

Noninvasive Diagnostic Devices for Diabetes through Measuring Tear Glucose

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Abstract

This article reviews the development of a noninvasive diagnostic for diabetes by detecting ocular glucose. Early diagnosis and daily management are very important to diabetes patients to ensure a healthy life. Commercial blood glucose sensors have been used since the 1970s. Millions of diabetes patients have to prick their finger for a drop of blood 4–5 times a day to check blood glucose levels—almost 1800 times annually. There is a strong need to have a noninvasive device to help patients to manage the disease easily and painlessly. Instead of detecting the glucose in blood, monitoring the glucose level in other body fluids may provide a feasible approach for noninvasive diagnosis and diabetes control. Tear glucose has been studied for several decades. This article reviews studies on ocular glucose and its monitoring methods. Attempts to continuously monitor the concentration of tear glucose by using contact lens-based sensors are discussed as well as our current development of a nanostructured lens-based sensor for diabetes. This disposable biosensor for the detection of tear glucose may provide an alternative method to help patients manage the disease conveniently.

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Introduction and Background

A statistical analysis by the World Health Organization (WHO) indicates that more than 220 million people have to live with diabetes.¹ Diabetes caused over 1.1 million deaths in 2005, and diabetes death is estimated to double by 2030.¹ Early diagnosis and continuous management are vital to patients to ensure a healthy life and to avoid circulatory problems and other diseases caused by diabetes, such as kidney failure, heart disease, and

blindness.¹⁻² Current practice for diabetes management relies on monitoring blood glucose. Patients have to prick their finger for a drop of blood multiple times a day, about 1800 times per year, to check glucose levels. Furthermore, the reports of blood-borne infection have been noted with these invasive glucose sensors. For these reasons, new techniques have been employed to develop a minorly invasive and/or noninvasive device for blood

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Abbreviations: (A) acceptor, (a.u.) arbitrary units, (BET) Brunauer-Emmett-Teller, (Con A) concanavalin A, (D) donor, (FITC) fluorescein isothiocyanate, (FMSN) fluorescent mesoporous silica nanoparticle, (FRET) fluorescence resonance energy transfer, (IR) infrared, (NP) nanoparticle, (SiO₂) silica, (TRITC-dextran) tetramethylrhodamine isothiocyanate-dextran, (UV) ultraviolet

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glucose monitoring, including infrared (IR) spectroscopy,³⁻⁵ fluorescence spectroscopy,⁶⁻⁷ Raman spectroscopy,⁸⁻⁹ optical polarization rotation measurement,¹⁰⁻¹³ photoacoustic probes,¹⁴ and surface plasmon resonance.¹⁵⁻¹⁶ However, results from these techniques need to be checked frequently against direct blood glucose measurements, that is, they cannot replace the direct measure of a blood glucose sensor.¹⁷⁻¹⁸ As an alternative, approaches to measure the concentration of glucose in an accessible body fluid, including urine, saliva, and tear fluid, have great potential for noninvasive diagnosis and management of diabetes. Compared to other body fluids, tears are more accessible than blood or interstitial fluid, more continuously obtainable, and less susceptible to dilution than urine.

Tear fluid is the aqueous layer on the ocular surface, and has many functions as part of the optical system, such as lubricating the eye and nourishing the cornea.¹⁹⁻²⁰ The average rate of tear product is in the range of 0.5–2.2 $\mu\text{l}/\text{min}$; about 0.72–3.2 ml of tears are secreted per day.²¹ Tear fluid normally consists of over 20 components, including salt water, proteins, glucose, some small metallic ions, etc.²¹ Tear glucose has been studied since 1930.²² Many reports have demonstrated that tear glucose is higher in diabetic subjects than in healthy ones. Furthermore, the correlation of tear glucose and blood glucose has been studied by different methods in both human and animals, e.g., rabbits.²³⁻²⁵

