Multiple Strategy Bio-Detection Sensor Platforms

Made from Carbon and Polymer Materials

Interim Report

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I. Introduction

This interim progress report will discuss the progress to date on the biosensors made from carbon and polymer composite micro-electromechanical systems (CPMEMS) and the multiple strategy bio-detection sensor platforms that incorporate these CPMEMS. Progress since the last interim report has involved steps in the individual CPMEMS layer fabrication. This includes an alternative sacrificial layer material, resolution of equipment issues, development of a test lithography mask set, and development of a wafer level packaging scheme. Progress reported within will also discuss active technology in the form of sense and release experiments, progress on the molecular bridge preparation of the CPMEMS surface, and the bio-collection instrument and technology.

II. Description of Technical Research Progress

a. Individual CPMEMS Layer Fabrication

i. Sacrificial layer, Unity 400 heat dissolvable polymer

As an alternative to a SiO_2 sacrificial layer, a thermal release polymer called Unity 400 from Promerus LLC is being investigated at Brewer Science, Inc. (BSI). With this material the processing steps to create the CPMEMS structures are as follows:

1) Deposit 1000Å of WTi alloy by RF magnetron sputtering on silicon wafers that have been coated with 2000Å of silicon nitride;
2) Deposit 3000Å of Au by thermal evaporation;
3) Deposit 1000Å of WTi alloy by RF magnetron sputtering;
4) Spin-coat Unity 400 at 2600rpm for 20 seconds;
5) Flash solvent at 100°C for 5 minutes;
6) Cure Unity 400 at 200°C for 120 minutes;
7) Mask wafer and perform RIE (Reactive Ion Etching) of Unity 400;
8) Electroplate Au pillars;
9) Spin-coat 1000Å of PSA and implant N^+ at a dose of $1.0x10^{16}$ions/cm^2;
10) Mask wafer and perform PE (Plasma Etching) of implanted polymer;
11) Decompose Unity 400 at 425°C for 120 minutes;
12) Wet etch WTi-Au-WTi stack to isolate Au pillars.

The wet etching process can induce a considerable amount of stress on the ion-implanted polymer bridge due to the capillary effect. It had been repeatedly shown that the ion-implanted polymer bridge significantly bowed after the wafer was rinsed and dried. A dry release approach is therefore highly desirable. Unity 400 completely decomposes into a low molecular weight hydrocarbon gas at 425°C, which can penetrate through the encapsulation layer, resulting in a residue-free air cavity. By controlling the temperature ramp rate there is little stress on the ion-implanted polymer bridge during the thermal release process.

The thermal decomposition kinetics of Unity 400 has been studied by using dynamic TGA (Thermal Gravimetric Analysis) at BSI. Prior to experiment the sample chamber was purged with high purity nitrogen (99.998%) so that the residual oxygen concentration was less than 5ppm. The Unity 400 sample was first heated to 200°C and held for 120 minutes to remove any residual solvent. This was followed by heating the Unity 400 sample at a ramp rate of 1°C/minute to 425°C and holding for 120 minutes. During the analysis the sample chamber was continuously purged with high purity nitrogen. The dynamic TGA results shown in Figure 1 verify that Unity 400 completely decomposed at 425°C, and there was only a negligible amount of residue left in the sample chamber. Another prominent property of Unity 400 is its good thermal stability up to 300°C. This ensures a sufficient processing window for the following fabrication steps.

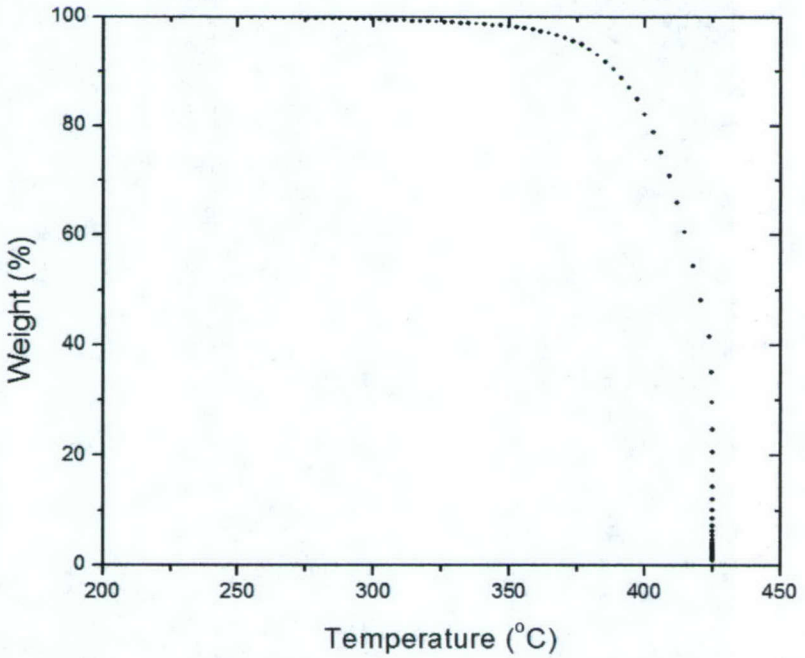

Figure 1: Dynamic TGA of Unity 400

The thermal decomposition kinetics of Unity 400 is highly dependent on the oxygen concentration. Nonvolatile oxides are a major source of the residue that is left after the thermal release process. Meanwhile in an environment with regular oxygen concentration, significant film shrinkage (>30%) was observed during the polymer curing process. The dynamic TGA experiments performed at BSI indicate that the oxygen concentration needs to be less than 5ppm. Currently there are three aspects being simultaneously pursued on the furnace at BSI in order to achieve this critical condition: (1) vacuum; (2) high purity nitrogen purge; (3) inline oxygen absorbing cartridge. Once such a system is implemented at BSI, we shall be able to routinely achieve the TGA result shown in Figure 1 on batch 3" wafers.

Another crucial process being investigated at BSI is step 7, which consists of the following sequences:

7.1. Deposit 1000Å of Al as a hard mask by thermal evaporation;
7.2. Spin-coat photoresist at 4000rpm for 60 seconds;
7.3. Pre-bake wafer at 115°C for 150 seconds;
7.4. Align wafer to mask and then expose wafer to UV on mask aligner;
7.5. Develop exposed wafer for 15 seconds;
7.6. Post-bake wafer at 115°C for 150 seconds;
7.7. Immerse wafer in an Al etchant for 100 seconds so as to transfer photoresist pattern to Al layer;
7.8. Strip photoresist with acetone;
7.9. Perform RIE so as to transfer Al pattern to Unity 400 layer;
7.10. Strip Al hard mask with Al etchant.

