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The effect of fibronectin on structural and biological properties of single walled carbon nanotube

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ABSTRACT

Despite the attractive properties of carbon nanotubes (CNTs), cytoxicity and hydrophobicity are two main considerable features which limit their application in biomedical fields. It was well established that treating CNTs with extracellular matrix components could reduce these unfavourable characteristics. In an attempt to address these issues, fibronectin (FN) with different concentrations was loaded on single walled carbon nanotubes (SWCNTs) substrate. Scanning electron microscope, atomic force microscopy (AFM), contact angles and X-ray photoelectron spectroscopy (XPS) were preformed in order to characterize FN loaded SWCNTs substrates. According to XPS and AFM results, FN could interact with SWCNTs and for this, the hydrophilicity of SWCNTs was improved. Additionally, SWCNT modified with FN showed less cytotoxicity compared with neat SWCNT. Finally, FN was shown to act as an interesting extracellular component for enhancing the biological properties of SWCNT.

1. Introduction

Carbon nanotubes (CNTs) had gained great attention as a versatile nanomaterial with various applications [1]. CNTs are graphitic tubular structures that have attracted a high interest due to their extraordinary physical, electrical and chemical properties [2]. CNTs are commonly divided into two geometries: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). MWCNTs have typically 2–40 layers of concentric tubular graphitic carbon while SWCNTs are a single-layer of tubular graphitic carbon [2,3]. CNTs have also a great potential in the field of tissue engineering [4,5], thermal ablation and drug delivery [6,7]. For instance, CNTs are able to support the growth of bone cells [8,9], cardiomyocytes [10] and neurons [11–15] as scaffolding materials. We have recently fabricated a nerve conduit based on silk fibroin (SF) containing SWCNT. SWCNT have improved the less electrical properties of SF and thus this suggests the application of SWCNTs in peripheral nerve regeneration [16]. Generally, SWCNTs have been reported more cytotoxic in comparison to MWCNTs [17–21]. It is well established that the surface properties of SWCNTs such as chemistry and roughness are responsible for biocompatibility and cytotoxicity features of SWCNTs. However, the effects of SWCNTs on cells are still controversial and the underlying mechanism for selective cell adhesion and growth is still obscure. In spite of cytotoxic effect, SWCNTs have highly hydrophobic surfaces that are unfavorable for cellular adhesion and growth. It was reported that extracellular matrix (ECM) proteins are able to improve the surface properties of polymeric substrates and thus could modulate the initial surface adhesion of cells [26]. Among various types of ECM proteins, fibronectin (FN) seems to be more attractive as a remarkable cell adhesive protein which is able to promote the cellular adhesion on hydrophobic polymer surfaces [22,23]. FN is a high-molecular weight (~440 kDa) glycoprotein of the ECM that binds to integrins, collagen, fibrin, and heparin sulfate proteoglycans. FN plays a major role in cell adhesion, growth, migration, and differentiation, and it is crucial for stimulating the processes such as wound healing and embryonic development. It was shown in our previous study that electrospinning of FN on SF/SWCNT substrates could support the cellular attachment and proliferation

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more than SF/SWCNT conduits without FN [16]. In addition, FN was used in same studies in order to improve the surface properties of CNTs. However, most of these studies were related to application of MWCNTs [22,24] and thus to our knowledge there are a few reports around the modification of SWCNTs with ECM proteins. In this regard, Namgung et al. have recently patterned SWCNTs on various substrates for patterning FN in order to control the directed adhesion and growth of cells [25]. Therefore, it was considered that FN could generate "moderately hydrophilic" surfaces on SWCNTs that are found to be most favorable to cell attachment presumably due to optimal nanosurface roughness, surface energy (wettability) and adhesion protein adsorption. In the present study, the structural properties of SWCNT/FN structures in terms of nanosurface roughness, surface topography, hydrophilicity and surface energy were investigated. Cells viability was also inspected in order to evaluate the ability of these structures to promote cellular adhesion and proliferation.

2. Materials and methods

2.1. Materials

Fibronectin (FN; human plasma, MW: 450 kDa), Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT), isopropanol were purchased from Sigma-Aldrich (USA). Carboxylated single walled carbon nanotube (SWCNT) was purchased from Neutrino Company of Iran. L929 cell line (Mouse fibroblast-like cell) was also obtained from National Cell Bank of Iran (NCBI).

