An Implantable Biochip to Influence Patient Outcomes Following Trauma-induced Hemorrhage

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Security and Privacy in Implantable Medical Devices
SPIMD, EPFL, ELA2, Lausanne, Switzerland. April 1, 2011
Clemson ranks 22nd among the USA's 162 public doctoral-granting universities.

*U.S. News and World Report, 2009*
Founded in 1889, Clemson University is a South Carolina land-grant institution dedicated to teaching, research, and public service, and to improving the quality of life through education.

Clemson's 1,400-acre main campus, located in the Northwestern corner of South Carolina on the shores of Lake Hartwell, is surrounded by 17,000 acres of University farms and woodlands devoted to research.

Approximately 17,165 students, including 3,096 graduate students are enrolled in five colleges offering Baccalaureate and Graduate degrees in over 70 fields of study.
Disclosure

Anthony Guiseppi-Elie, Sc.D.
Founder, President and Scientific Director
Founded 1995

*ABTECH Scientific, Inc.
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Richmond, Virginia 23219 USA

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http://www.abtechsci.com
ABTECH Scientific, Inc.

Founded in 1995 and located in the Biotechnology Research Park in Richmond, Virginia, ABTECH uses its platform electroactive polymer sensor technology (EPST™) to develop and deliver non-invasive, near-patient molecular diagnostic products of clinical significance.
ABTECH Scientific, Inc.

Laboratory Products Group

Microlithographically fabricated devices, related microelectrodes (pH and Ref), flow cell products (gas and liquid) and instruments that are used in research and development of electroconductive polymer sensor technology.

Advanced Products Group

Non-invasive, near-patient molecular diagnostic products of clinical significance and based on electroconductive polymer sensor technology.
Electrochemical Cell-on-a-Chip (ECC)
Electrochemical Cell-on-a-Chip (ECC)
Electrochemical Cell-on-a-Chip (ECC)

Unsilverized

Silverized

Counter Electrode
Interdigitated WE1 and WE2
Reference Electrode, Ag/AgCl
Si$_3$N$_4$ Passivation Layer

Unmodified

Polypyrrole modified
Electrochemical Flow Through Assay

Fig. 1. Schematic diagram of the IEB. (A) A test strip; (B) a cover; (C) a bottom and (D) a SPE.
 Electrochemical Cell-on-a-Chip (ECC) Integrated in a Microfluidic Cassette

An integrated biochip showing the microfabricated pattern of electrodes bonded and sealed into the Microncs’ microfluidic T-cassette.
Microfabricated Interdigitated Microsensor Electrodes (IMEs)

IME XX50.5 M, CD, FD; Au, Pt, ITO

XX = 05, 10, 15, 20 µm

IME XX50.5 M, CD, FD; Au, Pt, ITO
Schematic illustration of the IME XX25.3 chips
IME XX50.5 chips

XX = Digit width = spacing = 05, 10, 15 & 20 microns
25 Bands on each bus 50 Bands on each bus
3 mm line length 5 mm line length
Photomicrograph and AFM Images
IME XX25.3 chips
Sensor Arrays of VOC Responsive Polymers for the Electronic NOSE

- Chips of 05 µm, 10 µm, 15 µm and 20 µm with electropolymerized PPy, PTh or PAn
**Microband Electrodes**

