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Label-free *in vitro* prostate cancer cell detection via photonic-crystal biosensor

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Abstract

Prostate-specific antigen (PSA) biomarker assays are the current clinical method for mass screening of prostate cancer. However, high false-positive rates are often reported due to PSA's low specificity, leading to an urgent need for the development of a more specific detection system independent of PSA levels. In our previous research, we demonstrated the feasibility of using cellular refractive indices (RI) as a unique contrast parameter to accomplish label-free detection of prostate cancer cells via variance testing, but were unable to determine if a specific cell was cancerous or noncancerous. In this paper, we report the use of our Photonic-Crystal biosensor in a Total-Internal-Reflection (PC-TIR) configuration to construct a label-free imaging system, which allows for the detection of individual prostate cancer cells utilizing cellular RI as the only contrast parameter. Noncancerous prostate (BPH-1) cells and prostate cancer (PC-3) cells were mixed at varied ratios and measured concurrently. Additionally, we isolated and induced PC-3 cells to undergo epithelial-mesenchymal transition (EMT) by exposing these cells to soluble factors such as TGF- α . The biophysical characteristics of the cellular RI were quantified extensively in comparison to non-induced PC-3 cells as well as BPH-1 cells. EMT is a crucial mechanism for the invasion and metastasis of epithelial tumors characterized by the loss of cell-cell adhesion and increased cell mobility. Our study shows promising clinical potential in utilizing the PC-TIR biosensor imaging system to not only detect prostate cancer cells, but also evaluate prostate cancer progression.

Keywords

Optical Biosensors; Prostate Cancer; Label-Free Imaging; Cellular Refractive Index (RI); Photonic Crystal Biosensor; Cancer Diagnosis

1. INTRODUCTION

The prostate-specific antigen (PSA) has been widely used as a biomarker to screen men for prostate cancer, although there are many issues associated with this method including a high false-positive rate. This is because in addition to prostate cancer, infection and chronic inflammation or benign prostatic hyperplasia (BPH) may also cause elevations in PSA

levels.^[1–3] Therefore, there is a need in developing other biomarkers with higher specificity for improved detection of prostate cancer in recent years.^[4–13] Despite the significant progress in finding a number of new molecular biomarkers, none of them has been successful enough to replace the PSA test yet. On the other hand, using exfoliated prostate cancer cells isolated from urine specimens for diagnosing the carcinoma of the prostate has been proposed several decades ago.^[14–15] Although the examination at cellular levels provides excellent specificities in contrast to PSA tests, past attempts at diagnosing prostate cancer via traditional urine cytology were abandoned due to unacceptably low sensitivities.^[16] The challenge in increasing sensitivity mainly stems from lacking sensitive and specific markers that allow visualization and differentiation of malignant prostate cancer cells through immunofluorescence labeling for cancer-specific markers.

In this paper, we report the utilization of a unique approach to distinguish prostate cancer cells from normal prostate epithelial cells based on their different refractive indices (RI). Our approach is based on our previous research, where we demonstrated the feasibility of using cellular RI as a unique contrast parameter to accomplish label-free detection of prostate cancer cells via variance testing, but were unable to determine if a specific cell was cancerous or noncancerous.^[17] The PC-TIR biosensor has an innovative working mechanism that utilizes photonic crystal (PC) structure in a unique configuration to create a novel *open* optical microcavity that allows easy functionalization of the exposed sensing surface and direct access for analyte molecules and cells.^[17–26] Conventionally, a sharp resonant condition can be achieved with a high-Q optical microcavity with a cavity layer sandwiched by two pieces of PC structures. However, this conventional, *closed* configuration is not suitable for biosensing, because in practice it is impossible to put analyte cells in the closed microcavity. For quantitative measurements of cell RI values, we open up the closed microcavity structure and use only one piece of a PC structure in a total-internal-reflection (TIR) configuration (shown in Figure 1B). This unique configuration forms a PC-TIR biosensor with an *open* sensing surface, which allows for easy attachment of cells directly onto the biosensor surface.^[18–19] Compared with a surface plasmon resonance (SPR) sensor that has a typical bandwidth of ~40 nm,^[27–28] a PC-TIR biosensor possesses a much sharper resonant dip (~ 1 nm),^[22] thus allowing for precise measurements of the change in RI of attached cells by accurately monitoring the change in the sharp resonant condition of the *open* microcavity. Furthermore, in contrast to the surface wave of an SPR sensor, the cavity mode of a PC-TIR biosensor is localized, which makes it possible to measure individual cells attached atop of a PC-TIR biosensor chip.

