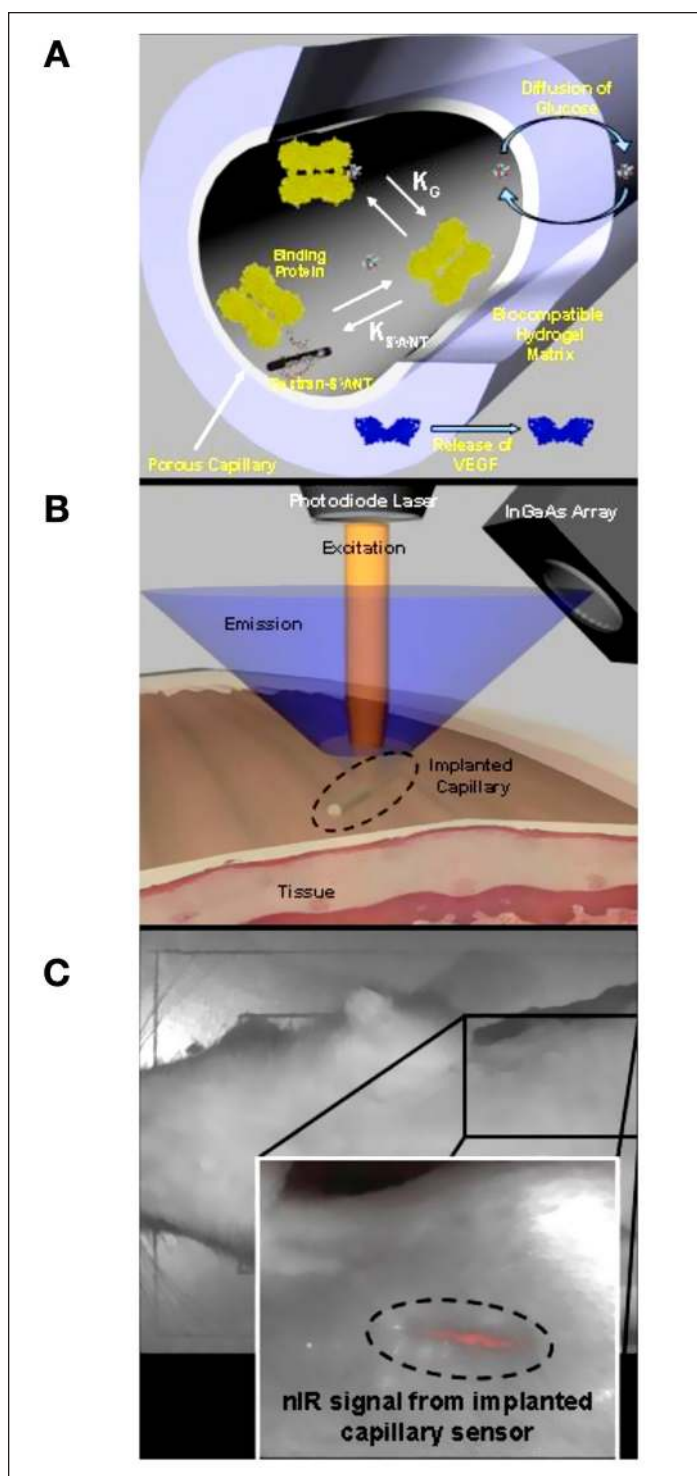


Figure 1. Implantable glucose sensor based on SWNT nIR fluorescence. **(A)** A porous dialysis capillary contains fluorescent glycosylated SWNT that shift their emission wavelength based on adsorption of a glucose-binding macromolecule. Free glucose diffuses into the capillary and replaces the bound entity, creating a reversible response. A porous, hydrogel allows slow release of angiogenic and anti-inflammatory factors to minimize fibrous encapsulation and biofouling. **(B)** The implanted device can be queried passively through the skin using near infrared light. **(C)** Image of SWNT-filled capillary embedded under rat skin acquired with the CRI Maestro *in vivo* imaging system with excitation from 649 to 690 nm, emission from 800 to 950 nm, a 5-second exposure, and 10 accumulations.

