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The Clinical Accuracy of Non-Invasive Glucose Monitoring for *ex vivo* Artificial Pancreas

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Summary

Diabetes is a serious worldwide epidemic that affects a growing portion of the population. While the most common method for testing blood glucose levels involves finger pricking, it is painful and inconvenient for patients. We compared the glucose levels measured non-invasively from tears to those obtained from capillary blood in 10 diabetic and non-diabetic patients. In addition, we assessed the clinical accuracy of the newly developed non-invasive tear glucose monitoring system using a Clarke Error Grid Analysis (EGA) and tested if such a relationship is individualized or universal. A glucose meter circuit was built to analyze capillary blood and right- and left-eye tear fluids, and the results were compared with those of a commercial glucose meter, which was used as the reference in the EGA. The prediction of glucose values from the voltages using linear regression showed the non-invasive system to be clinically accurate and universal. This result suggests that the coupling of a non-invasive glucose monitoring system, such as one that detects glucose in tears, with an insulin pump may be clinically feasible as an *ex vivo* artificial pancreas treatment. By employing this *ex vivo* artificial pancreas, many of the complications associated with frequent hyperglycemia and hypoglycemia events could be avoided, increasing the quality of life.

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Introduction

Diabetes is a serious worldwide epidemic affecting a growing portion of the population (1). According to the American Diabetes Association (2014), "In 2012, 29.1 million Americans, or 9.3% of the population, had diabetes." Diabetes is a disease where the blood glucose level in a person's body is elevated above and below the normal range due to metabolic issues (3). Insulin is a hormone that is responsible for signaling cells to take up glucose from the blood following the digestion of foods, such as starches and sugars, which elevate the blood glucose levels that the human body needs in order to function (4, 5, 6). Without insulin, the body would not be able to breakdown glucose to produce energy (7). The three types of diabetes are (i) Type 1 (T1D), (ii) Type 2 (T2D), and (iii) gestational diabetes (5). T1D, also labeled 'juvenile diabetes,' typically starts at early ages (children, teenagers, or young adults). While T2D is the most common type of diabetes and may develop at any age, both T1D and T2D require lifelong management and treatment (3). To maintain a stable insulin level, some T2D patients only take oral medication (8). However, a significant number of T2D patients and all T1D patients must test their blood glucose levels multiple times a day. They then inject insulin, based on the blood glucose levels, to artificially maintain their glucose level (8). The least common form of diabetes, gestational diabetes, affects women during pregnancy (5).

The most common and traditional method for testing blood glucose level is with finger-prick lancet devices, but this method is considered painful and inconvenient for the patient (9). Because of this inconvenience, many patients limit the measuring of their blood glucose levels, a habit that leads to ineffective diabetes management (10). While, over the years, the traditional method of drawing blood to assess blood glucose levels has been adjusted to require less blood, the process is still invasive (7, 11). Thus, there has been a significant investment in diabetes research over the past two decades to develop non-invasive methods to detect blood glucose levels (12). Recently, there have been advances in the development of nano-sensors that can detect glucose non-invasively. One such technological breakthrough is the development of contact lenses with nano-sensors that can detect the glucose level in human tears and operate using tear fluid as its energy source. Such a nano-sensor coupled with a Continuous Subcutaneous Insulin Infusion (CSII) device, also known as an insulin pump, will enable the development of a closed-loop system to emulate an *ex vivo* artificial pancreas (See **Figure 1**).

Tears contain a multitude of biomarkers (for example, glucose) that are also found in blood (13). However, overall tear fluid collection appears to impact the glucose concentration level (14), and attention has been drawn to the tears collection method done in previous studies. It is important to collect tears using a basal method, because irritating the eye increases tear production and may dilute

