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14. ABSTRACT

The goal of this project is to develop a new class of biosensors using carbon and polymer based micro-electromechanical systems (CPMEMS) and a platform that incorporates multiple bio-detection strategies. This progress report covers the development of a sense and release experiment, and further a proof of concept of actual drug release on a release experiment. Other points of progress include studies on alternate methods of developing the self assembled protein layers, specifically utilizing DNA-DNA binding to develop the biological detection method for incorporation into the CPMEMS sensor platform. Progress on the development and study of a Bio-collection system to capture airborne microorganisms and concentrate them in a water solution for detection. Progress on an alternate sacrificial layer (Unity 400) to develop the CPMEMS bridge structures will also be discussed in this report.

15. SUBJECT TERMS

CPMEMS, Unity 400 Thermal Release Polymer, Active Sense and Release, Ampicillin Drug Release, DNA-Based Sensor, Bio-Collection.

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Multiple Strategy Bio-Detection Sensor Platforms

Made from Carbon and Polymer Materials

Interim Report

ONR Award No. N00014-03-1-0893

for the work performed July 1, 2005 - Jan 31, 2006

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I. Introduction

This interim progress report will cover the progress to date on the development of a multiple strategy bio-detection sensor platform made from carbon and polymer based microelectromechanical systems (CPMEMS). Progress since the last interim report has primarily involved development of a polymer release system on a multi-microelectrode device. Work on the self-assembled protein layers has shifted to attaching oligonucleotides to a gold substrate due its high specificity, ability to readily attach to gold to form a uniform layer, and lower cost when compared to using antibodies. Other areas of progress that will be discussed in this report will include the Bio-Collection system and the sacrificial layer (Unity 400).

II. Description of Technical Research Progress

a. Active Sense and Release

i. Polymer based release system on a CPMEMS platform

Crosslink has been investigating the development of active sense and release experiments for incorporation into the broadband platform. In the last interim report, we reported the deposition of PPY/phenolred on the surface of microelectrodes fabricated on a silicon wafer and the release of phenolred into the solution when a potential was applied to the device. We also reported the deposition of PPY/Ampicillin on small carbon electrode and the release of ampicillin into the solution. In this report, we summarize progress of a polymer release system on a multi-microelectrodes device.

The design for controlled release devices on a chip is shown in Figure 1. The device contains 16 microelectrodes with four different sizes fabricated on a silicon wafer. The size of each microelectrode varies as follows: 250μm ×250μm, 500μm ×500μm, 750μm ×750μm and 1000μm ×1000μm. Gold is used as the electrode material and silicon wafer is used as the substrate. The gold pattern or microelectrode on a silicon wafer is prepared via the combination of a plasma etching and a thermal vapor deposition using a mask design to obtain the device pattern.

Figure 2 is a photograph of an actual device before and after it was deposited with PPY/Dopant. 14 of 16 microelectrodes in Figure 2(B) were coated with PPY/Phenol red on the surface. Figure 3 is a photograph of the apparatus for depositing PPY/Dopant on a microelectrode fabricated on a silicon wafer. A bi-potentiostat from GAMRY was employed for the electro-polymerization experiments and the release study.

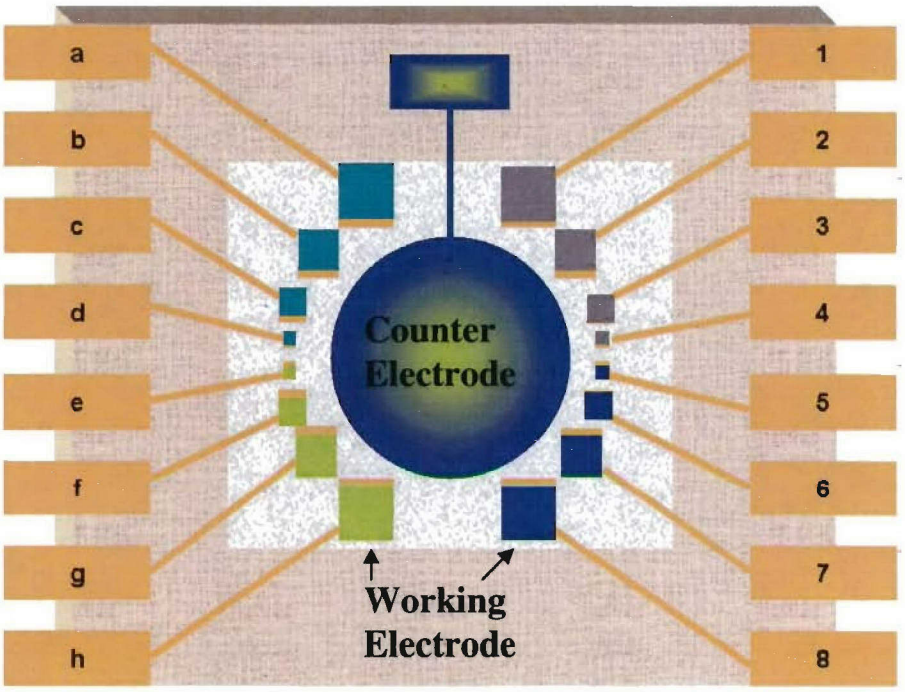
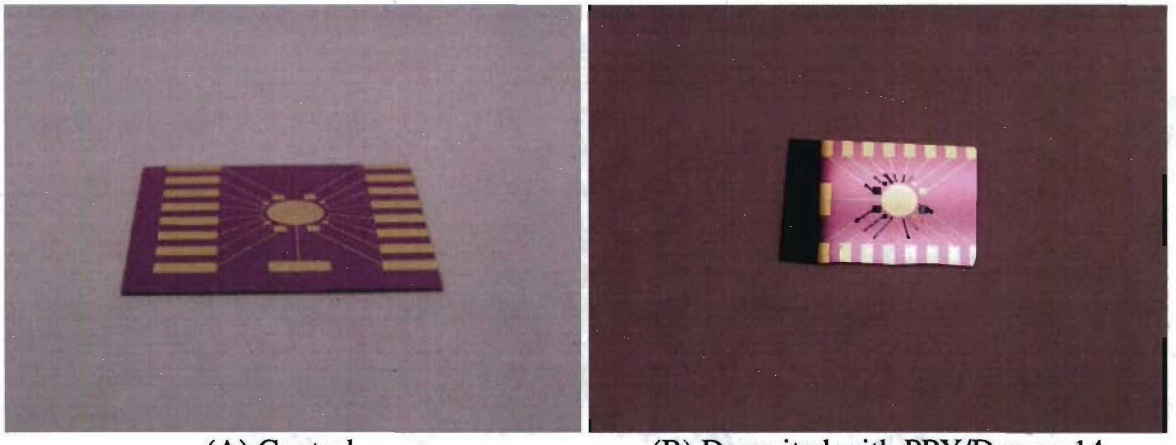


Figure 1. A Configuration of Multi-Microelectrodes on a Chip



(A) Control

(B) Deposited with PPY/Dye on 14 Microelectrodes

Figure 2. A Chip with 16 Gold Microelectrodes on a Silicon Wafer

After the multi-microelectrodes are fabricated on a silicon wafer, the properties of the electrode were tested using electrochemical techniques. Ferrocyanide is used as a probe to test the properties of the microelectrode. Thus the cyclic voltammerty was performed on individual electrodes with a scan rate of 10, 20, 40, 60 and 100 mV/S in a 0.5 M KCl aqueous solution containing 5mM Ferrocyanide. As shown in Figure 4, the

cyclic voltammograms of ferrocyanide at each Au microelectrode show the expected increase in peak current with increased electrode area.

For the cyclic voltammograms, the heights of redox peaks of ferrocyanide are proportional to the areas of the electrodes. The relation between the peak currents and the electrodes area are determined by equation (1):

$$Ip = (2.69 \times 10^5)\, n^{2/3} A Do^{1/2} Cv^{1/2} \qquad (1)$$

Where Ip reference the peak Currents (Ipa and Ipc)
n = Number of electrons transferred
A = Area of the electrode
Do = Diffusion coefficient
C = Concentration of the solution (mol/cm3)
v = Scan rate (V/s).

The effective area of the microelectrodes is calculated using the above equation. Since the surface of the microelectrode is rough, the effective size of the area is larger than that of its geometric area. Our calculated data indicated that the actual area of each electrode is 1.8~2.5 times its geometry area, which is in accordance with other studies. Figure 5 shows the linear relationship between electrode area and peak current.

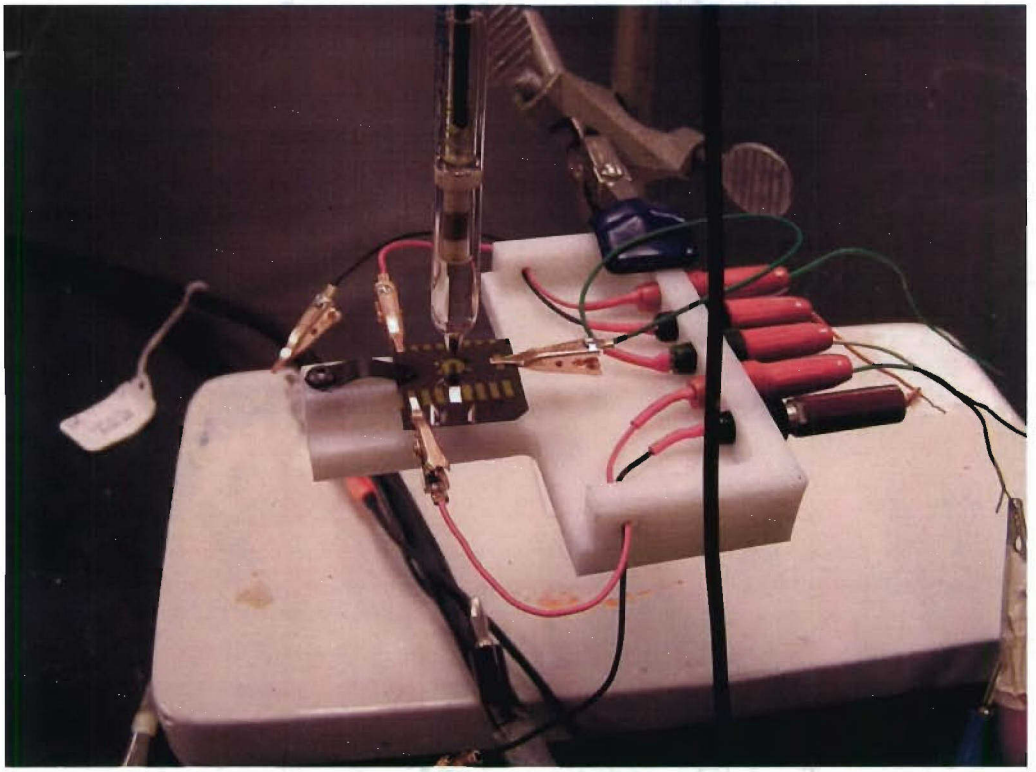


Figure 3. Apparatus for Electrochemical Experiments on the Surface of Microelectrode on the Chip