2.2. Film fabrication

In order to fabricate SWCNT film treated with FN, SWCNT was dissolved in chloroform (concentration 1/700 g/mL) using hemogenizer (15,000 rpm, 20 min). A thin film of SWCNT was prepared by placing 20 μL of the prepared solution on strill glass substrate. Afterward, the dried SWCNT coated on glass substrates were immersed in different concentrations of FN (1%, 3%, and 5%) for 24 h in order to stimulate the interactions between SWCNT and FN. The distribution of FN on the surface of SWCNT substrate was further evaluated using fluorescence microscopy as described by Hoffmann et al. In this regard, the coupling reaction was performed by diluting a small amount of FITC in a large excess volume FN solution in the carbonate–bicarbonate basic buffer at pH 9.0. The FITC solution was added very slowly with gentle and continuous stirring. The mixture was incubated for 1 h at room temperature in the dark [26].

2.3. Structural analysis

2.3.1. Scanning electron microscopy (SEM)

The morphology of SWCNT films before and after treating with FN was assessed using field emission scanning electron microscopy (FESEM; Quanta 200F, FEI, Oregon, U.S.).

2.3.2. Atomic force microscopy (AFM)

The topographical images of SWCNT whether neat or treated by FN were acquired using intermittent contact imaging mode of atomic force microscopy (AFM; JPK Nanowizard 2, JPK Instruments AG, Germany). Furthermore, atomic force microscopy was implemented to obtain topographical images of cells affected by SWCNT and SWCNT/FN films. Cells had been fixed by 3% glutaraldehyde and then washed by PBS three times. The tapping mode NCS-15Al cantilever oscillated with a frequency close to its resonance frequency over the surface to derive the images.

2.3.3. Contact angles

Wettability of SWCNT films before and after FN treatment was measured with the sessile drop technique using freshly purified water.

2.3.4. X-ray photoelectron spectroscope (XPS)

Surface analysis of SWCNT films before and after treating with FN was measured using XPS method (VG 220 i-XL ESCALAB spectrometer). The samples were fixed on the sample holder using carbon and aluminum tape to ensure a good electrical conduction hence preventing charge accumulation effects. Sample holder was then introduced and kept under vacuum for 12 h at room temperature. The radiation source was an Al mono-chromatized source (1486.6 eV). Silver Ag3d5/3 at the binding energies BE = 368.2 eV was chosen for the calibration of the spectrometer. Survey and high-resolution spectra were fitted with the Avantage processing program provided by Thermo Electron.

2.4. Biocompatibility

2.4.1. Cell viability study

MTT assay was performed for quantification of viable cells. Before performing MTT assay, L929 cells were cultured in DMEM at 37 °C with 5% Co_2. The cells were sub-cultured twice per week and were utilized for between 4 and 8 passages. After sterilization the films using ultraviolet (UV) light for 45 min, the samples were placed in 12-well culture plate (Three series of 12-well culture plates were used for MTT measurement in three different intervals) and then 2×10^4 L929 cells were seeded on each samples and kept in incubator for 1, 3, and 7 days. Afterwards, the culture media (DMEM/FBS) were removed at each intervals and the cells were stained with MTT solution for 4 h. The formed formazan was solubilized with isopropanol for 15 min. Absorbance was read at 570 nm. Moreover, tissue culture polystyrene (TPS) was used as a negative control group.

2.4.2. Cell morphology study

FESEM was conducted for evaluating the morphology of L929 cells on the substrates. The cells at a density of 2×10^5 were seeded on the substrates and incubated at 37 °C with 5% CO_2 for 3 days. The samples were fixed in 1.5% glutaraldehyde solution for 4 h and then dehydrated in graded alcohols; 10, 30, 50, 70, 80, 85, 90, 95, and 100%. Finally, the samples were sputter-coated with gold, and subsequently viewed using SEM at an accelerating voltage of 15-keV.

2.4.3. Cellular attachment

The cellular adhesion of L929 cells was studied by confocal fluorescence scanning microscopy (CFSM). Cells were cultured under 5% CO_2 and 95% relative humidity at 37 °C. The cells were allowed to adhere to a glass cover slip in 6-well plate for 24 h. The medium was removed and the cells were incubated with 100 μg/mL of each sample containing fluorescein isothiocyanate (FITC) for 4 h. Afterward, the samples were washed 3 times with PBS and were incubated with DAPI for 5 min in order to stain the nuclei and rewashed 3 times with PBS. The cells were then fixed with 1%formaldehyde for 10 min at 4 °C and analyzed by CFSM.

2.5. Statistical analysis

All of the quantitative data were expressed as means 6 standard deviation. Statistical comparisons were performed using one-way ANOVA with SPSS 16.0 (SPSS, USA). P values of less than 0.05 were considered statistically significant.