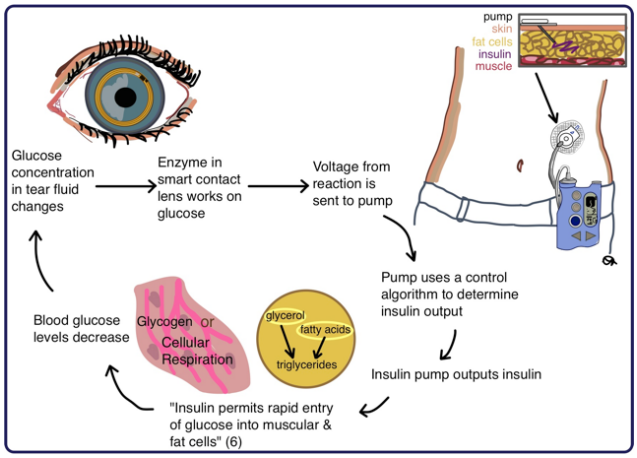

Figure 1. A diagram of the *ex vivo* artificial pancreas closed-loop system.

the glucose concentration in the tears (10). Past studies have collected tears using a capillary tube between the lateral canthus and the lacrimal punctum of the eye to avoid irritation. Moreover, measuring the glucose level in human tears appears to be challenging (15). Prior studies have used advanced chemical analysis tools, such as high-current liquid chronography and electrospray ionization mass spectrometry (15). However, these tools are very expensive as well as invalid when it comes to transforming the tool (or method) into a non-invasive glucose meter, like the "smart" contact lenses. Thus, Cha *et al.* (2014) performed an initial study using commercial glucose meter strips with traditional glucose detection enzymes (such as pyrroloquinoline quinone–dependent glucose dehydrogenase, PQQ-GDH).

While the typical blood glucose level of non-diabetic individuals is around 60 to 120 mg/dL, blood glucose levels in a diabetic individual can fluctuate from 40 to over 400 mg/dL. As a result, the current commercial glucose meters and commercial glucose meter strips are able to detect glucose levels between 20 to 600 mg/dL. However, Cha *et al.* (2014) found that the glucose level in tears is somewhere between 1 to 10 mg/dL for a non-diabetic and 2 to 35 mg/dL for a diabetic patient (17). Because of the low level of glucose in tears, if a commercial glucose meter were to assess tear fluid, the glucose meter would read 'Lo' (low reading). Moreover, Liao *et al.* (2012) noted, "Integrating biosensors on a contact lens would provide a noninvasive way for continuously sensing metabolites in tear fluid" (See **Figure 1**). Nevertheless, it was noted that "there are limited reports on the relationship between tear and blood glucose concentrations" (19). Additionally, such investigators have indicated that further research is needed to better understand the clinical accuracy of the correlation between the glucose level in tears to that in capillary blood (19, 20). Thus, we developed a glucose meter sensitive enough to assess glucose levels in tear fluid and investigated its accuracy in a clinical setting.

Clarke Error Grid Analysis (EGA) has been the "gold standard" for evaluating the accuracy of the correlation between the actual glucose level of the subject to the glucose level reported by the glucose meter device (21). As a matter of fact, the US Food and Drug Administration (FDA) has been using the EGA as their evaluation standard for new glucose monitoring devices. Therefore, the aim of this study was to compare the glucose in tears collected non-invasively to the glucose in capillary blood drawn from both diabetic and non-diabetic patients and to assess whether or not the clinical accuracy of a non-invasive tear glucose monitoring system is individual to each patient or is universal (such as in commercial glucose meters) using the EGA. We hypothesized that when we compared the glucose levels from tears to those in capillary blood drawn from diabetic and non-diabetic patients, the correlation would be universal, such as for a commercial glucose meter, and we found this to be true.

Results

EGA was used to measure the clinical accuracy of our glucose monitoring system, and a Pearson correlation measured the relationship between the predicted tear glucose values and the commercial glucose meter values. The Pearson correlation was computed between the four measured sets of data: (a) commercial glucose meter values of capillary blood; (b) the predicted values from the glucose meter circuit voltage peak of capillary blood; (c) the predicted values from the glucose meter circuit voltage peak of left-eye tear fluid; and (d) the predicted values from the glucose meter circuit voltage peak of right-eye tear fluid. In total, 160 experimental measures were extracted: a, b, c, and d, for four time periods: after fasting, 30 minutes, 60 minutes, and 90 minutes after consumption of a small breakfast sandwich, for each of the 10 patients (4 measures x 4 times x 10 patients). The constant or control variables were the room temperature, the humidity in the room, the commercial glucose meter (Accu-Check), the developed glucose meter circuit, the capillary blood collection method and location, the tear collection method and location, and the PQQ-GDH Enzyme used (Accu-Check Strips).

