Carbon nanofiber nanoelectrodes for neural stimulation and chemical detection: The era of “smart” deep brain stimulation

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A sensor platform based on vertically aligned carbon nanofibers (CNFs) has been developed. Their inherent nanometer scale, high conductivity, wide potential window, good biocompatibility and well-defined surface chemistry make them ideal candidates as biosensor electrodes. Here, we report two studies using vertically aligned CNF nanoelectrodes for biomedical applications. CNF arrays are investigated as neural stimulation and neurotransmitter recording electrodes for application in deep brain stimulation (DBS). Polypyrrole coated CNF nanoelectrodes have shown great promise as stimulating electrodes due to their large surface area, low impedance, biocompatibility and capacity for highly localized stimulation. CNFs embedded in SiO$_2$ have been used as sensing electrodes for neurotransmitter detection. Our approach combines a multiplexed CNF electrode chip, developed at NASA Ames Research Center, with the Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS) system, developed at the Mayo Clinic. Preliminary results indicate that the CNF nanoelectrode arrays are easily integrated with WINCS for neurotransmitter detection in a multiplexed array format. In the future, combining CNF based stimulating and recording electrodes with WINCS may lay the foundation for an implantable “smart” therapeutic system that utilizes neurochemical feedback control while likely resulting in increased DBS application in various neuropsychiatric disorders. In total, our goal is to take advantage of the nanostructure of CNF arrays for biosensing studies requiring ultrahigh sensitivity, high-degree of miniaturization, and selective biofunctionalization.

Biography

Dr. Jessica E. Koehne is a research scientist at the NASA Ames Center for Nanotechnology where she leads the Nano-Biosensors Group. Her research interests include the interface between nanoscale materials, electronics and biological systems with an emphasis on biosensing. Dr. Koehne has developed carbon nanotube and nanofiber based sensor platforms for the detection of DNA, rRNA, proteins and neurotransmitters with applications ranging from point-of-care to homeland security. Dr. Koehne has published over 40 peer reviewed articles in the field of nanotechnology and has received numerous awards for technical achievement including the 2011 Presidential Early Career Award for Scientists and Engineers.
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Outline

• Biosensing motivation
• Structure and fabrication of carbon nanofibers
• Application of carbon nanofiber electrodes in deep brain stimulation therapy
• Neurochemical recording by carbon nanofiber electrodes and Wireless Instantaneous Neurotransmitter Concentrator Sensor system
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
- Some of Ames Technical Areas
  - Astrobiology
  - Thermal protective systems
  - Simulation technology
  - Atmospheric science
  - Fundamental space biology
  - Human factors research
  - Nanotechnology
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
What are 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)

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**Why CNF as biosensor electrode material?**
1. Good conductivity
2. Wide potential window
3. Many active sites for electron transfer
4. Easy to pattern, grow and process on silicon devices

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 NH₃/50 sccm C₂H₂, 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

$\text{SiO}_2$ Encapsulated CNFs
30 devices on a 4” Si wafer

- 200 μm by 200 μm electrode dimensions
- 9 individually addressed electrodes
- potentially 9 different target molecules
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

Current 3x3 CNF device does not have an optimal geometry for implantation but can be used to preliminary in vitro investigations.
Goal:
Create a “smart” device based on a multiplexed CNF array for localized and efficient stimulation and neurochemical recording.

Stimulating Electrode:
Bare CNFs with high capacitance and low impedance.

Recording Electrode:
CNFs embedded in SiO₂ with ultrahigh sensitivity.
Neural Stimulation Using CNF Array

High Capacitance \( (C_0 = \Delta i/2v) \)
- Noble metal \( \sim 20 \mu F/cm^2 \)
- As-grown CNF array: 0.4 mF/cm\(^2\)
- Ppy-coated CNF array: 40 to 100 mF/cm\(^2\)

Low Impedance
- At 1 kHz, the impedance is negligible compared to the solution resistance

As-grown CNF Array

![Graph showing current density vs. potential difference](image1)

After Ppy coating

![Graph showing current density vs. potential difference](image2)

Biocompatibility
Optimization though chemical modification

Freedom for modification:
1. Changing anion dopants such as Cl\(^{-}\), ClO\(_4\)^{-},
poly(styrenesulfonate) (PSS), other polyelectrolytes, etc.
2. Loading with drugs such as antibiotics, anti-inflammatory molecules, etc. and biomolecules such as nerve growth factors.
3. Surface functionalization with cell adhesion molecules and specific ligands.

PC12 cells on PPy coated CNFs

Stimulation of Rat Hippocampal Brain Slices

**Experiment:** Measure voltage for a given stimulation current

**Stimulation by:**
- W wire
- Pt Microelectrode
- CNFs
- PPy coated CNFs

1) Only PPy coated CNFs were able to stimulate tissue under 1 mA stimulation current.
2) Only PPy coated CNFs did not induce the electrolysis of water (less than 1 mA and 1V)

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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

Glassy Carbon Electrode

- 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
WINCStrode for the Detection of Dopamine

The WINCS carbon fiber electrode (WINCStrode) is based on an approved human extracellular tungsten electrophysiology electrode that was modified by the addition of a short section of carbon-fiber to enable FSCV recordings.

Dopamine Detection:

3D Color Plots

Background Subtracted Cyclic Voltammogram

Calibration Curve

WINCS Carbon Nanofiber Electrode (WINCSnanotrode)

Scanning Electron Microscopy (SEM) Data:

Atomic Force Microscopy (AFM) Data:

Use a combination of SEM and AFM analysis to determine total electrode surface area

Dopamine Detection

Carbon Nanofiber Electrode

a) CNF BGS CV

b) CNF Calibration

\[ R^2 = 0.9870 \]

C) CNF 2.5 \( \mu \text{M} \) Dopamine

Carbon Fiber Microelectrode

d) CFM BGS CV

e) CFM Calibration

\[ R^2 = 0.9618 \]

f) CFM 2.5 \( \mu \text{M} \) Dopamine


*Analyst* 2011, 136, 1802-1805.
Implantable Style CNF Electrode Needle

Penetrating multiplexed array
- Ability to spatially resolve

[Diagram of CNF electrode needle with dimensions: 110 um x 170 um, 4800 um, 775 um]
Needle Assembly
Simultaneous Multichannel Oxygen Detection

Device: Needle

Instrument: WINCS Harmoni

Background Subtracted Voltamograms

- Channel 1: Oxygen
- Channel 2: Oxygen

Potential (V)

-1.1 V to 0.2 V

Time

-23 nA to 5 nA
Multichannel Detection: Dopamine and Oxygen

Channel 1: Dopamine

Channel 2: Oxygen

Background Subtracted Voltammograms

Dopamine Calibration

Oxygen Calibration

Increasing Oxygen with Constant Dopamine

Potential (V)

23 nA

-1.1 V

0.9 V

-30 nA

time

0 10 20 30 40 50

Current (nA)

Concentration (µM)

R square

channel 1 0.99681

channel 2 0.99689

Current (nA)

Concentration (µM)

R square

channel 1

channel 2

0.9847

0.9825

Current (nA)

Injection #
Summary

• 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
• High surface area carbon nanofibers have been demonstrated as effective stimulation electrodes
• Conductive polymer coatings have allowed us to tune the electrical properties and biocompatibility
• CNF sensors can distinguish between multiple electroactive analytes in a mixture using differential pulse voltammetry
• CNFs nanoelectrode arrays easily integrate with WINCS
• CNFs detect dopamine and oxygen with no channel cross-talk
• We have successfully fabricated a needle-like, implantable CNF electrode array for future in vivo investigations
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