Tear Glucose and Its Measures in Diabetes Patients

Since the 1930s, human models and animals have been studied to estimate the glucose level in tears and the correlation between tear glucose and blood glucose.²⁶⁻²⁸ The results indicate that the level of glucose in tears is often elevated in diabetes patients. Lewis and Stephens studied tear fluids of diabetic and nondiabetic subjects by using a commercialized blood test tool quantitatively and qualitatively, and concluded that tear fluid is a sample that can be used in the diagnosis of diabetes.²⁹ Gasset *et al.* and Motoji *et al.* found a definite relationship between tear glucose and blood glucose and concluded that hyperglycemia could be detected by measuring tear glucose levels.³⁰⁻³¹ Furthermore, Sen and Sarin compared the glucose levels in the tears and blood of both diabetic and nondiabetic subjects. Their study indicated that blood glucose was about two times higher in diabetic subjects than in nondiabetic subjects, whereas tear glucose was about five times higher in diabetic versus nondiabetic subjects.³² Dawn and Hill reported the correlation

coefficient (r) between blood glucose and tear glucose was +0.53.³³ Other studies indicate that diabetic and nondiabetic tear glucose mean values were 6.34 ± 0.7 mg/dl and 2.9 ± 0.5 mg/dl, respectively.³⁴ It should be noted that the discrepancy of the correlation coefficient (r) between blood glucose and tear glucose is caused by the different tear collection methods, e.g., filter paper or microcapillary.³⁵

Obviously, the development of an *in situ* ocular glucose sensor for diabetes control is heavily limited by the following factors: (1) it takes time to collect enough tear sample, i.e., at least 10 minutes is required to collect 10 μl of tear sample by using glass capillaries;³⁶ (2) the concentration of the glucose in tears is lower than that in blood;³⁷ and (3) very few suitable devices are able to compete against commercial blood glucose testers to quantitatively measure tear glucose in a short period of time. A couple of methods have been developed to overcome the difficulties of *in situ* testing of tear glucose. Coté and colleagues developed a polarimetric glucose sensor for monitoring ocular glucose.³⁸ It indicated that the time lag between blood glucose and anterior aqueous humor glucose concentrations was an average of five minutes. The other approach is related to the contact lens-based sensor, which has been receiving a lot of attention because the device is portable and disposable. It is likely that contact lens-based glucose sensors have great potential to realize continuous and noninvasive diabetes control. Furthermore, hydrogel-based soft contact lenses are approved as safe daily wear lenses in diabetes patients.³⁹⁻⁴⁰

Contact Lens Sensor for Diabetes

Contact lenses have applications beyond vision correction. They are being considered by at least three research groups who are working with such sensors as an alternative tool to continuously and noninvasively monitor the level of glucose in tears.⁴¹⁻⁴³ Luminescent/fluorescent contact lens-based sensors are a feasible technique because there are no electrodes or electric circuits. The fluorescence emission intensity (F) is described as

$$F = \Phi I_0 (1 - e^{-abc}) \quad (1)$$

where Φ is the quantum efficiency, I_0 the intensity of incident light, a the molar absorptivity, b the path length of the cell, and c the molar concentration of the fluorophore.⁴⁴ To our best knowledge, two different optical probes are used and incorporated into contact lenses, i.e., fluorescent labeled protein and glucose interactions,⁴¹

and boronic acid and glucose.^{42–44} The first one uses the competition reaction; the bonding between the glucose and enzyme is stronger than that between the fluorescent molecules and enzyme. The second one uses the direct reaction of analyte and fluorescent molecules. The amount of glucose is measured by using **Equation (1)**. Unfortunately, the boronic-acid glucose sensing is pH dependent. The interactions between the fluorescent protein and glucose also have limits to overcome because the intensity of fluorescence responds only to higher concentrations of aqueous glucose, which would not be an ideal method for accurately detecting tear glucose at lower amounts (0.5–2.2 $\mu\text{l}/\text{min}$). A sensitive transducer is required to detect the tear glucose. Existing methods of fluorescent glucose sensing apply fluorescence resonance energy transfer (FRET).⁴⁵ This method is based on the dual measure, i.e., the FRET and fluorescence intensity (I) measurements. FRET is a distance-dependent energy transfer from a fluorophore donor (D) to a fluorophore acceptor (A) in a nonradiative process.⁴⁵ **Figure 1** illustrates the FRET pair-labeled concanavalin A (Con A) and its fluorescence mechanism; Con A is a lectin protein and has specific bonding to the glucose molecule. The fluorophore donor is fluorescein isothiocyanate (FITC) and the fluorophore acceptors are derivatives of rhodamine-dextran [tetramethylrhodamine isothiocyanate-dextran (TRITC-dextran)]. When the distance of the two fluorophores is very close (<10 nm), the excited donor will transfer some of its energy to the acceptor, which becomes excited and emits light in a longer wavelength.⁴⁶ As the bonding between glucose and Con A is stronger than that between dextran and Con A, the FRET pair-labeled Con A will have changes in both its wavelength (λ) and fluorescence intensity (I) when the glucose molecule replaces derivatives of rhodamine-dextran