The two manipulated or independent variables were: (i) the glucose collection source (capillary blood vs. tears) and (ii) the presence of diabetes (diabetic vs. non-diabetic patient). The responding or dependent variable was the clinical accuracy as measured by the EGA, which compared the glucose levels measured by a commercial glucose meter to the glucose levels predicted from the voltage (V) in the glucose meter circuit; voltage which

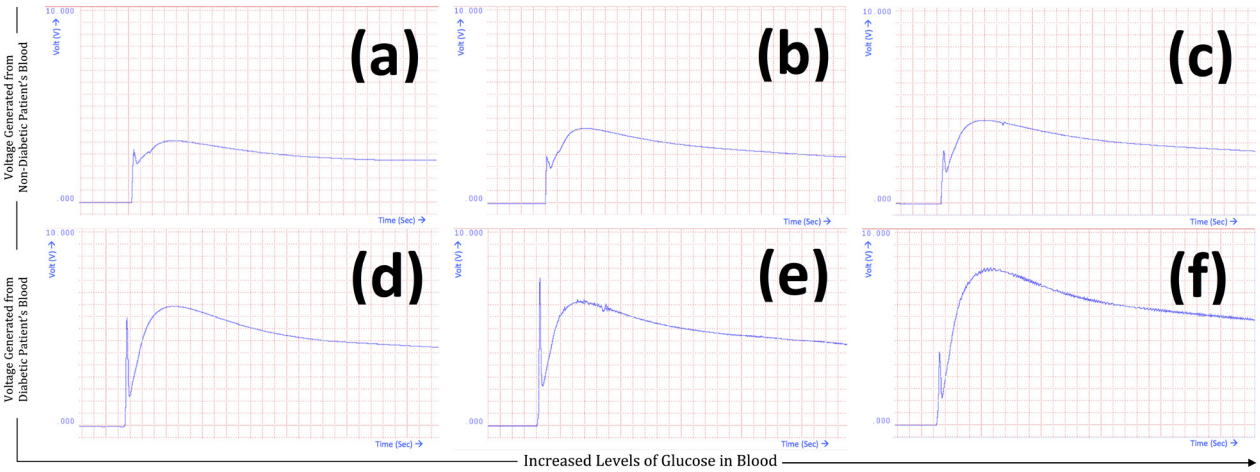

Figure 2. Samples results of capillary blood glucose meter circuit voltage graphs. The glucose meter circuit voltage graphs were collected with a DATAQ® data acquisition unit for the capillary blood of non-diabetic (a–c) and diabetic patients (d–f).

