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Cell Creeping and Controlled Migration by Magnetic Carbon Nanotubes

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Abstract Carbon nanotubes (CNTs) are tubular nanostructures that exhibit magnetic properties due to the metal catalyst impurities entrapped at their extremities during fabrication. When mammalian cells are cultured in a CNT-containing medium, the nanotubes interact with the cells, as a result of which, on exposure to a magnetic field, they are able to move cells towards the magnetic source. In the present paper, we report on a model that describes the dynamics of this mammalian cell movement in a magnetic field consequent on CNT attachment. The model is based on Bell's theory of unbinding dynamics of receptor-ligand bonds modified and validated by experimental data of the movement dynamics of mammalian cells cultured with nanotubes and exposed to a magnetic field, generated by a permanent magnet, in the vicinity of the cell culture wells. We demonstrate that when the applied magnetic force is below a critical value (about $F_c \approx 10^{-11}$ N), the cell 'creeps' very slowly on the culture dish at a very low velocity (10–20 nm/s) but becomes detached from the substrate when this critical magnetic force is exceeded and then move towards the magnetic source.

Keywords Cell creeping and migration · Carbon nanotubes · Magnetism

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Introduction

Carbon nanotubes (CNTs) [1] are molecular-scale tubes of graphite carbon with unique properties including extreme strength, electric properties and other characteristics [2], which account for their large scientific and industrial interest with thousands of original publications on nanotubes being reported every year. CNTs are either single-wall CNTs (SWCNTs) consisting of a single graphite lattice rolled into a perfect cylinder or multi-wall CNTs (MWCNTs) made up of several concentric cylindrical graphite shells. One characteristic that accounts for their scientific and clinical relevance to the biomedical field is the ability of CNTs to penetrate plasma membranes. This property has driven research and development, which has resulted in significant advances in CNT chemistry and functionalization specifically for the use of CNTs as vectors for the delivery of a spectrum of therapeutic substances, e.g., peptides, proteins, nucleic acids and drugs, to cells and tissues [3]. More recent reported biological studies have been based on work, which has exploited their unique physical properties. These include the strong near infrared absorbance by SWCNTs for tumour cell ablation [4], bacterial electroporation by field emission properties of MWCNTs [5] and localized heat release from SWCNTs following application of a radiofrequency radiation also for thermal ablation of cancer [6]. CNTs also possess intriguing magnetic properties, which derive from the metal catalyst impurities entrapped at CNT extremities during their manufacture, enabling them to react to external magnetic fields. This property has been utilized by Cai et al. to develop an alternative physical method of nanotube 'spearing' for in vitro and in vivo gene transfection of cells with plasmid DNA [7]. Monch et al. have shown that ferromagnetic filled carbon nanotubes can interact with human bladder cancer

EJ28 cells [8]. Based on this magnetic property, we have recently demonstrated that MWCNTs when exposed to a magnetic field are able to interact with cells and induce their migration towards the magnetic source [9]. Controlled migration of mammalian cells could have important potential clinical applications, e.g., in cancer therapy to curtail the metastatic behaviour of invasive cancer [10], accelerating regeneration after peripheral nerve injuries, etc. In the present paper, we report on the measurement and modelling of CNT-induced cell movement. Mammalian cells cultured with MWCNTs were tracked and studied for 3 days in order to document and measure their migration dynamics. These data were then used to develop and validate a model, which describes this CNT-induced cell movement in a magnetic field.

