ANTIBODY, CELL-BASED, AND TRANSGENIC FISH BIOSENSORS FOR AUTONOMOUS MONITORING OF ENVIRONMENTAL TOXICANTS

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Abstract

The Department of Defense (DOD) is concerned with the effects of the hazardous materials that arise from defense related operations. DOD is particularly interested in the effects of these materials on military personnel, civilian populations, and ecological systems. Five years ago, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities in New Orleans, LA, USA, initiated its Receptor-Based Hazard Monitoring (RBHM) Program to develop advanced biosensors. Through its basic and applied research, the CBR is developing cost-effective real-time sensor devices that will monitor potential and actual exposure of the general public or troops in the field to harmful chemical or biological agents. A major goal of the CBR is to assist faculty in making the transition from bench research to field implementation. In terms of autonomous platforms, the CBR has initiated development of an antibody-based biosensor to be deployed on an Autonomous Underwater Vehicle (AUV) for analysis of pollutants in the Mississippi River and the extended estuary of the Gulf of Mexico. This project will set the stage for the CBR's goal to develop a suite of advanced biosensors that can be used in autonomous real-time sampling networks for monitoring and risk assessment.

Introduction

Founded in 1989, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities is a partnership between a Historically Black College or University (HBCU) and a major research university that encourages scientists from multiple disciplines to work together to investigate and resolve environmental problems. The CBR's programs are organized around themes including: human and ecosystem health, water resources, communication and technology, and environmental education. Development of biosensors represents a true synthesis of most of these programs, harnessing the basic knowledge about how chemicals signal biological receptors for development of new technologies to monitor aquatic and terrestrial ecosystems.

Five years ago, the CBR initiated its Receptor-Based Hazard Monitoring (RBHM) Program through the DOD's Defense Threat Reduction Agency (DTRA) to develop advanced biosensors and biomarkers of exposure to toxicants. The primary objectives of the RBHM program are to assess the impact of hazardous materials through the development of biosensors and to conduct the basic research necessary to implement these technologies. Through the Office of Naval Research (ONR), substrate specific biosensors will indicate the presence and monitor the clean up of hazardous chemicals stemming from the development, testing, production, and operation or maintenance of military equipment and weapon systems. Through the U.S. Department of Energy (DOE), biosensors are being developed for new monitoring technologies and risk assessment approaches to the long-term stewardship of the DOE's Weapons Complex.

Through this basic and applied research, the CBR is developing sensor devices that will monitor potential and actual exposure of the general public or troops in the field to harmful chemical or biological agents. The CBR's research emphasis is on providing innovative cost-effective solutions to prevent, minimize, or remedy human health or ecological hazards. Defense-related hazardous materials being examined through these projects include:

- heavy metals (Darwish and Blake 2001; Khosraviani et al. 2000, 1998; Xia et al. 2000a,b,c,d, 1999);
- aromatic hydrocarbons;
- organophosphates (e.g., phosphorous-containing nerve gases);
- mycotoxins (i.e., toxins from fungi and molds);
- dinitrophenol (used in explosives);
- polyacetylenes;
- ionic uranium (Blake et al. 2000); and
- other chemicals that act as carcinogenic or endocrine disrupting compounds.

The CBR's biosensors developed to detect the compounds listed above employ a variety of biologically based receptors which utilize biologic reactions to assess and report the types and quantity of toxins in the field. While many of these technologies seek to mesh biologic receptors to "mechanical" devices, others seek to genetically engineer organisms and molecules (e.g., fish and antibodies) to provide cues (e.g., fluorescence) when exposed to environmental toxins. These projects employ a variety of methods including field studies and laboratory *in vitro* and *in vivo* research. Benefits of these biosensors include their cost-effectiveness when compared to traditional monitoring devices and their ability to assess the degree to which these biologic organisms can repair or reverse these adverse effects. Additional benefits include increased safety for Navy and the general public from exposure to a variety of defense-related toxicants.

For field applications, there is no single best technology for a biosensor. The best choice depends entirely on the desired response time, specificity, sensitivity, and durability and viability when exposed to field conditions. Cell-based biosensors, for example, are popular for assessments of both specific and general toxicity (families of chemicals) or other environmental parameters (e.g., biological oxygen demand). Their other primary strength is that they can be used to detect toxicant levels (e.g., bacteria fluorescence in response to exposure) as well as toxic endpoints of concern (e.g., gene expression utilizing gene-chip array technologies). However, cell-based sensors may not be as durable as other biosensors since one has to keep the cells alive during the experiment. Although bacteria cells are generally easier to keep alive than mammalian cells, mammalian cells generally respond more quickly to their environment than do bacteria.

