Towards optical fibre based Raman spectroscopy for the detection of surgical site infection

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ABSTRACT

Surgical site infections (SSIs) are common post-surgical complications that remain significant clinical problems, as they are associated with substantial mortality and morbidity. As such, there is significant interest in the development of minimally invasive techniques that permit early detection of SSIs. To this end, we are applying a compact, clinically deployable Raman spectrometer coupled to an optical fibre probe to the study of bacteria, with the long term goal of using Raman spectroscopy to detect infection *in vivo*. Our system comprises a 785 nm laser diode for excitation and a commercial (Ocean Optics, Inc.) Raman spectrometer for detection. Here we discuss the design, optimisation and validation of this system, and describe our first experiences interrogating bacterial cells (*Escherichia coli*) *in vitro*.

Key words: Raman scattering, SERS, spectroscopy, surgical site infection, bacteria, *Escherichia coli*.

1. INTRODUCTION

Surgical site infections (SSIs) – post-operative infections occurring in or near surgical sites within 30 days of the initial procedure – represent a significant clinical problem. In Europe, incidence rates can be as high as 20% depending on the surgical procedure [1] and SSIs remain a major cause of both morbidity and mortality [1-3]. Importantly, the transition from the original bacterial contamination to an established infection occurs early. However, SSIs are often detected much later (typically when the patient develops symptoms), meaning that further surgery can be required. This leads to increases in both the lengths of patients' hospital stays and the cost of treatment. Early detection of SSIs would permit more efficient treatment using antibiotics and would reduce the need for additional surgical interventions, thereby improving the experience of the patient and reducing the levels of both cost and mortality. As such, there is increasing interest in the development of minimally invasive techniques that can provide early diagnosis of SSIs, allowing effective antibiotic treatments to be administered. To this end, we are applying a compact, optical fibre based Raman spectrometer – that can be deployed in a clinical setting – to the study and detection of bacteria *in vitro*, with the aim of determining the potential of Raman spectroscopy for the early detection of SSI.

Raman spectroscopy and surface enhanced Raman spectroscopy (SERS) have been widely used in biomedical studies. In particular, Raman spectroscopy has been used for the detection and discrimination of bacteria, with applications in both hygiene and medicine. As early as 1980, Howard *et al.* reported the detection and identification of multiple bacteria in water using resonance Raman spectroscopy [4]. Since then many articles have been published discussing the use of Raman spectroscopy and/or SERS to detect bacteria and to discriminate between different species for a variety of applications (e.g. [5-9]). The use of optical fibres to deliver excitation and to collect the scattered Raman signal provides a route to minimally invasive medical use and Yang *et al.* recently reported the detection of the bacteria *Shewanella oneidensis* MR-1 in aqueous solution using a fibre-optic SERS probe [10]. Additionally, de Siqueira e Oliveira *et al.* [11] used an optical fibre based Raman probe to study multiple bacterial species that are involved in urinary tract infections (UTIs), a common type of SSI. Using this system, they were able to successfully discriminate between different pathogenic species using a diagnostic algorithm based on principal component analysis (PCA) of the acquired Raman spectra. Further investigation of the use of Raman spectroscopy in the study and diagnosis of UTIs has been performed by the research group of Costas Pitris [12-15]. In their research, the Pitris group used Raman spectroscopy to detect the presence of bacteria, to discriminate multiple bacterial species using PCA, and, finally, to

assess the bacteria's response to antibiotic treatment [12-15]. Overall, this implies that Raman spectroscopy has the potential to become a useful clinical tool for early diagnosis of infection as it can permit both the detection and identification of relevant bacteria.

In this article we report the use of a compact, clinically deployable, bench-top Raman spectrometer coupled to a bifurcated optical fibre probe for the study of bacteria *in vitro*. We discuss the design and validation of this system and present preliminary *in vitro* data from *Escherichia coli*. Further work will involve the study of additional bacteria and the application of our spectrometer to clinical studies investigating the potential for point-of-care diagnosis of SSI.

