Carbon Nanofiber Nanoelectrodes for Biosensing Applications

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NASA Ames Research Center

- Established in 1939 as the second laboratory of the National Advisory Committee for Aeronautics (named after NACA chair, Joseph S. Ames)
- Ames is 1 of 10 NASA field centers
- Located in the heart of the silicon valley
  - High-tech companies, start-ups, biotechnology
- Ames Technical Areas
  - Nanotechnology
  - Information technology
  - Fundamental space biology
  - Biotechnology
  - Thermal protective systems
  - Human factors research
Biosensor Motivation

NASA Applications

- Astronaut health monitoring
  - Lab-on-a-chip
- Water Quality monitoring
  - Pathogen detection on ISS and long duration missions
- Planetary exploration
  - Life on other planets

Outside Applications and Customers

- Medical Diagnostics
  - NIH, DARPA
- Environmental Monitoring
  - EPA, NIH
- Biowarfare agent detection
  - DHS, DARPA
- Food Safety
  - FDA
Biosensor Basics

Biomolecule + Transducer + Reader/Signal Processor

Type of Signal Transduction: optical, electrical, **electrochemical**, surface plasmon resonance, piezoelectric
Nanoelectrodes for Sensors

Nanoscale electrodes create a dramatic improvement in signal detection over traditional electrodes for small analyte concentrations.

Background: \( i_n \propto C_d^0 A \)

- **Scale difference** between macroelectrode and molecules is tremendous.
- **Background noise** on electrode surface is therefore significant.
- **Significant amount** of target molecules required.

- Nanoelectrodes are at the scale close to molecules.
- with dramatically **reduced background noise**.
- Multiple electrodes results in magnified signal and desired redundancy for statistical reliability.
Macroelectrode vs. Nanoelectrode

Critical dimension > 25µm

- Semi-infinite planar linear diffusion
- Glassy carbon

Critical dimension < 25µm

- Semi-infinite hemispherical diffusion:
  - Current exhibits a steady state
  - Diffusion layer is approximately 6r
- Carbon nanofiber

- Nanostructured Ensemble or Array Electrode

- Spatial Resolution: defined by \( r \)
- Sensitivity: signal to noise
  - \( \frac{i_s}{i_n} \approx nFC_0D_0/r \)
Carbon Nanofibers (CNFs)

**Edge Plane:**
1. High electron transfer rate (~0.1 cm/s)
2. Very high specific capacitance (>60 μF/cm²)

**Basal Plane:**
1. Low electron transfer rate (<10⁻⁷ cm/s)
2. Anomalously low capacitance (~1.9 μF/cm²)

(1) Coat silicon wafer with underlying Cr metal & Ni catalyst metal

(2) Growth of Vertically Aligned CNF Array by Plasma Enhanced Chemical Vapor Deposition (PECVD)

(2) Dielectric Encapsulation of silicon dioxide by TEOS Chemical Vapor Deposition (TEOS CVD)

(3) Planarization by Chemical Mechanical Polishing (CMP)

(5) Electrochemical Characterization

CNF Growth by Plasma Enhanced Chemical Vapor Deposition (PECVD)

Growth Process
- Heated to 650 C
- Plasma discharge 500 W, 530 V, 0.97 A
- 150 sccm NH$_3$/50 sccm C$_2$H$_2$, 5-6 torr
- Growth rate- 1000 nm/min
- Quality is good, alignment is good

Define CNF Placement by Catalyst Placement

Continuous Layer of Catalyst

Photolithography Defined Catalyst Spots

Electron Beam Lithography Defined Catalyst Spots

As Grown CNFs

SiO$_2$ Encapsulated CNFs
Fabrication of 3x3 Array

- 30 devices on a 4” Si wafer
- 200 μm by 200 μm electrode dimensions
- 9 individually addressed electrodes
- Potentially 9 different target molecules
Electrochemistry of CNF Arrays

As grown CNFs

High density

Low density

Embedded CNFs

Embedded high density

Embedded low density

All scans performed in 1mM $\text{K}_4[\text{Fe(CN)}_6]$ in 1M KCl at 20 mV/s

Chemical Mechanical Polish to Control CNF Density

Note:
For nanoelectrode behavior, diffusion layer is approximately 6\(r\)
Objective:
Test an ultrasmall biosensor for point of care diagnostics for astronaut health monitoring
Astronaut Heart Health Monitoring

Microgravity and Cardiovascular Health
• Fluid Shifts
• Changes in total blood volume
• Changes in heart beat
• Diminished aerobic activity

Need for on-flight diagnostics

Troponin-I
• biomarker: acute myocardial infarction
• normal levels: 0.4 ng/mL and lower
• risk of heart attack: 2.0 ng/mL and above

http://www.nasa.gov/exploration/humanresearch/areas_study/physiology/physiology_cardio.html
Electrochemical Impedance Spectroscopy of CNF Electrode

ultralow density CNF

<table>
<thead>
<tr>
<th>Fitting Parameters</th>
<th>Randomly Grown CNF</th>
<th>CNF (low density)</th>
<th>CNF (ultralow density)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I$ (A/mm$^2$)</td>
<td>$7.1 \times 10^{-6}$</td>
<td>$1.8 \times 10^{-6}$</td>
<td>$2.5 \times 10^{-7}$</td>
</tr>
<tr>
<td>$R_{ct}$ (KΩ)</td>
<td>N/A</td>
<td>1.8</td>
<td>17.3</td>
</tr>
<tr>
<td>CPE (μF)</td>
<td>906</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>$n$</td>
<td>0.79</td>
<td>0.89</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Surface Preparation of CNF Electrode

1. Sulfo-NHS
2. EDC
3. NH₂ anti-Troponin
4. Troponin-I
Troponin-I Detection

Blue: bare electrode
Pink: with anti-troponin
Black: with anti-troponin and protein

Increase in $R_{ct}$ observed upon anti-troponin immobilization and matching protein binding

Troponin-I concentration range: 100 ng/mL to 0.25 ng/mL
Detection down to 0.25 ng/mL

Troponin-I Concentration Study

Troponin-I concentration range: 100 ng/mL to 0.25 ng/mL
Detection down to 0.25 ng/mL

Motivation: Parkinson’s Disease

Parkinson’s disease is a neurodegenerative disorder in which patients have insufficient production of dopamine from dopaminergic cells in the substantia nigra.