For the deposition of PPY/Dopant on the surface of each microelectrode, a freshly prepared aqueous solution containing 0.1M of pyrrole and 0.1 M of dopant was used in all the experiments. The dopants included sodium sulfate, sodium phenolred, and other dye molecules. Two three electrode systems were used for the experiments. In the first system, both the working and counter electrodes are built in the chip and an external Ag/AgCl reference electrode was applied. In the second system, one of four largest of the microelectrodes($1000 \times 1000\mu m^2$) was converted to an internal reference electrode by depositing Ag/AgCl layer on the surface of the electrode while the working and counter electrodes remain the same and this system is what we call everything is built in the chip.

Both chronoamperometry and cyclic voltammetry are used for electropolymerization. When chronoamperometry is used, the applied potential was kept at 0.8V, and the others remained constant. When cyclic voltammetry is used, the scan rate is kept at 100mV/s with a scan range from -0.4V to1.0V. The thickness or the amount of deposited PPY can be controlled by varying the cycles for cyclic voltammetry and the times for chronoamperometry.

In a typical experiment, one drop of a freshly prepared aqueous solution containing 0.1M of pyrrole and 0.1 M of dopant was added to cover the microelectrode surface, and 0.8~0.9V vs Ag/AgCl reference electrode was applied to the working electrode. After 120 seconds, electropolymerization deposition was stopped and the microelectrode was washed with DI water, followed by immersing it into a DI water bath for 10mins to remove the non-doped dye molecules on the surface. Then the chip was ready for either the release experiment or the depositing of PPY/Dopant on the other microelectrodes after removing all traces of water from the chip. In fact the PPY/dopant film can be deposited on all of the microelectrodes all at once or one by one.

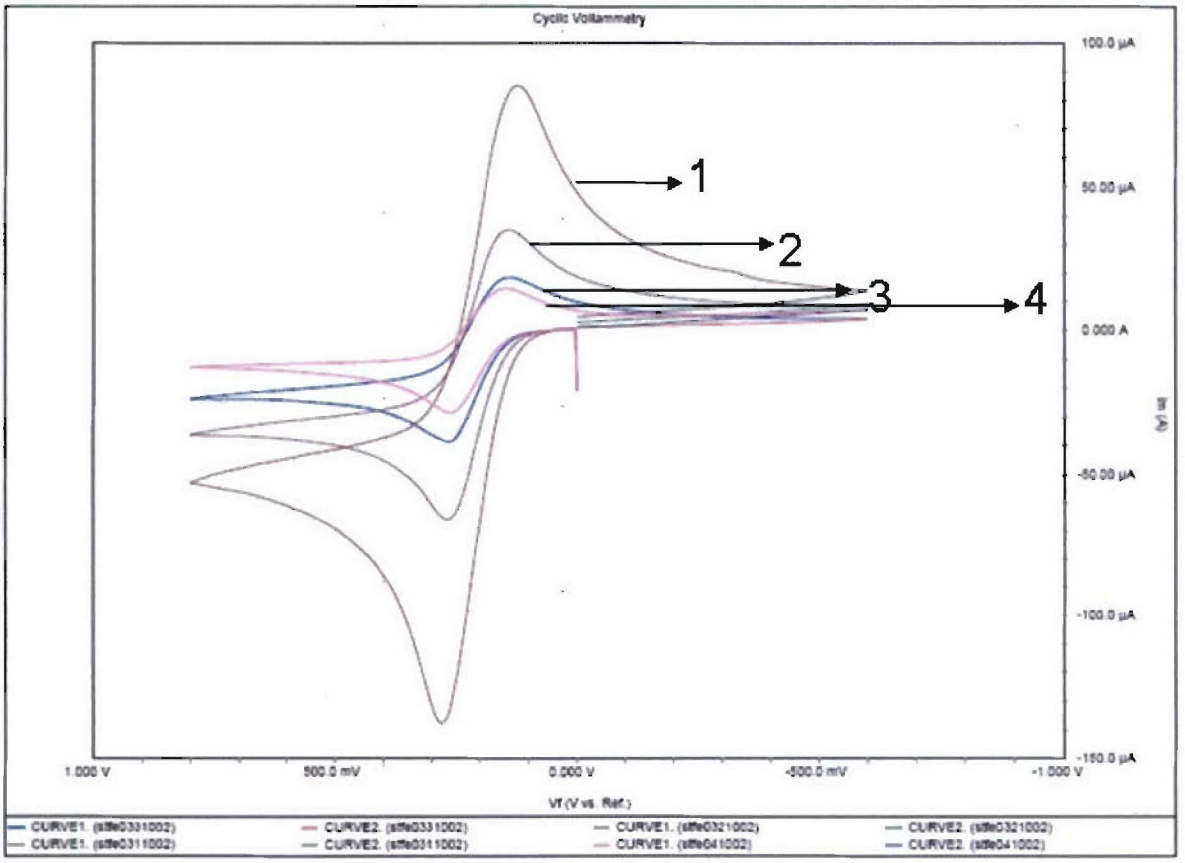


Figure 4. Cyclic Voltammograms of Ferrocyanide on Various Microelectrodes

The areas of 1, 2, 3 and 4 electrodes are 250µm ×250µm, 500µm ×500µm, 750µm ×750µm and 1000µm ×1000µm respectively.

Figure 6 shows one example where PPY/phenol red film was deposited on the surface of three microelectrodes with three different sizes. One can see that PPy/phenol red film is coated on the surface of each of the three microelectrodes and the growth of the film is on the surface of the microelectrode. The next step is to characterize PPY/phenol red film and find out how much of the dopant is incorporated into the film and can be released from the film.

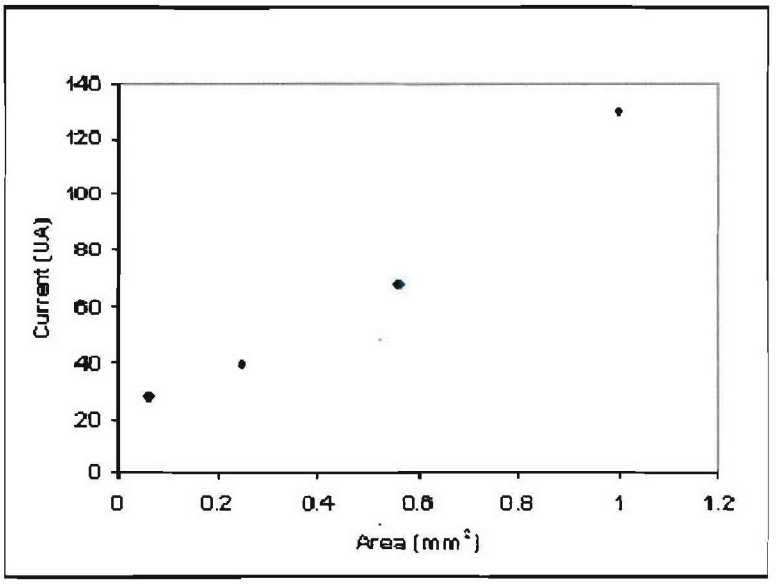


Figure 5. The Correlation between the Electrode Area and the Peak Current

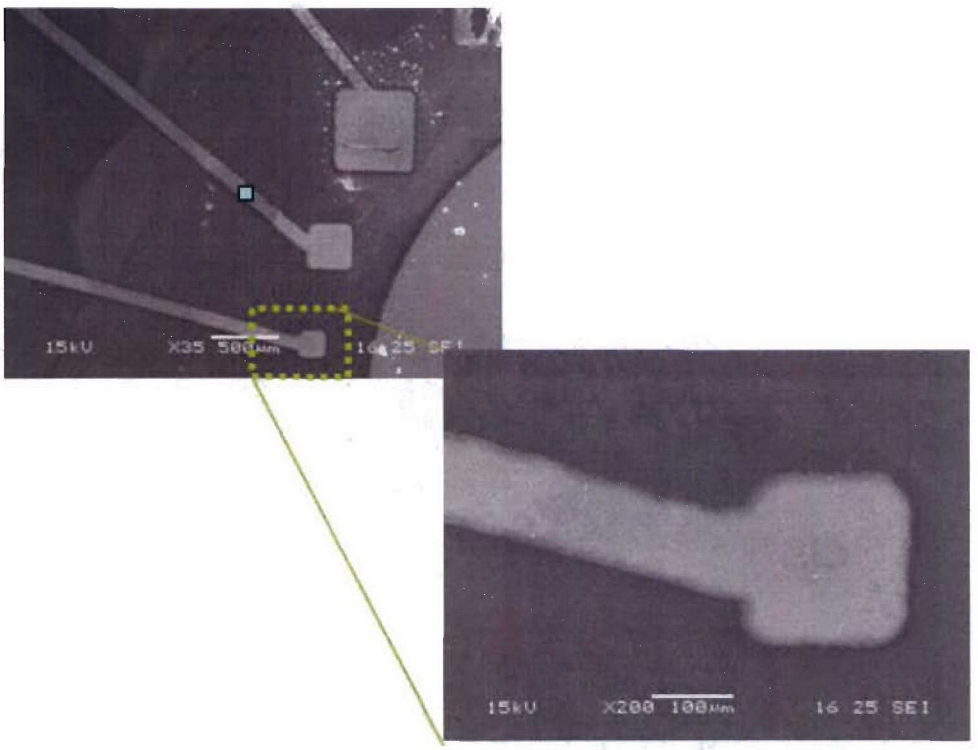


Figure 6. SEM Micrographs of the Deposition of PPY/Dye Molecule on the Surface of a selected Microelectrode

For a diffusion-controlled current, the current-time (i-t) curve is described by the Cottrell equation:

$$\mathbf{i = nFACD^{1/2}p^{-1/2}t^{-1/2}}$$

Where
n = number of electrons transferred/molecule
F = Faraday's constant (96,500 C mol^{-1})
A= electrode area (cm^2)
D = diffusion coefficient ($cm^2 s^{-1}$)
C = concentration (mol cm^{-3})

From the Cottrell equation, one can see the current passing through the electrode is proportional to the electrode area. Figure 7 shows the current-time curves obtained from electropolymerization on four microelectrodes with the electrode area varying from 250μm ×250μm to 500μm ×500μm to 750μm ×750μm to 1000μm ×1000μm. Since other factors were kept constant during polymerization, the current passed through each microelectrode is proportional to the microelectrode area.

