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SPR imaging study of DNA wrapped single wall carbon nanotube (ssDNA-SWCNT) adsorption on a model biological (collagen) substrate†

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The adsorption kinetics of single stranded-DNA dispersed single wall carbon nanotubes (ssDNA-SWCNTs) onto an immobilized collagen layer in a microfluidic channel was probed using surface plasmon resonance imaging (SPRi). The adsorption was measured for a range of both nanotube and solution parameters including the nanotube concentration, nanotube length, solution pH, and the type of medium (buffer and river water). The kinetic adsorption data suggests that the adsorption of the nanotubes to the collagen layer is irreversible in HEPES buffer, pH $\approx$ 7.4, at room temperature, with the nanotube adsorption displaying two different kinetic adsorption regimes for different concentration ranges. A stretched exponential function fit indicates that a transition of adsorption kinetics from single exponential (>40 $\mu\text{g/mL}$) to non-exponential kinetics (<40 $\mu\text{g/mL}$) occurs as the nanotube concentration decreases, suggesting that a diffusion-limited process was predominant at lower concentrations (<40 $\mu\text{g/mL}$). Adsorption measurements as a function of nanotube length also displayed differences in the apparent adsorption processes. Adsorption of shorter nanotubes (≈ 40 nm) was found to follow single exponential kinetics, while longer nanotubes (≈ 300 nm) adsorbed much more slowly, consistent with adsorption partially influenced by diffusion-limited processes. Finally, to evaluate the potential differences in adsorption for unintentionally released nanotubes in a riparian environment, nanotubes were stably dispersed in a sample of river water and exposed to the collagen layer. Compared to the adsorption under HEPES buffer the nanotubes in the river water were found to exhibit a reduced rate of adsorption. It is likely that passivation of the collagen layer by dissolved organic species could thus affect the fate of released nanotubes in a natural environment.

Introduction

Recently, there has been notable attention on using nanotubes for therapeutic applications and biotechnologies such as drug molecule carriers and as biosensors.^{1–3} Nanotubes are inherently advantageous materials for these applications due to their large uniform surface area, high mechanical strength, apparently low cytotoxicity and chemical/thermal stability; additionally, nanotubes have strong optical transitions in the near-infrared (NIR) wavelengths that can be used for sensing or for enabling therapeutic uses.^{4–7} Lastly, because the inner and outer wall of nanotubes can also be functionalized with different chemical functionalities, it is feasible to provide both stable dispersion in aqueous conditions and great flexibility in designing targeted and biologically useful systems.^{2,8}

A key barrier to enabling these technologies however is the lack of knowledge on how the dispersed nanoparticles will

interact with the various materials that comprise the biological environment. Nano sized materials can be introduced into the body, whether intentionally or not, through various paths such as lungs, intestines and dermis.⁹ In each of these pathways, the nanomaterials will interact with the surrounding biological environment in a manner mediated by the surface properties of the nanomaterial and its physical characteristics such as the aspect ratio. These interactions between nanostructured materials and biological proteins and interfaces are known for *in vivo* applications to greatly influence the transport properties of the nanomaterials including their circulating lifetime and bio-distribution.¹⁰ Consequently, our objective is to develop experimental methodologies that can measure the interactions of the nanomaterials with biologically relevant surfaces, in particular with cell membranes and tissues or model representations thereof.

In the present study, we measured the interactions between single-wall carbon nanotubes (SWCNTs) coated with single-stranded DNA and a collagen surface using surface plasmon resonance imaging (SPRi). This combination of nanoparticle and surface was chosen due to the likely application of SWCNTs for therapeutic use, and the ubiquity of collagen in the extracellular matrix (ECM) environment. To apply SWCNTs in a controlled manner consistent with possible applications however, they must first be individualized in solution using a dispersant and processed to remove non-nanotube impurities. The ss-DNA and processing used in this contribution has previously been shown

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to result in well dispersed and purified SWCNTs with a negative surface charge under physiological conditions. The strong binding of the ssDNA to the nanotube enables the robust individualization of SWCNTs in an aqueous environment.^{11,12} An additional benefit of the use of DNA as the dispersant is that the resulting dispersions can be separated into well defined length populations from the initially polydisperse length distribution using size exclusion chromatography (SEC).¹³ For the choice of surface, collagen is one of the most abundant extracellular matrix proteins, providing mechanical support to cells and tissues in a biological system, and can be repeatedly fabricated with ease,¹⁴ and is thus an ideal biopolymer for use as a biologically relevant surface for SWCNT adsorption.

