Impedance Analysis and Antibody Immobilization to Differentiate Methicillin-
Resistant Staphylococcus Aureus

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is an antibiotic resistant staph infection that impacts many individuals in the United States. At the moment, there is no revolutionary diagnostic tool for MRSA; either turnaround time is large or the procedure is expensive. Therefore, the goal of this project is to find a quicker cost effective diagnostic tool for MRSA screenings. Develop a quick and cost effective method to detect if a patient has (MRSA) is the need statement for this project. When researching different diagnostic tools, it was determined that micro-electromechanical systems (MEMS) would be the best method for MRSA diagnosis due to the quick turnaround times and inexpensive test costs. The MEMS device we propose to create will use changes in impedance to determine if MRSA is present. This process works by immobilizing antibodies on the surfaces of gold electrodes which have a high specificity towards MRSA. Everything that is not MRSA will not bind to the antibodies and will flow out of the MEMS device. When MRSA does bind to the antibodies this will cause a change in impedance which we can measure with an impedance analyzer.

Introduction

MRSA is a bacterial staph infection that is passed through skin contact. This bacteria has built up a resistance to antibiotics which makes it difficult to kill. If the bacteria is left untreated it can enter the bloodstream from a cut. At that point, it can infect the heart and liver which will ultimately lead to death (6). This is the main concern with MRSA in hospitals because many patients have open wounds and are more susceptible to catching infections. The most common way to test for MRSA takes 48 hours and in that time a patient can spread the disease to other patients or even health care professionals (6). Over the summer, a team met with Maureen Raab, Susan Allen, MD and Rocco Ottolino at the elderly care facility in Beaumont Hospital. One of the ideas that was discussed was a dip stick device for MRSA testing. They suggested that the development of an easier and quicker test would greatly improve their facility.

The unmet need we are pursuing is to develop a quick and cost effective method to detect if a patient has MRSA. There are many stakeholders in our project. Hospitals and nursing homes already test for MRSA and would buy our device even if it costs more as long as it functioned better. It could be used in school systems after an outbreak occurs like the recent outbreak at Novi high school. It could determine which students are infected in order to stop the spread of the bacteria. Some hospitals are even looking into screening all of their incoming patients and VA hospitals already screen all of their patients. In 2008 the VA treated 773,000 patients and if our device was set at the price on the standard test then there would be a market value of 30.94 million; just in VA hospitals alone (9).

There are currently only two diagnostic methods that are used to detect MRSA clinically; Chart 1 compares these methods. One method is plate based culture screenings where the samples are
placed in a customized dish of nutrients that encourage bacteria growth in order for the sample to grow large enough for analysis. This test is easy to conduct and doesn’t cost much but the detection time takes around 48 hours; it can even last up to 72 hours (7). A newer method utilizing a polymerase chain reaction (PCR) that only takes a few hours for detection results is now being used in some clinics (7). The downside to this technique is the extremely high cost of the equipment required to perform the PCR. Recent advances in micro-electromechanical systems (MEMS) have allowed the fabrication of what is referred to as lab-on-chip devices to be used in the detection of infections. Currently all these devices are in the research development stage and can be characterized into four different groups; magnetic, mechanical, electrochemical and optical. In general, when compared to the clinically used methods, MEMS devices are cost efficient, have high sensitivity, and have a one hour or less detection time (2).

Chart 1: Current MRSA Diagnostic Methods

<table>
<thead>
<tr>
<th>Device Design</th>
<th>Development Stage</th>
<th>Cost</th>
<th>Detection Time</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate Based Culture Screening</td>
<td>Clinically Used</td>
<td>$40 Per Test</td>
<td>48 Hrs-72 Hrs</td>
<td>&gt;85%</td>
</tr>
<tr>
<td>Polymerase Chain Reaction (PCR)</td>
<td>Clinically Used</td>
<td>~$30K Total</td>
<td>3 Hrs-4 Hrs</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>MEMS Methods</td>
<td>Research</td>
<td>$40 Per Test</td>
<td>&lt;1 Hr</td>
<td>&gt;95%</td>
</tr>
</tbody>
</table>

