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ABSTRACT

Our work focuses on the development of a medical Raman spectroscopy based platform to non-invasively detect and determine in-vivo molecular information deep inside biological tissues by monitoring the chemical composition of breast calcifications. The ultimate goal is to replace a needle biopsy which typically follows the detection of an abnormality in mammographic images. Here we report the non-invasive detection of calcium oxalate monohydrate in tissue through 40 mm of phantom tissues using our recently developed advanced Raman instrument complementing our previous detection of calcium hydroxyapatite through this thickness of tissue. The ability to detect these two key types of calcifications opens avenues for the development of non-invasive in-vivo breast cancer diagnostic tool in the future.

Keywords: Raman spectroscopy, breast cancer, hydroxyapatite, calcium oxalate
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1. INTRODUCTION

Advances in cancer screening to address outstanding clinical needs are often driven by the development of new diagnostic tools. One such need is the effective diagnosis of breast cancer. Ductal in situ carcinoma (DCIS), a subtype of breast cancer which stems from epithelial cells of milk ducts comprising 40% of the total number of cancers detected worldwide[1]. Due to large number of patients detected with breast cancer in late stage in the past, early screening programs have been implemented in a number of countries. Often, the marker for the presence of ductal carcinoma comes in the presence of calcifications in tissue revealed by mammography. These can be of two types; principally, calcium hydroxyapatite (HAP) and calcium oxalate (COM) with their detailed chemical makeup reporting on the nature of association lesion, i.e. whether benign or malignant[2]. Since X-ray imaging is not capable of providing chemical information from recorded images, the patient undergoes additional surgical procedure (needle biopsy) followed by histopathological examination. These recalls involve a large number of false positives [3]. Moreover the procedure is invasive, time consuming, costly for the healthcare system and above all come it constitutes a massive psychological burden to the prospective patients.

Availability of a non-invasive real time in-vivo medical diagnostic tool would provide a number of advantages as it could significantly reduce the medical costs, increased screening throughput and significantly improve psychological wellbeing of the patient. Both Raman and IR spectroscopies have shown the compositional changes associated with malignancies can be measured [4, 5]. Recently we have developed an advanced Transmission Raman spectroscopy (TRS) as a potential platform for a screening tool for patients undergoing mammographic screening [6, 7]. The instrument enabled us to diagnose HAP calcifications inside 40 mm phantom breast tissue[8]. Here we validate the system capability to detect also calcium oxalate monohydrate inside similar phantom tissue that replicates dimensions, compositions and consistency of breast.

2. MATERIALS AND METHODS

The breast tissue phantoms used in this study were made of porcine shoulder tissues composed of skin, fat and muscle to simulate much of the chemical composition of human breast tissue. The tissue samples were purchased fresh from a local supermarket and sliced to a thickness of around 40 mm (illumination path-length) x 35 mm x 50 mm (precision of +1.5/-1.5 mm) then were wrapped in a fine polyethylene cling film to avoid water evaporation and surface contamination during experiments.

Different amounts of calcium oxalate monohydrate (HAP) powder (Sigma Aldrich, St Louise, USA), representing type I breast calcifications, of 43, 83, 113 and 200 mg were smeared on tissue in the middle of the sample over a disk shape area (of approximately 2 mm diameter) oriented perpendicularly to the optical axis.
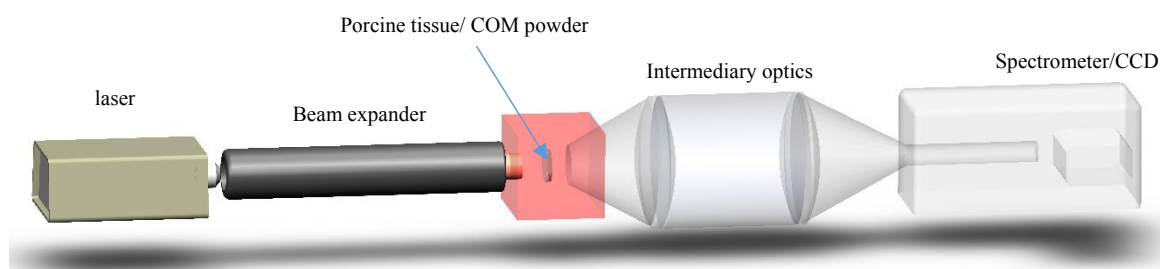

Figure 1. Schematics of the Transmission Raman setup.

