Enhancement of **electrochemical biosensor** performances using **redox cycling** at **3D sub-micrometer scale electrode architectures**

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   Electrochemical biosensors, Redox cycling, Carbon-MES

2. Approaches and Fabrication
   Sensor configurations, Morphology

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   Signal amplification, Sensitivity, Selectivity

4. Summary & Future works
Electrochemical Biosensors: Applications

• Health & safety
  - Clinical diagnosis, food control, environmental screening

• Biosensor classification
  - Transducer types
    • Electrochemical, Optical, Electrical, Piezoelectric, Calorimetric
## Electrochemical vs. Optical sensors

<table>
<thead>
<tr>
<th></th>
<th>Optical Sensor</th>
<th>Electrochemical Sensor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Excellent</td>
<td>High</td>
</tr>
<tr>
<td><strong>Selectivity</strong></td>
<td>High</td>
<td>Good</td>
</tr>
<tr>
<td><strong>Sample</strong></td>
<td>Clear sample only</td>
<td>No limitation</td>
</tr>
<tr>
<td><strong>Device configuration</strong></td>
<td>Complex</td>
<td>Simple</td>
</tr>
<tr>
<td><strong>Hand-held &amp; Disposable</strong></td>
<td>Difficult</td>
<td>Feasible</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>Relatively expensive</td>
<td>Cheap</td>
</tr>
</tbody>
</table>

- Amperometry
- Voltammetry
- Coulometry
- EIS
Enhancement of electrochemical sensing

3D sub-micrometer scale electrodes

- Redox sensor signal enhancement
  - 3D carbon IDEs
  - Sandwich electrodes

- Selective bioreceptor immobilization
  - Enzymes
  - Antibodies

- Simple microchannel integration

Redox cycling at 3D micro/nano-scale electrode architecture
- Large S/V
- Linear diffusion
- Signal amplification
  - > 30 in bulk
  - > 800 in μ-channel

Electrochemical reduction of aryl diazonium salt
- Bioreceptors
  - Selectivity ↑
- Efficient redox reaction
  - Sensitivity ↑

Redox in confined environment
- Irreversible species
  - Depletion
- Reversible species
  - Redox cycling
  - Selectivity enhancement
Redox cycling

• Sensitivity enhancement
  – Amplifying Faradaic current signal

Single mode

Dual mode (Redox cycling)

Interdigitated electrodes (IDEs)

Sandwich electrodes
Improvement of Redox cycling effect

- Diffusion enhancement via electrode reconfiguration

**Interdigitated electrodes (IDEs)**

- **Nano-gap**
- **High aspect ratio**

**Sandwich electrodes**

- **Large surface**
- **Nano-gap**

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Improvement of Redox cycling effect

- Limitations of previous approaches

**Interdigitated electrodes (IDEs)**
- Nano-gap: Expensive nanofabrication
- High aspect ratio: Complex MEMS process
- Limitation in electrode gap reduction with high aspect ratio

**Sandwich electrodes**
- Large electrode surface: Hassle alignment process
- Nano-gap: Sacrificial layer removal, Difficulty in chip integration, Small current signal

**Carbon-MEMS-based nanoelectrodes**
- Simple two-step nanoelectrode fabrication
- 3D architecture with complex design
- Electrode gap control via pyrolysis
- Comparable or even better current amplification
Carbon-MEMS

- Polymer patterning + Pyrolysis
  - Wafer-level simple fabrication of micro/nano carbon 3D structures
    - Conversion from polymer to glassy carbon
    - Conversion from insulator to conductor
    - Overcome limited manufacturability (brittleness)
    - Controllable geometry: photolithography, nano-imprint, e-beam lithography, electrospinning.