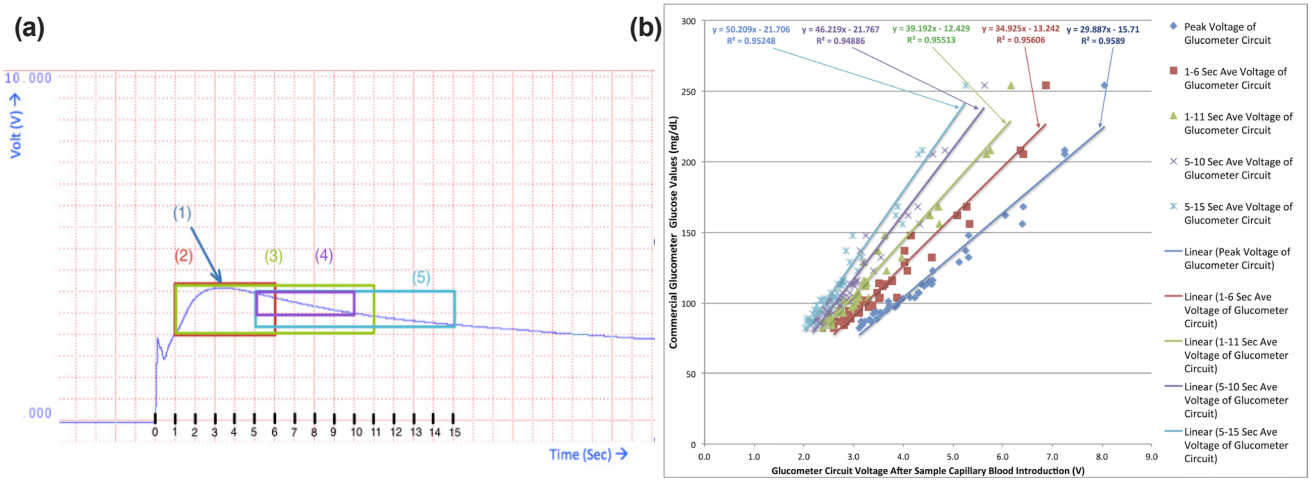

Figure 3. Glucose meter circuit calibration using capillary blood. (a) Five models of calibration for the glucose meter circuit using capillary blood. **(b)** The glucose meter circuit was calibrated across different aggregation techniques and linear regression models (n = 40 experiments). The model with the highest predicting accuracy was the peak voltage.

resulted from the introduction of tear fluid or blood. The sharp increase (spike) in voltage after the introduction of capillary blood to the glucose meter strip with PQQ-GDH Enzyme is due to the initial interaction of the enzyme with the capillary blood and was ignored. **Figure 2** shows our glucose meter circuit voltage graphs, collected with a DATAQ® data acquisition unit, for the capillary blood of non-diabetic (**a–c**) and diabetic patients (**d–f**).

To assess the clinical accuracy of the tear glucose monitoring system, the glucose meter circuit first needed to be validated using measurements of capillary blood. Measurements from the commercial glucose meter (the reference values on the y axis of **Figure 3**) and the glucose meter circuit voltage were collected and divided into the: (1) peak voltage (past the initial spike); and the average voltage (2) 1–6 seconds after introduction; (3) 1–11 seconds after introduction; (4) 5–10 seconds after introduction; and (4) 5–15 seconds after introduction. A

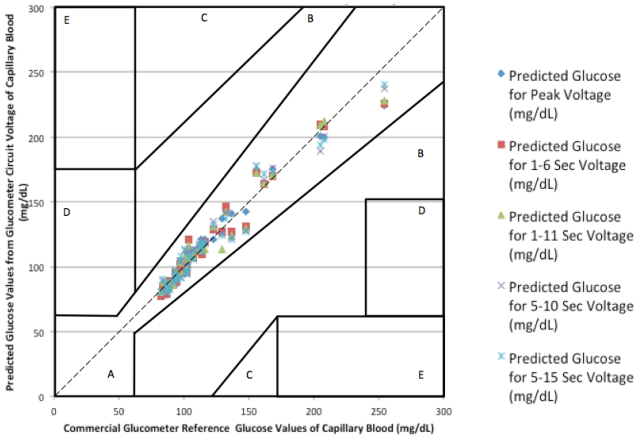

Figure 4. Clarke error grid analysis (EGA) for capillary blood in the glucose meter circuit. All 200 data points are within Region A, indicating that the developed glucose meter circuit has high clinical accuracy.

