RAPID ASSAY SYSTEM FOR INSULIN AND GLUCOSE IN WHOLE BLOOD BY USING A FULL AUTOMATED POSTAGE-STAMP-SIZE CHIP: POSSIBLE APPLICATION FOR A REALTIME FITNESS INDEX IN PEOPLE WITH METABOLIC SYNDROME

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ABSTRACT

This paper reports an automated compact immunoassay system with disposable, mass-producible, low cost, and postage-stamp-size microchip, for measurement of insulin and glucose from whole blood. The microchip consists of simple micro diaphragm pumps, and screen-printed electrodes. The chip is able to implement whole protocols for immunoassay including sample loading, washing, and loading of reagent for electrochemical measurement. It can measure glucose and sub-nanogram order insulin from 2 µl whole blood. This technology would extend clinical indices for insulin resistance (ie; HOMA-R, QUICKI) as indices for fitness not only to people with diabetes but also to the general population.

KEYWORDS

HOMA-R, user-friendly assay system, micro diaphragm pump, immunoassay.

INTRODUCTION

The amounts of glucose and insulin are important indices, due to its strong correlation with life style disease such as metabolic syndrome. From those amounts, we can calculate clinical indices for insulin resistance (ie; HOMA-R, QUICKI) as indices for fitness not only to people with diabetes but also to the general population. In those purposes, a compact, rapid and low-cost point-of-care testing (POCT) device is suitable. The measurement of insulin in blood, however, is not so easy because of its low concentration, a few ng/mL. Such measurements require the immunoassay with bind/free separation, which makes total system large and expensive. For self-testing application, it is also important to measure from a small amount of the blood, within 10 µL, which can be obtained by using lancet.

In this paper, an automated compact immunoassay system is reported with disposable, mass-producible, low cost, and postage-stamp-size microchip, for the measurements of insulin and glucose from 2 µl whole blood. The microchip consists of simple micro diaphragm pumps[1], and screen-printed electrodes. The chip is able to implement whole protocols for immunoassay including sample loading, washing, and loading of reagent for electrochemical measurement.

EXPERIMENT

Automated and compact (W115 x D150 x H105 mm) insulin and glucose analyzer, which consists of electrochemical analyzer, micro pump driver, and two syringe pumps system, was developed (see fig. 1). For the measurement of insulin and glucose, blood sampling was conducted by lancet. A specific amount of the blood sample (2 µl) was metered in the chip by the capillary action in metering chamber having specific volume. The sample was delivered to sensor electrode by diaphragm pump driven by integrated pressure control machine. In case of glucose measurement, the concentration of glucose in whole blood was simply measured by means of enzymatic method when the blood reached on the measurement electrode. Insulin measurement was conducted by previously developed electrochemical

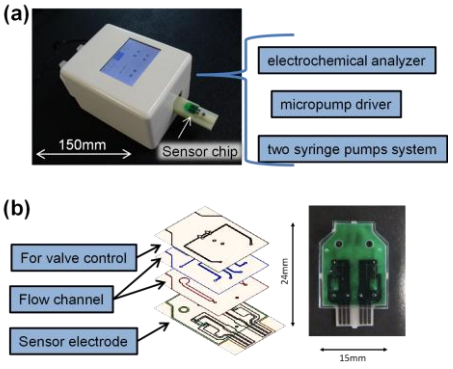

Figure 1: (a) Photograph of automated assay system. (b) Schematic illustration and photograph of a microchip for insulin and glucose measurement.

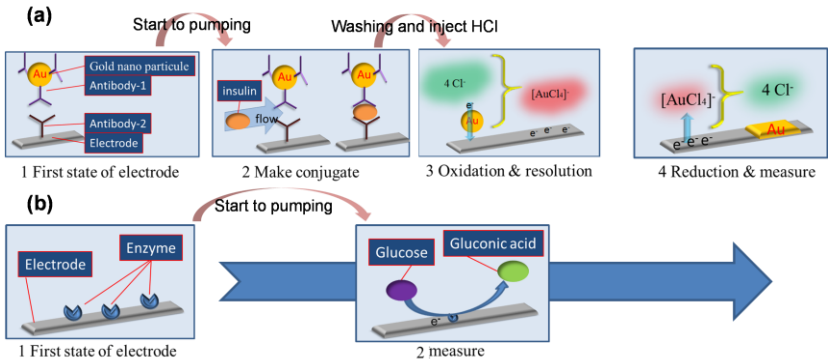

Figure 2: (a) principle of glucose detection. (b) principle of insulin detection

immunoassay[2]. In the step of immunoassay, the insulin in blood sample made complex with antibody labeled with gold nanoparticle conjugate, which already in the chip, during delivery to the sensor electrode. When the insulin reached on the electrode, it made sandwich complex on the electrode. Then free-gold nanoparticles and insulin sample were washed out by flowing washing buffer solution by syringe pump. Again the washing buffer was replaced with measurement reagent by flowing the reagent with syringe pump. For the detection of immunocomplex on the electrode, gold nano particles were oxidized by applying potential. Then reduction current was measured by means of DPV method.

