FACULTY SEED GRANTS APPLICATION

Funding Year 2010-2011

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Proposals with text exceeding the prescribed limits will not be considered

Applicant Information	
First Name	Kelly
Last Name	Reynolds
Email Address	reynolds@u.arizona.edu
Position Title	Associate Professor
Phone Number	520-626-8230
Department Name	Environmental Health Sciences
Department Number	4206
UA Mailing Address	PO Box 245210-5210
Proposal Title:	Lab-on-a-chip flow cytometer for the detection of enteroviruses
Requested Amount:	$9,835

Compliance/Research Risk Acceptance of Responsibility:

I acknowledge that if my project includes research risk item(s), I and my department are responsible for maintaining compliance in conjunction with institutional policies. Automatic notices for research risk items are not generated for gifts. Faculty Seed Grants submission implies acceptance and agreement with the University's Conflict of Interest Policy. To acknowledge please check the box (double click): ☒

Eligibility:

I certify that I am an eligible applicant and I have prepared my budget in conjunction with my department business office. To acknowledge please check the box (double click): ☒

Authorized Signers (Names and Titles)

Rod Gorrell, Senior Accountant Zuckerman College of Public Health

Linda Tumellie, Assistant Dean, Financial Affairs and Physical Resources

Abstract/Project Summary (100 word maximum):

Lab-on-a-chip systems are microfluidic devices that applicable for the detection of waterborne microbes at a fraction of the cost of conventional methods. We propose the design of a miniaturized flow cytometer for the detection of human viruses using engineered molecular beacons. These probes target unique microbial nucleotides, producing a fluorescent signal. Our novel approach integrates optical detection systems for fast, quantitative signal measurement. Microfluidic systems reduce reagent volumes, system cost, size and power requirements, while addressing the need for rapid, real-time environmental monitoring and disease diagnostics. Project results will provide data for larger funding opportunities with industry and government sponsors.

Project Description (two pages maximum)

Background information

Prevention of infectious diseases is a major challenge in a globalized world. Rapid detection of waterborne viruses and other microbes from environmental samples is of central importance for public health risk assessment, homeland security and environmental protection. Current methods for virus detection are based on mammalian cell culture and polymerase chain reaction (PCR) techniques. These methods require complex analyses and several days to complete, or fail to detect viable targets.

The development of microfluidic devices for the detection of pathogens and for use in other biological analyses has led to an increased research interest in lab-on-a-chip systems, in which many different analyses that would usually require a full laboratory setup can be performed on a miniaturized device. Some of the advantages of this type of microfluidic system are: reduced sample and reagent volumes, system cost, size and power requirements, as compared to conventional systems. In recent years, new microfabrication technologies for the microelectronics industry have been applied in chemistry and biotechnology for the miniaturization of different analytical devices. These technologies have also been utilized for the design of lab-on-a-chip platform flow cytometers. Conventional flow cytometers are extensively used bioanalysis tools in medical research and clinical diagnostics to study physical and chemical properties of cells or other biological samples (Cantera et al., 2010). Flow cytometers incorporate fluorescent detection to obtain detailed identification of cell types and functions based on fluorescence of antibody-conjugated dye labels. Lab-on-a-chip flow cytometers are promising devices for point-of-care and field applications because they are potentially much cheaper, portable and easier to use than bench-top systems.

Hsiao-Yun et al. (2008) developed a fluorescent reporter system for coxsackievirus infection, using engineered nuclease resistant molecular beacons (MBs) in buffalo green monkey kidney cells (BGMk). These MBs contain RNA bases that specifically target regions of the viral genome, producing fluorescence upon target binding. A cell-penetrating peptide is conjugated to the MB, facilitating nearly 100% the intracellular delivery within 15 min.

