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(DREAMS): Digital Emergency Medical Services and the Detection  
and Remediation of Chemical Threat Agents

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## INTRODUCTION

The Disaster Relief and Emergency Medical Services (DREAMS™) project is a consortium of scientists, medical professionals, and engineers from The Texas A&M University System and the University of Texas Health Science Center at Houston. The goal of DREAMS is to improve the diagnosis and treatment of critically ill or injured soldiers in the field by expediting their access to medical experts at trauma centers or field hospitals. Texas A&M University System researchers and engineers are working on two components of the DREAMS program:

1. Texas A&M University System (TAMUS) Digital EMS, and
2. Detection and Remediation of Chemical Threat Agents.

### Texas A&M University System Digital EMS

Texas A&M Digital EMS is the DREAMS component that allows trauma specialists to treat patients more quickly by providing the "virtual" presence of a physician on the battlefield or at the emergency scene. Digital EMS integrates multiple leading-edge telecommunications technologies, especially video processing, wireless communications, and digital signal processing. The Digital EMS ambulance phase one prototype, connects emergency medical personnel on the scene with trauma specialists in distant hospitals, allowing physicians to monitor patients using real-time video and vital signs data from a suite of advanced digital medical monitoring equipment.

Detection and Remediation of Chemical Threat Agents-The component of DREAMS dedicated to the detection and remediation of chemical threat agents is critical to the welfare of the soldier in the field and citizens at home. One of the newly emerging concerns regarding military and civilian disaster response scenarios is the involvement of chemical or biological weapons of mass destruction, which might range from noxious fumigants to neurotoxic, chemical warfare agents, or infective biological spores or viruses and their toxins. Texas A&M scientists are researching and developing new genetically engineered enzymes that recognize and decontaminate a host of chemical threat agents, and researching methods to integrate these new materials into detection and decontamination systems.

## **BODY**

### **Texas A&M University System Digital EMS**

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#### **Task 1: Work with University of Texas Health Science Center to Enhance Current Technologies within the Digital EMS Vehicle and Associated Hospital Systems**

##### **Medical Protocol Development/Implementation**

- Identified and acquired protocols to be implement in the system for initial operational testing
- Supporting efforts to restructure protocols for use in the digital environment
- Implemented enhanced methods for displaying medical protocols in the digital environment

##### **Physician's Workstation**

- Developed a mature prototype of the physicians workstation
- Implemented physicians workstation with dual monitor configuration

##### **Draft Hospital Database Schema**

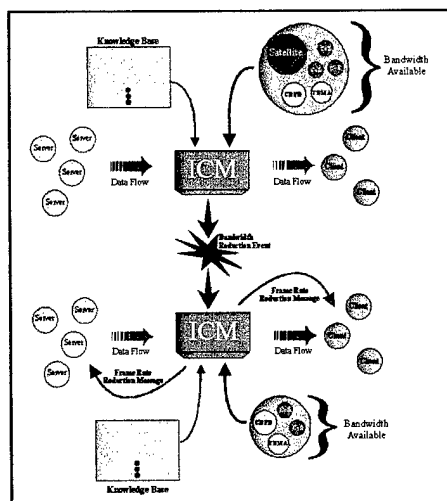
- Hosted preliminary discussions regarding communications bridge between digital ambulance and receiving hospital.
- Developed working database tailored to exercise hardware and software integration in the digital ambulance; i.e., integration of run record with a database.
- Monitoring HL7, HIPPA and other requirements to ensure compliance upon their implementation

##### **Satellite Activity**

During this reporting period, A&M staff attended meetings, received reports of technology development and supported the definition of system requirements. Texas A&M has not yet taken delivery of a satellite system for integration into the Digital EMS system. In the interim, and as a deployment solution for operational testing, the research team is exploring technologies which may provide service until development systems mature.

#### **Task 2: Enhance the Existing Digital EMS System to Accommodate Additional Functionality**

##### **Intelligent Communications Manager (ICM)**



Communications on the Digital EMS system is achieved by an Intelligent Communications Manager (ICM) that manages multiple data streams generated by the server and client applications running both on the remote vehicle and at the local hospital.

approach suffers from the inability to access the underlying inability to access and modify the communications protocol for eventual implementation on an ICM-based communications system.

The current focus of software development is in the transition from a RMI based client/server architecture to a mixed RMI/sockets approach. This approach retains the RMI benefits of remote invocation of local client/server pairs, but implements a UDP socket based approach when communication needs to be established between client/server pairs across the hospital-vehicle network.

### New Digital EMS Functionality

#### *Barcode reader integration*

A barcode reader was implemented on the Paramedic system for inserting medications into the treatment page of the run record panel. When a medication is scanned through the reader, a database lookup is performed on a set of stored medications already present in the system. If the barcode of the medication scanned can be matched to a stored barcode in the local database, the medication description is extracted and inserted into the next available location on the Run Record. A time is also inserted and marked in a Red color to signify to the user that the time needs to be verified and logged by the system. The user can then accept the specified time or modify it to the appropriate value. A "Set" button is provided to log the time to the run record. A dosage entry is also provided to record the appropriate amount of the medication given.