Antibodies by contrast are much more durable than cells once immobilized, often lasting days to weeks under average ambient conditions. They respond quickly to their environment (between 1 and 10 minutes) and have extremely high specificity and sensitivity with detection limits as low as parts per billion (ppb). This specificity makes them desirable for detection of toxicants but does not make them as useful for entire families of chemicals. Antibody-based tests have been developed to measure

cadmium, lead, and uranium at or near the EPA action levels (Darwish and Blake 2001; Khosraviani et al. 1998 and 2000; Blake, et al., 1998 and 2000). Immunoassays currently under development are being adapted to portable and autonomous field tests that can be used on-site. It is anticipated that these field tests can be exploited both in military and private sectors.

Customized models for aquatic bioavailability assessment have been designed by genetically engineering Japanese Medaka fish and zebrafish (Toscano and Mattingly, 1998). These customized fish were designed to bear the Green Fluorescent Protein gene, whose expression can be activated by the aquatic pollutant of interest. The chief benefit of this technology is that it can be used to monitor for various toxins' bioavailability, rather than the mere presence, in aquatic environments. As such, they act as an aquatic "canary in a coal mine", allowing the user to assess how life cycles of the organism interact with their environment for ultimate exposure and impact.

The major goal of this project is to transition the biosensor from bench research to field implementation through the engineering adaptation of the biosensor to the AUV. Specifically, The biosensors will be integrated into the Woods Hole Oceanographic Institutes' REMUS AUV. The REMUS vehicle is designed to be small, lightweight, low-cost, easy to use, and highly accurate in terms of navigational performance and sensor data. It has been optimized for large area, shallow water surveys. It can be equipped with a wide variety of sensor packages, most commonly including side-scan sonar, ADCP, OBS, and CTD. The vehicle is monitored and programmed using software that runs on a
laptop computer. A simple scripting language is used to program the vehicle for a desired mission. The same program may be used to simulate the mission profile before deployment.

Summary CBR Progress to Date for AUV Immunosensors

The CBR's efforts to develop and deploy an autonomous immunosensor on an AUV are being conducted by an interdisciplinary team led by Drs. Diane A. Blake (Tulane University Health Sciences Center), Robert C. Blake II (Xavier University, College of Pharmacy), and George Flowers (Tulane University, Department of Geology). Other team members come from Tulane's School of Engineering, NAVOCEANO, Sapidyne Instruments, Boise, ID, and COTS Technology, LLC.

The goal of this project is to develop a biosensor that will permit the rapid, automated identification and quantification of EDTA (ethylenediaminetetraacetic acid) in river water. EDTA is one of the more ubiquitous dissolved organic compounds in the Mississippi River. Because it is used to form water-soluble complexes with insoluble metals in a wide variety of domestic and industrial applications, EDTA concentrations can be used as an indicator of total domestic and industrial wastewater inputs. EDTA has low toxicity to humans and aquatic organisms. The chief water-quality concerns are its ability to mobilize toxic metals from sediments and to stimulate algal growth by increasing the availability of nutrients such as iron and zinc. EDTA is not biologically degraded during wastewater treatment and it thus serves as a relatively stable marker in environmental samples. As a consequence of the lack of biodegradation, its high solubility in water, and large rates of production and usage, EDTA is one of the most abundant organic contaminants in some surface waters. In general, EDTA concentrations in the Mississippi River range between 10 and 40 μg/L, concentrations well within the limits of detection we have achieved with other immunoassays.

A set of high-affinity, highly selective binding reagents (antibodies) will be used in the development of an immunosensor that can operate in an AUV to rapidly and accurately quantify EDTA in real-time in the field. Although more than a dozen antibodies that bind a variety of environmental pollutants have been developed in Tulane laboratories, the present project is focusing upon developing a biosensor that can detect EDTA in river and surface water.

Drs. Blake and Blake have isolated two monoclonal antibodies (clones 1B11 and 2D42) that recognize metal-free and metal-loaded EDTA with equal affinity. These antibodies are currently being further characterized to test their activity in the sample matrix (Mississippi River water) in which the AUV will ultimately be deployed. These biological reagents will be used to construct a prototype immunosensor that can be deployed on an AUV. Major activities include:

1) Demonstration of the feasibility of using a lab-based instrument and the 1B11 and 2D42 monoclonal antibodies to monitor EDTA in the environmental matrix of the test area.

2) Assembly, testing, and validation of a new field-portable prototype for EDTA detection based on the KinExA device manufactured by Sapidyne Instruments.
3) Comparison of the new biosensor with existing ONR sensors (e.g., evanescent wave fiber optic and continuous flow membrane biosensors).

Platforms that will be available for this biosensor will include immersible stationary buoys [as part of NAVOCEANO's Environmental Ambient Recording Systems (EARS)] and the REMUS AUV developed by Woods Hole Oceanographic Institute's (WHOI) Ocean Systems Lab (Percell et al. 2000) described above. It is anticipated that by the fall of 2001, we will have enough information to assemble a working prototype of an immunosensor for deployment on these platforms.
In order to move the EDTA antibodies to demonstration and validation as an AUV biosensor, we must first assemble and test a new analytical instrument for deployment for analytes on an AUV to conduct competitive immunoassays for environmental contaminants. The CBR will collaborate with Sapidyne Instruments in the research and development of a miniature version of the KinExAä instrument that can be deployed on an AUV.