Surface plasmon resonance imaging (SPRi) is a surface sensitive technique that is used to measure adsorption/desorption events by detecting refractive index changes at the liquid-solid interface.¹⁵ In this effort, we used SPRi in conjunction with a microfluidic system to simultaneously investigate the adsorption kinetics of ss-DNA wrapped SWCNT with regards to multiple parameters on the same chip. This was accomplished using PDMS microchannels fabricated *via* microfabrication to generate multiple channels across the observable field of view, which has additional benefits including reduction of the sample volume, efficient fluidic control, and enabling high throughput data accumulation *etc.*¹⁶ In any given experiment, it was possible to examine the adsorption kinetics within a range of nanotube concentrations and different length fractions in a parallel fashion. Observations were analyzed with a first order reaction model with power law dependence. From the data, two different adsorption process regimes were determined as a function of nanotubes concentration and nanotube length. Variation of solution pH of the dispersion medium on adsorption was also explored to provide further insight into the possible mechanism of adsorption. Finally, since nanomaterial related environmental and health concerns have arisen recently, we additionally measured the adsorption of nanotubes onto a collagen surface after both were incubated in harvested river water to simulate the adsorption of released SWCNTs in a natural environment.

Materials and methods§

Substrate preparation

Substrates for SPR were generated in-house in a multi-step process. Glass substrates (25 mm × 50 mm × 0.5 mm) were cleaned with piranha solution (H₂SO₄ : 30 vol. % H₂O₂ = 4 : 1) for 30 min, rinsed with deionized water (Milli-Q, 18.2 MΩ cm), dried with nitrogen, and stored in absolute ethanol. After cleaning, the Cr (0.5 nm) and Ag films were sequentially deposited on the glass substrate using a sputtering device (Denton Vacuum model DV-502A) at the pressure of $\approx 3 \times 10^{-6}$ torr. The Ag film was deposited at a deposition rate of 0.63 nm/s to a final thickness of 40 nm. Immediately after removal from the

sputtering chamber, each Ag substrate was stored in absolute ethanol. A hydrophobic self-assembled monolayer (SAM) was then applied to prevent Ag surface oxidation and to prepare the surface for collagen deposition. The 1-octadecanethiol (ODT), (Aldrich, used as received) SAM was formed on the Ag coated glass by overnight immersion of the substrates in absolute ethanol containing ODT (1 mmol/L) at room temperature, followed by thorough rinsing with absolute ethanol several times and drying with nitrogen. The equilibrium contact angle (CA) of the deposited SAM layer was measured to be $111.5^\circ \pm 0.6^\circ$, indicating the formation of a highly hydrophobic surface. The thickness measured by SPR was $\approx (1.8 \pm 0.2)$ nm and tapping mode AFM (Veeco, Dimension 3100) showed a planar morphology of the ODT layer with a root mean square surface roughness (R_{rms}) $\approx (1.54 \pm 0.08)$ nm. The contact angle and the roughness data were obtained by averaging the measured values from five different areas on the specimen. For both measurements, the reported uncertainty is equal to one standard deviation. The average value for the layer thickness from the SPR data was obtained from 3 different regions of interest in the SPR image and uncertainty is indicated by the standard deviation.

Microfluidic channel fabrication

Silicon wafers (diameter = 76.2 mm) were cleaned with solvents (acetone, methanol and isopropanol) and treated with O₂ plasma. SU8-50 (MicroChem, Newton, MA) was spun on the wafer using a spin speed of 104.7 rad/s for 35 s (SU8 thickness ≈ 100 μm), followed by a soft baking process at 95 °C for 45 min. Microchannels were patterned using a transparency mask under a UV lamp (600 mJ cm⁻², 365 nm UV line). Following exposure, a post expose bake (PEB) process was performed at 95 °C for 30 min. Finally, the SU8 film was developed in SU8 developer (MicroChem) for 10 min, followed by rinsing with isopropanol and drying in N₂. PDMS (Polydimethylsiloxane, Sylgard 184, Dow Corning) was mixed with a curing agent (10 : 1 ratio by mass) and the mixture was poured onto the SU8 structured wafer. Bubbles in the mixture were removed by use of a vacuum desiccator for 1 h after which the PDMS was thermally cured at 60 °C overnight. The PDMS was then carefully removed from the SU8 substrate, channel inlets and outlets were punched into the PDMS using a syringe borer, and the channel was sealed (van der Waals contact) against the ODT functionalized Ag substrate. The dimensions of the microchannels were measured by the contact profilometer (Dektak 6M) as 300 μm × 100 μm (width × height) and the length of the microchannels was 4 cm. After forming the channels, the microchannels and tubing (0.38 mm ID, Polyethylene) were cleaned with deionized water at a flow rate of 5 μL/min for at least 30 min using a micro pump (Programmable syringe pumps BS-9000-6, Braintree Scientific, Inc.) prior to collagen deposition.