Materials and Methods

Sample Preparation

MWCNTs fabrication is based on the synthesis of carbon nanostructures by catalytic chemical vapour deposition (CCVD) of hydrocarbon sources on substrates of alumina impregnated with metal catalysts (Fe) [11]. This fabrication process produces nanotubes with a diameter of 20–40 nm and a carbon content of 97.06%, less than 1% of which being amorphous [12] and bulk density of 0.15 g/cm^3. Non-covalent coating of the CNTs was performed with Pluronic F127 (PF-127, polyoxyethylene-polyoxypropylene block copolymer supplied by Sigma, St. Louis, MO), a water soluble surfactant with MW = 12,600 as previously reported [13]. Briefly, an aqueous solution of PF-127 (0.1%) containing 0.5 mg/mL of MWCNTs was heated (70 °C) on a hot plate with magnetic stirring for 4 h. The resulting mixture was sonicated (2510 Bransonic sonicator, CT, USA) at 20 W for 12 h. The mixture was then centrifuged at 900 g for 10 min to remove non-dispersed nanotubes. The concentration of the CNT solution measured by spectrophotometric analysis [14] at 270 nm averaged 100 μg/mL. For the evaluation of the effects of the surfactant, the sample was inspected by Focused Ion Beam (FIB) microscopy [15] (FEI 200 system delivering 30 keV beam of gallium ions). A drop of this solution was deposited on a silicon wafer fragment and dried for 2 h inside a laminar flow cabinet. FIB imaging revealed an average MWCNT length of 2 ± 0.6 μm (Fig. 1).

Magnetic Characterization

MWCNTs produced by CCVD have magnetic nanoparticles enmeshed in their tips [9], which enable their response to external magnetic fields. Analysis of metal impurities revealed the presence of Al and Fe, derived, respectively, from the alumina support and the catalyst used in the fabrication process. The total metal content (estimated by inductive coupled plasma mass spectrometry) was 2.94% (w/w), and the ratio Fe/Al is 7 ± 1 (w/w) [12]. The magnetic properties of the carbon nanotubes used in these experiments were analysed by the use of a SQUID magnetometer (MPMSXL-7, Quantum Design). The magnetization curve was recorded for solid samples of 5 mg of CNTs. Measurements were performed at 37 °C with an applied magnetic field up to 20,000 Oe. The analysis showed a magnetization curve typical of paramagnetic materials (Fig. 2), with a coercivity of about −400 Oe and a remanence of about 0.44 emu/g. Saturation magnetization (ms) was about 1.6 emu/g for a field of about 5,000 Oe.

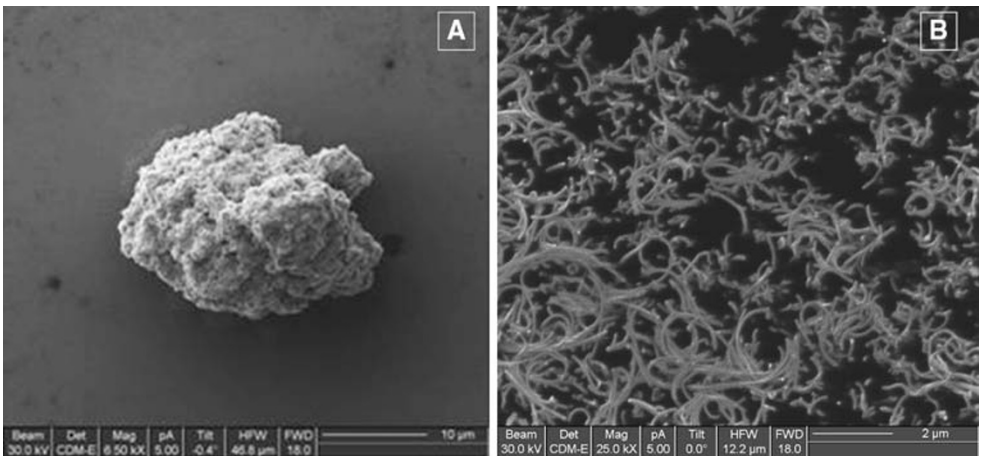

Fig. 1 FIB imaging of **a** as produced MWCNT and **b** a drop of MWCNT dispersion

