Development of point-of-care device for HbA_{1c} monitoring based on flow-immunoassay

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Point-of-care testing (POCT) has become centrally important in the monitoring of glycaemic control in the patient with diabetes. Much attention has been paid to lab-on-a-chip technology and analytical microsystems, due to the increasing applications in diagnostic fields and pharmaceutical industries. The lab-on-a-chip technology and analytical microsystems make a promise for fast, easy to handle and inexpensive assay for POCT of HbA_{1c}. Until now, we have developed a miniaturized flow immunoassay system using ion-exchange column for the detection of endocrine disruptors and allergen marker. In this study, an on-chip typed flow immunoassay system based on boronate affinity chromatography for the electrochemical detection of hemoglobin A_{1c} (HbA_{1c}), which is used to monitor long-term glycemic control and is the pre-eminent factor for quantifying the risk of complications in patients with diabetes, was developed. In general, the reference value of HbA_{1c} is given as the ratio of HbA_{1c} to total hemoglobin (Hb) ; (HbA_{1c} (%) = HbA_{1c} / (HbA_0 + HbA_{1c}) x 100). A simultaneously detection system of HbA_{1c} and total hemoglobin using blood cells was constructed.

Ferrocene labeled antibody were used for the electrochemical detection of HbA_{1c}. Ferrocene conjugated to anti Hb antibody (Fc-antibody) were prepared using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-Hydroxysulfosuccinimide (NHS). The oxidation potential of Fc-antibody was approximatively 600 mV (Fig. 1) and applied the potential for the detection in following experiments.

The assay procedure includes immunoreactions with ferrocene conjugated to anti Hb antibody (Fc-antibody) and Hb or HbA_{1c}. The reaction mixtures were injected into boronate affinity column. HbA_{1c}-antibody complexes were trapped to the column by the affinity of HbA_{1c} and boronic acid, while Hb-antibody complexes were eluted. The signal from eluted antibody was estimated as Hb quantity. Subsequently, the elution buffer including solbitol was applied to elute HbA_{1c}-antibody complexes. The signal from this elution was estimated as HbA_{1c} quantity (Fig. 2).

The flow immunoassay of HbA_{1c} was performed using blood cell samples to confirm the reliability of boronate affinity assay protocol. The immunoreaction mixture was applied to the column filling with *m*-aminophenyl boronic acid - agarose beads. The currents of eluted fractions after injection of washing buffer and subsequent elution buffer were measured by using flow cell. Two HbA_{1c} samples (10.65 ± 0.25 or 5.08 ± 0.25% measured by HPLC) were used as standards. The value obtained by this system proportionally corresponded to the ratio obtained by HPLC with an overall error of 1.5 ~ 3.0 %. This suggests that the reference value of HbA_{1c} were successfully measured by this assay protocol using blood samples.

On the basis of above results, on-chip typed flow immunoassay system on PDMS was constructed (Fig. 2). The current signal of HbA_{1c} was determined using on-chip typed boronate affinity column under differing HbA_{1c} concentration. The signal of HbA_{1c} increased as increasing HbA_{1c} concentrations (Fig. 3), indicating that HbA_{1c}-antibody complexes and Hb complexes was easily separated and HbA_{1c} were successfully detected by this on-chip typed flow immunoassay. The linear correlation was obtained in the range of 200-2000 μg/ml of HbA_{1c}. This on-chip typed flow immunoassay system will allow us to construct a novel POCT device for the monitoring glycated proteins including HbA_{1c}.

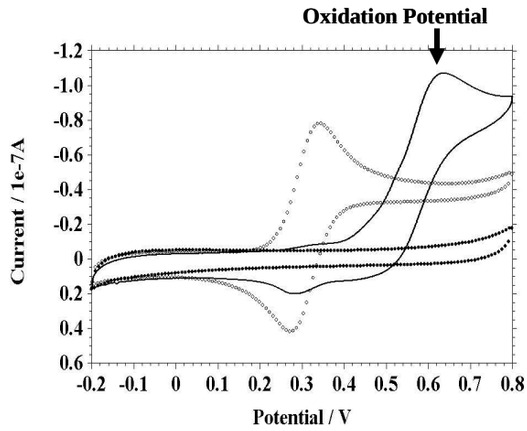

Fig. 1 Cyclic voltammograms at a scan rate of 20 mV/s using glassy carbon electrode. Frame circle ; Fc mono carboxylic acid. Solid circle ; Antibody, Solid line; Fc-antibody.

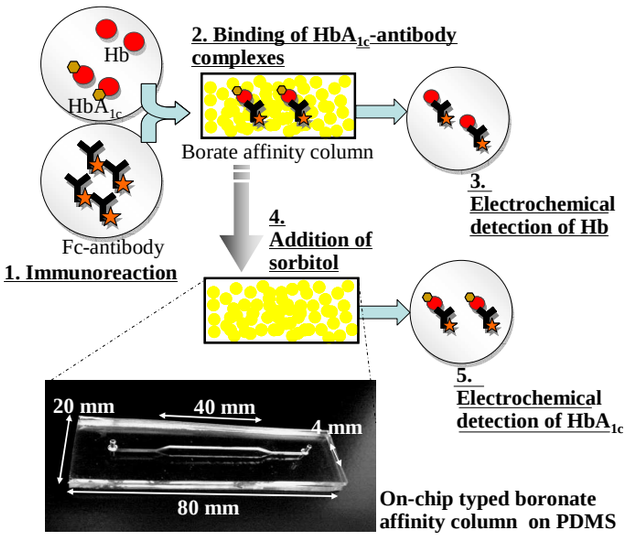

Fig. 2 Principle of flow type immunoassay system using Fc-antibody.

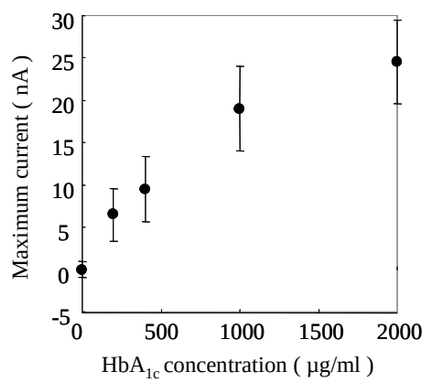

Fig. 3 Relationship between maximum currents and HbA_{1c} concentration. (Fc-antibody ; 1600 μg/ml)