

CLINICAL DIAGNOSTICS ON HUMAN WHOLE BLOOD, PLASMA, SERUM, URINE, SALIVA, SWEAT, AND TEARS ON A DIGITAL MICROFLUIDIC PLATFORM

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ABSTRACT

We present the use of an electrowetting-based “digital” microfluidic device for clinical diagnostics on human physiological fluids. Repeatable high-speed transport of microdroplets of human whole blood, serum, plasma, urine, saliva, sweat and tears, is first shown, to establish the compatibility of these fluids with the electrowetting system. A colorimetric enzyme-kinetic glucose assay is then performed on serum, plasma, urine, and saliva, and the results compared with those obtained using a reference methodology. The values obtained agree well, except for urine where there is a noticeable difference. The initial results demonstrate the feasibility of using an electrowetting-based lab-on-chip for clinical diagnostics.

KEYWORDS: clinical diagnostics, electrowetting, droplet, microfluidic

1. INTRODUCTION

Clinical diagnostics is one of the most promising application for microfluidic lab-on-a-chip technologies. All the benefits of miniaturization, such as reduced reagent consumption, reduced sample requirement, decreased analysis time and higher levels of throughput and automation, are realized in this application.

Currently, most microfluidic devices are based on continuous fluid flow, using electrokinetic phenomena for actuation. An alternative approach towards microfluidics is to manipulate the liquid as unit-sized discrete microdroplets. Due to architectural similarities with digital microelectronic systems, we refer to this approach as “digital” microfluidics. Digital microfluidic systems have several advantages over continuous-flow based devices, the most important being reconfigurability and scalability of architecture [1].

Electrowetting is one of several techniques, which have been used to actuate microdroplets in a digital microfluidic device. Electrowetting refers to the modulation of interfacial tension between a conducting liquid phase and a solid electrode, by the application of an electric potential between the two. The use of electrowetting for droplet actuation, splitting, merging and mixing, has been shown before [1][2]. We have also previously demonstrated the transport of enzyme laden droplets without loss in activity, and a complete colorimetric enzyme-kinetic glucose assay using standard glucose solutions on an electrowetting chip [3]. In this paper we extend the use of our electrowetting platform to actuate and analyze human physiological samples.

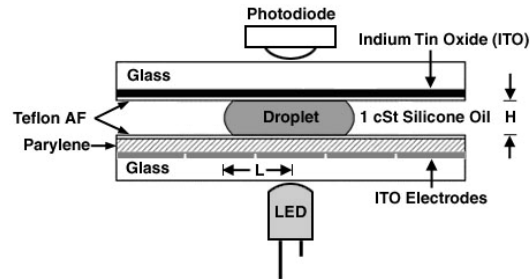


Figure 1 - Schematic of the electrowetting setup along with optical detection

2. EXPERIMENTAL SETUP

The experimental setup consists of the electrowetting chip on which the fluidic and biochemical processes occur, and a non-invasive optical absorbance measurement system, as shown in Figure 1. The details of the setup are described in [3]. In the experiments reported in this paper, we have used electrowetting chips with an electrode pitch of $L=1.5\text{mm}$ and a nominal gap spacing of $H=500\mu\text{m}$.

3. PHYSIOLOGICAL FLUID TRANSPORT

Transport of fluids containing proteins, such as enzyme-laden reagents and human physiological samples, on an electrowetting system can pose several challenges. Most proteins adsorb irreversibly to hydrophobic surfaces, contaminating and rendering them permanently hydrophilic. Therefore any contact between a liquid droplet containing proteins and the Teflon AF surfaces in the electrowetting system should be avoided. As a consequence, air is not a suitable filler medium for protein assays, since the droplet would always be in contact with the Teflon AF surfaces. Silicone oil, with its low surface tension and favorable wetting properties, is an ideal alternative. From visual observations and electrical capacitance measurements during droplet transport in silicone oil, we have inferred the presence of a thin film of oil, encapsulating the droplet at all times. This oil film isolates the droplet from the Teflon AF surfaces, minimizing adsorption and enabling transport.

In a droplet-based microfluidic system, the maximum switching frequency, which is the maximum rate at which a droplet can be moved across adjacent electrodes, is the transportability measure of interest. This is due to the digital nature of operation of the device. The maximum switching frequency is evaluated for droplets of different physiological fluids, as a function of applied voltage. The volumes of the droplets were between $1.3\text{-}1.5\mu\text{L}$. Figure 2 shows images of a heparinized whole blood droplet as it moves across 4 electrodes at 10Hz . Figure 3 plots the maximum switching frequency of droplets of whole blood, serum, plasma, urine, saliva, sweat, and tears, as a function of the applied voltage. All the fluids can be switched at frequencies of 20Hz using less than 65V . The transport is also sustainable for at least 25,000 individual droplet transfers.

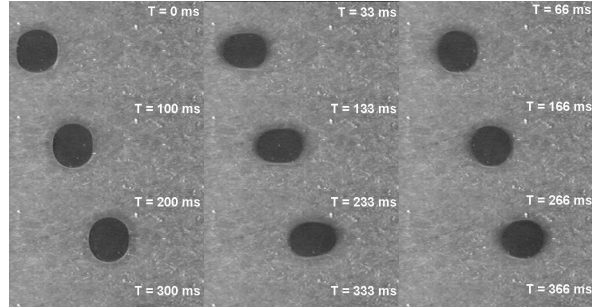


Figure 3 - High-speed transport of a 1.5 μ L droplet of whole blood across 4 electrodes.

