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Detection of Biomolecular Recognition Using Bio-transistors

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We have been investigating direct interaction between biomolecular charges and charged carriers in semiconductor materials. Field effect transistors have been used to detect biomolecular recognition based on electrostatic interaction[1-3] as shown in Fig. 1. In the case of genetic FET, oligonucleotide probes are immobilized on the surface of the gate insulator. The cycle of single-base extension and measurement of the V_T was repeated iteratively to determine the base sequence of the target DNA. As a result, the positive V_T shifts could be detected in accordance with the base sequence of the target DNA[1]. We also propose an oocyte-based field effect transistor (oocyte-based FET) for drug transport analysis, in which target transporters are expressed at the cell membrane of the oocyte. Non-invasive monitoring of the uptake kinetics of substrates mediated by membrane-bound transporters can be realized with oocyte-based FET. Discrimination of transporting ability among genotypes of the transporters could be realized using the oocyte-based FET[2].

We also develop a label free, potentiometric method to detect cell surface sialic acid (SA) using phenylboronic acid (PBA) compound integrated into the form of self-assembled monolayer (SAM) on a field effect transistor (FET) extended gold gate electrode. Conceptual scheme for label free detection of cell surface SA utilizing the reversible and covalent interaction with phenylboronic acid (PBA) compound is shown in Fig. 2. A self-assembled monolayer of 10-carboxy-1-decanethiol was first formed on a gold gate electrode followed by a condensation reaction with an amino group functionalized PBA (3-aminophenylboronic acid) in order to obtain a PBA-modified gold electrode. This was then lined to a field effect transistor (FET) gate for real-time monitoring of the charge density changes taking place on the electrode when binding with anionically charged SA. Due to predominant binding between undisassociated PBA and SA at pH 7.4, we found that carboxyl anions of SA were exclusively detectable among other glycan chain constituent monosaccharides, as the change in threshold voltage (V_T) of the PBA-modified FET, as shown in Fig. 3.

The PBA-modified FET was then tested for its ability to directly capture the glycan component SA present on the cell surface. As for proof-of-principle, erythrocyte was investigated, for which alternations of the surface SA content have been reported in diabetes mellitus. Fig. 4 display monitorings of the V_T changes on adding suspensions of (**a**) native rabbit erythrocytes and (**b**) those with enzymatically decreased surface SA (20% remains) with step-wise increase of the concentration onto the PBA-modified FET. The slopes of the V_T changes obtained in Fig. 4 reflect the altered amount of SA per cell. This suggests that once a cell number-V_T calibration line is determined for a healthy phenotype, the altered SA expression level on the erythrocyte can directly be monitored in a real-time manner, simply by placing the known-count living cell suspensions onto the device.

We demonstrated that a PBA-modified electrode with properly controlled pKa could differentiate the degree of tumor metastasis through perception of the cell membranes SA. The technique can be readily extended to other primary *vs* tissue systems if their cell number-V_T calibrations are predetermined. Such a capability may serve as a remarkably easy and quantitative adjunct to histological evaluation of tumor malignancy and metastatic potential during intra- or postoperative diagnoses[5].

Alternations of sialic acid (SA) contents on cell surface glycan chains have been implicated in numerous normal and pathological processes including developments, differentiations, diabetes and tumor metastasis. Techniques to conveniently monitor cell surface SA therefore have great relevance to handy ways of cytology. Ordinarily, cell surface SA density is assessed via multiple enzymatic and labelling procedures, which, however, involve severely invasive, in many cases lethal procedures. In the context of practical applications, these are very unlikely to provide a feasible diagnostic platform.

The method developed using bio-transistors has been achieved only by placing the known-count living cell suspensions on the electrode without any enzymatic, labeling and lethal procedures that are unavoidable in any other existing determination methods.

References

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(4) A. Matsumoto, N. Sato, H. Cabral, K. Kataoka, and Y. Miyahara, *J. Am. Chem. Soc*., 131, 12022–12023 (2009).
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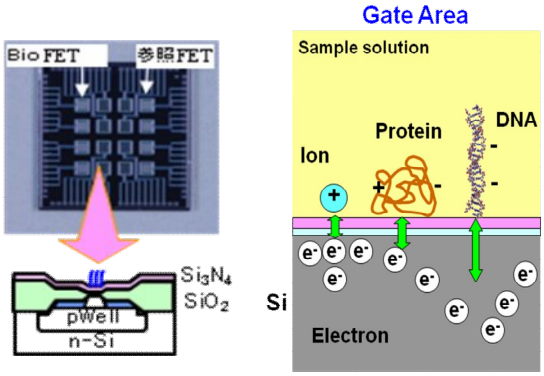

