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A Disposable Tear Glucose Biosensor—Part 2: System Integration and Model Validation

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Abstract

Background:

We presented a concept for a tear glucose sensor system in an article by Bishop and colleagues in this issue of *Journal of Diabetes Science and Technology*. A unique solution to collect tear fluid and measure glucose was developed. Individual components were selected, tested, and optimized, and system error modeling was performed. Further data on prototype testing are now provided.

Methods:

An integrated fluidics portion of the prototype was designed, cast, and tested. A sensor was created using screen-printed sensors integrated with a silicone rubber fluidics system and absorbent polyurethane foam. A simulated eye surface was prepared using fluid-saturated poly(2-hydroxyethyl methacrylate) sheets, and the disposable prototype was tested for both reproducibility at 0, 200, and 400 μ*M* glucose ($n = 7$) and dynamic range of glucose detection from 0 to 1000 μ*M* glucose.

Results:

From the replicated runs, an established relative standard deviation of 15.8% was calculated at 200 μ*M* and a lower limit of detection was calculated at 43.4 μ*M*. A linear dynamic range was demonstrated from 0 to 1000 μ*M* with an R^2 of 99.56%. The previously developed model predicted a 14.9% variation. This compares to the observed variance of 15.8% measured at 200 μ*M* glucose.

Conclusion:

With the newly designed fluidics component, an integrated tear glucose prototype was assembled and tested. Testing of this integrated prototype demonstrated a satisfactory lower limit of detection for measuring glucose concentration in tears and was reproducible across a physiological sampling range. The next step in the device design process will be initial animal studies to evaluate the current prototype for factors such as eye irritation, ease of use, and correlation with blood glucose.

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Abbreviations: (BG) blood glucose, (GDH-FAD) glucose dehydrogenase with flavin adenine dinucleotide, (LLD) lower limit of detection, (PBS) phosphate-buffered saline, (PDMS) poly(dimethylsiloxane), (pHEMA) poly(2-hydroxyethyl methacrylate), (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

As described in the article by Bishop and colleagues featured in this issue of *Journal of Diabetes Science and Technology*, there is a need for a less invasive method to self-monitor glucose levels for diabetes patients.[1] Tear glucose (TG) has been investigated since the 1930s. A vast majority of the studies demonstrated a correlation between blood glucose (BG) levels and tear glucose levels with a ratio of between 10 and 100 times less glucose in tears than in blood. While numerous studies have found correlation, discrepancies still exist between studies that can be explained by a variation in the collection technique.[2] A need exists for a sensitive, easy-to-use, low-cost, minimally invasive sensor for standardized measurements. Some specifications were established as highly critical for a practical device, including reproducibility and linearity over a broad dynamic range, as well as a suitable lower limit of detection (LLD). The feasibility of a device that meets these specifications was described previously. In the previous report, data from tear glucose studies were summarized,[3,4] providing insight into the integrated fluidics-capture system.

The medical device and diagnostics industry still follows the basic steps of standard engineering device design.[5,6] Steps of medical device design include determining the need for the technology, developing specifications from the user's needs, feasibility testing that examines the state of the art, developing alternative designs, investigating engineering models, and deciding how to translate the device to manufacturing.[7] In this case, a review of previous TG research highlighted a need for a disposable TG sensor that specifically filled the niche for a standardized research tool capable of direct translation into a medical device. By following the steps of engineering design, a robust device concept has been developed that avoids issues of quality control and failure modes.[8–11]

Described here is further progress in TG sensor development and testing of a fully functional prototype. Topics addressed include material selection[12] in relation to manufacturing, design and fabrication of the fluidics system, and testing integrated function of the working prototype. The progress here showcases the viability of the proposed concept and sets the stage for exciting new work in the future.

Methods

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). All solutions were prepared in phosphate-buffered saline (PBS) at pH 7.4 unless otherwise specified.