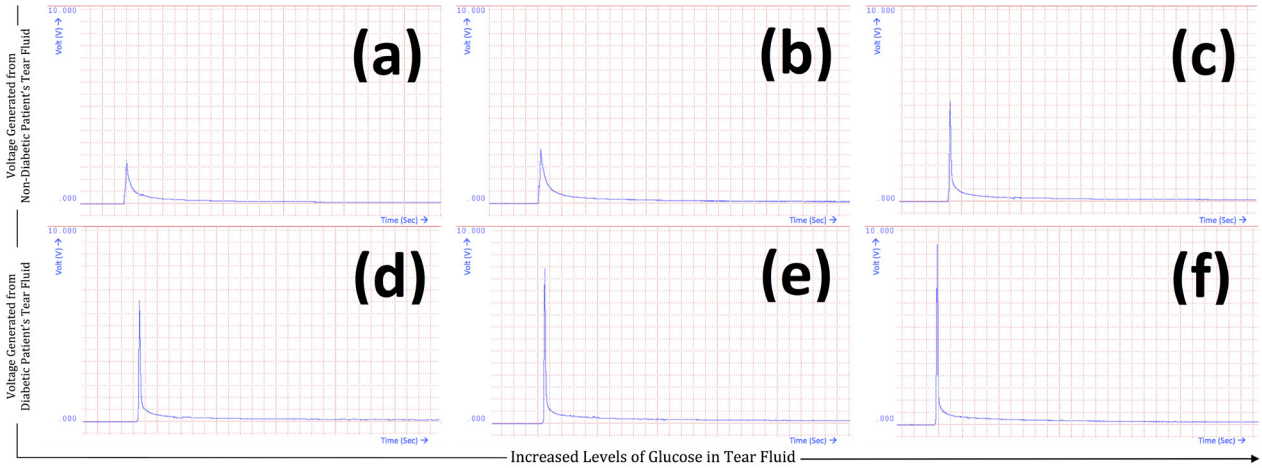

Figure 5. Sample results of tear glucose meter circuit voltage graphs. The glucose meter circuit voltage graphs were collected with a DATAQ® data acquisition unit for tears of non-diabetic (a–c) and diabetic patients (d–f).

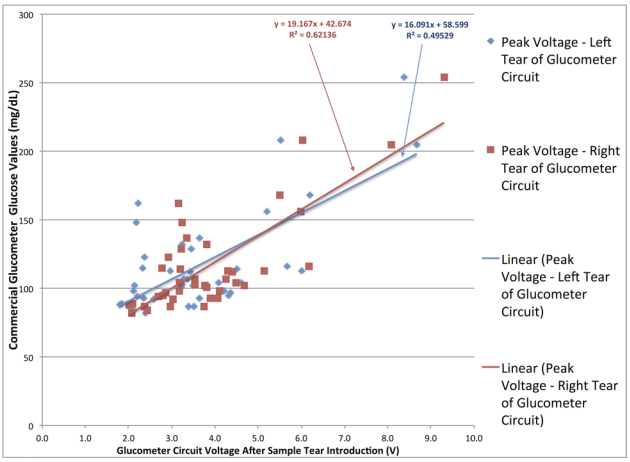

Figure 6. Circuit calibration for tears. The glucose meter was calibrated for tear samples using different aggregation techniques and linear regression models (n = 80, 40 Left + 40 Right Eye).

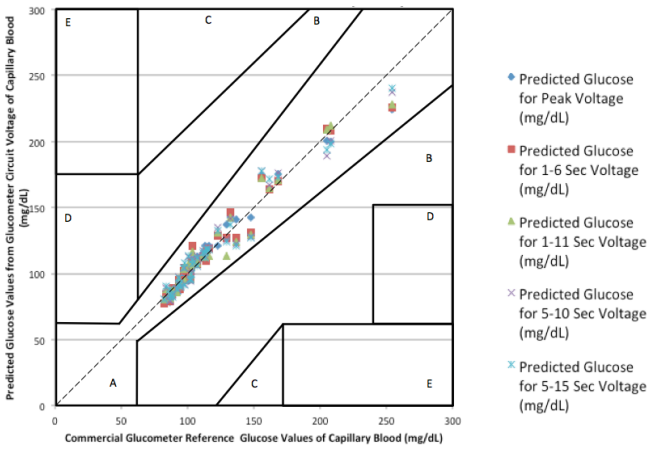

Figure 7. Clarke error grid analysis (EGA) for tears in the glucose meter circuit. All 80 data points (40 Left + 40 Right Eye samples) were within Region A and B.

linear regression was computed for each of these data sets (**Figure 3**). The model with the highest predicting accuracy (R^2 = 0.9589) was the peak voltage (Model 1), and the second highest predicting accuracy (R^2 = 0.9561) was the 1–6 seconds average of capillary blood (Model 2). The equations for all five linear regressions were used to compute the predicted glucose values of the capillary blood when measured by the circuit. The predicted glucose values for all five models were then plotted against the reference commercial glucose meter values, and all 200 data points (5 models x 40 measured points) were in Region A of the EGA (**Figure 4**), indicating the developed glucose meter circuit device had high clinical accuracy with capillary blood, which also indicates that both the circuit and the models are calibrated.

