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Method Article

Fabrication methods for a gel-based separation-free device for whole blood glucose detection

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A B S T R A C T

In this paper, we describe two fabrication methods (well array-based and biopsy punching-based) of gel disks to construct a gel-based point-of-care (POC) diagnosis device for direct colorimetric measurement of human whole blood glucose without any extra blood separation step. The gel disks are made of Polyethylene glycol (PEG) diacrylate (PEG-DA) containing immobilized glucose colorimetric assay reagents. The performances of three types of PEG-DA gel (molecular weight: 575, 3,400, and 10,000) based sensors as well as the two fabrication methods were investigated.

- The fabricated devices enabled colorimetric whole blood glucose sensing assay without the need for blood cell separation
- The biopsy punching-based gel disk fabrication method provided less variation on the fabricated gel disks

A R T I C L E I N F O
Method name: Fabrication technology to produce high-quality gel-based separation-free whole blood colorimetric glucose detection device
Keywords: Point of care diagnosis, Blood separation, Glucose detection, Hydrogel
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Specifications table

Subject Area	
More specific subject area	*Biomedical Engineering*
Method name	Fabrication technology to produce high-quality gel-based separation-free whole blood colorimetric glucose detection device
Name and reference of original method	A Gel-Based Separation-Free Point-of-Care Device for Whole Blood Glucose Detection http://dx.doi.org/10.1021/acs.analchem.0c03801
Resource availability	

Method details

Background

Poly(ethylene glycol) (PEG) diacrylate (PEG-DA) is a hydrogel material that has been used to immobilize enzymes and retain their activities inside the gel matrix [1]. Its nanometer-scale porous structure allows the transport of small molecules through the gel while blocking large molecules or cells [2]. The pore sizes can be adjusted by PEG-DA precursor concentrations or molecular weight, making it feasible to control the diffusion rate of molecules of interest [3]. Besides, its optical transparency and mechanical strength are tunable. These properties make it an excellent gel material to perform whole blood colorimetric based molecular assays for point-of-care diagnosis (POCT) applications. Colorimetric detection method has been extremely broadly utilized in POCT detection of glucose concentration in a variety of body fluids [4-7] In our previous work [8], we developed a POCT device using PEG-DA to immobilize enzyme and colorimetric assay reagent inside the gel matrix to perform colorimetric detection of glucose in human blood without any extra blood separation steps. In this work, we first describe two fabrication methods (well array-based and biopsy punching-based) to produce the gel-based POCT devices. The results show that the biopsy punching-based method improves the uniformity of the gel disk thickness, thus improving the reliability and reproducibility of the colorimetric assay.

Materials

Acetonitrile (Sigma-Aldrich)

3,3′-diaminobenzidine (DAB) (Sigma-Aldrich)
2,2-dimethoxy-2-phenyl acetophenone (Sigma-Aldrich)
D-Glucose (Sigma-Aldrich)
Glucose oxidase from Aspergillus niger (GOx) (BBI solutions)
Horseradish peroxidase, lyophilized powder, >250 units/mg solid (HRP) (Sigma-Aldrich)
Human whole blood (Biochemed Services)
PEG-DA (MW: 575; 3,400 and 10,000) (Alfa Aesar)
Phosphate-buffered saline (PBS) (Sigma-Aldrich)
Polydimethylsiloxane (PDMS) (Sylgard® 184, Dow Corning Corp., MI)
(Tridecafluoro-1,1,2,2 Tetrahydrooctyl) Trichlorosilane (United Chemical Technologies, Inc., Bristol, PA)
1-vinyl-2-pyrrolidinone (Sigma-Aldrich)

Fabrication and Detection procedure

The two fabrication methods of this gel-based POCT device is illustrated in Fig. 1.
Fig. 1a shows the fabrication steps of the biopsy punching-based method, as follows:
Step 1: 3D printing of master molds for Polydimethylsiloxane (PDMS) well replication
Step 2: Fabrication of PDMS master mold by soft lithograph procedure
Step 3: Solution preparation of DAB, HRP, and GOx
Step 4: Mixture of PEG-DA gel solution with enzymes and substrate

