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A Disposable Tear Glucose Biosensor—Part 1: Design and Concept Testing

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Abstract

Background:

Tear glucose has been suggested previously as a potential approach for the noninvasive estimation of blood glucose. While the topic remains unresolved, an overview of previous studies suggests the importance of a tear sampling approach and warrants new technology development. A concept device is presented that meets the needs of a tear glucose biosensor.

Methods:

Three approaches to chronoamperometric glucose sensing were evaluated, including glucose oxidase mediated by potassium ferricyanide or oxygen with a hydrogen peroxide catalyst, Prussian blue, and potassium ferricyanide-mediated glucose dehydrogenase. For tear sampling, calcium alginate, poly(2-hydroxyethyl methacrylate), and polyurethane foam were screened as an absorbent tear sampling material. A quantitative model based on the proposed function of concept device was created.

Results:

For glucose sensing, it was found that potassium ferricyanide with glucose dehydrogenase was ideal, featuring oxygen insensitivity, long-term stability, and a lower limit of detection of 2 μ*M* glucose. Polyurethane foam possessed all of the required characteristics for tear sampling, including reproducible sampling from a hydrogel-simulated, eye surface (4.2 ± 0.5 μl; $n = 8$). It is estimated that 100 μ*M* of glucose tear fluid would yield 135 nA (14.9% relative standard deviation).

Conclusion:

A novel concept device for tear glucose sampling was presented, and the key functions of this device were tested and used to model the performance of the final device. Based on these promising initial results, the device is achievable and within reach of current technical capabilities, setting the stage for prototype development.

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Abbreviations: (DM) diabetes mellitus, (GDH) glucose dehydrogenase, (GDH-FAD) glucose dehydrogenase with flavin adenine dinucleotide, (GO_x) glucose oxidase, (LLD) lower limit of detection, (PBS) phosphate-buffered saline, (pHEMA) poly(2-hydroxyethyl methacrylate), (PB) Prussian blue, (RSD) relative standard deviation, (SMBG) self-monitoring of blood glucose, (TG) tear glucose

Keywords: biosensor, diabetes mellitus, glucose monitoring, tear glucose monitoring

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Introduction

Diabetes mellitus (DM) now affects nearly 8% of the U.S. population.[1] Achieving near-normal glucose levels is vital for delaying or preventing the potentially debilitating microvascular complications that can result from the disease.[2–4] Self-monitoring of blood glucose (SMBG) is considered standard of care and an essential component of DM management.[5] While "finger stick" measurement methods are the current standard by which patients conduct SMBG, the pain and inconvenience associated with this approach can decrease patient compliance.[6] Hence, the development of convenient, noninvasive glucose monitoring systems for daily measurements has the potential to improve SMBG frequency and patient quality of life, allowing for better control of glucose levels.

Despite 70 years of research,[7] clinical studies have yet to resolve the relationship between tear and blood glucose concentrations. Disagreements have stemmed from both inconsistencies in tear glucose concentration and its correlation to blood glucose values. Reported tear glucose (TG) values have ranged across three orders of magnitude from single micromolar[8] to single millimolar.[7] Reviewing seven studies that tested the correlation between tear and blood glucose,[9–15] five found some correlation[9–13] while two found none or were indeterminate.[14,15] Initially, such an inconsistency in results observed in these various studies could discourage further exploration of TG for glucose monitoring, but a review of TG studies by Baca and colleagues[16] suggested that interesting relations between sampling approach and study results can be identified by considering the experimental context of previous TG studies. If differences in TG encountered between different studies can be attributed to technique, then disagreements between reports may not invalidate the possible correlation between tear and blood glucose. Regardless of the exact mechanism of glucose transport into tear fluid, the accuracy holds true for each set of experimental conditions described in the study. Thus, in order for these findings to be translated successfully into a viable clinical tool, these conditions must first be integrated into a simple approach that can be applied reproducibly in a variety of situations.

This statement frames the engineering design challenge at hand—to develop a technology to sample and sense TG that can be readily translated into a practical clinical tool. We posit that through a review of TG research, the technical requirements of such a device can be identified and used to construct a concept device. Once an acceptable device concept is created, engineering design and development allow for function optimization, cost reduction, and production scale-up.[17] Such a device could be adapted and developed over iterative testing to facilitate a more reproducible sample and analysis approach.

