University of Utah Research Foundation

PROPOSAL FOR TECHNOLOGY INNOVATION GRANT

1. Proposed Project Title:

Low Blood Volume Bioluminescent-Based Analysis Chip for Hemodialysis Patients

2. Amount Requested: $34,800/year for two years

3. Projected Period: July 1, 2000 to June 30, 2002

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4. Principal Investigator (PI):

Name of PI: Joseph D. Andrade **Date:** April 13, 2000

Signature:

Department: Bioengineering **Phone:** (801) 581-4379

5. Co-Investigators:

None

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6. Non-Confidential Abstract (approximately 250 words):

(Please note this non-confidential abstract will be made available to companies who are potential research sponsors unless you mark this abstract "Not for Distribution" and sign below.)

[XX] Yes, make this abstract available. [ ] Not for distribution.

Signature:

Joseph D. Andrade

The only effective treatment for end stage renal disease is hemodialysis, peritoneal dialysis, or kidney transplantation. Patients on dialysis and renal transplant patients should undergo regular measurement of those blood analytes which relate to kidney function and to the adequacy of dialysis treatment. We propose to develop a multi-analyte bioluminescent-based biosensor, using 10 to 100-μL of blood, analogous to those used for daily glucose measurement by millions of diabetics. In our case, however, the biosensor will measure a panel of analytes important in the assessment of the adequacy of dialysis and the health of a transplanted kidney. Such analytes include: creatinine, urea, uric acid, glucose, lactate, pyruvate, glycerol, and essential amino acids such as leucine. The biosensor will be a biochip consisting of ten micro-reaction chambers etched on a silicon substrate, one for each analyte and two for on board calibration chambers. Each of the micro-reaction chambers will be embedded with layers of lyophilized enzymes for bioluminescent reactions specific to the analyte to be measured. Microneedles will draw the sample fluid to the micro-reaction chambers via microchannels. The research will develop the miniaturization technology for bioluminescent analysis. The specific the technology derived from this research will be the design of the micro-reaction chambers for enhanced light output, rapid rehydration, and low-volume reagent layering for increased longevity and activity. These features will enable the low sample volume analysis to be performed by a computer controlled CCD camera that can easily be converted to a portable unit. This technology can also be applied to other analyte panels.

7. Detailed Project Description:

The only effective treatment for end stage renal disease is hemodialysis, peritoneal dialysis, or kidney transplantation. Patients on dialysis and renal transplant patients should undergo regular measurement of those blood analytes, which relate to kidney function and the adequacy of dialysis treatment. We propose to develop a multi-analyte bioluminescent-based biosensor, using 10 to 100-μL of blood, analogous to those used for daily glucose measurement by millions of diabetics. In our case, however, the chip will measure analytes important in the assessment of the adequacy of dialysis and in the health of a transplanted kidney. Such analytes include: creatinine, urea, uric acid, glucose, lactate, pyruvate, glycerol, and essential amino acids such as leucine [1].

GENERAL BACKGROUND

Bioluminescence-based analysis is 100 to 1000 times more sensitive than conventional chromogenic (absorbance) measurements and is accurate over a five or more orders of magnitude concentration range [2]. Firefly bioluminescence occurs by enzyme-catalyzed oxidation of luciferin utilizing adenosine triphosphate (ATP) [3]. Bacteria bioluminescence is closely coupled to nicotinamide adenine dinucleotide (NADH). Since most of biochemistry depends on ATP and/or NADH, nearly all metabolic reactions can be monitored by bioluminescence via one or more enzyme catalyzed and linked reactions [2,3,4]. During the production or consumption of a metabolite of interest, enzyme linked reactions will cause the production or consumption of ATP (or NADH). Detectable light is then produced via the following reaction (for ATP):

Equation 1: $ATP + O_2 + \text{D-Luciferin} + Mg^{++} \xrightarrow{Luciferase} \text{Oxyluciferin} + AMP + CO_2 + Ppi + \text{Light (560 nm)}.$

The change in light intensity will be stoichiometrically proportional to the time changing concentration of ATP (and thus proportional to the metabolite of interest). A photodiode or charge-coupled device (CCD) can be used for detection [4]. Depending on the instrument used to detect the luminescence, nanomolar, picomolar, and even femtomolar analyses are easily achieved [2]. With increased sensitivity, smaller amounts of sample fluid are needed for accurate analysis. The feasibility and protocol for using bioluminescence as a method for detecting galactose, creatine, creatinine, glucose, urea, pyruvate, glycerol, lactose, and other metabolites has already been established [2,3,4]. Our current research up until now has focused on bioluminescence. Only limited research has been done on developing the physical device for miniaturized bioluminescent chemical analysis.

