Simple Methods of Detecting Disease Factors in Tears and Bloods

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Abstract—This study developed simple methods to detect glucose in tears using paper based microfluidics. Correlation of tear glucose and blood glucose were studied. 11 volunteers participated in the experiments. After taking glucose tablets, tears and blood samples were measured at a given time intervals using a spectrophotometer or a paper diagnostics. As a result, glucose concentrations with increasing the glucose intake were increased in both tears and blood and it became the highest in 30 minutes. Later, the glucose concentration in tear and blood were decreased in about 60 minutes. Thus, we found that the tears and the blood glucose had the similar metabolic rate of decomposition showing a clear correlation between blood and tear.

Index Terms—tear glucose, paper microfluidics, SU-8, microfluidics

I. INTRODUCTION

As the approach of an aging society, Diabetes prevalence in elderly is increasing rapidly worldwide. Diabetes prevalence by 2040 compared to 2000 will be increased by more than 300% China, India and other Asian countries where the prevalence of diabetes is expected to increase by over 350%. [1]-[2]

As a way of identifying the diabetes symptoms, multiple tests and measurements of elements such as urine glucose, blood glucose, glycosylated proteins, have been attempted. Diabetic test yet which can satisfy all of the standardization, simplicity, sensitivity and specificity have not been established. [2]-[6] According to the World Health Organization (WHO), disease diagnosis devices including diabetes should be quick, accurate, not expensive, and user-friendly. The most widely used measure of diabetes, blood glucose concentration measurement is an invasive method causing a lot of inconvenience and complications. Therefore, for early diagnosis non-invasive diagnostics are needed for the development of affordable diagnostic methods.

There were studies associating tear glucose levels with blood glucose concentration and the possibilities that tear glucose concentration relative to the change in blood glucose concentration has been risen. [7] Lewis (1958) reported a correlation between the glucose and sugar tears. However it was not statistically valid. [8] This study measures the glucose concentration in a non-invasive method using a tear, which was compared to blood glucose levels.

II. MATERIALS AND METHODS

A. Choice of Paper

Whatman Chromatography Paper No. 1 (200 mm × 200 mm) was used without further adjustment of size. Whatman paper was chosen because of its uniform composition relative to other types of paper and it lacked additives that affected flow rate.

B. Designing and Printing Microfluidic Channels in Paper

Patterns in paper were created by two methods, using photoresist polymer2, 6-8 or wax materials.3, 4, 9 The photoresist polymer solution was composed of EPON SU-8 resin (52 wt%), triarylsulfonium hexafluorophosphate salts (photoacid) (5 wt%), and PGMEA (43 wt%). Whatman Chromatography Paper No. 1 was impregnated with the SU-8 photoresist. The paper prebaked on a hot plate set at 100 °C for 10 min. The paper was placed under the chemical fume hood to be cooled to room temperature and was exposed to UV light (405 nm) for 20 s under a transparency mask that was pre-made with designed pattern using CleWin® (PhoeniX Software, The Netherlands). Baking the paper for the second time on the hotplate set at 110 °C for 10 min, Acetone (3X) and 30% water in propan-2-ol (3X) were used to wash non-reacted polymer within the paper. The paper dried for 20 min under ambient conditions.

Or a Xerox Phaser 8560N color printer was used for depositing solid wax (Genuine Xerox Solid Ink Black) onto paper in defined patterns. Printing quality was set at the highest resolution for photo quality printing. Printed papers were placed on a hot plate set at 150 °C for 10 min. The paper was placed under the chemical fume hood to be cooled to room temperature and was exposed to UV light (405 nm) for 20 s under a transparency mask that was pre-made with designed pattern using CleWin® (PhoeniX Software, The Netherlands). Baking the paper for the second time on the hotplate set at 110 °C for 10 min, Acetone (3X) and 30% water in propan-2-ol (3X) were used to wash non-reacted polymer within the paper. The paper dried for 20 min under ambient conditions.

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a mixture of hydrophobic carbamates, hydrocarbons, and dyes. The patterned paper was cooled to room temperature, and was ready for further processing after 10 s. There was no significant difference in patterns created by one or another method.

C. Colorimetric Detection

At the end of the channel to measure the color change, glucose oxidase, horseradish peroxidase (HRP), N,N-Diethyl-p-phenylenediamine (DEPDA), 1-chloro-4-naphthol (4CN) were pre-deposited. Glucose oxidase (100U/mL) and HRP (5U/mL) solutions were mixed 1:1. DEPDA (10mM) and 4CN (10mM) were mixed 1:2 accordingly. Nanodrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA) was used to measure the light absorbance.

