Journal of Materials Chemistry B

RSC Publishing

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Carbon nanotube decorated magnetic microspheres as an affinity matrix for biomolecules†

Cite this: *J. Mater. Chem. B*, 2013, **1**, 1894

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Carbon nanotube (CNT) decorated magnetic microspheres were fabricated to develop a multimodal platform that utilizes non-covalent molecular interactions of CNTs to magnetically separate biomolecules. Hybrid CNT-microspheres prepared by a feasible method reported herein had a well-defined structure as characterized by Raman spectroscopy and scanning electron microscopy. Binding interactions of resulting magnetic CNT-microspheres with DNA oligonucleotides were studied to demonstrate that single stranded DNA (ssDNA) in a solution can be effectively recovered by magnetic CNT-microspheres through strong physical wrapping of DNA around CNTs' walls. The magnetic character of these CNT-microspheres combined with their capability to bind other molecules including DNA allows their use as an affinity matrix that can be utilized in affinity separation of biomolecules, and also as a platform to monitor non-covalent binding interactions of CNTs with other biomolecules. As a proof of concept, we report on the use of these CNT-microspheres in *in vitro* selection of ssDNA aptamers against carcinoembryonic antigen (CEA), a cancer biomarker, by Systematic Evolution of Ligands by Exponential Enrichment (SELEX). ssDNA aptamer candidates that have strong affinity towards CEA were successfully separated magnetically from a pool of ssDNA ($\sim 10^{14}$ molecules). Our results demonstrate that CNT-microspheres can serve as strong tools for affinity separation methodologies and can be utilized for various affinity pairs in solution.

Received 21st December 2012
Accepted 4th February 2013

DOI: 10.1039/c3tb00563a

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Introduction

Since their discovery in 1991,[1] CNTs have attracted wide attention as novel nanomaterials in multiple scientific disciplines ranging from materials science to medicine. As allotropes of carbon with a cylindrical nanostructure, they have unusual properties due to the unique combination of their dimensions, structure and topology. While CNTs were exploited for their excellent thermal, mechanical and electrical properties,[2–6] their more recently discovered ability to form non-covalent interactions with molecules and supramolecular complexes has diversified their utilization. CNTs' unique structure composed of only sp^2 hybridization allows strong van der Waals and π-stacking interactions with polyaromatic molecules. Together with their large surface area leading to high loading capacity, CNTs can act as perfect templates for self-assembly, the so-called "wrapping" by molecules such as surfactants,[7] polymers,[8–10] proteins[11] or aromatic molecular containers.[12] While such interactions have been useful for the utilization of CNTs as a tool in separation science,[13] they also formed the basis of more sophisticated sensing systems. Especially the discovery of CNTs' interactions with DNA extended their use to biotechnological platforms. CNTs and ssDNA can self-assemble through helical wrapping influenced by high affinity π-stacking interactions between DNA bases and CNT sidewalls.[14] The non-covalent nature of the bonding does not interrupt the inherent atomic structure and thus mechanical and electronic properties of CNTs are minimally disrupted. Additionally, the presence of DNA introduces additional recognition capabilities to the DNA–CNT hybrid structure. This unique interaction has been utilized for various potential applications including solubilization and sorting of CNTs,[15,16] DNA sequencing,[17,18] and sensing.[19,20]

Non-covalent interactions of CNTs with various molecules also allow formation of well defined, self-assembled microstructures with diverse morphologies allowing their unique properties to be better exploited. The combination of nano-scale diameter with micro-scale templates may create new unusual properties enabling new application areas. There have been attempts for the incorporation of CNTs onto polymeric matrices to form microspheres and microcapsules by using the layer-by-layer assembly method,[21–25] or dispersion polymerization.[26] Hollow-shell microstructures have been obtained by the final removal of the central colloidal particles. These microspheres create a synergistic effect bearing characteristic properties of CNTs exposed on the surface combined with designed

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† Electronic supplementary information (ESI) available. Photographs of CNT-microspheres demonstrating their stability at different washing conditions, supplementary SEM images of CNT-microspheres. See DOI: 10.1039/c3tb00563a

properties of the core matrix. Such CNT coated microspheres and microcapsules have been investigated for their potential use in catalysis,[27] membranes,[28] electrorheological fluids,[29] and drug delivery vehicles.[30] However, interactions of CNT adsorbed microspheres with other molecules like DNA, where CNTs bound to a core matrix non-covalently interact with that particular molecule, have not been studied yet. Such an approach would allow the use of CNT adsorbed microspheres as an affinity matrix that can be utilized in various applications based on the molecular recognition properties of functionalized CNTs.