The ability for a cost effective bioluminescent-based metabolite sensor to become available to the public depends on three things. The first is the fabrication method for creating an array of enzyme embedded micro-reaction chambers (μRCs). These reactions chambers must be designed to amplify light output and enhance mixing. Each μRC will house the unique enzymes for each of the analytes to be measured. The enzymes must be prepared for increased longevity and activity. The second element involves the integration of microneedles into the μRC array for convenient, low sample volume, minimally invasive metabolite analysis. Current micromachining technology enables the fabrication of hollow fabricated needles that are 30× smaller than a 30 gauge needle and can penetrate the stratum corneum of the skin without breaking (see Figure 1) [5]. The third parameter in developing a bioluminescent-based metabolite sensor depends on the implementation of a hand held luminometer for measuring the dynamic light output of each of the bioluminescent-analyte reactions.

SPECIFIC GOALS: Including Methods and Strategies

The first year of the grant will focus on developing the fabrication method of the needle-integrated biochip for an analyte panel for measuring renal function. Prototypes of the μRC array and channels have already been designed and built. The technology for the needle fabrication and etching arrays of micro-channel linked μRCs on silicon substrate has already been established using photolithographically [5]. Although the final biochip will have individual μRCs for the many

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7. Detailed Project Description (continued):

Figure 1: SEM of a multi-lumen, multiple output port micro needle. Shaft dimensions are 200 μm wide and 60 μm thick. Tip dimensions are less than 15 μm X 15 μm. Output ports are 30 μm².

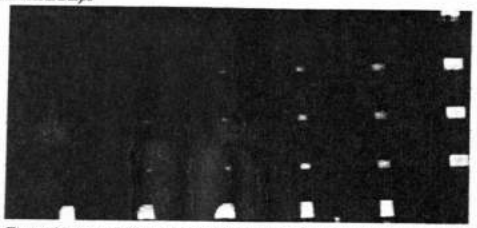

Figure 3: 20-sec integration of bioluminescence for the wafer that was etched 242.1-μm and coated with Ti/Cr. The μRCs seen here are the 750, 500, 400, 300, 250, and 200-μm wide squares. Volumes range from 3 to 85-nL. ATP concentration was 1-mM.

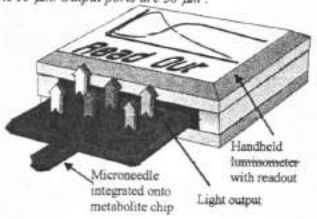

Figure 2: Integrated product design concept

metabolites of interest, initial tests will be done with glucose because of its high concentration in blood and the ease of calibration using standard glucose sensors that are already available in our lab. The prototype will have ten μRCs, eight for the analytes in question and two for onboard calibration μRCs designed to account for the existing ATP in the sample fluid and the activity of the enzymes. Initial designs will have μRCs ranging from 0.3 to 3-μL to accommodate blood sample volumes ranging from 10 to 100-μL blood samples. Commercially available sample blood standards will be used for testing the device. Red blood cells will be filtered from the sample fluid using commercially available plasma separation membranes. Preliminary tests during the first year will only involve CCD cameras (already available to our lab) in order to build a prototype. The final device would look something like the drawing in Figure 2.

The first issue for development will be to optimize design the μRCs design for maximal light output. Preliminary tests on an array of μRCs show the spatial resolution which a computer controlled CCD, with a close-up lens, can read a 1-mM ATP solution for volumes down to 0.75-nL. Chromium and silver coating of the μRCs have already proven to amplify the light intensity (see Figure 3) [6]. Building deeper, narrower μRCs with photolithographically built focusing lenses over the viewing windows can also enhance light. This issue will be addressed over the first 6 months.

The second issue will be to determine procedures for preparing and drying small volumes of enzymes within the small dimensions of μRCs while maintaining their effectiveness in bioluminescent activity. Initial enzymatic coating will be done by drying the enzymes with open face μRC in open air or lyophilization. Lyophilization machinery is available for free use from Protein Solutions, Inc. As more precise reagent layering is needed, ink-jet printing technology serves as an inexpensive method for dispensing enzymes with pL resolution [7].Contamination and nozzle clogging in the ink-jet can be reduced by coating the dispensing system with poly(ethylene oxide). Stepping motor platforms for precise positioning of the reagents are available from other funds listed in current projects.