Work description

The present research proposal is a collaboration between Dr. Marylynn Yates, Associate Professor of the Department of Environmental Sciences at the University of California, and Dr. Kelly Reynolds, Associate Professor of the UA College of Public Health. Drs. Mark Riley and Yeong-Yeol Yoon, at the UA's Department of Agricultural and Biosystems Engineeering will also lend assistance with the biosensor design and fabrication of the lab-on-a-chip. This project involves the use of MBs with a fluorescent reporter system combined with a portable and low cost microfluidic flow cytometer for the rapid detection of human viruses. The novelty of this device is the integration of microfluidic channels with hydrodynamic focusing flow, optics (waveguides, lenses and fiber-to-wave- guide couplers) and electronics into a single detection lab-on-a-chip platform that will permit the reduction in the size, cost and complexity of the system. This miniaturized device will use MBs with a fluorescent reporter in combination with living BGMk cells to detect viable human viruses in water (Hsiao-Yun et al. 2008).

Although the integration of MBs into a microfluidic system is new, detection schemes relying on integrated optics have been successfully demonstrated for micro flow cytometry (Lien et al. 2005) and protein analysis (Vieillard et al., 2007). Both fabrication and packaging are simple, cheap, and fast, resulting in devices that can be made and tested in a very short time frame. Following the setup of a conventional flow cytometer, our device will contain three main

components: a flow cell, a light source and a detector. A sheath fluid carries the target through the flow cells to be analyzed. The cell size and the fluid flow will be designed to separate out the targets to a level sufficient for detection.

Methods

This device will use a single line stream of monodispersed (single cell) suspension of BGMk cells previously incubated with the MB possessing a FAM fluorophore at one end and DABCYL quencher at the other. In this technique, probes are highly specific for a single enterovirus nucleotide sequence and upon target binding, the MB probe undergoes a conformational reorganization that forces the stem hybrid to dissociate and the fluorophore and the quencher are separated from each other, restoring fluorescence.

The stream of fluid containing the cells will be squeezed or hydrodynamically focused using two sheath streams to compress the central sample stream into a single cell line. Parameters such as scattered light and fluorescence light will be collected across the continuous laminar flow of a fine stream of the suspension in a sensing region. Waveguides will be constructed by filling microfluidic channels with a high refractive index liquid. These waveguides will be used to deliver excitation light (473nm) to the microchannel and the fluorescence will be collected by a waveguide at an angle of 45 degrees to the excitation waveguide using a highly sensitive photomultiplier tube that will provide important information about the sample under test. The basic components of the proposed system are available: the chip will be made from an existing mold available at the Sensors Lab (Heinze et al., 2010), University of Arizona, MBs will be provided by Dr. Yates and optics will be purchased.

Potential impact

A complete miniaturized flow cytometer combined with a MB based detection approach provides rapid and automated detection of infectious viruses compared to the conventional plaque assay. This biosensor can be constructed for a fraction of the cost of a conventional flow cytometer for the detection of different enteroviruses and have the advantage of portability and potential automation. This project is a step towards the future development of a real-time monitoring device for drinking water systems, among other environmental and clinical applications. Project results will provide data for larger funding opportunities with industry and government sponsors, such as the U.S. Environmental Protection Agency and the Water Research Foundation.

References

Cantera, JL, W Chen, MV Yates 2010 *Detection of Infective Poliovirus by a Simple, Rapid, and Sensitive Flow Cytometry Method Based on fluorescence Resonance energy transfer technology*. Appl Environ Microbiol 76:584-588

Heinze, BC, JR Gamboa, K Kim, JY Song, JY Yoon 2010 *Microfluidic Immunosensor with Integrated Liquid Core Waveguides for Sensitive Mie Scattering Detection of Avian Influenza Antigens in a Real Biological Matrix*. Anal Bioanal Chem 398: 2693-2700

Lien, V, K Zhao, Y Berdichevsky, et al. 2005 *High-sensitivity cytometric detection using fluidic-photonic integrated circuits with array waveguides* IEEE J Selected Topics in Quant Electron 11:827 2005

Vieillard, J, R Mazurczyk, C Morin, et al. 2007 *Application of microfluidic chip with integrated optics for electrophoretic separations of proteins*. J Chromat B 845:218-225

