

MicroSpy Biochip

Group # 7

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Abstract

In current hospital practice, when a newborn infant is noted to have symptoms of fever or infection, doctors immediately prescribe a cocktail of antibiotics to be administered. Simultaneously, a blood sample is taken from the infant to be cultured to determine any bacterial infections in serum. This culturing process takes an average of seven days. During this time, the infant is continuously pumped full of broad-spectrum antibiotics. Practices such as this have rapidly increased genetic mutations in pathogenic bacteria rendering it immune to further antibiotic treatment. This has given rise to the global phenomena of Antibiotic-Resistant Bacterial Agents (ARBA).

We propose engineering a biochip based on a protein-array format to be able to determine bacterial presence. Such a format would reduce delay time from 7 days to 2 hours, thus saving on excess therapy that only damages the patient and shortens lifespan of antibiotics. The proposed biochip would be comprised of nanohydrogels of Poly-Ethylene Glycol (PEG). Building on such a foundation would be a layer of biomarkers such as the surface proteins expressed by the target bacteria. Mode of operation would be somewhat analogous to current day glucose-meters.

[II-1] INTRODUCTION

Staphylococci are bacteria that are ubiquitous colonizers of human or animal skin and mucous membranes causing a variety of syndromes. *Staphylococcus aureus* can cause nosocomial and community-acquired infections ranging from mild conditions, such as skin and soft tissue infections, to severe, life-threatening sepsis (Lowy, 1998). *Staphylococcus aureus* is the staphylococcal species most commonly associated with human (nosocomial and community-acquired) and animal infections. The strains belonging to this species also cause food poisoning. They are routinely characterized by growth properties, specific surface constituents and their ability to coagulate blood plasma from various sources (staphylocoagulase), to produce a thermostable nuclease (TNase) and to form clumps in the presence of fibrinogen (clumping factor). These properties are shared by strains belonging to other species such as *S. intermedius*, *S. caprae*, *S. simulans* and *S. capitis*, (Kloos and Lambe, 1991) and consequently there is a need for methods to specifically discriminate *S. aureus* from other staphylococci as quickly as possible (Roberson et al 1992 and Guzman et al 1992).

Conventional identification methods are time-consuming and may yield false-positive or false-negative results, and misclassifications with automated susceptibility testing systems or commercially available latex agglutination kits have been reported recently. (Ribeiro et al 1999; Ruane et al 1986; Schwarzkopf et al 1993 and Wilkerson et al 1997). Most modern day detection schemes employ techniques of Polymerase Chain Reaction (PCR) as a detection process wherein the bacteria samples taken from clinical isolates are cultured via PCR (where the volume of the protein marks for *S. aureus* increase at a

doubling rate of every eight hours). Though this technique is faster than practices of culturing *S. aureus* using Agar plates (a substrate that promotes growth of bacteria by providing needed nutrients), it still requires medicinal treatment of people suspect for inspection. In current practices, PCR enabled technologies have a culture-to-result timescale of 3-4 days. During this time the patient needs to be doctored with numerous antibiotic concoctions in order to restrict growth of microbial entities. These antibiotics tend to be broad-spectrum drugs that are intended to kill any and all microbial life. However, repeated and prolonged use of these drugs reduces their ability of fighting these very microbes in the future. The human immune system, after a certain threshold of antibiotic exposure, is no longer able to protect against infections. Also, this results in a decrease of operational lifespan for the antibiotic drug.

The group intends to create a device that will serve as a lab-on-a-chip (LOC) for the rapid detection of *S. aureus* in a microarray format from human blood samples. A microarray format as opposed to PCR provides a platform with higher sensitivity. This could lead a fast detection time – on the order of a couple of hours – which would mean not having to expose patients to broad spectrum antibiotics. Also, since we envision the device to be built using LOC principles, the device would ideally make testing for *S. aureus* infections a household task. In a nutshell, it would act as a fast, cheap and, most importantly, a reliable test for answering the question of *S. aureus* infection in a binary manner of yes or no. Employing a generic microarray format also lends this project to be expandable. In the future, a cascaded series of questions could be asked such as determining sub-classes of *S. aureus*, strains resistant to specific antibiotics and testing affectivity of newer drugs.

[II-2] DESIGN REQUIREMENTS

The project must meet certain design objectives. The team would like to use the following objectives as guideline for measuring the success of the project.

a) Objectives:

- System must be able to be contained on a standard microscope glass slide
- Completed Lab-on-a-Chip must be compatible with existing microarray scanners
- LOC must provide easy system for inserting and disseminating clinical blood samples
- Soft-lithographically created polymer casing should provide correct sample loading.
- Valving control must be optimum for a fast bioassay.
- Must be easy to use since end-user may not be a trained professional.
- Must be a non-toxic and non-pathogenic.
- Must be in an easy to package form that will allow large customer release and also maintain viability after long shelf storage.

b) Performance criteria matrix:

The following matrix shall be used to evaluate the performance of the system. The met and failed column will be populated according to the results from the testing phase. However, the table below outlines the key factors that we feel that are crucial to the design.