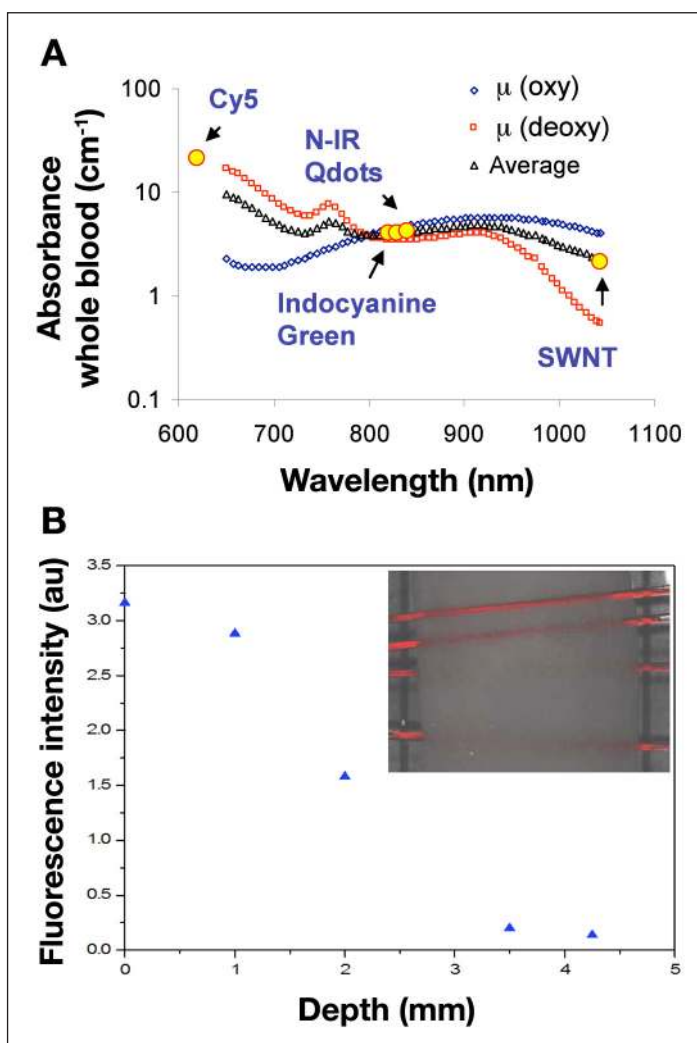


Figure 2. (A) Absorption coefficient of human whole blood (the dominant absorber for *in vivo* applications) for oxygenated, deoxygenated, and the average value. Potential fluorophores are compared. (B) Depth profile of a SWNT test sensor platform through chicken breast tissue. Measurement was made using the Maestro *in vivo* imaging system with excitation from 649 to 690 nm, emission from 800 to 950 nm, a 5-second exposure, and 10 accumulations. a.u., arbitrary unit. (Inset) nIR image of the SWNT-filled capillary proxy through chicken. The chicken breast is false colored in white, and the SWNT fluorescence is false colored in red.

fluorophores³² investigated to date, except for carbon nanotubes,³³ will limit the viable sensor lifetime.

For an optical sensor-implanted distance, d , into tissue with absorption coefficient, μ , and fluorophore quantum yield, ϕ , a one-dimensional, absorption and fluorescence model can be used to estimate the signal intensity and lifetime of the device:

$$I_s = I_0 \phi e^{-2\mu d - k\tau}, \quad (1)$$

where I_0 is incident excitation intensity, I_s is fluorescent optical signal outside of the body, k is pseudo-first-

order photobleaching rate constant, and τ is aggregate excitation exposure lifetime.

To maximize the signal detected from a sensor implanted a distance d into tissue, the fluorophore properties should be such that $\phi e^{-2\mu d}$ is maximized. Table 1 shows this value calculated for common organic and nanoparticle fluorophores. It is obvious that visible fluorophores are significantly attenuated in a tissue sample and are not appropriate for implantable devices. Attenuation of visible fluorophore emission offsets the advantage of their higher quantum yield when compared with near-infrared dyes. Additionally, this analysis discounts interference from tissue autofluorescence, which disproportionately suppresses signals in the visible. Figures 1C and 2B demonstrate the efficacy of measuring SWNT fluorescence through tissue. We should point out that the nanotube samples were not optimized for *in vivo* imaging.

The lifetime of any optical device is dependent on the stability of the fluorophore. For devices operating in a clinical setting, a maximum error of 20% can be tolerated for an *in vivo* glucose sensor before it is no longer viable.⁹ For an optical sensor, the resulting limit for the working lifetime (τ) of the device is

$$\tau = -\frac{\ln(0.8)}{k}. \quad (2)$$

Table 2 uses photobleaching rate constants taken from literature data to estimate sensor lifetimes for some common visible and nanoparticle fluorophores. Organic fluorophores photobleach rapidly and are impractical for long-term applications. Even quantum dots, which possess bleaching rate constants several orders of magnitude lower than those of visible fluorophores, exhibit observable photobleaching.³² Single walled carbon nanotubes are the only optical probe investigated to date that possesses no photobleaching threshold.^{33,34}

Failure Mechanisms of Implantable Sensors

Klueh and co-workers^{35,36} showed that placing cells that were genetically engineered to overexpress vascular endothelial cell growth factor induced significant neovascularization surrounding an implanted amperometric sensor for acetaminophen and later for glucose. The cells were placed in a hydrogel matrix, and the system was tested in an *ex ova* chicken embryo model. Results demonstrate that engineering neovascularization around the implant site can reduce undesired encapsulation effects dramatically.