**Independently Addressable Microband Electrodes (IAMEs)**

IAME XX04, Au, Pt or ITO

XX = 5, 10, 15, 20 mm

**Independently Addressable Interdigitated Microsensor Electrodes (IAIMEs)**

IAIME XX10, Au, Pt or ITO

XX = 05, 10, 15, 20 mm
## Microdisc Electrode Arrays 2

<table>
<thead>
<tr>
<th>MDEA DEVICES</th>
<th>Disc dimensions / Number of Discs</th>
<th>Active Area</th>
<th>Conductor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDEA 3600</td>
<td>D = 3,600 µm 1 disc</td>
<td>0.10 cm²</td>
<td>Au, Pt, ITO</td>
</tr>
<tr>
<td>MDEA 250</td>
<td>D = 250 µm 207 microdiscs</td>
<td>0.10 cm²</td>
<td>Au, Pt, ITO</td>
</tr>
<tr>
<td>MDEA 100</td>
<td>D = 100 µm 1,296 microdiscs</td>
<td>0.10 cm²</td>
<td>Au, Pt, ITO</td>
</tr>
<tr>
<td>MDEA 050</td>
<td>D = 50 µm 5,184 microdiscs</td>
<td>0.10 cm²</td>
<td>Au, Pt, ITO</td>
</tr>
</tbody>
</table>
Microdisc Electrode Arrays 3

5,184 microdiscs

1,296 microdiscs

207 microdiscs

1 macro disc
ABTECH’s Patents


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SPIMD, EPFL, ELA2, Lausanne, Switzerland. April 1, 2011
Improving Human Health Through Technology: Research at the Center for Bioelectronics, Biosensors and Biochips (C3B™)

The CU-C3B is a national model for advanced nano-bio electronics
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NIH Fogerty International Award
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Clemson C3B

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Mahammed Ashraf
Carolina Funkey
Chris Nixon

Louise Lingertelt
Gopakumar Sethuraman
Michael Zavatsky
Grand Challenge Problems

**The Bio-materials Interface:** Enabling chronically implantable bioanalytical devices - Bionics

**Bioelectronics:** Enabling direct electronic communication between solid state devices and the biology -- More than Moore
Goals of our Research

- Design, synthesis and characterization of soft polymeric biomaterials with low interfacial impedance, fast ion transport, biomolecule hosting, and in-vivo biocompatibility.

- Implantable Biotransducers (sonde) for continuous monitoring of interstitial glucose, lactate, pH and temperature.

- Implantable integrated biochips for interrogating and wireless reporting of physiologic parameters related to trauma induced hemorrhage and shock.
Opportunities for *in-vivo* Biosensors

**Lactate and Glucose Monitoring**

- **Manned Space Flights**
- **Diagnostics**

**Trauma Management**

- **Congestive Heart Failure and Transplanted Organ Health**

- **Battlefield Trauma outcomes**

- **Chronic Diabetes Care**

- **Insulin infusion pump**
- **Glucose monitor**

*Center for Bioelectronics, Biosensors and Biochips*
The Case for Monitoring

✦ Trauma - the No.1 killer of persons < 50 yrs old.
✦ Death from hemorrhage is implicated in 50-68% of battlefield trauma cases (Col. Erin Edgar)
✦ During hemorrhage induced trauma and following surgery, hemodynamics and physiology are delicate and can change rapidly
✦ Need to initiate immediate and continuous monitoring of molecular indicators of global physiologic stress.
Oxygen delivery: $\text{DO}_2 = \text{CO} \times \text{CaO}_2$

$= 460 - 650 \text{ ml/min/m}^2$

\(\text{CO} = \text{Cardiac Output}\)

\(\text{CaO}_2 = \text{Oxygen content of the arterial blood}\)
Lactic Acidosis: A Prognosticator in Trauma

Broder, G. and M. H. Weil (1964) "EXCESS LACTATE: AN INDEX OF REVERSIBILITY OF SHOCK IN HUMAN PATIENTS" Science, 143: 1457-1459
What motivates our focus on lactate?

- **Mortality/Morbidity**
  - Patients who have an arterial lactate level of more than 5 mmol/L and a pH of less than 7.35 are critically ill and have a very poor prognosis. The multicenter trials have shown a mortality rate of 75% in these patients.

