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Vertically aligned multiwall-carbon nanotubes to preferentially entrap highly metastatic cancerous cells

M. Abdolahad [a], Z. Sanaee [a], M. Janmaleki [b], S. Mohajerzadeh [a,*], M. Abdollahi [c], M. Mehran [a]

[a] Thin Film and Nano-Electronic Lab, School of Electrical and Computer Eng., University of Tehran, North Kargar Avenue, Tehran, Iran
[b] Nanomedicine and Tissue Engineering Research Center, Taleghani Hospital, Shahid-Beheshti University of Medical Science, North Chamran Avenue, Tehran, Iran
[c] Iranian Society of Pathology, Tohid Square, Tehran, Iran

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ABSTRACT

We have investigated the entrapment of colon cancer cells with two different metastatic grades on arrays of multiwall carbon nanotubes. It has been observed that the fraction of entrapment of higher metastatic cancer cells is significantly more than lower metastatic grades. The observed effects are due to the more deformability and softness of higher metastatic malignant cells in comparison with the lower ones both for live and fixed cells. Also cell fixation results in a decrease of their entrapment due to an increase in cell rigidity by the fixation process. The present phenomenon describes a new application of vertically aligned carbon nanotubes to distinguish the healthy and cancerous cells by means of their different deformability properties during entrapment on such arrays.

1. Introduction

The structural organization of living cells is characterized by their mechanical properties [1]. Elasticity and responses of living cells to external forces have attracted a great attention in the modern research of tissue engineering [2], as well as in cell biology and cancer investigations [3–6]. Living cells respond to mechanical stimuli in their native environments with biological changes such as shape alteration of membranes and nuclei [7], cell-spreading [8], actins and microtubule reorganization or cross-linking under cell membrane [9], and cell bursting/motility [10]. By investigating these responses, one can obtain important information on the tumoral grade of cancerous cells.

It is well known that a wide range of changes occurs during the cancerous transformation of a normal cell which have been classified in four different categories as; cytoskeletal changes, cell adhesion and motility, nuclei changes and enzyme production [11]. The latter two cases refer to the variation of the cell from inside where the shape and organization of the nuclei of cancer cells becomes different from that of normal cells of the same origin or special enzymes are secreted to invade neighboring cells. In the cytoskeletal changes, however, the distribution and activity of the microfilaments and microtubules may change. These alterations change the way in which the cell interacts with neighboring cells and alter the appearance of the cells. Changes in the cytoskeleton also affect the cell adhesion and movement (motility). In general, cancer cells are more deformable than normal cells.

Cancer cells exhibit a remarkable reduction in their cell–cell and cell–extracellular matrix adhesion which allows the formation of large masses. The alterations in cell adhesion also impact on the ability of the cells to move. Cancer cells must be able to move and migrate in order to spread out where cell adhesion plays a major role in

* *Corresponding author:* Fax: +98 21 88011235.
E-mail address: mohajer@ut.ac.ir (S. Mohajerzadeh).
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regulating this cell movement. Two of these main changes i.e. cytoskeletal and cell adhesion variations, are directly affect the mechanical properties of the cells which are being the center of focus for this investigation [11,12]. During the development of diseases such as cancer, the structures of the cytoskeleton and the extracellular matrix are often transformed [13,14]. With the cell progressing from a fully mature, post mitotic state to an immortal cancerous state, the cytoskeleton experiences a reduction in the amount of constituent polymers and accessory proteins and a restructuring of its biopolymer network [15]. Therefore, a direct correlation seems to exist between an increase in the deformability and progression from a non-tumoral cell to a tumoral and metastatic one [16–19].

Micro and nano-fabrication technology developments have profound contributions to cancer detection by measuring the changes in the mechanical properties of the cancerous transformed cells at its early grades. Many methods have been employed to investigate and model the mechanical properties of cells in living, dead and fixed forms such as, micropipette aspiration [17–19], atomic force microscopy [20–22], micro post array detectors (MPAD) [23] and the optical stretcher [24,25]. Carbon nanotube is also considered as a promising material for cancer therapy, as discussed in Refs. [26–34] owing to their exceptional electrical, mechanical and biological properties or even as an appropriate surface for cell growth [35].

