Introduction

There are a number of challenges in improving the diagnostic process in the healthcare industry. Least of which is finding ways to quickly and accurately test for particular compounds associated with different diseases and conditions. Traditional methods for detecting the presence of these compounds are both time consuming and costly. This is especially restricting in countries without well-equipped or maintained laboratories [1]. Having a method that provides quick, low-cost and reliable screening would save thousands of dollars annually and allow for quicker diagnoses. A detection method that satisfies these criteria is the Heat-Transfer Method (HTM), where changes in thermal resistance can be monitored in real-time to determine the presence of specific analytes [2]. This talk will discuss three classes of biomedical analytes (cardiac biomarkers, bacteria, and antibiotic drugs) that were studied using thermal detection, and will highlight artificial recognition elements we have developed and implemented in our sensors. Each analyte poses a unique challenge in terms of recognition and detection as they encompass a range of shapes, sizes and chemistry.

Methods

Two different cardiac biomarkers were studied using imprinted nanoparticles (nanoMIPs) specifically designed to have high binding affinity to the individual proteins [3]. The nanoparticles were subsequently deposited onto multiple thermocouples via dipcoating and the thermocouples were then inserted into a custom flow cell. To examine the use of thermal detection for bacteria, the growth of a model organism, *Staphylococcus aureus,* was monitored [4]. This was done in both buffered solutions and wastewater samples to determine the effect a complex medium has on the measurements. In combination with these measurements, imprinted polymers were developed for *S. aureus* using photopolymerization. The final analytes examined were beta-lactam antibiotic drugs, including nafcillin and amoxicillin. Imprinted polymers were used as the receptor for the sensor with their binding capabilities compared on various substrates. Polymers for each drug were generated in two different ways: microparticles formed after bulk polymerization, and thin films via photopolymerization. Both were deposited onto screen-printed electrodes and the thin films were also deposited onto borosilicate glass.

Results and Conclusions

The nanoMIPs showed comparable affinity to the biomarker proteins as commercial antibodies, and the multiplex design for the thermocouples allowed for detection in the 4-8 ng/mL range, well within the range of physiologically relevant concentrations. Optimization of the flow cell with the multiplexed array was conducted, showing that the positioning of the thermocouples with respect to the flow through the chamber will influence the measurement. When examining the behaviour of bacteria, a change in thermal resistance was measured as the bacterial colony grew even in the complex digestate solutions. Specific changes in their morphology were detected at higher temperatures, as determined by SEM and resistance changes. SEM images showed the presence of *S. aureus* binding sites in the imprinted polymer as well as partial rebinding after the introduction of a bacteria-containing solution. For the antibiotics, the thermal resistance increased upon target binding for both microparticles and thin films, which was translated to a LOD of 1.89 ± 1.03 and 0.54 ± 0.1 nM, respectively. Changes to the polymer films were then investigated, including a change in substrate and the introduction of a fluorescent moiety. Overall, these results show how versatile thermal detection can be in terms of sensor design while still providing fast, accurate, and easy-to-read results. In combination with low-cost detection elements, they could provide an avenue to accessible diagnostic tools for less developed areas.

[1] Kimani, F. W.; Mwangi, S. M.; Kwasa, B. J.; Kusow, A. M.; Ngugi, B. K.; Chen, J.; Liu, X.; Cademartiri, R.; Thuo, M. M. Rethinking the Design of Low-Cost Point-of-Care Diagnostic Devices. *Micromachines* 2017, *8* (11), 317.
[2] Eersels, K.; van Grinsven, B.; Peeters, M.; Cleij, T. J.; Wagner, P. Heat Transfer as a New Sensing Technique for the Label-Free Detection of Biomolecules. In *Label-Free Biosensing: Advanced Materials, Devices and Applications*; Schöning, M. J., Poghossian, A., Eds.; Springer International Publishing, 2018; 383–407.
[3] Crapnell, R. D.; Canfarotta, F.; Czulak, J.; Johnson, R.; Betlem, K.; Mecozzi, F.; Down, M. P.; Eersels, K.; van Grinsven, B.; Cleij, T. J.; Law, R.; Banks, C. E.; Peeters, M. Thermal Detection of Cardiac Biomarkers Heart-Fatty Acid Binding Protein and ST2 Using a Molecularly Imprinted Nanoparticle-Based Multiplex Sensor Platform. *ACS Sensors* 2019, *4* (10), 2838–2845.
[4] Betlem, K.; Kaur, A.; Hudson, A. D.; Crapnell, R. D.; Hurst, G.; Singla, P.; Zubko, M.; Tedesco, S.; Banks, C. E.; Whitehead, K.; Peeters, M. Heat-Transfer Method: A Thermal Analysis Technique for the Real-Time Monitoring of Staphylococcus Aureus Growth in Buffered Solutions and Digestate Samples. *ACS Appl. Bio Mater.* 2019, *2* (9), 3790–3798.