Immunologically Modified FETs for Protein Detection in Biological Fluids

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Introduction

Field effect transistors (FETs) are semiconducting devices that use electric fields to modulate current in a conductive channel. The application of an electric field proximal to the conductive channel causes either an increase or decrease in current depending on the sign and magnitude of the field.

- FETs can be modified to allow protein sensing by deploying receptors on channel surface
- FETs modified with immunological receptors (i.e., antibodies) are known as immunoFETs
- Binding of proteins to receptors brings layer of charge proximal to channel surface and modulates current
- Potential for real-time, label-free detection of proteins in physiologic buffers and in vivo
- Tool for physicians to monitor critical protein levels

ImmunoFET Device Layout

- = Analyte
- = Receptor
- = Silane

Silicone
Reservoir
Ohmic
Contact
AgAn
2DEG
GaN
Sapphire Substrate

Micro
Reservoir
Silicone
Ohmic
Contact

Figure 1. ImmunoFET device cross-section. Illustration of AgAnGaN heterojunction FET used.

- In an immunoFET, the metal gate is removed and replaced by a layer of receptors
- Reservoir is created with silicone rubber to allow liquid samples to be placed on device over the conducting channel
- Receptors (antibodies) are attached to conducting channel via silane layer and bind analyte of interest
- The 2-dimensional electron gas (2DEG) created between AlGaN/GaN layers provides electrons for current flow
- Current is modulated by electric field proximal to the AlGaN surface i.e. electrical charge of bound proteins cause a change in device current (Im)

ImmunoFET Device Function

- Receptors (antibodies) are attached to conducting channel via silane layer and bind analyte of interest
- 2DEG is sensitive to changes in electric potential at AlGaN surface
- Binding of positively charged proteins create a layer of charge and this electric field increases current in the device, while negatively charged proteins decrease current in the device
- Distance between analyte and channel is important as sensitivity drops of to 6th power of distance due to counter-ion shielding

ImmunoFET Selectivity

- ImmunoFET with receptors for human CXCL9 tested with protein samples of both human and murine CXCL9
- Demonstrated that device signal is driven by receptor specificity and able to distinguish between exceedingly similar proteins

CXCL9 Detection in Murine Serum

- Successful detection of 100ng/ml human CXCL9 in a complex physiologic buffer-murine serum

Figure 2. Basic function of immunoFET. This is for an N-type FET where electrons are the charge carriers.

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CXCL9 Detection in Patient Urine

- Preliminary immunoFET testing results detecting CXCL9 in the urine of renal transplant patients
- Samples from both patients undergoing acute rejection episodes and non-rejecting patients
- Differential CXCL9 levels confirmed by ELISA, initially blinded to patient allograft status

Figure 3. ImmunoFET specificity determined by receptor. Detection of huCXCL9 and muCXCL9 in PBS (pH 7.4)

Figure 4. ImmunoFET detection of huCXCL9 in murine serum.

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Figure 5. CXCL9 detection in urine of renal transplant patients.

ELISA Corroboration

- ELISA performed to detect CXCL9 in the urine of renal transplant patients and related to rejection
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- Samples from both patients undergoing acute rejection episodes and non-rejecting patients
- Initially blinded to patient allograft status
- From literature, CXCL9 levels of greater than ~330pg/ml experience acute rejection

Future Work

- Continue testing in complex physiologic environments (transplant patient urine, serum), move into tissue/tissue homogenates, detection of additional proteins of interest
- Working toward clinical device capable of real-time, label-free, point-of-care testing of protein levels in vivo

Figure 6. FET Sensor Optimization.

- Use of monoclonal antibodies or fragments thereof as affinity elements for immunoFET construction
- Anticipate improved consistency for charge detection and distance of charge from the sensing surface
- Increased sensitivity as result of reduced noise to signal ratio
- TEA (APTES type silane) vs. APDMES type silane as polymer film
- TEA has shown to create a meshwork while APDMES type silane would provide a monolayer and highly ordered surface and thus 4x10^6 increase in sensitivity

Figure 7. Conceptual illustration of APTES (a) and APDMES (b).

- Binding of CXCL9 to bring C-terminus (high density of positive charge) close to sensing surface to increase sensitivity

Figure 8. Secondary structure of CXCL9 with 1 depicting the first residue of the N-terminus [Cole, et. al. J Immunology 2001; 167: 623-627]

Opportunities for Optimization

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