RIE is a well-established technology in the semiconductor industry for high resolution etching of polymeric dielectrics. RIE of hydrocarbon polymers is usually performed in O_2 plasma and forms volatile but thermodynamically stable products such as CO, CO_2, and H_2O. Because Unity 400 contains silicon, nonvolatile silicon oxide is also formed during the etching process in O_2 plasma. The accumulation of this silicon oxide layer on the surface can slow down or even stop the etching process. The removal of the resulting silicon oxide can be achieved by addition of a small amount of fluorine containing molecules into O_2 plasma. Moreover the concentration of fluorine in O_2 plasma plays an important role on the smoothness of the etched polymer surface. The sidewall profile of the etched features is highly dependent on the ratio of physical etching to chemical etching during the RIE process. Physical etching can be enhanced by addition of Ar into O_2 plasma.

In particular, we started with the following recipe on the etcher at BSI:

Power (W)	Pressure (mTorr)	O_2 (sccm)	Ar (sccm)	CHF_3 (sccm)
50	300	15	40	5

Table 1: Recipe 0001

First the etch rate of Unity 400 by using this recipe was measured. A series of timed RIE were performed. At the end of each run, the thickness of the Unity 400 layer left on the wafer was measured by using a profilometer (Alpha Step 200). The results were plotted along with the RIE time and then a linear fitting was performed. The etch rate was thus obtained from the slope of the line. As shown in Figure 2 the etching process behaved in a very linear fashion and the etch rate was approximately 3.6nm/second when recipe 0001 was used.

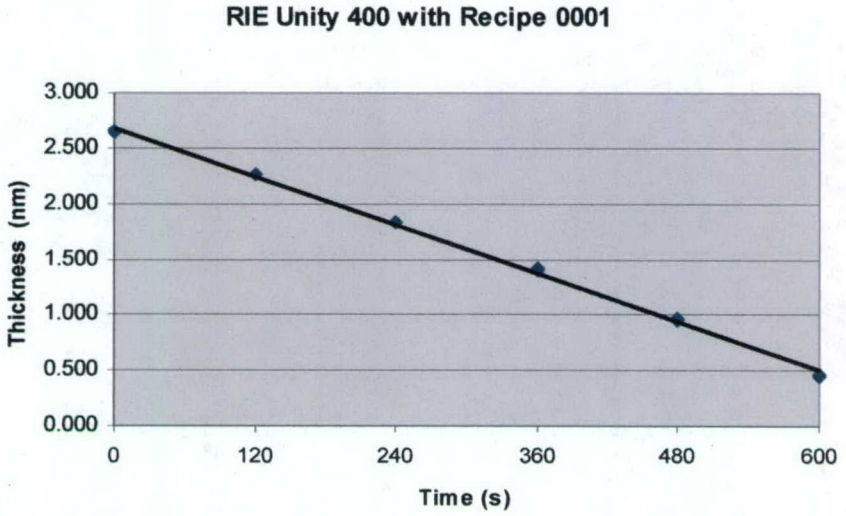

Figure 2: Etch rate of Unity 400 by using recipe 0001

After the characterization of the etch rate, a timed RIE was performed on a device wafer. On this wafer the thickness of the Unity 400 layer was 2.5μm. Thus approximately 690 seconds were required in order to etch through this layer. Additional 10 seconds were programmed into recipe 0001 to compensate for the loading effect. In another words the effective etching process ran for 700 seconds. The resulting patterns in the Unity 400 layer were inspected with Scanning Electron Microscopy (SEM). For the purpose of studying undercut and sidewall profile, the Al hard mask remained on the wafer during the SEM inspection. A typical etched feature is shown in Figure 3. Currently we are working on reducing the degree of undercut while retaining the vertical sidewall profile by tuning recipe 0001.

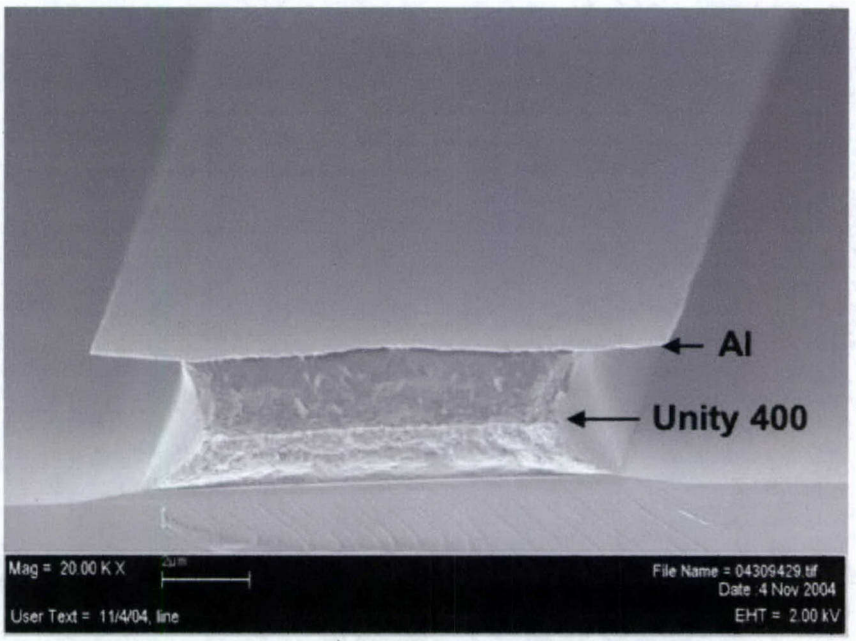

Figure 3: Unity 400 pattern after RIE with recipe 0001

ii. Equipment issues involved in layer fabrication

Equipment issues were discussed relative to the fabrication of the individual CPMEMS layer fabrication, in the previous report. These equipment issues involved the ion implanter and electron beam evaporation system at SMSU. Ion implantation is the primary step in creation of the metal mixed ion implanted polymers, which are the backbone of the biosensor CPMEMS. The equipment issue with the ion implanter was an unstable ion beam indicated by large fluctuations (arcs) in the system's acceleration voltage. It was determined that a new ion source would be needed. An ion source has since been purchased, customized in house, and installed. Programming of the new ion source controller is currently underway and testing of the complete system is scheduled for the upcoming months. During this downtime we have investigated a number of alternative options for ion implantation. An implant services company, Core Systems, was located and we have sent a couple of sample batches to them for implantation. The electrical properties of the samples turned out to be almost identical to results from the SMSU system. This implant services company has very good turn around and will be used until the SMSU ion implanter is completely operational.