Additionally, we report the use of cellular refractive indices (RI) of prostate cancer cells used as a label-free biophysical parameter to evaluate the cell membrane of cells undergoing epithelial-mesenchymal transition (EMT). EMT is a crucial mechanism for the invasion and metastasis of epithelial tumors characterized by the loss of cell-cell adhesion and increased cell mobility. In this study, we isolated and induced prostate cancer (PC-3) cells to undergo EMT by exposing these cells to soluble growth factors such as TGF- β 1.^[29] Utilizing the PC-TIR, as a label-free prognostic method for quantifying cellular RIs, allowed us to distinguish between EMT induced and non-induced PC-3 cells.

2. MATERIALS & METHODS

2.1 Cells and Cell Culture

Human noncancerous, benign prostate hyperplasia cells, BPH-1 prostate epithelial cells were obtained from Dr. Scott Lucia's laboratory at the University of Colorado Health Science Center and human prostate cancer PC-3 cells were obtained from ATCC. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% Penicillin in a humidified incubator with 5% CO₂ at 37°C. All cells used were passaged for 2 to 15 times (P2 - P15).

2.2 Experimental Set-up for quantifying Single Cell RIs

The PC-TIR biosensor was placed on a prism, coupled to a rotational stage (illustrated in Figure 2). The rotational stage is computer controlled and operated, allowing for precise control of the laser's angle of incidence (by an accuracy of .001°). Cell solution was placed atop the biosensor, within PDMS (silicon elastomer) borders. Acquired data represents the cellular membrane's resonance at varying angles, this cellular resonance being a direct variable of the cell membrane's refractive index, which is unique to each cell line.

Desired cells were trypsinized, re-suspended in cell medium, and dyed (with Vybrant DiD cell-labeling solution). Cell solution was diluted to approximately 5,000 cells/100 μ L. A PC-TIR biosensor was cleaned, and PDMS borders are attached onto its edges, and was placed atop the prism. Seventy-five microliters of cell solution was then placed atop the biosensor, and allowed to set for 30 minutes (to allow the cells to settle within the evanescent field). A Helium-Neon laser at 632 nm was then coupled into the PC-TIR biosensor with a prism to monitor the resonant condition of the biosensor. The rotational stage was rotated, allowing for the identification of the resonant angle (RA). The RA being the angle of incidence of the laser, at which the cells resonate the greatest. Once the angle was identified, images were acquired at sequential angles around the identified RA. A graph was then built with a MatLab program, with variables being the intensity of the cell membrane's resonance, and the angle at which said resonance was acquired. All frames (images) are separated by an angle change of .005°, all starting from an arbitrary 0 (or first image).

2.3 Experimental Set-up for Resonance Reflectance Analysis of EMT induced Prostate Cancer Cells

A broadband white light source was coupled into a single-mode optical fiber to obtain a good spatial mode. The output beam from the fiber was polarized and collimated to illuminate the PC-TIR biosensor placed on a prism. The reflectance spectrum of the biosensor was monitored with a high-resolution spectrometer (illustrated in Figure 3). The selected cells were then placed on top of the biosensor and allowed to settle onto the sensing surface. Our PC-TIR based method provides a direct and simple way to measure the RI of a well-defined layer of cells (of a single cell line). It should be noted that the cells are within the evanescent field of the probe light that has a penetration depth of $\sim$ 300 nm. Hence the detection with the PC-TIR biosensor allows the measurements to be focused on cell membrane and cytoplasm, which is expected to have a different molecular composition and nanostructures between PC-3 cells undergoing EMT, and PC-3 cells not undergoing EMT.

EMT induction: Transforming growth factor beta-1 was obtained from Thermal Fisher Scientific. TGF β -1 was diluted to a final concentration of 5ng/mL in Phosphate Buffer Solution (PBS). 50 μ L of TGF β -1 was added to one of the PDMS wells on the SiO₂ biosensor 30 minutes after the PC-3 cell solution (of ~ 1 million cells) was added.