The reader was integrated into the system by designing two Java classes which implement a device driver and a listener for device data. The driver class is responsible for initializing the device, configuring the serial port on which the

device is attached, and processing the input data received when a barcode is scanned. The listener class provides an interface which defines the method called by the device driver whenever a new barcode has been read.

Applications which require barcode scanning just instantiate a barcode device class and register for barcode events by implementing the barcode listener interface. When a barcode is scanned, a barcode action method is called in the registered classes with the number that was scanned.

#### *Magnetic card reader integration*

A magnetic card reader was integrated into the system for reading patient driver licenses, system users, and administrators. When a patient is brought on board the vehicle, a run record can be initiated by scanning the patient's driver license through the card scanner. Similarly, users of the system log into the system using a Dreams User Card which log the user into the system during each shift. An additional Dreams Administrator card is also used for accessing the administration functionality of the system which is not accessible to the normal user.

A card device class was developed for initializing the device on the attached port and processing the incoming data. When a card is swiped on the scanner port, a field delimited string is generated by the device. A parser class was created for parsing the generated string by card type and field. To access the card functionality, applications implement a card listener interface and register for card events in the device class. When a card is read by the port, all listeners are given the card string. Using the parser class, applications can then extract the field values for the card that was read.

#### Interface Engine for Hospital Informatics Database

Researchers developed the capability to access SQL compliant database. Given the schema and set-up for a unique environment, we have the ability to access a hospital database and query it.

A patient medical record database has been designed and implemented on the physician station. This database is implemented as a Microsoft Access database and is accessed by the system using a JDBC link via a Patient Record Server executing on the Physician station.

#### Updated servers/clients functionality

A new video server has been implemented with capability for supporting up to 4 cameras per frame grabber. Additionally, video streams can be modified based on compression rate, frame size, and frame rate. The new system is composed of 2 frame grabbers for a maximum of up to 8 cameras within the vehicle. This alleviates the need for additional PCI expansion busses required under the previous prototype.

Additional functionality on the Propaq system includes:

1. Support for executing a remote NIBP reading from the Physician Station.
2. Selection of Manual/Auto NIBP reading with multiple delays.
3. Support for remote streaming of ECG2 waveform.
4. Support for remote streaming of invasive pressures INVP1, INVP2.
5. Support for remote streaming of Respiration waveform.
6. Ability to dynamically detect when a Propaq device is connected and disconnected from the system.
7. Addition of display for status messages from server.
8. Ability to display status of NIBP measurement.
9. Dynamic scaling values for waveform data.
10. Freeze/Unfreeze waveform display

#### Virtual Collaboration Functionality

While the research team has a vision for how this capability might work within the digital ambulance, the requirements for this capability will be an outcome of the initial operational deployment.

### **Task 3: Integration New Technologies for Inclusion in the Digital EMS Vehicle to Support Additional Medical Functionality for Trauma Care at Remote and Hospital Sites**

#### Addition of Emergency Medicine Tools

The paramedic system on board the ambulance was enhanced with a set of emergency medical tools including: emergency calculators, tables, scores.

#### *Calculators Implemented:*

Alveolar-arterial oxygen gradient (A-a gradient)  
Anion gap  
Blood alcohol concentration  
Blood oxygen content  
Body mass index  
Burn  
Cerebrospinal white blood cell correction in traumatic lumbar puncture  
Factor IX dosing  
Factor VIII dosing  
Free water deficit  
Ionized calcium estimation  
Mean arterial pressure  
Predicted alveolar-arterial gradient (A-a gradient) for age  
Predicted endotracheal tube size for age  
Predicted peak expiratory flow (PEF, peak flow) - females  
Predicted peak expiratory flow (PEF, peak flow) - males  
Pregnancy Wheel

Room air alveolar-arterial gradient (A-a gradient)  
Serum osmolarity estimation  
Sodium bicarbonate replacement  
Sodium correction in hyperglycemia

*Tables Implemented:*

Endocarditis Prophylaxis - Antibiotic Regimens  
Endocarditis Prophylaxis - Which Cardiac Conditions Require It?  
Endocarditis Prophylaxis - Which Procedures Require It?  
Ethanol effects in non-alcoholics  
Opioid Potency Comparison  
Pediatric Resuscitation Equipment - Sizing by Age  
Pediatric Vital Signs  
Synovial Fluid Classification  
Systemic Glucocorticoids Comparison