Herein, we report the facile fabrication of CNT decorated magnetic microspheres and the DNA binding capability of these microspheres for potential use in affinity separation applications. As a proof of concept, we also demonstrate the utilization of CNT-microspheres for *in vitro* selection of aptamers against CEA, a cancer biomarker.

Experimental

Materials

Dynabeads® M-270 Amine (Life Technologies Corporation, Carlsbad, CA, USA) were used for the preparation of CNT-microspheres. They were 2.8 μm in diameter with a specific surface area of 2–5 $m^2\ g^{-1}$ and were positively charged at pH 2–9. Carboxylic acid functionalized multi-walled carbon nanotubes (MWCNTs) purchased from Arry® International Group Ltd. (Koln, Germany) with a diameter of 10–20 nm had a purity of >95 wt%, and were ~20 μm long. The functionalization degree was 0.5–3 wt% according to the manufacturer. The 76mer random DNA used for DNA binding studies and PCR primers were chemically synthesized by SynGen, Inc. (Sacramento, CA, USA). The sequence was designed to contain 40 random nucleotides in between two constant PCR priming regions. The 76mer DNA library used had the following sequence: 5′-TCTAACGTCAATGATAGAN40TTAACTTATTCGACC AAA-3′ and the primer sequences were as follows. Forward primer: 5′-fluorescein-TCTAACGTCAATGATAGA-3′ and reverse primer: 5′-TTTGGTCGAATAAGTTAA-3′. All washing steps of CNT-microspheres were performed using a magnetic stand (Magna-Sep, Invitrogen, Carlsbad, CA, USA). The cancer biomarker protein, CEA, was purchased from Fitzgerald Industries International (Acton, MA, USA).

Fabrication and characterization of CNT-microspheres

An aqueous MWCNT suspension (200 μL of 1 mg mL^{-1}) containing Tween (0.1%) was sonicated with alternating cycles of 10 s pulse with an interval of 10 s for a total of 5 min using an ultrasonicator probe (Vibra cell 75043). About 2×10^8 magnetic microspheres were washed thrice with water and concentrated using a magnetic stand. The sonicated MWCNT suspension was then mixed with washed microspheres and incubated for 4 h at room temperature on a vortex mixer followed by five times washing in each step with water, with 1 : 1 DMSO : water, and finally with water. CNT-microsphere conjugates were resuspended in water, and stored at 4 °C until use. Raman spectra were taken by using a Renishaw inVia Reflex Raman Microscope and Spectrometer equipped with a 532 nm excitation laser. To prepare samples, 10^6 CNT-microspheres were deposited on a silicon wafer and air dried. Scanning electron microscopy images were collected by using a LEO Supra 35VP Scanning Electron Microscope. The same samples on silicon wafers as for the Raman spectroscopy were imaged. FTIR spectra were taken with a Nicolet iS10 instrument.

CNT-microspheres–DNA interactions

For the titrations of CNT-microspheres into DNA, required concentrations of DNA solutions were prepared in 50 μL of PBS buffer. Aliquots of resuspended CNT-microspheres corresponding to the desired number of CNT-microspheres were taken in an Eppendorf tube on the magnetic stand and the supernatant was removed. The DNA solution was then added into the same tube containing CNT-microspheres, incubated on a vortex mixer for 5 min, and placed back on the magnetic stand. Once the fluorescence/absorbance intensity of the supernatant was read, CNT-microspheres were mixed again with the supernatant on a vortex, and transferred into the tube containing the next aliquot of CNT-microspheres. A_{260} was determined by using a Nanodrop 2000c Spectrophotometer. Fluorescence spectra were obtained with a Nanodrop 3300 Fluorospectrometer by blue LED excitation. The titration curve was generated by measuring the amount of DNA bound to CNT-microspheres against the number of CNT-microspheres by using the emission signal of fluorescein at 514 nm according to the following eqn (1):

$$\text{Amount of DNA bound} = (\text{amount of DNA in the parent solution}) \times (F_{max} - F)/F_{max} \quad (1)$$

where F_{max} is the fluorescence intensity of the parent DNA solution and F is the fluorescence intensity of the supernatant after incubation with CNT-microspheres.

