Applied Surface Science 351 (2015) 27–32

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

A new tool for differentiating hepatocellular cancer cells: Patterned carbon nanotube arrays

Gokce Kucukayan-Dogu[a,1], Damla Gozen[b,1], Verda Bitirim[b], Kamil Can Akcali[c], Erman Bengu[d,*]

[a] Materials Science and Nanotechnology Graduate Program, Bilkent University, Turkey
[b] Department of Molecular Biology and Genetics, Bilkent University, Turkey
[c] Department of Biophysics, Faculty of Medicine, Ankara University, Turkey
[d] Department of Chemistry, Bilkent University, Turkey

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 6 May 2015
Accepted 9 May 2015
Available online 18 May 2015

Keywords:
Carbon nanotube
Cancer cell
Differentiation
Collagen
Patterning
Toxicity

ABSTRACT

We aimed to develop a new approach to detect the invasiveness and metastatic degree of hepatocellular carcinoma cells (HCC) based on their epithelial mesenchymal transition (EMT) status by using patterned carbon nanotubes (CNT) without any further surface functionalization. We used well differentiated HUH7 and poorly differentiated SNU182 cells to examine and compare their adhesive features on patterned CNTs. We found that the well differentiated HUH7 cells attached significantly more on the patterned CNTs than the poorly differentiated SNU182 cells due to the difference in epithelial and mesenchymal phenotypes of these cells. Collagen coated patterned CNTs having less roughness resulted in a decrease in the number of attached cells compared to non-coated patterned surfaces indicating that surface topography playing also a vital role on the cell attachment. LDH testing indicated no adverse, or thereof toxic effect of collagen coated or non-coated patterned surfaces on the HCC cells. The results of this study clearly suggest that patterned CNT surfaces can be used as a diagnostic tool to determine the invasiveness and metastatic level of HCCs. Hence, CNTs could be considered as a promising diagnostic tool for the detection of differentiation and invasiveness of the HCC cells.

1. Introduction

Cancer is a devastating disease and responsible for high mortality rates [1]. The aggressiveness of a cancer cell depends on its interaction with neighboring cellular structure, and the metastasis of these cells to distant organs is one of the main causes of cancer mortality [2,3]. Hence, the factors that regulate the attachment of cancer cells are one of the most heavily studied areas of biology. Epithelial-mesenchymal transition (EMT) is one of these processes that has important roles in the regulation of the adhesion of cancer cells leading to an increase in invasiveness and metastatic potentials [4].

During development, EMT is a normal physiological and required process and has critical roles in embryogenesis. Epithelial and mesenchymal cells are two different cell types with distinct cell morphologies and functions [4]. The cells with epithelial characteristic are polarized immobile cells which interact with basement membrane. On the other hand, cells in mesenchymal phenotype become more migratory and invasive with distinct morphology, protein expression and gene signatures. EMT is initiated by several steps including; activation of transcription factors such as Twist and Snail, expression of specific proteins and microRNAs, reorganization of cytoskeleton and production of certain enzymes as a result of which an epithelial cell gain mesenchymal characteristic [5,6].

The cells undergoing EMT typically lose the expression of epithelial cell markers such as E-cadherin, and gain mesenchymal marker expressions, like α-SMA, N-Cadherin, vimentin and fibronectin [6]. These cells lose their adhesive features to neighboring cells or to a surface or substrate such as extracellular matrix. Adhesion occurs from the action of family proteins, called cell adhesion molecules (CAMs) which cadherin is a member of this family. The tumors in early stages undergoing EMT process become aggressive malignancies with increased invasiveness, metastasis and survival abilities [7,8]. Cancer cells undergoing EMT lose their adhesive properties. Thus, the relation between the tumor aggressiveness and EMT was clearly shown in hepatocellular carcinoma

* Corresponding author. Tel.: +90 3122902153; fax: +90 3122664068.
E-mail addresses: bengu@fen.bilkent.edu.tr, erbengu@gmail.com (E. Bengu).
[1] Equal contributions of authors.