Table 1.
Comparison of Commonly Utilized Visible and Near-Infrared Organic and Nanoparticle Fluorescent Probes Based on Quantum Yield (QY) and Absorbance in Human Whole Blood, (μ), the Dominant Absorbed in the Human Body

QY standards	QY (%)	Conditions for QY measurement	Excitation (nm)	μ (oxy) (cm^{-1})	μ (deoxy) (cm^{-1})	$\phi e^{-2\mu d}^a$
Cy5 ²⁶	27	PBS ^b	620	2	60	3.20×10^{-28}
Fluorescein ²⁸	95	0.1 M NaOH, 22°C	496	150	120	5.23×10^{-118}
Rhodamine 6G ²⁹	95	Water	488	200	105	3.30×10^{-133}
Rhodamine B ²⁹	31	Water	514	110	190	1.60×10^{-131}
Indocyanine Green ²⁷	0.266	Water (0.15 g/liter)	820	0.96	0.77	4.72×10^{-4}
Indocyanine Green ²⁷	1.14	Blood (0.08 g/liter)	830	1.01	0.7788	1.91×10^{-3}
Type II NIR QD ³²	13	PBS	840	1.05	0.778	2.09×10^{-2}
SWNT ²⁵	0.1	PBS	1042	0.889	0.12	3.65×10^{-4}

^a Based on a 1-cm-implanted sensor device, an average μ is utilized.

^b Phosphate-buffered saline.

Table 2.
Comparison of Photobleaching Tendency of Common Organic and Nanoparticle Fluorophores^a

Fluorescent probe	Photobleaching rate constant (h^{-1})	Fluence (mW/cm^2)	Nominal sensor lifetime
IR-Dye 78-CA ³²	250.92	600	3.2 s
Cy5 ³²	20.52	600	39.1 s
Indocyanine Green ³¹	0.0412	28	5.4 h
Type II NIR QD ³²	0.0827	600	2.7 h
SWNT ³³	0	1.0×10^6	α

^a Estimated optical sensor device lifetime is calculated from Equation (2).

Norton and co-workers³⁷ have proposed several antifouling coatings to circumvent these common failure mechanisms. The hydrogels serve multiple functions, including an overall resistance to cell adhesion and as a matrix to deliver anti-inflammation and angiogenic agents. Several different configurations were examined, and it was found that delivery of these agents through hydrogel decomposition was superior to embedded microspheres as delivery vehicles. Hydrogels³⁸ themselves are excellent implant coatings due to biocompatibility and overall resistance to biofouling.^{37,39}

While significant literature points to the clear advantage of this approach (combined hydrogel delivery of anti-inflammation and neovascularization factors),^{35–37,39,40} it has

not been conclusively demonstrated to work with an optical sensor device.

Concerns about Nanotube Toxicity

The toxicity of single walled carbon nanotubes is still in the early stages of exploration. While it has been shown that nanotubes implanted in the abdominal cavity of mice show a similar immunological response to asbestos, this toxicity was size dependent with a nanotube bundle length greater than 20 μm necessary for a response.⁴¹ Several researchers, including our group,⁴² have examined the cytotoxicity of SWNT in various eukaryotic cells. The majority of investigations have been concerned with drug or gene delivery where SWNT, suspended by a biomolecule, were tagged with a fluorophore for observation.^{43–45} Two of these studies^{43,44} used covalently functionalized SWNT, which exhibit a greatly reduced Raman and absorption cross section⁴⁶ and quenched photoluminescence.²⁴ One study³⁴ used the band gap fluorescence of surfactant-suspended SWNT to assess the toxicity of nanotubes in macrophages. The study showed nanotube fluorescence in cells only up to 24 hours after incubation, and the mechanism of intake was only applicable to cells that undergo phagocytosis. A larger study⁴² has demonstrated no cytotoxicity at a high concentration (100 mg/ml) after a 3-month time period.⁴² Additionally, nanotubes injected into rabbits demonstrated no toxic side effects over 24 hours⁴⁷ as did nanotubes injected into mice for a 3-month period.⁴⁸ There is even evidence of nanotube excretion once inside the body.⁴⁹

Nanotube-Based Glucose Sensor

Enzyme-Based Optical Glucose Sensor

The central challenge in the fabrication of SWNT-based optical sensors is designing a sensor that maintains the nanotube optical properties while providing a selective analyte-binding site. Covalent functionalization necessarily disrupts the electronic structure of the nanotube, and thus its optical properties.⁵⁰ Therefore, only noncovalent methods may be used. The first example of glucose detection using SWNT fluorescence was demonstrated with a glucose oxidase (GOx)-SWNT optical sensor using a recently developed dialysis method to assemble functional proteins onto solution-suspended SWNT.⁵¹ A target protein is combined with SWNT dispersed with a meta-stable surfactant and is dialyzed against surfactant-free buffer. Van der Waals forces alone can immobilize the protein monolayer, as demonstrated for glucose oxidase (Figure 3). Figure 3A shows nanotube fluorescence during the assembly process. The 10-meV shift in the emission maximum (Figure 3A, inset) confirms that the tightly packed surfactant phase has been replaced by an enzyme layer. Images show heights of 4.4 nm along the 1-nm-diameter nanotube consistent with an adsorbed dimer (Figure 3B).