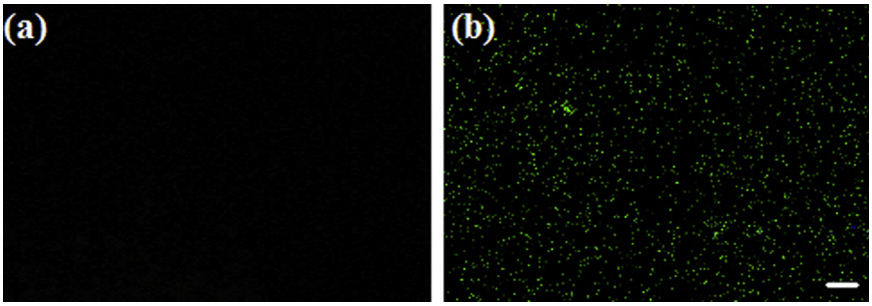

Fig. 1. FN distribution on SWCNT substrates using fluorescent microscopy: (a) non-labeled FN, (b) FITC-labeled FN (Scale bar = 50 μm).

3. Results and discussion

3.1. FN distribution on SWCNT substrate

The distribution of FN protein on the surface of SWCNT substrate is observed in Fig. 1. The green spots represent the homogenous distribution of FITC-labeled FN. However, there is no green spot in the sample that is not labeled with FITC. FITC usually interacts with amine groups of proteins. Fluorescent labeling requires basic conditions in order to activate amine groups. The isothiocyanate group could react with terminal and primary amines of the protein [27]. Therefore, it is suggested that FITC is successfully bonded to FN and Fig. 1 clearly shows the distribution of FITC-labeled FN on SWCNT substrates.

3.2. Surface morphology

Fig. 2 represents the morphology of SWCNT substrates before and after treating with FN. Here, some morphological features related to the presence of FN are observed. As shown, neat SWCNT had tubelike morphology with some bundled form fibers (Fig. 2a). After FN immobilization, the spaces among SWCNTs tubes become smaller and the SWCNTs phase showed continuous representation, resulting in more dense structures (Fig. 2b). This suggests the attachment of FN to SWCNTs.

3.3. Contact angle

Contact angle measurements and wettability are significant properties connected to the chemical and topographical nature of the surface and a key factor in governing the success of the engineered tissue, since the surface is the first part that comes into contact with biological fluids [28]. As SWCNTs are intrinsically not water soluble, modification through chemical functionalization using suitable dispersants and surfactants can enhance their solubility which is essential for controlled dispersion. For this, in the present work, we first examined the dispersion of SWCNTs in ethanol which lead to SWCNTs aggregation. In consistence with our observation, it was found that solubilization of SWCNTs in water and ethanol is very poor and thus form aggregates soon after sonication [29]. Therefore, chloroform was used as dispersion solution for SWCNTs. Additionally, researchers have introduced some effective methods for CNTs functionalization including covalent modification or noncovalent approaches such as polymer wrapping, hydrophilic biomolecule binding, and metal ion binding. Recently, noncovalent functionalization of CNTs has attracted certain attention because as it does not make any structural transformation and secondary structure. Park et al. [27] have described a noncovalent process for surface functionalization of SWCNTs using amphiphilic diblock copolymer (PEtOz-PCL), consisting of hydrophilic PEtOz and hydrophobic poly caprolactone (PCL), with remarkably enhanced solubility particularly in aqueous media. The PEtOz block on the nanotube surface provided possibility for controlling the assembly which could also be utilized as a template for Au nanoparticles formation on the surface of SWCNT. In this regards, Hu et al. [30] have also presented a new noncovalent process for the dissolution and the surface modification of SWCNTs by a commercially available diazo dye, Congo red (CR). The solubilized SWCNTs + CR were stable for more than two months due to π-stacking interaction between SWCNTs and CR [30]. Therefore, it is considered that noncovalent functionalization of SWCNTs with FN not only is a much simpler method compared with covalent functionalization but also has the advantage of preserving nanotube's sp2 structure while enhancing its water solubility [31]. The wettability of SWCNT is found to increase with introducing FN as it summarizes in Table 1. Here, it is represented that the wettability of SWCNT substrates containing 3% FN is higher than the other groups. The contact angles were found to exceed from ~95° on SWCNT substrates to ~121° on SWCNT/3%FN due to an increase in surface roughness and hydrophilicity. Although, adding 1% and 5% FN has also enhanced the contact angles of neat SWCNT substrates, while these variation was not significant in comparison to 3% FN. Generally, study of advancing and receding angles show a high hysteresis. As are reported here, different percentages