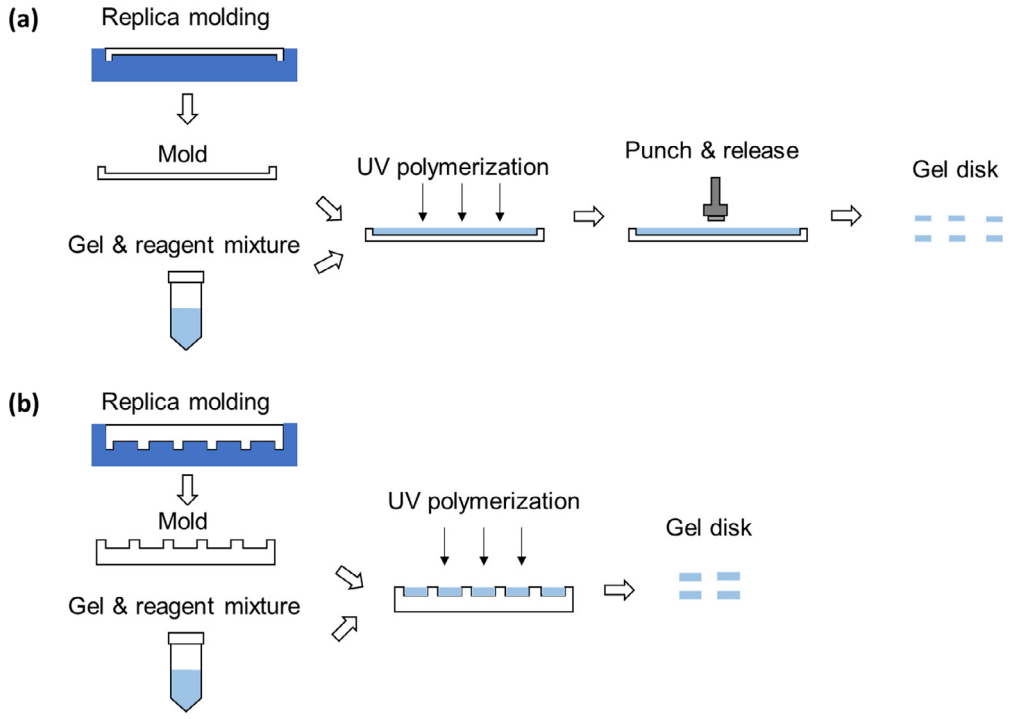

Fig. 1. Fabrication of gel disks through (a) punching out gel disks from a large gel sheet; (b) molding gel disks from a well array.

Step 5: Hydrogel polymerization by 365 nm UV
Step 6: Biopsy punching to create gel disks

Fig. 1b shows the fabrication steps of well array-based method, where Step 1 through Step 5 is the same as the first method, except for Step 6.

Step 1: 3D printing of master molds for PDMS well replication
Step 2: Fabricate PDMS master mold by soft lithograph procedure
Step 3: Solution preparation of DAB, HRP, and GOx
Step 4: Mixture of PEG-DA gel solution with enzymes and substrate
Step 5: Hydrogel polymerization by 365 nm UV
Step 6: Release gel disk from well array by forceps

The master mold for casting gel disks are fabricated by replica molding PDMS from a master mold printed by a 3D printer (EnvisionTEC Perfactory Mini). The 3D-printed plastic master mold was coated with (Tridecafluoro-1,1,2,2 Tetrahydrooctyl) Trichlorosilane for 20 min using a conventional desiccator to enable facile peeling off of the PDMS mold from the plastic mold. DAB solution (5 mg/ml) was prepared in acetonitrile due to the low solubility of DAB in water. GOx and HRP solutions were mixed at equal volume, then mixed with DAB solution at a 1:1 ratio to prepare the final colorimetric assay solution. This solution mixture was then mixed with an equal volume of PEG-DA precursor, and poured into the PDMS mold, followed by polymerization under 365 nm UV light exposure (EXCELITAS technologies) (45 mW/cm^2) for 2 min. The solvent acetonitrile could evaporate a few minites after gelation. After polymerization reaction, gel disks were punched and released from the polymerized gel disk using a biopsy tool, or released from an well array mold (Fig. S1). The gel disks were rinsed five times by PBS before use.