Figure 1 presents the schematics of the TRS setup, while a detailed description is given in our earlier paper[8]. The laser used in these experiments was a solid-state laser operating at 808 nm (Innovative Photonic Solution, Monmouth Junction, NJ, US) and the light was delivered to the sample using an optical fibre (550 μm diameter, 0.22 N.A., Thorlabs, USA). Prior to spectral filtering the laser beam diameter was expanded to 9-10 mm using a variable beam expander. The laser power, measured with the power meter after the laser-line filters was maintained constant at 1.3 W during all measurements. The power density ~15 mW/mm^2 delivered to the tissue was below the one used in previous experiments on tissue laser irradiation. Previously, we provided a safety working regime for power densities of 20 mW/mm^2 [9] with temperatures of subcutaneous tissue maintained below 43 ^{0}C, which is the thermal toxicity threshold for many live cells in-vitro [10]. The Raman signal was collected on the other side of the sample with a 50 mm diameter biconvex collection lens of 38 mm focal length (N.A. = 0.6) which directed the light towards focusing lens of the same diameter and a focal length of 60 mm to deliver the light into the spectrometer. A couple of long pass edge filters at 830 nm (LP02-830RU-25, Semrock, Rochester, US) were deployed to filter out the elastically scattered light, first being placed between collection and focusing lens and the second located inside the spectrometer. The spectrometer used was a Holospec 1.8i (Kaiser Optical Systems, USA) equipped with a custom made high dispersion grating covering a spectral range of 608-1243 cm^{-1}. A deep depletion CCD detector, Andor iDus 420BRDD (Andor, Belfast, UK) was coupled with the spectrometer to collect the Raman signals. The slit used inside the spectrometer was 1 mm wide, which, combined with a high dispersion diffraction grating, provided a spectral resolution of ≈ 15 cm^{-1}. The acquisition times of the spectra were 30 s with 15 accumulations chosen to obtain optimum signals whilst avoiding CCD detector saturation. All the recorded spectra were imported into Matlab for data pre-processing, which consisted of cosmic background removal, noise filtering using singular value decomposition, background subtraction (first order polynomial) and detector intensity offset removal.