The charge passed through each of the micro-electrodes can be integrated from the current-time (i-t) curve using a Gamry Echem Analyst program. Figure 8 displays a linear correlation between the charges passed through the microelectrode and the area of the micro-electrode. Since the deposited PPy can be calculated from the charges, we can use this method to control the film thickness as well as the amount of the dopant being incorporated into the film.

In general, the oxidative polymerization of pyrrole involves 2.0 electrons per-monomer unit, with 0.3 to 0.4 additional electrons per monomer required for partial oxidation of the film. Faraday's Law states that the number of moles of substance produced at an electrode during electrolysis is directly proportional to the number of moles of electrons transferred at that electrode. The law is demonstrated as below equation (2):

$$Q = nFN \qquad (2)$$

Where Q is the charges in coulombs, n is the charges equivalents/mole for the reaction, F is the Faraday (96486) coulombs/equivalent) and N is the number of the moles of reactant.

Pyrrole was polymerized onto electrode in the presence of counter ions, the counter ions were incorporated Polypyrrole film as a dopant. The oxidized monomer of pyrrole can be calculated from equation (3):

$$N = Q/(2.3 \times 96486) \qquad (3)$$

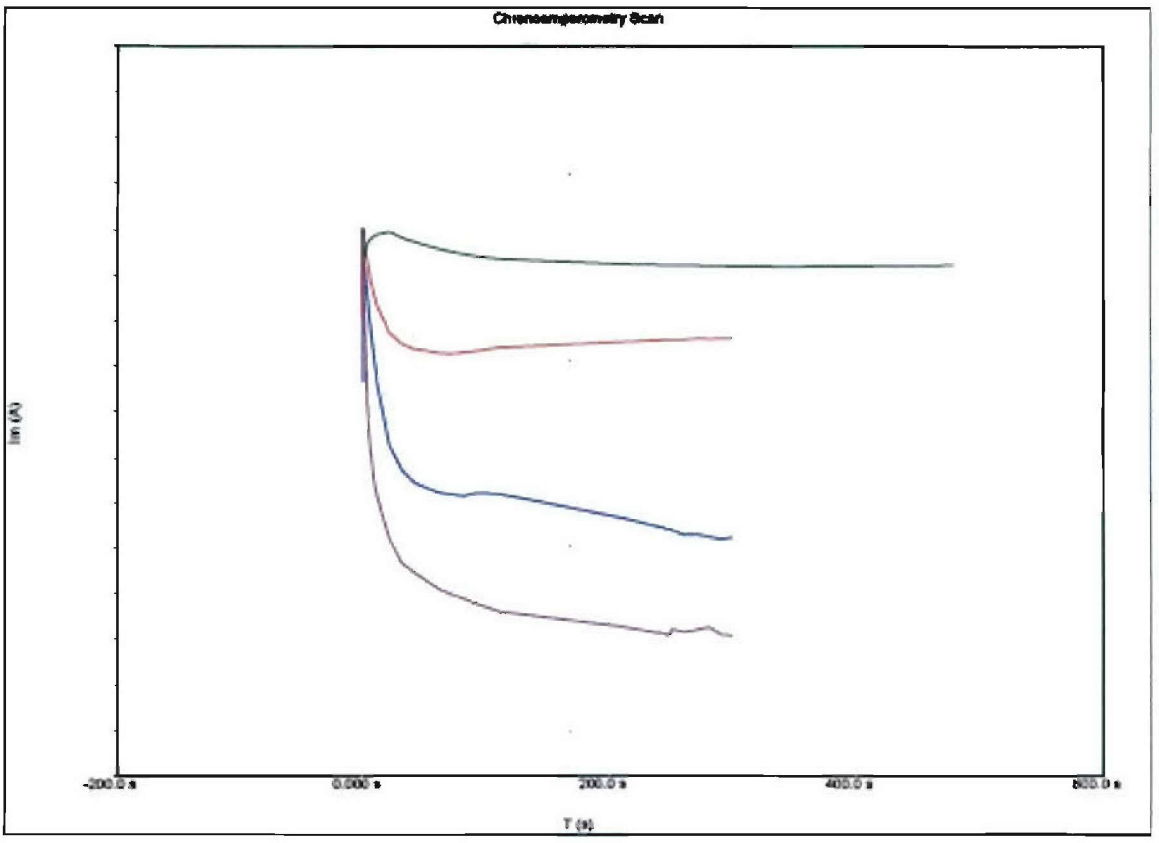


Figure 7. The Current~Time Curves of the Chronoamperometry in lectropolymerization of PY where green curve is for 250x250 μm^2, red curve is for 500x500 μm^2, blue curve is for 750x750 μm^2, and purple curve is for 1000x1000 μm^2

Q can be integrated from the current~time curves and thus the doped PPy can be calculated. There are 0.3 charge centers/one pyrrole unit, thus the amount can be calculated:

$$N_{dopant} = N \times 0.3 \qquad (4)$$

From the above equation, we can estimate the amount of dopant, and assume that all the dopants can be released where the released dopant is equal to Nx0.3. So, the theoretical released amount of dopant ($R_{thheoretical}$) can be calculated.

$$R_{thheoretical} = N_{dopant} \times 0.3 = ((Q/(2.3 \times 96486)) \times 0.3 = 1.35 \times 10^{-6} Q \qquad (5)$$

Since not all of the dopants could be released from the PPY film, the actual amount of released dopants is less than the theoretic data. The release ratio can thus be calculated from the following equation:

$$R_{ratio} = R_{actual}/R_{thheoretical} \qquad (6)$$

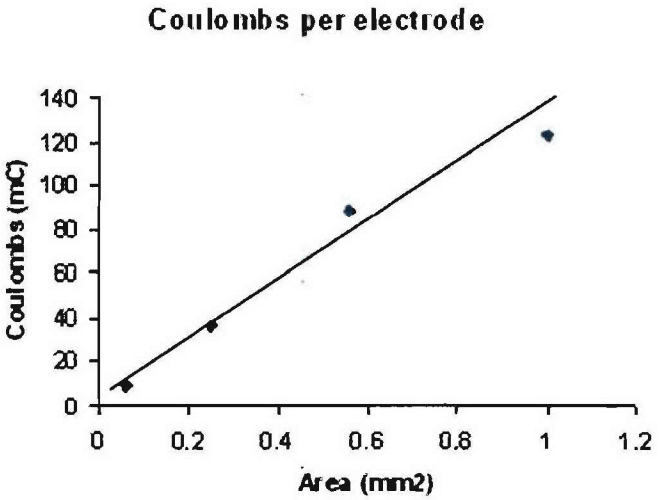


Figure 8. The Correlation between the Electrode Area and the Amount of Charges Passed Through the Electrode

In our experiments, the amount of the released dye molecules in a solution can be determined by using the combination of a Uv-vis spectrometer and the working curve and Q from equation (5) can be measured from the integration of i-t curves, R_{ratio} actually can be determined.

In one example, PPY/phenolred was deposited on the surface of the microelectrodes and the charges (Q) passed through the electrode during the polymerization was obtained from Gamry. Phenolred from the film was released into a tris-buffered solution for 60 minutes when a potential (-1.2v) was applied to the device and the amount of the released phenolred was obtained from a UV vis spectrometer and the working curve as shown in Figure 11. When the amount of the released phenolred is plotted against the charges (Q) passed through the electrode during the polymerization, a linear correlation was obtained and shown in Figure 9. This correlation can be used to estimate how much of the phenolred can be released.

The ratio of actual/theoretical released phenol red is between 0.12 and 0.25, indicated that up to 25% incorporated phenol red can be released from the PPy within 60 minutes.

One of the major goals with multi-microelectrode release device is to load different drugs on different electrodes and release each drug either separately or simultaneously. In order to prove the concept, we started to investigate three different dye molecules, which have different colors in aqueous solution. When the dye molecule is released into the solution from the microelectrode on the chip, it can be detected by not only a UV-Vis spectrometer but also through simple visual observation.