Tear Glucose

Many of the general challenges of tear sampling can be attributed to the delicate nature of the eye. Tear physiology and composition have been reviewed extensively elsewhere.[16] The aqueous layer of interest has a reported thickness of 3 μm[18] and a reported volume of 7 μl,[19] making it difficult to sample.

Experimentally, this challenge has been addressed through the three major approaches mentioned earlier: direct contact, minimal contact, and chemical sampling (e.g., absorbent fibers, microcapillary tubes, and lachrymators, respectively). Recent clinical studies have relied predominantly on the use of microcapillary tubes to collect single microliter samples. This approach offers the advantage of theoretically creating little or no eye irritation. However, this approach is not translated readily to SMBG devices. Also, some studies have still encountered variations, which may suggest mechanical irritation[16] or sample handling errors.

In addition to tear sampling, various approaches have been taken for quantifying glucose concentrations in tears. Glucose detection methods for clinical tear studies have included liquid chromatography–electrospray ionization mass spectroscopy,[15] high-performance capillary electrophoresis with pulsed amperometric detection,[13] enzymatic colorometry,[9–11] and enzymatic fluorometry.[20] Some notable efforts in TG device development include contact lens sensors with changing optical properties corresponding to glucose concentration[20] to be met with recent advances in integrating advanced circuitry and display technologies into soft polymer contact lens.[21] While this creative approach theoretically offers continual measurement, the need for regular calibration checks against strip sensors may pose a challenge as contacts have limited lifetimes. A disposable TG sensor offers the accuracy of electrochemical detection with the fabrication reproducibility of commercial screen-printed sensors.

Concept Development

Based on the discussion given earlier, design needs were identified (**Table 1**). These design needs address issues of concept functionality and usability, which must be achieved before transitioning to more rigorous formal device evaluation by accepted "standards" criteria for Food and Drug Administration-approved SMBG technologies.[22] It was determined that a modified approach to a "mechanical" sampling approach offered an excellent balance of capabilities and drawbacks. Specifically, the use of absorbent materials in direct contact with the eye allows rapid and simple sampling. Such material could be selected from soft polyurethane (PU) foams or absorbent hydrogels, which are widely used in the medical field. For glucose detection, electrochemistry offers many of the advantages found in current SMBG test strips, such as sensitivity, rapidity, reproducibility, and simplicity of instrumentation. The current prevalence of electrochemical systems on the market for SMBG provides a substantial body of research in the design and optimization of electrochemical glucose sensor on which to build.

Assuming an absorbent material will be implemented for sampling tears, one immediate challenge is the integration of such a system to an electrochemical sensor. Typically, SMBG test strips have adequate fluid to dissolve electrochemical assay components as well as create a conductive solution for electrochemical measurements. This presents a distinct challenge for an absorbent strip type system, as it is unlikely that adequate tears can be collected to hydrate an assay and it is not readily apparent how to extract absorbed tears for analysis.

To integrate these components, a small microfluidics system is proposed. This fluidics system features a sensing chamber connected by a channel to an external sampling feature in which an absorbent material could be placed (**Figure 1**). By prefilling this chamber with solution, the well can be compressed mechanically, driving the fluid to the absorbent material. Upon releasing the compression, the extracted tear sample is drawn back into the sensing chamber for analysis, also dissolving any dry reagents for detection. This new concept offers an approach to the sampling and electrochemical analysis of TG that addresses the needs identified earlier.

The next section presents initial results in the areas of glucose sensing, fluid sampling, sample extraction, and microfluidic design—the primary functions of the device.

