Introduction to BioMEMS & Medical Microdevices

Biosensors

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Topics

- Biosensor definition.
- Electrochemical methods.
- Recognition elements
- Nanotechnology and the Nano-Bio Labs
- Field effect transistors (FETs).
- Specific immobilization methods.
Biosensors

- “A device that uses specific biochemical reactions mediated by isolated enzymes, immune systems, tissues, organelles or whole cells to detect chemical compounds by electrical, thermal or optical signals.” (Int. Union of Pure and Applied Chemistry)

- A biological or biologically derived sensitive recognition element usually is immobilized on a transducer to measure one or more analytes.
Commonly combined with microfluidic systems for:

- High throughput processing,
- Enhanced transport for controlling the flow conditions,
- Increased mixing rate of different reagents,
- Reduced sample and reagent volumes (down to nanoliter), increase sensitivity of detection, and utilizing the same platform for both sample preparation and detection.
- Portability, disposability, real-time detection, unprecedented accuracies, and simultaneous analysis of different analytes in a single device.

Transition is underway from *electrochemical/optical to nano-electronic* technologies.

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Electrochemical Biosensing...

**Biomarkers**
**Pathogens**
**Pesticides**
**Toxins**
**Etc.**

**Analytes**

**Enzymes**
**Nucleic acids**
**Antibodies**
**Cells**
**Aptamers**

**Biorecognition elements**

**Electrode**
**Transistor**
**Arrays**
**Nanomaterial**

**Transducer**

**Potentiometry**
**Impedance**
**Voltammetry**
**Conductometry**

**Electrical signal**

Sample preparation – filtration, extraction, purification and enrichment.

Ideally retained in direct contact. The biomolecules change physical quantities, such as charge, mass or photons.

Measurement of current, potential, or field effect, realized through label-free or labeled approaches.

**Analytes** are detected with **biological recognition elements**, including enzymes, nucleic acids, antibodies, cells and aptamers. **Electrical signals** are derived from **transducers**, including electrodes, field effect transistors (FETs), arrays and nanomaterials.

**Steven S. Saliterman**

Enzymes
- Catalyst for biochemical reactions that act upon substrate molecules producing a product.

Nucleic acids
- DNA, RNA – composed of nucleotides. Adenine, Thymine, Guanine, Cytosine (DNA) or Uracil (RNA). A-T, G-C or G-U.

Antibodies (Immunoglobulins)
- Proteins produced in response to and counteracting a specific antigen (e.g. bacteria, virus, or foreign substance). IgG, IgM, IgA, IgE & IgD.

Aptamers
- Aptamers are artificial nucleic acid ligands or peptide molecules that can be generated against amino acids, drugs, proteins and other molecules. Function similar to antibodies.
Aptamers...

- Aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) molecules that *to bind to various molecular targets* such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms.
  - Aptamers bind because they fit their target. (Target recognition and binding involve three dimensional, shape-dependent interactions as well as hydrophobic interactions, base-stacking, and intercalation.)
  - Aptamers bind to its target site through *non-covalent interactions*.
- **Peptide aptamers** can bind cellular protein targets and exert biological effects, including interference with the normal protein interactions of their targeted molecules with other proteins.
## Comparison of Biological Recognition Elements

<table>
<thead>
<tr>
<th>Biological Recognition Element</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes [95,97]</td>
<td>High sensitivity</td>
<td>Possibility of losing their activity upon immobilization</td>
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<tr>
<td></td>
<td>High selectivity towards their targets</td>
<td>Most suitable for small analytes, e.g., glucose, urea and lactate</td>
</tr>
<tr>
<td></td>
<td>Suitable for oxidation reduction reactions</td>
<td></td>
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<tr>
<td>Antibodies [106,107]</td>
<td>Rapid analysis for direct immunoassays</td>
<td>Requiring labeling for indirect immune assays which can result in the increase</td>
</tr>
<tr>
<td></td>
<td>Suitable for bioaffinity interaction e.g., antibody-antigen interaction</td>
<td>cost and time required for analysis</td>
</tr>
<tr>
<td></td>
<td>Suitable for the detection of large targets e.g., bacteria and pathogens</td>
<td>Not suitable for detection of small targets using direct and sandwich immunoassays</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not suitable for oxidation reduction reactions</td>
</tr>
</tbody>
</table>
Comparison of Biological Recognition Elements…

| **Aptamers [108,109]** | Highly sensitive and selective  
Suitable for the detection of a wide range of analytes  
Long-term stability, inexpensive and rapid synthesis  
Flexibility to be modified with labels without losing their performance or binding properties | Higher toxicity than antibodies  
Faster excretion due to their small size  
Weaker binding to analytes |
1) Potentiometry