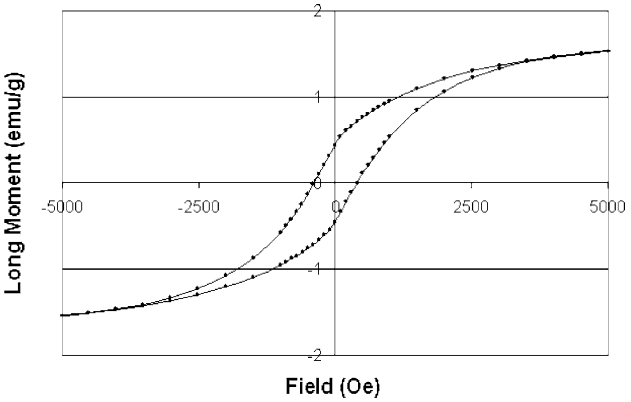

Fig. 2 Magnetization curve

Cell Culture

Human SH-SY5Y cells (ATCC CRL-2266) were grown using a mixture (1:1) of Ham's F12 and DMEM supplemented with 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin and 10% heat-inactivated fetal bovine serum (FBS). Cells were maintained at 37 °C in a saturated humidity atmosphere containing 95% air/5% CO_2. CNT-modified medium was obtained by adding PF-127 coated MWCNTs to the cell culture medium at a ratio 1:10 (v/v). Spectrometric and FIB analyses performed over 5 weeks from sample preparation revealed a great stability of the solution (no phenomena of nanotube aggregation or precipitation). Lipofectamine (GenePORTER 2; Genlantis, San Diego, CA) was used for the transfection of a plasmid DNA containing a green fluorescent protein (gfp) gene reporter in SH-SY5Y cells according to the protocol provided by the supplier. After transfection, cells were cultured without any experimental manipulation for 24 hours before any further experimental testing. The transfection efficiency was about 80% (percentage of GFP-positive cells counted by fluorescent microscopy). Optical and fluorescent microscopy was performed with a Nikon TE2000U fluorescent microscope equipped with Nikon DS-5MC USB2 cooled CCD camera. To determine the effect of carbon nanotubes and the surfactant on cell viability, we used WST-1 (tetrazolium salt 2-(4-iodophenyl)-3-(4-nitophenyl)-5-(2,4-disulfophenyl)-2H-tetrazoilium) cell proliferation assay. Then, 25×10^3 cells were seeded into each well of a 96-well plate and then incubated with the culture media for 72 h. The culture medium was then replaced with 100 μL of medium containing 10 μL of WST-1 solution (as described in quick cell proliferation assay kit, BioVision, USA) and incubated for 2 h in standard conditions. The absorbance was measured on a Versamax microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 450 nm with background subtracted at 650 nm.

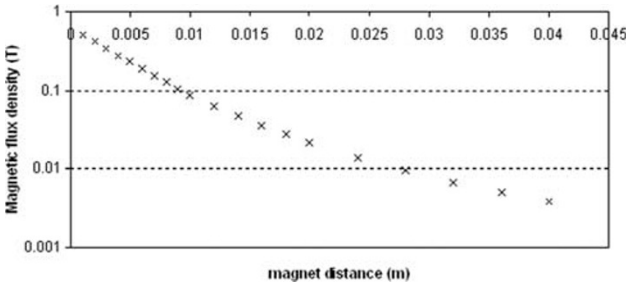

Fig. 3 Magnetic flux density $M(r)$ generated by the magnet

Cell Migration Assays

SH-SY5Y cells were mixed with GFP-expressing cells in a ratio 10,000:1. Cells were cultured in 6-well plates. A grid was applied at the bottom of the wells to measure the cell displacement. The grid, printed on a transparent sheet with a laser printer (30 μm resolution), had an inter-circle line distance of 500 μm. The cells were seeded at a concentration of 10^5 cells/well and incubated for 6 h to allow cell attachment. Cell culture medium was then replaced with CNT-modified medium. After 2 h of incubation, a magnet was placed close to the well. The magnet used was a neodymium cubic magnet (N48, Residual Induction $B_r = 1.41$ T, cube late $a = 12$ mm), which produces the magnetic flux density showed in Fig. 3.

Statistics

Values are reported as mean ± standard error of the mean (S.E.M.). The WST-1 experiments were carried out in triplicate. One-way statistical analysis of variance (ANOVA) followed by post hoc comparison test (Turkey test) was performed; a P value <0.001 was considered significant.