After calibrating the glucose meter circuit with capillary blood, a similar process was done with right- and left-eye tear fluid (**Figure 5**). The glucose meter circuit voltage, after the introduction of the tear fluid, was plotted against the commercial glucose meter values for capillary blood. Two linear regression models were computed (one for each eye, R^2_{Right}=0.622 & R^2_{Left}=0.495) and used as the equations to calculate the corresponding predicted glucose values from the glucose meter circuit voltage for tear fluid. Then, the commercial glucose meter values were plotted against the predicted glucose for the EGA (**Figure 7**). The 80 data points (40 left & 40 right) all were in Region A and Region B of the EGA.

In order to test for the universality of the non-invasive glucose monitoring system based on tears developed in this study, a Pearson correlation was calculated between the four measured sets of data: (a) commercial glucose meter values of capillary blood; (b) the predicted values from the glucose meter circuit voltage peak of capillary blood; (c) the predicted values from the glucose meter circuit voltage peak of left-eye tear fluid; and (d) the predicted values from the glucose meter

		Commercial Glucometer	Predicted Cap. Blood	Predicted Tear Left	Predicted Tear Right
ComGluc	Pearson Correlation	1	0.978**	0.711**	0.796**
	Sig. (2-tailed)	(N=40)	0.000	.000	0.000
PredCapB	Pearson Correlation	0.978**	1	0.709**	0.767**
	Sig. (2-tailed)	0.000	(N=40)	0.000	0.000
PredTearL	Pearson Correlation	0.711**	0.709**	1	0.812**
	Sig. (2-tailed)	0.000	0.000	(N=40)	0.000
PredTearR	Pearson Correlation	0.796**	0.767**	0.812**	1
	Sig. (2-tailed)	0.000	0.000	0.000	(N=40)

** Correlation is significant at the 0.01 level (2-tailed).

Table 1. Pearson correlations table of commercial glucose meter values vs. Predicted capillary blood, right tear, and left tear values (n=160 total measures)

circuit voltage peak of right-eye tear fluid. The Pearson correlations were all significant ($p < 0.001$) including the commercial glucose meter measurement of capillary blood vs. the predicted values from the glucose meter circuit voltage peak of capillary blood ($r_1 = 0.978$, $p_1 < 0.0001$), the commercial glucose meter measurement of capillary blood vs. the predicted value from the glucose meter circuit voltage peak of left-eye tear fluid ($r_2 = 0.711$, $p_2 < 0.0001$), and the commercial glucose meter measurement of capillary blood vs. the predicted value from the glucose meter circuit voltage peak of right-eye tear fluid ($r_3 = 0.796$, $p_3 < 0.0001$) (**Table 1**). Moreover, using Fisher *r*-to-*z* transformation, the results for the right- and left-eye correlations were compared, and we found that the two were not significantly different ($p = 0.1977$), which indicates that either eye can be used. So, while different people are naturally going to have different glucose levels, the correlations between their blood, left-eye tear fluid, and right-eye tear fluid appear to be significant. As such, the clinical accuracy of the non-invasive tear glucose monitoring system is universal, meaning that such technology does not have to be individually calibrated and is indeed a promising technology for improving the quality of life of diabetic patients.

Discussion

According to this clinical experimental research study, a non-invasive tear glucose monitoring system is clinically accurate and, therefore, can be universally accepted like a commercial glucose meter. Many diabetics find the most common method of glucose monitoring, finger pricking, inconvenient as well as uncomfortable. Findings from this study are significant, as they provide a contribution to the greater scientific community and explore the development of a non-invasive glucose monitoring system that may eliminate the need for diabetics to prick their fingers several times a day. Moreover, they may assist those with diabetes, endocrinologists, ophthalmologists, and biomedical scientific communities to advance one step closer to the development of the ultimate artificial pancreas (22).

The results of this study support the hypothesis that the glucose measurements collected non-invasively from tears agrees with those taken from capillary blood. According to EGA, the clinical accuracy between the tear glucose levels and capillary blood glucose levels is universally acceptable.

