Fluorescent Silica Beads for Detection of Cervical Cancer

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ABSTRACT

We present the use of self-assembled fluorescent silica (glass) beads for detection of cervical cancer. The cells from three different individuals (3 normal and 3 tumor) were tested for affinity by using the beads, a few microns glass nanoporous particles which contain florescent dyes sealed inside the pores. We have developed two different methods for detecting the affinity in a Petri-dish. After simple precipitation of the beads onto the cells, the unbounded beads removed by washing. The next method involved using centrifugation for the removal of the unbounded beads. Both methods show unambiguous identification between the normal and tumor cells.

Keywords: fluorescent beads, glass beads, flow cytometry, cell assays, detection, cancer detection

1 INTRODUCTION

The challenges for scientists working in area of biomedical technology are often multidisciplinary in nature. Recent advance in this field is a result of several inter-disciplinary research involving physics, chemistry, molecular biology, engineering and medicine [1]. Research efforts over the years have resulted in the development of DNA chips [2,3], miniaturized biosensors [4,5,6], and bioMEMS [7,8,9]. These smart microsystems have found enormous applications in gene expression profiling, drug delivery and clinical diagnostics. Most importantly, the development of high sensitive probes for detection of cancer has attracted considerable attention in biology and medical research fields.

Cervical cancer is the second commonest cancer in women worldwide and infection with oncogenic human papillomavirus (HPV) is the most significant risk factor in its aeitology [10]. HPV infection is a common sexually transmitted infection among both women and men. The objective of screening cervical cancer to prevent occurrence of and death from cervical cancer by detecting and treating high-grade squamous intraepithelial lesions (HSIL) (which are precursor lesions for invasive cancer) becomes of prime and utmost importance especially in developing countries. In United States alone an estimated 12,900 cases of cervical cancer and 4,400 deaths occur annually. Approximately $6 billion is spent each year on screening and cure of cervical diseases [11].

Papanicolaou (Pap) smear has proved to one of the most successful methods of cancer detection over the years.12 Although Pap test is the most widely used cancer screening method in the world and its impact in the incidence of cervical cancer is well known from a historical perspective, recent reports suggest that the sensitivity of Pap smear is only 50-60%, with the relative proportion of sampling to screening errors being 2:1 [13]. The tests may be further complicated by high unsatisfactory rates, preparation artifacts and unnecessary cost interventions. Each year in the United States alone approximately 3.5 million Pap smears are classified equivocal, out of which 75% of women do not have cancer. The economic constraints in developing countries have prompted alternative methods of screening cancer including visual inspection after application of 3-5% of acetic acid14 and Lugol's iodine [15]. The major disadvantage of these tests is its low specificity. Given the considerable variation in the way these tests are applied and interpreted in different settings, there is no standard universally accepted definition of the test results. It remains to be seen if the specificity can be improved by further developments in test definitions and training strategies.

Demands for the development of a universal testing and screening method have generated enormous scientific and industrial interest in the past. Several methods such as organic receptor molecules that undergo color changes,16 electrochemical [17], magnetic [18], and fluorescent tags [19,20] have been developed and reported. Among the mentioned techniques detection using fluorescence is an important non-isotopic method. The fluorescent biological labels have played an important role in the field of biomedicine, such as the detection of materials inside or outside of the cell [21,22], hybridization and sequencing of the nucleic acid [23] clinic diagnostic at the early stage [24]. These conventional fluorescent techniques have the following limitations: reduction in the emission intensity due to photobleaching, the toxicity of some of the fluorescent dyes may be harmful for living cells and detection sensitivity is not so high because only a few fluorophores can be coupled to one biomolecule in the conventional fluorescent label methods.

In the present study a non-conventional fluorescent silica probe is reported for detection of cervical cancer. The method is comparatively benign due to the encapsulation of

the fluorescent dye within the silica particles and does not use any complimentary modification of the silica surface for tagging. The reported method also has high specificity compared to conventional techniques. It is believed that this method can be standardized to make a commercial break through in screening of cancer. The method is simple, comparatively inexpensive and requires a short training period.

2 MATERIALS AND METHODS

2.1 Spectrofluorometric Measurements and Imaging of Cells

An efficient assembly was setup to record images of cells and record emission intensity of the fluorescent particles (Figure 1).

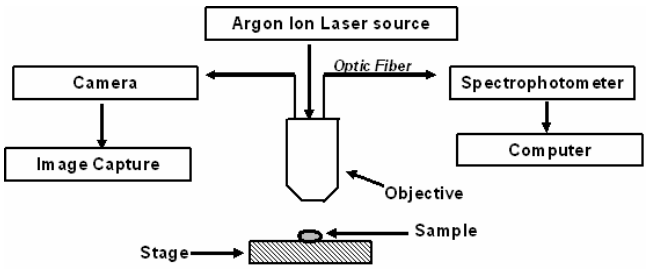

Figure 1: Schematic diagram of the optical measurement system.