High aspect ratio SU-8 photoresist → Pyrolysis 600 ~ 900 °C → Glassy carbon electrode
Carbon-MEMS

**Polymer patterning + Pyrolysis**
- Dramatic *volume reduction* up to 90%
- Conversion from *microstructures* to *nanostructures*
- Simple and easy fabrication of 1D carbon nanostructures

*Interdigitated electrodes (IDEs)*

*Sandwich electrodes*

- Post height: 10 ~ 20 μm; Electrode gap: 4 ~ 14 μm
- Post height: 3 ~ 7 μm; Electrode gap: 2 ~ 4 μm
Electrode configurations

3D sub-microscale electrode sets

Dual electrodes
- 1:1 aspect ratio IDEs
  - Generator: Comb 1
  - Collector: Comb 2

Sandwich electrodes
- Generator: Planar E
- Collector: Mesh

Triple electrodes
- Suspended mesh (Bioreceptors) + IDEs (Redox cycling)
  - Suspended carbon mesh
  - Substrate-bound IDA nanoelectrodes

Advantages
- Simple fabrication
- Large surface area
- Efficient Redox cycling
- Biocompatible material
Fabrication

• Carbon-MEMS
  3D sub-micro scale architecture

(A) Insulating layer deposition
(B) UV-lithography
(C) Pyrolysis

(E) Positive PR patterning
(F) SU-8 2025 photoresist spincoating
(G) Long UV exposure

(I) Short UV exposure
(J) Development
(K) Pyrolysis

Glassy carbon | Insulation layer (SiO₂) | PR (NR9-8000) | Photo mask | Exposed PR (SU-8) | Positive PR (AZ 4330) | RF sputtered SiO₂ | UV

1:1 aspect ratio IDEs

Sandwich electrodes

Triple electrodes

Suspended carbon mesh

Substrate-bound IDA nanoelectrodes
Electrode morphology

• Carbon-MEMS ➔ Dual electrodes
  - Interdigitated Array (top view)
  - Sandwich electrodes (side & top view)

Dimensions:
- Length: 100 μm
- Width: 620 nm
- Thickness: 650 nm
- Electrode gap ~ 1.9 μm

Dimensions
- Post height: ~ 4 μm
- Mesh thickness ~ 1 μm
- Mesh width ~ 300 nm
- Pad thickness ~ 600 nm
- Electrode gap ~ 2.4 μm
Electrode morphology

• Carbon-MEMS → Triple electrodes

Substrate-bound carbon IDEs

- 620 nm (line width)
- 650 nm (thickness)

Suspended carbon mesh

- 300 nm (line width)
- 1.2 mm (thickness)
Signal amplification via redox cycling

• Signal amplification
  – Amplification factor = \( \frac{I_{\text{Dual mode}}}{I_{\text{Single mode}}} \)
  (Current signal with Redox cycling)
  (Current signal without Redox cycling)
  – Linear diffusion between generator and collector
  – Bulk vs Microchannel
    • Volume confinement effect in microchannel
    • Cyclic voltammetry (CV) vs Chronoamperometry

Carbon nano-IDEs

Sandwich electrodes

Cyclic voltammetry

in bulk

Cyclic voltammetry

in microchannel

Chronoamperometry

in microchannel

Redox cycling : On
(Dual mode Anodic current)
Redox cycling : Off
(Single Mode)
Redox cycling : Off
(Single Mode)
Redox cycling : On
(Dual mode Cathodic current)
Redox cycling : On
(Dual mode Cathodic current)
Redox cycling : Off
(Single Mode)

~16X
~65X
~840X

Potential (V vs. Ag/AgCl)
Potential (V vs. Ag/AgCl)
Time (s)
Approaches for selective modification

- Diazonium salt reduction

Selective covalent modification

1. Diazotation
   NaNO₂, HCl, ice cold

2. Electrodeposition
   Carbon electrode

3. Using EDC/NHS linker
   Enzyme coating
   Enzyme-based biosensor

4. Antigen binding and sandwich formation
   Carbon electrode

Enzyme-based biosensor

Immunosensor

Myoglobin
β-galactosidase conjugated anti-IgG antibody
Secondary antibody
Enzyme
Enzyme-based biosensors