CNT-microspheres for *in vitro* selection

A series of different concentrations of CEA (0, 6, 17, and 28 pmol) was incubated with 300 pmol of pre-denatured 76mer random DNA library in 50 μL of binding buffer and the reaction mixture was incubated for 30 min on a vortex mixer at room temperature. The binding buffer contained 0.1 M Tris–Cl, 140 mM NaCl, 20 mM $MgCl_2$ and 20 mM KCl at pH 7.4. The contents were transferred to the tubes containing aliquots of 10^8 CNT-microspheres and incubated again for 30 min on a vortex mixer. The tubes were placed on the magnetic stand, and the supernatants were removed. Thus obtained supernatant solutions were ethanol precipitated to remove excess salts that might interfere with PCR, and resuspended in 25 μL of sterile distilled water. DNA in supernatant solutions was amplified by asymmetric PCR using 10 μL of the supernatant solution as the PCR template, 5 pmol forward primer and 0.1 pmol reverse primer in a total of 25 μL of the reaction mixture using Taq Mix (Qiagen, Hilden, Germany). In control reactions, no template, and 3 pmol untreated DNA library were used, respectively. The

following pre-optimized PCR conditions were programmed in the thermocycler (Mastercycler® 384): initial denaturation at 95 °C for 3 min followed by 25 cycles of denaturation at 95 °C for 30 s, followed by 49 °C for 30 s and amplification at 72 °C for 30 s and a final extension at 72 °C for 10 min. Precisely, 20 μL of PCR products were directly loaded into a 3% agarose gel pre-stained with ethidium bromide. Agarose gel images were obtained under UV-illumination.