An Olympus BH2-UMA microscope was connected to a JVC TK-1280U colour video camera. The images of cells were captured using FlashBus MV version 3.91 software. To record the fluorescent emission an optic fibre was used to connect the microscope to an Ocean Optics Inc, USB 2000 spectrometer. To calculate the average particle coverage per unit area of the cell surface a combination of Adobe Photoshop and SigmaScan image analysis software were used. A Cyonics air cooled argon ion laser head was used as the light source. A narrow 488 nm blocking filter (Omega Optical) was utilized to filter out the laser light. The spectrographs were recorded using OOIBase32 software.

2.2 Fluorescent Silica Labeling the Cells

The remove any residual surfactants on the surface of the silica particles that may have been present during synthesis, the particles were rinsed and centrifuged in distilled water thoroughly. The particles were then dispersed in HBSS solution to form a colloidal dispersion. The cells in the 60 mm culture Petri dishes were washed twice with HBSS solution for 2 min each. The cells were then exposed with 1 ml of the colloidal dispersion for 2 min. During these 2 minute period, the Petri dishes were subjected to initial 30 seconds of shaking on a Boekel Scientific Ocelot 260300F, to ensure uniform distribution of the colloidal solution and to minimize particle agglomeration. The cells were then washed with HBSS solution two times thoroughly, to remove excess particles that have not adhered to the cells. The Petri dishes were then dried in ambient air at room temperature.

2.3 Cell Cultures

Primary cultures of human cervical epithelial cells were prepared by a two-stage enzymatic digestion of cervical tissue as described[1] and cells were maintained in keratinocyte serum free medium (Invitrogen, Carlsbad, CA). Cervical cancer cells were isolated from primary cervical carcinomas as described[2]. All normal and cancer tissue was obtained from the Cooperative Human Tissue Network. Normal cervical cells were used at passages 2 to 4 when they were actively growing and carcinoma cell lines were used at passages 30 to 40. All cells were plated in 60 mm tissue culture dishes.

2.4 Centrifuge Experiments

In order to quantify the ease with which the particles could be detached from the cells, the culture Perti-dishes after exposure to silica particles were subjected to rigours washing using a spin coater (Headway Research Inc., PMW 202). The 60 mm Petri dishes were spun with an acceleration time of 1 minute from 0 to 1000 r.p.m, followed by constant spinning in HBSS for 1 , 2 and 4 minutes respectively. Total spin time being 2, 3 and 5 minutes respectively.

3 RESULTS AND DISCUSSION

3.1 Spectrofluorometric and Image Analysis of Cancer and Normal Cervix Cells

The cells exposed to the silica particles were imaged both with optical microscopy and fluorescent microscopy. Comparison of the emission spectrographs of the cancer and normal cells (Figure 2 and Figure 3, respectively) revealed a clear cut distinction between the two types of cells. The emissions from the cancer cells were on an average 50% stronger than that from normal cells. This increase in intensity was due to a greater number of silica beads per unit area attached to the cancer when compared to the normal cells. The optical images were analyzed to calculate the ratio of area of particles to the area of cells ($A_{PARTICLE}/A_{CELL}$). It was clearly evident from the histograms (Figure 4 and Figure 5) that the particles indeed adhered more to the cancer cells. This increase in intensity was due to a greater number of silica beads per unit area attached to the cancer when compared to the normal cells.

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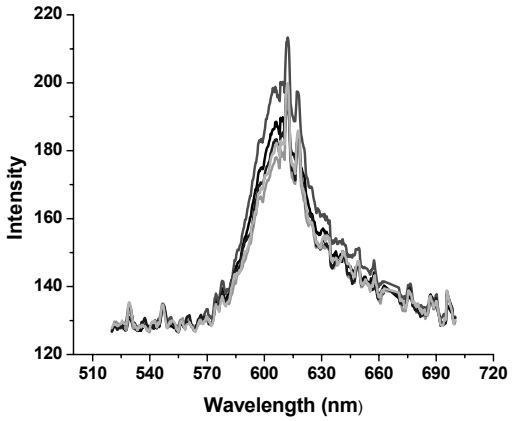

Figure 2: Spectrographs of cervical cancer cells.

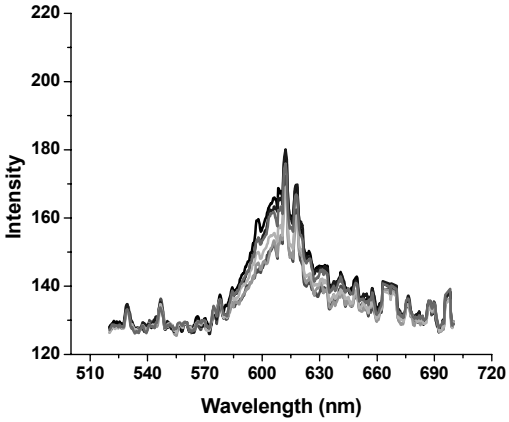

Figure 3: Spectrographs of normal cervix cells.