2. EXPERIMENTAL PROCEDURES

2.1 A compact optical fibre based Raman spectrometer

Our compact, clinically deployable Raman spectrometer was configured as shown in figure 1(a). The emission from a continuous wave (CW) 785 nm laser diode (L785P090, Thorlabs Ltd., UK) – which was housed in a temperature controlled mount (LDM9T/M, Thorlabs Ltd., UK) – was coupled into the excitation channel of a bifurcated optical fibre probe (InPhotonics RIP-RPB-785-FC-SMA, Ocean Optics Inc., The Netherlands). This probe contained two multimode fibres: one 105 μm diameter fibre for light delivery and one 200 μm diameter fibre for light collection. At the distal end of the probe (which was common to both fibres), the excitation light exited the fibre and passed through a collimation lens. A bandpass filter and a dichroic filter were then used to remove any background fluorescence or Raman signal caused by the optical fibre itself. Finally, a second lens brought the light to a focus at a distance of 7.5 mm from the tip of the probe. The scattered Raman signal was then collected by the second lens and directed toward the collection fibre by the dichroic beam splitter. A mirror directed the light through a long pass filter assembly (which blocked any residual elastically scattered excitation light) and a third lens coupled the light into the 200 μm collection fibre [16, 17]. The collection branch of the proximal end of the probe then delivered the light to a commercial Raman spectrometer (QEPro, Ocean Optics Inc., The Netherlands) for spectral detection.

Figure 1. Schematic diagram (a) and photograph (b) of the compact, optical fibre based Raman spectrometer used in this study.

All optical components were mounted on a 30 x 45 cm breadboard along with the driver for the laser diode (LDC 202 C, Thorlabs Ltd., UK) and a USB data acquisition box (NI USB-6009, National Instruments, USA) that allowed computer control of the on/off state of the laser. The entire system was contained within a light-tight black box and the only point at which laser light emerged from the system was at the distal tip of the fibre probe. For all measurements, the distal end of the probe was clamped above the sample in a custom made mount and fixed such that the laser emission was directed downward, allowing laser-safe use. The laser output and the spectrometer were both controlled by a laptop computer running software written in-house using LabVIEW 2014 (National Instruments, USA). A photograph of the fully housed system is shown in figure 1(b), where the compact, transportable nature of the device is evident.

To provide an acceptable signal to noise ratio (SNR), commercial SERS substrates (RAM-SERS-AU, Ocean Optics Inc., The Netherlands) were used to mount samples. For a typical measurement, a 10 μl liquid sample was placed

on the active area of a SERS substrate and illuminated with approximately 35 mW of optical power. Spectra were collected with acquisition times ranging from 0.1 s to several minutes. First, spectra from a 0.5 mM aqueous solution of Rhodamine 6G were recorded in order to validate the system (i.e. to confirm that we observed Raman peaks at the expected frequencies). Second, spectra from live *E. coli* were recorded at two concentrations (approximately 10^8 cells/ml and 10^9 cells/ml respectively) with a variety of acquisition times. These experiments served to confirm that the system provided sufficient sensitivity to detect and study the Raman signatures of bacteria.

2.2 *E. coli* sample preparation

E. coli (DH5 α strain, laboratory stocks) was streaked onto Luria Broth (LB) agar (15 g/l agar, Sigma-Aldrich, USA) and grown overnight at 37°C. A single colony was used to inoculate 5 ml of LB medium (5 g/l yeast extract, Fisher Scientific, UK; 5 g/l NaCl, Sigma-Aldrich, USA; 10 g/l tryptone, Fisher Scientific, UK), which was shaken overnight (220 rpm) at 37°C. 1.1 ml of the starter growth solution was then diluted in a 1:51 ratio with a further 55 ml of fresh LB medium (producing a total volume of 56.1 ml). This was then allowed to shake at 37°C at 220 rpm until OD600 (the absorbance at 600 nm) of 0.6 was reached, corresponding to a concentration of approximately 10^8 cells/ml. To concentrate the sample further, 50 ml of growth was centrifuged for 20 minutes at room temperature at 2500 x g (J6, Beckman Coulter Inc., USA) and the cells were then resuspended in 5 ml of LB medium to produce a sample with a concentration 10 times higher than that of the original (i.e. approximately 10^9 cells/ml). A 5 ml sample of the original growth was maintained at the initial concentration of 10^8 cells/ml.

3. RESULTS AND DISCUSSION

3.1 Surface enhanced Raman spectroscopy of Rhodamine 6G

To validate our system, we measured SERS spectra of Rhodamine 6G and compared them to those presented in literature. Figure 2 shows SERS spectra acquired from a 0.5 mM solution of Rhodamine 6G with acquisition times ranging from 0.1-10 s. In all cases, the observed peaks agree well with those reported elsewhere in the literature (e.g. [18]), indicating that our spectrometer successfully detects Raman signals from Rhodamine 6G at a concentration of 0.5 mM. Furthermore, all peaks are clear above the background with a good SNR even using the shortest acquisition time of 0.1 s (see figure 2(b)).

