Electronic Supplementary Material (ESI) for Integrative Biology


**Support information:**

# Evaluation of the shear force of single cancer cells by vertically aligned carbon nanotubes suitable for metastasis diagnosis

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## CTF of entrapped fixed Colon cancer cell

For more investigation about the effect of cell rigidity in the reduction of its traction force on CNT array, the CTF measurement was repeated for fixed HT29 cell (prepared by adding 5% glutardhyde to the cell solution for 5 min). Many papers reported that the cells in their fixed state are stiffer than their live state [1]. This increased stiffness is the result of cell microtubule cross linking after fixation. SEM image of an entrapped fixed HT29 cell on CNT arrays was shown in figure S1.

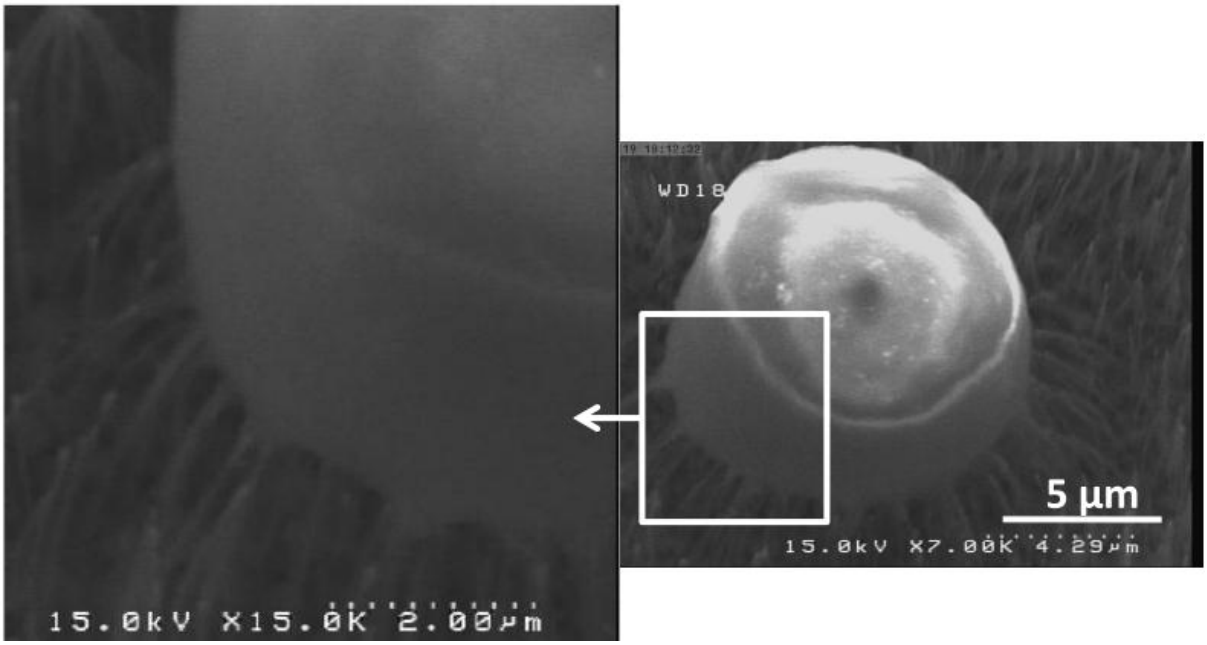


Figure S1. SEM image of entrapped of a fixed HT29 cell on CNT array. The metastatic grade and shape of the cell result in its lower deformation. Which is observable from the smaller deflection angles of nanotube beams?

The force applied by fixed HT29 cell was about 0.07 µN. The more rigid cytoskeletal structure (because of fixation) would be the reason of its lower CTF in comparison with live HT29 cell. In addition, primary metastasis as well as spherical and symmetrical shape of HT29 cell which was extracted from standard culture samples were parameters that result in their less deformation on CNT arrays in comparison with RC cell which were extracted from metastatic tumoral tissue and do not possess a symmetrical shape. In addition, cells removed from pathologic samples would have more deformability than cultured ones.

**Effects of materials of culture medium on the chemical states of the CNT**

Some CNT agglomeration may be occurred after the cell entrapment meanwhile during the cell entrapment as seen in SEM images of the cell occupied region in the paper (figure 5 and 7 of the paper), no agglomeration has been occurred for the CNTs which have involved in cell entrapment.