*Scores Implemented:*

Alvarado appendicitis score  
Apache II score  
Apgar score  
Bleeding probability after TPA for MI  
Cardiac arrest: likelihood of mortality and neurologic recovery  
Community-acquired Pneumonia  
Mortality risk for adults  
Coronary disease probability  
Crotalid snakebite severity score  
Croup score (pediatric)  
Geriatric depression scale(Koenig)  
Glasgow Coma Scale  
Mangled extremity severity score  
Mini mental status exam  
NIH Stroke Scale  
Ranson's criteria for pancreatitis mortality prediction  
Strep pharyngitis probability score  
TWEAK alcoholism score  
Trauma score for survival probability

ISTAT and SpaceLabs Systems Integration

Texas A&M researchers continue to attempt to work with the ISTAT and SpaceLabs vendors to gain the insight required to integrate the system with the Digital EMS device. The research team is actively seeking alternative solutions providing the same functionality with more willingness on the part of vendors to provide the necessary protocols to support integration.

Texas A&M received upgraded support software for the ISTAT blood analysis system.

**Task 4: Integrate new U. S. Army, NASA, and DARPA Technologies Such As Medic-CAM and WARP into the Existing System for Enhancing System Functionality**

- Medic-CAM was integrated successfully into the system. No additional requirement exists to support/integrate the system beyond the current capability.
- The WARP device provided does not function properly. The software upgrade from NASA has not yet been provided.
- Awaiting TATRC's issue of the PIC hardware and software. The system will be integrated upon receipt.
- CSTAT system has not yet been provided by government.

**Task 5: Enhance the Existing Infrastructure for Supporting a Network of Multiple Digital EMS Vehicles and Hospital Systems in an Integrated Environment**

The major efforts toward this objective focused on the development of a mapping and navigation system that facilitates the tracking of multiple ambulances in a system. The navigation system consists of the following modules: a GPS receiver, a map display tool, a route calculation engine, a street database, and a friendly graphical user interface (GUI). Our approach to designing the navigation system is to use as much as possible commercially available software development tools for map display and route calculation in order to shorten the development time.

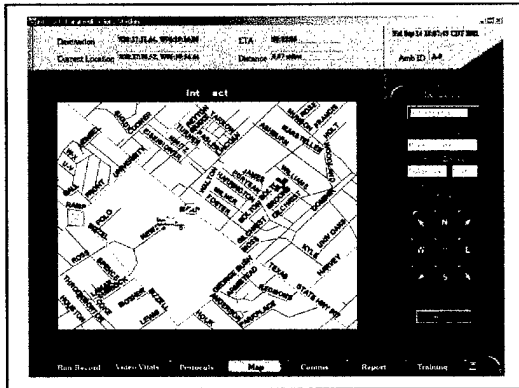
The navigation system follows the server-client architecture that is the basic software architecture of the Digital EMS system. A navigation server resides on the ambulance and receives the location data from the GPS receiver via a serial connection. The server broadcast this location information to all the map clients residing on the ambulance and in the hospital. When the map client on the ambulance chooses a destination and calculates a route, the route information such as destination latitude / longitude, distance, and estimated time of arrival is uploaded to the server. The server then sends it to the map client in the hospital.

The GUI of the Digital EMS system is developed in pure Java, requiring that the GUI of the navigation system must be pure Java. It puts a very strict requirement on the development of the navigation system since almost all map display tools and route calculation engines are not developed in Java. That poses a great challenge in integration.

Selection of Individual Mapping Modules

### *The GPS Receiver*

After a broad market research of commercial GPS receivers, we chose the Placer GPS 450/455 mobile unit, which is one part of Trimble's vehicle tracking line of products.



Paramedic Workstation map display.

### *Map Display Module*

After a thorough search of mapping development software tools, we have found the following four candidates: MapObjects 2.0 from ESRI, MapX from MapInfo, MapOCX Pro from Chicago Map, and GeoEngine from Etak. All of them are Microsoft Windows based products. The first three are ActiveX controls, which are based on Microsoft's COM (Component Object Model) technology. GeoEngine is a set of Windows DLLs (Dynamically Linked Library), which is easier to integrate with Java

compared to an ActiveX control. In terms of functionalities, all four of them except MapOCX Pro meet our requirements. We finally chose MapObjects 2.0 because ESRI provides good development supports and is the number one in development of GIS software. Also we have an educational site license from ESRI making MapObjects 2.0 more cost effective than MapX and GeoEngine.

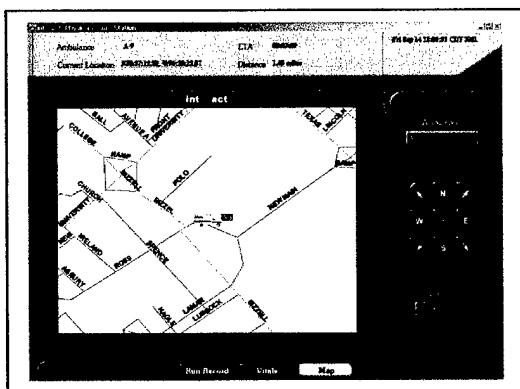
### *Route Calculation Module*

MapObjects 2.0 comes with an automation object for route calculation, but it is very inefficient. ESRI has also developed NetEngine - a tool for programmers to define, store, traverse, and analyze networks. NetEngine is very versatile and provides ready-to-use algorithms such as hierarchical shortest path algorithm that leads to realistic though not optimal paths. This is essential for efficient route calculation with a large street network.