3. RESULTS

3.1 Prostate Cancer Cell Detection

Utilizing the experimental setup described in Figure 2, data was collected and analyzed for 3 samples, pure BPH-1 cells, pure PC-3 cells, and a mix of 30% PC-3 cells and 70% BPH-1 cells. Figure 4 demonstrates the uniformity of a pure BPH-1 cell solution sample test across 74 analyzed cells. Figure 5 demonstrates the uniformity of a pure PC-3 cell solution sample test across 35 analyzed cells. Figure 6 demonstrates two distinct resonate peaks, each corresponding to either the RI of the 20 PC-3 cells measured or RI of the 42 BPH-1 cells measured (across a total of 65 analyzed cells).

3.2 Epithelial-Mesenchymal Transition Analysis

Utilizing the experimental setup described in Figure 3, we measured the Reflectance Spectra of PC-3 cells, and PC-3 cells undergoing EMT. A well distinguished blue-shift of ~ 1.1 nm was observed when PC-3 cells were exposed to TGF β -1 (shown in Figure 7).

4. DISCUSSION

The presented study was conducted in order to demonstrate the preliminary success of using the PC-TIR biosensor as a novel label-free method for the detection of prostate cancer cells. As shown in Figure 6, detection of a 30% prostate cancer sample was successful, where two RA peaks are distinguishable and at corresponding percent total (measured 30.8% PC-3 cells, expected 30%). While there is some noise present (shown in Figure 6A (1)), it is negligible as it was less the 5% of the total RA events.

Furthermore, the study demonstrates the feasibility of using the PC-TIR biosensor to quantify EMT within prostate cancer cells. As shown in Figure 7, there is a clear and well distinguishable shift in the resonance dip, of ~ 1.1 nm. The observed shift is due to the loss of cellular membrane proteins (thus lowering the cellular RI). This corresponds to previous prostate cancer EMT research, that states that EMT is characterized by the loss of cell-adhesion proteins (that reside in the cell membrane).

Future research will focus mainly on detecting smaller percentages of prostate cancer cells (20%, 10%, and 5%), as well as being able to quantify a single cancers cells prognostic stage (via EMT progression analysis).

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6. REFERENCES

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Figure 1.

The PC-TIR biosensor: (a) Schematic structure of a PC-TIR biosensor, (b) The PC-TIR biosensor, and its use of TIR to form an open microcavity, offering a unique sensing interface open for direct interactions with cells.

Figure 2.
Schematic diagram of the experimental set-up for quantifying cellular RI values with a PC-TIR biosensor.

Figure 3.
Schematic diagram of cell RI measurements via resonant reflectance spectroscopy of a PC-HR biosensor.

Figure 4.

Pure BPH-1 test sample: (A) Compiled graph of all the cells relative intensity against the relative angle of incidence, where a single cell's RA is the angle at which its intensity is the brightest. (B) Is the average of all compiled cells. (C) Histogram of RA events against corresponding Angle of Incidence. (D-F) Demonstrates samples of images specified in C by numbers (1–3).

Figure 5.

Pure PC-3 test sample: (A) Compiled graph of all the cells relative intensity against the relative angle of incidence, where a single cell's RA is the angle at which its intensity is the brightest. (B) Is the average of all compiled cells. (C) Histogram of RA events against corresponding Angle of Incidence. (D-F) Demonstrates samples of images specified in C by numbers (1–3).

Figure 6.

30% PC-3 and 70% BPH-1 test sample: (A) Is the average of all compiled cells. (B) Histogram of RA events against corresponding Angle of Incidence. (C-F) Demonstrates samples of images specified in A by numbers (1–4). (D) Demonstrates the RA image of the PC-3 cells, measuring 20 PC-3 cells or ~ 30.8% of the total number of cells. (E) Demonstrates the RA image of the BPH-1 cells, measuring 42 BPH-1 cells or ~ 64.6% of the total number of cells.

Figure 7. Reflectance spectra for PC-3 cells (red) and PC-3 cells exposed to TGF β -1 (yellow) attached on a PC-TIR biosensor in comparison with PBS (blue) on the biosensor surface. PC-3 exposed and not exposed to TGF β -1 can be well distinguished due to the sharp resonance dip of the biosensor.