- A potential difference between two half-cells with negligible current flowing. The cathode is the *indicator* and the anode the *reference electrode*.
- The most prominent potentiometric sensors are *ion-selective electrodes* in which a membrane provides for the ion-selective response.
- In most cases potentiometric sensors are chemosensors; however, when combined with a bioselective separation process they can also be assembled to be full biosensors.
- In FETs the same principle is being applied through the measurement of ions present in the *gate electrode* area of the FET.
2) Voltammetry

- Application of a potential between a working electrode (WE) and reference electrode (RE). A current is flowing and measured between a counter electrode (CE) and the WE as a result of reduction/oxidation processes at the surface of the electrodes.

- In its most simple form, a constant potential is applied and current is either measured at a specific time (amperometry) or integrated over a period of time (coulometry).

- Other approaches to improve S/N: differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) or to obtain analytical information on the redox reaction (cyclic voltammetry, CV).
Simple Cyclic Voltammetry Setup…

Keithley Model 2450-EC Source Meter (SMU)

When an SMU is programmed to source voltage in the remote sense (4-wire) configuration, internal sensing provides a feedback voltage that is measured and compared to the programmed level. The voltage source is adjusted until the feedback voltage equals the programmed voltage level. Remote sensing compensates for the voltage drop in the test leads and analyte, ensuring that the programmed voltage level is delivered to the working electrode.

Voltammogram from using a cyclic voltammetry script. (V vs. I)
Example of Voltammetry Use…

Nickel oxide nanoparticles (nNiO) and multiwalled carbon nanotubes (MWCNT). ITO – Indium Tin Oxide WE and RE.

Bi-enzyme (cholesterol oxidase (ChOx) and cholesterol esterase (ChEt) functionalized nanocomposite microfluidic-based biosensor developed for the detection of cholesterol.

3) Electrochemical Impedance Spectroscopy (EIS)
   - Monitoring the impedance, a frequency dependent resistance, after an electrical stimulation (voltage or current) in the ac mode.

4) Conductometry
   - Conductance is the inverse value of resistance measured in dc mode.
   - The resulting sensors are often referred to as chemiresistors and typically serve to measure conductivity changes within the bulk of an electrochemical cell, for gas sensing or enzyme-based strategies.

Nanotechnology

Classification of Nanomaterial Dimensions
“Enzyme-Like” Activity of Nanoparticles…

- Nanomaterials like gold nanoparticles (AuNPs), Fe₃O₄, Pd, NiO, TiO₂ have intrinsic enzyme-like activity.
- Metals and metal oxides are well-known catalysts driving many catalytic reactions.
- They are also widely used in electrochemical biosensors enabling nonenzymatic detection of metabolites such as sugars and reactive oxygen species, and enabling catalyst-enhanced signal amplification.

Graphene, carbon nanotubes (CNTs), and carbon nanofibers (CNFs), have been extensively studied and applied in electrochemical sensors.
Nano-Bio Lab…

Fume hood with spin coater.

Nano Materials Lab

Jim Marti with microcentrifuge.

CO₂ incubators.

Stirring incubator.

Chemical reagents.
Field Effect Transistor Biosensors

Schematic of an FET-based biosensor, based on complimentary metal oxide semiconductor (CMOS) technology.

Different nanomaterials embedded onto the gated region of the FET-based biosensor.

Principles of FETs…

• When the gate metal potential ($\Psi_m$ - psi) is changed, the electric field induces the “band bending” of the semiconductor channel accordingly.
• This results in channel carrier concentration changes, such as inversion, depletion or accumulation (negative gate).
• The gate potential can be given by other factors such as pH, ions or charge of biomolecules.