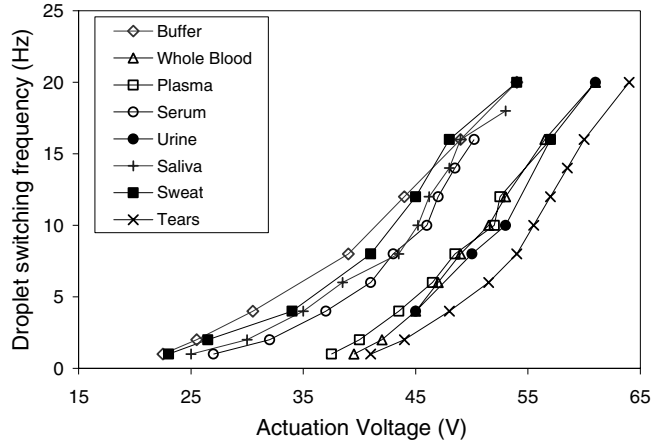


Figure 2 – Maximum droplet switching frequency as a function of applied voltage.

4. GLUCOSE ASSAY

Serum, plasma, urine and saliva were assayed for glucose on the electrowetting device and the value compared with results obtained using a reference methodology. A colorimetric enzyme-kinetic method (based on Trinder's reaction) is used to measure the concentration of glucose in both the cases. The details of the colorimetric glucose assay are described in [3]. The reference reading is done on a spectrophotometer, using a dilution factor of 100 as compared to a dilution factor of 2 on the electrowetting-chip. The linear range for the on-chip assay is 9-100mg/dL. Saliva and urine samples were spiked with glucose since the original concentrations were too low to be measured in our system. On both the systems 100mg/dL glucose was used as the calibrant. Figure 4 compares the results from the on-chip assay with the reference values. The concentrations measured agree well with the reference values, except for urine where there is a significant deviation. This is likely due to interference by uric acid which reacts with hydrogen peroxide

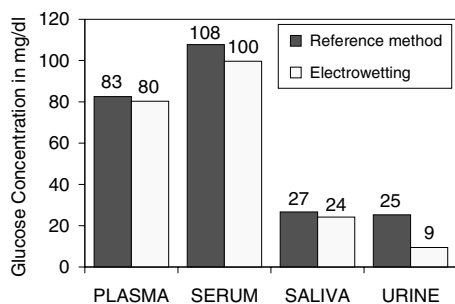


Figure 4 - Comparison of glucose concentrations obtained by the reference method with the electrowetting system.

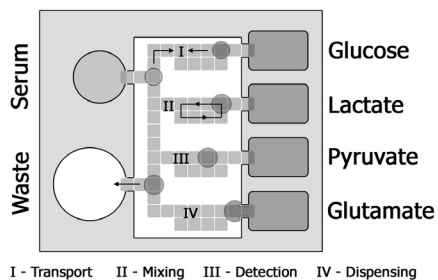


Figure 5 - Architecture of a digital microfluidic lab-on-chip for multiple assays on serum.

(an intermediate product) in the Trinder’s reaction. The interference is significantly reduced in the reference method because of the 100 fold dilution.

In addition to glucose we have also demonstrated the detection of other metabolites such as lactate, glutamate, and pyruvate using our system. These assays can be integrated on a single chip, along with an on-chip droplet dispenser, to create a fully automated lab-on-a-chip for clinical diagnostics on serum (Figure 5).

5. CONCLUSIONS

In this paper we demonstrate for the first time the use of electrowetting to actuate human physiological fluids. Microdroplets of human whole blood, serum, plasma, urine, saliva, sweat and tears, were transported at frequencies of 20Hz using less than 65V. The transport is also sustainable for at least 25,000 individual transfers. A glucose assay was also performed on serum, plasma, urine, and saliva. The values agree well with reference measurements, expect for urine where there is a significant difference, due to interference by uric acid. Future work involves the integration of a reliable and repeatable on-chip droplet dispenser, and the evaluation of clinical applicability of the device based on statistical performance parameters, including analytical accuracy and precision.

Blood samples were obtained with IRB clearance and the research is not subject to the Common Rule (45CFR46.102(f)) and HIPAA (45CFR164.500(a)).

REFERENCES

- [1] M. G. Pollack, A. D. Shendorov, and R. B. Fair, “Electrowetting–based actuation of droplets for integrated microfluidics,” *Lab on a Chip*, v.2, no.1, pp.96-101, 2002.
- [2] P. Paik, V. K. Pamula, M. G. Pollack, and R. B. Fair, “Electrowetting-based droplet mixers for microfluidic systems,” *Lab on a Chip*, v.3, no.1, pp.28-33, 2003.
- [3] V. Srinivasan, V. K. Pamula, M. G. Pollack, and R. B. Fair, “A digital microfluidic biosensor for multianalyte detection,” *Technical Digest IEEE MEMS 2003*, pp. 327-330.