Table 1.
List of Identified Needs for a Tear Glucose Device

Design need
Reproducibly sample from tear film
Accurately analyze glucose concentrations (1 to 1000 μ*M*)
Capture adequate fluid for analysis technique
Minimal tear sampling time
Simple tear sampling
Simple glucose analysis
Integrated sampling and sensing
Low cost and scalable fabrication

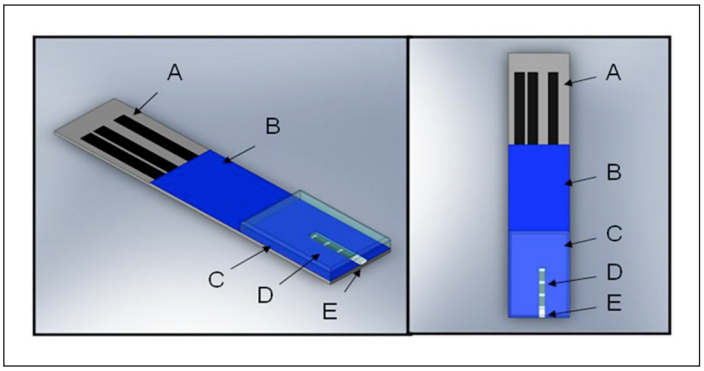

Figure 1. An integrated device concept for sampling and sensing of tear glucose, featuring screen-printed electrical leads **(A)**, an insulating layer **(B)**, a silicone fluidics piece **(C)**, a sensing well covering the three electrode system **(D)**, and an absorbent sampling material **(E)**.

A quantitative model is also presented, which is used to estimate final device feasibility based on initial results. These results indicate that a low-cost, sensitive, easy-to-use TG device is achievable and within reach of current technical capabilities.

Materials/Methods

Model Development

Before initiating any actual experiments, the presented concept was translated into a series of systematic steps, which could be modeled mathematically to predict system outputs and error propagation. Using standard spreadsheet software (Microsoft Excel 2007, Microsoft Corporation, Richmond, VA), the equations were organized sequentially by the order each modeled step would be performed in the operation of the device. Thus, the output of each step (i.e., glucose concentration and sensor current) and their corresponding variation could be followed through the entire device operation for the input parameters determined during initial bench testing.

Chemicals

All reagents were obtained from Sigma-Aldrich unless otherwise specified. Glucose dehydrogenase with flavin adenine dinucleotide (GDH-FAD) cofactor with an activity of 207 U/mg was donated generously by Amano Inc. (Japan). The glucose oxidase (GO_x) used had an activity of 155.6 U/mg. All solutions were prepared in phosphate-buffered saline (PBS) at pH 7.4 unless otherwise specified.

Electrochemical Detection

For electrochemical glucose sensing, a disposable, commercial screen-print sensor (Zensor, Taiwan) was selected. The sensor featured a working (71.0 mm^2) and counter electrode made of conductive carbon ink, a pseudoreference electrode made of silver ink (–72 mV vs Ag/AgCl), and a nonconducting insulating layer. A CHI 1230A potentiostat (CHI, Austin, TX) connected to a desktop computer was used to make electrochemical measurements. Chronoamperometric measurements were made by applying a potential of +0.45 volt for 10 seconds with a sampling rate of 10 Hz unless otherwise specified.

Glucose Assay Development

Three different assay approaches were evaluated, including the combination of GO_x and potassium ferricyanide, GDH-FAD and potassium ferricyanide, and GO_x with O_2 and a H_2O_2 catalyst, Prussian blue (PB). For the ferricyanide-mediated systems, assay solutions were prepared with 1 mg/ml of the enzyme and 100 m*M* mediator in PBS. Assay solutions were spiked with glucose stock solutions before making chronoamperometric measurements. Briefly, PB was prepared using a previously developed protocol[23] in which a solution of 100 m*M* ferric chloride in 10 m*M* HCl was combined with 100 m*M* potassium ferricyanide in 10 m*M* HCl on the working electrode surface, allowed to set for 60 minutes, and then washed thoroughly with distilled water before using.