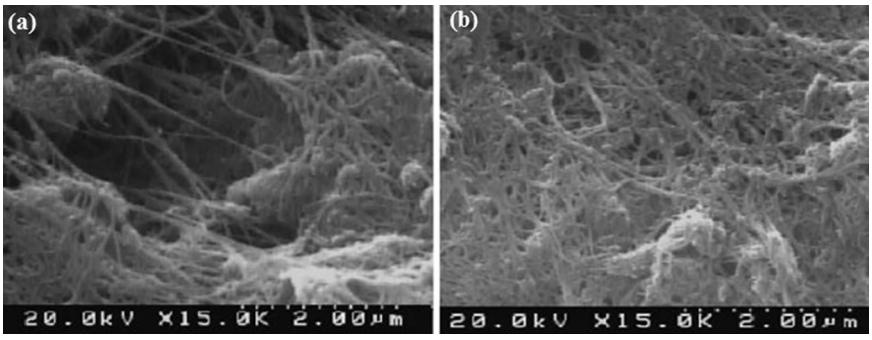

Fig. 2. SEM images of (a) SWCNT fibers, (b) FN loaded SWCNT substrate. After FN absorption, the fibers became denser.

Table 1
Wettability of prepared SWCNTs films with different FN concentration.

	SWCNT	SWCNT/0.1FN	SWCNT/0.3 FN	SWCNT/0.5 FN
Left Contact Angle	95.8°	100.8°	121.3°	106.9°
Right Contact Angle	96.7°	105.3°	121.5°	107.6

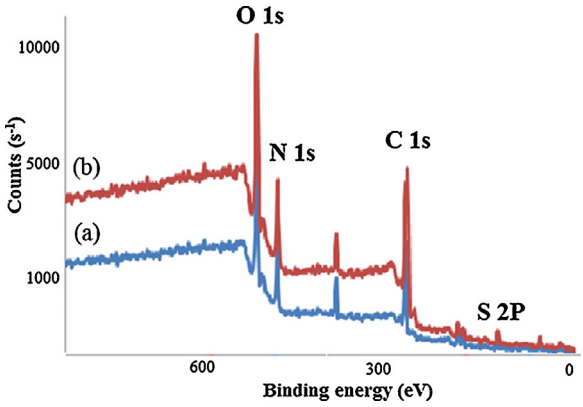

Fig. 3. XPS spectra of SWCNT samples before and after FN absorption. Blue and red peaks indicate SWCNT and SWCNT/FN samples, respectively.

of FN have changed the contact angles of neat SWCNT, inversely. This observation may indicate that there might be an optimal percentage for efficient behavior of FN and other proteins and growth factors. It is suggested that the primary functionalized nano carriers using carboxyl, amine, thiol groups are able to deliver several hydrophobic biomolecules (proteins, peptides, nucleic acids, enzymes) and poorly water-soluble drug molecules (doxorubicin, fluorescein, etc.) [29,32]. In addition to primary functionalization of nanotubes, secondary functionalization can be done by coupling with amino acids and bioactive peptides, allowing enhanced solubility in hydrophilic agents. This strategy is applied in drug, vaccine and gene delivery as a basis where the vehicles enter damaged cells and release biomolecules in order to hasten the process of repair [33]. Therefore, carboxylated SWCNTs coated FN protein could be applied in biomedical applications extensively as a result of its potent capabilities. In consistence with this study, Vidal et al. have shown that chemical treatment of MWCNTs with RGD peptides have increased the wettability of these papers which results in enhancing the spreading and attachment of cells [24]. It is suggested that an increase in the concentration of oxygen atoms is a critical parameter in exceeding the hydrophilicity [34,35].

3.4. XPS

XPS was performed toward the development of a useful mechanism for enhancing the chemical properties of SWCNT after treating with FN. As are shown in Fig. 3, carbon (C), oxygen (O), and nitrogen (N) were the main elements detected by XPS. An increase of oxygen and carbon peaks are noted after treating SWCNT films with FN (Fig. 3b). This oxygen and carbon contribution might be the signature of adsorbed FN. These observations established an increase in hydrophilicity of SWCNT after FN treatment due to an intensification in the amount of oxygen atoms. When FN was adsorbed on the surface of SWCNT, nitrogen and sulfur signals were also detected due to cysteine components. In this regard, a study has demonstrated that addition of RGD peptide and fibronectin to annealed CNPs (carbon nanoparticles) could strengthen the XPS spectra related to C and O element. They also have shown that nitrogen and sulfur elements do not exist in purified CNPs while they appear after RGD peptide and fibronectin treatment [24].

