Microelectrochemical Assays: from Academic Research to Business

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OVERVIEW OF BROADER IMPACTS

Research funded by the National Science Foundation at the University of Arkansas led to several broader impacts. One was the stimulus for two startup companies, Vegrandis, LLC, and SFC Fluidics, LLC (SFC not discussed). Meanwhile, cooperation between the University and the Company has continued, leading to expansion of applications of benefit to society: environmental, clinical, drug and vaccine development, genetically modified plants, among others. Other broader impacts include research training of graduate and undergraduate students, production of Ph.D. degrees, and *return of advanced degree recipients to the State of Arkansas*.

IMPACTS OF NSF FUNDING TO UNIVERSITY AND HOW THEY LED TO INITIAL STARTUP COMPANY SUCCESS

via NSF Grants

Direct Academic Outcomes from NSF Grants

- Degrees completed: 4 Ph.D.s
- Degrees in progress: 3 Ph.D.s and 1 M.S.
- Undergraduate research students: 11
- Publications: 10 in peer-reviewed journals
- Patents and Patents Pending: 6
- Student research awards: 4 national, 2 statewide, 2 campus-wide
- Intellectual property led to two startup companies: Vegrandis, LLC, and SFC Fluidics (not discussed here)

via Startup Company, Vegrandis, LLC:

Economic Impact to State of Arkansas:

- Contribute to growth of high tech industry
- Employment of scientifically-trained staff
- Brought back to state: three U of A Ph.D.s as employees

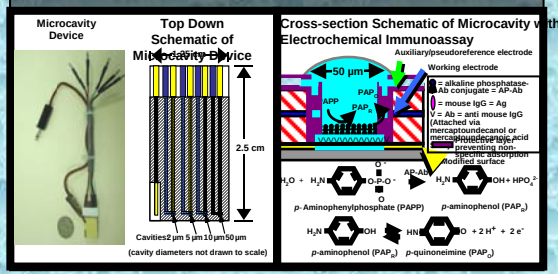
Commercial Research and Development:

Awards to Vegrandis, LLC, Based on Licensed Intellectual Property Originating from NSF-funded Academic Research

- **EPA**: Two, SBIR Phase I Awards, Total: $140,000.
- **NIH**: One, STTR Phase I Award, $100,000 (in partnership with SFC Fluidics, LLC).
- **NSF**: Three, SBIR Phase I Awards, Total: $300,000.
- One, SBIR Phase II Award, Total: $500,000, plus additional $500,000 IIb opportunity.

THE SCIENCE: BENEFITS OF SELF-CONTAINED MICROELECTROCHEMICAL ASSAYS

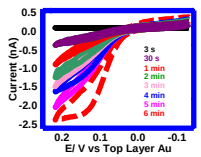
- analysis in turbid samples possible
- use of opaque device materials o.k.
- manipulation of external electrodes not needed
- small volume analysis (pL)
- low waste volumes
- high sensitivity (close proximity of electrodes to assay site)
- low background signal (low charging current)
- fast response times (short diffusion lengths)
- minimization of interferences (through redox cycling)
- amplification possible (through small volumes and/or redox cycling)



INITIAL RESULTS AT UNIVERSITY OF ARKANSAS

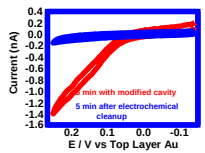
Proof-of-Concept Detection of Mouse IgG[1]

Response to PAP$_R$, in 200 nL PAPP Solution after Incubation with Recessed Microdisk-Modified Microcavity



- Fast response: as soon as 30 s incubation with PAPP.
- Signal continues to increase as additional enzyme-generated product is produced.

Response to PAP$_R$ in 500 nL PAPP Solution, Before and After Cleaning Modified Recessed Microdisk



- The active immunoassay is only at the recessed microdisk. Thus, large signals at the working electrode for PAP$_R$ are due to enzymatic generation at modified recessed microdisk only.
- This leads to possibilities for development of assay arrays.