- Carbon IDEs
  - Glucose biosensor

Gluconic acid

- GOx
- Fe[CN$_6$]$^{3-}$
- Fe[CN$_6$]$^{4-}$
- Pyrolytic carbon
- SiO$_2$
- Si

Sensitivity enhancement at non-functionalized comb (Comb 2)
- More electrochemically active area
- Efficient Redox cycling
- 2-fold higher sensitivity vs Comb 1
- 295-fold better LOD vs Comb 1

Graph showing current vs glucose concentration for Comb 1 (functionalized) and Comb 2 (non-functionalized).
Enzyme-based biosensors

- Carbon IDEs
  - Glucose biosensor

- Carbon IDEs with AuNPs
  - Glucose biosensor

**Sensitivity enhancement at non-functionalized comb (Comb 2)**
- More electrochemically active area
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- 295-fold better LOD vs Comb 1

**Sensitivity enhancement with electrode modification (AuNPs)**
- More electrochemically active
- Larger surface area
- 30% better sensitivity vs carbon IDEs
- 2-fold better LOD vs carbon IDEs

**AuNP electrodeposition**
Enzyme-based biosensors

- Carbon IDEs
  - Glucose biosensor

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Selectivity enhancement in microchannel

• Microchannel-integrated sandwich electrodes
  – Alignment-less process
  • Flat PDMS plate + Sandwich electrodes
  • Carbon posts work as channels walls
Selectivity enhancement in microchannel

- Confined volume in microchannel
  - Electrochemically reversible species → Redox cycling
  - Electrochemically irreversible species → Depletion

Reduction of interference from irreversible species
Selectivity enhancement in microchannel

- Selective detection of dopamine
  - Electrochemically reversible species: Dopamine (DA)
  - Electrochemically irreversible species: Ascorbic acid (AA)

(A) Bulk solution (w/o PDMS ceiling)

(B) Microchannel (w/ PDMS ceiling)

Interfering current signal from AA ➔ No interference effect
Selectivity enhancement in microchannel

• Selective detection of dopamine
  – Electrochemically reversible species: Dopamine (DA)
  – Electrochemically irreversible species: Ascorbic acid (AA)

• Dopamine sensing (Bulk vs Microchannel)
  – LOD enhancement in microchannel
    : 3.3 μM (Bulk) → 0.2 μM (17 fold)
  – Sensitivity enhancement in microchannel
    : 0.32 nA/μM (Bulk) → 0.63 nA/μM (2 fold)

Dopamine sensing in a microchannel
  ✓ Dopamine: 100 nM – 10 mM
  ✓ Ascorbic acid: 1 mM
  ✓ PBS: 0.1 M
Triple electrode-based immunosensors

- Electrochemical immunosensors
  - Sandwich immunoassay
  - Redox cycling
  - Generation of redox substrate near the adjacent electrode

(a) Parallel IDA electrodes
(b) PDMS with electrodes
(c) Magnetic-beads

Limitations of reported immunosensing schemes
1. Large gap between the bottom and ceiling electrodes; (a) and (b)
2. Required proper alignment; (a) and (b)
3. Beads can block and inhibit the diffusion of electroactive species; (c)

Require new sensor configurations
Triple electrode-based immunosensors

- Suspended mesh + Substrate-bound IDEs
  - Selective sandwich formation at suspended mesh
  - Generation of redox substrate (PAP) near carbon IDEs
  - Efficient Redox cycling (PAP ↔ PQI) at IDEs

Mesh-to-IDA distance

Diazenium salt | Monoclonal antibody
---|---
Bio-marker (e.g., Myoglobin) | Secondary antibody
β-galactosidase conjugated anti-IgG antibody

PAPG: 4-aminophenyl β-D-galactopyranoside
PAP: p-aminophenol
PQI: p-quinoneimine

Generator comb
Collector comb
Triple electrode-based immunosensors

- Suspended mesh + Substrate-bound IDEs
  - Effect of mesh-to-IDE distance
  - Simulation on diffusion-limited current signal

- Mesh-to-IDEs distance > 5 μm → small distance enhances diffusion of PAP
- Maximum current at 5 μm
- Mesh-to-IDEs distance < 5 μm → disturb diffusion among IDEs
Triple electrode-based immunosensors

- Suspended mesh + Substrate-bound IDEs
  - Mesh-to-IDEs distance ~ 3.3 μm
    - Efficient production of redox species near IDEs
  - Cardiac biomarker (Myoglobin) in human serum:
    - LOD ~ 0.48 pg/mL
    - High selectivity against interfering species

![Image of triple electrode-based immunosensor setup with suspended mesh and substrate-bound IDEs.](image)

- Graph showing the relationship between spiked cMyo concentration in serum and current (nA). The equation $y = 7.356\ln(x) + 67.392$ with $R^2 = 0.973$.