Device Design

There are specific parameters and stages that are needed to consider when diagnosing MRSA. Before one can detect if a patient has the infection they need to separate the MRSA bacteria from every other type of cell. The reason concentration is a factor is because we need to consider how it will impact sensitivity. When you pull a swab from either the nares or blood there is only a set amount of MRSA bacteria in that sample. This is why amplification is needed in clinically used methods. The last consideration needed is how to detect the actual MRSA cells. This can be related to concentration because with detection you either need a large amount of MRSA cells or you need to have a device that is very specific in order to detect a smaller amount of cells (4). Our goal for the project is to focus on the detection mechanism of this diagnostic process. In order to understand detection, we must first understand the distinctive biology that MRSA cells possess. The presence of MRSA can be isolated using a highly specific binding interaction. Research shows that a protein known as PBP-2a controls the mecA gene and this is precisely what differentiates MRSA from all other staph bacteria (3). Because of this specificity, anti PBP-2a proteins could be used to bind to the cell wall of MRSA cells. Antibodies work well in this application because they only accept the antigen or protein that fits into the antigen-binding site. When an antigen reaches an antibody that does not have a matching antigen-binding site no polypeptide bond will form. This is the principle we will be applying to our project (5).

Our team, MRSA Diagnostics, propose to design and fabricate a MEMS Biosensing device for the detection of MRSA. This device from the bottom-up contains a glass slide with two electrodes on its top surface and is shown in Image 1. The electrodes will have antibodies present on their surface. PDMS is also used and comprises of a flow channel that is bonded directly to the glass surface and will comprise of the electrodes in its channel. The device will
contain an inlet followed by a flow channel that flows over highly sensitive electrodes in which PBP-2a antibodies will be contained on its surface. In order to get our sample into the device we will inject a sample into the inlet with a needle and syringe. When the solution flows over the antibody covered electrodes the MRSA bacteria will bind to them. Since this binding isn’t visible to the naked eye we will then use an impedance measurement to detect the impedance change resulting from the bound antibodies to the antigen. The gold electrodes are precisely what allow us to measure impedance. The specific impedance values and differences will be determined during the testing stages of our device. Our concept is novel in that no impedance measurement method exists to detect MRSA. This concept is extremely cheap and detection time is almost immediate since no sample amplification is needed.

Image 1: 3D image of the concept

In order to construct the prototype there are several steps that will be conducted. First we will fabricate our device. The next step involves immobilizing the antibodies on the electrodes’ surfaces. We will also simultaneously run flow simulations using COMSOL to mimic the fluid dynamics that will occur within the device channel due to the channels geometry. The last steps involve testing device functionality. We are going to fabricate our device beginning with the electrode containing glass slide, the process can be found in Diagram 1. To begin this fabrication process, a glass slide will be obtained. The slide will undergo sputtering thin-film deposition. The next step involves a lithography process in order to define specific areas for the gold electrodes. The last step utilizes a wet etching technique to clear out the unwanted gold sections. The specific mask that will be used during the lithography process, will be provided to us per Dr. Auner’s lab, since it already expresses the design needed for our application. After we fabricate the electrode containing glass slides, we will create the PDMS substrate that will ultimately bind to the glass slide; the process is shown in Diagram 2. We will use a soft lithography protocol to create the channel containing PDMS. The first step is to create a master mold which is resembled in the first three steps on Diagram 2. This is accomplished by applying a thin layer of negative photoresist and applying a lithography process in which unwanted photoresist is removed. After this process is complete, we will have a master mold which is
when we would pour the PDMS solution. Finally, we will cure the PDMS and peel it off the master mold. This is when we will be left with just the blue layer. Before bonding the PDMS to the glass slide, we will immobilizes antibodies with the antigen-binding site vertically positioned. The main issue scientists run into is having too low or too high of shear stress meaning the bacteria flows over the electrodes’ surfaces without binding to the antibodies and ends up being washed away to the outlet. Flow simulations will determine the size and speed at which the sample should be inserted into the device. After successfully immobilizing the antibodies onto the electrode the two fabricated pieces, PDMS and the glass plate, can be bonded together. We decided to bond them by oxygen plasma treating the PDMS and then compressing the two layers together to make them adhere.

Diagram 1: Electrode containing glass slide fabrication process

Diagram 2: PDMS channel fabrication process
Methods

After completing fabrication, antibody immobilization, and device bonding, we will inject the staph solution into the inlet and allow flow dynamics to push the solution towards the electrodes. For this project, we will be testing with MRSA, which is a bio level 2 hazard. Due to its infectious nature, we will be receiving training from Wayne State University and working with a trained professional at all times. We will also be testing with methicillin-susceptible *Staphylococcus aureus* (MSSA) as a control. Our goal is to show that our device can detect MRSA even if other strains of staph are present. After we input a sample of MRSA and MSSA into our chip, MRSA will react to the Anti PBP-2a because of the Penicillin-Binding Protein 2 (PBP2) in the cell walls of the MRSA (4). This will cause a change in the impedance which we will be able to measure. Since the current through our electrodes alternates based on the bonding that occurs, it is crucial to measure impedance rather than just resistance. This will allow us to factor in the phase changes that occur. When we are using MSSA, there should be no capturing involved resulting in very minimal to no change in the impedance. We will use an impedance analyzer to measure impedance and plot the impedance versus the concentration. Graph 1 represents one of the types of graphs we plan on creating during our project but instead of plotting growth we will be plotting the different strains of staph. In order to validate our testing, we will be using Raman Spectroscopy. With this technique, we will be able to identify our sample and quantify them. This technique uses a laser to scatter particles and detects the Rayleigh or elastic scattering (1). With this machine, we will be able to look closely at our sample to see if there truly is a difference after undergoing the binding process. We will use the same sample concentration of staph as we do when testing our MEMS device. This way we will be able to compare our results.