- **However, if lactate levels normalize in:**
  - 24 hrs = 90-100% survival
  - 24-48 hrs normalization = 75% survival
  - >48 hrs = 13%


HYPOTHESIS: Clinical Outcomes Related to Peripheral Perfusion Following Trauma:

The case for continuous lactate monitoring

Survivors without Complications
Survivors with Multisystem Organ Failure
Nonsurvivors

Baseline

Lactate

VO₂

Time
An Implantable Biochip for Physiologic Status Monitoring

Glucose, Lactate, pH and Temperature
Implantable Biochips

Design of a Subcutaneous Implantable Biochip for Monitoring of Glucose and Lactate
Anthony Guiseppi-Elie, Sean Brahim, Gymama Slaughter, and Kevin R. Ward

Fabrication and Packaging of a Dual Sensing Electrochemical Biotransducer for Glucose and Lactate Useful in Intramuscular Physiologic Status Monitoring
Abdur Rub Abdur Rahman, Gusphys Justin, Adilah Guiseppi-Wilson, and Anthony Guiseppi-Elie, Member, IEEE

Anal Bioanal Chem
DOI 10.1007/s00216-010-4271-x

An implantable biochip to influence patient outcomes following trauma-induced hemorrhage
Anthony Guiseppi-Elie
Front-end biotransducer for discrete component prototyping of the PSMBioChip

Design of the microdisc electrode array (MDEA)

1. Individual small diffusion layer: linear diffusion
   - Insulator
   - Au microdisc

2. Individual small diffusion layer: radial diffusion
   - Overlapping diffusion layers

3. Overlapping diffusion layers: linear diffusion

MDEA50 and MDEA50*|hydrogel qualified by CV in 1.0 mM FeCOCOOH in TRIS Buffer

\[ I_p = 2.687 \times 10^5 n^{3/2} v^{1/2} D^{1/2} A_{Cox} \]

Reduced voltametric peak currents

Reduced apparent area of the electrode

Performance enhancement of the microdisc electrode array format of the PSMBioChip

\[ I_p = 2.687 \times 10^5 n^{3/2} v^{1/2} D^{1/2} A C_{ox} \]

Un-Coated

Hydrogel Coated

Enhanced effective area with reduction of disc diameter

Enhancement maintained for 50 µm device beneath hydrogel

Conferring biological specificity to a multi-analyte bioanalytical biochip – How?

- Micro-solenoid, non-contact printing
- Micro-contact quill or pin printing
- Ink-jet printing
- Spin-coating and electropolymerization
Custom Spot Production

Microarray Array Fabrication
BioDot AD 3400

- Hydrogel
- Enzyme
- Cocktails

- Silicon Pins
- Microsolenoid
Custom Spot Production on Die

1” x 3”
Die Carrier
Fits Plattern
Electroconductive Hydrogels: Properties of Polypyrrole-Poly

Sean Brahim, Anthony Guiseppi-Elie

Molecularly engineered p(HEMA)-based hydrogels for implant biochip biocompatibility

Sheena Abraham, Sean Brahim, Kazuhiko Ishihara, Anthony Guiseppi-Elie

Electroconductive hydrogels: Synthesis, characterization and biomedical applications

Anthony Guiseppi-Elie

Analysis and characterization of dielectric properties of polypyrrole-based hydrogels

M. R. A. Smith, M. L. Thompson, J. H. Smith

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Review

Electroconductive hydrogels: Synthesis, characterization and biomedical applications

Anthony Guiseppi-Elie
In vivo Physiological Status Monitoring biosensors

Electrostimulated drug delivery devices

Clinical Applications for Electroconductive Hydrogels

Deep Brain Stimulation Devices

Implantable Biofuel Cells

Neural prostheses

Deep brain stimulation

The Deep Brain Stimulation system is used to help control tremors and chronic movement disorders. Tiny electrodes are surgically implanted in the brain and are connected via a suture to a stimulator (or two, for some disorders) implanted under the skin near the clavicle.