- Bar chart comparing currents for different substances: BSA, CK-MB, cTnl, Glu, cMyo, and Mix, with cMyo spiked at 0.1 ng/mL. cMyo is much higher than other substances, with BSA, CK-MB, and cTnl being 10x higher than cMyo, and Glucose 900,000x higher than cMyo.
Summary & Future works

• Summary
  − Wafer-level batch fabrication via C-MEMS
    • 3D sub-micrometer scale electrode architectures
    • Simple and cost-effective processes
  − Electrochemical sensor performance enhancement
    • Sensitivity
      • Selective bioreceptor immobilization near sensing area
        [Efficient Redox cycling at 3D electrodes]
      • Electrode surface modification with AuNPs
    • Selectivity
      • Bioreceptors (Enzymes, Antibodies)
      • Depletion effect in microchannel

• Future works
  − Multiple biomarker detection in a single chip
    • Microchannel integration
    • Integration of multiplex sensor array
    • Cholesterol, Glucose, CK-MB, Myoglobin, Troponin, etc.
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**Micro / Nano Integrated Systems Lab**
Thank you for your attention
AuNPs/Carbon IDEs

• Electrochemical characterization
  – Cyclic voltammetry
    • 10 mM [Fe(CN)₆]²⁺ in 0.1 M KCl
  – Carbon nanoelectrode
    • Width = 650 nm; Thickness = 650 nm
  – Current signal enhancement in dual mode
Triple electrode-based immunosensors

- **AuNPs/carbon IDEs**
  - Cholesterol sensors
    - Sensing range: 0.005-10 mM
    - Sensitivity $\approx 994 \, \mu A \, mM^{-1} \cdot cm^{-2}$
    - LOD $\approx 1.28 \, \mu M$
Triple electrode-based immunosensors

- Effect of selective immobilization
  - Low current signal deviation after immobilization (IDEs)
  - Spontaneous physical binding reduces signal significantly.
Triple electrode-based immunosensors

- Effects of various sensor preparation conditions on the detection of 0.1ng/mL cMyo
Immunosensing performance

• Carbon 3D triple electrode
  - 3D triple electrode set + selective immobilization
  - Reversible redox cycling (PAP ↔ PQI)
  - Generation of redox substrate (PAP) near the IDA electrode
  - Characterization according to antibody binding site

### Configuration (I)
- Antibody binding site: suspended mesh generator & collector
- IDA nanoelectrodes

### Configuration (II)
- Antibody binding site: one comb of IDA generator
- the other comb of IDA collector
- suspended mesh collector

### Configuration (III)
- Antibody binding site: one comb of IDA generator
- suspended mesh collector
- the other comb of IDA collector
Immunosensing performance

- Carbon 3D triple electrode
  - 3D triple electrode set + selective immobilization
  - Reversible redox cycling (PAP ↔ PQI)
  - Generation of redox substrate (PAP) near the IDA electrode
  - Characterization according to antibody binding site

Sensitivity enhanced at sensor configuration (I)

- Small inter-electrode gap between IDEs
- Efficient redox cycling
- Large surface area of mesh enabling substantial biomolecule binding
- Efficient mass transfer

(I) Antibody site: suspended mesh, generator and collector: IDA nanoelectrodes;
(II) Antibody site: one comb of IDA nanoelectrodes, generator: the other comb of IDA nanoelectrodes, collector: suspended mesh;
(III) Antibody site: one comb of IDA nanoelectrodes, generator: suspended mesh, collector: the other comb of IDA nanoelectrodes)