Graph 1: Data collection example graph
Timeline

Due to the fact that we only have five months to work on this project we decided our first goal is to create a working prototype. Also, we need to test the functionality of our device to prove it works as well as or even better than standard methods. After we have a prototype completed, we will submit a provisional patent to protect our idea. Our last goal is to submit an abstract to the National Biomedical Engineering Conference in order to share our ideas with other scientists in the field. We will be doing an impedance study and analyzing how it affects the detection of MRSA cells. We will be using a mask provided from Wayne State University which will give us the design of our MEMS device. We will be testing an application of the device to see how we can repurpose it for detecting MRSA.

Our main priority at the moment is to have materials ordered in December. Then in the first weeks of January, our team will undergo three different types of training and preparation including OSHA training, Biosafety training, and equipment use training. We will also start fabricating in January and have our first device completed by the end of January. We will undergo testing in February and create design revisions when necessary throughout the month of March. Also, we have included a buffer week in case something pushes our schedule off course. Timeline 1 shows our course of action for this project. Because there are only two of us working on this project we plan on doing most parts of the project together. Our takes are outlined in the M diagram shown in Diagram 5. We are both required to complete the OSHA training and we want to collaborate on our device design. Even though we are working together on all aspects of this project each one of us is an expert on a certain part of the project and will oversee that aspect. Amanda will be in charge of fabricating and understanding how the fluid dynamics will impact our device. This will include looking at flow simulations to determine the fluid dynamics. Kaitlyn will be in charge of the testing procedures which includes validation testing. She will be in charge of the biochemical side of the project and will make sure that we achieve the desired results. We will be communicating through text, emails and our shared calendar; we will be going to Wayne State together.

Timeline 1: Project timeline
Our budget consists of our base material which is glass, our PBP-2a antibodies, our travel expenses to Wayne state for fabrication and testing, and other unexpected expenses. All in all they total roughly $1,660 dollars and the full budget can be found in Budget 1. LESA has funded us 1,500 dollars for our project and all other materials and resources will be provided to
us by Wayne State University. This includes any fabrication material, electrodes, staph bacteria, tools and equipment use. The majority of our budget is going towards antibodies because they are the most crucial part of our design because without them they would be no binding to cause a change in impedance.

Conclusion
Overall this project will greatly improve the health care industry. MRSA is a growing problem in many hospitals. Quicker diagnostic times will help health care providers determine who has MRSA and treat them appropriately in order to stop them from spreading the disease. The low cost of a MRSA device would allow many people access to our device. This is the impact our device will have once it is complete.
Appendix

Acknowledgements

We acknowledge the individuals who have helped us in pursuing our project goals. That includes Wayne State’s Smart Sensors and Integrated microsystems lab for providing us with training, machinery and raw materials in order to work on our project. Also, our advisors who have been guiding us throughout this project. Lastly, the LESA foundation for providing us with the grant necessary to complete our project. Our team will continue this development process using an impedance MEMS Biosensor for the detection of MRSA.

Clinical application

Even though we are only working on the detection mechanism of MRSA, there are still unique steps that need to be taken in a clinical setting to obtain a testable sample for our device. These steps will be outside the scope of our project but we envision the steps to be included in our devices future revisions. In order to complete our first separation a blood sample can be taken from the patient as shown in part one of Diagram 4. In step 2, filter paper could be used to only allow cells that are smaller than 1.5 microns to flow through leaving a solution of bacteria because human cells are the order of 2-120 microns (8). Step 3 is when Antibiotic coated magnetic beads at the nano-scale size would be used to capture and control all the bacteria with the exception of staph bacteria. The magnetic beads containing the bacteria will then be disposed of leaving us with the staph bacteria solution. These three steps are steps that would be taken in an actual clinical laboratory setting to have a testable sample of strictly staph bacteria to insert into our detection device.

Diagram 4: Clinical/Laboratory steps
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