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Abstract: Endoscopic point sensing of bacterial infections in the distal lung *in vivo* is challenging. Here fluorescence imaging agents are combined with time-resolved fluorescence spectroscopy to overcome limitations from tissue fluorescence by measuring the fluorescence lifetime.

1. Introduction

In the dawn of multiple drug resistant (MDR) infections, it is important to identify lung infection and inflammation *in situ* rapidly, accurately and less invasively. One approach to tackle this challenge is by combining optical imaging agents with imaging or sensing through optical fibres. This has previously been approached with different imaging modalities - spectral, ratiometric, and time resolved. These techniques can each be limited by distortions from the imaging fibre bundle and tissue autofluorescence, however, time-resolved fluorescence spectroscopy (TRFS) is able to the overcome inherent drawbacks of intensity-based spectroscopy such as photobleaching, detector distortions, fibre background and tissue autofluorescence by utilizing the fluorescence lifetime [1,2]. Moreover, complementary metal-oxide semiconductor (CMOS) single photon avalanche diode (SPAD) line sensors are becoming increasingly popular for applications such as TRFS due to their high multiplexing capabilities [3,4,6]. The time-correlated single photon counting (TCSPC) capabilities of these line sensors allow the simultaneous generation of arrival time histograms for all 256 pixels. The pixels are correlated to different wavelengths enabling the recording of a full spectrum in one shot. This allows the investigation of the response from excited exogenous fluorophores in three dimensions to enhance selectivity and sensitivity: fluorescence intensity, fluorescence emission spectral shape and fluorescence lifetime. This enables further development of endoscopic point sensing of exogenous fluorophores and optical imaging agents with low excitation powers (< 1 mW) and short exposure times (< 10 s).

Presented here is an approach for optical fibre-based sensing of bacterial infections with clinical molecular probes for direct detection of gram-negative bacteria [5]. While this has been previously demonstrated in fibred imaging modalities [5,6], we demonstrate selectivity without reliance on spatial information offering faster interventions and cheaper disposable fibre probes.

2. Methods and Material

A single standard optical fibre (diameter 50 μm, NA 0.22, Thorlabs) is combined with a time-resolved spectrometer and used for localised point sensing of fluorescently labelled *E. coli* (strain ATCC 25922) on human lung tissue (NHS Lothian Bioresource, REC 13/ES/0126). The robust and versatile time-resolved spectrometer [2,7] is based on four components: a pulsed laser diode (wavelength 485 nm, repetition rate 20 MHz, pulse duration < 1 ns, avg. power 20 μW, PicoQuant), a coupling and collection system based on the design of an epifluorescence microscope, a transmission spectrometer (spectral range 500 nm to 800 nm, spectral resolution 3 nm), and a CMOS SPAD line sensor [2,4,7].

Our bespoke optical molecular reporters are composed of a targeting moiety, a linker and a fluorophore. The receptor molecule allows for identification of gram-negative bacteria by targeting the lipid A in the outer membrane of the bacteria [5]. The fluorophore, nitrobenzoxadiazole (NBD), is quenched in its quiet state and is activated in the presence of gram-negative bacteria (Fig. 1).

3. Results and Discussion

We are able to detect gram-negative *E. coli* bacteria on lung tissue, in fluid extracted from the lung tissue and in an *ex vivo* lung perfusion (EVLP) model through a single optical fiber probe, in measurements with low excitation power ~ 20 μW (which is safe for *in vivo* applications), and with short integration times (10 s). Fluorescence intensity alone is not a reliable parameter when attempting to observe bacteria on lung tissue due to the

heterogeneity of tissue fluorescence, concentration dependency of exogenous fluorophores in the field of view and patient heterogeneity. However, the spectral shape quantised as the mean wavelength combined with the fluorescence lifetime offers a reliable method to detect the presence of labelled gram-negative bacteria (Fig. 2a,b). Furthermore, utilising the same two parameters, we are able to distinguish between live and heat-killed bacteria (Fig. 2c), potentially due to NBD being environment sensitive.

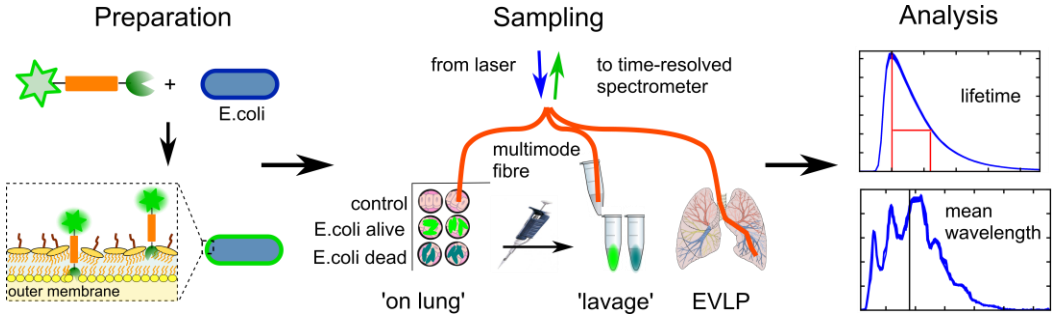

Fig. 1. Schematic of the preparation of the fluorescent labelled bacteria, the sampling on lung tissue an in 'lavage' condition, and the analysis with fluorescence lifetime and mean wavelength determination.

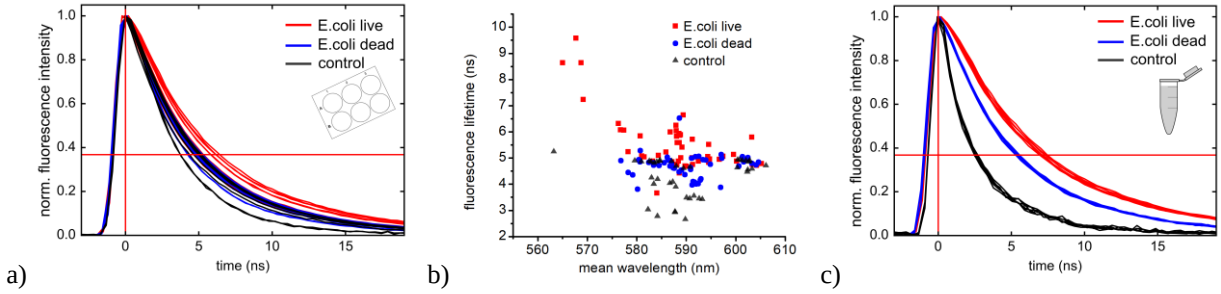

Fig. 2. a) Fluorescence decays of labelled live (red) and dead (blue) bacteria on lung tissue. b) Fluorescence lifetime and mean wavelength of the fluorescence emission of labelled live and dead bacteria on lung tissue showing grouping of conditions. (c) Fluorescence decays labelled live (red) and dead (blue) bacteria in 'lavage' condition showing distinct lifetime differences.

4. Conclusion

We present here the endoscopic point sensing of bacterial infections in size restricted regions such as the alveolar space of the distal lung. We additionally present promising further testing in a pre-clinical model (*ex vivo* human lung perfusion). Early results support the potential use of fluorescence lifetime and the spectral shape of the fluorescence emission to confirm the presence of bacteria with the aid of optical imaging agents and overcome the known challenges from tissue autofluorescence. Changes in both parameters might also indicate changes in the morphology of bacteria introduced through treatments.

Time-resolved spectroscopy also allows the use of off-the-shelf optical fibres and makes complex fibre probe development unnecessary, hence enabling deployment of miniaturised disposable fibre probes.

4. References

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