The ability of this instrument to accurately quantify analytes of interest will be assessed by comparing the performance characteristics of the miniature KinExA with those of an established 96-well microtiter plate reader, and an evanescent wave fiber optic immunosensor. The format, sensitivity, precision, and reproducibility of the EDTA immunoassay will be determined on each assay platform (i.e., comparing AUV results with other platforms).

Minimal quality control assurance requirements include the analysis of field reagent blanks, field fortified blanks, and field fortified matrix samples. Certified AA grade Pb standards will be utilized to verify method performance. Field reagent blanks (FRB's) will be included with field sample collection activities and will also be included with field samples being sent for confirmatory analysis. Before processing any sample in the field, at least on FRB must be analyzed to demonstrate that no interferences are introduced by equipment and reagents. FRB's must also be analyzed every time reagents are changed. If the FRB produces a positive response, the source of the contamination must be determined and the interference eliminated before proceeding with the analysis.

In addition, we plan to add known concentrations of EDTA (to a minimum of 10% of that in the actual sample) and analyze this field fortified matrix sample (FFM). The spike levels will be equivalent to or greater than the background concentration in the samples selected for fortification. The mean percent recovery (%R) will be calculated after correcting concentration measured in the fortified sample (C_f) for the concentration measured in the unfortified sample (C_t) according to the equation:

$$\%R = \frac{C_f - C_t}{C_a}$$

Where C_a is the concentration of analyte used to fortify the sample (the known concentration added). These values should be compared to the control limits of the FFB. If the method accuracy falls outside the 50% to 150% range, the accuracy problem encountered with the FFB may be judged to be matrix-related. The results for unfortified samples must be labeled suspect due to matrix effects.

Future Applications

The benefits of this research include new technologies for low-cost, near real-time and real-time monitoring and communication of presence and concentrations of environmental pollutants. Sample analysis costs, often the major cost involved in the environmental monitoring, can be reduced by 50% or more through the use of antibody-based assays. In addition, real-time or near real-time data provided by antibody-based sensors can also be of significant advantage in protection of personnel in the field.

Adaptation of the immunosensors as a collector on AUVs has direct application in support of the Naval Oceanographic Office's military surveys in the Littoral Zones. Another significant area of support is the pre-invasion mission to detect mines and obstacles for clearance/avoidance in the Very Shallow Water (VSW) and Surf Zone (SZ) approaches to the amphibious landing areas.

Specifically, the biosensors role during military surveys conducted by the Naval Oceanographic Office will be to collect the natural "background" environmental harmful agents to

personnel that work in the waters of the Littoral Zones. Development of this definitive database will support the intelligence requirements of the SEAL, EOD, and amphibious assault teams. Moreover, immunosensors will improve the probability of mission success, endurance and survivability of SEAL swimmers through detection of harmful agents during the initial AUV environmental survey. This health-risk assessment will involve the prediction and monitoring of waters polluted [either naturally or by intention (or both) by the opposing forces] with heavy metals, microbial hazards, anthropogenic chemical, lipophilic environmental chemicals, toxic dinoflagellate, and the detection of outflow from waste treatment plants prior to the hunt for mines and obstacles. In order to implement this program, the CBR has partnered with NAVOCEONO, COTS Technology, LLC and other academic, agency, and industry partners in a program called the Long-Term Estuary Assessment Group (LEAG) (Warrenfeltz, et al. 2000). Through creation of technical working groups for 1) sensors and platforms, 2) modeling, data, and processes, and 3) informatics, the LEAG consortium intends to establish a network of autonomous biosensors and communication technologies for real-time monitoring and predictive modeling, using the Mississippi River and Gulf of Mexico as its natural laboratory.

Meeting the challenging new requirements of "Ship-to-Objective Maneuver" of the Amphibious Ready Groups will demand a new level of intelligence that places greater emphasis on real-time and near real-time environmental data of the VSW/SZ arena. Through integrated biosensor development and field research in aquatic environments, the CBR's goal is to bridge knowledge from molecular to ecosystem level analyses for holistic assessments of the environment and health.

We believe that this project supports strategic goals of the National Oceanic & Atmospheric Administration/National Ocean Science (NOAA/NOS) including: (1) Habitat, (2) Hazards, and (4) Coastal communities. To date the nations coastal communities have not had low-cost sensor that can rapidly and efficiently collect the baseline to track the impacts in the areas of habitat and hazards. This project provides the unique tools to commence the longitudinal studies that will establish the baseline data sets for measuring and assessing the impacts on a realtime and near-realtime basis. Through the application of this science and new engineering tools we can improve the ability of coastal communities to mitigate the impacts of all natural and anthropogenic hazards.

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