### *Street Map Database*

Before comparing the available digital map databases, it is beneficial to list the requirements and needs of the navigation system being developed for the DREAMS project. The main purpose of the navigation system, including a GPS receiver, is to locate the ambulance, display the map, and safely guide the ambulance back to a hospital in a time-saving manner. In order to meet these needs, the digital map databases must be highly accurate, up-to-date, contain geocoding information, and fully routable. Also, the database should be available in the format compatible with our software. Thus the map database should be readable by MapObjects 2.0 since we chose MapObjects 2.0 for map display.

For the coverage of continental US, there are three proprietary digital map databases: EtakMap Premium from Etak, Dynamap/Transportation from Geographic Data Technology (GDT), and the NavTech database from Navigation Technologies (NavTech). Etak and GDT are ESRI's data provider partners and thus are highly recommended by ESRI. Though quite accurate in most major metropolitan areas, the coverage of NavTech database is limited and not good in inter-town areas.



Physician Workstation map display.

EtakMap Premium and Dynamap/Transportation match very closely in terms of coverage, accuracy, currency, and geocoding accuracy. Both are created from multiple sources and available in ESRI's shapefile format, which is accepted by MapObjects 2.0. Both may be updated semi-annually or annually and can be purchased by state or county.

A one-year license for EtakMap Premium costs \$10,000 for the state of Texas. That license is for 1 to 5 users. For a three-year license agreement, the first year costs the same while the second and the third cost half of the first year, for a total of \$20,000. A license from Dynamap/Transportation costs \$9,000 for the state of Texas and the license term is indefinite. It costs 10% of the original price to update Dynamap/Transportation semi-annually. Therefore Dynamap/Transportation is more cost-effective in the long run.

#### *Functionalities and GUI*

We developed three map clients residing on Paramedic Station, Driver Station, and Physician Station. They have different functionalities and thus different GUIs. The GUI on the Driver Station is sized 800 by 600 while the other two are sized 1024 by 768.

The map client on the Physician Station is designed primarily for map display and manipulation. It has the following functionalities:

- Map zooming from 0.5 mile to 150 miles;
- 8-directional map scrolling;
- Tracking the ambulance and displaying EMS icon with the ambulance ID;
- Displaying the ambulance ID, the current location, date, time, distance and ETA.

Besides all the functionalities for map display and manipulation, the map client on the Paramedic Station offers various options to choose a destination and then calculates a route. Different locations are shown with various icons. It also automatically updates the route and thus distance and ETA as the ambulance is moving. It has four options for destination selection:

- Preset locations
- Street address
- Intersection address
- Latitude/longitude coordinates

The map client on the Driver Station is even more powerful and versatile. It has five options for selecting a destination. With the exception of the last option, "Mark Destination on Map," a pop up window will appear when anyone of these buttons is pressed, prompting the user for destination information.

**Task 6: Develop and Test a Prototype Digital EMS Vehicle in Diverse Urban and Rural Settings for Evaluation and Performance Analysis of Integrated Digital Technologies**

Testing activities have been delayed due to unanticipated human use requirements. These activities have been reevaluated, redefined and rescheduled and are anticipated to begin in Spring 2002.

**Task 7: Develop Methodologies for Using New Local, State, and National Network Infrastructures for Providing the Digital EMS Vehicle with High Speed Terrestrial Connectivity to the Hospital Nodes**

Researchers worked actively with the State of Texas for utilization of existing networks (K-12, public utilities, municipalities) to support emergency use of their high performance networks. One potential scenario involves ambulances physically connecting to the network via portals installed on the exterior of public buildings. The networks would be used to transfer data, images and video between the ambulance and remote hospitals. Such connectivity might support physician mentoring of emergency medical personnel through life saving procedures.

**Task 8: Publish Findings and Results in Appropriate Conference Proceedings and Journals and Demonstrate Capabilities of the Digital EMS Ambulance**

The Digital EMS System was demonstrated and/or the project was presented at the following meetings and conferences:

- Internet2 Conference, March 28-29, 2000
- DREAMS On-Site Visit, Texas A&M University, May 4, 2000
- ATA Annual Meeting, May 21-24, 2000
- Texas EMS Conference, November 19-22, 2000

## **KEY RESEARCH ACCOMPLISHMENTS**

- A working prototype systems have been developed, including
  - Physician's Workstation
  - Interact Ambulance
  - Intelligent Communications Manager
- Identified site for initial field testing
- Initiated field test preparations
  - Communications studies planned
  - End-user workshops conducted