During the enzyme placement and enhancement process, we anticipate difficulty in maintaining enzyme integrity due to the high temperature, UV light, and/or chemicals involved in bonding glass

7. Detailed Project Description (continued):

windows over the μRCs on silicon wafers and low temperature pressure adhesives to bond the glass to the silicon. We propose to address this issue with enzymes that have been genetically engineered for thermal stability. We also anticipate a decrease in light output accuracy due to a decrease in activity due to the drying and slow mixing during the rehydrating process of the enzymes. In order to enhance the bioluminescent activity, a variety of additives are available to minimize enzyme denaturation and inactivation due to drying and rehydration processes. Such additives include sucrose, trehalose, polyethylene glycol, dextran, and antioxidants, including vitamin C, vitamin E, and glutathione [8]. Piezoelectric actuators can also be added to the chip to enhance accelerate the mixing process for increased bioluminescent activity [9]. This issue will be addressed from the 7th month to the 12th month.

During the second year of the project, we will optimize the μRC characteristics and enzyme layering and preparation of the remaining analytes for the renal function analysis chip. The remaining analytes include creatinine, urea, uric acid, lactate, pyruvate, glycerol, and leucine. Calibration and verification of the device will be compared with HPLC tests using equipment available to J. Andrade in the U of U's Department of Pharmaceutics. We will also use the partnership companies established during the first year to develop a computer controlled CCD camera with simple user interface and the proper analyte calibrating algorithms for the final prototype production.

SIGNIFICANCE OF RESEARCH

The technology derived from this research will be the discovery of methods for miniaturization and long-term stabilization of bioluminescent reactions for chemical analysis. This novel research will create an analytical panel designed specifically for testing renal function and rejection for kidney transplant patient and adequacy, functionality and hypoglycemia diagnosis for dialysis patients. However, the application of this miniaturized bioluminescent technology is only limited to the number of μRC placed on a chip. The same technology can be applied for panels designed specifically for newborn intensive care. Such a low-blood volume analysis system would allow frequent measurement of many analytes for these infants who don't have the volume of blood to analyze. Other custom panels can be designed for athletic clubs (for the metabolite analysis service they offer), obstetricians and gynecologists, and diabetics (so they can measure insulin in addition to glucose).

REFERENCES

[1] E. Riedel, H. Hampl, M. Nundel, D. Bushe, H. Fuchs, "Severity of Anemia Influences Pattern of Amino Acids and α-Keto Acids in Hemodialysis Patients," *Metabolic and Nutritional Abnormalities in Kidney Disease*, Contrib Nephrol. Basel, 1992, Vol. 98, pp. 98-104.

[2] S. Brolin and G. Wettermark, *Bioluminescence Analysis*, VCH Publ., 1992.

[3] A. Campbell, *Chemiluminescence*, VCH Publ., 1989.

[4] J.D. Andrade, C.Y. Wang, D.J. Min, et al. "Toward Dollar Devices for Measuring Metabolic Biochemistry," *Anti-microbial, Anti-infective Materials*, S.P. Sawan and G. Mirivannan, eds. Technomic Publishing Co., 1999.

[5] Frazier, B. A., et al., U.S. Patent 5,876,582. Methods for Preparing Devices Having Metallic Hollow Microchannels on Planar Substrate Surfaces.

[6] J. D. Brazzle, D. A. Bartholomeusz, R. Davis, J. Andrade, R. VanWagnen, B. Frazier, Active Microneedles with Integrated Functionality," *Solid State Sensors and Accuators Conference*, Transducers Research Foundation, June 4-8, 2000.

[7] A. L. Hart, A. P. Turner, "On the use of Screen and Ink-jet Printing to Produce Ampermetric Enzyme Electrodes for Lactate," *Biosensors & Bioelectronics*, Vol. 11, No. 3, pp. 263-270, 1996.

[8] D. J. Min, J. D. Andrade, R. J. Stewart, "Specific Immobilization of *In Vivo* Biotinylated Bacterial Luciferase and FMN:NAD(P)H Oxidoreductase," *Analytical Biochemistry*, 270, 133-139, 1999.

[9] S. Fischer-Fruhholz, "The Handling of Nanoliter Samples on a Chip," *American Laboratory*, 46-51, Feb. 1998.