The detection of glucose concentration using the gel disk sensor is conducted through the following steps. The detection mechanism of colorimetric assay used in this work has been described in our previous work [8].

Step 1: Place gel disk into a standard glucose solution or human whole blood
Step 2: Wait for 5 min, allowing the reaction to be completed
Step 3: Wash off residual blood on the gel disk surface using PBS
Step 4: Place the gel disk in an environment with stable ambient light
Step 5: Use a smartphone camera to obtain images of the gel disk
Step 6: Analyze the image with a smartphone application or PC software (e.g. ImageJ) to acquire grayscale intensity of the gel disk area

The reaction time can be slightly shorter or longer than 5 min; however, as long as the certain reaction time is selected, this same amount of reaction time should be applied to all sample assays. Variation of ambient light can significantly affect the background intensity of the captured gel disk image, potentially introducing measurement error [5]. There are two approaches to minimize the effect of ambient light: (1) providing a much stronger illumination, for example, place the gel disk on top of a lightbox when capturing images of gel disks; (2) using a 3D enclosure structure to block ambient light [4,6]. During gel disk imaging, the exposure time, light sensitivity (ISO), and white balance needs to be a fixed setting (for instance, 1/1000 second, 100, and 5,000 k, respectively) for all images to be captured. The camera applications that can manually adjust and fix these three parameters are preferred. Any change to these parameters may lead to a variation in the background light intensity. Image analysis can be conducted either using a smartphone application that can provide image intensity information on-site or various free image analysis software on PC (e.g., NIH ImageJ) to obtain the grayscale value of the imaged gel disks. Only the circular sensing area is selected for the gray value calculation. The grayscale value of the black-white inverted image is expected to have a positive correlation with the blood glucose concentration.

Method validation

Thickness evaluation of the fabricated gel disk

We evaluated the geometry of the fabricated gel disks made from the two aforementioned methods. The gel disks were either punched out from a large gel sheet (2 cm diameter and 1 mm thick) using a biopsy puncher having a diameter of 5 mm, or released from a well array (5 mm diameter and 1 mm thick). Both methods allow mass production if necessary. Fig. 2 shows the colorimetric assay result uniformity and thickness of gel disks fabricated by the two methods. The gel disk made by the biopsy punching-based method (Fig. 2a) shows uniform color change after immersed in 1 mg/ml glucose solution. However, for the gel disk made by the well array mold (Fig. 2b), the color at the edge region (denoted by A′) of the disk was darker than that at the center region (denoted by A). This is due to the uneven thickness of the gel disk, where the edge region is thicker than the center region (see cross-section view). Fig. 2c shows the thickness differences between the center and edge of the gel disks made of PEG-DA with molecular weights of 575, 3,400, and 10,000, respectively. The gel disk made from the biopsy punching-based method has a more uniform thickness, while the gel disk made replica-molded from the well array has edges that are on average 142 μm thicker (approximately 20 % thicker) than the center region.

Whole blood test

As shown in Fig. 3a, glucose detection from whole blood was performed by dropping a gel disk containing colorimetric assay reagent into whole blood for 5 min. Glucose diffuses into the gel matrix and causes a colorimetric reaction while blood cells are prevented from entering into the gel so that no blood separation step is required prior to detection. After the reaction, the gel disks were rinsed to remove residual blood on its surface, and then images were taken and analyzed to obtain a colorimetric readout. We also confirmed that the blood cells had no interaction with the gel materials to affect the assay outcome (Fig S2).