Current treatments include L-dopa, dopamine agonists, MAO-B inhibitors, surgery (ablation and deep brain stimulation)

http://knight.noble-hs.sad60.k12.me.us/context/exploringLife/text/chapter28/concept28.2.html
http://www.profelis.org/webpages-cn/lectures/neuroanatomy_1ns.html
Deep Brain Stimulation (DBS)
- Started in the 1960’s
- Over 80,000 successful surgeries
- Has been demonstrated to be an effective neurosurgical treatment for several pathologies including:
  
  • tremor
  • epilepsy
  • Parkinson’s disease
  • depression
  • Tourette syndrome
  • chronic pain

How DBS Works
- Brain pacemaker, electrical impulses to different areas of the brain
- Stimulation 24/7

Potential Improvements
- Time consuming and difficult to program without feedback
- Want real-time monitoring of the neurochemical output
- Development of chemically-guided placement of DBS electrodes in vivo.

Clinical efficacy is not questioned, but mechanisms are very poorly understood
History of DBS

• DBS used for pain control since 1960s
• DBS for tremor began in Europe (1987)
• Europe: CE mark approval for
  – Activa® Tremor Control Therapy in 1993
  – Activa® Parkinson’s Control Therapy in 1998
• USA: FDA approval for
  – Activa® Tremor Control Therapy in 1997
  – Activa® Parkinson’s Control Therapy in 2002
Deep Brain Stimulation Electrodes

DBS Electrodes from Medtronic

Electrode modèle 3387
4 plots de 1,5 mm espacés de 1,5 mm

Electrode modèle 3389
4 plots de 1,5 mm espacés de 0,5 mm

Surface de contact de 1,5 mm

10,5 mm
7,5 mm

CNF Electrodes

Current 3x3 CNF device does not have an optimal geometry for implantation but can be used to preliminary in vitro investigations.
Goal:
Develop a multiplexed CNF array for localized, fast, and efficient and neurochemical recording
Electrochemical Detection of Neurotransmitters

- Molecules of Interest
  - Dopamine
    - Movement disorders, addiction
  - Serotonin
    - Depression, hunger
  - Adenosine
  - Oxygen
  - Hydrogen Ions (pH)

- Techniques
  - Differential Pulse Voltammetry
    - More sensitive
  - Fast Scan Cyclic Voltammetry
    - Better temporal resolution
## Simultaneous Detection of Neurotransmitters

<table>
<thead>
<tr>
<th>Glassy Carbon Electrode</th>
<th>Carbon Nanofiber Electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascorbic Acid</td>
</tr>
<tr>
<td></td>
<td>Dopamine</td>
</tr>
<tr>
<td></td>
<td>Serotonin</td>
</tr>
</tbody>
</table>

- CNF electrode has ability to distinguish multiple electroactive brain chemicals in a mixture!
- Detection limits 50nM for DA and 100nM for 5-HT
Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS)

The Mayo Clinic-developed WINCS is a microprocessor-controlled, MRI-compatible, battery-powered instrument that combines Bluetooth® digital telemetry with fast scan cyclic voltammetry and constant potential amperometry.

WINCS was designed in compliance with FDA-recognized standards for medical electrical device safety.

Experimental Setup

Custom-Designed Flow Cell

Cross-section:
- Solution in (2 mL/min)
- Solution out
- Electrical lead
- Polycarbonate
- Sample

WINCSware User Interface

WINCSware allows viewing of the data in nearly real-time
Dopamine Detection

Carbon Nanofiber Electrode

a) CNF BGS CV

b) CNF Calibration

R² = 0.9870

Carbon Fiber Microelectrode

d) CFM BGS CV

e) CFM Calibration

R² = 0.9618

NASA
In the Works

**Want to Combine:**

1) Penetrating multiplexed array
   - Ability to spatially resolve chemical release events

2) Array of individual carbon nanofiber nanoelectrodes
   - High sensitivity (increased signal to noise)
   - Rapid detection (increased cell time constant)
   - Wide potential window of carbon
Summary

- Carbon nanofibers can be used to as nanoscale electrodes to reduce background noise while maintaining large sampling volume
- Carbon nanofiber nanoelectrode arrays are easily fabricated using standard silicon processing
  - CNF spacing defined by photolithography, e-beam lithography or top layer dielectric polishing time
- Carbon nanofibers have been used as sensitive nanoelectrodes for cyclic voltammetry and electrochemical impedance spectroscopy investigations
- Changes in $R_{ct}$ are measured after antibody immobilization and protein binding
- Carbon nanofiber nanoelectrode arrays have been used to detect down to 0.25 ng/mL troponin-I
- CNFs can distinguish between multiple electroactive analytes in a mixture using differential pulse voltammetry
- CNFs nanoelectrode arrays easily integrate with WINCS
- CNFs detect dopamine with similar performance to a standard carbon fiber microelectrode
- The flexible multiplexing capability of CNF devices will be used in the future for in vivo studies of neurotransmitter detection
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