3.5. AFM

AFM was performed as a complementary of SEM technique in order to assess the morphology of SWCNT fibers before and after treating with FN. SWCNT and FN are observed in the form of straight lines and spheres (connecting strings of SWCNT) in AFM nanotopography images, respectively (Fig. 4a and b). The average length of SWCNT fibers was estimated around 10 nm. As previously described, SWCNT powder was sonicated in chloroform in order to prepare a hemogen solution before preparing the films on glass substrates. In AFM photographs, the homogeneity of SWCNT fibers length and diameter is distinguishable which confirms the process of making SWCNT solution. The diameter of FN spheres were also about 200–500 nm. Generally, AFM of proteins is frequently difficult and needs exactitude consideration [36]. Due to mobile nature of proteins, taking individual AFM images of them are very difficult. However, some membrane and non-membrane proteins including fibrinogen [37,38], RNA polymers [39], collagen [40], actin [41], immunoglobulin [42,43] and etc. are investigated by AFM. In addition, Lamprecht et al. had also used AFM for evaluating the ability of bovine serum albumin (BSA) and a phospholipid-linked polyethylene glycol chain immobilized on double-walled carbon nanotubes [44]. In order to take photographs of proteins with high resolutions, they are usually conjugated to specific substrates [36,45]. In the present work, the interaction between SWCNT and FN was lead to immobilization of FN spheres on SWCNT fibers and taking successful images of FN spheres. Generally, there are two types of CNTs functionalization including covalent and non-covalent interactions based on adsorption, wrapping, hydrophobic interaction or covalent bonding of different molecules and chemical reagents. All of these modifications usually make CNTs more hydrophilic [46–51]. However, non-covalent modifications are more attractive in comparison to covalent interactions. This is because non-covalent interactions usually do not disturb the π system of the graphene sheets after adsorption of polymers and surfactants [46]. It was suggested that FN could interact with the SWCNT via electrostatic and hydrophobic bonds (non-covalent interaction). Parallelly, in a study, it was observed BSA and human serum albumin (HSA) could absorb on carbon nanotubes via hydrophobic and electrostatic interactions [52,53]. Moreover, It is appeared that the morphology of SWCNT fibers was independent of concentrations of FN (data not shown). It was reported in a similar study that different types of macromolecules such as DNA did not affect the morphology of SWCNT fibers after conjugation [54]. Despite of morphology consistence of SWCNT fibers after treating the substrates with FN, the nanosurface roughness of these substrates was changed. The hydrophobic nature of SWCNT is responsible for high surface energy of these substrates which results in high nanosurface roughness. On the contrary, hydrophilic structures could smoothed the nanosurface roughness [55–58]. In consistence with contact angle results, treating SWCNT substrates with FN has increased the wettability and hydrophilicity of these structures. Therefore, it was not surprising that FN could smoothed the surface of SWCNT substrates that is beneficial for cellular adhesion and proliferation [58]. For this purpose, images of cells affected by pure SWCNT and SWCNT/FN substrates were captured. Fig. 5a exhibits the tail of a cell on SWCNT substrates. As it can be seen, SWCNT spread and covered the cell entirely. In Fig. 5b, FN spheres which spread around a cell are

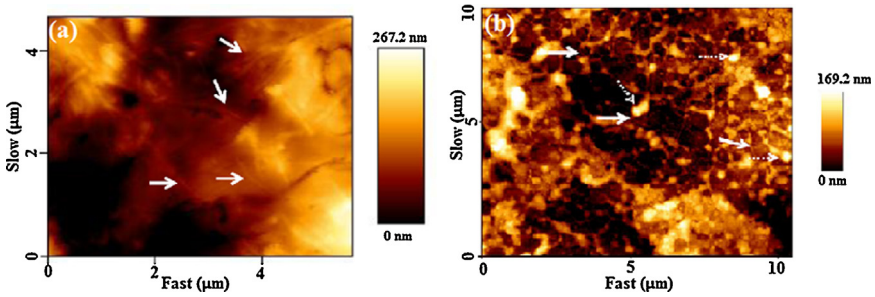

Fig. 4. AFM nanotopography of (a) SWCNT substrate, (b) SWCNT/FN substrate. Dense arrows and break arrows indicate SWCNT fibers and FN spheres, respectively.

shown. SWCNT fibers are also marked by white arrows. Therefore, it is confirmed that the presence of FN could smoothed the SWCNT substrate. This strategy is also useful for increasing the biocompatibility of cytotoxic nanomaterials such as carbon nanotubes [22].

