Determination of glycated albumin using boronic acid-derived agarose beads on paper-based devices

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(Received 4 January 2018; accepted 22 January 2018; published online 1 February 2018)

Self-monitoring of glycated albumin (GA), a useful glycemic marker, is an established method for preventing diabetes complications. Here, the paper-based lateral flow assay devices were developed for the sensitive detection of GA and the total human serum albumin (tHSA) in self-monitoring diabetes patients. Boronic acid-derived agarose beads were packed into a hole on a lateral flow channel. These well-coordinated agarose beads were used to capture GA through specific cis-diol interactions and to enhance the colorimetric signals by concentrating the target molecules. The devices exhibited large dynamic ranges (from 10 $\mu\text{g/ml}$ to 10 mg/ml for GA and from 10 mg/ml to 50 mg/ml for tHSA) and low detection limits (7.1 $\mu\text{g/ml}$ for GA and 4.7 mg/ml for tHSA), which cover the range of GA concentration in healthy plasma, which is 0.21–1.65 mg/ml (0.6%–3%). In determining the unknown GA concentrations in two commercial human plasma samples, the relative percentage difference between the values found by a standard ELISA kit and those found by our developed devices was 2.62% and 8.80%, which are within an acceptable range. The measurements of GA and tHSA were completed within 20 min for the total sample-to-answer diagnosis, fulfilling the demand for rapid analysis. Furthermore, the recovery values ranged from 99.4% to 110% in device accuracy tests. These results indicate that the developed paper-based device with boronic acid-derived agarose beads is a promising platform for GA and tHSA detection as applied to self-monitoring systems. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5021395>

INTRODUCTION

Diabetes mellitus is a fast-growing chronic disease related to high levels of glucose in the blood.¹ The diabetes complications are caused by glycated proteins that non-enzymatically react with reduced sugars, and they are prevented by controlling the blood glucose concentration.² There are multiple indicators of a patient's diabetes progress, including blood levels of glycated hemoglobin (HbA1c), 1,5-anhydro-D-glucitol (1,5-AG), and glycated albumin (GA). Although HbA1c level has been considered the gold standard in glycemic control over the past 2–3 months in clinical practice,³ HbA1c detection may not be reliable if the patient undergoes hemodialysis. 1,5-AG indicates blood glucose over the previous days to weeks because it is unaffected by basal metabolism^{4,5} but some research groups have investigated whether irrelevant decreases in blood 1,5-AG concentration are observed in pregnant women or in patients who have chronic kidney disease.⁶ Meanwhile, the GA level is an index that indicates glycemic levels accurately for the prior 2–3 weeks and also provides stable estimates for patients with chronic kidney disease, hemoglobinopathy, or those who are pregnant.⁷ Quantified GA levels also sensitively reflect postprandial glycemia, a risk factor for cardiovascular disorders and multiple neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.^{8–10} The

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GA level is defined as the ratio of GA to the total human serum albumin (tHSA; both glycated and non-glycated albumin) and is a good marker of glycemia in self-monitoring diabetes patients.

Point-of-care devices for diabetes provide an easy and useful method to prevent diabetes complications through self-monitoring. Recently, the paper-based devices have attracted significant interest in point-of-care detection due to their rapid, portable, user-friendly, and low-cost diagnosis capabilities.¹¹ In particular, the paper-based lateral flow assays (LFAs) can detect biochemical targets using the colorimetric method without the need for highly trained personnel, complicated data analysis, or expensive equipment.^{12,13} To improve the accessibility of self-monitoring for end-users, scanners, cameras, and smartphones have been enabled to screen and read the lateral flow strips. These electronic devices are equipped with high-resolution imaging systems and are within the easy reach of many patients. They are, therefore, very practical tools that allow for the quantitative analysis of multiple target analytes and enhance the advantages of LFAs, such as cost-effectiveness, rapid detection, simple operation, and portability.

In this study, we developed a novel paper-based LFA device for the sensitive detection of GA and the total human serum albumin (tHSA) in self-monitoring diabetes patients. In the device, bromocresol green (BCG) dye was used to quantify the albumin (GA and HSA) and boronic acid-derived agarose beads were packed into a detection hole in a lateral flow channel to capture GA. The reaction between the BCG dye and the albumin produced blue-colored albumin-BCG complexes, which could be distinguished by the naked eye. A crucial part of this proposed device is the detection hole filled with the boronic acid-derived agarose beads, which enables an intense colorimetric assay of GA by the high-surface-area agarose beads. At the detection hole, boron-carbohydrate interactions could occur between the sugar moieties of GA and the boronic acid in the agarose beads. This enabled a strong binding with the blue-colored GA-BCG molecules, while the HSA-BCG complexes passed through the hole without interaction. Compared to the standard ELISA method, the proposed device showed highly sensitive detection with good precision for unknown GA concentrations in human plasma samples.