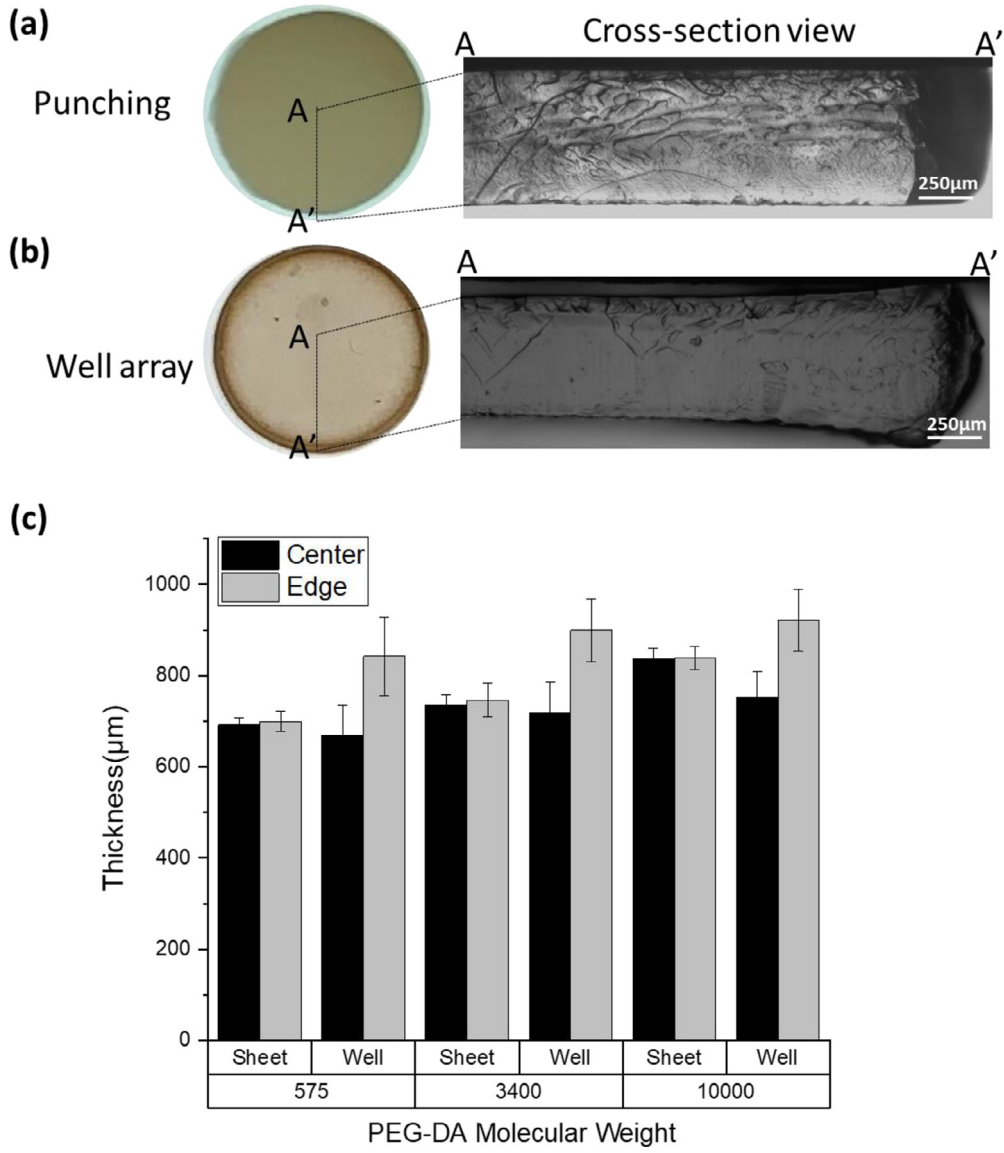

Fig. 2. Colorimetric assay result, uniformity and thickness of gel disks made by the biopsy punching-based and well-array-based fabrication methods. (a) Top view and cross-section view of a gel disk made from the biopsy punching-based method after reaction with 1 mg/ml glucose. (b) Top view and cross-section view of a gel disk made from a well-array mold after reaction with 1 mg/ml glucose. (c) Center and edge region thickness of the gel disks made of PEG-DA with molecule weight of 575, 3,400, and 10,000, respectively. Each data point shows the mean ± standard deviation, $n = 5$.