We have investigated the role of BSA (bovine serum albumin) as an anti adhesive agent in the entrapment process. For this purpose, the cell entrapment test was carried out with two separate live HT29 cell solutions with the same cell concentration on two separate CNT-holding surfaces where BSA was added in just one of the cell solutions. As illustrated in figure S2, the fraction of cell entrapment on CNT arrays was essentially the same for both cases although some minor variation has been measured which is of the order of 3%.

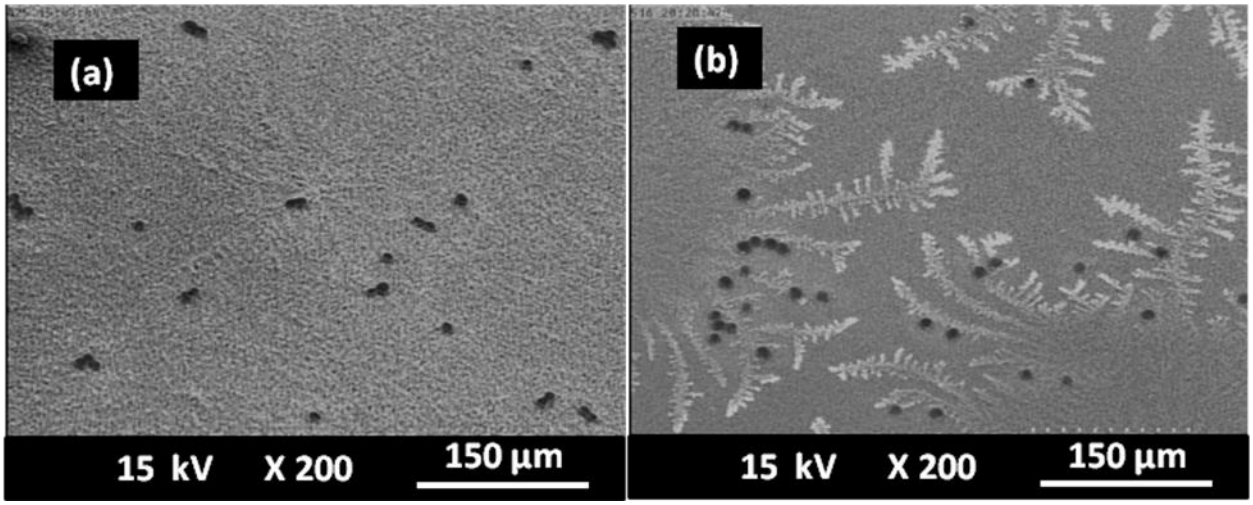


Figure S2. SEM image from entrapped live HT29 cells: a) with and b) without BSA added to the cell solution. The fraction of cell entrapment hasn't been changed so much.

The culture media seems to have a sort of agglomeration in the structure of CNT arrays as shown in the next AFM image as well as SEM images of CNT arrays after cell entrapment process (figure S3)

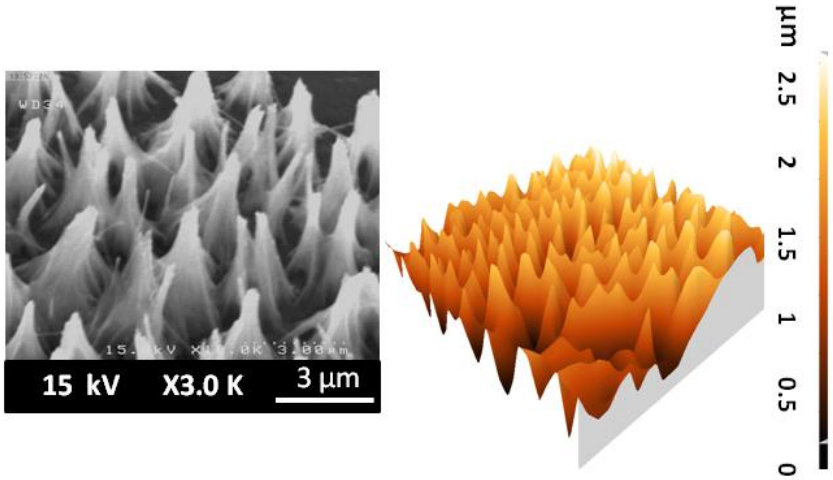


Figure S3. SEM (Right) and AFM (Left) images of agglomerated CNTs after the flowing of cell solution media.

