

L. Confalonieri¹, S. Direito¹, S. Titmuss¹, S. Minett², A. Sheward², R. Allen^{1,3}.

¹School of Physics and Astronomy, The University of Edinburgh, Edinburgh, UK; ²KIMAL, Bromsgrove, UK; ³Institute of Theoretical Microbial Ecology Institute, Friedrich Schiller University, Jena.

l.confalonieri@sms.ed.ac.uk

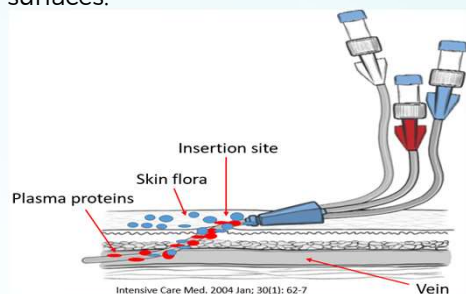
Abstract

The formation of bacterial biofilms on venous catheters is a major cause of failure, resulting in the need for invasive procedures to remove and replace the catheters, as well as the risk of lethal bloodstream infections.

A standardised method for real-time biofilm detection in hospital settings is still missing. Working with Kimal, a leading manufacturer of medical devices, we are investigating the role of pH for in-situ biofilm detection.

Introduction

Colonising bacteria from the skin flora are forming biofilms on the catheter surfaces.



Gradual pH changes have been reported to occur in biofilms as a result of their metabolism. By embedding pH-sensitive dyes in the catheters' coating, we are aiming to reveal the bacterial colonisation of plastic catheters by a simple colour change.

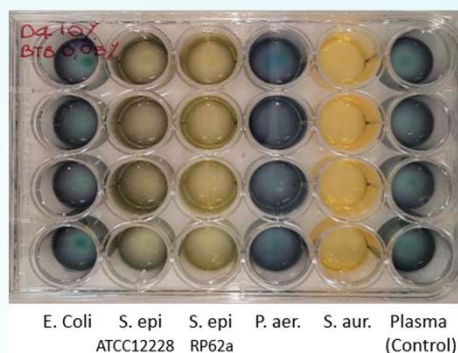
Materials and Methods

Biocompatible polyurethane hydrogels were combined with pH-sensitive dyes to obtain pH-sensitive films which are not prone to leaching. Some of the most relevant bacteria for catheter-related infections were cultured in human plasma from healthy donors from the Scottish National Blood Transfusion Service.

Results

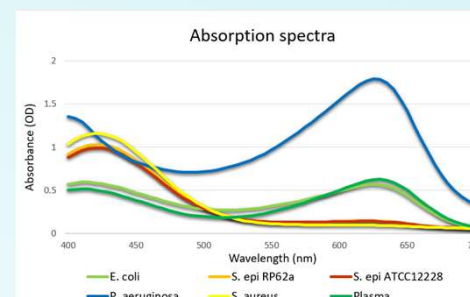
24-well plates were coated with a pH-sensitive film (~100µm thickness), filled with plasma (control) and different bacterial cultures, then incubated at 37°C for 24h.

The observed colour change in response to bacterial cultures' growth is shown:

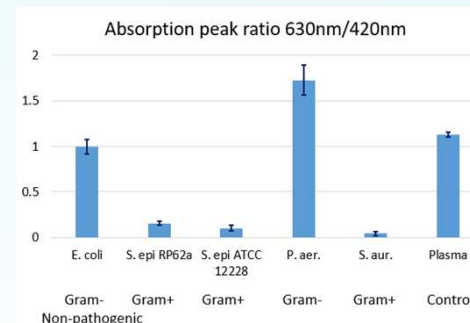


- The film turns green in contact with plasma, which is at physiological pH.
- *S. epidermidis* ATCC 12228, RP62a and *S. aureus* NCIMB 12702 cultures are more acidic (yellow).
- *P. aeruginosa* PA01 one is more alkaline (blue).
- *E. coli* MG1655 reference strain is non-pathogenic; it does not grow in plasma and does not modify its pH, so this culture appears green.

The colour change was quantified via absorption spectra acquisition.



The peak ratio of the red and the blue components of the spectra is almost binary. It could be a promising tool to identify classes of growing pathogens (Gram+, Gram-).



Further Work

The pH change was found to be dependent on bacterial growth conditions. This stimulated further studies of metabolic pathways simulations via Flux Balance Analysis.

Confocal microscopy imaging and ratiometric pH sensing of biofilms grown in static conditions, to mimic the catheter insertion site, are in progress.

Conclusions

This work aims to provide a proof of concept for the potential use of pH-sensitive dyes for in-situ biofilm detection on medical devices. It opens the perspective for future applications in the health sector for a prompt identification of pathogens and a rapid selection of the proper antibiotic treatment.

Acknowledgments