Absorptive Sampling Development

A soft, absorbent, eye-like surface was prepared by polymerizing a thin (1-mm) sheet of poly(2-hydroxyethyl methacrylate)(pHEMA). Briefly, pHEMA was prepared by combining the monomer, 2-hydroxyethyl methacrylate (98% purity, 200 ppm hydroquinone monomethyl ether stabilizer), the cross-linker, ethylene glycol dimethacrylate (98% purity, 100 ppm hydroquinone monomethyl ether stabilizer), and the thermal initiator, ammonium persulfate, at a ratio of 30:0.5:6 wt% in distilled water. The solution was then poured into a small container of proper dimensions before heating at 60°C for 6 hours. The final sheet was washed in heated ethanol (80°C) and then water (80°C) to remove any unreacted monomers or contaminants. Calcium alginate was prepared in a sheet form by pouring 1 wt% sodium alginate into a petri dish. A concentrated 2 *M* calcium chloride solution was misted gently onto the dish using a small atomizer, cross-linking the alginate solution. Initial screening of the materials involved testing of their ability to absorb water and then release the captured sample. This entailed placing dried, cylindrical segments of the material 0.5 mm in height and 1.0 mm in diameter in water and evaluating the rate of absorption qualitatively. The release of the absorbed sample was evaluated by deforming the material mechanically and evaluating fluid loss qualitatively. For the evaluation of commercial PU foams (Studio Tools, Minneapolis, MN), circular segments approximately 0.5 mm in height and 1.0 mm in diameter were cut from larger foam squares. A small foam holder was cast in silicone rubber poly(dimethylsiloxane) and used to hold each segment for testing. In the experiment, a small sheet of 5 × 5-cm pHEMA was placed flat on a glass dish and soaked in water. Prior to sampling, the disk was tilted to pour off all excess fluid, leaving a soft, hydrophilic surface with a very thin layer of water dispersed across its surface in simulation of the surface of the eye. The foam segment was pressed carefully against the pHEMA for 20 seconds, and the change in mass was used to estimate the amount of fluid absorbed.

Results and Discussion

Quantitative Model

A model was developed based on the functional steps of tear sampling, sample dilution, and glucose detection. This model begins with the sampling of tear fluid with glucose concentration, C_t, into an absorbent material. This first step can be modeled as a captured fluid volume, V_c, with an associated standard deviation, σ_c. Next, this tear sample is extracted by an extraction solution with volume, V_e (σ_e). The final result of this extraction is dilution of the glucose concentration of the sample based on the calculated dilution factor (X_{dil}, σ_{dil}). The dilution factor thus becomes a ratio of tear sample volume to total volume of tear sample and extraction solution [**Eq. (1)**]. The propagation of error contributed to $X_{dilution}$ by the two variables V_c and V_e can be calculated by taking the square of the partial derivatives of each term multiplied by the corresponding variance term [**Eq. (2)**], shown in its expanded form in **Eq. (3)**:

$$X_{dilution} = V_c/(V_c + V_e) \quad (1)$$

$$\sigma_{dil}^2 = (\delta(X_{dil})/\delta(V_c) * \sigma_c)^2 + (\delta(X_{dil})/\delta(V_e) * \sigma_e)^2 \quad (2)$$

$$\sigma_{dil}^2 = (-V_c/(V_c + V_e)^2 * \sigma_c)^2 + (V_e/(V_c + V_e)^2 * \sigma_e)^2. \quad (3)$$

For simplification, the extraction efficiency is assumed to be 100%. The concentration of extracted glucose in the sensing well, C_{glc}, can then be calculated [**Eq. (3)**]. Again, error propagation can be calculated in the same fashion as **Eq. (2)**, yielding a simplified form shown in **Eq. (5)**:

$$C_{glc} = X_{dil} * C_t \quad (4)$$

$$\sigma_{glc}^2 = (C_t * \sigma_{dil})^2 + (X_{dil} * \sigma_t)^2. \quad (5)$$

Finally, the linear regression of the electrochemical assay must be determined and used to estimate the output current of the extracted glucose concentration. Assuming a linear relationship with slope, m, and y intercept, b, the output current, I_o, can be calculated [**Eq. (6)**], as well as its corresponding variance [**Eq. (7)**], using the same approach as described earlier:

$$I_o = m * C_{glc} + b \quad (6)$$

$$\sigma_o^2 = (C_{glc} * \sigma_m)^2 + (m * \sigma_{glc})^2 + \sigma_b^2 \quad (7)$$

Thus, each equation in this model represents a device functionality, which can be tested quickly for feasibility in an isolated experiment.

