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## Paper integrated microfluidic contact lens for colorimetric glucose detection†

Pelin Kubra Isgor, <sup>a</sup> Taher Abbasiasl, <sup>a</sup> Ritu Das, <sup>b</sup> Emin Istif, <sup>c</sup>  
 Umut Can Yener<sup>b</sup> and Levent Beker <sup>\*abd</sup>

Contact lenses offer a simple, cost-effective, and non-invasive method for *in situ* real-time analysis of various biomarkers. Electro-chemical sensors are integrated into contact lenses for analysis of various biomarkers. However, they suffer from rigid electronic components and connections, leading to eye irritation and biomarker concentration deviation. Here, a flexible and microfluidic integrated paper-based contact lens for colorimetric analysis of glucose was implemented. Facilitating a three-dimensional (3D) printer for lens fabrication eliminates cumbersome cleanroom processes and provides a simple, batch compatible process. Due to the capillary force of the filter paper, the sample was routed to detection chambers inside microchannels, and it allowed further colorimetric detection. The paper-embedded microfluidic contact lens successfully detects glucose down to 2 mM within ~10 s. The small dimension of the microfluidic system enables detection of glucose levels as low as 5  $\mu$ L. The results show the potential of the presented approach to analyze glucose concentration in a rapid manner. It is demonstrated that the fabricated contact lens can successfully detect glucose levels of diabetic patients.

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## 1. Introduction

Wearable technologies have gained great attention for health monitoring in recent years.<sup>1–5</sup> Being capable of non-invasive analysis of sweat, interstitial fluid (ISF), saliva and tears, wearable sensors pave the way to personalized healthcare. Amongst various bodily fluids, tears contain biomarkers such as glucose, amino acids, peptides, lipids, and electrolytes<sup>6</sup> and could provide information about ocular infections, keratoconus,<sup>7</sup> and dry eye disease.<sup>8</sup> Anomalous glucose levels might be an indicator of diabetes and subjects who have a higher tear glucose level than 0.67 mM can be considered diabetics.<sup>9–12</sup>

Controlling glucose levels might prevent further complications and help diabetes management. Finger prick blood measurement is a well-known and widely used technique for analyzing glucose levels.<sup>13,14</sup> However, it might cause discomfort for the user and

increase the possibility of unwanted complications.<sup>15</sup> Current studies have shown approximately 10 min lag time for tear glucose being behind blood glucose.<sup>15–17</sup> Sensor devices consistently demonstrate a notable correlation between blood glucose and tear glucose levels.<sup>18–21</sup> Moreover, based on blood and tear sample collection from 30 subjects, there is a strong correlation between tear and plasma glucose concentrations.<sup>22</sup> Therefore, tear fluid is an important study object for continuous glucose monitoring as a non-invasive option.

Conventional methods for tear collection rely on paper strips and capillary tubes followed by benchtop analysis which requires trained personnel and expensive laboratory equipment. Moreover, paper insertion activates eye irritation and lowers biomarker concentration due to the overflowing of tears which may result in compositional variations leading to false positive results. Also, contaminants introduced during extraction lead to unreliable results.<sup>23</sup> Recent advancements in smart contact lenses (SCLs) and sensing technologies have led the way to overcome existing limitations in traditional tear sampling methods and offer reliable solutions, providing point-of-care analysis, rapid response time, and continuous monitoring. Thus, various SCLs have been developed to monitor intraocular pressure, cholesterol, and cortisol, and detect exosomes, analytes, and pH.<sup>6,24–30</sup>

Besides intraocular pressure monitoring, SCLs that are capable of continuous glucose monitoring have been demonstrated by embedding MoS<sub>2</sub> transistors, cerium-oxide nanoparticles, electrochromic electrodes, and electrochemical