Once the glucose oxidase is assembled, an electroactive species such as potassium ferricyanide, $K_3Fe(CN)_6$, can irreversibly adsorb at the surface of the nanotube and

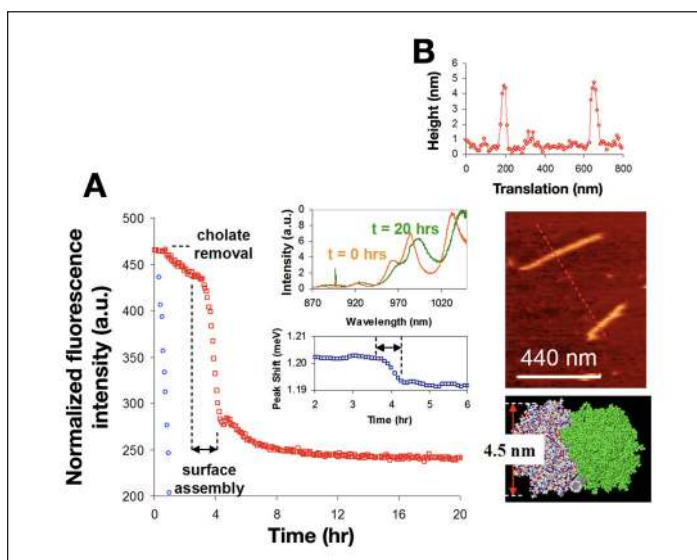


Figure 3. (A) Dialysis in the presence of glucose oxidase yields protein-stabilized nanotubes as monitored optically using transient emission. In the absence of enzyme (blue), the system aggregates rapidly. Assembly can be tracked using the 10-meV shift in wavelength (inset). a.u., arbitrary unit. (B) Atomic force microscope images of enzyme-suspended nanotubes on mica reveal average heights of 4.4 nm that correspond to a monolayer of dimers.

shift the Fermi level into the valence bands or quench the emission after photoexcitation.⁵¹ This functionality can be partially reduced, as glucose oxidase catalyzes the reaction of β -D-glucose to the D-glucono-1,5-lactone with a H_2O_2 coproduct. The partial reduction of potassium ferricyanide at 37°C and pH 7.4 can reversibly couple the carbon nanotube fluorescence to the glucose concentration.⁵¹ The solution can be loaded into a sealed dialysis capillary where glucose is free to diffuse across the capillary boundary while the nanotube-sensing complex is retained (Figure 4). This capillary is imaged easily in the nIR through a human epidermal tissue sample. The fluorescence of one nanotube is shown to respond to glucose after an 80-second transient, even in a

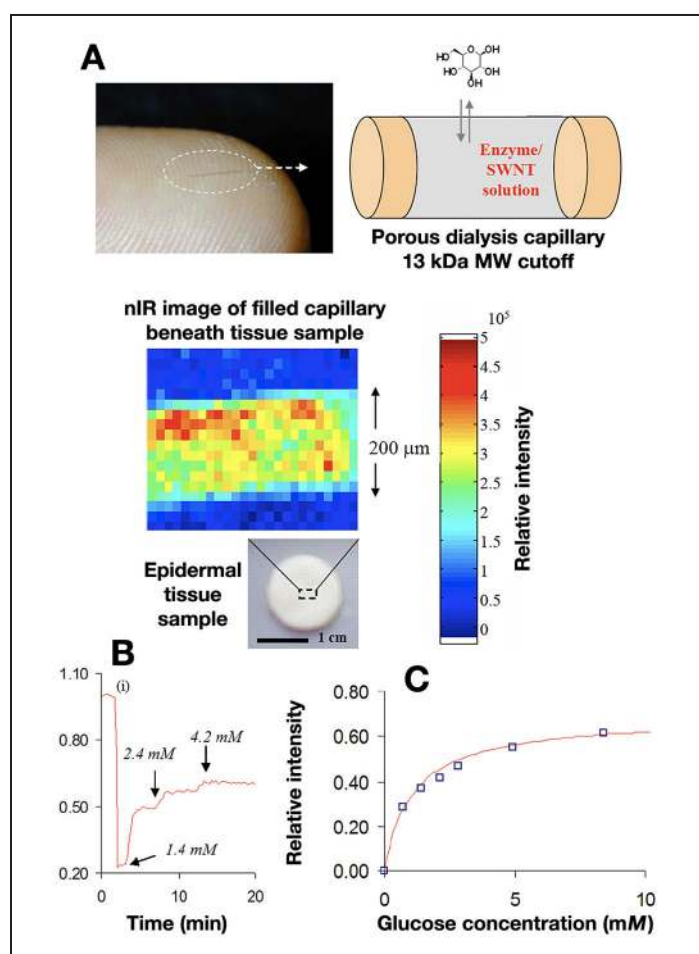


Figure 4. (A) A $200 \mu\text{m} \times 1 \text{ cm}$, 13-kDa microdialysis capillary, shown to scale on a human finger, is loaded with the nanotube solution allowing glucose to diffuse through the membrane with containment of the sensing medium. Placing the capillary beneath a human epidermal tissue sample (above), we can clearly map the nanotube fluorescence from the capillary, seen in the two-dimensional profile. (B) After ferricyanide surface reaction at 37°C and pH buffered at 7.4 (i), the fluorescence response to the addition of 1.4, 2.4, and 4.2 mM glucose is scaled by the difference between minimum and maximum intensities. (C) The response function relates the normalized intensity to the local glucose concentration in the range of blood glucose detection with a type I absorption isotherm.

whole blood specimen maintained at 37°C and pH 7.4. The detection limit is approximately 2.2 molecules per nanometer of nanotube length.