## **REPORTABLE OUTCOMES**

- Draft Schemas for Hospital Database
- Mechanical drawings for standard ambulance
- Mechanical drawings for digital ambulance
- Power/environmental monitoring system design
- ICM System Architecture

## **Detection and Remediation of Chemical Threat Agents**

An experienced team of research scientists at Texas A&M University provides support for the development of new chemical and biochemical technologies that will permit on-site detection and remediation of chemical contamination associated with the presence of chemical threat agents. In particular, this program develops the collaboration of three research groups, which already have extensive experience with the detection and detoxification of chemical neurotoxins and pesticides. Ongoing research studies in the laboratories of Drs. Richard M. Crooks (Chemistry, TAMU), James R. Wild (Biochemistry, TAMU), and Frank M. Raushel (Chemistry/Biochemistry, TAMU) have developed and demonstrated prototype detectors, both chemical and enzyme-based biosensors, for the detection of chemical toxins, which include organophosphate neurotoxins such as agricultural pesticides and chemical warfare agents. In addition, extensive characterization of the broad-spectrum organophosphate has been ongoing in our laboratories since 1987 (Raushel and Wild; over 60 publications). Finally, we have demonstrated that it is possible to utilize this enzyme in a variety of applications, which should be able to protect and decontaminate both patients and medical personnel, sites, and equipment. (Wild: Hong et al. 1998; diSioudi et al. 1998; LeJune et al. 1997; 1998; Xu et al. 1996; Pei et al, 1994) (Raushel: Tuovinen et al, 1994, 1996; Pei et al.).

Following is a summary, by task, of the research activities of the DREAMS Detection and Remediation of Chemical Threat Agents subproject.

### **Task 1: Determine the Catalytic Limits for the Existing Organophosphate (OP) Hydrolases**

The investigators have expanded a large collection of native and mutant enzymes whose kinetic characteristics will be evaluated with a library of OPs with a full range of molecular constituents attached to the phosphate core.

- A better understanding of the structure of the active center of the enzyme has been developed by the evaluation of relative catalytic rates of native enzymes toward different stereo-isomers of Soman and Sarin analogues
- The catalytic limits of the existing enzymes have been expanded in animal model studies which have demonstrated that rats can be protected against over 400 x LD<sub>50</sub> paraoxon (a neurotoxic agricultural pesticide) and multiple fold resistances against CW surrogates (e.g. Demeton-S) in a collaborative toxicology study with Dr. Mark Bitensky (Boston University)
- A wide range of OP hydrolases have been evaluated by new structural genomic comparisons and additional enzymes with sequence similarities but lacking functional similarities (the "Phosphotriesterase Family") as well structural non-identical, but functionally related enzymes are being included in materials evaluation.

### **Task 2: Investigate Mutation of Individual Residues and Creation of Rational Combinations**

Individual amino acids have been chosen to be changed in ways proposed to change their chemical nature in a rational attempt to modify the catalytic activity of the enzymes to alter their catalytic rate profiles.

- Enzymes with modified characteristics toward chemical warfare neurotoxins, particularly with enhanced detoxification of Soman and Sarin, as well as VX and R-VX, have been genetically constructed by a series of sequential modifications. Select changes have resulted in a multiple-fold increases (or decreases - which are not of application interests for this project) in hydrolysis rates against various CW agents and their surrogates.
- There is often a trade-off in stability of the OPH dimers when the active sites of the enzyme are altered in order to enhance catalytic efficiencies. The free energy required to denature the active dimers has been observed to drop as much as 50-75% which may affect certain protection applications.

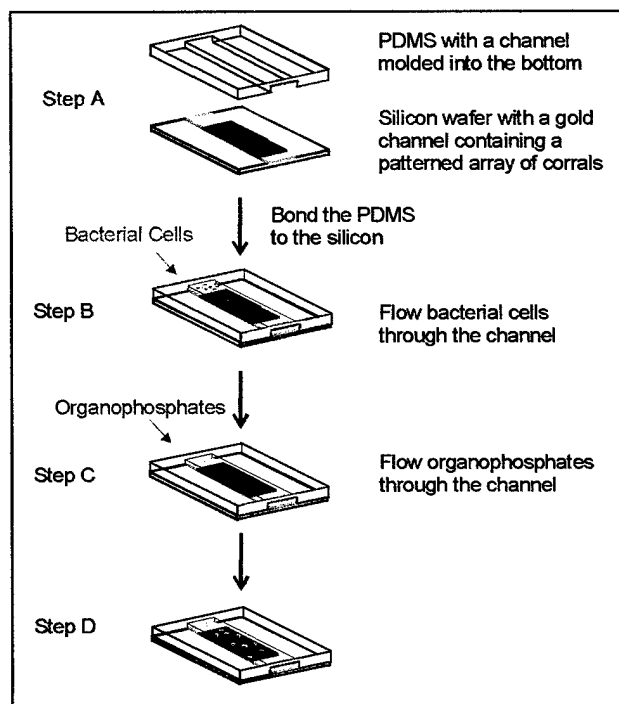
### **Task 3: Investigate Combinatorial Mutagenesis of Amino Acids Affecting the Active Site of the Enzyme**

In order to identify unpredictable structural changes that could lead to the enhancement of V-agent destruction and/or protein stability, novel combinatorial, mutagenic approaches are being developed to create and select semi-random mutations in the enzyme.