Clinical Applications for Electroconductive Hydrogels
Generalized Synthesis of Electroconductive Hydrogels

Monomer Solution → Hydrogel network

UV-polymerization of acrylates

Chemical Oxidative polymerization of Py

Electro polymerization of Py

0.7 V vs Ag/AgCl

FeCl₃

Hydrogel-PPy co-network

**In-vivo Implantation in Sprague Dawley Hemorrhage Model**

After implantation for 2 weeks in the trapezius muscle

**A**
un-modified p(HEMA)

Significant encapsulation and accumulation of foreign body material

**B**

1 mol % MPC

Thin band of encapsulation and much reduced residual inflammation

---

**Muscle**

**Area of Moderate Fibrosis and inflammation with foamy histocytes around the site where hydrogel was placed**

**Residual area of Hemorrhage**

**Area where hydrogel existed**

**Muscle**

**Rim of new connective tissue forming a capsule and some residual inflammation around hydrogel**

**Residual area of Hemorrhage**

**Hydrogel**
Electrochemical Impedance of Electroconductive Hydrogels – **Planar** and **Transverse** Interrogation

Electropolymerized PPy films grown on IME* (left) and on IME* | Gel devices (right).
**Electrochemical Impedance of Electroconductive Hydrogel of P(Py-co-PyBA) vs. PPy - Planar**

IME*IPPy (Planar EIS)

IME*IGel-PPy (Planar EIS)

IME*|PPy and IME*|Gel-P(Py-co-PyBA) : Planar EIS (co-planar counter and working electrodes). Bode impedance magnitude and phase plots for the IME*|PPy (left) and IME*|Gel-P(Py-co-PyBA) (right) measured using the co-planar arrangement of counter and working electrodes.
Substrate surface modification, derivatization, monomer casting and electroconductive hydrogel synthesis

Cell viability as a function of Au electrode surface composition (after 4 days)

PC12 - Pheochromocytoma

RMS 13 – Human Muscle fibroblasts

Cell densities following trypsinization (5 min) and enumeration of RMS13 and PC12 pre- (blue bars) and post- (red bars) incubation for 4 days on Au*, Au*|hydrogel, Au*|PPy, Au*|hydrogel-PPy surfaces
Initial cell density at seeding (4.9+/−0.7 \times 10^5 \text{ cells/ml})

Comparison of PC12 cell densities post-incubation (for 4 days) on Au*, Au*|hydrogel, Au*|PPy, Au*|hydrogel-PPy (5, 25 and 50 second electropolymerization times). Initial seeding cell density was 4.9+/−0.7 \times 10^5 \text{ cells/ml} (broken line). * Indicates a p-value greater than 0.05.

EGylationation of LOx at Lysine Residues

LOx with Primary Amines

Enzyme activity via kinetic assay: $v_{\text{max}}$, $K_M$, $k_{\text{cat}}$, $k_{\text{cat}}/K_M$

MW and MW distribution via capillary gel electrophoresis:

L - Lactate + O$_2$ $\rightarrow$ Pyruvate + H$_2$O$_2$

$\beta$ - D - glucose + O$_2$ $\rightarrow$ glucono-$\delta$ - lactone + H$_2$O$_2$
Schematic illustration of the molecular constituents of a poly(HEMA-co-PEGMA-co-HMMA-co-SPMA)/P(Py-co-PyBA) electroconductive hydrogel containing an oxidoreductase enzyme and bioactive hydrogel topcoat containing phosphoryl choline (MPC).