3.6. Biocompatibility

3.6.1. Cell viability

In spite of attractive features, the biological properties of CNTs is a prime concern. Many factors including length, type of functionalization, concentration, duration of exposure, method of exposure and type of disparant are responsible for CNT toxicity [59]. In this regard, many studies have focused on biological behavior of carbon based biomaterials. For example, it was shown by Girase et al. [60] that grapheme oxide reinforced silicon elastomers have induced the function of osteoblasts cells and cell–substrate interactions. This suggested the positive effect of graphene on cellular behavior. In addition, the antimicrobial activity of a nanohybrid structure comprising of silver nanoparticles (AgNPs) anchored to the thiol-group of functionalized polymer that was directly crystallized along the long axis of CNT was evaluated. It was reported that the nanohybrid structure provides the stability of AgNPs and thus retain their large effective surface area and this leads to high antimicrobial activity of silver ions [61]. Misra et al. [62] have also represented a novel carbon based nanostructures for enhancing the osteoblast cells activity for tissue engineering applications. They have shown that silicone–SWCNH nanostructure could support long-term stability and osseointegration in terms of high mechanical strength and superior bone-bonding properties of the implant for soft tissue reconstruction. The enhanced osteoblast functions and cellular response together with higher expression level of proteins were attributed to physicochemical properties of the hybrid nanostructured system, notably, chemistry and hydrophilicity. However, there is still less knowledge about the cytoxicity, fate, and behavior of carbon-based nanomaterials in the aquatic environment. This issue limits the application of carbon based nanomaterials in many fields without safety consideration. Therefore, it was crucial to study their toxicity under different conditions [63]. MTT assay was performed in order to evaluate the cytotoxic effect of SWCNT substrate through their metabolic activity. As are shown in Fig. 6, SWCNT substrates have liberated cytotoxic effect on L929 cells in the culture media while the cells have proliferated normally on FN treated films after 1, 3, and 7 days. Generally, the application of SWCNTs in tissue engineering and drug delivery applications is limited due to its inherent cytotoxicity and aggregation in culture media. It is necessary to mention that carboxylated SWCNTs (the type of applied SWCNT in this study) is presumably more compatible than other functionalized SWCNTs due to its more water-solubility. However, in the present study, carboxylated SWCNT has shown a cytotoxic feature compared with negative control group and FN treated SWCNTs. This issue suggested the presence of toxic materials in the carboxylated SWCNTs preparation that is not filtered during purification process. Moreover, aggregation of SWCNT nanoparticles is the other main cue that should be considered for SWCNT cytotoxicity [64]. This observation confirms the agglomeration of carbon nanostructures in aqueous media because of their hydrophobic nature [65]. Although, all FN treated SWCNTs samples had cytotoxicity effect in comparison to negative control group, but the cytotoxicity had reduced in comparison to neat SWCNT. It is suggested that modification of SWCNT with FN could reduce the SWCNT toxicity. It should be noticed that no significant differences were observed for cell viability in samples containing different concentrations of FN. The toxic effects of CNTs on cells are mainly focused on cell apoptosis, overt toxic reactivity, ROS production, membrane perturbations and cell signaling, etc. [66]. The cytoxicity of CNTs is also depends on the types of involved cell lines. For example, it was reported that SWCNTs induced cytotoxicity in alveolar macrophages of the guinea pig with SWCNTs dose increasing [17]. MWCNTs also induced necrosis and degeneration in the alveolar macrophages of guinea pigs [17]. Additionally, Kisin et al. reported lung fibroblast (V79) cell line lost the viability and was induced with DNA damage in a concentration and time dependent manner after exposure of cells to SWCNTs [67]. Another

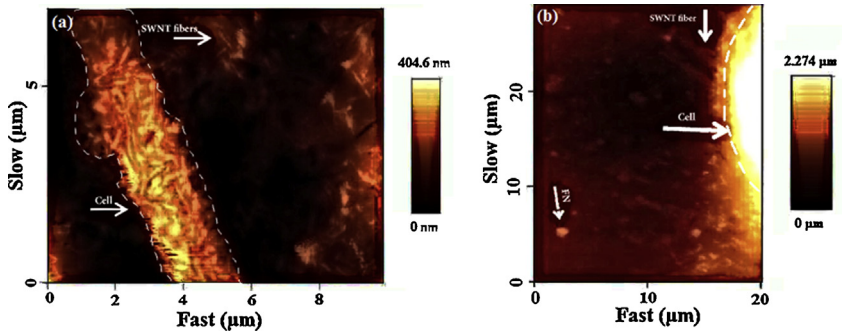

Fig. 5. AFM topography of cells seeded on FN loaded SWCNT substrates (a) SWCNT substrate, (b) FN loaded SWCNT substrate. In image (a) and (b), luminous cells (the margin of cell is identified in break-line) with SWCNT fibers and FN spheres are observed.