- A collection of recombinant, randomly mutagenized genes directed toward expanded catalytic hydrolysis for VX is in the process of being developed.

It will be necessary to adapt existing assays for robotic screening of thousands of genetic variants.

- A procedure for the screening of randomly mutagenized recombinant genes for the production environmentally-stable proteins has been developed for in vivo selection with varying concentrations of denaturant in gels.



Flow cell design.

#### Task 4: Introduce the Best Currently Existing Enzymes into Existing Biosensor Detectors

Enzyme-based biosensors will be constructed to utilize an array of genetically modified enzymes which will allow discrimination between chemical warfare agents and other chemical neurotoxins.

- It has been possible to develop patterned surface arrays for the attachment of living cells in manipulable “corrals” which serve as biological markers for the presence of chemical (and potentially biological) toxins.
- An important step toward developing a sensitive and robust sensor based on proton release upon enzymatic hydrolysis has been accomplished by the use of a pH-sensitive fluorophore incorporated into hyperbranched polymer films.
- It has been possible to incorporate several different enzymes into amperometric enzyme-based “FETs” and

provide for the discriminative detection of G-agents by one enzyme (OPAA which is specific for phosphonofluoridates) compared to another enzyme (OPH which has broad substrate specificities).

- The sensitivity of biosensor recognition has been evaluated relative to the minimal limits of stochastic variation which is anticipated relative to the rates of chemical turnover. It is important to consider the accuracy with low levels of poorly hydrolyzed substrates (e.g. VX or RVX)

**Task 5: Select and Purify Mutants with Specifically Desired Catalytic Characteristics** Recombinant proteins will be screened and selectively purified. Their ability to detoxify G-agent and V-agent surrogates will be determined by the use of novel micro-total screening systems which are expected to allow for the optical screening of thousands of recombinant enzymes.

- Selected Enzymes have been synthesized and in the process of purification and characterization.
- New genes are being synthesized and their DNA sequences are being verified.
- A large number of enzymes are available for evaluation with actual chemical agents; however, it has been difficult to get surety agent data due to backlogs at DOD collaborators.

**Task 6: Introduce the New Genetically Engineered Proteins into New Biological/Chemical Sensors**

Technical Milestones 4 and 5 (above) will come together to produce a new generation of discriminating detectors that are designed for stability and environmental tolerance as well as chemical agent sensitivity.

Enzymes are available for the introduction into biosensors but the complex arrays are just being developed.

**Task 7: Introduce Genetically Designed Enzymes into Decontamination Applications**

The new class of enzymes developed in Task 4 (above) will be introduced into a variety of on-going application studies, including the formation of chemical decontamination wipes, in the formulation of chemical decontamination solutions, in enzyme-based protection filters, in bioreactors, etc. characterization.

The application modifications are being developed and included in several of the applied enzyme functions. Collaboration with LynnTech Inc. has resulted in the development of enzyme-active surgical swabs.

**Key Research Accomplishments**

- Enzymes have been shown to effectively protect animals from OP neurotoxicity
- The existing enzymes have varying activities for stereo-isomers of Sarin and Soman
- Other native enzymes are being evaluated in parallel to OP
- New genetically modified enzymes have been made
- There is a trade-off in stability and enzymatic capabilities
- Procedures to screen new combinatorial libraries are being developed
- An effective environmental screening procedure for enzyme stability has been directed
- Patterned surface arrays have been synthesized
- New pH sensitive fluorescent detectors have been developed

- The discriminating capacity of enzyme biosensors for G-agents has been established
- Select enzymes have been purified and are ready for evaluation with CW agents
- Select enzymes have developed for establishing a discriminating biosensor array
- Enzyme-based decontaminating surgical gauze materials have been developed
- Enzyme has been shown to attach to human and animal skins
- Enzyme-based fogging systems have been effective in surrogate decontamination
- Patterned array of corrals
- Grow *E. coli* on patterned surface
- Fabricate smaller corrals for patterning bacteria
- Design new stamp
- Developed a new method to control and direct cell growth using photoacid patterning.
- Showed that the patterned hyperbranched polymer surfaces can be addressed individually due to the unique chemical composition of each region.
- Detected pH changes in bulk solution via pH-sensitive fluorophore incorporated in the hyperbranched polymer films.
- Achieved micro-contact printing on plastic
- Successfully imaged micro patterned plastic surfaces
- Performed controlled cell growth on patterned plastic surfaces