Prototype expectations	Met	Failed
Sensitivity to 10^3 cfu of S.aureus		
Correct fluorescent signaling		
Correct test controls: positive / negative		
Must perform in -10 to 38 celcius (In order to deploy with first-response EMT teams that		

face harsh outdoor weathers)		
Clinical correctness / success percentage of >85%		

[II – 3] DESIGN APPROACHES

The primary design of the LOC-device will have 3 distinct phases: (1) Surface treatment to convert the initial device substrate into a feasible and biocompatible entity (2) Creating/ Patterning structures on this substrate suitable for capturing *S.aureus*. (3) Detecting the captured bacteria in a fluorescent manner that provides high signal-to-noise ratio (SNR). Of primary importance is step #2 as the success of the project hinges on this parameter. Therefore, the group did most extensive research on it than other requirements. We propose to work with an FDA-approved polymer – Poly Ethylene Glycol [PEG] - whose use is well established in the field of bioengineering. It is well established in the micro-devices industry that patterning invokes the ability to structure a material in two or three dimensions at various length scales. Patterning of PEG at length scales of nanometers and microns further affords the opportunity to control interactions with specific proteins and with individual cells (Voldman, 1999) and there has been tremendous development of patterned hydrogels and PEGylated surfaces over the past decade in the context of both device fabrication and *in vitro* models of cell-material interactions.

Perhaps most well known among surface-patterning methods used for bio-related applications is soft lithography or contact printing (Prime, 1993). Patterned elastomeric stamps can ink a surface with particular cell-adhesive oligomers. Often the surfaces are gold coated, and the adhesive oligomers are thiolated. Cell adhesion to surface regions

not directly modified by the stamping process can then be blocked using PEGylated thiols.

New methods for patterning using thin-film hydrogels have recently been proposed (Liu, 2002; Mellott, 2001; Nguyen, 2002; Revzin, 2003). These take advantage of the fact that photolithographic methods can be combined with the photopolymerization of difunctional molecules like PEG diacrylate. Thin films of functional PEG macromer can be patterned by selective exposure to UV light through a photomask. Unexposed regions can subsequently be washed away using a good solvent for PEG leaving behind patterns of crosslinked PEG hydrogel. This technique can be employed in high throughput screenings and clinical diagnostics, interfacing biomedical microsystems, and for studying interactions of cells with their environment in tissue engineering applications.

The minimum feature size achievable via photolithographic patterning on PEG macromeric precursor is on the order of a few microns and is ultimately limited by small-molecule diffusion in the precursor thin film. An alternate method of PEG surface patterning, which can create nanoscale hydrogel feature sizes – namely, electron-beam patterning – again borrows from the electronic-device industry. As in device-processing applications, e-beam patterning brings with it the ability to generate nanoscale features without the need of a mask. Furthermore, because energetic electrons directly create radicals, that can lead to crosslinking, patterned gels can be made from pure polymer films coated onto a substrate. E-beam processing thus brings the ability to create surface-patterned features whose structure can be modulated at length scales on the order of tens of nanometers up to tens/hundreds of micrometers. This is particularly significant for applications that require spatial control of small groups of proteins, whose characteristic dimensions are typically on the order of ten nanometers or more, as well as for bacteria, which have characteristic dimensions on the order of one micrometer.

[II-4] FINANCIAL BUDGET

Budget					
Item	Description		\$/part	Amnt Needed	Total
Monitor	a used monitor		\$50.00	1	\$50.00
RAM	184 Pin 512MB DDR		\$48.00	1	\$48.00
Hard-drive	120 GB drive		\$90.00	1	\$90.00
RS32 cables			\$15.00	4	\$60.00
NI DAC	National Instrument		\$200.00	1	\$200.00
Switch	A Port Switch		\$50.00	1	\$50.00
Software			\$100.00	1	\$100.00
PEG polymer	5g of PEG		\$93	1	100
Antibodies	Antibodies for S.Aureus detection		\$128	1	\$128
Pluronics	Surfactant for chip treatment		FREE	1	0
			FREE		
			(provided by		
			sponsor)		
S.Aureus samples	Bacteria for actual trials			-	0
				TOTAL=	\$826.00

[III] CONCLUSION

Current day techniques used in hospitals for identification of *Staphylococcus aureus* are time-consuming and often incorrect. Most modern day detection schemes employ techniques of Polymerase Chain Reaction (PCR), a process that itself requires several hours of lab-time thus making rapid detection impossible. In current practices, PCR enabled technologies have a culture-to-result timescale of 3-4 days, which still leaves suspected patients of *Staphylococcus aureus* to be doctored with numerous antibiotic

concoctions in order to restrict growth of microbial entities. These antibiotics tend to be broad-spectrum drugs that are intended to kill any and all microbial life. Repeated and prolonged use of these drugs reduces efficacy and life-span of these drugs as well as numerous medical side-effects in patients.

We propose a device that will serve as a lab-on-a-chip (LOC) for the rapid detection of *S. aureus* in a microarray format from human blood samples. Prototyping will be performed using techniques of surface modification, surface patterning and fluorescent detection. Viability of the device will be answered via trials using *Staphylococcus aureus* samples from clinical isolates. The proposed format will provide high sensitivity and fast detection while keeping costs low. It will be efficient as a reliable test for answering the question of *S. aureus* infection in a binary manner of yes or no. Employing a generic microarray format also lends this project to be expandable. In the future, a cascaded series of questions could be asked such as determining sub-classes of *S. aureus*, strains resistant to specific antibiotics and testing affectivity of newer drugs.

[IV-REFERENCES]

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