Glucose Assay Selection

The three glucose detection approaches utilized different pathways of electron flow, yielding assay performance characteristics as shown in **Figure 2**. First, the combination of GO_x and potassium ferricyanide was evaluated for glucose detection (**Figure 2A**). While used commonly in glucose assays, there is a distinct lack of sensitivity (1.8 nA/μ*M*) and a poor lower limit of detection (LLD) of 500 μ*M* at low concentrations in tears. This effect could be attributed to the competitive oxidation of the enzymatic cofactor by O_2, resulting in decreased signal and increased variance. To address this competitive reaction, a second approach was to utilize only O_2 to detect the production of H_2O_2 during the enzymatic oxidation of glucose by GO_x. By incorporating a H_2O_2 catalyst, PB, this product could be measured readily using low magnitude potentials. Repeating the same experiment, it was found that iterative uses of a single sensor within a short time span showed an improved LLD with decreased sensitivity (0.50 nA/μ*M*). This improved LLD is likely attributed to the undiverted flow of electrons into H_2O_2. **Figure 2B** shows the unreplicated response of a single PB assay, which

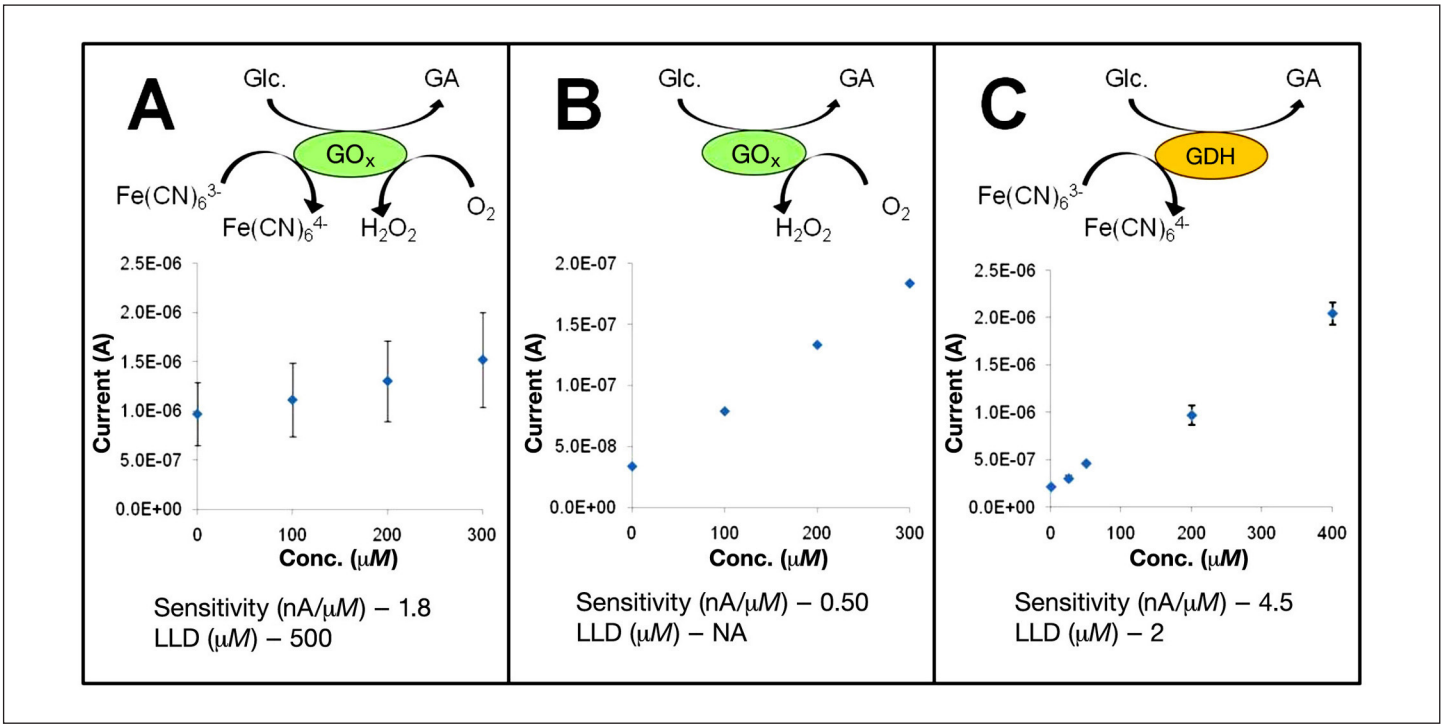

Figure 2. Diagram of the enzymatic reaction and sensor performance, including sensitivity and estimated LLD, for GO_x/ferricyanide **(A)**, GO_x/O_2/PB **(B)**, and GDH/ferricyanide **(C)**. Glc., glucose; GA, gluconic acid. Chronoamperometric measurements were carried out at +0.45, –0.1, and +0.45 volt vs the silver pseudoreference 10 seconds after applying the potential. Error bars represent one standard deviation.

pointed to a promising approach to enhanced glucose LLD. However, it was found that PB sensors lost variable sensitivity over time in aqueous solution, a critical problem for a sensor design that requires prefilling the well with extraction fluid. This lack of stability is noted in some literature, especially in basic solutions, and it was found that the same effect was encountered even in acidic buffers (pH 5.5).