Results and Discussion

Cell Migration on Exposure to a Magnet Field

In our previous work, we demonstrated that SH-SY5Y cells, cultured with a cell culture medium modified with PF-127 coated CNTs, are able to migrate under the effect of an external magnetic field [9] towards the magnetic source. No such displacement was detected in control dishes when cells were cultured in a CNT-free cell culture medium. In the present study, in vitro assays were performed in order to follow the migration dynamics of isolated cells. The fluorescent (target) cells were identified in the well and their exact position determined at 0, 24, 48 and 72 h after placement of the magnet. Figure 4 compares the displacements of the cells treated with the CNTs and exposed to the magnetic field (a) to control cells that are not exposed to the

magnetic field but have been treated with the CNTs (b) and control cells without CNTs but with the magnetic field applied (c). Experimental data confirm, in agreement with our previous work [9], that all the cells treated with the CNTs move towards the magnet while the cell displacement is negligible for control cells (resolution of the measure 50 μm). The cell proliferation assays confirmed that PF-127 and PF-127 coated MWCNTs have no deleterious effect on cell viability at the concentration used (Fig. 5), in agreement with data reported in the literature [16]. Experiments were also undertaken to quantify nanotube 'capture' by SH-SY5Y cells incubated for 2 h in the CNT-modified cell culture medium. The nanotube concentration in the culture

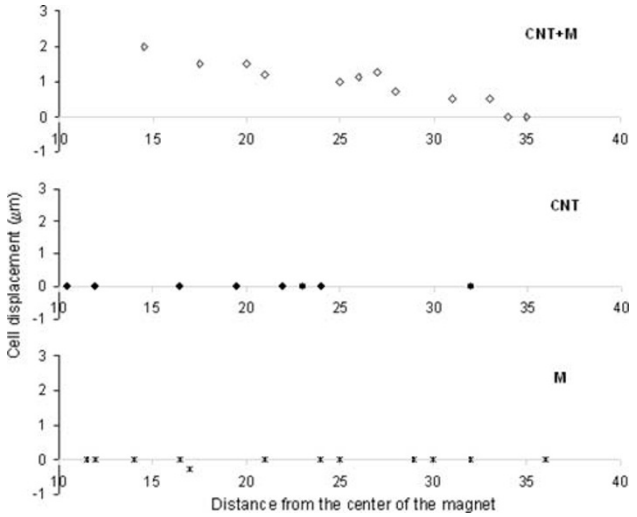

Fig. 4 Migration assays 24 h after the application of the magnet. **a** Cells treated with the CNTs and exposed to the magnetic field **b** control cells not exposed to the magnetic field but treated with the CNTs and **c** control cells without CNTs but with the magnetic field applied

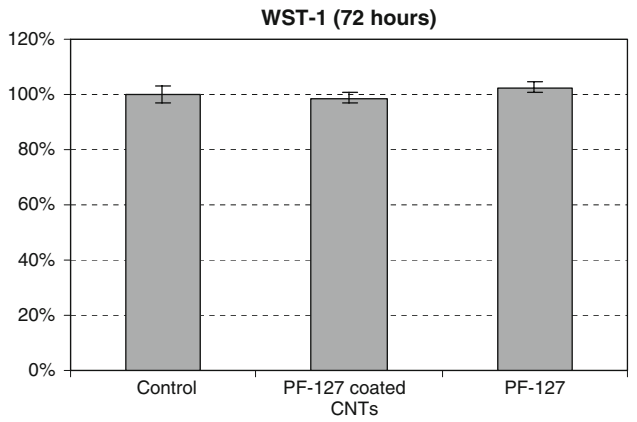

Fig. 5 WST-1 cell proliferation assay after 72 h of incubation in complete medium (control), in complete medium added with CNT solution (PF-127 coated CNTs) and in complete medium added with 0.01% of surfactant (PF-127)

fluid was measured before and after the incubation. The results from 4 replicate experiments confirmed that each cell entraps $m_{\mathrm{CNT}} = 0.76 \pm 0.2$ pg of CNTs. Repeated experiments with cells cultured with a phosphate-buffered solution (PBS) modified with the PF-127 coated CNT solution (ratio 10:1 v/v) gave the same result. We then used such experimental data to corroborate a simple model, which describes mathematically the dynamics of movement of such CNT-tagged cells under the influence of the external magnetic field.