Protein Affinity-Based Optical Glucose Sensor

The prototype described in **Figures 3** and **4** is based on GOx and is limited by cofactor regeneration. Additionally, the GOx–SWNT sensor operates in a flux-based manner, with the same disadvantages of flux-based sensors discussed later. A more robust scheme would be both reversible and not require regeneration. As such, the next generation in SWNT sensor design is focused on utilizing protein competitive binding to make a SWNT-based glucose affinity sensor. **Figure 5** shows the general sensor design. The nanotube is first coated in dextran, which is a glucose analogue. The addition of concanavalin A (Con A), a lectin with four glucose-binding sites, will induce protein-controlled aggregation and a SWNT fluorescence decrease, as the number of nanotubes free in solution is decreased. The introduction of glucose will cause the equilibrium to shift and dissolution of the aggregate in conjunction with fluorescence recovery.⁵²

As nanotubes are inherently hydrophobic and dextran is a very hydrophilic polymer, it is first necessary to functionalize the dextran with hydrophobic phenoxy moieties. Increasing the weight percent phenoxy content

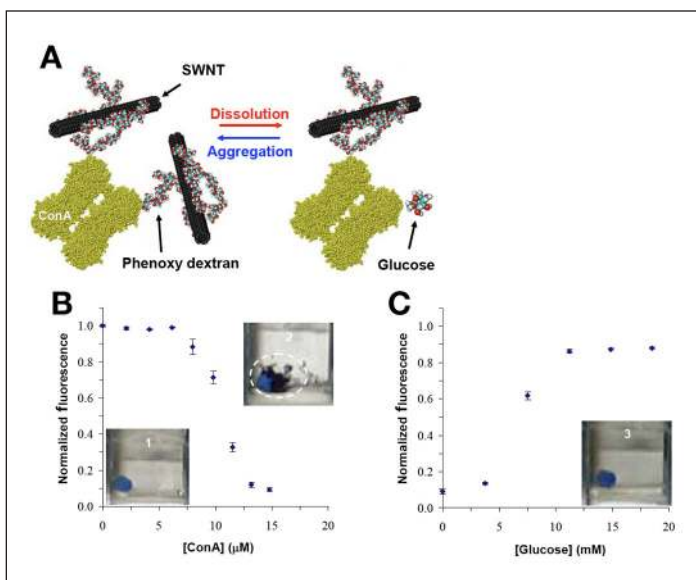


Figure 5. (A) Schematic of affinity glucose sensor operation. Dextran-coated nanotubes will aggregate in the presence of Con A, decreasing measured fluorescence. The addition of glucose results in aggregate dissolution and fluorescence recovery. (B) The addition of Con A to dextran-suspended nanotubes results in a 90% decrease in SWNT fluorescence and visible nanotube aggregation. (C) The subsequent addition of glucose causes nanotube aggregate dissolution and fluorescence recovery.

from 0 to 8 wt% results in the increase of the number of nanotube in solution, where dextran alone is not capable of suspending nanotubes in solution. Again, a gentle dialysis method is used to assemble dextran on the nanotube surface.

The addition of Con A to the phenoxy–dextran-stabilized nanotubes results in nanotube aggregation and a fluorescence decrease. A minimum amount of Con A is necessary before large enough nanotube aggregates are formed to cause a decrease in fluorescence. At a Con A concentration between 0 and 5.4 mM, the aggregate size is small enough to remain in solution and add to the total fluorescence signal, but at concentrations larger than 5.4 mM the aggregates attain a size such that they begin to settle out of solution and are no longer excited by the laser. Additions of glucose reverse this effect. This is the first demonstration of a reversible affinity sensor using single walled carbon nanotubes.⁵² We note that the current sensor design is not sensitive to glucose over a wide enough range and would need to be designed to cover 2–30 mM.⁹

Effect of Sensor Architecture

Modeling Optical Sensor Dynamics

To compare the dynamics of optical and electrochemical glucose sensors, we consider the transient glucose response of a model, a well-controlled patient with basal insulin provided in response to three meals per day with $t = 0$ at 6 a.m.: a 40-gram breakfast at $t = 60$ minutes (7 a.m.); a 50-gram lunch at $t = 360$ minutes (12 p.m.); and a 100-gram dinner at $t = 720$ minutes (6 p.m.). The model of Lehmann and Deutsch⁵³ was used to calculate meal metabolism dynamics. **Figure 6A** compares blood glucose dynamics predicted by Sorensen,⁵⁴ with 80–349 mg/dl glucose, and Bergman and colleagues,⁵⁵ with concentration ranges of 80–172 mg/dl. The mass transport model of Schmidtke and colleagues⁵⁶ was used to calculate the resulting subcutaneous glucose dynamics based on the blood glucose response (**Figure 6B**) and resulted in a time lag of about 30 minutes between blood and glucose concentrations due to mass transfer resistance.