We have previously investigated the biological applications of vertical MWCNT arrays on interaction with micro organism [36,37]. In this paper, for the first time, different mechanical properties of higher metastatic colon cancer cells from lower ones are employed to observe the significant differences in the entrapment fraction of such cancerous cells onto vertically aligned carbon nanotubes. Colon cancer cells were prepared in a cell culture. For higher metastatic live colon cancer cells, we have observed an entrapment fraction more than 75% whereas for lower metastatic grades, this entrapment fraction has been lower than 30%. In addition, the entrapment fraction of fixed colon cancer cells is considerably lower than live cells which could be due to their more rigid structure. The detection of epithelial cells such as colon cells at higher metastatic cancerous transformation by means of their different entrapment on CNT arrays can be of great application in laboratory studies for cancer cell diagnosis.

2. Experimental setup

Growth of vertically aligned MWCNT arrays is achieved using a direct-current plasma enhanced chemical vapor deposition as reported elsewhere [38]. The CNT length and diameter are 12 μm and 75 nm respectively and they could act as elastic beams which are sensitive to deflection. Highly ordered CNTs have been demonstrated which could be achieved on desired patterns and geometries. For biological tests we use colon cancer cells from HT-29 and SW-48 cell lines which were isolated from grade 1 and 4 Human colon tumors. HT-29 is a lower metastatic grade of colon cancer (grade 1) whereas SW-48 is at grade 4. These cells were obtained from the standard cell banks and they were maintained at 37 °C (5% CO_2, 95% air) in RPMI-1640 medium (Sigma 8758) supplemented with 5% fetal bovine serum (Gibco), and 1% penicillin/streptomycin (Gibco). The fresh medium was replaced every other day. HT-29 cells were harvested with 0.25% trypsin–EDTA solution (Invitrogen) and the resulted suspended cells with augmented medium were used to expose onto CNT arrays. In order to conduct individual tests on CNT-holding substrates, separated solution of HT-29 and SW-48 cells were poured onto a target substrate surface, held by an angle of 45° using a peristaltic pump (Watson–Marlow Bredel Pumps Co., Model 323E/D) with a tube diameter of 0.8 mm. Various flow rates from 2.5 cc/min to 20 cc/min have been examined for pouring the cell solution. The volume of cell solution was about 0.5 mL and the surface of the target substrate was about 0.5 cm^2.

A special closed loop circulating mechanism was implemented to control the cell solution flowing process. A peristaltic pump was used to pour the media consisting cells from a reservoir with different flow rates. To prevent cells being laid on the bottom of the container, the whole solution was placed on a shaker system. As time duration and cell counts were identical for all experiments, one can deduce this

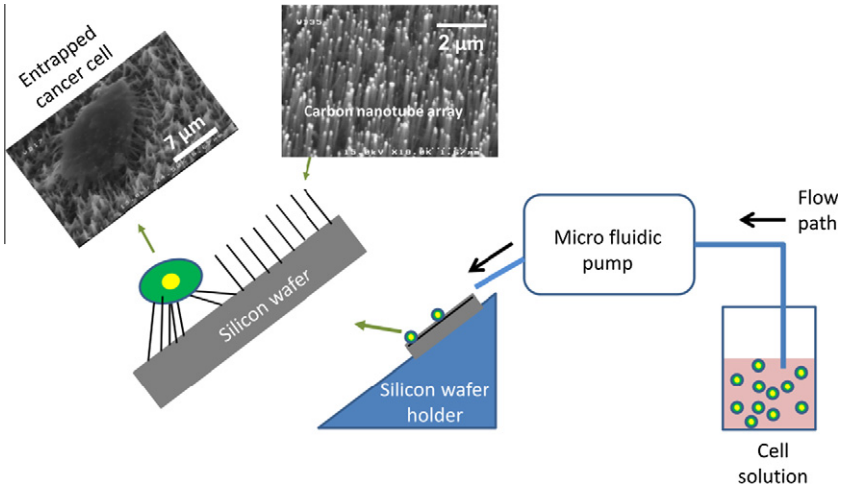

Fig. 1 – A diagram of cancer cell entrapment process on CNT arrays. The cell solution is flown onto the CNT arrays by a microfluidic pump. The time duration of the experiment was of the order of few seconds.