8. Commercial Potential:

The Foundation funds only projects that are likely to produce commercial results in a two-year time frame. Thoughtful, realistic answers and consultation with industry is important in completing this section.

a) Describe the commercial applications of the proposed research, giving attention to patentability, market size, and comparisons with currently available products of similar application or use.

b) Indicate at least two or three specific companies that might be interested in the results. How would this fit into their current offerings or their research and development programs? Please recall that the Foundation seeks to foster interactions with Utah companies where possible.

c) We encourage you to summarize your discussions with a representative of a company regarding research support for this project or the licensing of research results. If you would like assistance in identifying such a company, please telephone the Technology Transfer Office at (801) 581-7792.

The technology derived from this research will be the discovery of methods for miniaturization and long-term stabilization of bioluminescent reactions for chemical analysis. The possible patentability of specific technology derived from this research will be the design of the micro-reaction chambers for enhanced light output, rapid rehydration and low-volume reagent layering for increased longevity and activity. This novel research will create an analytical panel designed specifically for testing renal function and rejection for kidney transplant patient and adequacy, functionality and hypoglycemia diagnosis for dialysis patients. However, the application of this miniaturized bioluminescent technology is only limited to the number of μRC placed on a chip. The same technology can be applied for panels designed specifically other low blood volume panels such as newborn intensive care, athletic clubs (for the metabolite analysis service they offer), obstetricians and gynecologists, and diabetics (so they can measure insulin in addition to glucose).

Renal transplant patients have to have their serum creatinine monitored regularly in order to detect possible kidney transplant rejection. Discussions with Dr. John Holman, kidney transplant surgeon in the University of Utah Department of Surgery, indicates that a simple, convenient, accurate method to measure blood analytes relevant to kidney transplant patients, which can be performed by patients at home, is needed. Whole blood creatinine is the classic measure of renal function used for transplant monitoring. The more than 50,000 renal transplant patients in the United States would save a great deal of time and effort if a simple and effective home test for creatinine were available. The over 2 million creatinine determinations per year for

8. Commercial Potential: (continued)

this population, at an average cost of perhaps $20 per test, leads to a significant overall cost to the patients and to the health care system.

In addition to kidney transplant patients, hemodialysis patients who often suffer hypoglycemic episodes, have deficiencies in both water and fat-soluble vitamins, and exhibit inadequate amino acid levels. Often these deficiencies are not recognized, leading to malnutrition and inadequate nitrogen balance. Dialysis and kidney transplant patients should be regularly monitored for glucose, creatinine, urea, uric acid, and the essential amino acids. Currently, hematocrit, urea, and uric acid are monitored, but only during dialysis. Glucose can be regularly monitored by minimally invasive glucometers used by diabetics. However, our technology would provide doctors a minimally invasive, inexpensive, multi-analyte sensor for millions of dialysis patients to monitor the adequacy of the hemodialysis treatments.

Two local companies have an interest in this technology: Protein Solutions, Inc. (PSI), located in the University of Utah Research Park, and Frezenius Medical, Ogden, Utah. Protein Solutions is involved in the development of devices for the measurement of common metabolites, initially for the management of inborn metabolic diseases, including phenylketonuria (PKU) and galactosemia. PSI is using traditional paper based sensing devices. The multichannel silicon based chip technology proposed here would likely lead to far more effective and competitive biosensors for health related applications.

Frezenius is one of the world's major manufacturers of hemodialyzers, the machines used in the treatment of end stage renal disease when kidney transplantation is not available or indicated. Mr. Eric Stroup and Dr. C.H. Ho, both University of Utah alumni, have expressed considerable interest in the developments of sensors for hemodialyzers and for the management and monitoring of dialysis patients.

Shortly after the work begins on this project, we will be meeting with these 2 firms, and perhaps others suggested by the Technology Transfer Office, and begin discussions regarding the potential commercial interest in licensing of the technology developed in this research.

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Provide the following information for each current and pending research project for each principal investigator and each co-investigator named.

9. Current Projects:

Project No. and Agency	Total or Annual Rate	Duration	% of Effort Committed	Title of Project
Whitaker Foundation	$769,327	3 ½ years 7/97 to 12/00	25%	Personal Sensors for Management of Chronic Metabolic Disease

10. Pending Projects:

Project No. and Agency	Total or Annual Rate	Duration	% of Effort Committed	Title of Project

None

12. Attach curriculum vitae for the principal investigator and each co-investigator (limit 2 pages each). The preferred style is a simple two-page biographical sketch using the NIH format.