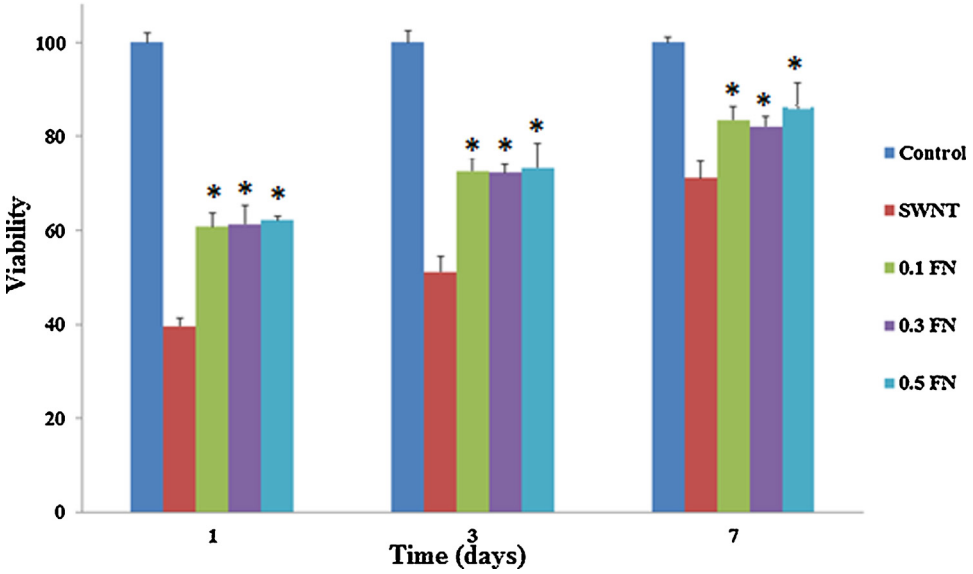

Fig. 6. Cell viability of SWCNT substrates before and after loading FN. * indicates p value ≤ 0.05 compared to neat SWCNT substrates.

study showed that adsorption of fibronectin or serum proteins on CNTs allowed cell attachment and spreading well [24]. It is useful to note that short-term in vitro test cannot be taken as a good method for predicting long-term toxicity. Moreover, the interaction between CNTs and the biological system is so complicated that in vitro methods are not enough to confirm the CNTs-induced toxicities. Instead, in vivo methods are adopted to probe the deep toxicities of CNTs [68].

3.6.2. Cell morphology

SWCNT substrates have provided synthetic environments to bring L929 cells in close proximity so that they can assemble while retaining their normal morphology (Fig. 7). It is suggested that FN loaded SWCNT substrates could impart and accelerate the growth of cells in comparison to bulk SWCNT substrate. FN loaded SWCNT membranes form a supportive environment around cells and provide anchorage to the cells. In this well-defined architecture, the adhesion and proliferation of cells are influenced.

3.6.3. Cell attachment

Fig. 8 shows the cellular attachment of adherent L929 cells after 3 days of culture. A difference was observed between cells grown on SWCNT film before and after FN treatment. While cells spread on hydrophilic FN containing films, they did not adhere

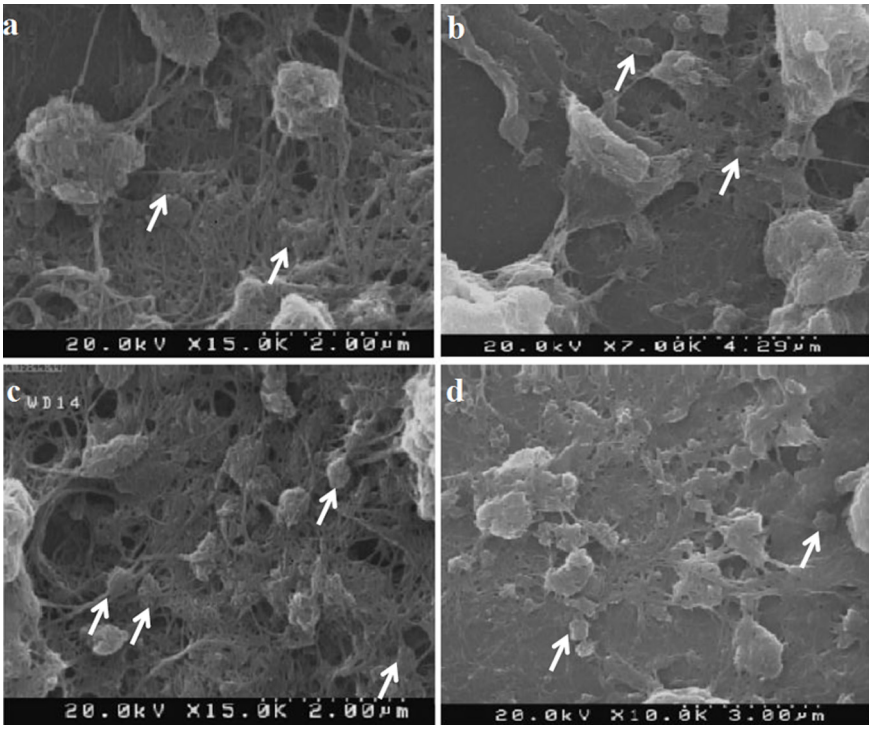

Fig. 7. Cellular morphology on (a) SWCNT, (b) SWCNT/1% FN, (c) SWCNT/3% FN and (d) SWCNT/5% FN substrates. It is clear that cells spread and attached more on SWCNT films containing 3% FN.

