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Diagnosis of diabetes mellitus using sialic acid expression of erythrocyte and a microfluidic resistive temperature detector (micro-RTD)

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ABSTRACT

Sialic acid (SA, N-acetylneuraminic acid), a negatively charged monosaccharide, is expressed less frequently in diabetes patients than in normal people. A simple method for differentiating abnormal erythrocytes from normal erythrocytes based on SA-expression can be a useful diagnostic tool for diabetes. This study developed such a system by first modifying a sensor surface with SA recognition molecules to capture SA-expressing erythrocytes. Second, the sensor surface was thoroughly washed to remove abnormal erythrocytes while the normal erythrocytes remained. The remaining cells from the patient's and normal samples were heated with a 532 nm wavelength laser and temperature changes were measured using a resistive temperature detector (RTD). Hemoglobin molecules in the erythrocytes absorb a specific wavelength of photons (532 nm), which is reflected as a temperature change. In total, 17 samples from diabetes patients and 9 samples from normal patients were applied to the study system. The average temperature difference (0.32 °C) between diabetics and non-diabetics was significant, indicating that the study system can be utilized for diagnosis of diabetes mellitus. Moreover, a microfluidic channel using PDMS (poly-dimethylsiloxane) was fabricated and integrated onto the RTD to precisely deliver the cells. The process described above was repeated and almost identical results were obtained, suggesting that the microfluidic RTD has promising clinical applications.

1. Introduction

Diabetes is a metabolic disorder characterized by persistent hyperglycaemia that causes major health problems. Glucose levels in diabetic patients are higher than the normal, which results from defects in insulin secretion, insulin action or both. It is estimated that by 2025, there will be over 300 million individuals with diabetes worldwide [1]. Monitoring concentrations of blood glucose has been instrumental in the management of diabetes for three decades [2]. Although conventional glucose sensors are highly selective and accurate, they depend on fluctuations in blood glucose levels at the time of hypoglycemia or rapid glucose changes [2]. Therefore, alternatives are needed to overcome these limitations.

Sialic acid (SA, N-acetylneuraminic acid), a negatively charged monosaccharide, is usually expressed at the termini of glycan chains on erythrocyte membranes [3]. Since SA expression in diabetes mellitus patients is 38% lower than in healthy people [4], quantitative analysis of SA on the cell membrane could serve as an alternative indicator of diabetes mellitus [4–6]. It has also been demonstrated that SA plays a role in the aging of erythrocytes [7,8] and accelerated aging of erythrocytes is reportedly related to human diabetes mellitus [9]. Therefore, differentiation of diabetic vs. non-diabetic erythrocytes based on SA expression on the cell membrane would be a useful diagnostic tool for diabetes.

In the current study, the SA recognition molecule phenylboronic acid (PBA) was first immobilized on the sensor surface to capture SA-expressing erythrocytes from both normal individuals and patients with diabetes. Washing with a buffer solution removes more diabetic erythrocytes than non-diabetic erythrocytes, as SA is expressed less frequently on erythrocytes from diabetic subjects. The remaining erythrocytes can then be differentiated by measuring total hemoglobin content, which is a reliable indicator for the diagnosis of diabetes (Scheme 1).

As in previous research, this study developed a micro-thermal biosensor that can measure temperature changes in erythrocytes

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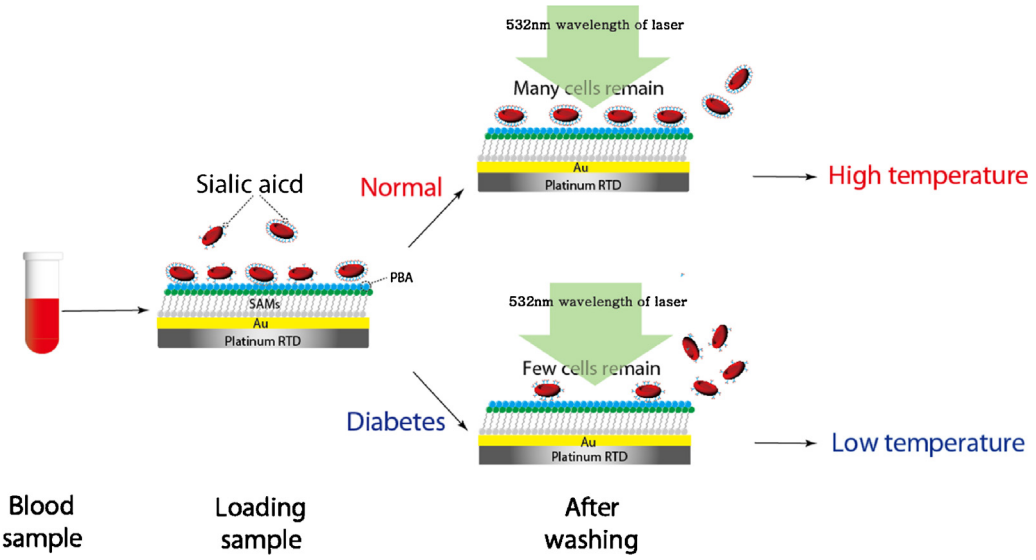