Figure 2. SERS spectra recorded in a 0.5 mM sample of Rhodamine 6G. (a) Spectra recorded with acquisition times of 1, 3, 5 and 10 s. (b) Spectra recorded with acquisition times of 0.1 s and 1 s. The y-axis scale in (b) is smaller than that used in (a), revealing that the spectral peaks are clearly resolvable even using a 0.1 s integration time. Also note the clearer presence of background peaks in the spectra presented in (b), for example at approximately 1050 cm^{-1} and 2160 cm^{-1} . These were attributed to the SERS substrate itself and were observed to decay over time. Hence, their relative intensities are weaker in the longer acquisitions shown in (a), which were recorded later.

3.2 Surface enhanced Raman spectroscopy of *E. coli*

After validating our spectrometer using Rhodamine 6G we recorded a series of spectra from *E. coli* to assess the sensitivity of our system for the detection of bacteria. Figure 3 shows Raman spectra from *E. coli* recorded at two concentrations (approximately 10^8 cells/ml and 10^9 cells/ml) and using multiple acquisition times (1 s, 10 s, 60 s and 300 s). Background spectra recorded from a bare SERS substrate and the bacterial growth media are also presented. As shown in figure 3(a), while there are clear background signals from the SERS substrates and the growth media, spectral peaks from the *E. coli* are evident above this background. In particular, a series of 3-4 Raman peaks are clearly observed in the frequency range $1250\text{--}1500\text{ cm}^{-1}$ (indicated by the black arrows in figure 3). These peaks are just discernible at 10^8 cells/ml and are clearly detected at 10^9 cells/ml. Additionally, the spectral locations are in qualitative agreement with previously published data (e.g. [7, 19, 20]), even though there is considerable variation within the literature. Importantly, the characteristic shape of the *E. coli* spectra can be resolved even at acquisition times as low as 1 s. This is demonstrated in figures 3(b) and 3(c), which show spectra recorded from *E. coli* at a concentration of 10^9 cells/ml with varying acquisition times. Using a 1 s integration time (figure 3(c)), the characteristic *E. coli* peaks in the $1250\text{--}1500\text{ cm}^{-1}$ frequency range are still clearly detected above the noise. This indicates the high sensitivity of our system, which will be vital when deploying this spectrometer (or a similar system) to clinical diagnostic studies of SSI where the concentration of bacteria will be considerably lower.

Figure 3. SERS spectra recorded in live *E. coli*. (a) Normalised spectra from a bare SERS substrate, blank bacterial growth media, and *E. coli* at concentrations of 10^8 cells/ml and 10^9 cells/ml (see key; 10^9 cells/ml concentration is labelled as 'x10 conc.'). Normalised spectra are shown in (a) to account for variations in intensity caused by fluctuations in laser power and differences in the spectral intensities observed when using different SERS substrates. (b) SERS spectra from *E. coli* at a concentration of 10^9 cells/ml with acquisition times of 1, 10, 60 and 300 s respectively. (c) The 1 s acquisition shown in (b) presented on a smaller y-axis scale revealing that spectral peaks are clearly resolved even with this short integration time. The black arrows in (a-c) indicate the spectral peaks corresponding to *E. coli*.

Overall, these results indicate that our compact, fibre-coupled Raman spectrometer provides sufficient sensitivity for the detection of *E. coli in vitro*. This is true when using acquisition times as low as 1 s and with a moderate laser excitation power of approximately 35 mW. Further work will now involve the interrogation of a series of additional bacterial species in order to ascertain whether Raman spectroscopy has merit in the detection and identification of bacteria that are relevant to SSI.

4. CONCLUSIONS

We have presented preliminary data from an *in vitro* study of the Raman spectral signatures of live *E. coli*. This data was collected using a compact, fibre-optic Raman spectrometer that has been designed for clinical use. The use of optical fibres for light delivery and collection is important as it allows interrogation of remote samples, which is highly beneficial in medical applications. Additionally, Raman spectra (or SERS spectra) have rarely been recorded in bacterial samples using fibre-optic systems [10, 11] due to the reduced signal collection efficiencies and high background levels that such devices often suffer from. Here, using a fibre-optic spectrometer, we recorded Raman spectra from live *E. coli* and observed qualitative agreement with literature. Furthermore, we were able to discern the relevant spectral peaks over the background with acquisition times as low as 1 s, indicating that our setup may be useful in the detection of bacteria at low concentrations. Further work will now involve the investigation of additional bacterial species to assess the merit of fibre-optic Raman spectroscopy in the diagnosis of SSI.

5. ACKNOWLEDGMENTS

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6. REFERENCES

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