(TRITC-dextran) to react with Con A. The change of the fluorescence properties (the shift of the fluorescence as a function of time and intensity, etc.) can be used to calculate the amount of glucose reacting with Con A. FRET is an inexpensive and very sensitive method to apply to molecule imaging.⁴⁶ However, barriers to secure a feasible contact lens sensor include the photobleaching of fluorescence molecules; low concentration of tear samples or analyte; low fluorescence intensity or signals; and vision influence. In addition, a safety concern is that the boronic acid and Con A may be released from the lens into the body. Thus, a lens sensor should be able to monitor a wide range of glucose levels quickly and accurately; the fabrication of the lens sensor should be reproducible and the lens sensor should have an extended lifetime. In addition, the ideal sensor would be biocompatible and be able to keep the biorecognizer stable. We have developed a new nanostructured optical sensor embedded in a contact lens to detect the glucose in tears continuously and quantitatively.⁴⁷ The key processes and results are as follows:

(1) Encapsulation of FRET pair-labeled protein within mesoporous nanoparticles

Porous nanostructures are developed to encapsulate FRET pair-labeled protein to act as the optical probe, which afterward become incorporated or embedded into the contact lens. Three of the most significant advantages of using porous nanostructures are as follows: (1) The porous structures of nanoparticles act as the analyte (tear glucose) collector and achieve high concentration of analyte because of the large ratio of surface area to volume and interporous structures. (2) The porous nanostructures exhibit stable optical signals. Our results indicate that the porous nanoparticles protect

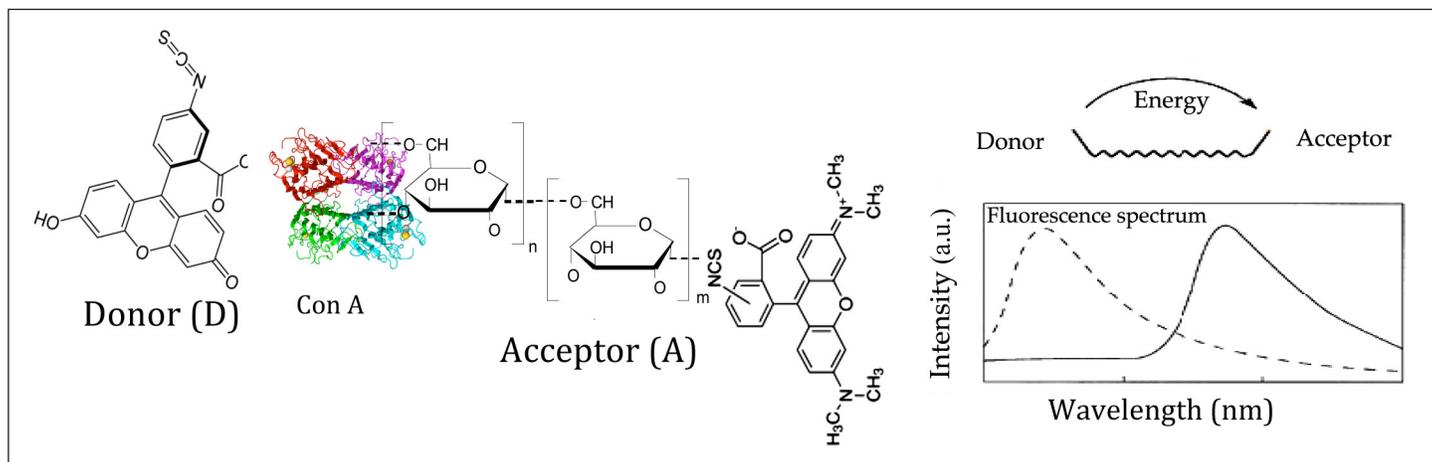


Figure 1. An example of the FRET pair-labeled Con A.