The magnetic flux generated from the magnet is given by the Eq. (1) [17]:

$$B = \frac{1}{\pi} B_r \cdot \operatorname{arctg}\left(\frac{a^2}{2r \cdot \sqrt{4r^2 + 2a^2}}\right) - \operatorname{arctg}\left(\frac{a^2}{2(r+a) \cdot \sqrt{4(r+a)^2 + 2a^2}}\right) \quad (1)$$

where r is the distance from the magnet.

A single cell entrapping magnetic particles is subjected to a translational force F_m in the presence of a gradient field according to:

$$F_{\mathrm{m}} = \frac{1}{\mu_0} \cdot \chi_{rp} \cdot V \cdot B \cdot \frac{\mathrm{d}B}{\mathrm{d}r} \quad (2)$$

where μ_0 is the magnetic permeability of free space, χ_{rp} and V are, respectively, the magnetic susceptibility and the total volume of magnetic particles attached to the cell given by:

$$V = m_{\mathrm{CNT}}/\rho \quad (3)$$

The non-dimensional value (SI) of magnetic susceptibility χ_{rp} was estimated about 1.5 (see "Magnetic Characterization"). The gradient field is given by:

$$\frac{dB}{dr} = \frac{1}{\pi} B_r \cdot \frac{-\frac{1}{2}\frac{a^2}{r^2 \cdot (4r^2+2a)^{1/2}} - \frac{2a^2}{(4r^2+2a^2)^{3/2}}}{1 + \frac{1}{4}\frac{a^4}{r^2 \cdot (4r^2+2a^2)}} + \frac{\frac{2a^2}{(2r+2a)^2 \cdot (4r^2+8ar+6a^2)^{1/2}} + \frac{2a^2}{(4r^2+8ar+6a^2)^{3/2}}}{1 + \frac{a^4}{(2r+2a)^2 \cdot (4r^2+8ar+6a^2)}} \quad (4)$$

By substituting our experimental data, we obtain the function $F_m(r)$ plotted in Fig. 6.

We have observed experimentally that such a magnetic force induces cell migration. The adhesion of a cell to a surface is mediated by reversible bonds between specific receptor-ligand molecules [18]. The magnetic force operates by overcoming the receptor-ligand bonds, which are responsible for the very slow movement (quasi-stationary) state of the cell.

The model predicts cell migration as a function of time by calculating the bond lifetime τ when the cell is stressed by the external force $F_m(r)$. The Bell theory [19, 20]

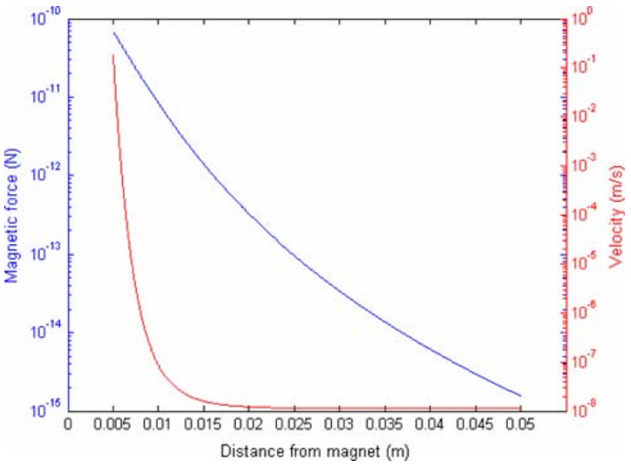

Fig. 6 Magnetic force $F_m(r)$ on a cell (blue line) and cell velocity $|v(r)|$ from the model (*red line*)

predicts that bond survival decreases exponentially with the level of pulling force, according to:

$$\tau = \tau_0 \cdot \exp\left(\frac{E_0 - r_s \cdot f}{kT}\right) \quad (5)$$

where E_0 is the free energy change on binding, r_s is the binding cleft, f is the force applied per bond and $kT = 4.1 \times 10^{-21}$ J is the thermal energy. In the literature, for a representative antigen–antibody bond, E_0 is estimated about 5.9×10^{-20} J, the binding cleft $r_s = 0.5$ nm within a factor of 2 and τ_0 in the order of 10^{-8} s [18].