Collagen substrate formation in microchannels

Collagen type I solution sourced from rat tail tendon was purchased from Upstate (Lake Placid, NY), from which 10 μg/mL collagen solution was prepared in 25 mmol/L of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution. At deposition, the pH of the HEPES buffer was 5.5 and the collagen solution was maintained at 4 °C to minimize gel formation of collagen in the microchannels. To deposit the

§ Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

surface layer with collagen, the collagen solution was introduced into the microfluidic system at a flow rate of 2.5 $\mu\text{L}/\text{min}$ for 60 min, followed by a HEPES buffer (pH 5.5) rinsing step for 30 min to remove excessive collagen molecules loosely attached on the deposited collagen layer.

ss-DNA wrapped SWCNT preparation

SWCNT dispersions in aqueous solution were prepared by sonication (1.5 h, $\approx 1 \text{ W}/\text{mL}$ power) of raw nanotube soot (Southwest nanotechnologies, SP Grade, Lot: NI6-A001) at 1 mg/mL concentration in the presence of 1 mg/mL (GT)₁₅ ss-DNA followed by centrifugation at $21\,000 \times$ gravity for 2 h to remove non-nanotube material. During sonication the charged DNA wraps around the nanotubes preventing reaggregation.^{11,12} Average length of nanotubes dispersed in aqueous solution is found to be approximately 200 nm^{13,17–19} and nanotubes of this length were used for most of the experiments except length dependant adsorption study. This preparation has previously been shown to produce dispersion of individual nanotubes.^{13,20} Length separation in size range from 40 to 300 nm was performed *via* size exclusion chromatography as previously reported.¹⁷ Exchange of the aqueous medium and concentration of the nanotube dispersion was performed using forced dialysis stirred cells (Millipore), and repeated exchange of the working solution. Concentrations and extent of dispersion were determined using UV-vis-NIR spectroscopy; a previously established extinction coefficient of $2.6 \text{ A m}^2 \text{ g}^{-1}$ at 775 nm was used to calculate the nanotube concentration.^{13,21} The mass per volume measure of concentration refers solely to the nanotube mass; our research (not shown) and that of others^{11,22} indicates that the nanotube and bound oligonucleotide mass are approximately equal. The absorbance spectra of the nanotubes used in this study is shown in Fig. 1.

River water

River water was collected directly from Great Seneca Creek (Gaithersburg, MD), and was sequentially filtered to remove

Fig. 1 UV-vis-NIR absorbance as a function of wavelength for 0.1 mg/mL SWCNT wrapped with ssDNA in HEPES buffer (pH 7.4) and river water.

large sediment and particles prior to use through filters primed with deionized water. The filters (Millipore) sequentially used have pore size of 1.6 μm (glass fiber), 0.45 μm (HV Type), and 0.22 μm (GV Type). After the last filtration, approximately 50 mL of nearly clear solution remained. This liquid was used without further processing for incubation of the collagen surface, and for exchanging the medium of the DNA-SWCNTs.