Fluidics System Fabrication

For fabrication of the fluidics system, a two-part mold was created for casting. A design was made in SolidWorks (Dassault Systèmes SolidWorks Corp., Concord, MA) software and then used to fabricate the two-piece mold on a MAXNC 10 CL-EC, three-axis CNC mill (MAXNC, Gilbert, AZ). The mold pieces were made from a ½-inch-thick acrylic plastic sheet (Desert Star Plastics, Phoenix, AZ) or aluminum T-6061 (Online Metals, Seattle, WA) for faster heating. For casting, a 10:1 (elastomer:curant) mixture of poly(dimethylsiloxane)(PDMS), Dow Corning Sylgard 184 (Ellsworth Adhesives, Germantown, WI) was mixed thoroughly and then degassed in a vacuum to remove air bubbles. The two-part mold was sprayed with a silicone mold release (Ease Release 200, Mann Release Technologies, Easton, PA) and then clamped together. The PDMS was then injected into the mold using a syringe with a 16-gauge needle and cured at 70°C for 15 minutes. The "soft" cured parts were then removed and cured further at 60°C for 12 hours. For assembly, the fluidics systems were washed with a detergent (Alconox, VWR International, White Plains, NY), then water, then ethanol, and then air dried. The clean fluidics systems were fixed to a screen-print sensor by applying a thin layer of uncured PDMS to the piece before pressing the components together carefully in a custom jig and heating at 60°C for 8 hours. Small segments were punched out of a sheet of absorbent commercial polyurethane foam, and one segment was inserted carefully into the sampling well of each fluidics system. Finally, an enzymatic assay containing 1 mg/ml GDH-FAD and 100 m*M* potassium ferricyanide in PBS was injected into the sensing well of the device at a flow rate of 0.1 ml/hr for controlled amounts of time to dispense a fixed fluid volume and then immediately tested.

Tear Sampling Simulation

In order to evaluate the performance of the prototype, a simulation of tear fluid on the eye was created. A thin sheet of 5 × 5-cm poly(2-hydroxyethyl methacrylate) (pHEMA) was soaked in PBS and then placed on a petri dish. Prior to sampling, the dish was tilted to pour off all excess fluid, leaving a soft, hydrophilic surface with a very thin layer of fluid dispersed across its surface. This setup roughly approximates the soft tissue of the eye with a thin distribution of tears across it. For sampling, a prepared device was pressed gently against the surface of the pHEMA to saturate the foam. The device was then set flat on the bench, and the sensing well was depressed repeatedly to extract the tear sample. A chronoamperometric measurement was made immediately. The entire process from sampling to sensing took approximately 120 seconds. Sheets were soaked in PBS with various concentrations of glucose to test different concentrations. In the reproducibility study, new seven sensors at each of the concentrations (0, 200, and 400 μ*M*) were tested. For the dynamic range study, one new sensor was used at each concentration to measure the response from 0 to 1000 μ*M* glucose in 200 μ*M* increments.

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.

Results and Discussion

Fluidics System

Initially, the well area also served as the sensing area, so this dimension was fixed. An initial design in computer-aided design was made (**Figure 1**), but this was later redesigned to hold the adsorbent PU foam. Next, the mold design was fabricated in acrylic in two pieces to facilitate separation and removal of the casted parts (**Figure 2**) and prototypes were cast (**Figure 3**). This initial design had a relatively large channel for fluid movement, which resulted in a 41.8-μl volume. This was found to cause an unsatisfactory dilution factor, and the next design decreased both the length and the width of the channel. For further iterations, the sample volume was reduced further by decreasing the heights as seen in **Table 1**. The final fluidic device had a volume of 5.8 μl, which resulted in a dilution factor of 3.5. Referring to the most recent large-scale study of over 100 diabetic and nondiabetic individuals, mean tear glucose concentrations

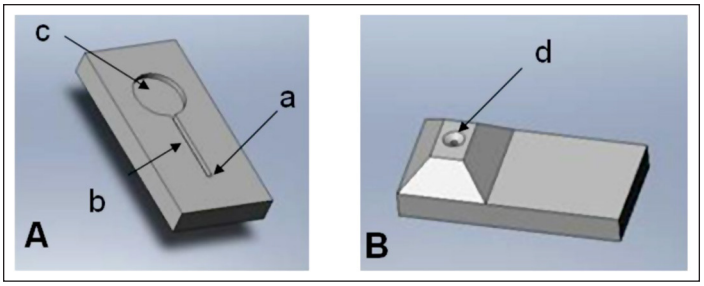

Figure 1. A computer-aided design schematic of the proposed fluidics portion **(A)** of the device showing underlying fluidics from the sample inlet (a), channel with length, width, and height varied (b), and reservoir (c) and **(B)** interface to be in contact with the conjunctiva (d).