To examine the repeatability and stability of the developed sensing method, the gel disks (PEG-DA, MW: 575, 3,400, and 10,000, respectively) made from the biopsy punching-based method were immersed into human whole blood samples with unknown glucose concentration. Fig. 3b shows that the gel disk made from PEG-DA 575 had the highest background intensity but also the highest signal intensity. The gel disk made from PEG-DA 3,400 had the highest intensity difference (41 %), which makes it best for colorimetric assay applications. Since the individual gel disk thickness from the

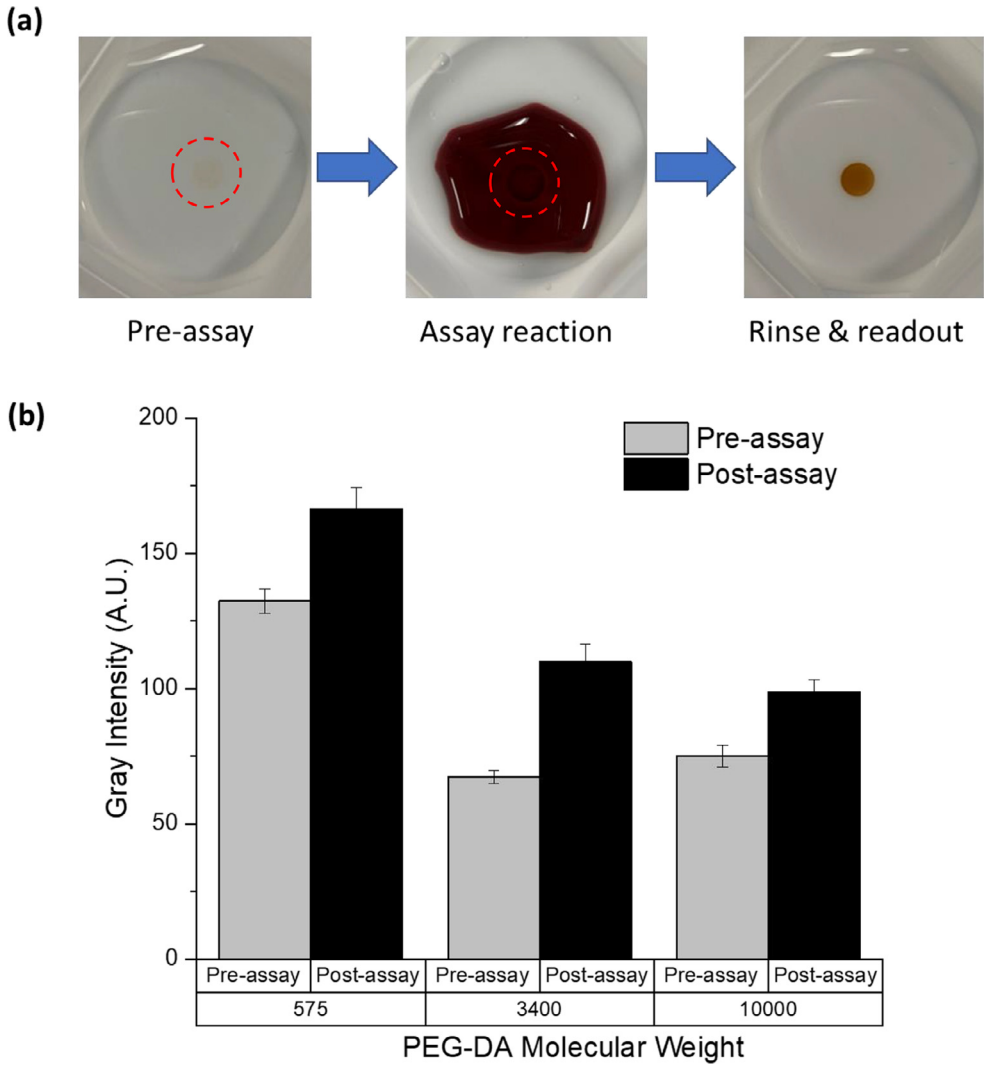

Fig. 3. Blood glucose detection with unknown glucose concentration in human whole blood. (a) Detection procedure; (b) grayscale intensity of the gel disks made from the molecular weight of 575, 3,400, and 10,000, respectively, after detection. Each data point shows the mean ± standard deviation, $n = 9$.

same batch is relatively constant when using the biopsy punching-based method, the relative standard derivation (RSD) is small for all PEG-DA materials tested (ranging from 3 % to 6 %).

Characterization of the fabricated sensors using standard glucose solutions

The analytical performance of our gel-based device was evaluated using a standard glucose solution at a clinically relevant concentration range (0 - 5 mg/ml) (Fig. 4). For all data shown in this section, the gel disk was fabricated using PEG-DA 3,400 and