Finally, the enzyme GDH-FAD was evaluated. GDH-FAD offers the advantage of oxidizing glucose; however, its FAD cofactor cannot be oxidized by O_2. Furthermore, unlike other forms of GDH with different cofactors, GDH-FAD does not show sensitivity to other ions in solution or cross-reactivity with other sugars as seen with the pyrroloquinoline quinone and nicotinamide adenine dinucleotide cofactors.[24] **Figure 2C** shows evaluation of the assay using seven different disposable sensors at each concentration. A wider range of glucose concentrations was selected to highlight the improved LLD and range of the assay. Improved reproducibility and sensitivity (4.5 nA/μ*M*) were observed over the other two approaches. This assay enjoys the simplicity and stability of GO_x/ferricyanide and the undiverted electron flow of GO_x/O_2/PB without the drawbacks of either of the other approaches. Through further studies (data not shown), an estimated limit of detection of 2 μ*M* was calculated.

Sampling Material Selection

One of the key functions of the proposed device is the ability to sample tears from the eye. To achieve this, an absorbent polymer featuring biocompatibility, scalability in fabrication, high absorption volume and rate, and reproducible performance would be ideal. Accordingly, three material candidates were identified: calcium alginate, pHEMA, and PU foam. The first material, calcium alginate, is a natural hydrogel created from sodium alginate, a polysaccharide obtained from sea algae that is cross-linked ionically by divalent cations such as calcium. In its wet state, calcium alginate is already saturated and fails to absorb significant volumes of additional fluid. In its dry state the hydrogel matrix collapses and fails to reabsorb similar volumes again. A second material, pHEMA, showed excellent water absorption characteristics. Unfortunately, the rate of absorption was on the order of minutes rather than seconds. While rapid-absorbing forms of pHEMA have been achieved by creating microporous hydrogels,[25] the trade-off in mechanical stability is undesirable. A commercial PU foam was identified that fit all of the required material characteristics and could absorb fluid rapidly into its porous structure. Pressing small cylindrical segments 1 mm in diameter and 0.5 mm in height to the simulated pHEMA eye surface, capture was rapid (<20 seconds) and reproducible on the correct volume scale (4.2 ± 0.5 μl; n = 8). **Table 2** shows a summary of the evaluations of these three materials.

Table 2.
Summary of Material Characteristics for Calcium Alginate, pHEMA, and PU Foam

Material requirement	Calcium alginate	pHEMA	PU foam
Biocompatibility	Yes	Yes	Yes
Scalability	Yes	Yes	Yes
High absorption ratio	No	Yes	Yes
Rapid absorption	No	No	Yes
Reproducibility	No	No	Yes