Characterization techniques

A previously described²³ angle scanning imaging SPR based on the Kretschmann configuration and equipped with a 514 nm wavelength LED laser source (WT&T inc. LE-1G), polarizer, sapphire prism ($n = 1.7850$) and CCD detector (Q imaging, Retiga 2000RV) with 1600×1200 pixels was used for this study. Refractive index matching oil (Cargill, $n = 1.5220$) was applied between the prism and the Ag coated glass substrate ($n = 1.5220$). Reflectivity of the P or S-polarized light is detected by the CCD detector and the image is displayed with the imaging software (NorPix, StreamPix). During data capture, a SPR curve is obtained by an angle sweep and is fitted with polynomial parabolic curve ($n = 6$), with the derivative of the fit function providing the minimum angle of the SPR curve. The SPR minimum angle, θ , was measured to be 73.05° for the ODT coated Ag/water interface and experimental data was fitted with Fresnel equations to estimate the thickness of film layers.²⁴ In SPR, incident light is selectively polarized into components either parallel (p-polarized) or perpendicular (s-polarized) to the plane of incidence. Since a surface plasmon is excited only by the p-polarized component and the attenuation of the reflectivity corresponds to the surface plasmon resonance, percent reflectivity (%R) is defined as $\%R = I_P/I_S \times 100\%$, (I_P and I_S refer to the reflected intensity of p- and s-polarized light, respectively). Analysis of SPR images for all experiments was performed using ImageJ software (National Institutes of Health).

UV-Vis-NIR measurements were taken in transmission mode on a PerkinElmer Lambda 950 UV-Vis-NIR spectrophotometer over a wavelength range of 2500 to 185 nm. The incident light was circularly polarized prior to the sample compartment, and the spectra corrected for both dark current and background. The optical absorption spectrum was recorded at 1 nm increments with an instrument integration time of 0.16 s per increment using a 1 mm or 2 mm pathlength quartz cuvette. The reference beam was left unobstructed, and the subtraction of the appropriate reference sample was performed during data reduction. For some samples an ultrafiltration cell (Millipore 8003, 8010, or 8050) was used to concentrate the nanotubes prior to measurement.

Unless stated otherwise, the standard uncertainty throughout this contribution is denoted by error bars equal to one standard deviation.

Results and discussion

Description

Collagen is the most abundant protein in the extracellular matrix protein (ECM) and thus is a good candidate for use as a model biological substrate. However, uniform collagen layers can be difficult to form, with the result being various structures ranging from a monolayer to thick gelatinous networks, depending on

the surrounding conditions such as pH, temperature, underlying surface chemistry of substrate and concentration, unless the deposition conditions are tightly controlled.^{25–28} In this study, an ODT functionalized surface was selected as an underlying substrate for collagen layer formation, and all collagen layers were deposited at a solution pH = 5.5 to minimize collagen fibrilization. Adding this step to the process results in the formation of a uniform and homogeneous collagen surface during deposition on which the nanotube–collagen interaction can be controllably measured.

Deposition of the collagen was monitored using the SPR imaging technique, and the thickness of collagen layer was estimated using the Fresnel equation (refractive index of collagen is 1.43); the estimated layer thickness from the SPR measurement was 4.0 ± 0.6 nm, consistent with the thickness of a collagen protein monolayer.^{25,29} A schematic of the collagen and SWCNT adsorption processes is shown in Fig. 2 (a)–(d). Use of the microfluidic system allowed us to form uniform collagen films in a controlled environment and monitor that the collagen morphology in the microfluidic system was well retained at the surface during both solution rinsing and SWCNT adsorption processes. An analysis of SWCNT adsorption in a microfluidic channel, in conjunction with SPR imaging, allows high throughput observations and enables adequate experimental process control such as flow rate and solution exchange. The change in the SPR signal from the collagen layer formation on the ODT coated substrate, followed by adsorption of nanotubes on the collagen film in a microchannel device is shown in Fig. 3 (a). In the figure the effects of the multiple processing steps can be observed. First the ODT functionalized substrate was rinsed with DI water (5 μ L/min for 30 min) to stabilize the baseline reflectivity. After the baseline stabilized, a solution of collagen (pH 5.5 and 4 $^{\circ}$ C) was injected into the microchannel; the formation of the collagen layer on the ODT SAM is shown by the large increase in the reflectivity signal ($\Delta\%R$). That this signal does not significantly change upon subsequent rinsing with two HEPES buffers, pH 5.5 for rinsing and pH 7.4 to equilibrate the surface to physiological pH conditions indicates that the collagen layer was fully attached

Fig. 2 Schematic drawing of the fabrication process of (a) Octadecanethiol (b) collagen layer formation (pH 5.5, 1 h) and (c) ss-DNA wrapped SWCNT adsorption on the collagen surface. (d) Schematic process design of SWCNT–collagen interaction in a microfluidic system.