**Role of mechanical interactions on cell entrapment on CNT array.**

Cells attachment to CNT beams may return to many parameters: It may be initiated from biochemical adhesion of cell surface receptors to CNT. But binding process of cells integrins (trans-membrane adhesion receptors of the cell) to a surface need to ECM protein secreting from the cell (named as RGD domain proteins) meanwhile (in case of renal cancer cells) fixed cancer cells do not have any adhesive sites and cant secrete any adhesive proteins. Fixed cells are died cells with cross linked cytoskeletal structure with no vital biochemical signal transduction. We repeat the entrapment process for live SW48 and HT29 cancer cells. The entrapment has been occurred in about 30 sec (as shown in below SEM images), which is too rapid to lead to any biological interaction. In fact, surface protein secreting from the cell is not a fast process which need a sufficient time ranged between 20 min to several hours depending on the cell type or surface as discussed in ref [2].

For chemical interactions, it must be born in mind that cells membrane is hydrophilic (as stated in the main manuscript) while CNT arrays are hydrophobic [3]. So the attachment of a hydrophilic surface to a hydrophobic one could not be immediately related to the wettability or chemical interactions. For more investigation about the role of mechanical parameters in cell-CNT interaction in comparison with chemical and biological ones, two metastatic grades of live colon cancer cells (SW48 and HT29 as high and primary stage of colon cancer respectively) have been compared by the fraction of their entrapment on CNT arrays. We know that higher metastatic cancer cells have more deformable cytoskeletal structures [2, 4], meanwhile they have less adhesive walls compared to lower metastatic ones [5, 6]. Therefore, if the chemical and biological adhesion happens to have the main contribution in the cell entrapment on CNT arrays, the fraction of low metastatic cell entrapment must be higher. But if the deformability and mechanical parameters have the main role in cell entrapment, the high metastatic cell should be further entrapped. The result of our experiment confirms the role of mechanical interaction between cell and CNT. The SEM images of figure S4 corroborate the role of cell deformability and mechanical parameters in their entrapment on CNT arrays.

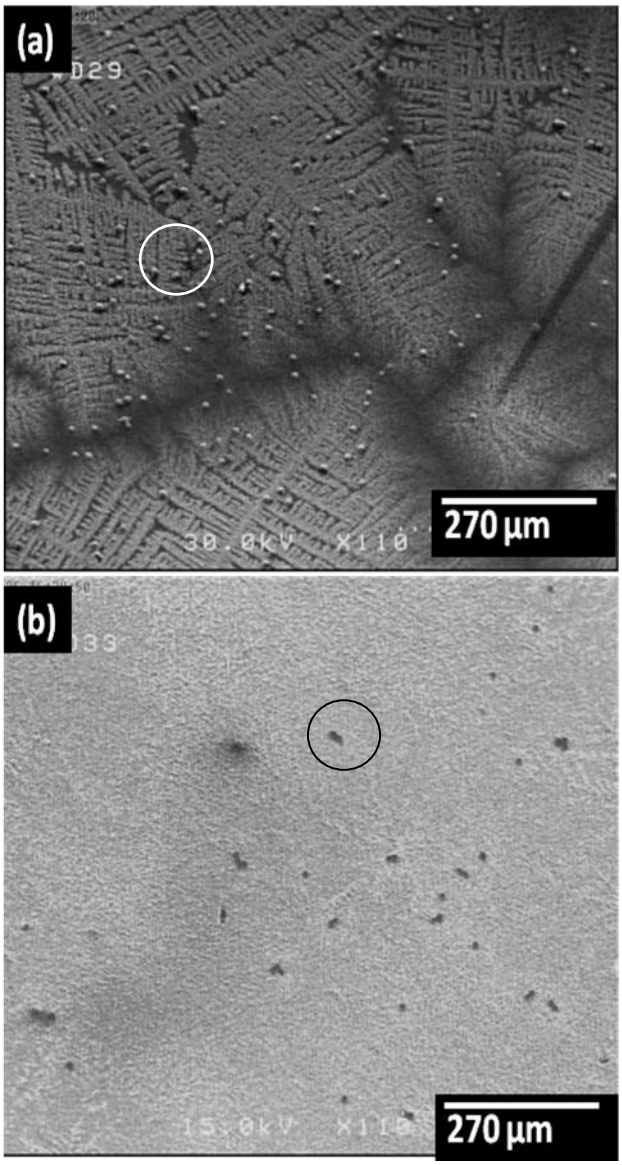


Figure S4. (a) SEM images of trapped SW48 and (b) HT29 cells on CNT arrays with the same manner and the same solution concentration. The fraction of entrapped highly metastatic cancer cells is significantly more than the one corresponding to a lower metastatic grade. The solution flow rate was 2.5cc/min and the area of the arrays was 0.5 $cm^2$. The flowing occurred in less than 30 sec. Circles in both sub-figures show a place that one or more cells have been trapped.