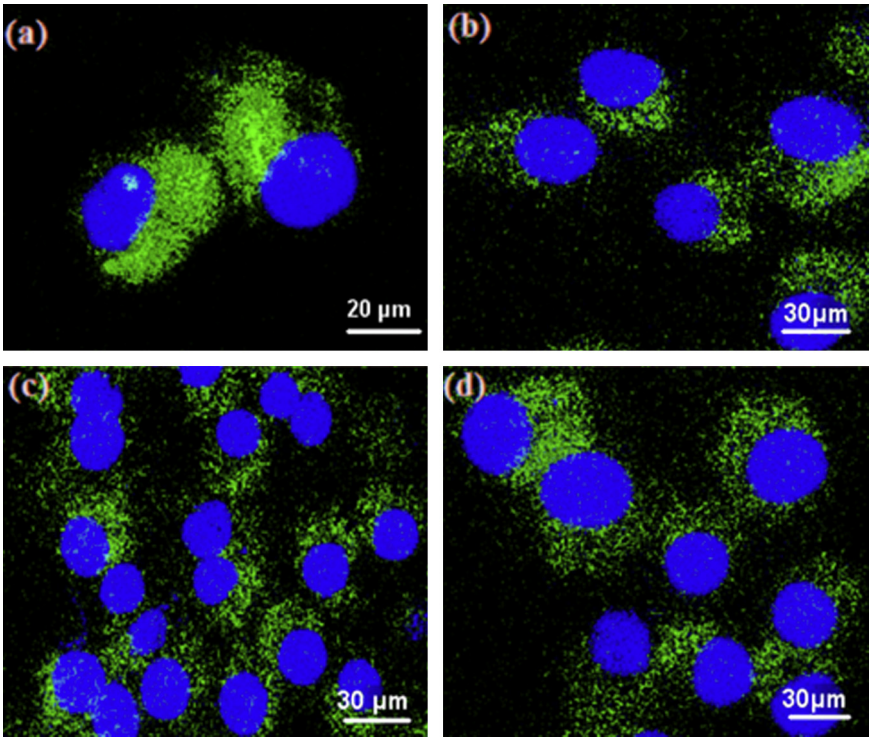

Fig. 8. Confocal micrographs concerning cellular attachment on (a) SWCNT, (b) SWCNT/1% FN, (c) SWCNT/3% FN and (d) SWCNT/5% FN substrates.

properly on hydrophobic neat SWCNT substrates. Generally, both too hydrophilic and too hydrophobic surfaces are not suitable for cellular attachment while well-balanced hydrophilic-hydrophobic surfaces promoted cell adhesion. As are clearly observed in Fig. 7, SWCNT film with 3% FN showed higher cellular adhesion in comparison to control and other FN treated films. It is cited in many studies that immobilization of extracellular matrix components such as fibronectin, laminin, and collagen on synthetic surfaces gives the best substrate for cell spreading and adhesion [69,70]. In our previous study, It was shown that aligned FN nanofibers on SF/SWCNT substrates were not only able to arrange the cells along with their direction but also helped the cells to maintain their normal morphology while spreading [16]. Martyn et al., have also displayed that fibroblast cells attachment on silicon substrates could be enhanced by depositing fibronectin. Thus, fibronectin is appeared as a functional protein for enhancing the biological properties of polymeric substrates [71]. Moreover, it was reported that fibronectin-coated scaffold mimetics promoted cell adhesion. Fibronectin was also capable of directing matrix assembly by mesenchymal stem cells when cultured inside those matrices. Therefore, fibronectin as a key component of the ECM contributes to both the structural integrity as well as the functional properties of live tissues [72].

4. Conclusion

In this work, we studied the absorption of FN protein on carboxylated SWCNTs and proposed that FN could improve the SWCNT properties in order to use in biomedical applications. It is suggested that FN could interact with SWCNTs via electrostatic and hydrophobic bonds. Morphology of FN treated SWCNTs showed that the space among SWCNTs becomes smaller, resulting in more dense SWCNTs. FN protein could improve hydrophilicity of SWCNTs. From point of cytotoxicity view, absorption of FN on SWCNTs could reduce nanotube toxicity and thus enhance cellular attachment in comparison to untreated SWCNTs. No significant differences were observed for cell viability in samples containing different concentrations of FN. Generally, we speculate that the nanofibrous structure of SWCNTs allows FN adsorption without severe change in conformation, thus providing an appropriate sites for cell growth.

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