Principle of operation of the amperometric biotransducer

Chronoamperometry (CA)

- Cottrell’s Equation

\[ i(t) = \frac{nFACD_{o}^{1/2}C_{o}^{*}}{\pi^{1/2}t^{1/2}} \]

- \( E_1 \)- No redox activity
- \( E_2 \)- \(|E| > E^\circ\)
- \( \tau \)- step size (determined experimentally)
- Quiescent solution
Fabrication of the electrochemical p(HEMA)/Glucose and Lactate TYPE I biotransducers
Fabrication of the electrochemical p(HEMA)/Glucose and Lactate TYPE II biotransducers
Evaluation of the electrochemical p(HEMA)/Glucose and Lactate Dose-Response of TYPE I biotransducers

Glucose \rightarrow \text{Gluconolactone}

PPy-hydrogel composite

Platinum

H_{2}O_{2} \rightarrow O_{2}

GOx_{ox} \rightarrow GOx_{red}
**In vitro** calibration of the dual responsive glucose and lactate biotransducer – different lactate sensitivities

MDEA 5037 lactate and glucose biosensor incorporating electroconductive polymer bio-smart hydrogel membrane of composition 80:10:2.5:2.5:5.0 mol% (HEMA:TEGDA:PEGMA:MPC:Py) in 0.1 M PBKCl, pH 7.0 at RT.
A catheterized and instrumented Sprague Dawley rat under controlled hemorrhage conditions with intramuscularly (trapezius) implanted PSM Biochip.

Christian Kotanen and Anthony Guiseppi-Elie “Development of an implantable biosensor system for physiological status monitoring during long duration space flights” Gravitational and Space Biology (2010), 23(2) 55-63
In vivo amperometric performance of the implanted PSMBioChip during hemorrhage – Sprague Dawley rat model.

Amperometric response of an intramuscularly implanted lactate biosensor during hemorrhage, the mean arterial pressure (MAP) and the systemic blood lactate obtained using a YSI Biostat Bioanalyzer.

Discrete component prototyping of the PSMBioChip

TI /ChipCon CC1110
• 8051 Microprocessor
• 9-14 bit ADC
• RF Transceiver
  • MICS: 402-405 MHz

A Sprague Dawley rat equipped with a head mounted wireless transmitting dual potentiostat to support intramuscular bioanalytical measurements of lactate and glucose in the trapezious muscle.
Status

✦ CDMRP funded small vertebrate animal studies ongoing at Clemson University

✦ IP ownership released by Clemson University to Guiseppi-Elie, successfully transferred to ABTECH Scientific, Inc.

✦ Collaborative program established with Tripler Army Medical Center – Dr. Catherine Uyehara, Chief, Dept. Clinical
Nanotube filaments penetrate the glycoprotein shell and attain tunneling proximity to the cofactor. Impact on bioactivity via denaturation is minimum.

- Highly conductive
- Strong
- Large surface area
- Chemically stable
- Inert

Carbon nanotubes
Courtesy: CNST

Enzyme subunit

1.2-1.4 nm

~10 nm

Glucose oxidase
Courtesy: NCBI

A sphereoidal shape:
6.0 nm x 5.2 nm x 3.7 nm
Amperometric enzyme biosensor - Direct

Direct Biosensor
No mediator molecule needed
Oxidation possible
Reduction possible

GOD-CNT (Annal. DC9909)

Scan rate (mV/s): 10, 25, 35, 50, 65, 80, 100, 120, 140

Data files: 012002-012006 012008-012011

E (Volts)
I (Amps)

Sean Brahim, Nikhil K. Shukla and Anthony Guiseppi-Elie

Molecular Bioelectronics
Direct Electronic Control of Enzyme Function

“Bio-smart” materials by design; combining molecular biorecognition (enzymes), biocompatibility (PEG and MPC), interference shielding (PPy) and redox mediation (M) within p(HEMA)-based hydrogel

Polymers are non-cytotoxic and support excellent viability and restricted proliferation

*In vitro* measures of biocompatibility are highly correlated with extent of hydration.

Biotransducer design using E’Cell-on-a-Chip (ECC) microlithographically fabricated microdisc arrays

Systems integration and form-factor

Demonstrated physiologic status monitoring
2011 International Conference on Frontiers of Characterization and Metrology for Nanoelectronics

MINATEC Campus, Grenoble, France
23 - 26 May 2011

Registration Deadline: 01 May 2011
www.nist.gov/pml/semiconductor/conference

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