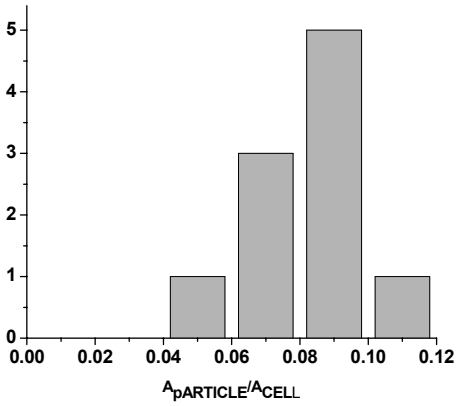

Figure 4: Statistics of ($A_{PARTICLE}/A_{CELL}$) distribution for cancer cells

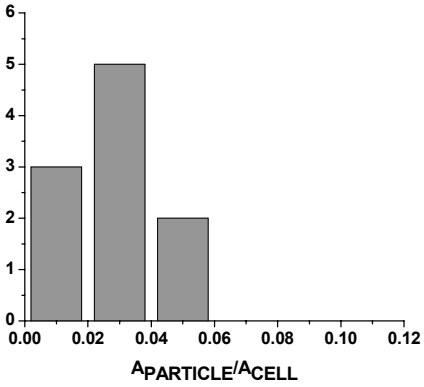

Figure 5: Statistics of ($A_{PARTICLE}/A_{CELL}$) distribution for normal cells

It must be noted that the distribution in Figure 4 and Figure 5 is distinct with no ambiguity. Indeed this method can be used very effectively to screen cancer. Complementary experiments were performed on cells cultured in a serum containing medium. This was done to show that this novel method of cancer screening was independent of the presence of the serum in the culture medium (not shown).

3.2 Detachment of the Probes from the Cell Surface

It was evident that the fluorescent silica particles attached effectively to the cancer cells and the emission spectrographs and optical images could be very efficiently used to distinguish between the cancer and normal cervix cells. The ease with which the probes can be detached from the cell surface is a direct measure of the particle adhesion to the cell surface. This was quantified in our centrifuge experiments. It can be concluded from the spectrographs (Figure 6) that the emission intensity decreased along the Petri-dish diameter for the cancer cells while it remained relatively constant for the normal cells, Figure 7. It can also be inferred that the emission intensity decreased with spin time in case of the cancer cells while it remained comparatively constant for the normal cells. Indeed the increase in the centripetal force along the radius of the Petri dish resulted in the detachment of the particles in case of cancer cells. The area under the emission curves can be used as an estimate of the number of particles per unit area of the cells.

The 3D histogram (Figure 7) shows that indeed the number of particles decreased along the radius of the Petri dish for the cancer cells. It may be now stated that the normal cells are more adhesive than the cancer cells and it is relatively easier to detach the particles from the cancer cells when compared to the normal cells. It is now hypothesized that the adhesive nature of the normal cells together with the dominant long range repulsive forces

resulted in maintaining a constant number of particles attached to the cells.

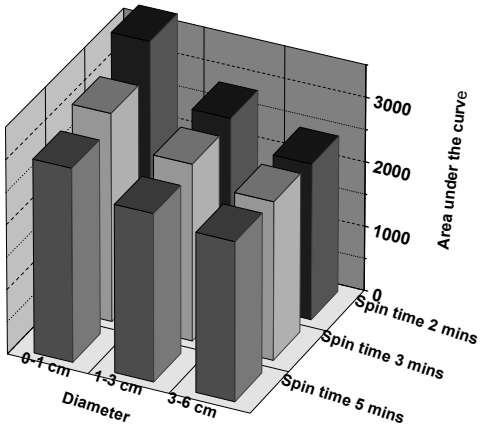

Figure 6: 3D histogram depicting area under spectral curve for different spin times and for measurements along the diameter of the culture Petri-dish for cancer cells.

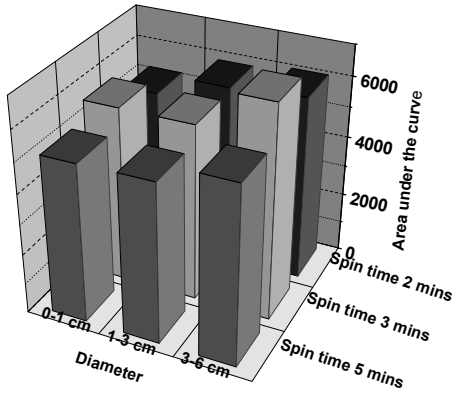

Figure 7: 3D histogram depicting area under spectral curve for different spin times and for measurements along the diameter of the culture Petri-dish for normal cells.

The number of particles attached to normal cells was independent of the exposure time to the particles and rigorous washing using high speed spinning. It is interesting to note that the effectiveness of the probe to distinguish between cancer and normal cells still prevails, but the role is now switched. Under mild washing conditions the fluorescent silica particles adhered more to cancer cells, but under rigorous washing in a high speed spinning environment the particles adhered more to normal cells. Irrespective of the nature of the washing environment the particles could be very effectively used to distinguish cancer and normal cells with minimum ambiguity.

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