SiO_2 deposition is a critical step in the CPMEMS fabrication. SiO_2 is used for electrical insulating layers as well as deposition of a sacrificial layer. The sacrificial layer for fabrication of the CPMEMS is 25,000 Å or 2.5 μm. The sputtering system at SMSU is not designed for deposition of 2.5 μm thicknesses on 3 inch substrates. It would take many hours to deposit layers 2.5 μm thick utilizing the sputtering system. This led to the investigation of alternate deposition techniques. It was determined that electron beam evaporation would be the most economical and efficient choice for the growth of SiO_2 films of this nature. An electron beam evaporation source has now been purchased and incorporated into a current SMSU vacuum system. This was completed in early January 2005 and is currently undergoing calibration and testing.

b. Platform Fabrication

i. Lithography mask set – 16x16 array

The previous interim report discussed the individual CPMEMS layer fabrication process. This process has been taken one step further to create a 16x16 array of CPMEMS devices. This is the initial step prior to the development of the multiple strategy platforms. The mask set to develop the 16x16 array consists of 7 masks, and will allow for several arrays to be fabricated on one wafer. Each array will be enclosed in a die, where on a single 3 inch diameter silicon wafer, 12 dies can be fabricated. Electrical contacts will protrude through the sides of the individual die for testing and connection purposes. Figure 4 shows a schematic diagram of a single test die with each mask superimposed on one another.

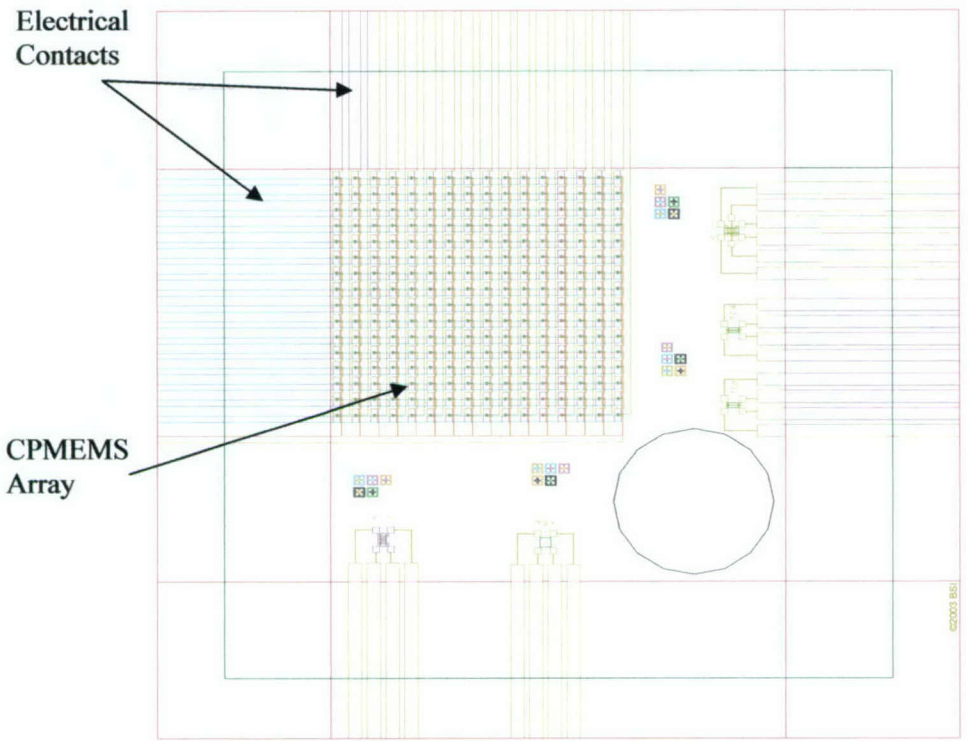

Figure 4: A 16x16 CPMEMS array prototype test die

ii. Development of a wafer level packaging scheme

The fabrication of the multiple strategy platforms will incorporate a wafer level packaging process, which is a cost-effective approach to manufacturing MEMS devices. The wafer level packaging process is unique by its stages of development, where several dies are created on a single silicon wafer, and are then packaged at the same time. This allows for multiple dies to be manufactured at once, and then tested both pre and post-production. After production the wafers are cut into individual dies for testing or application. Initial design work on a packaging scheme of this type is underway and will be discussed below.

The first stage of the wafer level packaging process, as discussed in the previous section, is the fabrication of the 16x16 arrays of devices on a silicon wafer. The second stage is the development of a metal grid which is used to form the wells that serve as part of the enclosure of each die. Several grid materials will be investigated; metals such as brass, chrome, and stainless steel will be examined. The geometry of the grid will encompass the twelve dies on a 3 inch wafer. The grid geometry is shown in Figure 5. The grid will be machined using a micro machining laser. To attach the grid to the silicon wafer, an indium solder pattern (or some other adhesive) will be placed on the grid. The grid will be positioned on top of the silicon wafer and aligned to a metal pattern exactly matching the grid dimensions. The entire structure will be

heated until the solder melts. Once the solder cools it will bind the metal grid to the silicon wafer.

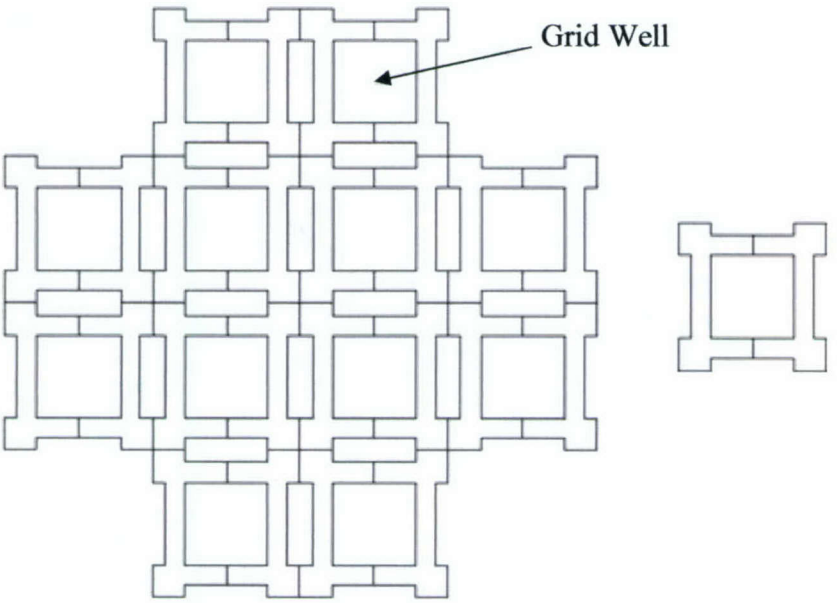

Figure 5: Metal grid geometry for 12 dies on the left and a single cut die on the right

The third stage will be to enclose the dies from the top using a double-sided polished silicon wafer. The wafer will be attached in the same manner as the metal grid. Finally each individual die will be cut out using the micro machining laser. The three mentioned stages are depicted in Figure 6; first the arrays of devices are created, then the metal grid is positioned and attached, and lastly the dies are encapsulated using a silicon wafer. In the future a re-circulating liquid flow through system will be designed and integrated to allow for analyte to be sent to the arrays of CPMEMS devices for detection.