Biofouling Stability

The two major limitations to sensor operation *in vivo* are foreign body encapsulation^{36,57–60} and membrane biofouling of the sensor surface.^{39,61} Foreign body encapsulation, such as fibroblast encapsulation at the site of implantation, has been reviewed elsewhere,^{58,59} and a number of possible solutions have been proposed.^{35,36,39,40,61–66}

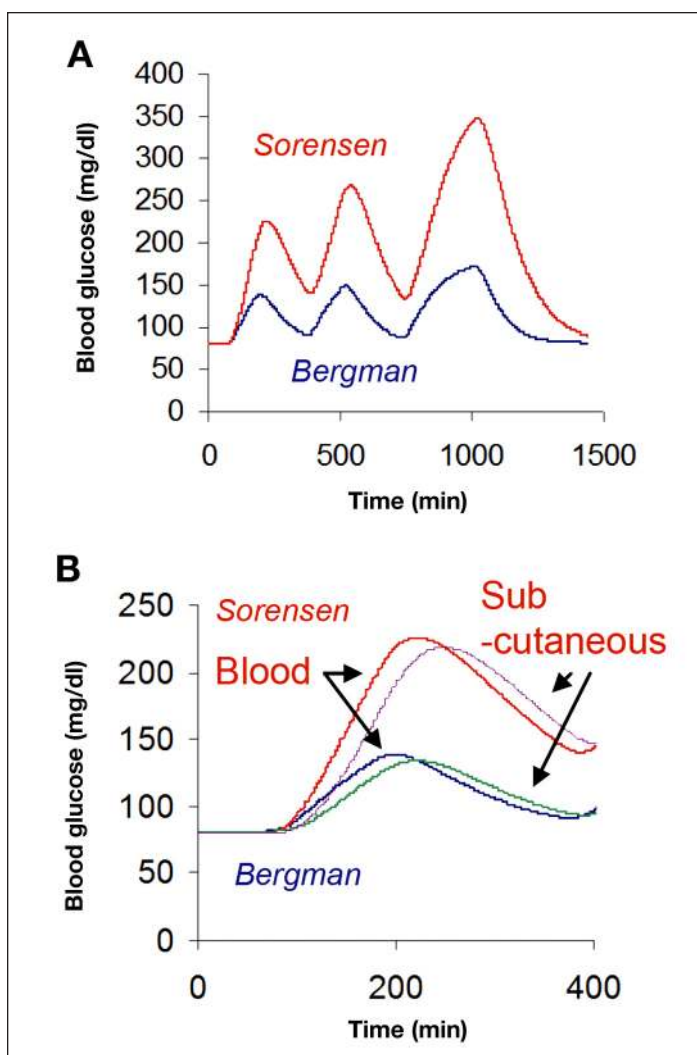


Figure 6. (A) Simulation of blood glucose levels of a healthy patient using the Sorensen (red) and Bergman (blue) models and a three-meal protocol described in the text. (B) Calculated subcutaneous glucose profiles using a mass transport model with a rate constant of 0.04 min^{-1} showing a 30-minute lag for both models

Biofouling of the sensor membrane, via either cell or protein adhesion, is the second problem with adherent bodies either blocking glucose transport or consuming glucose locally.^{39,61} In doing so, the measured concentration is decreased relative to the value in the blood. As biofouling worsens over time, the difference between actual blood glucose, C_B , and measured glucose, C_S , increases until the sensor is no longer viable.

Sensor architecture can play a significant role in exacerbating or ameliorating the effect of biofouling on the sensor. Biofouling at the sensor membrane necessarily limits the flux of glucose to the sensor as cellular adhesion becomes more pronounced. A review has been published elsewhere.³⁴ The simplest possible biofouling model is a first-order process that decreases the sensor

membrane area, $A(t)$, from its initial value, A_0 , with rate constant k_f :

$$A(t) = A_0 e^{-k_f t} \quad (4)$$

Electrochemical sensors do not measure glucose directly but instead measure the flux of glucose through a limiting membrane. The signal, S , is proportional to the flux, J :

$$S \propto J = \frac{A(t)D}{L} (C_B - C_S) \quad \text{electrochemical sensor (5)}$$

Here, D is the membrane diffusivity of glucose [$6.7 \times 10^{-10} \text{ m}^2/\text{s}$ for Alltech semipermeable membrane (Netherlands)⁶⁷] and L is the membrane thickness. Fouling decreases the measured signal immediately and can be corrected only by frequent recalibration and eventual replacement.