EXPERIMENTAL

Materials

Albumin from human serum (A9511), human glycated albumin (A8301), human plasma (P9523), m-aminophenylboronic acid-agarose (A8312), bromocresol green, and sodium azide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The human glycated albumin ELISA kit (Catalog No: E-EL-H1993) was obtained from Elabscience (Houston, TX, USA). Grade 4 Chr cellulose chromatography papers (filter paper) and Grade 3 MM Chr cellulose chromatography papers were supplied by GE Healthcare Worldwide (Amersham, United Kingdom). The cellulose fiber pad was provided by Merck Millipore (Darmstadt, Germany). Phosphate-buffered saline (PBS, pH 7.0) was obtained from Biosesang, Inc. (Sung Nam, South Korea). A laminating pouch film for the support film was purchased from Hands Korea (Goyang, South Korea). All reagents were of analytical grade and prepared with deionized (DI) water with a resistivity of 18 M Ω cm.

Fabrication of the paper-based LFA device

The paper channels were prepared on filter paper using the wax printing method with a Xerox ColorQube 8870 printer, which is an effective and practical technique for fabricating the paper-based devices. The wax-patterned sheet was heated at 90 °C for 10 min to form a hydrophobic wall around the paper channels by melting the wax. A hole 2 mm in diameter was then punched in each channel. As shown in Fig. 1(a), the paper channel was immobilized on a support film with a sample pad and an absorbent pad using a laminating machine (SKY-325R6, SKY-DSB CO., Ltd., Seoul, South Korea). An 8 μ l of 50% (v/v) aqueous suspension of agarose beads derived from m-aminophenylboronic acid was dropped on the hole to form a detection zone, then dried at 4 °C for 5 min. 0.5 mM of BCG in a 0.2 M succinic acid buffer (pH 4.2)

FIG. 1. (a) Schematic illustration of the paper-based LFA device and optical/FE-SEM images of the boronic acid–agarose beads. BCG was absorbed on the paper channel, and boronic acid–derived agarose beads were packed into a hole on the device. (b) The cis-diol binding reaction between boronic acid and the sugar in glycated albumin.

was applied to the paper channel and dried at 4 °C for 3 min for the BCG-assisted colorimetric detection. Each paper-based GA and tHSA chip was prepared before use.

Quantification of GA and tHSA levels using the paper-based LFA device

The entire detection process was carried out at room temperature with a humidity of 45%. The tHSA and GA solutions in a 1× PBS buffer (pH 7.0) containing 0.9% NaCl and 0.05% NaN₃ were introduced onto the as-prepared paper channel. The blue-colored BCG-albumin (HSA and GA) complexes were generated and streamed to the detection zone by dropping 60 μl of PBS buffer as a running buffer [Fig. 1(b)]. The test was performed for 20 min at room temperature to allow the reactions of all molecules to complete, followed by drying in an oven at 60 °C for 1 min to obtain maximum color intensity. The images of the detection spot following the reaction were obtained using a Samsung SCX-4650 digital scanner and image analysis software (Image J, National Institutes of Health, USA). The scanned optical image of the detection zone was split into red, green, and blue channels by Image J. The red channel and the

green channel were used to calculate the mean pixel intensity for GA and tHSA detection, respectively, because they provided the highest contrast in the grayscale images.

Determination of GA in human plasma samples by the ELISA method

Samples of plasma from pooled human blood were reconstituted in DI water with two different dilution factors, 2 and 4. The ELISA assays were performed according to the product's user manual, and the optical densities of the standard samples and the unknown concentrations of GA in the human plasma samples were measured using a Synergy Mx multimode microplate reader from Biotek (Winooski, VT, USA) set to 450 nm.

RESULTS AND DISCUSSION

Figure 1(a) shows a schematic of a paper-based LFA device. The device is composed of four components: (1) the sample pad, (2) the wax-patterned filter paper for the lateral flow channel, (3) the hole packed with m-aminophenylboronic acid-derived agarose beads, and (4) the absorbent pad. These four components were combined with a support film. In this approach, the boronic acid-derived agarose beads with diameters of less than 50 μm were packed into a 2 mm diameter hole in the lateral flow channel by dropping the beads into an aqueous suspension. These well-coordinated agarose beads functioned as support materials for the immobilization of boronic acid moieties on the device, allow the selective detection of GA through boronic acid–cis-diol interactions of the sugars. Moreover, the high surface area of the agarose beads enhanced the colorimetric signal by concentrating the target molecules.