The demonstration of automatic operation of the developed system was carried out using whole blood sample. After filling chamber with the sample, the sensor chip was set on the eternal system then diaphragm pump was started. The behavior of the all steps were observed under microscope.

It has been reported in previous studies that the reaction was accelerated by using micro-scale reaction chamber [3-4] . The immunoreaction in microchannel was tried to be compared with conventional method. In brief, the calibration curve of insulin was measured by different incubation time. In the case of microchannel reaction 2µl of sample was flown in 10 min however 2 µl of sample droplet was just put on the electrode and incubated for 60 min in case of conventional method.

From view point of further miniaturization of whole system, cost reduction, and commercial product development, it is favorable to operate all liquid operation with diaphragm pump. For this, the less volume of washing reagent is advantageous. In this purpose, we evaluated the effect of washing by signal to noise ratio as a function of volume of washing reagent.

RESULT & DISCUSSION

Figure 3 (a)-(d) shows automatic operation of the insulin and glucose analyzer. (fig. 3 (a)) shows electrode before pumping. When the process is started via touch panel, diaphragm pump is operated to deliver the whole blood to the electrode (fig. 3 (b)). During the operation, any kind of problems such as back flow of the sample from inlet port was not found. Then series of reagent injections are automatically implemented for the washing of electrode and electrochemical measurement. For washing process, 10 µl of washing buffer was injected by 2 µl/min. During this process, replacement blood with washing buffer was done and the blood smear was paled out. Back flow or leakage of washing buffer was not found in this process also. HCL injection was properly done (fig. 3 (c)-(d)). Again, we confirmed that leakage and backflow of the reagent was not found. Whole process is operated within 15-20 min without handling help.

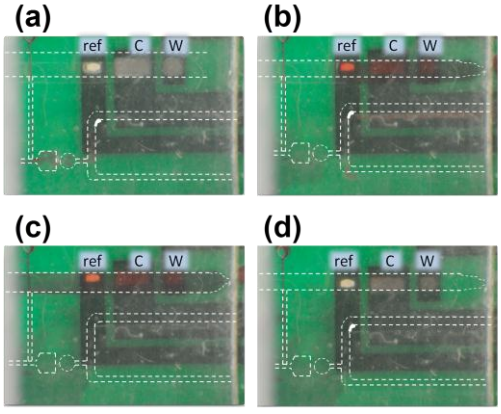

Figure 3. Photograph of whole blood and reagent handling in automated system. (a) Initial state of the blood pumping. (b) Blood reached to electrode surface (c) washing injection (d) washing complete and measurement reagent (HCl) injection.

Figure 4 compares the result of insulin detection using screen-printed electrode. It is found that the calibration curve in microchannel exhibit close profile as that of conventional method. Therefore it suggests that immunoreaction accelerated and that the signal level in micro channel with 10 min reaction become comparable with conventional reaction system with 60 min reaction.

Fig. 5 shows the effect of washing volume on the signal and background. Clear difference in the signal between positive control (sample include 2 ng/ml of insulin) and negative control (sample include 0 ng/ml of insulin). While signal and background is slightly increasing with decreasing of the washing buffer volume, however S/N is remains to be same in any volumes, suggesting packaging of the washing reagent is expected to be possible for further miniaturization and cost reduction of whole system is promising.

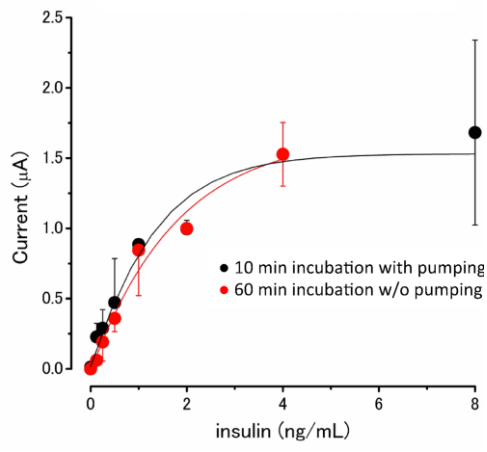

Figure 4. A measured result of insulin using screen-printed electrode.

CONCULUSION

By using this system, whole process is operated within 15-20 min without handling help. The signal level become comparable with conventional reaction system within 10 min reaction, when the immunoreaction is carried out under continuous flow in micro channel. And even the volume of the washing is decreased down to 3 ul however it does not result in significant degradation of S/N. Therefore packaging of the washing reagent is expected to be possible and further miniaturization and cost reduction of whole system is promising. Our technology would extend clinical indices for insulin resistance (ie; HOMA-R, QUICKI) as indices for fitness not only to people with diabetes but also to the general population.

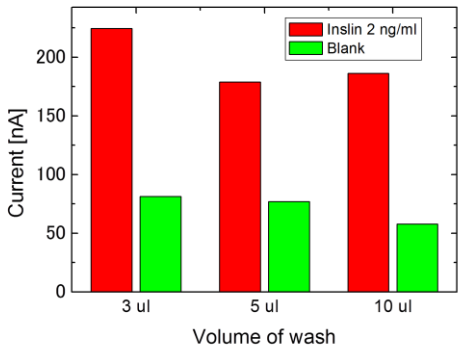

Figure 5. Effect of washing buffer volume on signal to noise ratio.

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