This exploratory experimental study had a few limitations. One limitation was that, while acceptable relative to similar studies in the field, the number of patients was still relatively small, with only three diabetic participants. A larger pool of diabetics might have provided a more compelling argument and may have provided additional information on how accurate the measurement of glucose from tears can be for highly fluctuating levels. Moreover, the data collected in this clinical study included multiple measurements from 10 participants, thus the data is not independent, and independence is a fundamental assumption of many statistical methods. Therefore, future research could benefit from accounting for these repeated measurement situations, especially if the sample of participants permits it. Another issue is the randomization of the eye stimulation. While this study did not randomize the order of eye of collection (L/R, R/L, etc.), future research should consider the randomization, as it may have an effect on the results. Another limitation is that the study did not include patients using contact lenses. The use of contact lenses may have affected the study, because people who wear contact lenses may have had drier eyes than people who do not wear contact lenses. Additionally, another limitation of the study is with the enzyme used. While the PQQ-GDH enzyme has been widely used for glucose meter strips, it is very expensive (~$1/strip) and disposable (*i.e.*, can only be used once). Therefore, using a different enzyme that can provide glucose voltage from tears continuously, even for few days, might have provided different results then the PQQ-GDH enzyme and may require additional investigation for its clinical accuracy. However, the findings of this study appear to provide a promising avenue for additional clinical and non-clinical research studies.

The findings of this study also indicate that monitoring blood glucose level from tears in a non-invasive way is clinically acceptable. Because the universal model for

monitoring blood glucose level from tears appears to be clinically acceptable, it would be easier for manufacturing companies to pre-program this non-invasive glucose monitoring system, much like commercial glucose meter manufacturers currently do. The coupling of such a device along with an insulin pump would form a closed-loop *ex vivo* artificial pancreas. This coupling would insure not only the timely injections of insulin, but also the automatic, appropriate, and frequent adjustments to the amount of insulin injected (**Figure 1**). This would significantly lower the number of complications that arise from frequent hypoglycemic and hyperglycemic events, leading to weight gain or blindness, and therefore, would increase the quality of life of diabetic patients. Moreover, given that a non-invasive glucose monitoring system is feasible if integrated into a "smart" contact lens, this *ex vivo* artificial pancreas would not only be able to be commercially available, but also would significantly help diabetic patients.

This *ex vivo* artificial pancreas would furthermore be advantageous for the diabetic patient, because they would not need to prick their fingers (less invasive), and they will most likely only have to wear one "smart" contact lens. Given that the Pearson correlation coefficient between the glucose levels measured in the right eye and those in capillary blood was higher than that of the left eye, although not significantly different, it is recommended that only a contact lens on the right eye will be sufficient. Additional work should be done in this area of research to investigate other factors that have not been explored in this study including people who wear contacts, additional diabetic patients, and the testing of different continuous enzymes and non-enzymatic methods.

Materials and Methods

Prior to experimentation, we constructed the glucose meter circuit. The glucose meter circuit was constructed using a 3-panel breadboard, 20 breadboard cable jumpers (red, black, and green), one LM358 microprocessor amplifier, one LF356 microprocessor amplifier, one 100-kΩ resistor, two 3.3-kΩ resistors, one 82-kΩ resistor, two 1-μF capacitors, one multimeter, and two miniature test hook clips (black and red). The glucose meter circuit was connected to a low voltage AC/DC power supply that was set to 5V DC, attached to a data acquisition unit (DATAQ®), and also to a computer via a USB port (**Figures 8a & 8b**). The glucose meter circuit has a sensitivity level of 0.1 mV, which is equivalent to ~0.195 mg/dL in right-eye tear fluid detection.

Prior to the data collection, the research underwent an Institutional Review Board (IRB) and Student Research Committee (SRC) approval. All patients used in this study first signed a Human Informed Consent Form, agreeing that they understand the procedure, benefits, and minimal risks of the study, along with their ability to quit the study at any time. No patients used were from vulnerable populations (minors, pregnant women, prisoners, mentally disabled, or economically disadvantaged) or had prior or current respiratory and nasal cavity issues (Diseases/Problems/Disorders/Pain/etc.). The 10 participants in this study included 4 females and 6 males. Three of the participants were diabetic patients and seven were not. Out of the diabetic patients, two were insulin dependent and one was being treated with oral medication (250 mg Metformin). Average age of the participants was 39.6 years old, ranging from 18 to 68 years old (Std.Dv = 17.2).