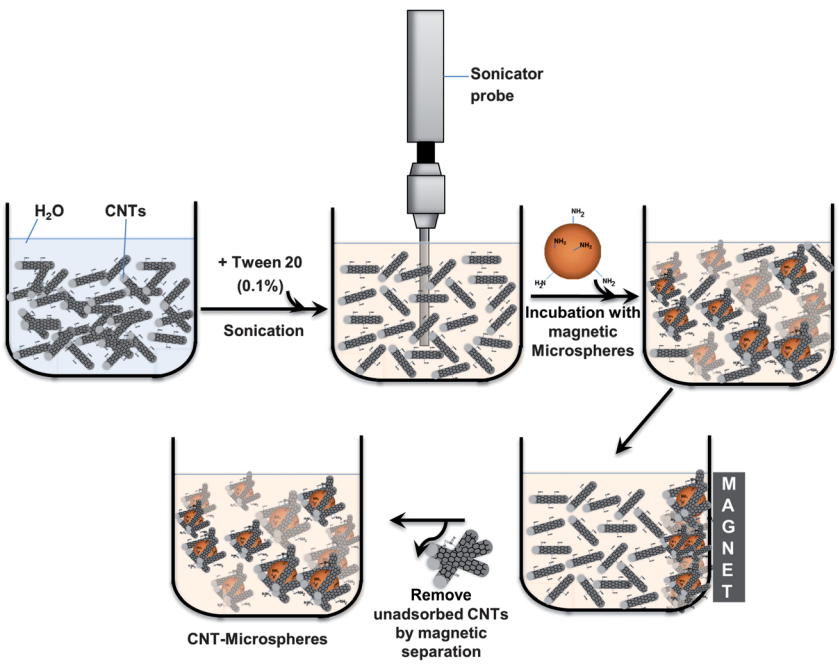
Results and discussion

Preparation and characterization of CNT-microspheres

Magnetic microspheres were used as the core matrix that served as a template for the self-assembly of CNTs. These microspheres were composed of highly cross-linked polystyrene with free amine groups and magnetic material precipitated in polymeric pores. These microspheres were chosen because of their great potential to non-covalently interact with CNTs. Strong electrostatic interactions of amine groups with CNTs containing carboxylic acid defects have been reported previously.[31] Similarly, strong non-covalent interactions of polystyrene with CNTs through π–π bonds have also been demonstrated.[8,32] Magnetic microspheres chosen for this study possessed both of these potential binding elements for the optimization of a strong non-covalent interaction with CNTs. Carboxylic acid functionalized MWCNTs at a concentration of 1 mg mL^{-1} were dispersed in the presence of 0.1% surfactant Tween 20 by ultrasonication. The suspension was homogeneous and stable for at least several months, as no sedimentation was observed. Dispersed MWCNTs were simply incubated with magnetic microspheres by shaking, and thus formed CNT coated microspheres were then isolated by the application of an external magnetic field. The magnetic nature of microspheres allowed extensive washing of CNT-microspheres for the effective removal of unattached CNTs and also the excess surfactant (Scheme 1).

After extensive washing, the inherent brown color of magnetic microspheres turned black indicating the strong attachment of CNTs on the surface. Raman spectroscopy was performed to analyze hybrid CNT-microsphere structures. Fig. 1a shows the spectrum of dispersed MWCNTs before being treated with microspheres. The disorder induced D-band at 1339 cm^{-1} and the tangential stretch G-band at 1580 cm^{-1} with a shoulder at 1604 cm^{-1} were visible along with the second harmonic G′ band 2684 cm^{-1}, which are typical Raman bands for MWCNTs.[33] The spectrum in Fig. 1b represents magnetic microspheres after being incubated with CNTs, and washed extensively. The presence of the same spectral features indicated adsorbed CNTs on microspheres with no perturbation to their atomic structure. The typical upshift of the G band that is mostly seen when CNTs are wrapped[34] with molecules was not detected. This lack of the shift of Raman peaks might indicate an electrostatic and/or H–bonding interaction between carboxylic acid functionalized MWCNTs and amine functionalized magnetic microspheres rather than π-stacking or van der Waals interactions. Moreover, overlapping Raman spectra suggested that CNTs were not disturbed by being adsorbed on magnetic microspheres, and that they retained their inherent electronic properties.

The stability of CNT-microspheres was investigated by treating them under various conditions to verify if the adsorbed CNTs were strongly attached to microspheres. For this purpose, the prepared CNT-microspheres were treated with various organic solvents and mild denaturation agents of different compositions, and the supernatant obtained after the removal



Scheme 1 Fabrication of CNT-microspheres. An aqueous CNT suspension containing 0.1% Tween is dispersed by ultrasonication, followed by incubation with magnetic microspheres. CNTs that are not adsorbed on microspheres are removed magnetically.

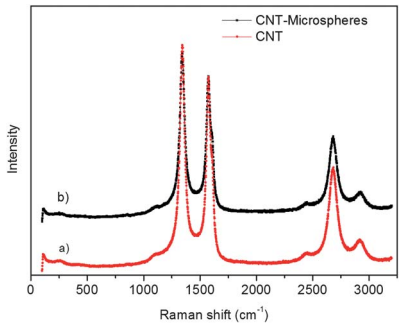


Fig. 1 Raman spectra of dispersed MWCNTs (a), and magnetic microspheres incubated with MWCNTs followed by washing (b).

of microspheres in each case was analyzed for the release of bound CNTs. The supernatant exhibited a grayish color in the presence of CNTs that were released from the CNT-microspheres, whereas a clear supernatant was obtained when CNTs remained intact on microspheres. Surfactant concentrations of up to 2% and solvents of different polarities/H^+ donating abilities like methanol and DMSO did not lead to any dissociation of CNTs when CNT-microspheres were incubated in these solutions. Similarly, pyridine, an aromatic organic solvent, which has a strong potential for interacting with sp^2 hybridized molecules, also failed to remove adsorbed CNTs from the microspheres, neither did increasing the temperature up to 95 °C have any effect on the stability. However, a minor degree of CNT dissociation was visibly observed only when microspheres in 0.1% Tween were sonicated (Fig. S1, ESI†). These data suggested that the interaction between CNTs and magnetic microspheres was very strong at the molecular level and that it can only be destructed by applying an external ultrasonic pressure in the presence of a surfactant. This type of stability over a wide range of perturbations can permit utilization of these CNT-microspheres in various applications.