The reaction between the BCG dye and albumin is renowned as a cost-effective albumin detection method, which is distinguishable by the naked eye due to the easily formed blue albumin-BCG complexes. The albumin-BCG complexes were generated by dropping 0.5 mM BCG in a 0.2 M succinic acid buffer (pH 4.2) on a paper channel and drying at 4 °C for 3 min. When the tHSA and GA in 1× PBS buffer (pH 7.0) were added, the BCG specifically bound to the albumin, resulting in a color change from yellow to intense blue at acidic pH. After the formation of albumin-BCG complexes, the coupling between the GA-BCG complexes and the boronic acid was accelerated by dropping 60 μl of PBS buffer containing 0.9% NaCl and 0.05% NaN_3 on the sample pad as a running buffer. The boronic acid-derived agarose beads strongly bound to the GA-BCG complexes and produced a highly concentrated color intensity, while the HSA-BCG molecules migrated to the absorbent pad without interacting with the boronic acid. The boronic acid interact with the sugar groups in glycosylated albumin through a cis-diol binding reaction known as boronate affinity [Fig. 1(b)].¹⁴ Moreover, we performed the interference study with glucose, galactose, and fructose, which contain cis-diol groups that can interact with boronic acid in the agarose beads. The color intensities of these molecules were similar to that of the buffer solution because the BCG binds to the amino acid group of the albumin. The GA levels quantified by the boronate affinity are in the range of 0.6%–3.0% for normal individuals,¹⁵ while those for a typical patient with diabetes are 2–5 times higher.¹⁵ The GA levels are calculated by the following equations:

$$\text{tHSA (mg/ml)} = \text{GA (mg/ml)} + \text{non-glycosylated albumin (mg/ml)}, \quad (1)$$

$$\% \text{GA} = \frac{\text{GA (mg/ml)}}{\text{tHSA (mg/ml)}} \times 100. \quad (2)$$

The assay was completed in 20 min to maximize the color intensity, and a digital scanner equipped with an image analysis software (Image J) quantified the color intensity of the device.

To investigate the effects of pH on the albumin-BCG chromophore formation and the cis-diol binding reaction of GA with boronic acid, the two reactions were carried out in running buffers with different pH [Fig. 2(a)]. At acidic pH, the color intensity was low because the optimal pH for cis-diol binding between the GA and boronic acid is weakly basic. On the other hand, an acidic pH was appropriate for the formation of albumin-BCG complexes due to the

FIG. 2. (a) Effect of pH on albumin-BCG chromophore formation and the cis-diol binding reaction of GA with boronic acid. The GA concentration was 10 mg/ml. (b) Selective detection of HSA and GA with boronic acid-agarose beads. (c) Calibration curve for GA quantification.

anionic form of BCG and the cationic charge of albumin ($pI=4.7$). When an alkaline solution was used as the running buffer, the signal intensity was also low because the albumin-BCG complexes decomposed easily. Therefore, the maximum signal intensity was obtained at pH 7.

Figure 2(b) shows the selective detection of HSA and GA with the boronic acid-derived agarose beads. As expected, the color intensity for 10 mg/ml of GA was clearly higher than that

for 10 mg/ml of HSA. In fact, the color intensity of 10 mg/ml of HSA was similar to that of the buffer solution. This indicates that the system was successful operating by the specifically binding GA molecules to the boronic acid–agarose beads in the device. Figure 2(c) shows a calibration curve for the GA quantification, which was obtained by color scanning. The color intensity linearly increased between 10 μ g/ml and 10 mg/ml, with a correlation of 0.994, indicating that the system has a large dynamic range. The limit of detection (LOD, defined as $3\sigma/\text{slope}$) was 7.1 μ g/ml, much lower than the GA concentration in healthy plasma, which is 0.21–1.65 mg/ml (0.6%–3%).

To validate our paper-based devices for GA sensing, the unknown GA concentrations in human plasma samples with dilution factors of 2 and 4 were quantified and compared with the values found by the standard ELISA method. A commercial ELISA kit was used to quantify human GA *in vitro* using the recommended protocol (the detectable range: 1.30–83.2 μ g/ml, the sensitivity: 0.78 μ g/ml). As indicated in Table I, the GA levels determined by the ELISA method were very similar to those found by our devices. To evaluate the precision of the device for GA sensing, the relative percentage difference (RPD) was calculated using the following equation:

$$\% \text{ RPD} = \frac{|(x - y)|}{(x + y)/2} \times 100. \quad (3)$$

Here, x and y are the experimental (proposed method) and reference (ELISA) values, respectively. The RPD values were 2.62% and 8.80%, within the acceptable range of $\text{RPD} \leq 50\%$.¹⁶ These results show that the proposed devices have high accuracy and great potential to measure GA in biological samples.