Licensed medical providers conducted all data collection of bodily fluids in a medical office/clinic in Miami. After confirming that the patient fasted, the data acquisition software was opened on the computer, and Channel 1 of the data acquisition unit was set to 60 samples per second (60Hz). Then, we assigned a participant number ([Gender][DB/ND]P# - *i.e.*, FNDP01, MDBP02, etc.) on the data log Excel file and attached the black miniature test hook clip to the reference electrode of a commercial glucose meter test strip and the red clip to the working electrode (**Figure 8b, g**). Another commercial glucose meter strip was attached to the commercial glucose meter (**Figure 8e**). Following that, a new sterile lancet was placed in the commercial finger-pricking unit, and we started the recording on the data acquisition software.

After the physician or registered nurse (RN) pricked

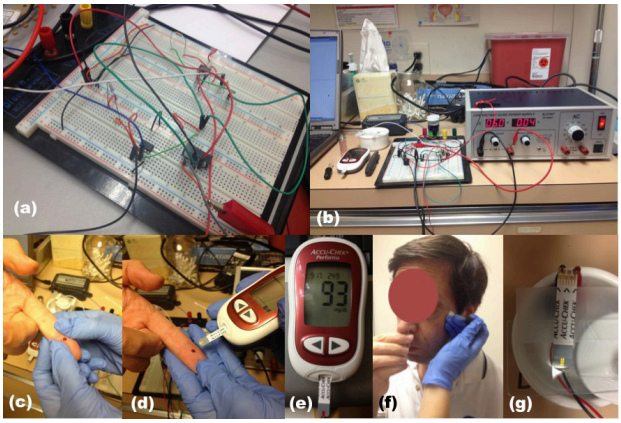

Figure 8. Glucose meter circuit and sample collection methods. (a) The glucose meter circuit developed and used during experimentation; **(b)** the setup, at the medical office, of the materials prior to experimentation; **(c)** the physician squeezing the patient's finger lightly to help obtain a small amount of capillary blood; **(d)** the blood being introduced to the commercial glucose meter; **(e)** the commercial glucose meter after the introduction of capillary blood; **(f)** one of the patients inserting a cotton swab into his left nasal cavity to stimulate their nasolacrimal duct, while the physician collects tear fluid; **(g)** a glucose meter strip attached to the glucose meter circuit.

the patient's finger using the finger-pricking unit, they squeezed the participant's finger lightly to help obtain a small amount of capillary blood (~2 µL), and the blood was introduced to both the glucose meter strip attached to the commercial glucose meter and then to the glucose meter strip attached to the glucose meter circuit (**Figures 8c, d, e**). The glucose meter strips were then disposed of in a red bio-hazard bin, a new recording file was titled "[date][Gender][DB/ND][P#]TearL[Exp#].wdq (*i.e.*, 20140905P01FNDTearL01.wdq)," and the glucose meter strip attached to the glucose meter circuit was replaced. The patient then inserted one cotton swab into his/her left nasal cavity and stimulated their nasolacrimal duct (**Figure 8f**). After the participant accumulated around 2 µL to 5 µL of tear fluid between the lateral canthus and the lacrimal punctum, the physician or RN used a sterile disposable glass micropipette to collect ~2 µL tear fluid (**Figure 8g**). The tear fluid was then quickly brought to the commercial glucose meter test strip, and the procedure was repeated for the tear fluid from the right eye.

The glucose meters (commercial and circuit) were readied with new glucose meter strips (**Figure 8b**), and data was collected from each participant following the same procedure noted above three more times to a total of four data collections from each participant during 90 minutes of experimental time. After the first data collection was completed, the patient was provided with a small breakfast sandwich (134 kcal, 50% carbohydrate, 25% protein, and 25% fat). Then, the second data collection took place 30 minutes after consumption of the sandwich, the third data collection took place 60 minutes after consumption of the sandwich, and finally the fourth data collection took place 90 minutes after consumption of the sandwich. Later, the data from the glucose meter circuit were used to create predicted values for capillary blood, right tear fluid, and left tear fluid; as well were compared to the commercial glucose meter values in a series of EGA's and Pearson correlation tests.

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