Immunologically Modified FETs for Protein Detection in Biological Fluids

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Introduction

Field effect transistors (FETs) are semiconductor 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

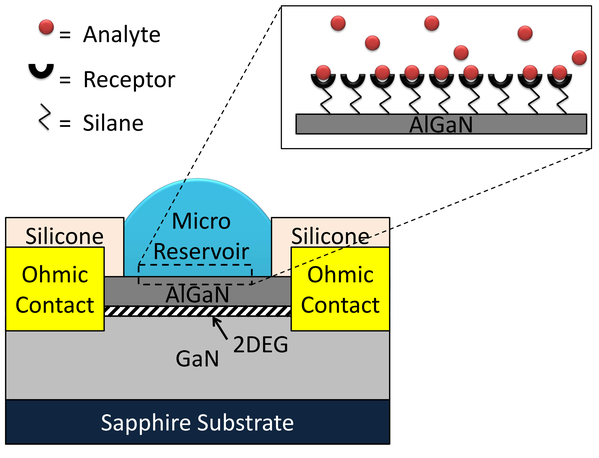

Figure 1. ImmunoFET device cross-section. Illustration of AlGaN/GaN heterojunction FET used.

- 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 (I_{ds})

ImmunoFET Device Function

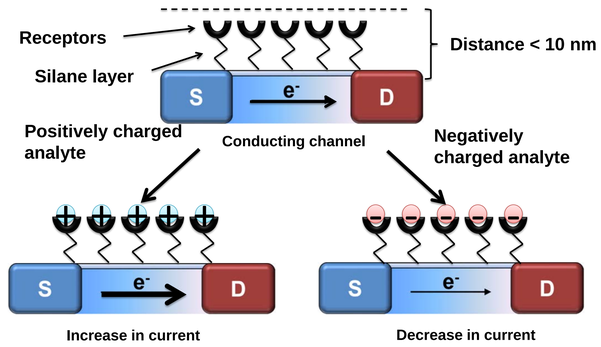

Figure 2. Basic function of immunoFET. This is for an N-type FET where electrons are the charge carriers.

- 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

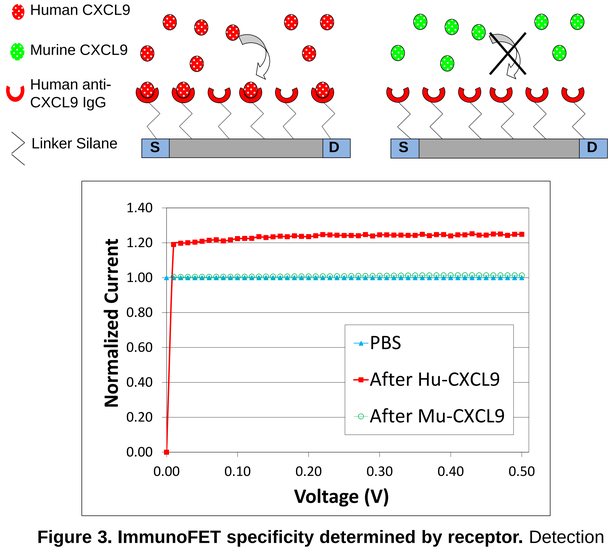

Figure 3. ImmunoFET specificity determined by receptor. Detection of huCXCL9 and muCXCL9 in PBS (pH 7.4).

CXCL9 Detection in Murine Serum

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

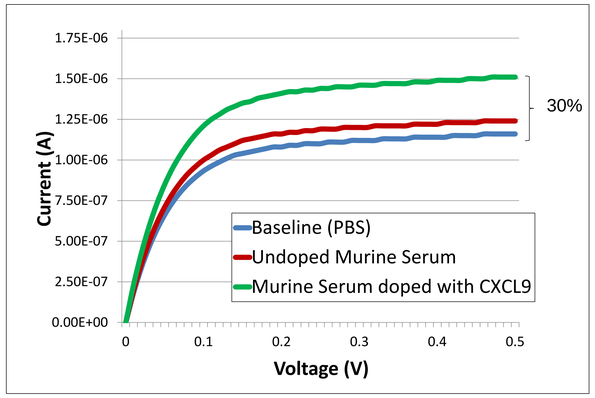

Figure 4. ImmunoFET detection of huCXCL9 in murine serum..

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

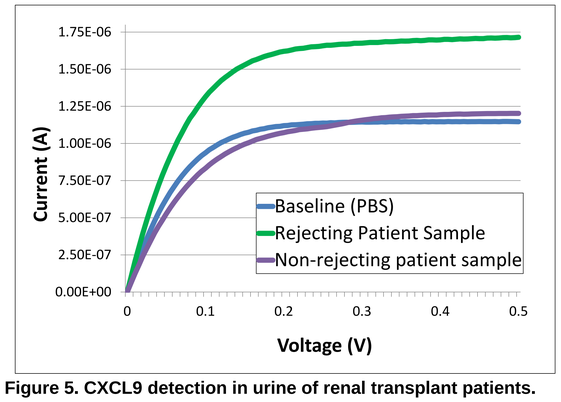

Figure 5. CXCL9 detection in urine of renal transplant patients.

ELISA Corroboration

- ELISA performed to detect CXCL9 in the urine of renal transplant patients and related to rejection.
- 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.

Opportunities for Optimization

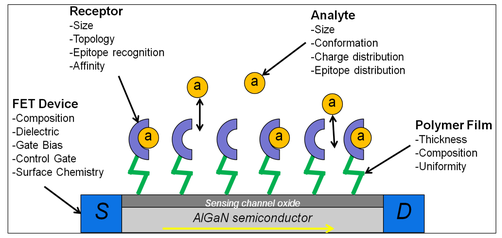

Figure 6. FET Sensor Optimization.

- 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**

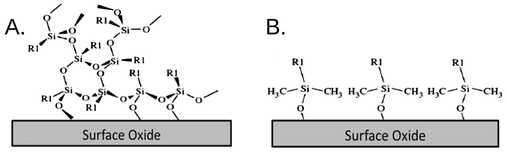

Figure 7. Conceptual illustration of APTES (a) and APDMES (b).

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

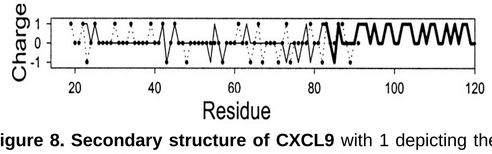

Figure 8. Secondary structure of CXCL9 with 1 depicting the first residue of the N-terminus [Cole, et. al. *J Immunology* 2001; 167: 623-627]

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*

1. Casal et al. *Phil Trans R Soc A*. 2012; 370: 2474-2488.
2. Gupta et al. *Biosen & Bioelec*. 2008; 24: 505-511.
3. Nicholson et al. *J Nanoeng Nanosys*. 2010; 223: 149-161.
4. Bhushan et al. *J R Soc: Interface*. 2009; 6: 719-733.
5. Wen, X et al. *IEEE Sensors*. 2010; 11: 1726-1735.
6. Lit et al. *Ann Rheum Dis*. 2006; 65:209-215.

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