A quantitative tHSA assay on a paper device was carried out by scanning the blue color intensity of tHSA–BCG complexes. Various tHSA concentrations (~ 50 mg/ml) with 2% GA were used. As shown in Fig. 3(a), the blank sample had a yellow color that originated from the BCG at acidic pH. On the other hand, high concentrations of tHSA showed a dark and vivid blue color due to the larger amounts of tHSA and BCG chromophores. There was a linear correlation between the tHSA concentration and the color intensity between 10 mg/ml and 50 mg/ml, with a LOD of 4.7 mg/ml, covering the reference range of tHSA in blood [Fig. 3(b)].

The accuracy of the sensing system was evaluated by analyzing the GA content in the tHSA. Five different concentrations of GA and tHSA were analyzed by averaging five time measurements. The GA and tHSA concentrations were determined from the calibration curves, as shown in Figs. 2(c) and 3(b), respectively. The GA contents were calculated using Eq. (2). As summarized in Table II, the recovery values were between 99.4% and 110%. These paper-based LFA devices required a total of 20 min for sample-to-answer diagnosis, fulfilling the demand for rapid analysis. As shown in Fig. 4, the blue color of the GA on the boronic acid–agarose beads could be observed by the naked eye in the detection zone. The tHSA color density detection also increased with the higher concentrations of tHSA. This confirms that the boronic acid–agarose beads in our paper-based LFA device can sense GA levels with enough sensitivity to enable facile diabetes monitoring.

TABLE I. Determination of GA level in human plasma samples using a commercial ELISA kit and the paper-based LFA devices.

Human plasma Sample	ELISA measurement (μ g/ml)	Paper-based GA sensor			Relative percentage difference (%)
		Found (μ g/ml)	Scanned image	Grayscale image	
1	77.7	75.7			2.62
2	9.61	8.80			8.80

FIG. 3. (a) Optical images of tHSA–BCG complexes formed at various concentrations of tHSA. (b) Calibration curve for tHSA quantification.

TABLE II. Analytical assay results from the simultaneous detection of GA and tHSA on the paper-based LFA devices.

Added			Found			Recovery (%)
tHSA concentration (mg/ml)	GA concentration (mg/ml)	GA value (%)	tHSA concentration (mg/ml)	GA concentration (mg/ml)	GA value (%)	
33	0.66	2	32.27	0.71	2.20	110
	1.65	5	33.62	1.76	5.23	105
40	0.80	2	39.44	0.84	2.12	106
	2.00	5	38.57	2.01	5.22	104
50	2.50	5	48.55	2.41	4.97	99

CONCLUSIONS

A paper-based LFA device used to self-monitor GA and tHSA levels was developed using boronic acid-conjugated agarose beads. The quantification of GA and tHSA levels was performed in two steps. First, albumin-BCG complexes were formed, changing the color from

**2% GA
in 33 mg/mL tHSA**

**5% GA
in 40 mg/mL tHSA**

**5% GA
in 50 mg/mL tHSA**

FIG. 4. Optical images for simultaneous detection of GA and tHSA on paper-based LFA devices. The pH of the running buffer for GA and tHSA detection was 7 and 4, respectively.

yellow to blue. Second, GA was bound to the boronic acid–agarose beads by boronate affinity, resulting in an increased color intensity. Boronic acid–agarose beads were used to capture GA through specific cis-diol interactions between the boronic acid and the reduced sugars in GA. Moreover, the agarose beads in the detection zone functioned as support materials to increase the colorimetric signal by concentrating target molecules. The color intensity increased linearly over a broad GA concentration range (10 $\mu\text{g/ml}$ to 10 mg/ml) with a correlation of 0.993 and an LOD of 7.1 $\mu\text{g/ml}$. These results include the reference range from boronate affinity chromatography. When used to determine the unknown GA concentrations in human plasma solutions, the proposed device was in good agreement with a standard ELISA. These results show that the proposed device has great potential for measuring GA in biological samples. To determine the GA content, the tHSA quantification was performed on the paper device using BCG. There was a linear correlation between tHSA and the color intensity between 10 mg/ml and 50 mg/ml, with an LOD of 4.7 mg/ml, which covers the reference range of tHSA in blood. The recovery values ranged from 99.4% to 110% in accuracy tests of the device. Moreover, the paper-based LFA device required a total of 20 min for sample-to-answer diagnosis, fulfilling the demand for rapid analysis. Based on these results, the developed devices have great potential for the sensitive detection of GA in self-monitoring patients with diabetes.

ACKNOWLEDGMENTS

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2008-0061891).

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