## Reportable Outcomes

1. "Poly(ethylene glycol) Hydrogel-encapsulated Fluorophore-Enzyme Conjugates for Direct Detection of Organophosphorus Neurotoxins. 2001. Russell, R.J., M.V. Pishko, A.L. Sinoomina, and J.R. Wild. 2001. *Analytical Chemistry* 71:4909-4912.
2. "An Enzyme-based Biosensor for the Direct Detection of G-type Neurotoxins." (2001) A.L. Simonian, J.K. Grimsley, A.W. Flounders, J. S. Schoeniger, Tu-Chen Cheng, J.J. DeFrank, and J. R. Wild. *Biosensors and Bioelectronics* 16:69-72.
3. "A Novel, Enzyme-based Method for the Wound-Surface Removal and Decontamination of Organophosphorus Nerve Agents." 2001. J.K. Grimsley, W.P. Singh, J.R. Wild, and A. Giletto. In Bioactive Fibers and Polymers. American Chemistry Society. 35-49.
4. "The Influence of External Environment Fluctuations on the Signal Formation of Microbiosensors." 2001. V.B. Arakelian, J.R. Wild, and A.L. Simonian. *Biosensors and Bioelectronics*, 16.:69-72.
5. Enhanced-Rate Biodegradation of Organophosphate Neurotoxins by Immobilized Non-Growing Bacteria. 2001. J-W Kim, E.I. Rainina, W. W. Mulbry, C.R. Engler, and J.R. Wild. *Biotechnology*, In Press.

6. "Microbial Biosensors Based on Potentiometric Detection." 2000. A.L. Simonian, E.K. Rainina, and J.R. Wild, Enzyme and Microbial Biosensors: Techniques and Protocols. Methods in Biotechnology, Humana Press. Eds. A. Mulchandani and K.R. Rogers. 6:237-248.
7. "Utility of OPH Enzyme for the Remediation of Mutagenicity of Methyl Parathion." 2001. T-H Cho, J.R. Wild, and K.C. Donnelly. Environ. Toxicology and Chemistry.
8. "Ancillary Function of Housekeeping Enzymes: Fortuitous Degradation of Environmental Contaminants." 2000. R. Shane Gold, Melinda E. Wales, Janet K. Grimsley, and James R. Wild. Enzymes in Heteroatom Chemistry. NATO\_ASI Publication, p263-286.
9. "Active Site Modifications of OP Hydrolase for Improved Detoxification of OP Neurotoxins." 2000. J.K. Grimsley, B. di Sioudi, T.R. Holton, J.C. Sacchettini, and J.R. Wild. Enzymes in Heteroatom Chemistry. NATO\_ASI Publication, p 223-242.
10. "Stereoselective Detoxification of Chiral Sarin and Soman Analogs by Phosphotriesterase." (2001). W. -S. Li, K. T. Lum, M. Chen-Goodspeed, M. A. Sogorb, and F. M. Raushel. *Bioorganic & Medicinal Chemistry*, 9, 2083-2091.
11. "Stereochemical Specificity of Organophosphorus Acid Anhydrolase Toward *p*-Nitrophenyl Analogs of Soman and Sarin." (2001). C. M. Hill, W.-S. Li, T.-C. Cheng, J. J. DeFrank, and F. M. Raushel. *Bioorganic Chemistry* 29, 27-35.
12. "Substrate and Stereochemical Specificity of the Organophosphorus Acid Anhydrolase from *Alteromonas* sp. JD6.5 Toward *p*-Nitrophenyl Phosphotriesters" (2000). C. Hill, W. Lu, T. Chen., J. DeFrank, and F. M. Raushel. *Bioorganic and Medicinal Chemistry Letters*, 10, 1285-1288.
13. "Growth of Mammalian Cells on Micropatterned Surfaces of Weak-Acid, Polyelectrolyte Hyperbranched Thin Films on Gold." 2001. M. L. Amirpour; P. Ghosh; W. M. Lackowski; R. M. Crooks; M. V. Pishko *Anal. Chem.* 73, 1560-1566.
14. "Two New Approaches for Patterning Polymer Films using Templates Prepared by Micro-Contact Printing." 2001. P. Ghosh; W. M. Lackowski; R. M. Crooks *Macromolecules*. 34, 1230-1236.
15. "Biosensors Based on Arrays of Living Cells." Gordon Research Conference on Microfluidics (Oxford, UK, July, 2001).
16. "A General Approach for High Throughput Screening of Mutant Enzymes for Remediation of Chemical and Biological Agents Using Arrays of Living Cells." Army Research Office Agent Water Monitors Workshop (Aberdeen Proving Grounds, MD, August, 2000).
17. "Patterned Arrays of Cells." American Chemical Society National Meeting (Washington, DC, August, 2000).
18. "A Simple Approach for Preparing Patterned, Micron Scale Corrals for Controlling Cell Growth." American Chemical Society National Meeting (San Francisco, CA, March, 2000).