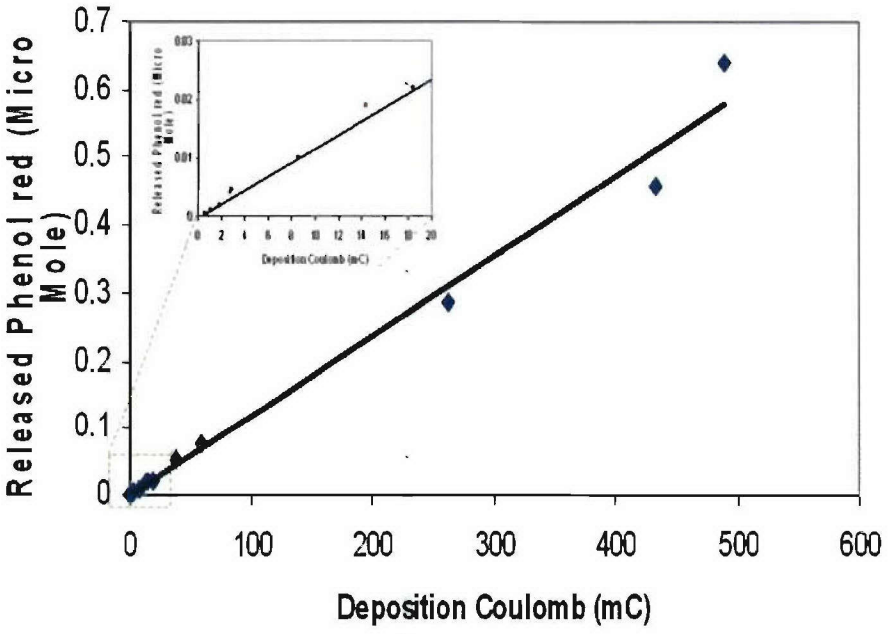
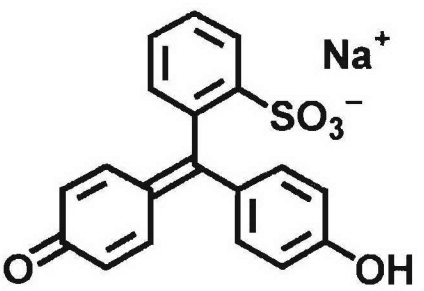


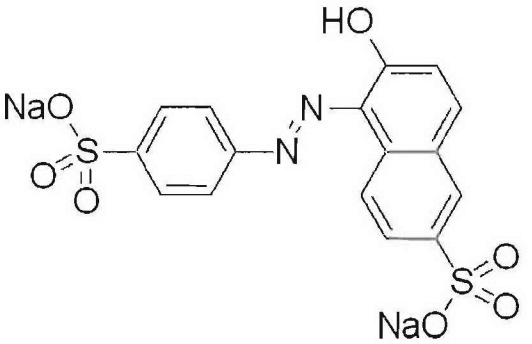
Figure 9. The Linear Correlation between the Charges (Coulomb) in Electropolymerization and the Amount of Phenolred Released in the Solution

As shown in Figure 10, each of the water soluble dye molecules has one or two sulfonate functionalities attached to the aromatic rings and thus in principle can be incorporated into the PPY film via electropolymerization. Since λ_{max} for each of three dye molecules is quite different, the color of the aqueous solution for each dye molecule is different, which varies from yellow to red to green.

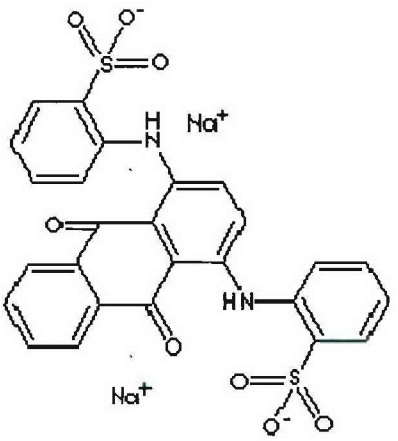
The working curves of the above three dyes molecules were obtained with a Hitachi-2100 Uv-Vis spectrometer. The solutions with various concentrations of dyes ranging from 1μM to100μM were prepared in tris-buffered solution. The peak absorbance of the solutions at λmax for each dye was recorded. Figure 11 displays three working curves of absorbance versus concentration giving a straight line passing through the origin. The amount of the released dye molecule from each microelectrode on a chip can be determined.



Phenolred
λ_{max} = 557 nm



Sunset Yellow
λ_{max} = 482 nm



Acid Green
λ_{max} = 642 nm

Figure 10. Chemical Structures of Three Dye Molecules Used in Electropolymerization

For sunset yellow, a correlation between the charges (Coulomb) passed through the microelectrode during electropolymerization and the amount of sunset yellow released in the solution was also observed. The ratio of actual/theoretic released sunset yellow is around 0.18, which indicated that 18% of incorporated sunset yellow can be released from the PPy within 60 mins. For acid green, the study is currently underway. Results from those release characterizations of the microelectrodes on the chip will help us guide the study when incorporating the drug molecules.

In our initial experiments, both PPY/phenolred and PPY/sunset yellow were coated on different microelectrodes on the same chip. The release of phenolred or sunset yellow from different microelectrodes into the same solution was observed when a potential (-1V) applied to the related microelectrode. Currently, we are working on the quantitative release of those two dyes with a UV-Vis-spectrometer.

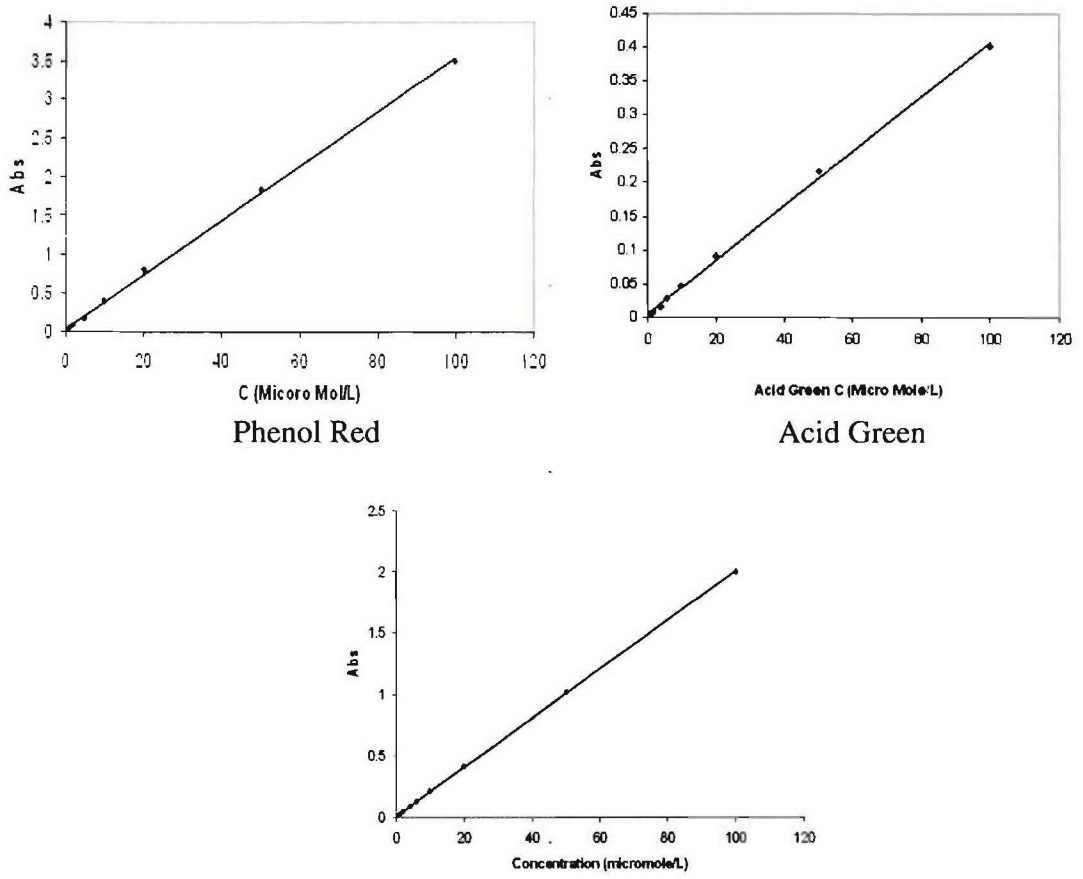


Figure 11. The Working Curves of Three Dyes used in Electropolymerization

ii. Fabrication of a Gel layer and release of dye molecules

Previous release experiments have focused on the release of dopant in aqueous solution. Although the release of phenolred as well as other dopants has been demonstrated earlier in this report, the release of the dopants in other media is unknown but may have potential applications in many fields.

Many hydrogel materials are commercially available. Currently our focus is on an alginate based hydrogel because an electrolyte is in hydrogel after the preparation. In a typical experiment, the hydrogel layer of alginate is prepared by adding 0.1M Calcium chloride solution onto wet coating of sodium alginate, followed by cooling it at -4°C for 30 minutes.

In our initial study, the PPY/Phenol red layer was deposited onto the surface of either small carbon electrodes or the Au microelectrodes on the chip via electropolymerization. Then alginate hydrogel was fabricated on the surface of the device to cover both the working and counter electrodes and the device was then ready for the

release experiment. A bipotentiostat from Gamry Instruments was used with a two or three electrode system in the release study and different potentials ranging from -1.0 to -2.5 V were applied to the device to initiate release of the dye molecule, which was monitored with an Olympus C-740 Ultrazoom digital camera.

As shown in Figure 12, the appearance of red color in the gel between the center carbon working electrode and the ring carbon counter electrode indicated that phenolred was released into the gel after a potential was applied to the device for 1 hour. Similar release of phenolred from PPY/phenol red on four of the Au microelectrodes into the gel was also demonstrated as shown in Figure 13 after the potential was applied to the device.

Compared with the released process in aqueous solution, the release process within the gel layer is slower and all released molecules remain within a small area. This is because the diffusion of released dye molecules within the gel layer is slower than that in aqueous solution.

iii. Drug release study

In our initial study, ampicillin (amp), an antibiotic, was incorporated into the PPY film on our small carbon electrode via the chronopotentiometry method where the current was kept constant. Although our previous results showed that amp can be released into the buffer solution when a potential is applied to the PPY/AMP coated electrode, we faced several difficulties including (1) making a smooth film on the electrode, (2) the loading of amp in PPY/amp film, (3) the stability of ampicillin in PPY, and (4) the release of amp in a controlled manner.