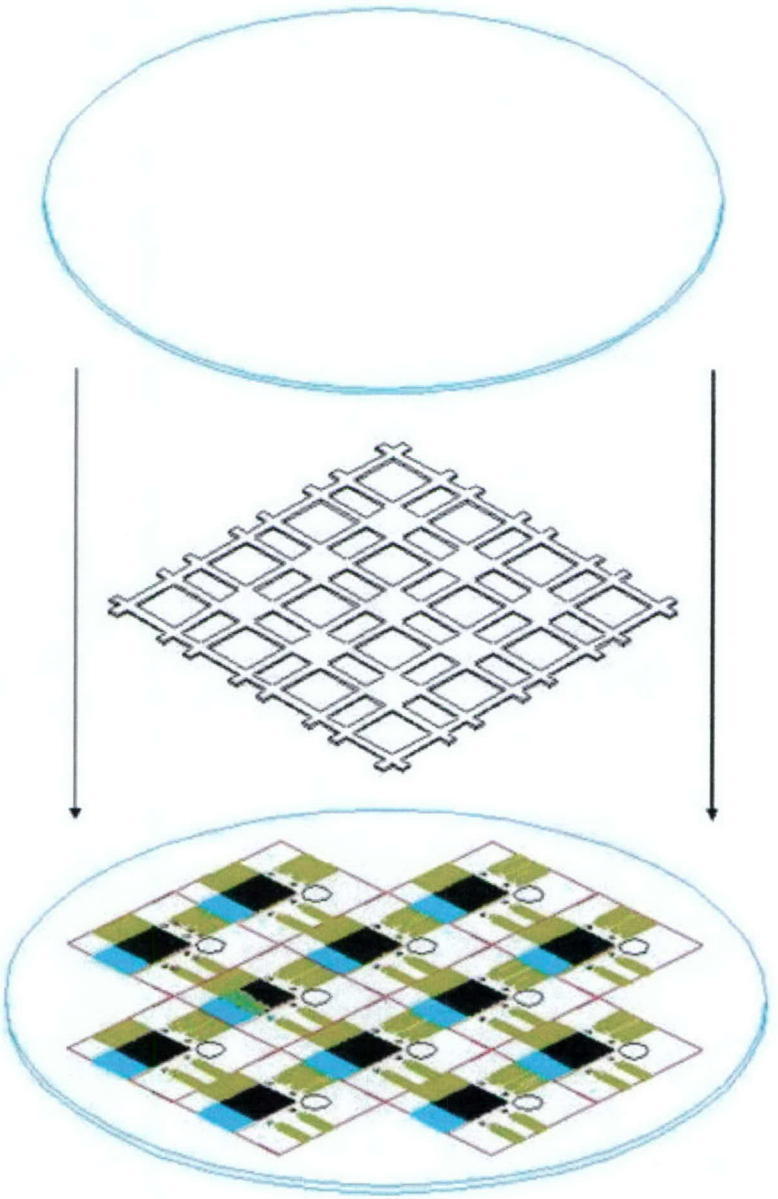

Figure 6: 3 step process; bottom (CPMEMS array), middle (metal grid), top (Si Cover)

c. Developing Active Sense and Release Experiments

The primary objective of Task III is to develop active sense and release experiments for incorporation into the broadband platform. Polypyrrole, a conducting polymer, has been chosen for this experiment due to its redox switching property. Phenolred, Ampicillin and Green fluorescent protein were selected for the initial study to prove the release concept. The following five antibiotic drug candidates currently used in treatment regimens have been chosen for future studies: (1) Aminoglycosides, (2) Penicillins and Cephalosporins, (3) Macrolides, (4) Quinolones

and Fluroquinolones, and Tetracyclines. These drugs are complicated molecules with a variety of different functional groups, and were selected to demonstrate the robustness of our technology.

Initially, glass was used as the substrate with gold as the electrode material. A single gold electrode (Figure 7) with a center diameter of 1" was produced. A Polypyrrole based film was prepared on the surface of the electrode through an electropolymerization method. However, the adhesion of gold film was poor resulting in delamination of the electrode material from the substrate during the release process. The problem was solved by replacing the glass substrate with a polycarbonate sheet. Also, the size of the electrode was reduced by decreasing the diameter of the center electrode to 0.25". The significance of this size reduction not only provided for a better fit for the electrode in the reaction container, but also provided valuable design information for the microelectrodes that will be employed on the MEMS platform. Another change that was made was the gold electrode was replaced with a carbon electrode. Carbon is an inexpensive material, which is inert to many of the chemicals used in the experiment, and can be readily made with our (Crosslink) in house printing methods. Figure 7 also shows a design of the multi electrodes where four single working electrodes and one counter electrode were combined to make 4 devices on one substrate.

