Squeezing, Hearing and Illuminating Prostate Cancer: Searching for Diagnostic Signatures

Citation for published version (APA):

Citing this paper
Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights
Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy
If you believe that this document breaches copyright please refer to the University of Manchester’s Takedown Procedures [http://man.ac.uk/04Y6Bo] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.
**Squeezing, Hearing and Illuminating Prostate Cancer Searching for Diagnostic Signatures**

Elsa Correia Faria§, Tim J. Harvey§, Caryn Hughes§, Andrew D. Ward†, Alex Henderson§, Noel W. Clarke‡, Mick D. Brown‡, Richard D. Snook§ and Peter Gardner§

**Abstract**

Currently, the diagnosis of prostate cancer is achieved by annual PSA screening and digital rectal examination (DRE). However, the lack of sensitivity and specificity of PSA as a tool to diagnose prostate cancer, as well as its inability to predict the clinical aggressiveness of a tumour has limited its utility. In our work, we search for diagnostic signatures for prostate cancer which might also provide information on the aggressiveness of the cancer. To this end we have used reflection mode Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS), Raman Laser Tweezers and atomic force microscopy (AFM) to study the applicability of these techniques to discriminate between prostate cancer cells of differing invasiveness and non-cancer prostate cells. Results of our work have shown that the cells can be discriminated spectroscopically using FTIR-PAS2 and Raman Laser Tweezers. 3. Sensitivities and specificities of >95% and >98% were achieved using Raman Laser Tweezers and Principal component-linear discriminate analysis (PC-LDA).

In addition, we have investigated the hypothesis that the mechanical properties of cells might be a useful marker for cancer progression. It has been suggested that cancer cells are less stiff than non-cancerous cells and have determined the stiffness of non-cancerous prostate cells (BPH), non-invasive prostate cancer cells (LNCaP) and highly invasive prostate cancer cells (PC-3) using an atomic force microscope (AFM). We have shown that prostate cancer cells were more easily deformed than non-cancer cells. However, the highly invasive PC-3 cells were stiffer than the non-invasive LNCaP.

**Hearing Prostate Cancer FTIR – Photoacoustic Spectroscopy**

Photoacoustic spectroscopy (PAS) is an alternative to conventional IR spectroscopy and relies on the detection of acoustic waves generated as a result of the absorption of modulated light. (See schematic in Fig. 4) The amplitude of the PAS signal depends on the amount of absorbed energy that is converted to heat through non-radiative decay routes.

The elastic modulus (Young’s modulus, E) was then obtained from the force curves using the Hertz model, which was found to fit the curves well for different indentation ranges as exemplified in Fig. 2 for a BPH cell.

**Illuminating Prostate Cancer Raman Laser Tweezers**

Laser Tweezers enable the trapping of micron-sized particles, such as cells by virtue of radiant forces (see Fig. 7). We have combined Laser Tweezers with Raman spectroscopy to analyse single urological cells with reduced interference from the substrate and other cells.

Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were then used to classify spectra obtained for the different cell types. The results shown here were obtained for formalin fixed cells.

**Squeezing Prostate Cancer Atomic Force Microscopy**

Several studies have shown that cancer cells are more easily deformed than healthy cells and that highly aggressive cancer cells are more easily deformed than less aggressive cancer cells. It has also been suggested that the elastic properties of cancer cells play a major role in the metastatic process. Consequently, it has been hypothesised that elastic properties such a deformability of the cells could be used as a marker for metastatic potential.

To determine whether prostate cells of different stages in the disease progression can be discriminated based on their elastic properties, the elastic moduli of whole cells were obtained for BPH (benign prostate hyperplasia), and two well-characterised and widely used prostate cancer cell lines LNCaP clone FGC (non-invasive) and PC-3 (highly invasive), by indentation with an atomic force microscope (Fig. 1).

The results revealed that the Young’s moduli decrease with indentation depth, but for each indentation range the trend is maintained and BPH, PC-3 and LNCaP can be discriminated based on their apparent Young’s modulus (Fig. 3). As hypothesised, the benign BPH cells are less easily indented and therefore exhibit a higher Young’s modulus than the tumorigenic LNCaP and PC-3 cells.

However, contrary to the hypothesis, PC-3 cells exhibited a higher Young’s modulus than LNCaP cells. The reasons for this behaviour are not yet known, but are currently being investigated.

**References**


**Acknowledgements**

The authors would like to acknowledge financial support for TIR from the Prostate Cancer Research Foundation (PCRF) and for RF and SB from the Association for International Cancer Research. The Science and Technology Facilities Council is acknowledged for facility access time.

**Fig. 4:** Schematic representing a photoacoustic spectrometer.

**Fig. 5:** Example of Hertz model fitting to a force curve obtained on a BPH cell for 1000 nm indentation range, and the Hertz equation. After indentation: E = Poisson ratio; a = Tip–half angle.

**Fig. 6:** PCA score plots of the background-subtracted, vector normalized, first derivative spectra.

The results for different combination of cell lines can be seen in Fig. 9. Good clustering can be observed using the first two LDFs for the four cell type model and using the first three LDFs for the six cell line model. Sensitivities of >93% and specificities of >96% were obtained. Exposure to urine for up to 12 hours does not affect the ability to classify these cells using this method.