Model Validation

By isolating each key functional step of the proposed device, values and variances could be estimated for tear sampling, dilution, and glucose sensing. Input parameters for the model included terms defining how glucose would be captured and diluted (V_c, V_e, C_t) and the response of the assay (m, b), as well as associated standard deviation for the terms. Fluid volumes and standard deviations were based on initial tests of capture and fluid injection into the devices. Based on initial sampling testing, V_c was assigned a value of 4.2 μl with a 12% relative standard deviation (RSD). Initial testing using a syringe pump indicated that volumes of fluid on the order of 10 μl could be dispensed reproducibly with 10% RSD. The sensor response from the replicated test in **Figure 2C** was used to obtain values for m (4.5 nA/μ*M*, 10% RSD) and b (220 nA, 8.0%). It was assumed that there was no variation in C_t, as standard stocks were used. These values were entered into the model to estimate the system output for 100 μ*M* glucose using **Eqs. (1)** and **(3)–(7)**(**Figure 3**).

These results indicate two important points. For the first point, it is calculated that an integrated device based on these initial results would have a dilution factor of about 2.4. With a calculated LLD for the glucose sensor of 2 μ*M*, this would put the theoretical LLD of the proposed integrated device at about 5 μ*M*. This LLD is an excellent

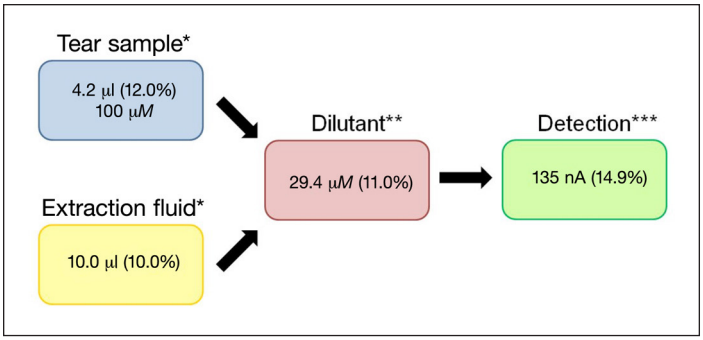

Figure 3. Model of conceptual device function based on isolated experiments. Note that % RSD is shown in parentheses. Initial values (*) were obtained experimentally or calculated using **Eqs. (1)** and **(2)–(5)**(**) or **Eqs. (6)** and **(7)** (***).

level of sensitivity, as many clinical studies have reported glucose micromolar concentrations ranging from the tens to thousands. However, the average volume of tear fluid on the eye is only 7 µl. Thus, it can be anticipated that a final device would need to be reduced in volume to the level of many commercial blood glucose sensors (1 µl or less of fluid).

Second, based on the model it is estimated that 100 µ*M* of glucose tear fluid would yield 135 nA (14.9% RSD). This level of variation is promising. Currently, blood glucose sensor variances in the United States typically range from 3 to 10% for disposable and continuous monitoring systems.[26] With a model estimated 14.9% RSD, the proposed conceptual device is promisingly close for an initial estimation. Once a prototype is constructed, it is likely that system variance could be reduced further.

Conclusion

The topic of glucose in tears has been discussed, and the implications of recent studies have highlighted a need for the development of a disposable device for sampling and sensing glucose in tears. A conceptual device has been presented that integrates an electrochemical sensor and absorbent sampling material with a fluidics system for onboard sample extraction. A mathematical model of the conceptual device has been created, and through initial, isolated experiments the model has been used to estimate the LLD of the device to be 5 µ*M* and the variance at 100 µ*M* to be 14.9% RSD.

There are still significant challenges to overcome in translating a conceptual device based on isolated experiments into a viable integrated sensor. In particular, it will be necessary to address issues of sensor storage stability, sensitivity, and selectivity within the guidelines of accepted "standards" criteria for SMBG devices. Also, single-use sensors will require a scaled fabrication setup capable of reproducibly creating a supply to meet demand. However, advances in electrochemistry, microfluidics, and scalable screen printing and casting fabrication techniques offer promising solutions. Based on these findings, it is concluded that the proposed conceptual device is technically viable. With future work, it can be realized as a potential tool for the study of tear glucose and possibly as a means for diabetes patients to assess their glucose levels.

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Disclosure:

Authors Daniel K. Bishop and Jeffrey T. La Belle have filed a U.S. patent relating to this research.