<sup>a</sup> Department of Biomedical Sciences and Engineering, Koç University Rumelifeneri Yolu, Sarıyer, Istanbul 34450, Turkey. E-mail: [lbeker@ku.edu.tr](mailto:lbeker@ku.edu.tr)

<sup>b</sup> Department of Mechanical Engineering, Koç University Rumelifeneri Yolu, Sarıyer, Istanbul 34450, Turkey

<sup>c</sup> Faculty of Engineering and Natural Sciences, Kadir Has University, Cibali Mah., Kadir Has Cad., Fatih, Istanbul, 34083, Turkey

<sup>d</sup> Koç University Research Center for Translational Research (KUTTAM), Koç University Rumelifeneri Yolu, Sarıyer, Istanbul 34450, Turkey

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and colorimetric sensors in commercial contact lenses, polyHEMA hydrogels or similar hydrophilic polymer materials.<sup>6,31–35</sup> The present SCLs offer continuous glucose monitoring up to 3 weeks;<sup>16</sup> however, fabrication requires access to cleanroom facilities where trained personnel use multi-step fabrication processes including photolithography and deposition. As a result, it takes several hours to fabricate one contact lens. Besides being time-consuming, the clean-room processes require dedicated infrastructure. Moreover, an electrical read-out circuit and an external device must be implemented on contact lenses for data transfer and communication. Therefore, these SCLs are not compatible with the eye because of rigid electronic components.<sup>36–38</sup> As an attempt to reduce tedious and time-consuming cleanroom fabrication, colorimetric paper-based sensors have been integrated into contact lenses.<sup>23,39</sup> However, laser-inscribed commercial contact lens fabrication requires a 3-axis femtosecond laser which might result in several hour fabrication for only one contact lens. Moreover, synthesizing a UV-curable methacrylated poly(dodecanediolcitrate) polymer for contact lens molding is a cumbersome process.<sup>40</sup>

3D printing for fabricating molds has been newly introduced with respect to clean room fabrication. With advancements in 3D printer technology, it is now possible to take high-resolution prints and smoother 3D printed structures and have biocompatible resins. Stereolithography (SLA) which is a photopolymerization based technique grants more complicated 3D structures with high-resolution. Soft lithography is a well-known technique that provides rapid prototyping of polydimethylsiloxane (PDMS) which is a transparent and flexible material. To harness the best sides of these techniques, we combined SLA 3D printing and soft lithography techniques for contact lens fabrication. They provide a batch-compatible process and print complex microfluidic structures on molds for further experimentation.

Paper microfluidics has been used for sweat monitoring in wearable devices; however, paper integration to contact lenses for glucose monitoring is under-explored.<sup>40,41</sup> Paper-based microfluidic systems lower the fluid volume that is needed to fill the chamber and make contact with the sensing system. Moreover, they minimize leakage and facilitate capillary flow due to the quick absorption of liquid. The filter paper inside microchambers ensures electrode free sensing and maintains minimum backflow.

Herein, we present a simple, reproducible and batch compatible method to fabricate microfluidic channel integrated SCLs for colorimetric paper-based glucose detection. Employing 3D printing and soft lithography techniques provides an alternative to clean room fabrication. Utilization of contact lenses prevents overflowing of tears and contamination risks. For colorimetric analysis, optical images were collected using a smartphone camera, followed by extraction of the red, green, and blue (RGB) content of the images using ImageJ software. *In vitro* experiments reveal that the proposed system can detect glucose concentration down to 2 mM. The proposed device has a response time of ~10 s for colorimetric glucose detection. The sensitivity of the system can be improved by utilizing different

functionalization chemicals. The proposed method holds great potential in simple and robust fabrication of SCLs, which can be integrated with complex microfluidic structures.

## 2. Materials and methods

### 2.1 Materials

Glucose oxidase (GOx) (G2133-10KU), potassium iodide (KI) and chitosan were purchased from Sigma Aldrich (MO, USA). Glucose powder (D(+)-glucose, 1191GR500) was purchased from neoFroxx (Germany). Phosphate buffer solution was purchased from Biowest (France). PDMS (Sylgard 184 silicone elastomer kit) was purchased from Dow Corning (USA). Filter paper was purchased from Macherey-Nagel (Düren, Germany).