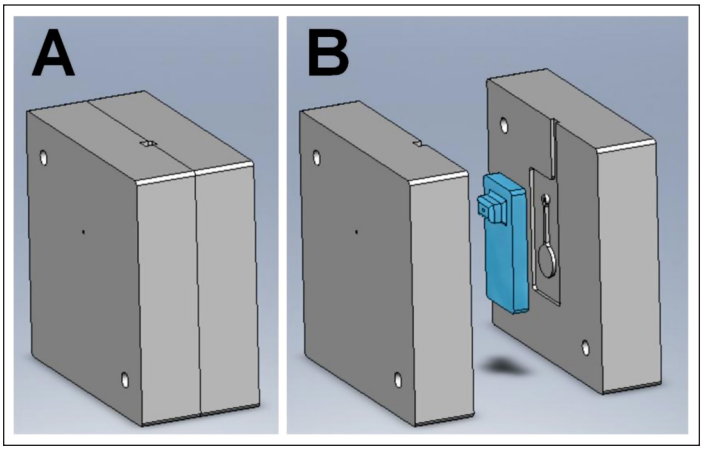

Figure 2. A computer-aided design (CAD) schematic of the mold assembly showing both halves of the mold **(A)** and CAD of the actual part fabricated **(B)**.

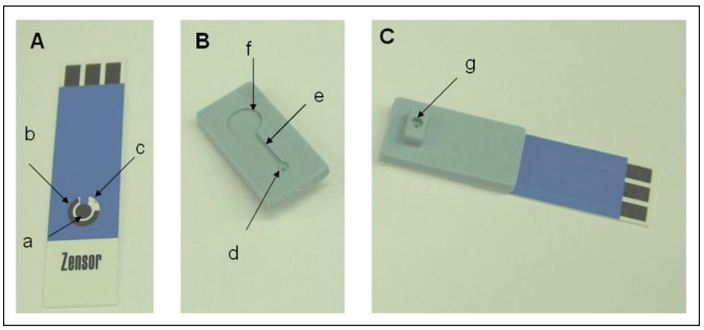

Figure 3. (A) Screen-print electrode with working (a), counter (b), and reference (c) electrodes. **(B)** The microfluidic capture system can be seen with the sample inlet (d), channel (e), and sensing well (f), which also acts as the pump. **(C)** The inset (g) used to hold the biocompatible capture material is shown.

after carbohydrate load were 0.35 ± 0.04 and 0.16 ± 0.03 m*M*, respectively.[13] A dilution factor of 3.5 puts these mean concentrations well within the sensing limits of our assay. From these prototyping results, it has become apparent that fluidic design is a primary challenge. In order to reduce variation in fluid sampling, sampling sponges must be allowed to fully saturate at a volume below the anticipated range of tear volumes on the eye. This may call for further optimization in the future to reduce dilution volumes as sample size continues to scale down.

Electrochemical Detection

Operation of the device once assembled is simple (**Figure 4**). Simply touching the device to a moist surface allows for the adsorbent layer to absorb a fixed volume of fluid. Then, by applying and removing slight pressure onto the reservoir-pump region, mixing of reagents and the sample occurs and the sensor is ready for detection. In **Figure 4**, an ultraviolet light shows the clean dispersal of the "sample," here a fluorescent dye, rhodamine 6G. The simple functionality of this device is critical for a successful design. Approaches to tear glucose measurement have been hampered by the challenge of integrating a sensitive sensing technology with an easy approach to sampling.

A reproducibility (n = 7) study (**Figure 5A**) was performed at concentrations of 0, 200, and 400 μ*M* glucose in the fully integrated system. Current levels were recorded over time, and the current at 9.9 seconds was recorded (time to reach ~95% response time) and plotted against the concentration. A relative standard deviation (RSD) of 15.8% was measured at near physiological levels of TG (200 μ*M*) using seven separate devices. Comparing these results with the error estimation model developed in Part 1 of this work, the estimated RSD for the system was 14.9%, supporting the accuracy of the model. A test was performed over the dynamic range of 0 to 1000 μ*M* in increments of 200 μ*M* (**Figure 5B**). A linear regression was calculated with an R^2 of 99.56%. This result demonstrates that the device is capable of glucose within the concentration range reported by the majority of previous tear glucose research. Next, the baseline (0 μ*M*) standard deviation was later used to calculate limits of detection. From these data and previous estimates of the baseline standard deviation (×3), a LLD of 43.4 μ*M* was calculated. This result was eight times higher than expected. Comparing the linear regression from our previous work with this result, there was a 7.2 times decrease in the response slope for new data. This accounts for the majority of the error in the estimation of LLD. One possible reason for this decrease in sensitivity is nonideal sample extraction. Also, the slight increase in RSD can likely be attributed to the several steps in sensor assembly, which require manual

Table 1.
Fluidics Dimensions from Fabricated Systems and Estimated Total Volumes

Well area (mm^2)	Channel length (mm)	Channel width (mm)	Channel height (mm)	Total volume (μl)
37	9.4	1.9	0.76	41.8
37	5.6	1.5	0.76	34.6
37	5.6	1.5	0.51	23.1
37	5.6	1.5	0.25	11.5
37	5.6	1.5	0.13	5.8