Summary for Mouse IgG Studies

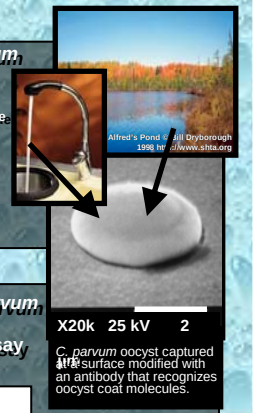
- Time for assembly (Ag & Ab-AP & rinsing) and detection (PAPP): ≤ 30 min.
- Small volumes (~1/50 commercial): 1 µL for Ag and Ab-AP, & 200 nL for PAPP
- Detection limits for PAP$_R$: 4.4 nM or 880 fmol.
- Detection limits for IgG (~1/100 commercial): 56 fM (9 pg/mL) or 56 zmol (9 fg).
- Not optimized for incubation times, geometry, or electrochemical technique
- Solved nonspecific adsorption problems to polyimide: minimize false positives
- Controlled location of immobilized immunocomponents: arrays possible

Benefits to Society: Expansion of Applications

- *Cryptosporidium parvum*: mRNA assay for heat shock protein 70 (indicates viability)[2] and immunoassay for coat protein of oocyst.
- *Plasmodium falciparum* (malaria): both DNA hybridization and immunoassay for liver-stage parasite (to be used in drug discovery and vaccine development)

EXPANDED APPLICATION TO *Cryptosporidium parvum*

- Waterborne pathogen in 80% of surface waters in USA
- One of top 5 pathogens causing 5 million deaths each year worldwide (13,000 children/ day)
- Infectious dose: 1-132 oocysts
- Thick-walled oocyst: difficult to kill (heating, chlorination, ozone)
- EPA detection methods: *inadequate!* (5 days-4 weeks, $450-$750 / sample, inaccurate and imprecise, >100 oocysts/L detection)
- No effective treatment for cryptosporidiosis

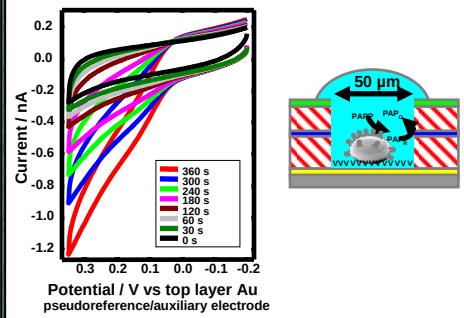


C. parvum oocyst captured at surface modified with an antibody that recognizes oocyst coat molecules.

INITIAL RESULTS AT VEGRANDIS, LLC on *C. parvum*

Self-Contained Microelectrochemical Immunoassay for *Cryptosporidium parvum*

Timed Response after Addition of 4 mM PAPP
(250 oocysts / L, 0.3 - 1.5 µL each step, 50 mV/s, humid chamber, assay time from addition of oocysts to PAPP: 30 min)



Further Expansion of Applications

E. coli, ovarian cancer, genetically modified plants, allergens, interfacing with different microfluidics platforms, interfacing with both bench-top instruments and portable hand-held instruments.

REFERENCES

1. Aguilar, Z. P.; Vandaveer, W. R.; Fritsch, I., "Self-Contained Microelectrochemical Immunoassay for Small Volumes using Mouse IgG as a Model System", *Anal. Chem.*, 2002, *74*, 3321-3329.
2. Aguilar, Z. P.; Fritsch, I., "Immobilized Enzyme Linked DNA-hybridization Assay with Electrochemical Detection for *Cryptosporidium parvum* hsp70 mRNA", *Anal. Chem.*, 2003, *75*, 3890-3897.

ACKNOWLEDGMENTS

***Academic funding from National Science Foundation*:** CHE-0096780 and CHE-9624114; Center for Sensing Technology and Research through NSF EPS-9977778 (and Arkansas Science and Technology Authority, 00-ARMF-06); University of Arkansas' Innovation Incubator through NSF-0090596.

***Assistance with science*:** Ms. Brigitte E. Factor, Ms. Mali Sirisena, and Ms. Eyitayo Fakunle.

***Business contributions*:** Dr. Calvin Goforth, President of Virtual Incubation Company, LLC (VIC) and of Vegrandis, LLC, and all support staff at VIC.

***Encouragement to begin startup companies*:** Research and Sponsored Programs Office of the University of Arkansas.