Our model considers a small adherent cell with area ≈ 200 μm^2 (square section, $l = 10$ μm, see Fig. 7) and 200 bridged receptors for μm^2 of surface. The cell 'creeps' on the surface under the effect of the external force F_m. By considering the cell displacement as the sum of the successive displacements r_s, we get:

$$r(t) = r(0) - \sum_{1}^{n} r_s \quad (6)$$

with

$$t = \sum_{1}^{n} \tau_i(f(r)) \quad (7)$$

The model results plotted in Fig. 8 were achieved with $r_s = 1$ nm (corresponding to one bond broken for each elementary displacement) and $\tau_0 = 5 \times 10^{-8}$ s. The overlap between the model and experimental data is quite good ($R^2 = 0.967$), and the parameters are in the range of values referred to above.

This model can be used to calculate the velocity vs. position of the cell at each point. Figure 6 shows clearly that the cell 'creeps' at a roughly constant velocity about 10–20 nm/s until the magnetic force reaches a critical value $F_c \approx 10^{-11}$N [19], which is sufficient to detach the cell from the substrate and initiate cell migration towards the magnetic source.

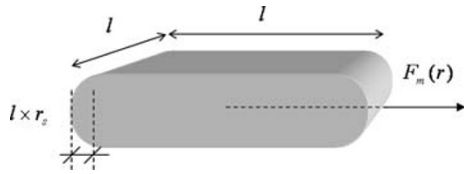

Fig. 7 Model of an adherent cell walking on the substrate by individual displacement r_s under the effect of $F_m(r)$

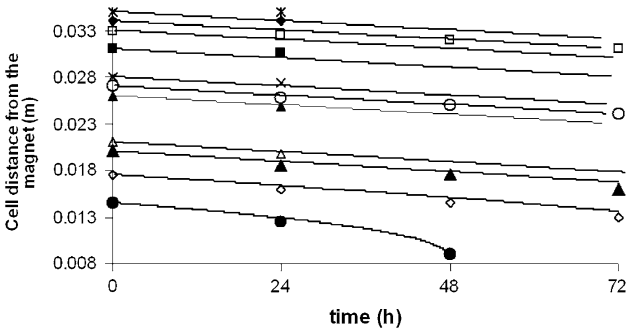

Fig. 8 Migration of isolated SH-SY5Y cells under the external magnetic field $B(r)$. Experimental data (*markers*) and model fitting (*line*). $R^2 = 0.967$

Conclusion

Carbon nanotubes produced by CCVD of hydrocarbon sources on substrates impregnated with metal catalysts entrap at their extremities metal particles, which confer them magnetic properties. We have previously reported that such nanotubes when added to the cell culture medium induce cell migration towards a permanent magnet. This paper proposes a model of cell movement based on the theory of bond survival postulated by Bell in 1978. The model parameters, i.e., nanotubes magnetic susceptibility and the amount of CNTs entrapped per cell were estimated experimentally. In vitro tests of cell migration dynamics confirmed goodness of fit with the proposed model. The model predicts that SH-SY5Y cells cultured with 10 μg/mL of nanotubes 'creep' on the culture disk (at a velocity about 10 nm/s) until the applied magnetic force reaches a critical value ($F_c \approx 10^{-11}$N), which causes cell detachment and migration towards the magnetic source. In view of the potential clinical application, further studies are needed to study the in vivo behaviour and function of mammalian cells tagged with CNTs and subjected to the effect of an external magnetic field.

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