Wang, Z, J El-Ali, M Engelund, et al. 2004 *Measurements of scattered light on a microchip flow cytometer with integrated polymer based optical elements*. Lab Chip 4:372-377

Yeh, H-Y, Y-C Hwang, M.V. Yates, et al. (2008) *Detection of Hepatitis A Virus by Using a Combined Cell Culture-Molecular Beacon Assay*. Appl Environ Microbiol 74:2239–2243

Yeh, H-Y, Y-C Hwang, M.V. Yates, et al. (2008). *Visualizing the dynamics of viral replication in living cells via Tat peptide delivery of nuclease-resistant molecular beacons*. PNAS: 17522–17525

FACULTY SEED GRANTS
Budget and Budget Justification

A. Project Budget

EXPENSE ITEM	FSG Requested Amount
Wages & ERE	4,550
Travel	0
Capital Equipment (>$5k)	0
Operations	
<$5k equipment	2,960
materials and supplies	2,325
printing/duplication/marketing	0
Postage	0
Consultant Fees	0
Participant Payments	0
Other	
TOTAL EXPENSES	9,835

B. Total Project Budget Justification (be concise)

Expense Items
Wages & ERE: Funding is requested for one graduate student, working 220 hours (~1/4 time) at $20.00 per hour and a fringe benefit rate of 3.4%, for a project total (wage + fringe) of $4,550. The student will be involved with the growth of adenovirus stocks and BGMk cells and collecting quantitative measurements of the optical signals.
Travel: NA
Operations (include justification for each subcategory): Total budget for operations is $5,285. Specific items include: optics equipment (photomultiplier tube, $795; power supply, $1,595; and fiber optic adapter, $175); laser system, $395; lab-on-chip supplies (silica fibers, saline solution, syringes) $300; cell culture supplies (serum, antibiotics, media) $1400; molecular beacon, $350; enterovirus stocks, $275
Postage: NA
Consultant Fees: NA
Participant Payments: NA
Other: NA

Applicant Brief CV (two pages maximum)

Kelly A. Reynolds
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Tucson, Arizona 85724 email: reynolds@u.arizona.edu phone: 520-626-8230

Professional preparation

The University of Arizona	*B.S., Microbiology*	*1989*
The University of South Florida	M.S., Public Health	1992
The University of Arizona	*Ph.D. Agriculture*	*1995*

Appointments

July 2006- present, Associate Professor, Environmental Health Sciences, Zuckerman Arizona College of Public Health. The University of Arizona
July 2007- present, Adjunct Faculty, Department of Soil, Water and Environmental Science
1995-2006, Assistant Research Scientist/Appointed Faculty, Department of Soil, Water and Environmental Science, The University of Arizona.

Related Publications

Storrer, S., K. Verweire, M.T. Walter, K.A. Reynolds. 2010. Water Quality Impact of Recreation in Natural Tinajas. *In review*. Journal of Environmental Quality.
Mahalanabis, M., K.A. Reynolds, I.L. Pepper, C.P. Gerba. 2010. Comparison of Multiple Passage Integrated Cell Culture-PCR and Cytopathogenic Effects in Cell Culture for the Assessment of Poliovirus Survival in Water. Journal of Food and Environmental Virology. *Accepted*.
Yang, Z., M.K. Fah, K.A. Reynolds, J.D. Sexton, M.R. Riley, M. Anne, B. Bureau, P. Lucas. 2010. Opto-electrophoretic detection of bio-molecules using conducting chalcogenide glass sensors. Optics Express. 18 (25): 26754-26759.
Vargas, CA, AA Wilhelm, J Williams, MR Riley, P Lucas. 2009. Integrated capture and spectroscopic detection of viruses. Appl Environ Microbiol. 75(20): 6431-6440.
Miles, S, CP Gerba, IL Pepper, KA Reynolds. 2008. Point-of-use drinking water devices for assessing microbial contamination in finished water and distribution systems. Environ Sci Tech. 43(5): 1425-1429.
Wilhelm, AA, P Lucas, KA Reynolds, MR Riley. 2008. Integrated capture and spectroscopic detection of viruses in an aqueous environment. Optical Fibers and Sensors for Medical Diagnostics and Treatment Applications VIII. Proc. Of SPIE. Vol. 6852, 68520K-1.
Reynolds, KA, KD Mena, CP Gerba. 2008. Risk of waterborne illness via drinking water In the United States. Rev Environ Cont Toxicol. Vol. 192(4):117-158.
Hurst, CJ, KA Reynolds. 2007. Detection of viruses in environmental waters, sewage, and sewage sludges. *In:* CJ Hurst, RL Crawford, JL Garland, DA Lipson, AL Mills, LD Stetzenbach (eds.) Manual of Environmental Microbiology. 3rd Edition. ASM Press, Washington, D.C. ISBN-10: 1-5581-379-8