Fig. 3 (a) SPR monitoring of collagen deposition and unsorted ss-DNA wrapped SWCNT adsorption (b) A representative plot captures the SPR reflectivity changes for 20 μ g/mL of SWCNT adsorption onto a collagen surface at pH 7.4 and 25 $^{\circ}$ C.

on the substrate. Finally, the nanotube solution in HEPES buffer (pH 7.4) was introduced at a lesser flow rate (2.5 μ L/min) for 30 s to fill the entire microchannel with nanotube solution, after which the pump was stopped, and the nanotube adsorption measurements were obtained under quiescent flow condition.

Fig. 3 (b) shows the kinetic adsorption of the dispersed SWCNT (20 μ g/mL, pH 7.4) onto the collagen layer. Once the nanotubes are introduced, an abrupt reflectivity change ($\Delta\%R$) occurs as the nanotubes adsorb onto the collagen surface. Steady-state, as measured by $d\Delta\%R/dt = 0$, was achieved after approximately 10 min. Post adsorption (30 min) rinsing with HEPES buffer (pH 7.4) was found not to affect $\Delta\%R$, indicating that the ss-DNA wrapped SWCNTs were irreversibly adsorbed onto the collagen surface at neutral pH. As a control experiment, nanotubes were also introduced onto ODT surface (no collagen layer) and almost no adsorption was observed compared to when the collagen layer is present (data not shown). Thus, the large signal increase in Fig. 3(b) is solely due to the interaction between functionalized nanotubes and collagen layer and is not representative of the nanotubes binding directly to the underneath ODT surface. Poor adsorption onto the ODT surface can be rationalized by a weak attractive van der Waals interaction.³⁰

Adsorption kinetics of ss-DNA wrapped SWCNT on collagen layer

SWCNT adsorption kinetics onto the collagen layer were then studied for different concentrations of the SWCNT dispersion

ranging from 7.5 to 160 $\mu\text{g/mL}$ (all at 25 $^{\circ}\text{C}$ and pH 7.4). For these experiments the collagen layers were prepared simultaneously in multiple, parallel, microchannels on single substrates. With this methodology nearly identical layers of collagen were deposited in all channels (substrates). This was verified by measuring the thickness of the collagen layers using the SPR angle; variation in the SPR angles was $< 0.06^{\circ}$. This allowed for comparison of the absolute adsorption rate differences for the multiple SWCNTs dispersions measured on a given device. Adsorption kinetic data for each of the five channels (plus a blank) in a single device for the first 35 min of SWCNT adsorption is shown as a function of time in Fig. 4 (a). We see that the initial increase is steeper for higher SWCNT concentrations and that the asymptotic value ($\Delta\%R$) also increases with increasing concentration. Since the interaction between the SWCNT and the collagen layer is strong and irreversible, the equilibrium method can't be applied to estimate a binding constant. A first order reaction model with a power law was applied to describe nanoparticle adsorption on a biological surface²³ and polymer layer formation previously.³¹ Douglas *et al.* demonstrated a transition from single exponential kinetic to non-exponential kinetics using this model for temperature and molecular weight dependent polymer sorption behaviors.^{31,32} Therefore we analyzed the nanotube adsorption kinetics quantitatively with the modified first order reaction model, considering two different kinetic regimes as the nanotube concentration is increased. The first order reaction model used is shown in eqn (1).

$$\Delta\%R = \Delta\%R(\infty)[1 - \exp(-(t/\tau_{\text{on}})^{\beta})] \quad (1)$$