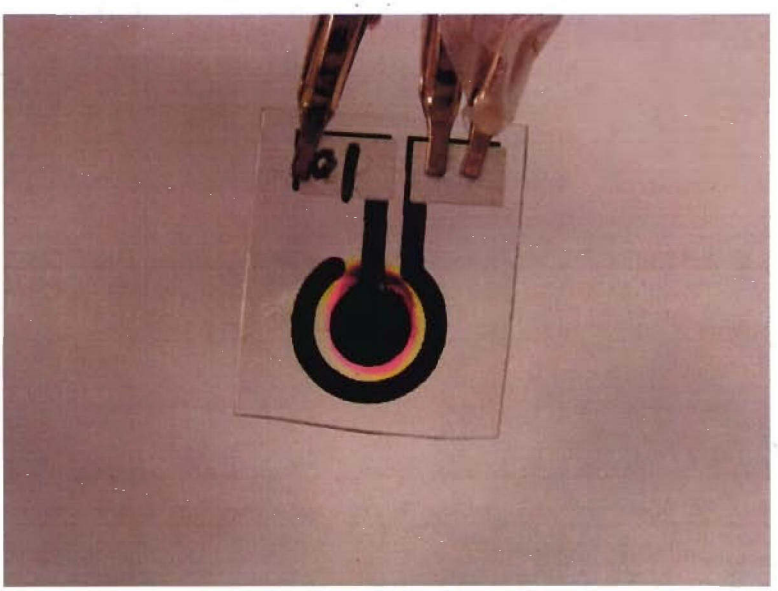


Figure 12. The Release of Phenol Red from PPY on the Surface of a Single Carbon electrode into the Gel

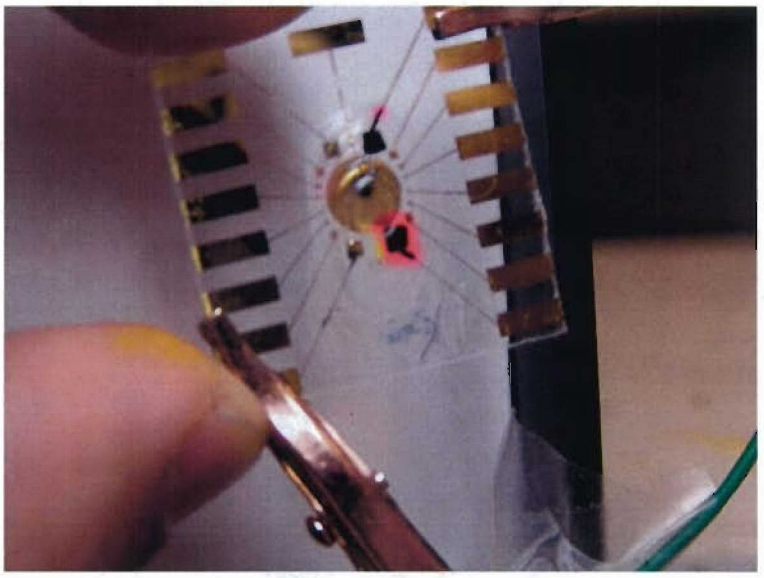


Figure 13. The Release of Phenol Red from PPY on the Multi-Electrodes into the Gel

Several methods including chronopotentiometry, chronoamperometry, and cyclic voltammatry have been used to prepare PPY/amp on the surface of small carbon electrodes. Our results showed that none of the above methods provides a smooth PPY/amp film on the surface of the carbon electrode.

We also continued our effort to establish a protocol to well detect the released ampicilin in a buffered solution. Initially tris-buffered solution was used for the experiment, however we have found from the literature that ampicillin in solution is not very stable at pH above 7. Since pH for tri-buffered solution is about 7.4, which is above 7, we decided to switch to phosphate buffered solution where the pH can be changed from acidic to basic. HPLC has been used for detecting ampicillin in the buffered solution and the working curve at a pH equal to 4.3 was established by monitoring the absorbance of ampicilin at 204 nm. Currently, the method to deposit smooth a PPY/amp film on the surface of the electrodes has yet not been established; we decided to delay the detection of ampicilin with HPLC at other pH levels.

b. Progress on Self Assembled Protein Layer

i. Update on antibody study

The primary objective of Task II – Antigen Platform Feasibility is to develop the appropriate process to bond the proteins, antibodies, or oligonucleotides to the carbon surface of the CPMEMS.

In previous studies, we demonstrated that we could use antibodies bound to a layer of nitrocellulose on a glass slide to capture bacteria in solution. While this method worked in preliminary studies, the use of antibodies was determined not to be feasible for detecting many different types of bacteria in solution. As the number of bacteria in a solution increased, it becomes necessary to wash the slides more extensively to favor specific antibody-bacteria interactions. Unfortunately, the number of captured bacteria decreased dramatically when using the more stringent wash procedure. Thus, by trying to minimize nonspecific binding of bacteria to the antibodies, we greatly reduced the number of captured bacteria. To circumvent this problem that is characteristic of antibody arrays, we will now utilize DNA molecules as the bound capture molecules for the reasons detailed below.

ii. Oligonucleotide study

During the past several months, we have succeeded in attaching oligos (short pieces of DNA) to a gold substrate and have established a method to monitor the density and binding capacity of the DNA. At this time, we have chosen to focus our efforts on DNA as a binding substrate due to its high specificity, ability to readily attach to gold to form a uniform layer, and lower cost when compared to using antibodies. The use of oligos will allow us to design multiple probes to specifically detect bacteria and fungi in air, water, or soil samples. In addition, this methodology, which involves DNA-DNA binding between the oligo DNA and the organism's DNA, will increase the likelihood of positively identifying a particular organism. A model of the DNA sensor is shown in figure 14. Initially, a chemically modified piece of DNA (Cy3-labeled oligo) that contains a sulfhydrol (SH) group is allowed to attach on a gold coated surface. The SH group facilitates uniform binding of the DNA to the gold surface and also helps to orient the DNA on the surface so as to increase the likelihood of binding the target DNA. In addition, the Cy3 label allows us to monitor the density of oligo binding using fluorescent microscopy as shown in figure 15. We have optimized the binding conditions and have begun testing the ability of the attached oligo to bind other DNA (antisense oligos and bacterial DNA). In addition, we have designed oligos that are specific for detecting bacteria versus fungi.

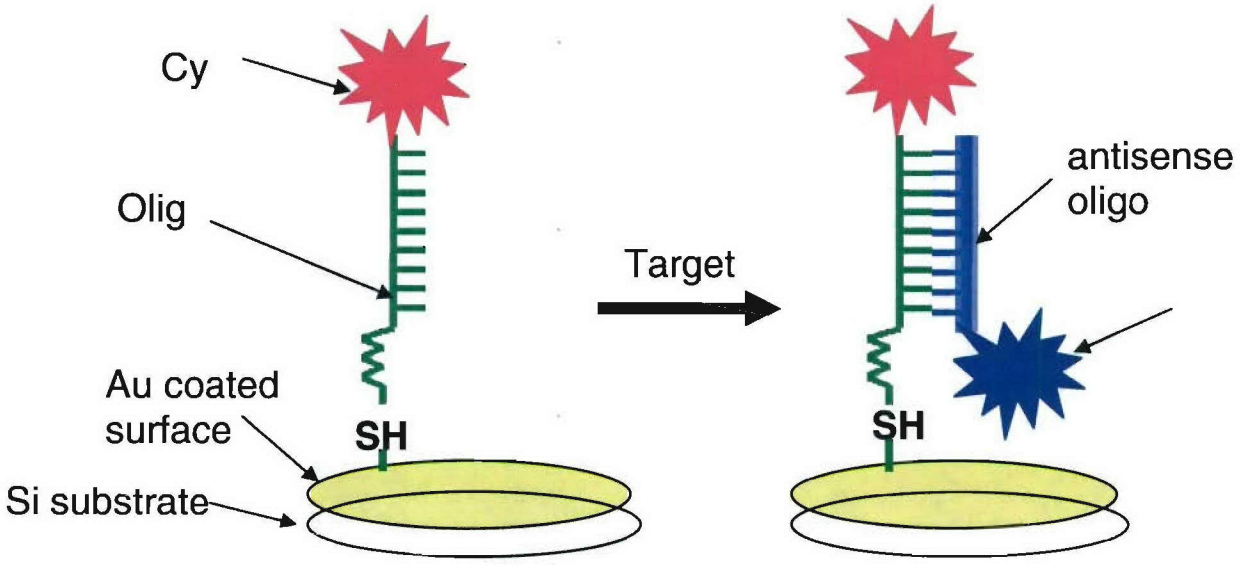


Figure 14. Model of DNA Biosensor

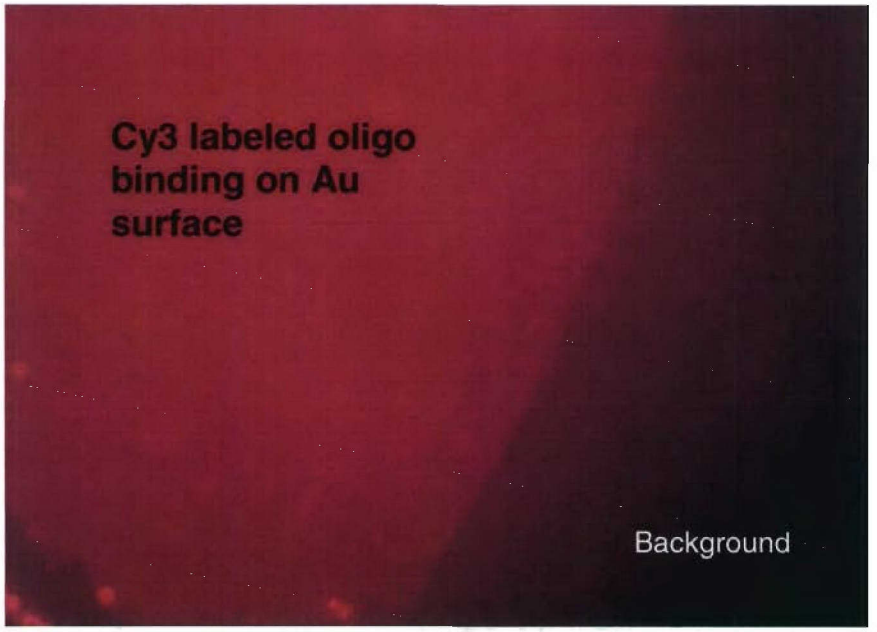


Figure 15. Attachment of DNA to Au Surface

One of the obstacles that had to be overcome involved the isolation and preparation of the organism's DNA. A developed a bio-collection system has been developed that is able to concentrate the number of bacteria found in the air using a water capture method. To be able to gain access to the DNA in the bacteria it is necessary to break open the bacteria. This can be done in a number of ways including heating,

addition of detergents, or sonication. We chose the method of sonication for it has the advantage of not only breaking open the bacteria but also shears the DNA into small fragments that can be used in our DNA-DNA binding assays. As shown in figure 16, sonication of bacteria for 5 mins (left lanes) yields DNA that is only several hundred base pairs in length, which is optimal for our experiments. We will use this method in all future experiments.