Reynolds, KA. 2006. Drinking water Quality and Water Security. *In*: RM Maier, IL Pepper, CP Gerba (Eds.). Environmental and Pollution Science. Academic Press, New York.
Reynolds, KA 2004. Integrated cell culture/PCR for detection of enteric viruses in environmental samples. *In:* Spencer, J.F.T and Ragout de Spencer, A.L. (Eds.). Public Health Microbiology: Methods and Protocols. Methods in Molecular Biology, Vol. 268. pp. 69-78. Humana Press. Totowa, New Jersey.
Tanner, BD, S Kuwahara, CP Gerba, KA Reynolds. 2004. Evaluation of Electrochemically Generated Ozone for the Disinfection of Water and Wastewater. Wat Sci Tech. 50(1):19-25. IWA Publishing.
Reynolds, KA, CP Gerba, M Abbaszadegan, IL Pepper. 2001. ICC/PCR detection of enteroviruses and hepatitis A virus in environmental samples. Can J Microbiol. 47(3): 153-157.
Gerba, CP, KA Reynolds, SE Dowd, IL Pepper. 2001. Polymerase Chain Reaction for the Detection of Parasites and Viruses. *In*: Rapid Detection Assays for Food and Water. S. Clark, K.C. Thompson, C.W. Keevil, and M. Smith (eds.), The Royal Society of Chemistry, Cambridge, United Kingdom.
Reynolds, KA, CP Gerba, M Abbaszadegan, IL Pepper. 2001. Rapid PCR-Based Monitoring of Infectious Enteroviruses in Drinking Water. AWWA Research Foundation.
Blackmer, F, KA Reynolds, CP Gerba, IL Pepper. Use of Integrated Cell Culture-PCR to evaluate the effectiveness of poliovirus inactivation by chlorine. Appl Environ Microbiol. 66(5) 2267-2268. May 2000.

Recent Honors and Awards

Water Conditioning and Purification International's Award of Appreciation for the most requested article reprints in journal history, 2009-2010
Water Quality Association, Honorary Lifetime Member Award, 2009. In recognition of exceptional service given to the water quality improvement industry.
William B. Fritzsche Memorial 2009 Top 50 Award, for service and dedication to the water treatment industry. Sponsored by Water Conditioning and Purification International.

Recent Related Synergistic Activities and Grants

"Evaluation of the Suez Canal Environments for toxin Producing Cyanobacteria" USDA/FAS/OCBD 58-3148-7-168; $60,000; 2007-2010
Arizona Public Health Association, Member
Water Quality Association, Member
International Association on Water Quality, Member
American Water Works Association, Member
The American Society for Microbiology, Member
Institute for the Study of Planet Earth, Faculty advisor
Executive Faculty Council, Graduate Certificate in Water Policy, University of Arizona,
Member, Research Advisory Council, The University of Arizona
Advisor. American Water Works Association Project Advisory Committee
Technical Review Council, *Water Conditioning and Purification International*