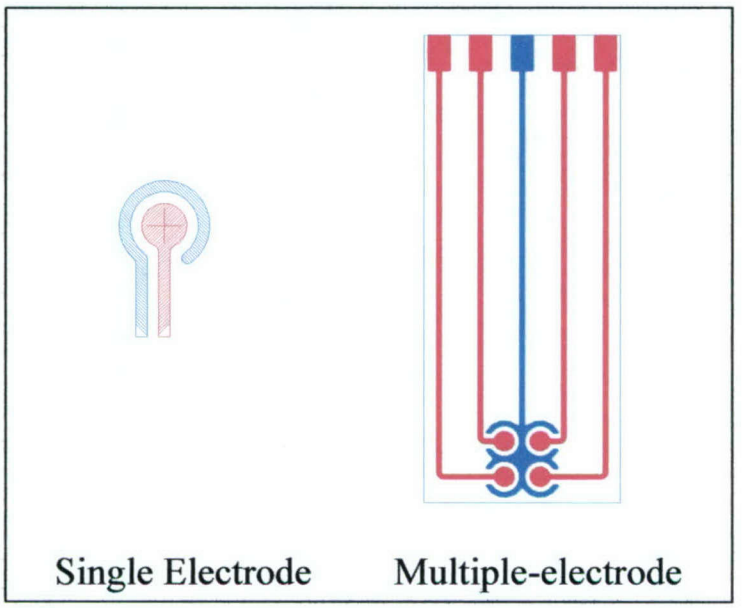

Figure 7: Configurations of both Single and Multi Electrodes

The method to deposit polypyrrole/phenolred based films on the surface of the electrode has been developed. A number of polypyrrole/phenolred and polypyrrole/ampicillin films have been successfully deposited on the electrode surface. The film properties can be determined mainly by (1) the amount of the current passing through the electrodes, (2) the concentration of pyrrole monomer in the reaction media, (3) the reaction time, (4) the type of the dopant molecule, and (5) the concentrations of both pyrrole and the dopant. At this stage, the applied electric current and concentrations of pyrrole and the dopant were kept at 0.5 mA and 0.1 M, respectively. The film thickness is simply determined by the reaction time and the type of the

dopant employed. In fact, the reaction time ranged from 90 to 1800 seconds when phenolred or ampicillin was used as the dopant. After each reaction, a smooth green film formed on the surface of the electrode. No cracking in the film was observed under an optical microscope. However, increasing the reaction time over 1800 seconds or the electric current over 0.5 mA resulted in the formation of an irregular, uneven film. If the size of the electrode is further reduced, the experimental condition for making the ideal film will be impacted.

Figure 8 shows the UV-Vis spectrum of phenolred in Tris-buffered saline recorded with a UV-Vis spectrometer from Ocean Optics. The spectrum showed an absorption maximum at 537 nm. The analytical method for measuring the released phenolred was developed by measuring the absorbance of the released phenolred at 537 nm. According to Beer's law ($A = \varepsilon bc$), the absorbance (A) of the absorbing substance is directly proportional to the concentration (c) of the absorbing substance. The constant ε is called the molar absorptivity when the concentration c is in moles per liter and b, the absorbing length, is in centimeters. Therefore, the concentration of phenolred in the solution can be obtained from measuring its absorbance in solution. The absorbance of phenolred at 537 nm in Tris buffered solution was measured at different concentrations including $5x10^{-5}M$, $1x10^{-5}M$, $5x10^{-6}M$, $1x10^{-6}M$, and $5x10^{-7}M$. As shown in Figure 9, the working curve that a plot of absorbance versus concentration gave a straight line passing through the origin with a slope equal to εb. The concentration, as well as the amount of the released phenolred, can thus be detected from the release study. In principle, this method can detect any released absorbing molecules in solution.