## CONCLUSIONS

The ultimate vision of the DREAMS program is the integration of both medical and telecommunications advancements into a system of medical protocols and engineered capabilities that will facilitate the administration of lifesaving care sooner and keep people alive. Those people may be citizens in our communities, soldiers on a battlefield or people in remote nations who are in need of military and medical assistance. Regardless of the venue, the challenge remains one of time and distance. The convergence of the three DREAMS research projects—Digital EMS, Detection & Remediation, and Science, Triage and Treatment (STAT)—will result in the realization of the vision.

We continue to make steady progress. This report focuses on the Digital EMS and Detection and Remediation projects, but the coordination with UT Health Science Center at Houston continues. As medical advancements developed in the STAT project mature, they will be evaluated for their potential relevance to emergency medical support and integrated into systems as appropriate.

Likewise, developments resulting from the Detection & Remediation of Chemical Threat Agents subproject will be integrated into the Digital ambulance or similar system as the program evolves. We have prepared arrays of viable bacterial cells within microfluidic devices. As we continue to develop this technology, these devices will be shown to be useful for rapidly screening new biological agents (including enzymes useful for detoxifying hazardous materials) and for sensing chemical and biological agents in the environment. Potential advantages of the approach include the following factors. (1) Because microfluidic devices can be multifunctional, it will be possible to expand the versatility of the present microfluidic systems to incorporate additional functions. (2) These systems are light-weight and require little power, so they should be easily adapted to portable applications. (3) The approach is quite general, so that once perfected for one application, it should be easily expandable to others.

### Challenges

The challenges associated with the DREAMS vision are many. The Digital EMS project seeks to provide telemedicine capabilities on board a mobile ambulance in a rural environment. The dependence upon wireless communications is obvious. The availability of wireless connectivity is often scarce. This combination of circumstances provides the basis upon which the Digital EMS research began and continues to present the greatest challenge to the research team. The most significant challenges to the Digital EMS project are:

**Science, Triage and Treatment (STAT)**

Study methods to speed diagnosis, apply cardiac treatments, test methods to limit reperfusion

**Detection & Remediation**

Biochemical analysis, biosensor construction, development and integration of detection system

**Digital EMS**

Systems engineering and integration, telecommunications, protocol development

**THE RESULT:**

**An integrated system of devices and protocols that enhance the survivability of traumatic injury.**

The DREAMS Vision: A convergence of medical and engineering systems that will save lives.

- Managing high-bandwidth applications in a wireless environment
- Providing a common user interface while integrating many systems
- Human subjects approval
  - DOD-specific requirements
  - DOD/local human subjects requirements
- Lack of standard medical protocols
- Lack of support for multimedia (video, voice, etc.) in electronic medical records
- HIPPA compliance

These challenges present research opportunities and shape the technical approach to Digital EMS project. While keeping a constant eye on the status of pending human use legislation, DREAMS

researchers continue to make the transition from functional prototype to field ready ambulance. Specifically, the project team is preparing to deploy field tests in Liberty County, TX in partnership with Liberty County EMS. The magnitude of the technical effort required to transition from laboratory/demonstration capability is significant. However, the process is well underway. Near-term activities specific to the operational test deployment include a telecommunications survey, which will provide insight into the wireless communications infrastructure available in the test area. In addition, training sessions for emergency medical personnel will be held and the software system will be refined to support site-specific requirements.

## REFERENCES

- Voit, B. *J. Polym. Sci.* **2000**, *38*, 2505-2525.
- Zhou, Y.; Bruening, M. L.; Bergbreiter, D. E.; Crooks, R. M.; Wells, M. *J. Am. Chem. Soc.* **1996**, *118*, 3773-3774.
- Zhou, Y.; Bruening, M. L.; Liu, Y.; Crooks, R. M.; Bergbreiter, D. E. *Langmuir* **1996**, *12*, 5519-5521.
- Bruening, M. L.; Zhou, Y.; Aguilar, G.; Agee, R.; Bergbreiter, D. E.; Crooks, R. M. *Langmuir* **1997**, *13*, 770-778.
- Lackowski, W. M.; Ghosh, P.; Crooks, R. M. *J. Am. Chem. Soc.* **1999**, *121*, 1419-1420.
- Aoki, A.; Ghosh, P.; Crooks, R. M. *Langmuir* **1999**, *15*, 7418-7421.
- Ghosh, P.; Amirpour, M. L.; Lackowski, W. M.; Pishko, M. V.; Crooks, R. M. *Angew. Chem. Int. Ed.* **1999**, *38*, 1592-1595.
- M. L. Amirpour; P. Ghosh; W. M. Lackowski; R. M. Crooks; M. V. Pishko *Anal. Chem.* **2001**, *73*, 1560-1566.