The conventional ion-selective FET (ISFET) is comprised of a MOSFET with the metal gate replaced by a dielectric layer as a sensing membrane. This dielectric is normally silicon dioxide.
Biosensor FETs…

- Types:
  - Ion selective FET (ISFET) – conventional and double gate.
  - Silicon nanowire biosensors.
  - Organic FET and graphene FET biosensors.
- Miniature, ultra sensitive and fast response time.
- Respond to electrostatic charges and potential changes.
  - Detection of nucleotides, amino acids, cells (e.g. bacterial and viruses).
- Arrays may allow parallel processing.
- Suitable for integration with other electronics.
- Excellent for point-of-service devices of the future.

Solid-Liquid EDL Model…

- Due to local coulomb force, ions in solution are separated into three layers:
  - **Stern layer** (stationary layer), forms at the solid surface. Ions in Stern layer are attracted by charges in solid and bound to the surface firmly. Therefore, the electric potential decreases linearly from the interface to the solution.
  - **Diffuse layer** has ion concentration changing as a function of the distance from the interface. The ion concentration function follows Maxwell-Boltzmann statistics and the electric potential in the diffuse layer decreases exponentially with the distance from the surface of solid-liquid interface.
  - **Bulk solution**, where coulomb force from the interface has no significant impact.
- **Debye length** – the distance the solid-liquid interface to the boundary of the diffusion layer and bulk solution.
  - The longest distance where charges of analytes can affect surface characteristic.
  - This is the minimum sensing distance.
  - The boundary of diffusion layer and bulk solution is defined by the decay of electric field at the ratio of $e^{-1}$.

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a) Effect of charge screening on the electrical potential of biomolecules and b) the device.

c) An aptamer-based immobilizer compared with an antibody-based immobilizer by Debye length. Shorter immobilizers yield better conductivity.
• Potential diagram for a general electrolyte-insulator-semiconductor (EIS) model, (analogous to MOS model with the replacement of metal by electrolyte).

• A sufficient difference of surface potentials ($\Delta \Psi$) changes the status of channel into inversion.

• The potential difference is originated from the change of charge concentration on the surface ($\Delta \sigma_0$) and it is attributed to the contribution of intrinsic charge from biomolecules or ions released from enzymatic reactions.

Silicon Nanowire Biosensor...

a) An illustration of silicon nanowire; a wire-like channel connects to source and drain electrodes.

b) The SEM image of a silicon nanowire.

d) Positive charges accumulate on the surface. The electrostatic attraction force to electron carriers results in higher conductance.

e) The original state of SiNW.

f) Negative charges accumulate on the surface. The electrostatic repulsion force to electron carriers results in lower conductance.


Biomolecule Detection…

a) Attachment of hydroxide groups on the surface.

b) Surface modification of the first linker from the silane group and the second linker from the carboxyl group.

c) Antibody immobilization.

d) Binding of antigen. Measurements occur between the source and drain.
ai) SiNW  
aii) PANI nanowire  
aiii) Graphene.

(b) Changes of majority carrier numbers of the nanowire and the current flow of device before and after the immobilization of:  
i) negatively charged  
ii) positively charged biomarkers both on n-type substrates with the impact of back-gate biasing.
The functionalization process required more complex process than immobilizing the probes only. This is because the functional groups of biomolecules need cross-linkers to form covalent bonds with the sensing film (dielectric layer) of FET-based biosensors.

The most commonly used cross-linking process for ISFET and SiNW (i.e. the oxide based sensing dielectric) is the APTES-GA method. (3-aminopropyltriethoxysilane)

APTES is a silane molecule, which is able to bond with sensor dielectric surface, such as SiO₂.

This self-assembled organic monolayer, APTES, is formed on the sensor surface and provides a good platform for the second stage linkers by its amine group in the other end.

The second stage cross-linker is glutaraldehyde, which is a bifunctional reagent connecting the APTES and bio-probe by imide bonds.
Immobilization of Antibodies and Enzymes on 3-Aminopropyltriethoxysilane-Functionalized Bioanalytical Platforms for Biosensors and Diagnostics

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Innovative Chromatography Group, Irish Separation Science Cluster (ISSC), Department of Chemistry and Analytical, Biological Chemistry Research Facility (ABCRF), University College Cork, Cork, Ireland
APTES can be deposited on solid materials, electrode materials, nanomaterials, and nanocomposites. It reacts with the free hydroxyls of an oxidized substrate by $S_N^2$ exchange with loss of ethanol.