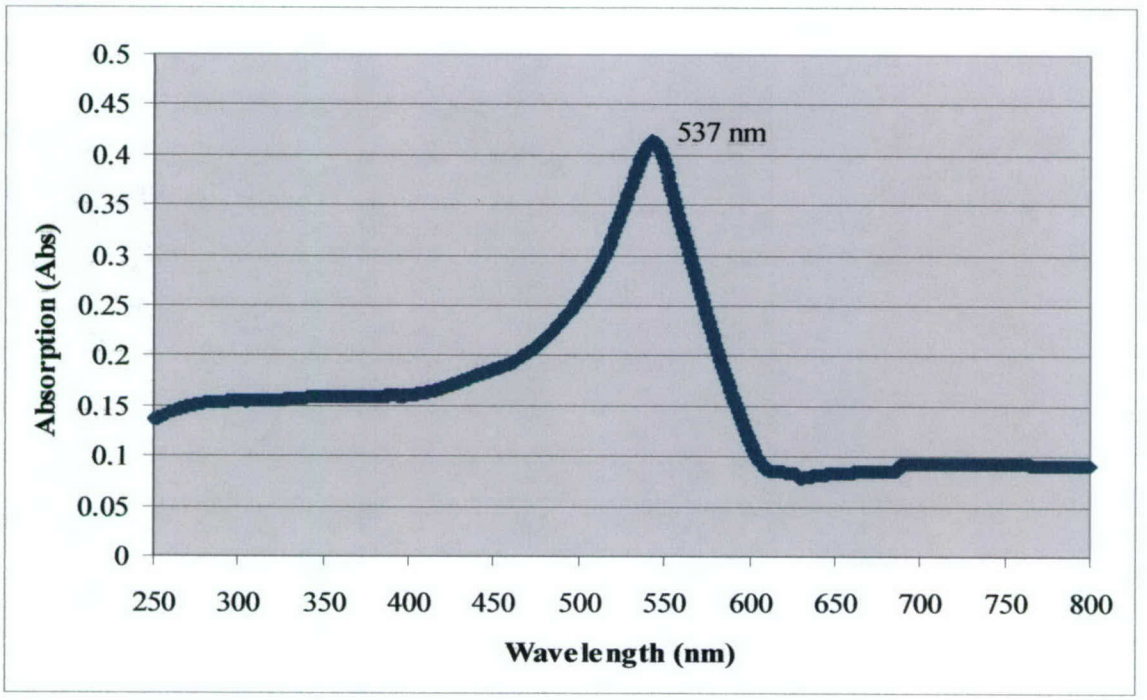

Figure 8: UV-Vis Spectrum of Phenolred in Tri-Buffered Saline

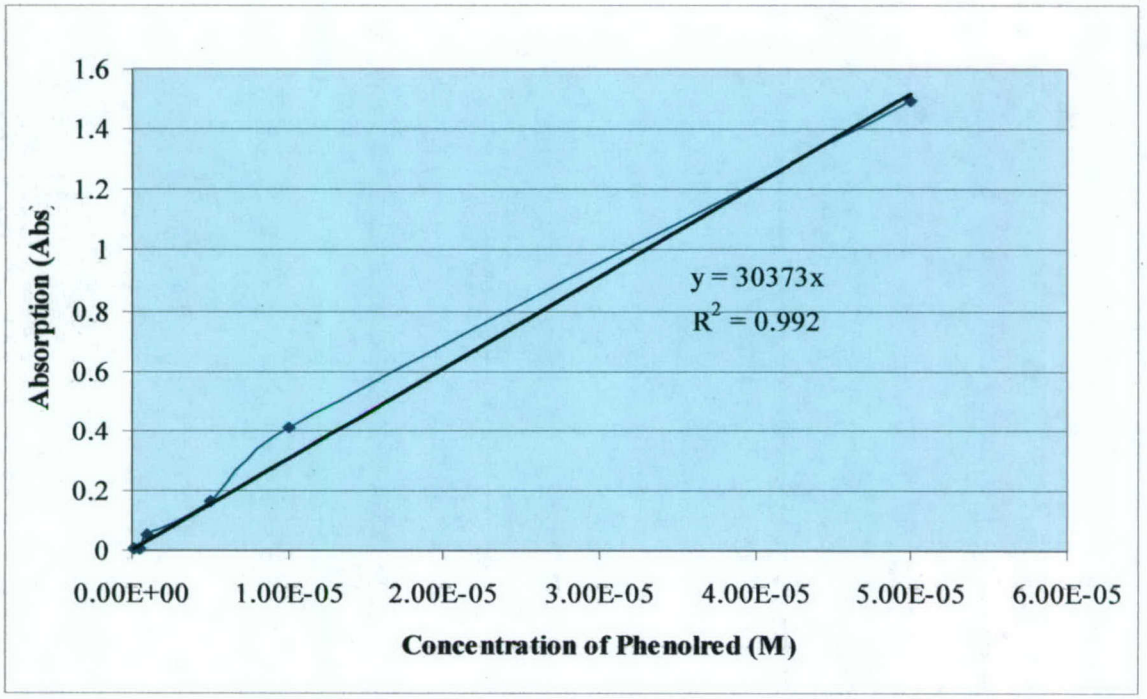

Figure 9: The Working Curve for Phenolred in Tris-Buffered Saline

The release profiles of phenolred from a PPY/phenolred film in solution were obtained with the developed analytical method. The thickness of PPY/phenolred films increases from sample (2) to (5). Curve (1) for sample (1) in Figure 10 shows that phenolred was not released when the circuit was open. However, phenolred (see curve (2)-(5) for sample (2)-(5)) was released when a voltage (-2V) was applied with the release profile depending on the film thickness. As one can see, curve (2) for the thinnest PPY/phenolred film shown solely in Figure 11 demonstrated that 70% of the releasable phenolred was released in 20 minutes. This is considered a burst release.

However, the release behavior changed with the thicker film and the burst release profile disappeared as indicated by curves (3) to (5) in Figure 10. As discussed in the proposal, a negative voltage applied to a PPY/Phenolred film will reduce the PPY film from a positive stage to a natural stage where phenolred, the negative counter ion, will be expelled from the PPY film by the negative voltage. For the thin film, phenolred was released into the solution immediately and a burst release was observed. For the thick film, most of the phenolred within the polymer film in principle has to migrate to the film surface and then get released. This migration will take a much longer time, which explains why the burst release disappears and a diffusion controlled release is observed.