Scheme 1. Schematics of diagnostic strategy for diabetes mellitus. First the sensor surface was chemically modified by SA recognition molecules (phenylboronic acid) to capture the SA-expressing erythrocytes. Then the sensor surface was thoroughly washed to remove erythrocytes from diabetics without removing erythrocytes from non-diabetics. The remaining cells from diabetic and non-diabetic samples were heated with a 532 nm wavelength laser and temperature changes were measured using a photo-thermal biosensor. Hemoglobin molecules on the erythrocytes absorb a specific wavelength of photons (532 nm) and convert them to thermal energy, which is reflected as a temperature change. The temperature changes provide diagnostic results.

heated by laser irradiation [10].Chromophores absorb photons of a specific wavelength and convert them to thermal energy [11]. The hemoglobin molecule is a typical chromophore. A single erythrocyte contains 270 million hemoglobin molecules and each hemoglobin molecule has four heme groups. Each heme group consists of an iron atom at the center of a large heterocyclic porphyrin. These iron ions absorb photons at wavelengths of 418 nm, 530–545 nm, and 577–595 nm, and convert them to thermal energy. Specifically, the absorption peak for light is at a wavelength of 532 nm, and the photothermal parameters of erythrocytes at this wavelength have been well established [12–14]. The microfluidic channel was fabricated using PDMS (poly-dimethylsiloxane) and placed on the sensor surface in order to uniformly transport erythrocytes to the sensor surface and generate accurate shear stress inside the channel. This study explored the ability of the resistive temperature detector to accurately diagnose diabetes by measuring the temperature difference between normal erythrocytes and those under-expressing SA.

2. Materials and methods

2.1. Fabrication and surface modification of the photothermal sensor

Details of the fabrication and surface modification of our micro-thermal sensor have been described elsewhere [10]. Briefly, the sensor chip consists of three layers deposited on a Pyrex glass wafer. The bottom layer is a resistive temperature detector (RTD), which is a thermometer that functions by correlating the resistance changes of the RTD element with temperature changes. The middle layer consists of silicon dioxide for electrical insulation between the top and bottom layers. The top layer is made of gold, on which the self-assembled monolayer (SAM) and phenylboronic acid (PBA) are immobilized to capture SA on the membrane surfaces of the erythrocyte. To capture the erythrocytes using SA-mediated cross-linking, the gold surface on the micro-thermometer with silicon dioxide was chemically treated to allow biomolecular interaction between PBA and SA (Fig. 2b).

2.2. Design and fabrication of a microfluidic channel on the surface of thermal sensor

The microfluidic channel has a single construct which is rectangular and has a low aspect ratio to facilitate uniform flow through the cross section of the channel [15]. The inlet and the outlet were configured at the both ends of the channel for tube connections. When the PDMS channel is bonded on the thermal sensor, the chemically treated sensing region of the thermal sensor is located at the center of the channel to capture erythrocytes. The microfluidic channel placed on the thermal sensor was fabricated using PDMS rapid prototyping technique [16]. First, the photo-mask was designed using computer-aided design (CAD) program and printed on a high resolution transparency. A negative photoresist (SU-8 3025, MicroChem Corp., MA, USA) was spun onto a 4-in silicon wafer at rate of 1600 rpm for 30 s after pre-baking. The silicon wafer was subsequently exposed to UV light through the previously designed photo-mask. After developing, the channel mold remained on the silicon wafer. The heights of the SU-8 mold on the silicon wafer were examined by microscope at different random locations, and the average height was 50 μm. Then, PDMS (Sylgard 184, Dow Corning Corp., MI, USA) was poured over the silicon mold and the air bubbles were removed using a vacuum pump. After being cured on a hotplate for 2 h at 95 °C, the thickness of the cured PDMS cast was measured at 2 mm. Simultaneously, a cured PDMS with the same thickness was prepared for the photothermal experiments. Punching holes were created by a PDMS punch for tubing interconnects. Finally, both the PDMS replica and the thermal sensor were treated with air plasma (Cute-B Plasma, FEMTO Science Co., Korea) for 90 s and bonded together. After bonding, the surface modification was performed on the microfluidic channel using the same methods described in previous works [10].

2.3. Preparation of blood samples from diabetes patients

Blood samples were collected from 17 patients with diabetes mellitus and nine healthy individuals. Among of them, 5 samples from healthy individuals and 13 samples from diabetics were firstly tested with manual operation to verify the feasibility of the sensor