CNT-microspheres were further characterized by scanning electron microscopy (SEM). Homogeneous coverage of microspheres with CNTs (Fig. 2A–C and S2, ESI†) was clearly visible as

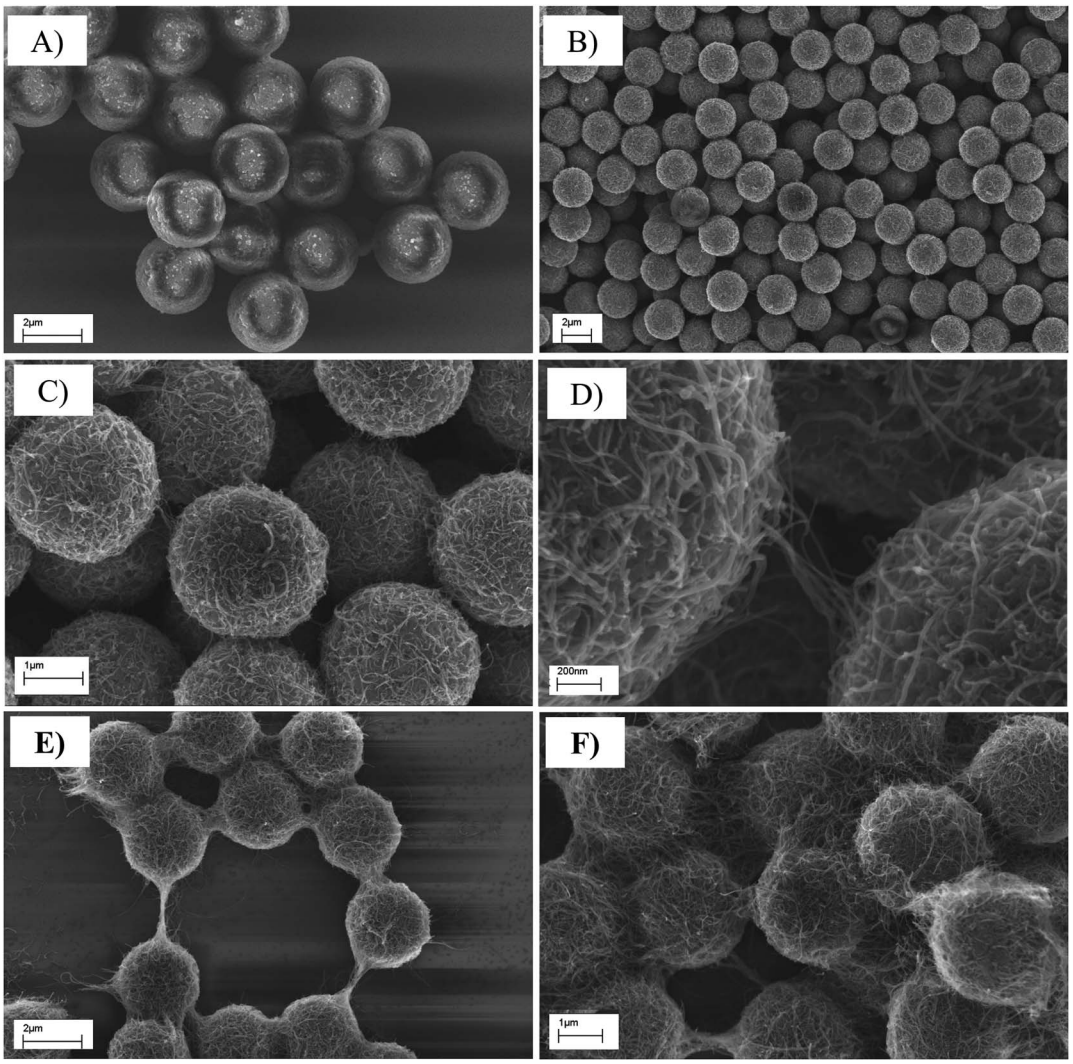


Fig. 2 SEM images of naked magnetic microspheres (A), microspheres coated with 0.1 mg CNTs/10^7 microspheres at different magnifications (B–D), and microspheres coated with 1 mg CNTs/10^7 microspheres at different magnifications (E and F).

compared to naked microspheres that had a smooth surface (Fig. 2A). CNTs formed networks on the microspheres which occasionally extended to form bridges between microspheres (Fig. 2D).