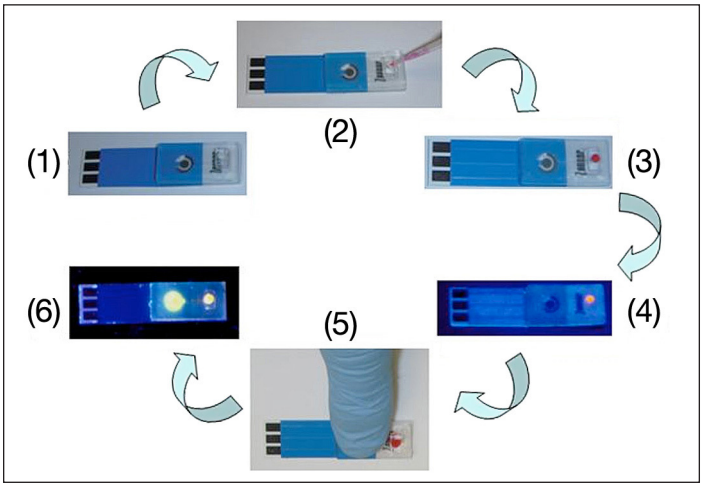

Figure 4. Schematic of operation. **(1)** A sensor is prepared, and the sample is pipetted **(2)** into the foam capture material **(3)**. Under 365-nm [ultraviolet (UV)] stimulation the dye can be visualized **(4)**. When pressure is applied to the sensing region **(5)** and released **(6)** under UV stimulation, the dye can be seen to flow down the channel and into the sensing region itself.

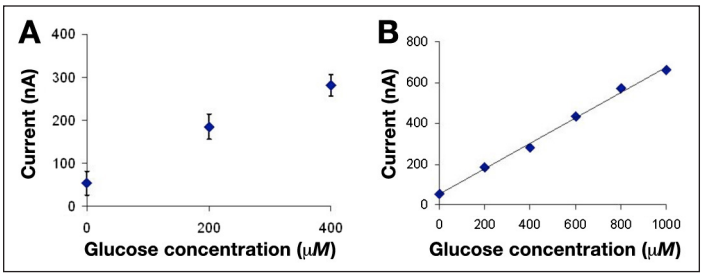

Figure 5. (A) Experimental results demonstrating the reproducibility of sampler electrodes at 0, 200, and 400 μ*M* glucose concentrations (n = 7). **(B)** Experimental results demonstrating dynamic range with 0 to 1000 μ*M* of glucose in 200 μ*M* steps with the line representing a linear regression of data with an R^2 of 0.9956.

assembly and may introduce variation unaccounted for in the model.

Conclusion

The prototype presented herein is now capable of detecting physiological glucose concentrations within the ranges commonly presented in the literature. Namely, linearity over the range of 0 to 1000 μ*M* (R^2 of 0.9956) and 15.8% RSD reproducibility of the device have been demonstrated. Assessment of device variability matches predicted models presented previously. Further, the disposable prototype is manufactured readily in modest quantities using standard fabrication technologies.

Looking forward toward future testing, it is important to recognize the limitations of metrics such as RSD for assessing the functionality of self-monitoring of blood glucose (SMBG) technology. Measurement errors only become significant when they change the user's decision to manage their BG; perhaps a "take reading again" error message could be used to avoid potential and harmful erroneous errors, if any occur. Much like a standard Clarke error grid used currently to assess SMBG technologies, it will be necessary to consider not only the precision and accuracy of this TG biosensor, but also how data gathered will be used. Developing an understanding of this process will require studies of TG/BG correlation and kinetics in animal or human models.

A second important consideration for the device will be the anticipated acute and chronic effects and complications of sampling tears from the eye. Future testing in animal models is warranted and will include an investigation of other factors, such as irritation on the eye with both acute and chronic use. Furthermore, animal studies will help determine the ergonomic design of the sensor to permit eventual self-testing in human trials. In addition, tissue analysis will identify any local histologic changes when using the sensor on a chronic basis. Due to the vast neurosensory innervations of the ocular surface, methods will need to be developed to minimize tissue trauma and assure a painless sampling for the tear film. Histologic analysis will determine any detriment to long-term, chronic use of the sensor. A correlation to BG will need to be evaluated and improvements will need to be made to reduce artifacts from interferents found commonly in complex solutions represented by tear fluid. This novel device offers a promising new tool for future research to expand our understanding of TG toward the ultimate goal of painless BG measurement.

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Disclosure:

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

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