the fluorophores from leaching and photoquenching (**Figure 2**). This indicates the difference in the photobleaching behavior of free fluorophore versus fluorescent mesoporous silica nanoparticles (FMSN).⁴⁸ After 300 minutes of continuous exposure under an electromagnetic field with a wavelength of 490 nm, the photobleaching behavior of the FMSN is reduced only by ~9% compared to free FITC, which is reduced by 30%. (3) The large surface-to-volume ratio of nanostructures may lead to higher selectivity for glucose sensing.

(2) Synthesis of nanostructured optical sensor by encapsulation of FRET pair-labeled protein within mesoporous nanoparticles

Different FRET pair-labeled proteins, i.e., Con A, have been developed. The FRET pair with a longer emission wavelength (λ_{em}) in a visible to near-IR wavelength (550–650 nm), and the color of the sensor shows red. The TRITC-Con A-FITC, as an example, can be entrapped in porous nanoparticles, e.g., silica (SiO₂). Compared to other nanoparticles (NPs), FMSNs have good thermal-mechanical properties, a functional surface that can be derivative via silylation reactions, and greater biocompatibility.^{49–50} A modified water-in-oil microemulsion method was used to encapsulate the FRET pair-labeled protein within FMSNs. The average diameter of the nanoparticles is around 55 ± 10 nm as measured by a high resolution transmission electron microscope as shown in **Figure 3**. The overall reaction of producing FMSNs is



The Brunauer-Emmett-Teller (BET) surface area and pore size were determined from nitrogen adsorption and desorption isotherm data obtained at 77 K. The BET measurement results indicate the average pore size is approximately 4–5 nm.⁵¹

(3) Fabrication and characterization of nanostructured optical probe embedded in hydrophilic hydrogel contact lens

A two-step photopolymerization method was applied in casting optical nanoparticles into soft hydrogel lens materials. First, fluorescent NPs were assembled on a pre-treated polydimethylsiloxane slice through spin-coating. The substrate acts as an ultraviolet (UV) shield to protect the eyes. Following that, the optical probe was embedded in a hydrophilic hydrogel lens materials through deep UV irradiation. Deep UV [source: 1 kW Hg(Xe),

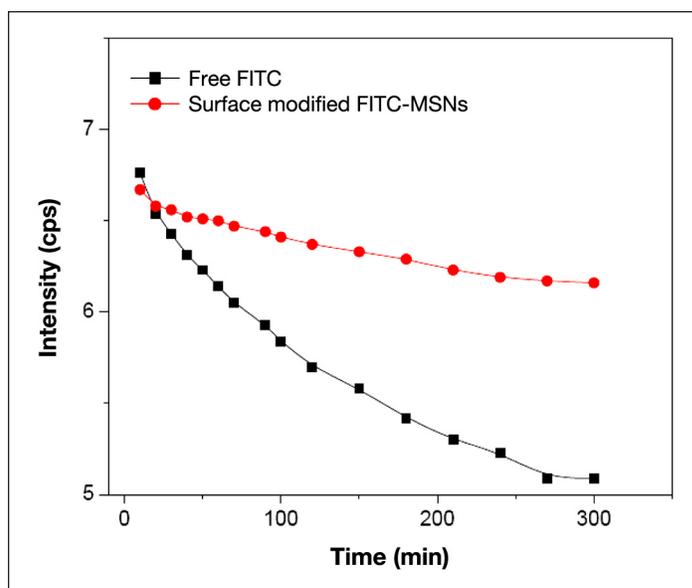


Figure 2. Photobleaching behavior of FITC: free FITC (black) and foam-structured FMSNs (red).⁴⁸