Fig. 1: *Biologically coupled field effect transistors (FET) are based on direct transduction of surface density change of charged biomolecules into electrical signal by the field effect. Since biomolecules such as DNA are negatively charged in an aqueous solution, electrons are expelled from the surface of silicon by electrostatic interaction. Thus, a specific binding of charged biomolecules at the gate surface can be detected as a shift of the threshold voltage V_T*

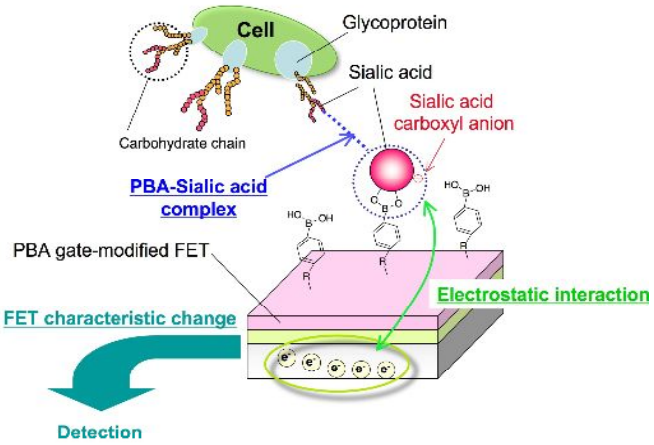

Fig. 2: *Conceptual scheme for label free detection of cell surface SA utilizing the reversible and covalent interaction with phenylboronic acid (PBA) compound, assisted by field effect transistor (FET) based charge detection.*

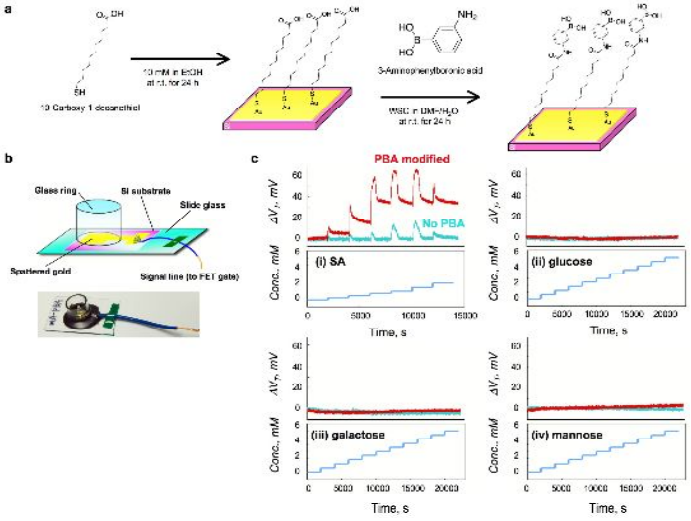

Fig. 3: *PBA gate modified FET specifically detects SA under the physiological pH and ionic strength conditions.* ***a****, PBA modification of a FET extended gold gate electrode.* ***b****, Schematic (upper) and photographic (bottom) representations of the PBA-modified gold gate electrode.* ***c****, Time courses of the V_T of the PBA-modified FET (red plots) for stepwise changes in various monosaccharide concentrations investigated at pH7.4 (155 mM NaCl). Light green colored plots denoted as "No PBA" represent control FETs without PBA modifications. Light blue colored lines displayed beneath each graph indicate patterns of concentrations with which each monosaccharide was added to the FET gate.detection.*

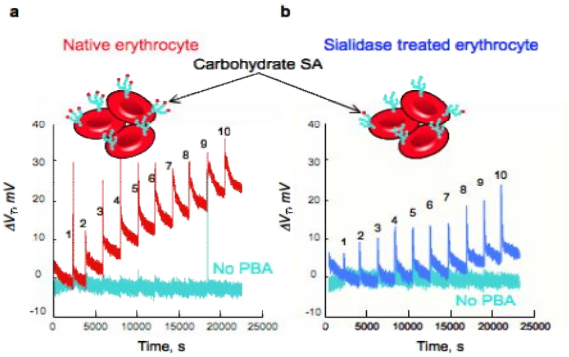

Fig. 4: *PBA-modified FET discriminates altered SA expressions on rabbit erythrocytes.* ***a*** *Monitoring of V_T response on increasing erythrocyte concentration with a step of 10^6 cells/mL added on a PBA-modified FET. Numbers denoted in the graph indicate concentrations of the cell suspensions (million cells/mL) and time points of each addition.* ***b****, The same experiment as* ***a*** *but using erythrocytes that were partially treated with sialidase. The remaining SA content was 20 % of the native (86 ± 8 pmol/10^6 cells vs 405 ± 15 pmol/10^6 cells) based on a colorimetric assessment using a commercial SA quantification kit (n = 3).*