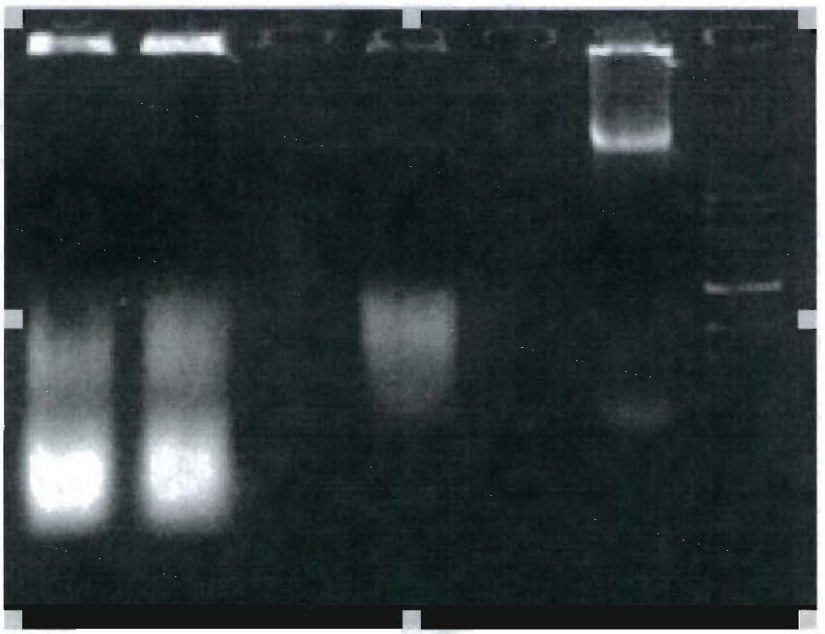


Figure 16. Sonicated Bacteria E.coli DNA on agarose gel

c. Progress on Bio-Collection System

A more extensive study was conducted on the ISAVAC collection system. It was reported previously that the intention of this study was to convert an air purification system into a bio-collection unit. The system was originally designed to remove airborne particulates such as pollen, bacteria, smoke, fumes, dust, plant spores, etc from a given environment. Work on a scaled down version of the ISAVAC system was completed, but is yet to be tested. Simultaneous studies were conducted on the larger scale system for rate of collection and micro-organism collection.

Figure 17 below shows an image of the modified air purification system along with a schematic diagram of the process of collecting biological particulates from the environment. Figure 1 a) is the actual picture of the ISAVAC system with a closed system attachment and HEPA filter. Figure 1 b) is the schematic diagram of the ISAVAC system with the closed system attachment HEPA filter and arrows showing the

flow of air (red indicating contaminated incoming air and green indicating clean outgoing air).

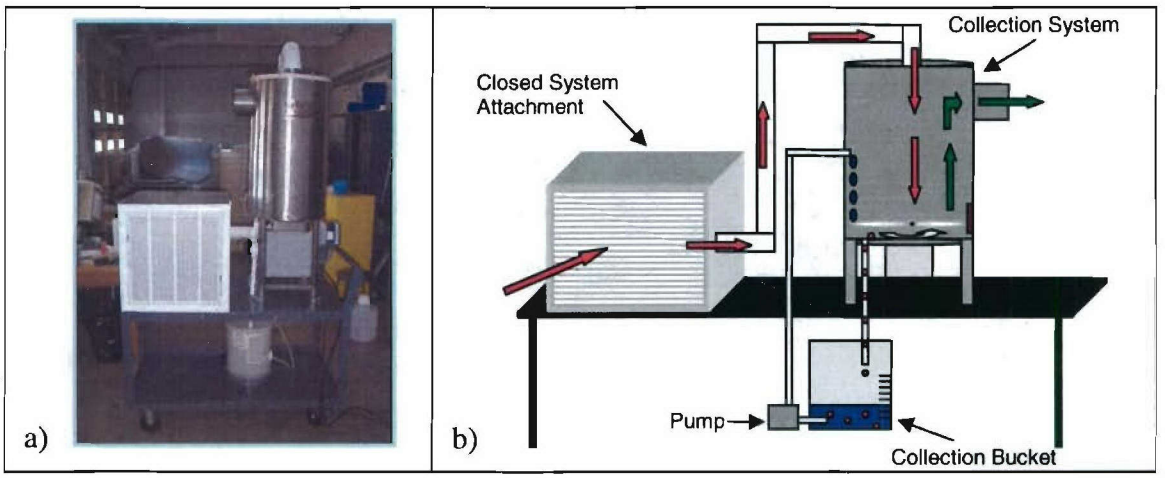


Figure 17. a) Actual picture of the ISAVAC System b) Schematic of the ISAVAC System

The water from the collection bucket is recycled by being pumped back up into the system, therefore concentrating the collected solution over time. The square box with the vent and HEPA filter is called the closed system attachment. The attachment is connected to the input opening of the ISAVAC system by a series of piping that is connected to a clear acrylic box with a 2 micron HEPA filter and a vent. The air is drawn in the vent and passes through the HEPA filter into the box and on through the piping to the ISAVAC's main compartment. The importance of this attachment is that particles 2 micron and larger cannot pass through the filter, and thus the air that enters the box is for the most part contaminant free. We can then only collect nonpathogenic bacteria that we purposely introduce into the system to create a control collection study.

Before each experiment was conducted, the system was cleaned by running it for 20 minutes with 70% ethanol and rinsed by running it for another 20 minutes with autoclaved deionized water. After the cleaning procedure was completed, the cleaning solution was emptied from the collection bucket. The system was then run with a desired volume of liquid to begin the collection process for each study conducted.

The first study that was conducted was to determine the rate of evaporation of the collection solution. It was noted that the humidity at the exit of the system was rather high and therefore autoclaved deionized water used to collect the airborne particulates evaporated at a certain rate. The study to determine the evaporation rate was done both in an enclosed environment as well as outdoors. 10.25 L of distilled water was added to the collection bucket, and the system ran for 4 hours. The room humidity, exhaust humidity, temperature, and amount of water in the collection bucket were recorded every 30 minutes.

The rate of evaporation was determined by finding the slope of the volume of water versus time graph in figure 18, which was determined to be -2.0850 L/hr. A high rate of evaporation meant that the particulates that were being captured were evaporating at a large rate and therefore the concentration of the final collected solution was not very effective. We determined that a large amount of the water used to collect the particulates was evaporating due to the humidity caused at the exit of the system. It was determined after further review that an isothermal condenser would need to be installed on the output of the system to decrease the rate of evaporation.

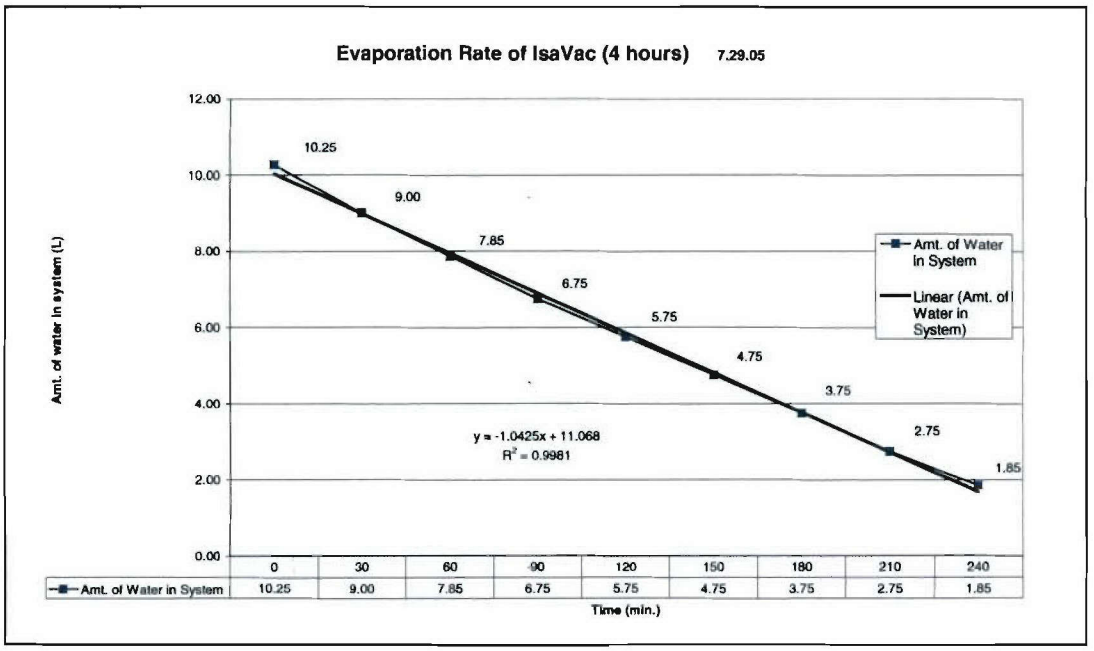


Figure 18. Evaporation rate of the System Collection Solution

For the study that was conducted in a closed environment, 4.25 L of autoclaved deionized water was added to the collection bucket. Initially, 5 ml of water from the collection bucket was removed with a new disposable syringe and stored in a sterile 15mL conical vial. The system was run for 6 hours with a 5 ml sample collected every hour. After each sample was collected, the humidity and temperature of the room was recorded and 2.0 L of autoclaved deionized water was added to the collection bucket. After collection, 60 μL of each sample was plated on an LB agar. The remaining samples in the vials were then centrifuged for 5 minutes at 3000 rpm at 4°C where all but 100 μL of the supernatant was removed and the pellet was re-suspended, and 60 μL was spread onto new LB plates. All plates were incubated overnight at 37.5°C and colony forming units were then counted.

This study was done to determine the number of colonies that could be grown from the collected solution after a 6 hour run. Each sample was plated on an LB agar and the remaining sample solution was centrifuged then plated to determine if centrifuging the samples was necessary to obtain a higher number of colonies per plate to get a more

accurate count. Figure 19 shows bacteria collected by the system over time. The growth of colonies on the LB agar showed that the system did in fact collect some form of bacteria. The graph also shows that the concentration of biological material in the collected solution decreased over time. This decrease in concentration is directly correlated to the loss of solution due to humidity issues with the system.