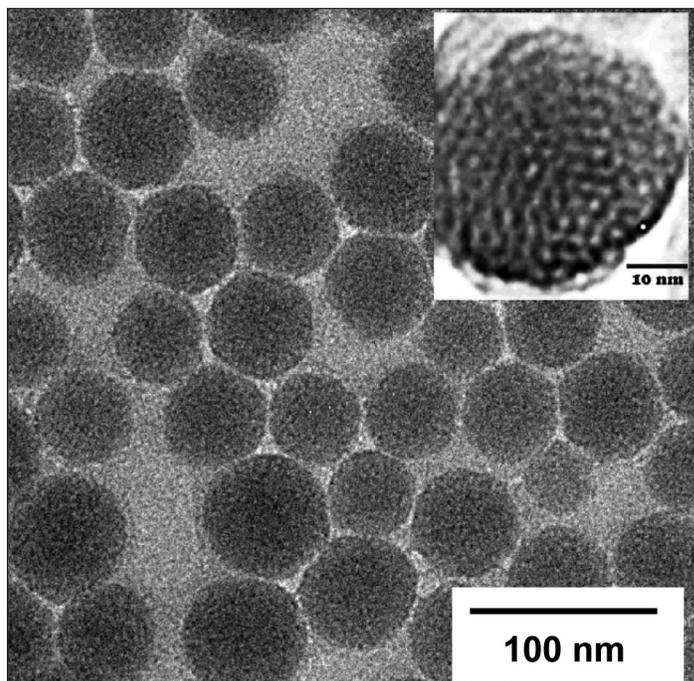


Figure 3. Transmission electron microscopy micrograph of FMSNs.

220–260 nm] was used to polymerize the 2-hydroxyethyl methacrylate solution to integrate the sensor into the soft hydrogel contact lens.⁵¹ Our lab-produced hydrophilic soft lens sensors have been tested to characterize their oxygen permeability and light permission. The small image inset in **Figure 4** is the scanning electron microscope micrograph of the hydrogel lens. However, it is noted that the low wavelength of photopolymerization may give an adverse effect to the fluorescent pair-labeled protein.

(4) Detection of fluorescence intensity and resonance energy transfer as a function of aqueous glucose

To find out how fast the sensor can be, laser confocal scanning microscopy was used to investigate the color change before and after adding 10 μl of glucose (1 mg/dl). The change at the microscale can be detected in less than 1 minute as shown in **Figure 5**. To investigate how sensitive the sensor can be, both fluorescence intensity (I) and resonance energy transfer (RET) as a function of time and as a function of the concentration of glucose (3.6–78 mg/dl), respectively, were investigated through a fluorospectrometer. The linear relation of $I(D)/I(A)$ and the concentration of glucose was found. Furthermore, the shift of $\lambda(D)/\lambda(A)$ is also related to the concentration of glucose due to the effect of FRET. Meanwhile, the lens sensor can be used to monitor aqueous glucose continuously for 5 days as shown in **Figure 6**. It is noted that time lag appears in the case of low concentration. Furthermore, FRET requires an external illumination to initiate the fluorescence transfer, which may cause an obvious background noise and bring up the concern of safety. A new design has been developed to overcome the shortcoming.

Conclusion

It is clear that testing ocular glucose has great potential for noninvasive diagnosis of diabetes. The challenges have been addressed in this article. The advantages of the nanostructure-laden contact lens are (1) the porous nanostructure is able to bind to the desired bioassay for conjugating the glucose in tears; (2) the porous nanostructures act as an analyte reservoir, which helps to achieve the high loading of analyte for target sensing (e.g., glucose sensing); (3) the nanostructures embedded in the contact lens will not interfere with patient vision and will likely enhance oxygen permeability because of the porous structures; (4) the nanostructures are able to load high concentration of bioassay to secure better sensitivity; and (5) the nanostructures keep the loaded fluorescent molecules from photobleaching. Further efforts are needed to improve the resolution and sensitivity of the new device and to determine a physiologically relevant and baseline tear glucose concentration.