APTES bonds to a substrate in three different ways:
1. Surface attachment and condensation lead to horizontal polymerization when a surface-bound APTES molecule forms siloxane with its neighboring surface-bound APTES.
2. In vertical polymerization, the surface-bound APTES reacts with a nearby APTES in solution.
3. Where the substrate possesses surface hydroxyl groups, the amine of APTES forms a hydrogen bond with the metal surface or becomes protonated by abstracting protons from the surface.
Silanization occurs on any substrate, normally with chemically active hydroxyl groups for silane grafting.

Some substrates (e.g., silica, agarose, etc.) already have hydroxyl groups, while others require a pretreatment step using KOH/NaOH, acid, piranha solution, or plasma treatment to introduce hydroxyl groups.
The amine group of APTES enables the covalent bonding of biomolecules based on the use of a functional linker. GLD (glutaraldehyde), a homobifunctional agent (same reactive groups on each end of the crosslinker), cross-links the amino groups of APTES-functionalized surfaces to the biomolecule amino groups.

Aldehyde groups form imine bonds with amine groups of lysine common to almost every protein or enzyme to form reversible Schiff bases.

The most widely used hetero-bifunctional cross-linker combination is EDC and N-hydroxysuccinimide (NHS)/N-hydroxysulfosuccinimide (SNHS), where EDC of the EDC-NHS/SNHS complex first binds to the carboxyl-terminal of the Abs.
Most commonly used enzyme immobilization strategies on APTEs-functionalized bioanalytical platforms.
The specific molecular interaction between the avidin and biotin pair is a well-known phenomenon and has been extensively advocated for biomolecule immobilization as the cubic shape avidin possesses four biotin binding sites.

Conceptually, avidin is covalently attached to APTES-surfaces by either GLD-mediated or EDC-mediated reactions as previously described.

Avidin then acts as a biocompatible linker between APTES-surfaces and biotinylated biomolecules.
a) Synthesis of protocol Ppy-NDFLG on flexible substrate. Polypyrrole-converted nitrogen-doped few-layer graphene (PPy-NDFLG) was fabricated by chemical vapor deposition combined with vapor deposition polymerization.

b) Reaction steps for the fabrication of the aptasensor platform based on Ppy-NDFLG conjugated with an anti-VECF aptamer. The FET biosensor was fabricated by the adsorption of 1,5-diaminonaphthalene (DAN) on the surface of PPy-NDFLGs, and the aptamer was introduced onto the DAN/PPy-NDFLGs because of defect minimization.

Nitrogen-doped few-layer graphene converted from 2D polypyrrole nanomaterials and integrated into FET aptasensors for antivascular endothelial growth factor (VEGF) detection.


High-performance integrated field-effect transistor-based sensors


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HIGHLIGHTS
- Performance of FET-based biosensors for the detection of biomolecules is presented.
- Silicon nanowire, polyaniline and graphene are the highlighted nano-scaled materials as sensing transducers.
- The importance of surface material interaction with the surrounding environment is discussed.
- Different device structure architectures for ease in fabrication and high sensitivity of sensing are presented.
Summary

- Biosensor definition.
- Electrochemical methods.
- Recognition elements
- Nanotechnology and the Nano-Bio Labs
- Field effect transistor (FET)-based biosensors.
- Specific immobilization Methods – Review Vashist et al.

Excellent paper for a team presentation!
Appendix

- Comparison of Immobilization Methods
- Biosensor Electrode Material
- Label Free vs Labeled Assays
- Examples of Droplet-Based Systems
- Conventional MOSFET
- Fabrication Process of FETs
- Additional FET Configurations
- NANO-flex Dynamic Light Scattering
- Measuring Particle Size and Concentration
### Table 2. Comparison of Standard Immobilization Methods