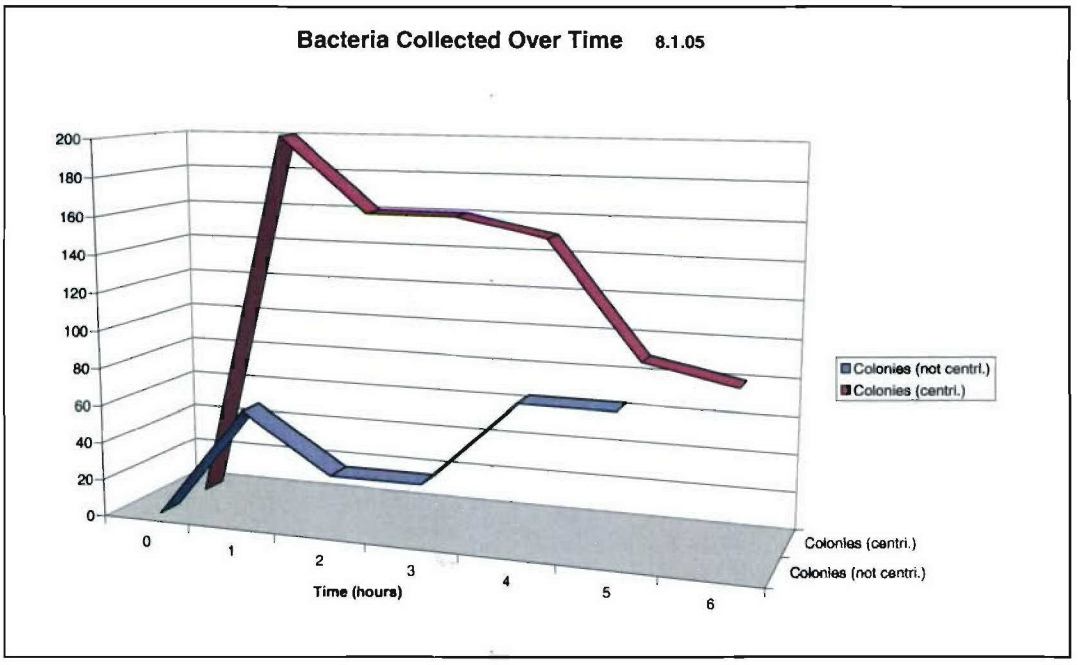


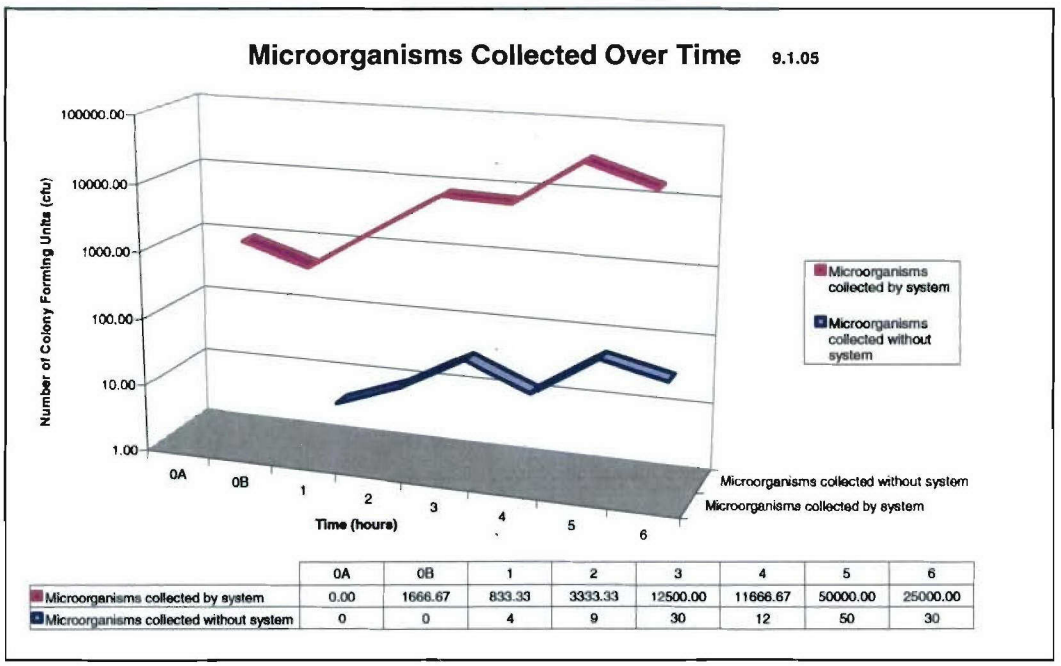
Figure 19. Bacteria Collected by the System over Time

The plates that contained the centrifuged samples had many more colonies than the plates with the non-centrifuged samples. Also, no colonies appeared at time "0" where the samples taken were of the autoclaved deionized water that had ran through the system for a short interval of time. This gave us a starting point, which signified that at time "0" the system was clean prior to collection, and all microorganisms that were collected after time "0" had been collected by the system.

Another similar study was conducted outdoors where 5.0 L of autoclaved deionized water was added to the collection bucket. To measure the effectiveness of the system, seven uncovered LB plates were set outside on a table next to the system as it was running. A comparison was made to determine the amount of growth of microorganisms on each plate that sat in an ambient outdoor environment compared to the rate of growth for plates that contained solution that was collected by the system. The system ran for 3 hours with a 10 ml sample collected every 30 minutes. After every sample was collected, one LB plate was covered and refrigerated, the humidity and temperature of the room was recorded, and autoclaved deionized water was added to the collection bucket to maintain a 5.0 L water level. Each plate that sat out in ambient air was left out for the same time period that corresponded to the time interval between which a sample was taken from the collection system. After collection, the samples were

centrifuged for 5 minutes at 3000 rpm at 4°C where all but 150 μL of the supernatant was removed and the pellet was re-suspended, and 60 μL was spread onto new LB plates for the collected solutions. All plates, including the plates set outside during collection, were incubated overnight at 37.5°C and colony forming units were then counted.

The number of colony forming units counted on the LB plates after incubating overnight at 37.5°C for both the plates that sat outside in ambient air and the plates that had the collected solution is shown in figure 20. It was noted that the number of colonies on each plate that had the collected solution was larger by a multiplied factor of approximately 830 compared the number of colonies on each of the time correspondent plates left out in ambient air. This clearly confirms that this system is far more effective in collecting microorganisms rather than trying to collect them without any sort of collection device.



	0A	0B	1	2	3	4	5	6
Microorganisms collected by system	0.00	1666.67	833.33	3333.33	12500.00	11666.67	50000.00	25000.00
Microorganisms collected without system	0	0	4	9	30	12	50	30

Figure 20. Number of Colony Forming Units Counted

The next study that was conducted was with the closed system attachment, where a 2 micron HEPA filter was connected to the system and ran for 2 hours with a constant volume of 4.5 L of autoclaved deionized water. 15 ml of water from the collection bucket was removed with a new disposable syringe and stored in a sterile 15mL conical vial every 30 minutes. After every sample was collected, the humidity and temperature of the room was recorded, and more autoclaved deionized water was added to the collection bucket to maintain a volume of 4.5 L. After collection, the samples were centrifuged for 5 minutes at 3000 rpm at 4°C where all but 100 μL of the supernatant was removed and the

pellet was re-suspended, and 60 µl was spread onto LB plates. The plates were incubated overnight at 37.5°C and colony forming units were then counted.

It was determined that since the cleaning protocol that was followed had proven to have been highly effective in freeing the system of contaminants, the small amount of bacteria that was collected originated inside the closed system attachment. A more effective cleaning protocol of the closed system attachment is currently being established. Figure 21 shows that at each time interval there were indeed some colonies of bacteria that did form during the time 2 hour time period that the system ran with the closed system attachment.