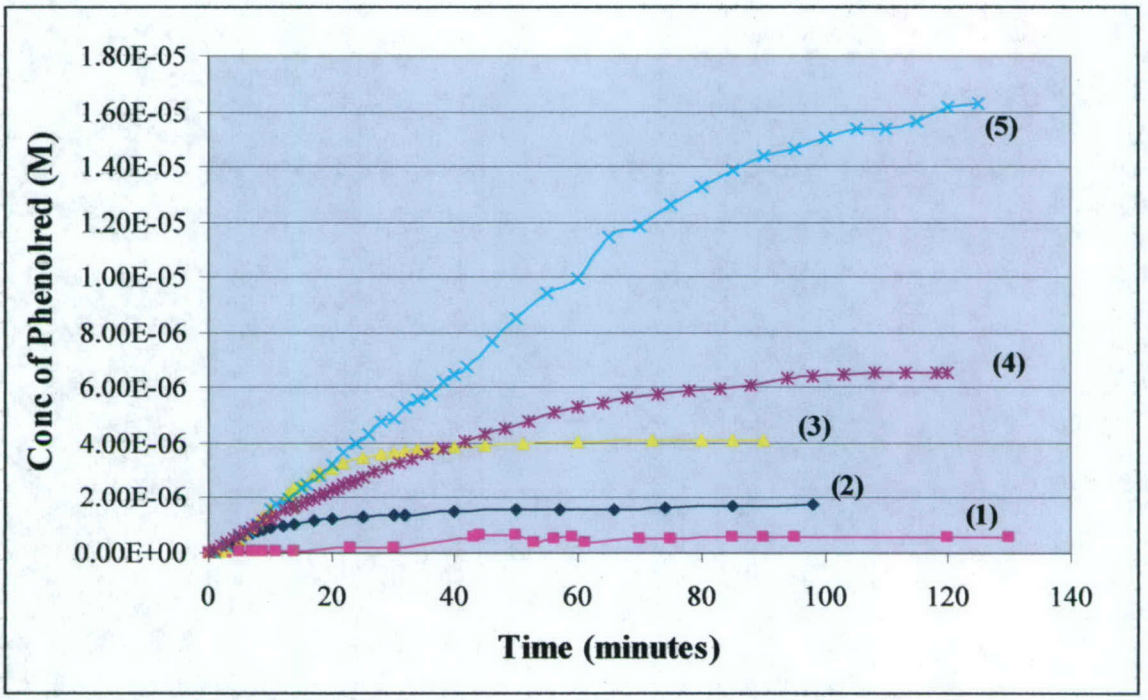

Figure 10: Release Profiles of Phenolred from PPY in Tris-Buffered Saline

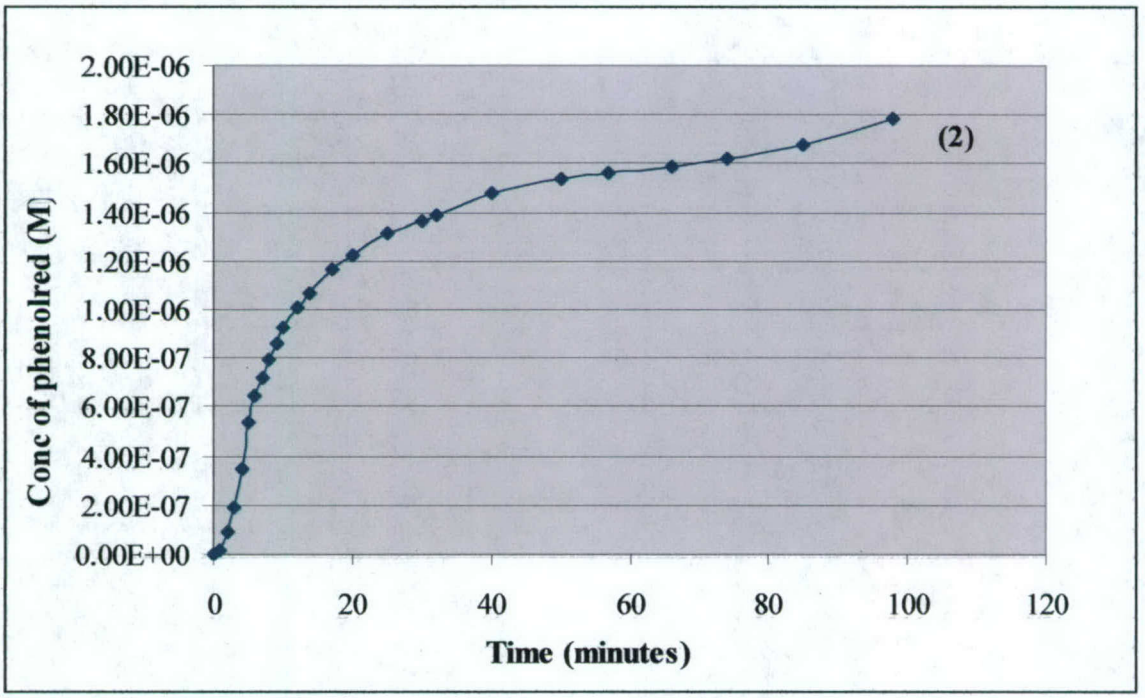

Figure 11: Release Profile of Phenolred from Thin PPY Film in Tri-buffered Saline

This new result has not been discussed in any related literature and is also critical to the design of our triggered release device. For example, if the burst release is required for our device, the film has to be thin enough to achieve the goal. Further experiments are required to fully understand this result.

In conclusion we have demonstrated that small carbon based electrodes were developed and prepared via our in house printing methods. The depositions of Polypyrrole/Phenolred or Ampicillin films on the carbon electrode have been established and films with different thickness have been made via an electropolymerization method. The analytical method for measuring the released dopant such as phenolred was developed. The measured absorbance of the released dopant by a UV-Vis spectrometer in solution was used to determine the amount of the released dopant. The release profiles of PPY/Phenolred in solution at different conditions were obtained with the developed analytical method. The results showed the release mechanism under the applied voltage is associated with the film thickness and the burst release changed to the diffusion controlled release when the film thickness increased. A small multi-carbon electrode device was designed and prepared where the deposition of PPY/dopant film on each electrode is underway.

d. Progress on Self-Assembled Layers

The primary objective in this section is to develop the appropriate process to bond the proteins, antibodies, or oligonucleotides to the carbon surface of the CPMEMS. Work on this objective to date has focused on building the molecular bridge that will allow us to detect the binding of an agent such as bacteria, viruses, or proteins to antibodies that are anchored to a glass slide. The same technology will be used to attach antibodies to the carbon-coated slides. The glass is first cleaned with water, ethanol, and then diethyl ether before spotting a thin coating of nitrocellulose (0.5 μl), which provides a substrate to which antibodies bind. This step is necessary to allow other molecules to be bound since few biological molecules can stick to the glass. To determine whether antibodies would bind to our nitrocellulose-coated slides, we utilized antibodies that have a fluorescent dye attached to allow for detection (Figure 12). UV light causes the antibodies to emit green light that we can detect using a fluorescent microscope. In our studies we have found that the amount of light detected is directly proportional to the amount of antibodies added to the nitrocellulose.

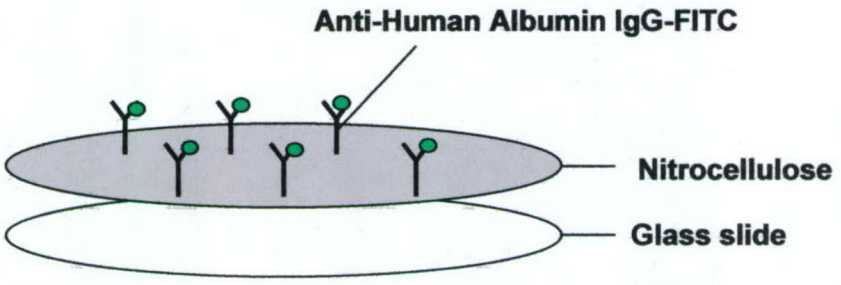

Figure 12: Attachment of Antibodies to Nitrocellulose

Recently, we have shown that if antibodies that recognize E. coli bacteria are bound to the nitrocellulose, it is possible to capture bacteria with the antibodies (Figure 13). In this experiment, we utilized bacteria that express green fluorescent protein which allows for easy detection if bound to antibodies. Binding of the fluorescent bacteria was detected using an Olympus fluorescent microscope and photographed using a Nikon digital camera. Thus, these data provide evidence that antibodies bound to a layer of nitrocellulose on a glass slide can be used to capture bacteria in solution (Figure 14). Thus, we have achieved one of the main goals of this subaim to create a molecular bridge to detect the presence of bacteria in solution. In future studies, we will build this molecular bridge on a slide that is coated with the carbon biopolymer and design an array such that we can quantitate the number of bacteria binding to a range of antibody concentrations.

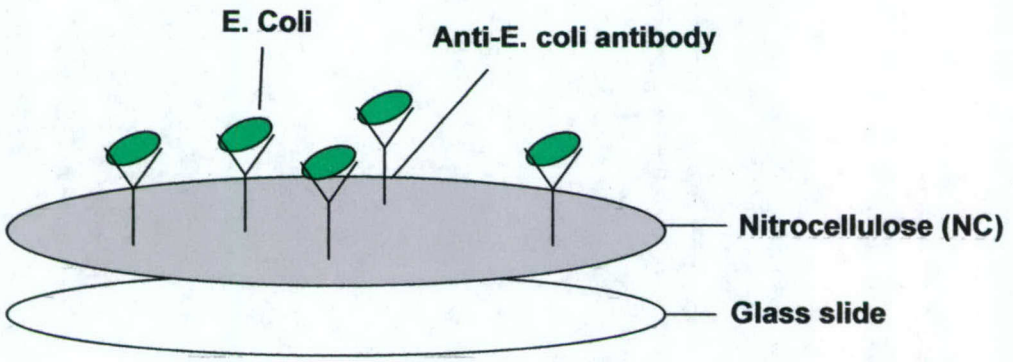

Figure 13: Captured E. Coli bacteria with antibodies. Note: E. coli bacteria are expressing green fluorescent protein.

