

Public Abstract

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Title:Rapid detection and characterization of Mycobacteria using microchannel Electrical Impedance Spectroscopy

The presence of virulent, pathogenic bacteria in our body, food, water and other consumables is harmful and causes enormous economic and personal losses. Here we will be looking at developing a platform technology that can rapidly detect pathogenic bacteria. The platform will be based on an electrical spectroscopic method called microchannel Electrical Impedance Spectroscopy (m-EIS). Initially, we will look at the growth of bacteria as our primary method to detect the presence of bacteria and find the time-to-detection. Following this, we will study the death of bacteria in a suspension using our technique of m-EIS to reduce the time-to-detection further. We want to detect the pathogenic Mycobacteria Tuberculosis (that causes Tuberculosis in humans), which according to WHO, is the second leading cause of death due to infectious diseases. As proof of concept, surrogates like *Mycobacterium bovis* BCG and *Mycobacterium smegmatis* will be taken up for detection using our technique of m-EIS. However, our first focus is to prevent surgical site infections.

Transmission of contagious infections like tuberculosis or infections occurring post-surgery like surgical site infections can be reduced and prevented, to some extent by proper treatment and correct use of antibiotics. For example, surgical site infection, occurring after insertion of implants can be reduced by use of coatings and other surface modifications. Many of these amendments ensure prevention of bacterial adhesion and kill surrounding bacteria near the implant. Further, in some cases, they promote the growth of desired cells that warrants proper integration of the implants with the bone. Surgical site infections occurring post-surgery can be reduced by following proper preoperative skin preparations. Several techniques are applied in the hospitals like 2-step scrubbing and painting, 2-step scrubbing and drying, and 1-step painting with a drying time. However, most of these techniques are time-consuming and labor-intensive. Here, we have demonstrated that the antimicrobial efficacy of a spray-on formulation containing Betadine is comparable to the existing techniques. The spray-on Betadine formulation is significantly less time-consuming and is not labor-intensive.

Though prevention is necessary, often time, the pathogens have to be detected and identified as early as possible. In recent times, several molecular, serological and proteomic-based methods have been developed. However, these methods have several disadvantages such as they are expensive, labor-intensive, bulky, among others. The culture-based techniques are considered the gold standard. Though sensitive, they too suffer from inherent disadvantages of long time-to-detection. Hence, there is an urgent need for the development of rapid and cost-effective techniques for detection and identification of bacterial pathogens.

Here, we present an approach that can detect the presence of viable microorganisms in suspensions, much faster than culture-based technique. The existing automated culture-based systems detect the metabolic changes in the growth media and the environment of the bottles containing the media (parameters monitored like pH, Oxygen, Carbon Dioxide among others) that change as the bacteria proliferates. As these changes in pH, Oxygen and others can be minute, a large number of bacteria is needed before the changes can be detected. This increases the time-to-detection significantly for culture-based techniques. Our technique, microchannel Electrical Impedance Spectroscopy (m-EIS), relies on the fact that on the application of an AC electric field to a bacterial suspension, the viable bacterial cells become polarized and store charges due to the presence of intact cell membranes. As a result, they behave as electrical capacitors. Any change in the number of bacterial cells in the suspension (like growth or death of cells) is

reflected by a concomitant shift in the storage of bacterial charges and capacitance of the bulk. Due to the unique geometry of the measuring device (microfluidic cassettes) used by us, we can distinguish between the capacitance arising due to the bacterial cells and that of the parasitic double layer capacitance.

A practical application of this technique has been found to be useful for detection of clinically significant slow-growing mycobacterial cultures. Most of the automated culture-based systems available in the market are based on the measurement of the growth dynamics of the microorganisms. However, as the generation time of the slow growing bacteria is long, these systems take a long time (6-8 weeks) to generate results. With the use of our m-EIS measurement technique, we can reduce the times-to-detection by ~50%.

Further, we observed that the time-to-detection is further reduced by monitoring cell death in real time using our technique. As only living entities can be killed, our technique can detect the presence of viable cells in a suspension by monitoring their death. It has been observed that using our technique following the death dynamics is much faster than growth dynamics. The death of microorganisms that have long generation times like mycobacteria can be achieved by use of antibiotics (depending on which antibiotics and its concentration) at a much faster rate than their growth in a nutrient media. Real-time monitoring of death shows a decrease in the bulk capacitance values which provides us the time-to-detection much more rapidly.