Within this model, $\Delta\%R$ is the percent reflectivity changes, $\Delta\%R(\infty)$ is a steady state value of reflectivity at long times, τ_{on} is an adsorption time constant and β is a parameter related to the time dependant rate. The values of $\Delta\%R(\infty)$, τ_{on} and β were obtained by fitting the kinetics curve with eqn (1) and the values of τ_{on} and β are plotted with nanotube concentration in Fig. 4 (b). τ_{on} can be considered physically as the time to adsorb to $(1 - 1/e)$ of $\Delta\%R(\infty)$. The figure shows a clear transition of adsorption kinetics around a nanotube concentration of 40 $\mu\text{g/mL}$. τ_{on} tend to show slow increase as concentration decreases from 320 $\mu\text{g/mL}$ and at concentration below 40 $\mu\text{g/mL}$, τ_{on} value significantly increases, indicating the adsorption rate becomes slowed. At approximately this concentration, the rate of adsorption changes from non-exponential adsorption kinetics ($0.5 < \beta < 1$) to single exponential kinetics ($\beta \approx 1$). For diffusion limited adsorption to a planar surface the rate of adsorption should scale as $\approx t^{-1/2}$, which when inserted into the first order reaction equation for the nanotube adsorption gives eqn (1) with $\beta \approx 1/2$. This is consistent with the β values for concentrations $< 40 \mu\text{g/mL}$, and indicates that if the nanotube concentration is low, the adsorption rate is determined primarily by a diffusion limited process. The implication is that the adsorption process occurs much faster than the rate at which the incoming nanotubes arrive at the collagen surface due to diffusion, such that the adsorption rate of nanotube is dominated by the contribution from the nanotube diffusion kinetics.

Fig. 4 (a) SPR reflectivity changes for the unsorted ss-DNA wrapped SWCNT (≈ 200 nm) adsorption onto a collagen surface in different nanotube concentration ($\mu\text{g/mL}$) at pH 7.4 and 25 $^{\circ}\text{C}$. The solid lines represent the fit from eqn (1). (b) Open square: τ_{on} and open circle: β . The values of τ_{on} and β were calculated for different concentrations of the nanotubes adsorbed onto a collagen substrate with a 95% confidence interval (C.I.). The dotted lines through the data points are the first order exponential fittings to aid the reader's eyes (c) Adsorption isotherm of ss-DNA wrapped SWCNT adsorbed onto the collagen surface at pH 7.4 and 25 $^{\circ}\text{C}$. The exponential fitting through the data points is drawn to aid the reader's eyes and the error bars represent standard deviations.

At higher nanotube concentrations, however, the adsorption occurs at a constant rate, which indicates that $\beta \approx 1$, and that the process is not limited by the nanotube diffusion rate processes. Furthermore, the experimental observation that the adsorptions follow single exponential kinetics while the β and τ_{on} values are nearly stable with increasing nanotube concentration above 40 $\mu\text{g/mL}$, additionally indicates that the adsorption rate is maintained by the higher concentration of incoming nanotubes.

To study the effects of nanotube concentration on the total adsorbed amount, the steady state of SPR angle shift ($\Delta\theta$) was measured after 60 min of adsorption as a function of the nanotube concentration [Fig. 4 (c)]. SPR profiles were obtained before and after the SWCNT adsorption for each concentration and then the parabolic curves of the SPR profiles were fit to a 6th order polynomial regression and the derivatives of these functions were calculated to yield a corrected $\Delta\theta$. The value of $\Delta\theta$ is proportional to the amount of the SWCNT adsorbed on the collagen surface. The figure shows that initially the slope of the adsorption isotherm increased abruptly. Because the adsorption is irreversible, depletion of the nanotube concentration in bulk is expected in low concentration regime. Therefore, the adsorbed amount of nanotubes is dependent on the bulk concentration. As nanotube concentration is increased ($>100 \mu\text{g/mL}$), the adsorption on the collagen surface becomes saturated, indicating that the adsorbed concentration approaches a jamming coverage of the available reaction sites.³³

Adsorption of ss-DNA wrapped SWCNTs of different lengths onto collagen layers

Applying simple scaling arguments to the above results, we would expect that the concentration at which the adsorption process shifts should be strongly affected by the average length of the SWCNT in the dispersion. To explore this parameter, DNA wrapped SWCNTs dispersions of different average lengths that had previously been separated from equivalent heterogeneous batches of nanotubes, and made from the same parent material to those used above, using size exclusion chromatography (SEC)^{13,17} were introduced to the collagen surface. Measurement of the adsorption rate for two different lengths of SWCNTs (avg. lengths: 300 nm and 40 nm) at 20 $\mu\text{g/mL}$ concentration, demonstrated significantly different kinetic behavior [Fig. 5 (a)]. Faster kinetics was observed for shorter nanotubes than for the longer average length and unsorted samples. We applied the eqn (1) to the adsorption kinetics data and the values of τ_{on} and β were obtained and plotted as a function of average length as shown in Fig. 5 (b). The figure shows that the adsorption of ≈ 40 nm nanotubes follows single exponential kinetics ($\beta \approx 1$). As might be expected, the adsorption of the longer nanotubes (≈ 300 nm) shows a slower process that is more strongly determined by diffusion ($\beta \approx 0.78$), as the equivalent sphere size increases, albeit sublinearly, with the rod length. The values for the average 200 nm long nanotubes were also included in the figure and the trends of β and τ_{on} , showing monotonic behavior. These results can be explained by length dependant diffusion rate of nanotubes. Longer nanotubes diffuse more slowly than shorter nanotubes; in the comparison between the two data sets, the lower rate of flux to the surface for the longer SWCNTs is apparently slower than rate at which the adsorption process can remove