We observe that the fraction of entrapment for SW48 cells is considerably more than that for HT29 one, supporting the suggested method for CTF measurement of cancer cells by their entrapment on CNT arrays. In addition, as shown in the SEM image of figure 9 of the main paper, the more rigid cells (healthier cells) exert lower CTF and the more deformable cells (higher stage cancerous ones) apply more force (CTF) which would be a cancer diagnostic profile.

**Biocompatibility investigation of VACNT arrays**

Singhal et al. report a stable live state for the cell after incubation with CNT for a long period of time [7]. Moreover, entrance of vertically aligned nanotubes into the inner parts of the cell does not induce any membrane rupture or cell death under the experimental conditions as we reported elsewhere [8]. The florescent microscope images from SW48 cancer cells (which were tagged by green florescent protein (GFP)) 5 minutes after their entrapment on CNT surface have been shown in figure S5-a. The expression of GFP (green image of the cells) confirms the cell viability after their entrapment on nanotubes. In addition, to investigate the effect of nanotubes on making toxicity to culture media we experimented the MTT (M2128, Sigma) assay which was utilized to investigate cytotoxicity of the MWCNTs based on a procedure according to the ISO10993-5 standard test method in indirect contact manner [9]. The colorimetric 3-(4,5-dim ethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test evaluates cytotoxicity on cells on the basis of reduction of yellow tetrazole to purple formazan. The metabolic activity of cells is proportional to the color density development. For the experiment, SW48 cells were obtained from the National Cell Bank of Iran, Pasteur Institute. Cells were maintained at 37 °C (5% $CO_2$) in Ham's F12+DMEM (1/1V) medium supplemented with 10% Fetal Bovine Serum (FBS), and 1% penicillin/streptomycin (All from Gibco).

For the MTT assay the vertically aligned CNT arrays were sterilized using UV irradiation and alcohol to disinfect. After rising with sterile deionized water, the arrays were incubated with complete medium without cells at 37°C for 24 hours. Meanwhile, SW48 cells were seeded in 12-well plate at a density of $5\times10^4$ cells per well in triple. After 24 hours of incubation the culture medium were replaced with the supernatant solution from vertically aligned CNT arrays samples and were incubated for another 24 h. Then the MTT reagent was added to the wells and was incubated for another 4 h. Thereafter the formazan crystals formed, they were solubilized using a solubilizing buffer (DMSO). The absorbance of the samples was read at 570 nm in a spectrophotometer (CECIL, USA). Figure S5-b presented the MTT diagram of SW48 cancer cells which their media solution was exposed to VAMWCNT arrays. MTT results indicated that more than 95% of the cells remained alive. As a result, MWCNT wouldn't make any toxicity in cells media solution.

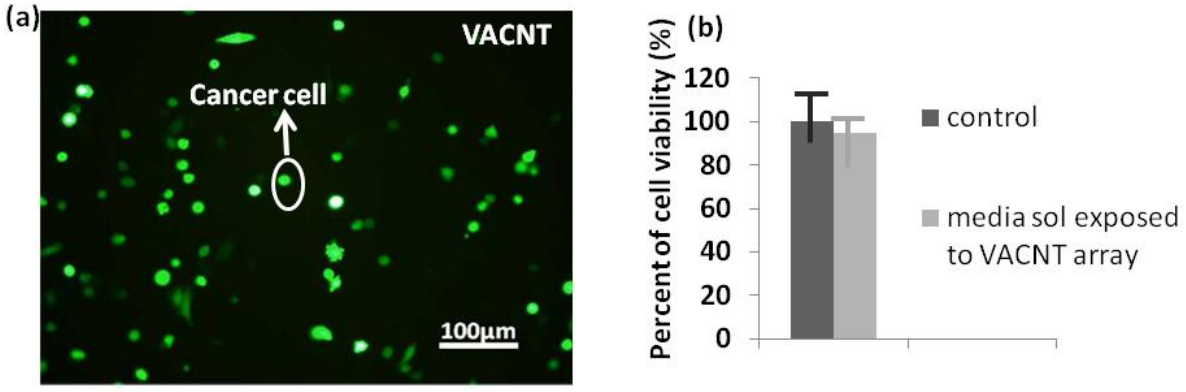


Figure S5. a) Florescent microscope image from the SW48 cancer cells entrapped on VACNT array after 5 min. The cells were tagged by GFP before seeding. The green color of the cells is the sign of their viability after culturing on nanowire surface. b) MTT diagram of cells which their media solution has been incubated with VACNT array in comparison with control sample.

## References:

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