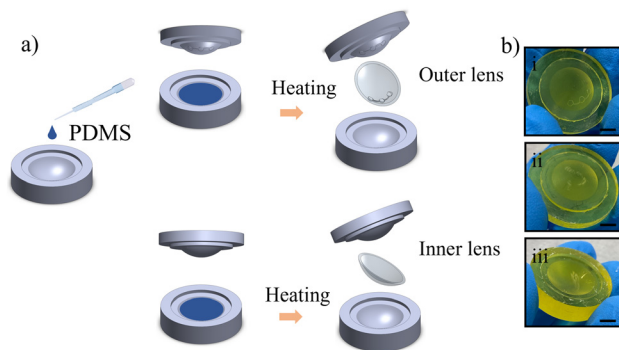
### 2.2 Colorimetric assay preparation

The chemical reaction of glucose oxidase (GOx) and potassium iodide (KI) gives a color change when subjected to glucose. Glucose solutions were prepared by dissolving glucose in PBS. 2 mg of glucose oxidase was dissolved in 1 mL phosphate buffer saline (PBS) to prepare GO<sub>x</sub> solution. 2% (w/w) chitosan was prepared by dissolving it in acetic acid solution. The glucose assay was formed by dispensing 5 µL of 2% (w/w) chitosan, 2 mg mL<sup>-1</sup> glucose oxidase (GOx) solution and 500 mM potassium iodide onto 4 mm diameter filter paper, consecutively.<sup>42</sup> After the filter paper was fully dried, 5 µL of 1 mM, 2 mM, 3 mM, and 5 mM glucose solutions were pipetted onto functionalized filter paper. Following color change, the RGB of each assay was analyzed for calibration. The assessment was done over five replicates per measurement.

### 2.3 Device fabrication

The wearable microfluidic contact lens is composed of three layers: the outer contact lens including microchannels, two 1.5 mm diameter microchambers and an inlet for glucose introduction; the paper-based analytical zone embedded in microchambers of the outer lens for detection of glucose to give a color change; the inner lens that is in contact with the cornea which encloses microchannels and reservoirs. Microchannels were placed at the outer region of the cornea to provide clear vision. Reservoirs have a 1.5 mm diameter and a 200 µm height. Two male molds and one female mold to fabricate inner and outer lenses were designed to create a unique shape of the human eye using CAD software (SOLIDWORKS). The molds were printed with stereolithography using a high resolution 3D printer (MicroArch BMF, the USA). Following printing, the molds were parylene coated to facilitate the detachment of the PDMS replica. PDMS was prepared using a silicone elastomer base and curing agent in a 10:1 ratio. After mixing and degassing of the PDMS mixture with a desiccator, the prepared PDMS was poured into the female mold, and the male mold was pressed onto it. The PDMS mixture containing molds were put on a hot plate at 100 °C for 35 min. Following curing, PDMS replicas were peeled off (Fig. 1a). The photographs of both female and male molds can be seen in Fig. 1b. 1.4 mm diameter





**Fig. 1** Fabrication process of both inner and outer layers of the contact lens. a) Contact lens molding and b) 3D printed contact lens molds: i) male mold containing a microfluidic channel (scale bar 4 mm), ii) male mold (scale bar 3 mm) and iii) female mold (scale bar 5 mm).

filter paper was cut using a laser (LPKF Laser & Electronics, Germany) to properly fit inside microchambers. Functionalized paper for colorimetric glucose detection was inserted inside microchambers in the outer lens. Following insertion of paper, the inner and outer lenses were plasma bonded for 50 sec and left on a hot plate at 80 °C for 15 min to secure bonding. Plasma bonding ensures enclosing of microchannels and prevents fluid leakage inside the contact lens.