However, it is noted that there is a discrepancy in the tear glucose levels with different methods of collecting tear samples. In addition, FRET normally requires an external illumination to initiate the fluorescence transfer, which may cause obvious background noise. More efforts are required to develop noninvasive diagnostic devices for testing tear glucose. We expect

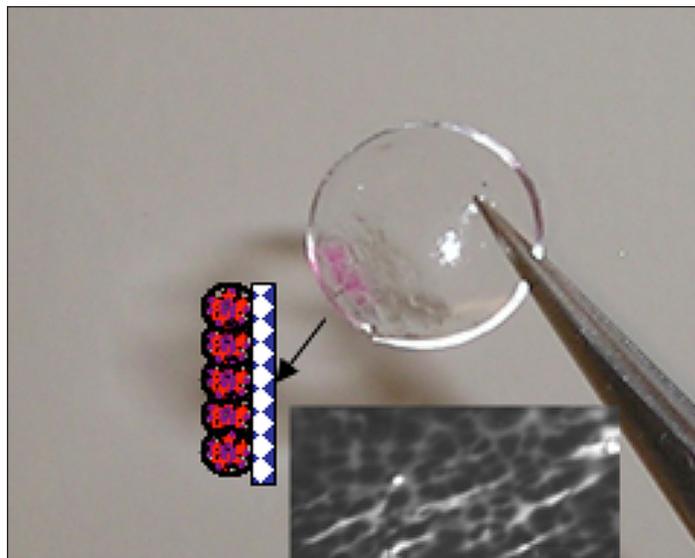


Figure 4. Lens sensor developed in Zhang's lab at the University of Western Ontario, London, Ontario.

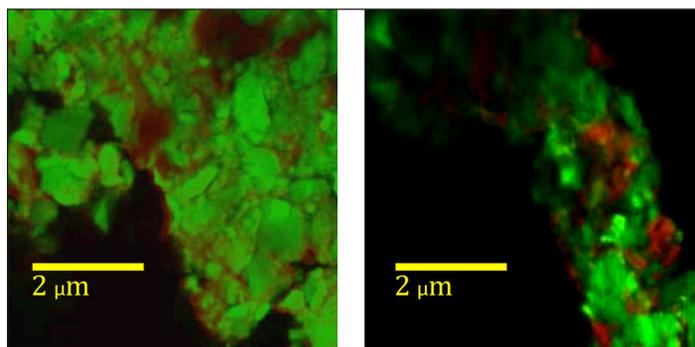


Figure 5. LCSM image: before (right) and after (left) adding 10 μl aqueous glucose (1 mg/dl).

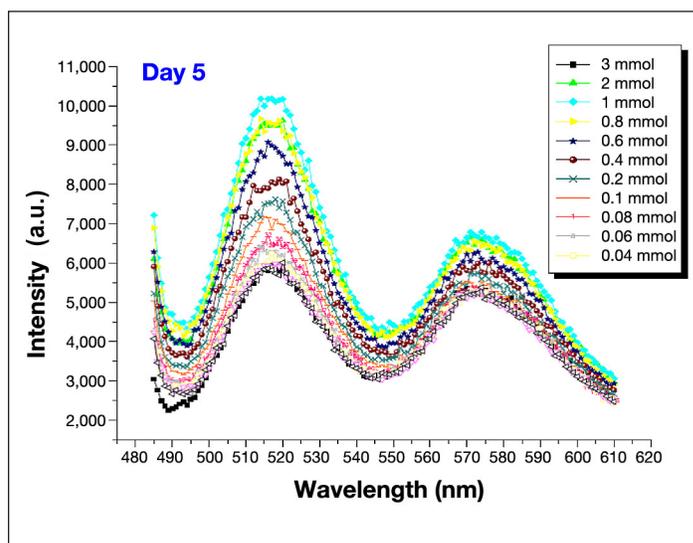


Figure 6. Fluorescence responses as the function of the concentration of glucose, which can be continuously monitored for 5 days.

that the nanostructured contact lens-based device will be a safe, sensitive, and noninvasive glucose monitoring solution for patients, and will have a significant impact on point-of-care technologies.

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