D. Clinical Test.

A total of 11 people participated in the experiment. Collected the blood and tears of men and women without eye disease or previous history were studied. Smoking and drinking alcohols were prohibited during clinical test period. Commercial glucose candies sold in pharmacy stores were used for glucose intake. Glucose candies were consumed from 3g to 12g. Levels of glucose concentration were measured at every 15 min.

TABLE I. COLORIMETRIC SPECTRUM OF GLUCOSE SOLUTION WITH INCREASING CONCENTRATIONS

<table>
<thead>
<tr>
<th>Glucose Concentration (mM)</th>
<th>0.05</th>
<th>0.1</th>
<th>0.25</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

III. RESULTS AND DISCUSSIONS

A. Colorimetric Spectrum of Glucose

Table 1 shows the spectra of the color change of glucose samples (0.05 ~ 10mM) using spectrophotometer. Generally the higher the concentration of the color of the glucose concentration was deepened.

Clinical trials showed that greater glucose intakes, glucose concentration in tear and blood increased rapidly exhibiting the body metabolism accelerated. (Fig. 1) Before the sugar intake, blood glucose levels were 95.73 ± 1.23 mg/dL in average. After sugar intake, the levels were increased at maximum 190.95 mg/dL. Tear glucose measured by a paper microfluidics were 5.23 ± 2.12 mg/dL and by a spectrophotometer were 8.47 ± 2.61 mg/dL before sugar intake. The maximum sugar levels in tear were 12.75 and 13.72 mg/dL (paper microfluidics and spectrophotometer, respectively).

![Figure 1. Relative glucose concentration over time tested in blood, tear A (spectrophotometer), tear B (paper microfluidics) with various glucose intakes A: 3g, B: 6g, C: 9g, D: 12g.](image)
B. Comparison of Tear Glucose with Blood Glucose

According to previous related studies, the glucose concentration present in the tears was 10 times lower than in bloods. The amount of tear glucose was estimated as 50 ~ 1000 µM that is consistent with the study. [9]-[10] Monitoring tear glucose for diabetes mellitus has been studied to develop noninvasive detection of blood glucose levels. Investigators have tried to collect nonstimulated tears for objective glucose concentrations. Typically, capillary tubes or paper was inserted in tear film meniscus or paper was used. [11]-[12] We used dry eye teat paper to collect nonstimulated tears.

Clinical trials also showed that after 30min. of the sugars uptake, the relative glucose concentrations were the highest in both blood and tear_A (measured by a spectrophotometer) and Tear_B (measured by paper microfluidics. after figures showed the highest per person after 30 minutes (Fig. 1). It was assumed that the amount of glucose tablet in this clinical test was small enough to be metabolized in 60 min. by insulin.

Overall, the degree of relative glucose concentration changes over time, depending on the sugar intakes. For instance, with 3g of sugar intake, as shown in Fig. 1A, the digestion of glucose in blood is fast and relative glucose concentration were slightly changed, however in tears which was measured by paper microfluidics, with small amount of glucose intake, 3g, the relative glucose concentration were dramatically changed with maximizing 170% at 30 min after the intake. Tear glucose levels measured by a spectrophotometer did not significantly changed meaning that the spectrophotometer could be less sensitive compared to optimized paper microfluidics.

With 12g of sugar intake, glucose digestion patterns over time in blood and tear are similar and confirms that blood and tear sugar levels are somewhat correlated. In both cases, after initial uptake, the glucose concentrations increased upto 30 min. and rapidly decreased in 60 min. When Blood glucose levels decrease, similarly tear glucose levels (Tear_B case) decrease. However, tear glucose levels measured by a spectrophotometer (Tear_A case) didn’t show the same pattern potentially due to its low sensitivity.

A series of methods for collecting tear glucose has been reported that may have an individual impact. [13]-[16] For instance, wireless contact-lens for tear glucose monitoring has been studied. The wirelessly powered biosensor was designed to gain the concentrated range of glucose levels in tears. [17]-[19] To understand the glucose levels in tears, one must understand the correlation between tear and blood glucose levels. In this study, we confirmed that the glucose concentration in tears appear in the same pattern with blood sugar degradation.

IV. CONCLUSIONS

This study developed a diagnostic test applicable to tear glucose measurements using paper microfluidics and compared glucose levels in tear and blood. As a result, paper microfluidic device could provide sensitive assays of measuring glucose in small amount of tears. Also, there was correlation between blood glucose and tear glucose concentrations. We assumed that glucose digestion rates in blood influence tear glucose concentrations. We found the possibility of application of paper microfluidics to measure disease factors in tears non-invasively.

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REFERENCES


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