## 2.4 Image analysis

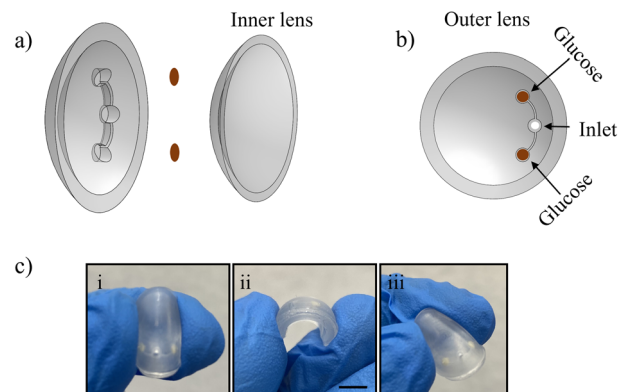
Following color change on paper, images were captured under white light conditions *via* a smartphone camera for each concentration. JPEG images were transferred to a computer for extracting R, G, and B channels using ImageJ software. The intensity values of the entire filter paper were measured. The correlation between glucose concentration and intensity was decided by analyzing the G channel of the images.

# 3. Results and discussion

## 3.1 Device design

A simple assembly method was used for device realization. The device consists of an inner lens, a colorimetric detection layer, and an outer lens (Fig. 2a). There are holes at the inlet of the outer lens and at the detection regions for glucose entering and exiting, respectively. The detection layer has two detection zones, one of which is for verification, and they are connected to microfluidic channels (Fig. 2b). Specific enzymes and chemicals for glucose detection were confined in filter paper, which was fixed within the detection chamber. Microfluidic channels connecting two detection zones ensure glucose guidance to chambers. The colorimetric glucose sensor is flexible, since all layers are made of soft materials (Fig. 2c).

In Fig. 3, the color change of functionalized paper from white to dark brown can be seen in the images taken from a video. During this video (Video S1†), 5 mM glucose is introduced to the microchannels inside the contact lens



**Fig. 2** Schematic illustration of different layers of the SCL. a) Schematic of the microfluidic contact lens, b) inner side of the outer lens and c) contact lens under mechanical stress, bending, and from different angles (i–iii).

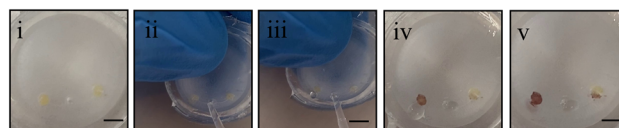
using a pipette tip (Fig. 3i and ii). After 10 s, a dark brown color is observed due to glucose detection (Fig. 3iv). The color becomes more brownish after 1 min (Fig. 3v).

## 3.2 Utilization of a 3D printer

Contact lens molds were printed with a high resolution 3D printer. The proposed method provides robust and simple fabrication of SCLs. The system holds great potential to fabricate complex microfluidic structures on contact lenses for further experimentation. The 3D printer accommodates a rapid turnaround time between design iterations. The printed molds can be used repetitively, and it is a batch compatible process.