http://dx.doi.org/10.1016/j.apsusc.2015.05.054
0169-4332/

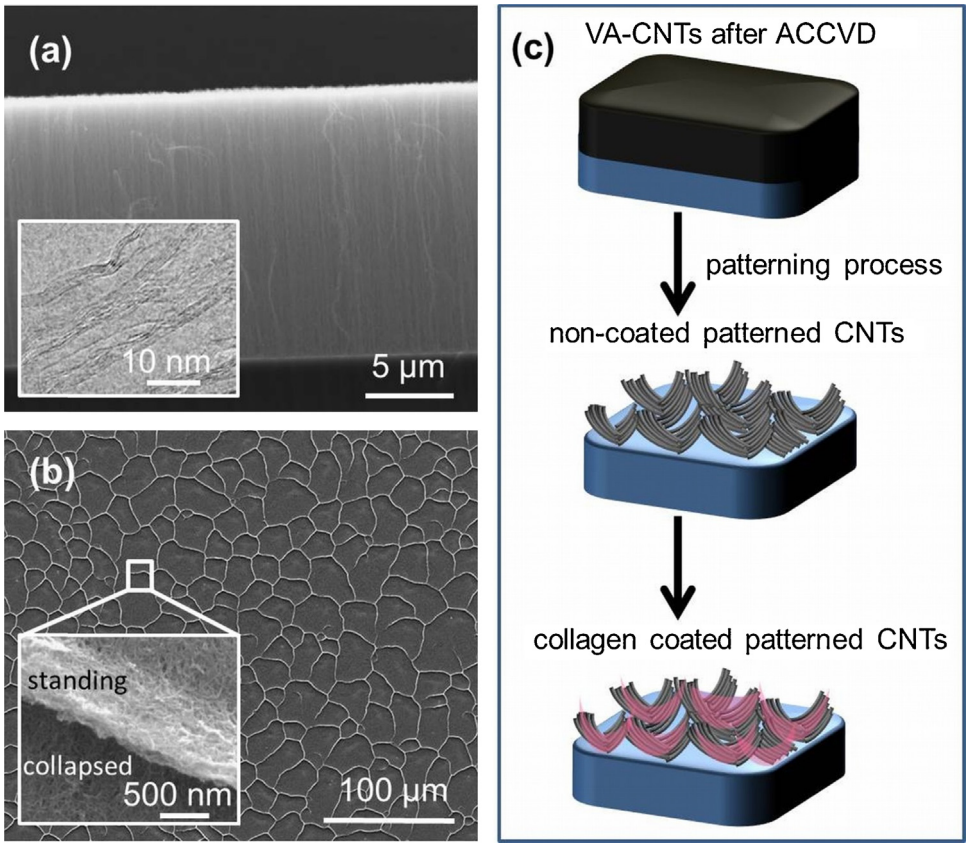

Fig. 1. (a) Side view SEM images of VA-CNTs. Inset shows TEM image of CNTs. (b) Top view SEM image of patterned CNT surface with the inset showing a high magnified image from the wall of cavity. (c) Schematic representation of CNT surfaces after patterning and collagen coating.

(HCC) which is the third leading cause of world's cancer related mortality [9]. HCC is an epithelial cancer with four stages including well differentiated, moderately differentiated, poorly differentiated and undifferentiated tumors. The poorly differentiated cells gain mesenchymal phenotype through EMT to become more independent from the underlying tissue, with increased capacity to invade and metastase [10].

Novel strategies have been developed to classify and characterize the stages of cancer cells. The nanostructured materials such as nanowires [11], metal nanoparticles [12,13] and quantum dots [14] have been widely investigated both for cancer treatment and diagnosis applications. These recent advances in the field of biomaterials also triggered research on carbon nanotubes (CNTs) for the investigation of various cellular interactions [15]. There are number of studies reporting the use of CNTs as a nano-fibrous scaffolds for living cells [16–21]. Furthermore, the unique properties of CNTs prompted their application as a potential new tool for the detection of different types of cancer cells (oral, prostate and lung cancers) not only by taking advantage of their functionalization (e.g. binding specific markers on CNTs) [22–27] but also through exploiting their rigid surface properties for entrapping cancer cells [28,29]. For instance, improvement in the sensitivity and detection limits of cells have been achieved by the integration of CNTs in immunosensors [22,24,25].

In this study, we have employed patterned vertically aligned CNTs (VA-CNTs) as a tool to assess the invasiveness and metastatic degree of cancer cells based on their EMT properties. Our results showed that well differentiated HCC cells attached more on the patterned CNTs compared to the poor differentiated HCC cells due to their EMT characteristics without displaying any cytotoxic effects. Hence, our results suggest that VA-CNTs could be developed in to a promising tool for the detection of differentiation and invasiveness of the HCC cells.

2. Materials

2.1. Synthesis and patterning of VA-CNTs

VA-CNTs were grown by alcohol catalyzed chemical vapor deposition (ACCVD) method on oxidized Si (100) surfaces as described before in our previous study [18]. Sandwich catalyst layers (Al/Co/Al) were prepared for the growth of VA-CNTs by using the electron beam and thermal evaporation techniques [18]. Si substrates with the aforementioned layers were introduced into the ACCVD furnace for the growth of VA-CNTs through reduction and reaction steps using ethanol as a carbon source at temperature of 625 °C under flowing H_2 and Ar gases (20 sccm and 100 sccm, respectively). After the growth, patterning was induced to the VA-CNTs by using a dropper filled with deionized water. Following this step, some of the patterned CNTs were treated with 1 µg/µl sterilized collagen solution for every cm^2 (approximately 10:1 weight ratio of collagen to CNT) resulting in two separate groups of patterned CNT arrays; one non-coated and the other collagen coated.

2.2. Cell culture

We used two human HCC lines (SNU182 and HUH7) in our experiments. SNU182 is poorly differentiated and HUH7 is well differentiated hepatocellular cancer cell line. SNU182 cells were cultured in RPMI medium (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum, 1% non-essential amino acids and