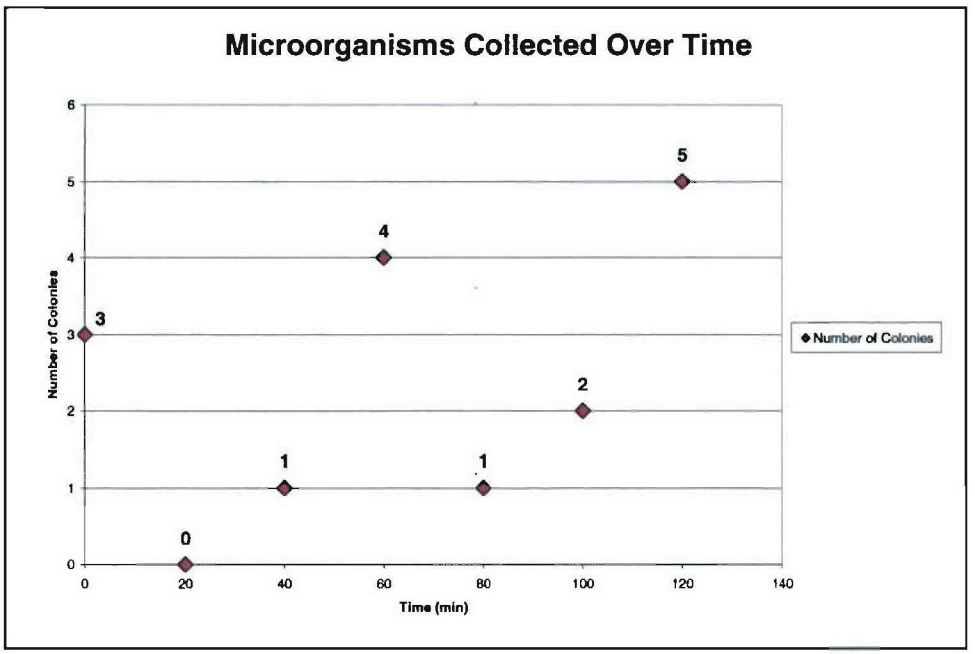


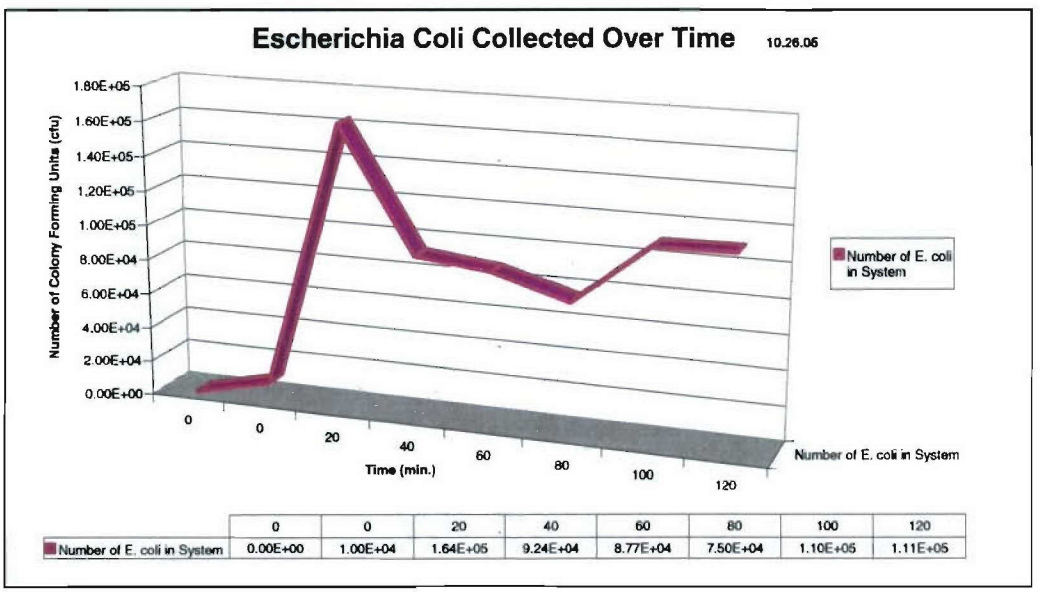
Figure 21. Number of Colony Forming Units Formed with Closed System Attachment

The last study that was conducted was the controlled collection experiments, where Escherichia coli HB101 K-12 was used as a test microorganism. This strain of E. coli is classified as enteric meaning it is naturally found in the body's intestinal tract where it helps provide a source for Vitamin K and B-complex vitamins. This microorganism was chosen because its doubling time is about 20 minutes in ideal conditions, making it is easy to get large amounts of bacteria that we need, it is nonpathogenic, and its genome is well understood and easily modified.

For this study, 4.0 L of autoclaved deionized water was initially added to the collection bucket. The closed system attachment with a 2 micron HEPA filter was connected to the system and 140 µL of Escherichia coli in an LB solution at a concentration of 3.0×10^9 bacteria/ml was added to a small ultrasonic humidifier, which was placed inside the closed system attachment box. The ultrasonic humidifier was then turned on, allowing the bacteria to be aerosolized such that the collection system could

collect the bacteria. This was also done to determine whether or not a biological particle of known size can be collected. Initially, 15 ml of water from the collection bucket was removed with a new disposable syringe and stored in a sterile 15mL conical vial. The system ran for 3 hours with a 15 ml sample collected every 20 minutes. After every sample was collected, the humidity and temperature of the room was recorded and autoclaved deionized water was added to the collection bucket to maintain a constant volume of 4.0 L. The samples were centrifuged for 5 minutes at 3000 rpm at 4°C where all but 100 μL of the supernatant was removed and the pellet was re-suspended, and 60 μL was spread onto LB plates. Plates were incubated overnight at 37.5°C and colony forming units were then counted.

Figure 22 does show that nonpathogenic E coli was captured by the system. It was determined that the lack of linearity from the graph of the collection can be attributed to the high rate of evaporation of the solution in the system. Some of the collected bacteria is re-aerosolized and then expelled from the system lowering the concentration of bacteria in the solution. Prior to conducting these experiments it was known that the requirement to add autoclaved deionized water to maintain a constant volume in the system would dilute the solution, which in turn would lower the concentration of bacteria. This study as well as the preceding studies confirmed this by showing that for longer time periods that the system ran, the lower the concentration of microorganisms in the solution. As was stated earlier these problems may be solved by installing an isothermal condenser on the output of the system and draining the condensed liquid back into the collection bucket.



	0	0	20	40	60	80	100	120
Number of E. coli in System	0.00E+00	1.00E+04	1.64E+05	9.24E+04	8.77E+04	7.50E+04	1.10E+05	1.11E+05

Figure 22. Number of colony forming units per plate in the controlled collection experiment

d. Progress on Unity 400 Heat Dissolvable Polymer

i. Patterning of Unity 400 films

The thermal release polymer, Unity 400 (Promerus LLC) being investigated at BSI as a means to relieve stress during the construction of air bridges has been demonstrated in terms of compatibility, shrinkage, and residue-free thermal decomposition when residual oxygen is properly controlled. A crucial step being investigated at BSI in the Unity 400 process to create CPMEMS structures is the masking and reactive ion etching (RIE) of the cured Unity 400 polymer. This process consists of the following sequence:

- Deposit 1000Å Al as hard mask by thermal evaporation;
- Spin-coat photoresist Shipley 1813 at 4000rpm for 60 seconds;
- Pre-bake at 115oC for 150 seconds;
- Perform UV exposure at 25mW/cm2 for 4 seconds on mask aligner;
- Develop for 15 seconds;
- Post-bake at 115oC for 150 seconds;
- Perform wet etch of Al for 100 seconds to transfer photoresist pattern to Al;
- Strip photoresist Shipley 1813 with acetone;
- Perform RIE of Unity 400 to transfer Al pattern to Unity 400 layer;
- Strip Al hard mask with Al etchant.

The detailed results of the preliminary efforts on patterning Unity 400 films by RIE were shown in previous interim reports. The major issue encountered therein was considerable undercut due to the dominance of the isotropic etching. In order to solve this problem, a new RIE system with greater capability of anisotropic etching is being set up at BSI. This new system is being installed at BSI facilities and is expected to be operational at the end of January 2006. Anisotropic etching studies will begin following operator training on the new system.

ii. Status of the e-beam evaporator

BSI was also in the works of purchasing an e-beam evaporation system that would be utilized in the process of developing the CPMEMS air bridge structures. The e-beam evaporator is being installed at the BSI facilities and is expected to be operational the week of in mid January 2006. Representatives from the manufacturer will be providing operational training in February, 2006.

III. Conclusions

a. Accomplishments and Future Work

During the time frames that this interim report covers we have:

1. Successfully designed and built a new multi-microelectrodes based device on a chip.
2. Characterized the electrochemistry properties of those multi-microelectrodes and each of them behave like a regular electrode.
3. Successfully demonstrated a release system of polypyrrole/dopant on this multi microelectrode device.
4. Demonstrated the growth of a thin film of PPY/Dopant on the surface of the microelectrode and characterized the film properties including the amount of dopant that can be released into the solution.
5. Established the method to coat the multi-microelectrodes with different PPY/Dopant films and the analytical protocol to study the released dye molecules.
6. Established a method to fabricate hydrogel on carbon electrodes and demonstrate the release of phenolred from PPY/phenolred film into the gel when a potential is applied to the device.
7. HPLC method for detecting ampicillin in phosphate buffered solution at acidic condition was established.
8. Determined that the use of antibodies was not a feasible detection method for many different types of bacteria in solution.
9. Began to utilize DNA molecules as the bound capture molecules and succeeded in attaching oligos (short pieces of DNA) to a gold substrate.
10. Established a method to monitor the density and binding capacity of the DNA.
11. Determined that using DNA as a binding substrate would allow for high specificity, readily able attach to gold and form a uniform layer, and lower cost when compared to using antibodies.
12. Established that using oligos will allow us to design multiple probes to specifically detect bacteria and fungi in air, water, or soil samples.
13. Optimized the binding conditions and have begun testing the ability of the attached oligo to bind to other DNA (antisense oligos and bacterial DNA).
14. Designed oligos that are specific for detecting bacteria versus fungi.
15. Conducted studies on a modifying an air purification system into a bio-collection system.
16. Demonstrated that the collection system was able to capture airborne particulates out of the air and concentrate in them a liquid medium.
17. Demonstrated the growth of bacteria captured from an enclosed environment as well as an outdoor ambient environment.
18. Demonstrated the capture of a known nonpathogenic strain of E. coli.
19. Determined that the humidity levels in the system were direct cause of the decrease in concentration of the collected microorganisms.
20. A new RIE system with greater capability of anisotropic etching is being set up at BSI.

21. BSI purchased an e-beam evaporation system to be utilized in the process of developing the CPMEMS air bridge structures.

In the months ahead, our work will include:

1. To continue the work on a release system of polypyrrole/dopant on the multi-microelectrodes device with different dopants.
2. Investigation of an alternative biologically active dopant molecule for release experiments.
3. To continue the work on the release of three or four dopants into hydrogel and optimize the fabrication of the hydrogel on the surface of the electrodes.
4. Complete the design of a sensor for attaching oligos.
5. Testing of the binding of complementary strands for detection purposes using the cyclic voltammetry (impedance) method.
6. Design of an actual set (Au patttern) to best detect the DNA binding.
7. Demonstrate proof of concept by utilizing arbitrary collected samples to detect different types of bacteria.
8. Establish the difference between a pathogenic and non-pathogenic strain to determine different level of specificity.
9. Attach an isothermal condenser to the output of the collection system to decrease the loss of microorganism rich solution.
10. Conduct studies on the effects of attaching the isothermal condenser to the output
11. Determine the shortest length of time for sufficient collection for biosensor detection,
12. Determine the upper and lower size limits of collected particles by the system.
13. The RID system will be installed at BSI facilities and is expected to be operational at beginning of February 2006.
14. Anisotropic etching studies will begin following operator training on the RIE system.
15. The e-beam evaporator will be installed at the BSI facilities and is expected to be operational during mid January 2006. Representatives from the manufacturer for the e-beam will be providing operational training in February, 2006.
16. Continue work on developing CPMEMS air bridges using alternate methods once required systems have been installed.