Analysis of prostate and bladder cells using Raman tweezers

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Introduction

The prostate specific antigen (PSA) test for prostate cancer (CaP) suffers from low sensitivity, resulting in a high rate of false positive tests[1]. There is a clear need, therefore, to develop new diagnostic methods for this disease. Here we report on an initial investigation into developing alternative diagnostic methods for the analysis of CaP cells in urine, which builds on earlier work within our group, in which infrared spectroscopy has been used to discriminate cancer cell lines and grade prostate tissue[2-4]. We have utilised Raman tweezers, which have been used previously to differentiate normal from cancerous cells[5]. Raman tweezers were used to trap and analyse both live and alcohol fixed CaP cells, benign prostate cells and malignant bladder cells. Preliminary investigations concentrated on discriminating a malignant CaP cell line (PC-3) and a malignant bladder cell line (MGH-U1). This was followed by analyzing alcohol fixed PC-3 and MGH-U1 as well as another metastatic CaP cell line (LNCaP) and benign prostatic hyperplasia (BPH). The role of the alcohol is to preservative the cells for an extended period of time with out autolysis (enzymatic cell decay).

Experimental

All cells were prepared according to standard methods[2]. For live cells trapping and analysis took place in phosphate buffered saline solution (PBS). For fixed cell analysis, cells were suspended in SurePath™ alcohol preservative (BD Diagnostics), and stored at <4°C until required.

514.5 nm light was delivered from an Argon ion laser to an inverted microscope via a 1.2 NA objective. Back-scattered Raman light from cells was collected through the objective to fall on a CCD. Live and fixed cells were analyzed in PBS and SurePath respectively, over a 1 minute duration.

After collection, each spectrum had its background contribution removed, followed by extended multiplicative signal correction (EMSC). Live cell spectra were subjected to principal components analysis (PCA). Fixed cell spectra were subjected to principal components-linear discriminate analysis (PC-LDA)[6]. For PC-LDA the data set was divided into a training and test (validation) set.

Results and discussion

A typical Raman spectrum of a trapped cell is shown in figure 1(a). Figure 1(b) shows the PCA plot obtained from live PC-3 and MGH-U1cell spectra. The PCA plot shows some degree of separation of PC-3 and MGH-U1, demonstrating that these cells can be discriminated based on their spectra. Figure 1(c) displays the PC-LDA training plot of alcohol fixed PC-3, LNCaP, BPH and MGH-U1 cells (60 spectra per cell type). Unlike PCA which attempts to capture as much variability in a small number of new variables, PC-LDA optimizes separation between groups[6]. Separation of the cell types into 4 distinct clusters is observed. Table 1 shows the sensitivities and specificities, using the test set of spectra, projected blind into the PC-LDA plot.

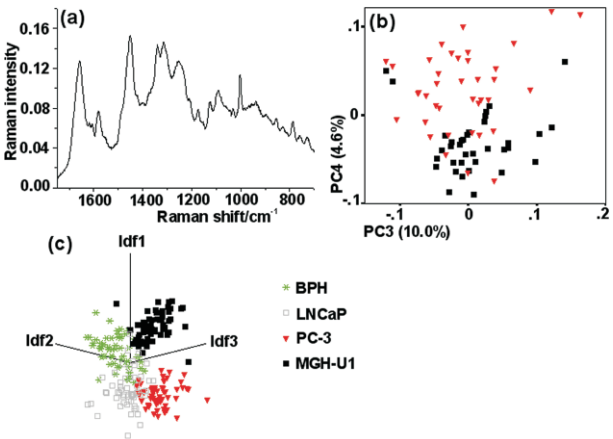

Figure 1. Raman cell spectrum (a), PCA plot of live cells (b) and PC-LDA plot of fixed cells (c). Each data point in (b) and (c) represents one spectrum, which in turn represents one cell.

	BPH	LNCaP	PC-3	MGH-U1
Sensitivity (%)	93.8	90.2	72.7	91.7
Specificity (%)	97.1	94.5	98.2	96.4

Table 1. Sensitivities and specificities achieved by the PC-LDA model.

Conclusion

We have shown that Raman tweezers can be used to: (i) analyse and discriminate between live prostate and bladder cells and (ii) analyse and classify fixed prostate and bladder cells with high sensitivities and specificities. These results reveal the diagnostic potential of Raman tweezers for prostate cancer.

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