References:

1. Centers for Disease Control and Prevention. 2007 National Diabetes Fact Sheet. Prevalence of impaired fasting glucose in people aged 20 years or older, United States, 2007 [cited 2009 May 11]. Available from: http://www.cdc.gov/diabetes/pubs/factsheet07.htm.
2. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329(14):977-86.
3. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352(9131):837-53.
4. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352(9131):854-65.
5. American Diabetes Association. Standards of medical care in diabetes mellitus--2009. Diabetes Care. 2009;32 Suppl 1:S13-61.
6. Wagner J, Malchoff C, Abbot G. Invasiveness as a barrier to self-monitoring of blood glucose in diabetes. Diabetes Technol Ther. 2005;7(4):612-9.
7. Michail D, Vancea N, Zolog N. Lacrimal elimination of glucose in diabetic patients. C R Soc Biol Paris. 1937;125:194-5.
8. Leblanc JM, Haas CE, Vicente G, Colon LA. Evaluation of lacrimal fluid as an alternative for monitoring glucose in critically ill patients. Intensive Care Med. 2005;31(10):1442-5.
9. Chen R, Jin Z, Colon LA. Analysis of tear fluid by CE/LIF: a noninvasive approach for glucose monitoring. J Capillary Electrophor. 1996;3(5):243-8.

10. Daum KM, Hill RM. Human tear glucose. Invest Ophthalmol Vis Sci. 1982;22(4):509-14.

11. Daum KM, Hill RM. Human tears: glucose instabilities. Acta Ophthalmol (Copenh). 1984;62(3):472-8.

12. Gasset AR, Braverman LE, Fleming MC, Arky RA, Alter BR. Tear glucose detection of hyperglycemia. Am J Ophthalmol. 1968;65(3):414-20.

13. Lane JD, Krumholz DM, Sack RA, Morris C. Tear glucose dynamics in diabetes mellitus. Curr Eye Res. 2006;31(11):895-901.

14. Sen DK, Sarin GS. Tear glucose levels in normal people and in diabetic patients. Br J Ophthalmol. 1980;64(9):693-5.

15. Taormina CR, Baca JT, Asher SA, Grabowski JJ, Finegold DN. Analysis of tear glucose concentration with electrospray ionization mass spectrometry. J Am Soc Mass Spectrom. 2007;18(2):332-6.

16. Baca JT, Finegold DN, Asher SA. Tear glucose analysis for the noninvasive detection and monitoring of diabetes mellitus. Ocul Surf. 2007;5(4):280-93.

17. Ulrich KT, Eppinger SD. Product design and development. 3rd ed. New York: McGraw-Hill; 2004

18. King-Smith PE, Fink BA, Hill RM, Koelling KW, Tiffany JM. The thickness of the tear film. Curr Eye Res. 2004;29(4-5):357-68.

19. Mishima S, Gasset A, Klyce SD Jr, Baum JL. Determination of tear volume and tear flow. Invest Ophthalmol. 1966;5(3):264-76.

20. Badugu R, Lakowicz JR, Geddes CD. Ophthalmic glucose monitoring using disposable contact lenses--a review. J Fluoresc. 2004;14(5):617-33.

21. Parviz B. Augmented reality in a contact lense. IEEE Spectrum. 2009; September.

22. Mahoney JJ, Ellison JM. Assessing glucose monitor performance--a standardized approach. Diabetes Technol Ther. 2007;9(6):545-52.

23. Ricci F, Amine A, Palleschi G, Moscone D. Prussian Blue based screen printed biosensors with improved characteristics of long-term lifetime and pH stability. Biosens Bioelectron. 2003;18(2-3):165-74.

24. Tsujimura S, Kojima S, Kano K, Ikeda T, Sato M, Sanada H, Omura H. Novel FAD-dependent glucose dehydrogenase for a dioxygen-insensitive glucose biosensor. Biosci Biotechnol Biochem. 2006;70(3):654-9.

25. Oxley HR, Corkhill PH, Fitton JH, Tighe BJ. Macroporous hydrogels for biomedical applications: methodology and morphology. Biomaterials. 1993;14(14):1064-72.

26. Heller A, Feldman B. Electrochemical glucose sensors and their application in diabetes management. Chem Rev. 2008;108(7):2482-505.