Fig. 5 (a) SPR reflectivity changes of adsorption of 20 $\mu\text{g/mL}$ of ss-DNA wrapped SWCNT in different length fractions (300 nm and 40 nm) on the collagen surface as a function of adsorption time. (b) Open square: τ_{on} and open circle: β . The values of τ_{on} and β were calculated for different average SWCNT length adsorbed onto the collagen substrate with a 95% confidence interval (C.I.). The dotted lines through the data points are the sigmoidal and second order polynomial fittings to aid the reader's eyes and the error bars represent one standard deviation.

the nanotubes from the solution. This results in the measurement of a slower adsorption rate for the longer SWCNTs and fitting coefficients more indicative of a diffusion limited process. It is unknown whether the small difference in $\Delta\%R$ after 30 min adsorption for both nanotubes in Fig. 5 (a) is due to a slight difference in the bulk nanotube concentration or a different factor such as a length effect on $\Delta\%R$.

pH effect on adsorption of nanotubes

The effect of pH on the adsorption rate of nanotubes is also a critical parameter for both cellular and unintended release environments. To explore this effect using our model system, adsorption experiments using the polydisperse length nanotubes (avg. length ~ 200 nm) were performed at different pH environment. In each experiment, 80 $\mu\text{g/mL}$ of ss-DNA wrapped SWCNTs were dispersed into different pH HEPES buffers ranging from pH 5.5 to pH 10.1 using the forced concentrating cell. pH 10.1 was

chosen as the highest pH as we have previous results (unpublished) showing that the DNA-SWCNT dispersion becomes unstable beyond pH 11, resulting in rapid aggregation of the SWCNTs. All solutions were made by adjusting the pH of 25 mmol/L HEPES buffer from 5.5 using aliquots of 0.1 mol/L KOH. After pH adjustment, the SWCNT solutions were introduced as previously into the microchannels, which had also been equilibrated with the respective and corresponding pH buffers, and the adsorption kinetics were again measured using SPR imaging [Fig. 6 (a)]. Larger changes in $\Delta\%R$ were observed under high pH conditions compared to neutral and lower pH conditions, indicating that more nanotubes were adsorbed to the collagen surface at the higher solution pH. $\Delta\theta$ in Fig. 6 (b) also shows a similar trend along the adsorption kinetic data. The 30-mer strand (GT)₁₅ ss-DNA used to wrap nanotube has an isoelectric point at approximately 4.5 so the DNA wrapped nanotubes were negatively charged throughout the range of the pH conditions (5.5 to 10.1). Collagen on the other hand possesses amine containing amino acids having a $pK_a \approx 11$, which become more positively charged at low pH values.^{34,35} Therefore, mutual

electrostatic interaction is weak under pH conditions near the pK_a value of amine group of collagen and the isoelectric point of DNA, resulting in less adsorption of nanotube on collagen under the pHs. More adsorption can be found between these values and the result in Fig. (6) shows that the adsorption of nanotubes on collagen surface is maximum under pH 9 due to their strong electrostatic interaction. The result demonstrated trends in the strength of SWCNT binding by varying the pH but possible swelling and disintegration of collagen substrate under varying pH are factors that complicate any attempt at a quantitative study of the effect of electrostatic interactions on binding strength. Collagen molecules are comprised of repeating amino acid sequences, forming a triple helix structure and the molecules undergo self assembly process to form large fiber structures under appropriate conditions of (pH and temperatures).^{36,37} Another possibility is a rearrangement of the DNA on the nanotube surface at higher pH leading to increased attractive surface-SWCNT interactions. This clearly shows the need for nanotube adsorption experiments on an amino-SAM model substrate that would identify pH dependant adsorption or morphological effect. We plan to conduct these in future studies.