3. RESULTS AND DISCUSSIONS

The purpose of the experiments was to determine the detection limits of the setup for COM calcifications in conditions similar to a potential in-vivo medical device. The protocols replicated the previous experiments when calcium hydroxyapatite was introduced in the middle of the phantom tissue in different amounts while the spectra were recorded after each sample change. The setup retained the same optimization as previously reported [8]. With this new series of measurements we intended to obtain a comprehensive picture of detection limits for both calcification types COM / HAP and spectrometer ability to spectrally differentiate the calcifications within the sample. The Raman spectra are illustrated in Figure 2 after background removal and normalization. Visible are large Raman peaks attributed to phospholipids (860–890 cm^{-1} / blue star), HAP/lipids (963 cm^{-1} / brown star) and protein/carbohydrates (1072 cm^{-1} /green star). COM peak is not readily visible - its position is highlighted by the red line.
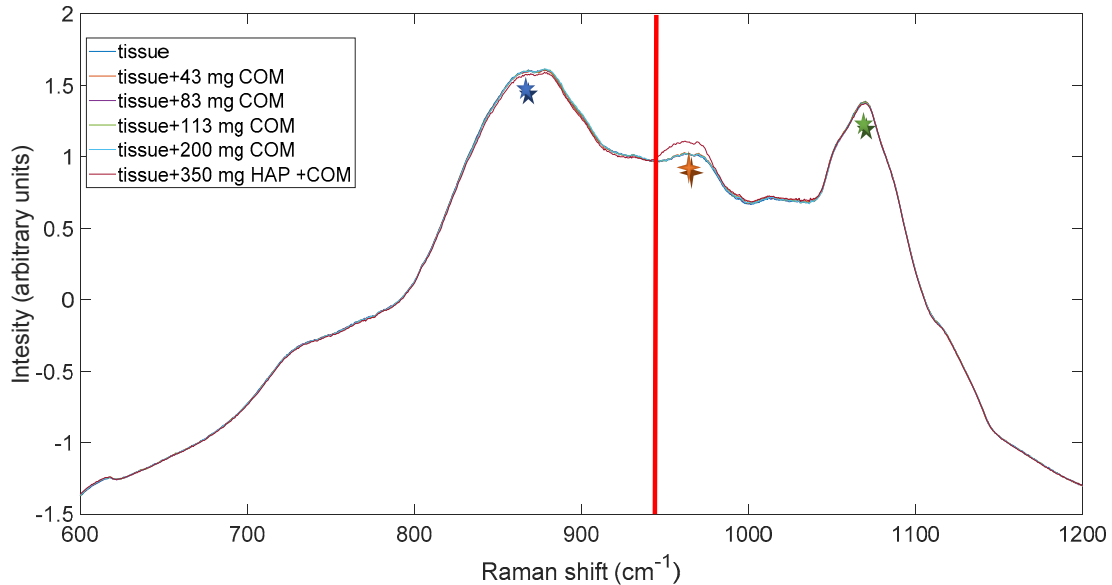

Figure 2. Normalized Raman spectra of the porcine tissue with and without calcification powders added. The red line highlights the COM peak position.

The usual procedure of peak detection is to measure the same sample with and without chemicals inserted inside the tissue. Afterwards a spectral difference is performed to cancel most of the tissue background. The procedure was successfully used before[7]. The first measurements of COM and HAP powder inside in similar amounts (350 mg each) are display in figure 3 alongside with pure spectra of COM and HAP. The two calcification types can be discerned.
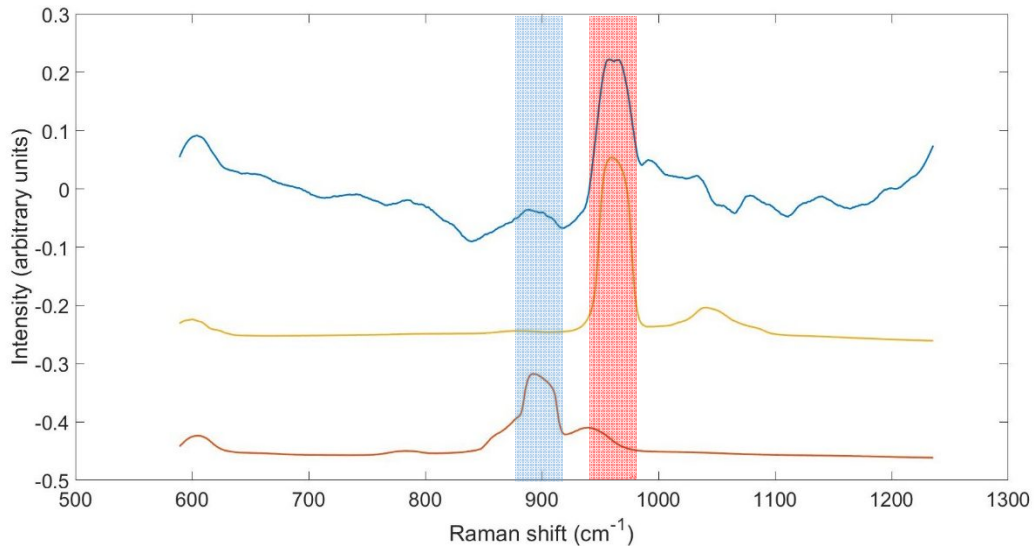
Figure 3. Raman spectrum of both COM and HAP inside 4cm tissue (top) obtained after performing spectral difference. The pure spectra of COM (bottom) and HAP (middle) are plotted below. Highlighted are the 945 cm^{-1} and 960 cm^{-1} bands of COM and HAP.