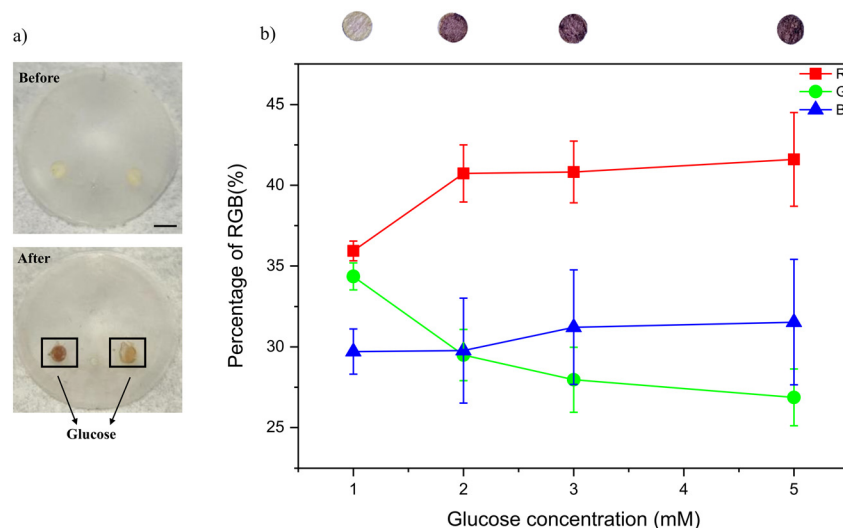
## 3.3 Detection of glucose

Glucose concentration was determined based on the colorimetric response of chemicals condensed on filter paper. During glucose detection,  $\text{GO}_x$  oxidizes glucose and produces hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The produced  $\text{H}_2\text{O}_2$  oxidizes KI to  $\text{I}_2$  which enters branched chitosan chains to create a brown color.<sup>43</sup> Sensors were characterized after a color change of the sensing agent was observed with the naked eye. When glucose was in contact with the colorimetric agents in the detection unit, the paper color changed. Under white light conditions, photos were taken *via* a smartphone. Depending on the R, G, and B values, glucose concentration can be obtained. The R, G,



**Fig. 3** Images taken from a video. i) Photo of the paper integrated microfluidic contact lens (scale bar 6 mm), ii) 5 mM glucose introduction, iii) glucose introduced to the chambers, iv) color change after 10 s and v) color change after 1 min, scale bars denote 8 mm and 5 mm, respectively.





**Fig. 4** a) Photo of the paper integrated contact lens before glucose introduction and after glucose introduction. b) RGB percentage with respect to glucose concentration. Decreasing G level indicates increasing glucose concentration.

and B values were analyzed with ImageJ software for each concentration after the colorimetric reaction occurred. The color change from white to brown showed glucose detection and the intensity of the brown color is correlated with the detected glucose concentration. When the glucose concentration increased from 1 to 5 mM, the sensing reagent color became darker. The tear glucose interest range is higher than 0.67 mM. Therefore, upregulated glucose levels result in darker brown colors. Subjects who have a higher tear glucose level than 0.67 mM can be considered diabetics. Therefore, our system can detect tear glucose of diabetic patients.<sup>12</sup> The G channel values decreased with increasing glucose concentration (Fig. 4). Therefore, we correlated the G channel value with the detected glucose concentration. Note that color change can be observed down to 2 mM. Moreover, the detection was fast since color change was observed within 10 seconds.

## 4. Conclusions

In this study, we have designed and fabricated a clean room free, batch compatible wearable PDMS contact lens for non-invasive colorimetric detection of glucose within ~10 seconds being faster than reported studies. 3D printing provides an alternative method to clean room for contact lens fabrication. The conformality of the contact lens was increased with respect to electrode-integrated counterparts. The results reveal the capability of the designed device in glucose sensing down to 2 mM, which could be even higher by applying different chemicals. The proposed system can detect tear glucose levels of diabetic patients. The transparency of the contact lens can be increased by decreasing the layer thickness during 3D printing. Colorimetric analysis of glucose solutions at different concentrations showed that the presented approach could serve as a simple method to monitor glucose levels from tears.

## Data availability

The data supporting this article have been included as part of the ESI.† Further data are available upon request from the authors.

## Author contributions

PKI developed the device, designed and performed the experiments, acquired and analyzed the data, and wrote the manuscript. TA guided through the experiments and data characterization. RD and EI provided feedback for the experiments. UCY printed the lens molds. LB supervised the project and reviewed the manuscript. All authors approved the final manuscript.

## Conflicts of interest

There are no conflicts to declare.

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