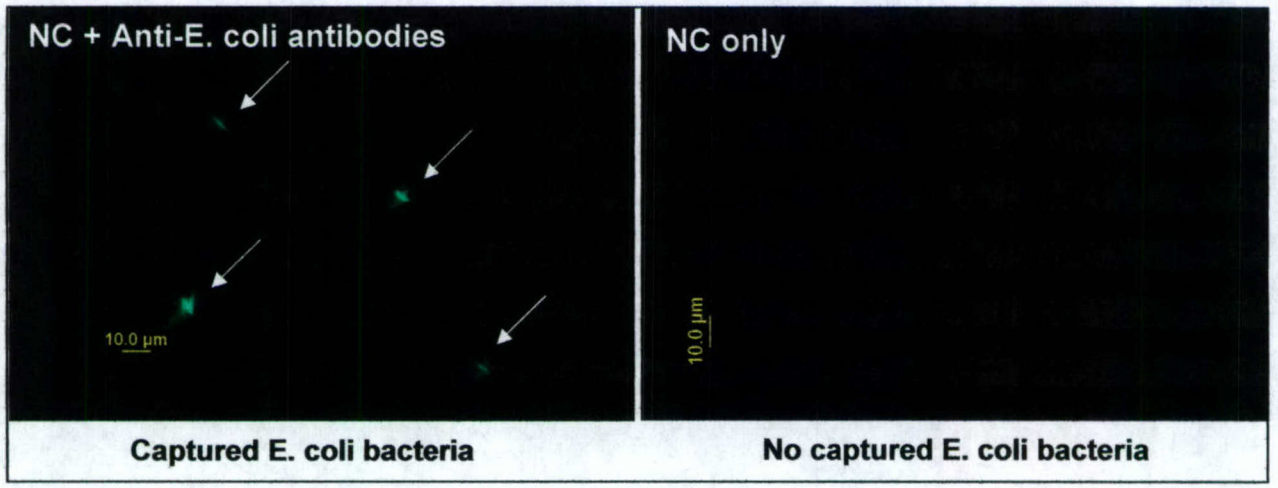

Figure 14: Fluorescent microscope photographs

e. Progress on the Bio-Collection System

The previous interim report discussed the utilization of an alternate bio-collection technology. The air purification system called ISAVAC was purchased and was investigated to determine whether or not it could be developed into a viable bio-collection system. The system was designed for environmental control by means of airborne particulate removal including pollen, bacteria, smoke, fumes, dust, plant spores, etc. The system was modified such that the original function of removal of airborne particulates was converted to collection of those same airborne particulates. Current work on this portion of the research is to scale the system to a smaller version of the main collection system to make it more portable.

Other systems apart from the ISAVAC system were also investigated. Sceptor Industries Inc, has a collection system called Spincon Advanced Air Sampler. The system has a collection rate of about 16 cfm. Dimensionally it is very portable with a size of L x W x H of 18" x 15" x 8" and a weight of 46 lbs. The system has the ability to collect, retain, and concentrate particles in solution, as well as collect particles sizes down to 0.2 microns. The ISAVAC system has a collection rate of approximately 600 cfm. Current work on design and construction of a small scale version of the system will allow for an approximate collection rate of 100 cfm. Particle size collected is estimated to be similar to the Spincon system. The system will be geared to be a collection system rather than an air purification system as was its original function. This would include the ability to collect, retain, and concentrate particles in solution.

III. Conclusions

a. Accomplishments and Future Work

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

1. Began investigation of Unity 400 heat dissolvable polymer (at Brewer Science, Inc) as an alternative to a SiO_2 sacrificial layer. BSI has studied the thermal decomposition kinetics of Unity 400 as well as began a study to determine the appropriate RIE recipe.

2. Located and been using outside ion implantation services to accommodate the current delay during the downtime of the SMSU ion implanter.

3. Finished development of an electron beam deposition system, as an alternate growth method of SiO_2, apart from the current RF magnetron sputtering method. Testing and calibration of the new system is currently underway.

4. Developed a lithography mask set with a 16x16 array of devices for testing the steps in the CPMEMS fabrication process.

5. Began development of a wafer level packaging scheme that allows for multiple dies to be packaged at once.

6. Began development of the active sense and release experiments (Crosslink) including: electropolymerization of films on carbon electrodes; developed an analytical method for measuring the released dopant; measured absorbance of released dopant; and obtained release profiles at different conditions.

7. Demonstrated that antibodies bound to a layer of nitrocellulose on a glass slide can be used to capture bacteria in solution. Indicating that we have created a molecular bridge to detect the presence of bacteria in solution.

8. Began design work on scale down and modification of the air purification technology (ISAVAC) as an antigen collection system.

In the months ahead, our work will include:

1. Continued work on refining and optimizing the microlithography process to fabricate the CPMEMS devices. Including the development of Unity 400 as an alternative to SiO_2.

2. Completion of the programming and testing of the ion implanter ion source and testing completion with regards to the electron beam system for SiO_2 growth.

3. Continued development of a wafer level packaging scheme.

4. Continued development of the active sense and release experiments including: the testing of biological activity from a drug released by the electrode; the incorporation of green fluorescent protein into the film and the characterization of the release; and the demonstration of an addressable electroactive polymer-drug release device coupled to a BioMEMS sensor.

5. Future studies in the area of the self-assembled layers will be to build a molecular bridge on a substrate that is coated with an ion implanted polymer (carbon) layer and design an array such that we can quantitate the number of bacteria binding to a range of antibody concentrations.

6. Continued development and scale down of the antigen collection system using ISAVAC technology.

REPORT DOCUMENTATION PAGE

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Research in collaboration with Brewer Science, Inc. (Rolla, MO) and Crosslink (St. Louis, MO)

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 report discusses the progress to date on the biosensors made from CPMEMS and the multiple strategy bio-detection sensor platforms that incorporate these CPMEMS. An alternative sacrificial layer in the individual CPMEMS layer processing in being developed using a thermal release polymer. Development of a test lithography mask set and a wafer level packaging scheme in underway. Advancements in the areas of active technology in the form of sense and release experiments and the molecular bridge preparation of the CPMEMS surface have been made.

15. SUBJECT TERMS

CPMEMS, Ion Implantation, Unity 400 Thermal Release Polymer, Active Sense and Release, Platform Fabrication, Wafer Level Packaging, Collection System (ISAVAC).

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