The next set of measurements included the detection of COM inside the tissue at different amounts. The experiments were performed on the same tissue sample starting from 0 mg COM and then subsequently gradually increasing its amount. Figure 4 shows the results of the measurements after performing spectral subtraction, note the spectra are smoothed using Savitzky-Golay filtering. A definitive evidence of setup sensitivity to COM presence in the tissue is presented by peak evolution with amount added to tissue. From 200 mg to 43 mg there is clear evolution of the 945 cm^{-1} peak height, while the peak position can be easily traced back to pure spectra of calcium oxalate presented above. The 43 mg seem to be below the detection limit, compared to previous measurements of HAP inside tissue where 20 mg were slightly visible in the Raman spectra. It is worth mentioning that measurements of HAP inside were performed with a higher laser power of 3.4 W (x2.6) with the beam collimated to 4-5mm diameter spot.
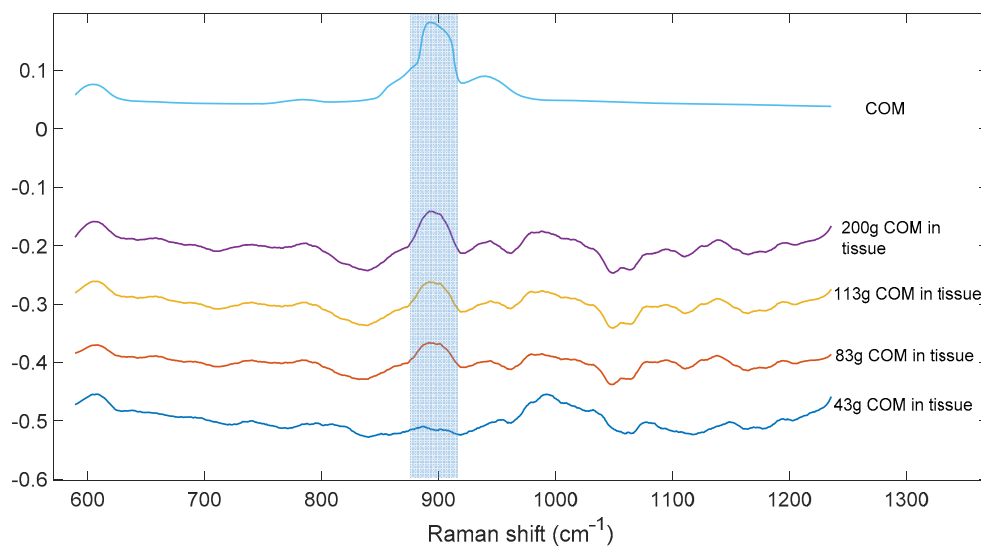

Figure 4. Results of TRS measurements of HAP powder inside 40 mm thick block of tissue at different concentrations of HAP: reference spectrum of HAP and difference spectra. The marker COM peak is highlighted.

4. CONCLUSIONS

In this paper, we evaluated the potential of the TRS instrument to detect COM inside a 4 cm thick tissue block. The device was capable of detecting calcifications in soft tissue at clinically relevant concentrations inside 40 mm of biological tissue. The instrument prototype performance was tested with COM inside porcine tissue at different concentration levels at clinically relevant levels. According to our data, the limit of quantitation for COM inside the biological tissue is ~80 mg. The setup was capable of differentiating spectrally COM and HAP. The ability to detect these two key types of calcifications opens avenues for the development of non-invasive in-vivo breast cancer diagnostic tool in the future

Note that we have previously shown [11] that when fatty rich tissues are used at room temperature for deep Raman, the transmission of signals achieved is 30% lower than that at body temperature. Therefore the detection limits outlined here are highly conservative.

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