In contrast, for an optical sensor that directly measures glucose concentration at the sensor, C_S , fouling only adds a mass transfer resistance with an ultimate delay in sensor response. However, this delay is not realized until the rate of glucose across the sensor membrane becomes commensurate with the rate of change of blood glucose:

$$S \propto C_S$$

$$\frac{dC_S}{dt} = \frac{A(t)D}{LV} (C_B - C_S) \quad \text{optical sensor (6)}$$

Figure 7A compares a model electrochemical sensor and an optical sensor subjected to a moderate biofouling rate constant of $5 \times 10^{-5} \text{ min}^{-1}$ and an initial sensor area of $2 \times 10^{-6} \text{ cm}^2$. Biofouling distorts the signal measured by the electrochemical sensor immediately, with a loss of 20% of its accuracy after 3.7 days. However, a sensor that instead measures concentration directly is stable for more than 76 days after implantation, under identical conditions.

Sensor Biocompatibility and Fabrication

Evaluation of Dialysis Capillary Biocompatibility

Initial studies of capillary sensor biocompatibility have been performed using a chicken embryo chorioallantoic membrane (CAM) tissue model.⁵⁷ A shell-less culture was developed whereby chicken embryos were transplanted to plastic-covered cups after an initial 3-day incubation. The implant was then coated in egg white, to enhance incorporation, and placed onto the CAM after an additional 4 days of incubation. **Figure 8** shows the histological response after a 9-day incubation for an uncoated and polyethylene glycol (PEG) hydrogel-

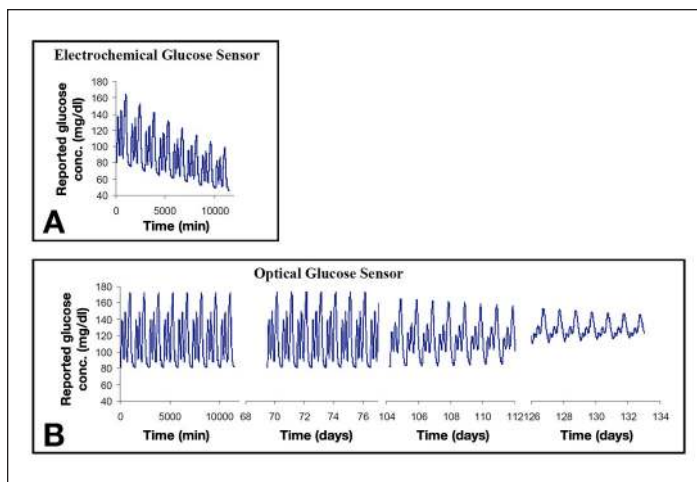


Figure 7. Effect of biofouling on the model responses of two types of glucose sensors. (A) An electrochemical sensor measures the flux of glucose to the electrode and exhibits signal distortion immediately as the effective membrane area is reduced. The sensor produces a 20% error after 3.7 days. (B) The same biofouling rate and initial area yield no diminution in an optical sensor response for more than 76 days. Constant parameter values were assumed for each sensor: $D = 6.7 \times 10^{-6} \text{ m}^2/\text{s}$, $L = 10^{-5}$, $A_0 = 2 \times 10^{-6} \text{ m}^2$, $V = 3.1 \times 10^{-10} \text{ m}^3$, $k_f = 5 \times 10^{-5} \text{ min}^{-1}$.

coated regenerated cellulose capillary and a polyester thread, used as a negative control. The hematoxylin and eosin-stained sections show that for the tissue surrounding the uncoated dialysis capillary, a high density of inflammatory cells, such as heterophils and giant mast cells, was present, whereas there was very little inflammatory response for the PEG-coated capillary. A severe inflammatory response and fibroblast encapsulation was observed around the thread. This shows that inflammation due to an implant in the CAM tissue model progresses from acute to chronic with associated fibrosis, similar to mammalian models, and can be used to prescreen the biocompatibility of implant materials.

Potential Interference from Protein Components in Whole Blood

False positive or negative readings from such a sensor may be caused by glucose-bearing proteins of low molecular weight (MW). **Table 3** outlines the principal components in plasma and blood with average molecular weight, carbohydrate content, and concentration.

The current sensor design utilizes a 13-kDa MW cutoff membrane separating the ambient tissue and fluid from the functionalized carbon nanotube. Excluding components above the cutoff and focusing on those with nonzero carbohydrate content leaves β -globulins as potentially interfering. Among this fraction, however, only