<table>
<thead>
<tr>
<th>immobilization method</th>
<th>biorecognition element</th>
<th>principle</th>
<th>examples</th>
<th>advantages</th>
<th>disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>adsorption</td>
<td>small molecules</td>
<td>van der Waals force, hydrophobic force, hydrogen bonds, ionic interaction</td>
<td>proteins, antibodies/antigens</td>
<td>simple, quick</td>
<td>weak forces, leakage, low reproducibility</td>
</tr>
<tr>
<td>covalent binding</td>
<td>proteins</td>
<td>electron sharing (functional groups)</td>
<td>$-\text{NH}_2$, $-\text{COOH}$, $-\text{SH}$, $-\text{OH}$, imidazole, phenol, phosphate</td>
<td>irreversible (strong), mild</td>
<td>conformation change of BRE, molecular architecture difficult</td>
</tr>
<tr>
<td>cross-linking</td>
<td>proteins, enzymes</td>
<td>two functional groups of cross-linker connect BRE to transducer</td>
<td>carbonyldiimidazole, SAM glutaraldehyde, EDC, NHS</td>
<td>many cross-linker options, simple, quick, mild conditions</td>
<td>inter/intramolecular linking, sizing restrictions</td>
</tr>
<tr>
<td>entrapment</td>
<td>cells, proteins</td>
<td>restrain due to steric hindrance in matrix or membranes</td>
<td>polyacrylamide, gelatin, polyvinyl alcohol, alginate</td>
<td>simple, quick</td>
<td>leakage, permeability of interferences, diffusion barrier</td>
</tr>
</tbody>
</table>
## Biosensor Electrode Materials…

**Table 1. Examples of Electrode Materials and Their Properties**

<table>
<thead>
<tr>
<th>Electrode Material</th>
<th>Potential Window/V</th>
<th>Conductivity/S cm⁻¹</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>gold</td>
<td>−0.1 to 1.3</td>
<td>10⁷</td>
<td>inert, reusability, preparation of SAMs, easy to clean</td>
</tr>
<tr>
<td>ITO (Indium tin oxide)</td>
<td>−0.4 to 1.9</td>
<td>10⁴</td>
<td>electrochemical stable, cheap, transparent</td>
</tr>
<tr>
<td>carbon</td>
<td>−0.4 to 1.7</td>
<td>10³</td>
<td>solvent resistance, reproducible, mechanical stability, biocompatible</td>
</tr>
<tr>
<td>Conducting polymers</td>
<td>−1.0 to 1.0</td>
<td>up to 10³</td>
<td>low cost, adjustable redox activity, transparent, flexible</td>
</tr>
</tbody>
</table>

*The potential window and the conductivity are given for glassy carbon. The potential window and the conductivity are given for PEDOT:PSS (poly(3,4-ethylenedioxythiophene) polystyrene sulfonate).*

- Lessening conductivity
- High stability and inertness.
- Unstable in acidic environments
- Glassy carbon commonly used.
- Poly(3,4-ethylenedioxythiophene) (PEDOT), polyaniline (PANI), or poly-pyrrole (PPy)
Label-Free vs Labeled Assays...

- Strategy depends on the BRE-analyte interaction, type of analyte and the expected electrical signal.

- Impedance and potentiometric sensing is usually label-free.

- Voltammetry can be used when signals are generated from oxidative or reduction of intrinsic electroactive compounds.

Examples of Droplet-Based Systems…

Monitoring glucose using glucose oxidase.

Detecting E. coli with magnetic beads conjugated with antibodies as the BRE.


Conventional MOSFET...

- A metal or polysilicon gate covers the region between the source and drain.
- The flow of electrons from the source to the drain is controlled by the voltage applied to the gate.
- A positive voltage applied to the gate, attracts electrons to the interface between the gate dielectric and the semiconductor.
- These electrons form a conducting channel between the source and the drain, called the inversion layer.
- No gate current is required to maintain the inversion layer at the interface since the gate oxide blocks any carrier flow.
- The net result is that the current between drain and source is controlled by the voltage which is applied to the gate.
From silicon on insulator (SOI) to three different devices: silicon nanowire, polyaniline nanowire and graphene biosensor.
a) Back-gated FET on an SOI wafer.

b) Underlap FET.

c) Double gate FET.

Laser light comes into the sample. Measures particles ranging in size from 0.8 to 6,500 nanometers.
Measuring Particle Size and Concentration…

Nano tracking particle analyzer.

Sample stage fluid cell with laser light.

Material microscope, measuring down to 0.5 micron.