Similarly the effects of ionic strength and ion composition are planned for future studies as our silver SPR surface degrades in the presences of chloride ions, and changing the salt composition is expected to have non-trivial effects on the stability of the collagen layer.³⁸

Comparison of CNT adsorption in river water *versus* HEPES buffer

Lastly, we compared the adsorption behavior of ss-DNA wrapped SWCNT dispersed in different medium (River water *versus* HEPES buffer) onto a collagen surface to explore the effect that dissolved natural organic matter could have in determining the distribution of released nanoparticles. For this experiment, natural river water was harvested from a local water source (Great Seneca creek Gaithersburg, MD). The river water was filtered several times with syringe filters (with a final filter pore size of 0.22 μm) to remove suspended particles while retaining dissolved organic matter such as humic substances.³⁹ A comparison of the UV-Vis-NIR absorbance spectra (Fig. 1) for the nanotubes taken in HEPES buffer and in the river water in Fig. 1 shows that the nanotubes exchanged with the river water display effectively identical spectra, indicating that the dispersion state of the nanotubes was not significantly altered by the exchange. As nanotubes are very sensitive to their local environment,⁴⁰ this indicates that the ss-DNA layer on the SWCNT was unaffected, over the short time scale of this experiment, by the dissolved ions in the river water. To mimic a natural setting, the collagen layer was also incubated with the river water prior to nanotube adsorption. During this incubation, an increase in the SPR signal ($\Delta\%R$) was observed indicating that adsorption of organic species to the collagen surface occurred. As previously described, the adsorption of the nanotubes to the collagen was measured as a function of SWCNT concentrations ($\mu\text{g/mL}$) [Fig. 7]. Dramatically less adsorption was observed for the nanotubes in river water compared to the adsorption under HEPES buffer. Our hypothesis is that the adsorbed organic matter alters the electrostatic potential of the collagen layer,

Fig. 6 (a) Adsorption kinetics curves ($\Delta\%R$) and (b) SPR angle shift ($\Delta\theta$) measured for the adsorption of 80 $\mu\text{g/mL}$ of unsorted ss-DNA wrapped SWCNT on the collagen surface in different pH conditions (pH 5.5 to pH 10.1). The line is an eyeguide and the error bars represent one standard deviation.

Fig. 7 Adsorption of unsorted ss-DNA wrapped SWCNT dispersed in different medium (River water *versus* HEPES buffer) on the collagen surface as a function of different SWCNT concentrations ($\mu\text{g/mL}$). The amount of adsorption in the presence of the river water is dramatically decreased. The lines are eyeguides and the error bars represent standard deviations.

leading to a more effective repulsive interaction potential with the SWCNTs. In general, the surface functional groups of the humic substances in the natural organic matter are mostly phenolic and carboxylic groups that could electrostatically interact with collagen surface under neutral pH.⁴¹ Due to increased electrostatic or steric repulsion effects, the passivated collagen surface has demonstrably fewer available adsorption sites for the incoming nanotubes, and that could have resulted in less adsorption than the nanotube adsorption in HEPES buffer. This indicates that the amount and type of organic matter in a natural environment will likely influence the stability and end points of released nanomaterials, and that these effects should be taken into account when determining the fate of nanoparticles in ecosystem.

Conclusions

Adsorption of ss-DNA wrapped SWCNT on collagen biopolymer substrate was investigated using SPR imaging. The adsorption kinetic data suggests that binding between the SWCNT and collagen substrate is irreversible at room temperature and physiological conditions ($\text{pH} \approx 7.4$). Furthermore we showed that analysis of the adsorption kinetics over the range of nanotube concentrations allowed for the determination of a diffusion limited binding regime. Nanotube adsorption was also found to be dependant on the nanotube length, with the longer nanotubes (≈ 300 nm) displaying much slower adsorption compared to the shorter nanotube (≈ 40 nm). pH was also found to have a significant effect on the adsorption, with more adsorption of SWCNT observed under basic conditions ($\text{pH} > 9$) than acidic and neutral pH conditions. Lastly, SWCNTs were dispersed in the filtered river water, followed by adsorption to the collagen layer to mimic the adsorption in a natural environment in which both surfaces are coated with dissolved organic matter. The results show that much less adsorption occurs in the presence of the organic humic substances, which has implications for metrology in determining the fate of nanoparticles in natural environments.

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