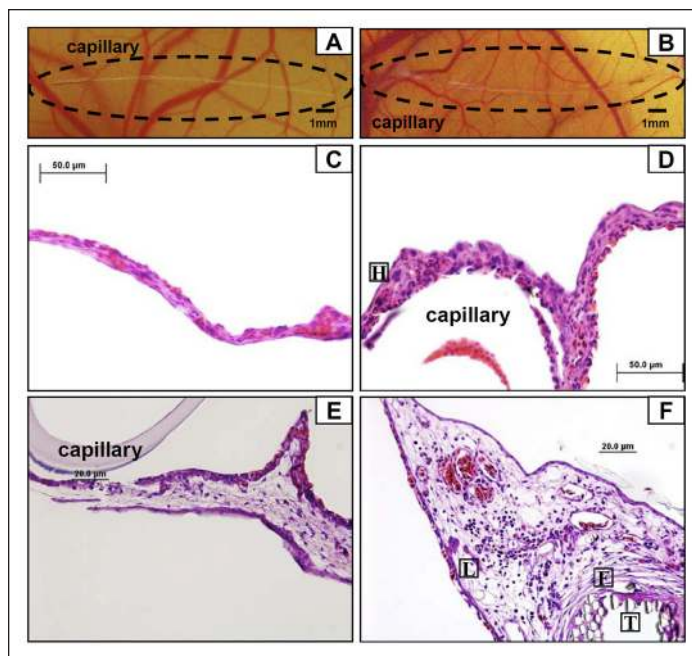


Figure 8. (A) A capillary placed on CAM for 2 hours. (B) A capillary placed on CAM for 9 days. (C) Hematoxylin and eosin (H&E) stain of normal CAM morphology. (D) H&E stain of capillary in CAM. A high density of heterophils and other acute inflammatory cells was observed around the capillary. (E) H&E stain of PEG-coated capillary in CAM. A very little inflammatory response was observed. (F) H&E stain of thread in CAM. Several layers of fibroblast encapsulation and severe inflammatory response were observed around the thread. H, heterophils; L, leukocytes (mostly macrophages and giant cells); F, fibroblast encapsulation induced by the thread; T, thread fibers within the tissue.

β_2 -microglobulin has a MW below the cutoff (11.7 kDa) of the sensor membrane. It is present in trace amounts and has no carbohydrate content. Thus, interference from such components in blood will be nonexistent. Testing in unseparated blood components is necessary, however, to demonstrate this fact.

Anticipated Device Fabrication Cost and Reproducibility

The final design of the 1-cm-implantable device utilizes 10 ng of carbon nanotube material. A conservative estimate of \sim \\$1000/gram for this material and that of the hydrogel and dialysis coatings yield \\$0.17 per device. For excitation and collection optics, a number of vendors already offer hand-held, palm-based spectrofluorometers for under \\$2000 each. This provides an upper bound for the cost of data readout.

There has recently been significant progress in the ability to separate and prepare homogeneous carbon nanotube solutions, including methods from our group and others. Such methods will allow the production of a sensor based on SWNT fluorescence ratios. Different SWNT

**Table 3:
Potential Interfering Components of Plasma and Blood Proteins**

Protein	MW (Da)	Amount in Serum (mg/100 ml)	Percent Carbohydrate
Prealbumin	54,980	10 – 40	0
Retinol-binding protein	21,000	3 – 6	0
Albumin	66,500	0	0
Galactoglycoprotein	81,000	–	76
α -Globulins	26,000 – 725,000	Trace – 420	3 – 42
β -Globulins	11,730 – 440,000	Trace – 320	0 – 22
Low MW proteins	5,100 – 14,000	0.1 – 1.5	0
Complement components	71,000 – 400,000	1 – 120	0 – 9.4
Complement factors	24,000 – 540,000	2 – 35	0 – 10
Coagulation proteins	58,000 – 340,000	1 – 450	1.5 – 15
Immunoglobulins	23,000 – 950,000	Trace – 1800	0 – 10
β_2 Microglobulin	11,730	Trace	0
Lysozyme	14,000	0.5 – 1.5	
Basic Protein B ₁	11,000	–	
Basic Protein B ₂	8,800	<1	0
0.6 S γ_2 -Globulin	5,100	<1	0
2 S γ_2 -Globulin	14,000	0.1	0
Post γ -globulin	13,260	–	0

types fluoresce at distinct wavelengths. Combining SWNT with one emission wavelength, prepared to be nonanalyte responsive, with SWNT of another emission wavelength, prepared as the sensor, will give the sensor a method of internal calibration. Ratios between the peaks should yield accurate analyte concentrations regardless of changes in sensor depth or presence of local scatterers.

Conclusions

Single walled carbon nanotubes are an exciting class of materials that, as demonstrated, have a potential niche in *in vivo* sensor technology. A sensor based on nanotube fluorescence would avoid a number of complications that current sensing technology faces. Single walled carbon nanotube nIR fluorescence and photostability avoid *in vivo* transmission and sensor lifetime issues that most conventional fluorophores face. Additionally, a SWNT sensor that detects glucose directly should not be as affected by biofouling as a flux-based sensor.

We have demonstrated two prototype SWNT-based glucose sensors: an enzyme-based sensor and an affinity sensor. The SWNT-based affinity sensor avoids inherent problems faced by flux-based sensors. Finally, initial biocompatibility testing regarding the sensing device has been carried out. These results form a promising beginning toward a nanotube-based *in vivo* continuous glucose monitoring device.

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