the biopsy punching-based method. According to the calibration curve shown in Fig. 4a, the grayscale intensities of the gel disks are proportional to glucose concentration. The assay result displayed a linear profile from 0 mg/ml to 0.5 mg/ml (Fig. 4b), with a correlation coefficient R^2 value of 0.984.

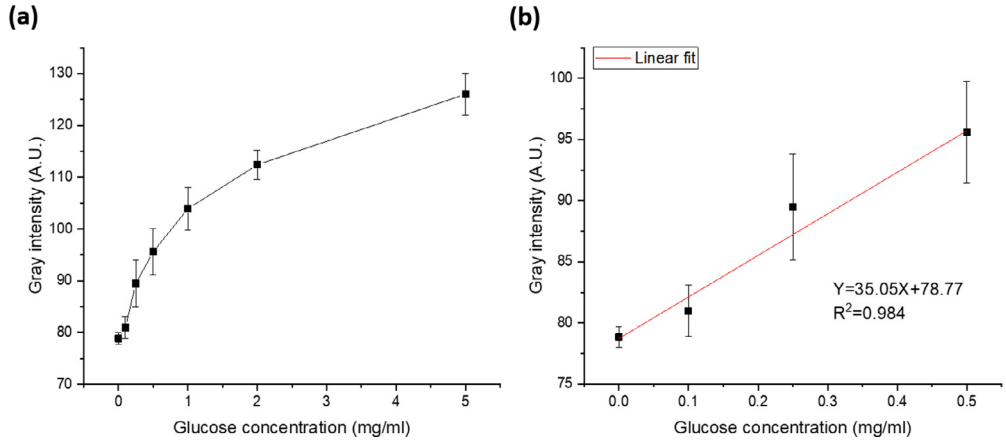

Fig. 4. Glucose assays performed on the fabricated gel disks. (a) Correlation between glucose concentration (0 to 5 mg/ml) and colorimetric assay readout. (b) Correlation has a linear relation range up to 0.5 mg/ml of glucose concentration (gray intensity (A.U.) = 35.05 × glucose concentration (mg/ml) + 78.77(A.U.), R^2 = 0.984). Each data point shows the mean ± standard deviation, $n = 5$.

Conclusion

The materials needed and procedures for the fabrication of gel disk-based separation-free colorimetric device for whole blood glucose detection are described in detail. The colorimetric intensity response of gel disks made by PEG gels having different molecular weight was evaluated. The gel disks fabricated through the biopsy punching-based method showed less thickness variation and more uniformity of color development during glucose detection. In conclusion, the presented method provides a route for mass-production of gel disk-based whole blood colorimetric assays.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2021.101236.

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