The effect of the concentration of CNTs during microsphere coating was also studied by SEM. CNT-microspheres at a concentration of 0.1 mg/10^7 microspheres were coated uniformly with a single layer of CNTs on the surface of microspheres, leading to a pool of individually separated microspheres (Fig. 2B–D). However, homogeneity of the microsphere coating decreased upon increase in the initial CNT concentration by ten-fold (1 mg CNTs/10^7 microspheres), where CNTs on the microspheres formed webs that connected microspheres forming aggregates (Fig. 2E and F). The SEM images showed that the method applied to prepare CNT coated microspheres in this study provided a controllable process that can be optimized depending on the desired application. When surfactant-dispersed CNTs were incubated with magnetic polystyrene-coated amine-functionalized microspheres, a competition between potential non-covalent interactions was expected to occur due to the following: (i) interaction between individual CNTs, (ii) interaction between CNTs and the surfactant, (iii) interaction between CNTs and the polystyrene coating of microspheres and (iv) interaction between amine functional groups of microspheres and carboxyl functional groups of CNTs. SEM images of uniformly coated microspheres at low concentrations suggested that microspheres–CNT interactions were more favored than CNT–CNT interactions. However, at higher concentrations of CNTs at which the loading capacity of microspheres was exceeded, CNT–CNT interactions prominently lead to the formation of aggregates of CNT-microspheres that appeared as a web-like structure.

Interactions of CNT-microspheres with DNA

Interactions of CNT-microspheres with DNA were investigated to assess the potential of these microspheres in affinity separation. Increasing numbers of CNT-microspheres were titrated into a solution of 76-base-long single ssDNA. The nucleotide sequence of the ssDNA used in this study was composed of a 40 base-long random sequence region flanked on both sides by constant sequence regions, resulting in a pool of ssDNA with 4^{40} different nucleotide sequences. The binding between CNT-microspheres and ssDNA was evaluated by measuring the decrease in the absorbance at 260 nm. The increase in the number of CNT-microspheres was proportional to the depletion of ssDNA by adsorption from the ssDNA solution (Fig. 3). At every titration point, DNA bound CNT-microspheres were pulled to the walls of the tube by applying a magnetic field followed by the absorbance reading of the unbound DNA supernatant solution. All DNA in the solution was removed when the number of CNT-microspheres reached ~10^8. At this point, every single magnetic microsphere was loaded with approximately 2×10^6 ssDNA molecules. This high DNA loading efficiency leading to the complete removal of the parent DNA was attributed to its interaction with CNTs present on the surface of microspheres rather than binding to magnetic

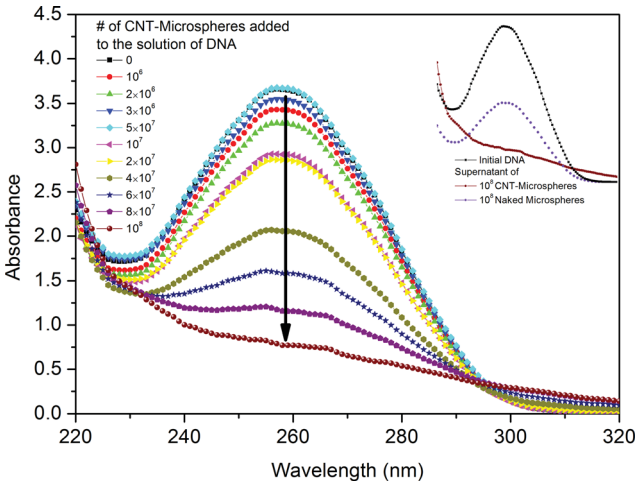


Fig. 3 Absorbance spectra of the 76mer DNA supernatant solution at different concentrations of CNT-microspheres. The initial concentration of DNA was 6 μM. Inset: spectra of the DNA solution after being treated with naked microspheres (red), compared to the initial DNA solution (black), and spectra of the DNA solution after being treated with the same number of CNT-microspheres.

microspheres, as the same number of naked microspheres failed to remove/adsorb the same amount of DNA from the solution (Fig. 3, inset). This interaction between CNTs and ssDNA was not sequence specific since the presence of diverse ssDNA of different sequences in the pool did not affect the binding.

To see if the size of ssDNA has any effect on the binding ability of CNT-microspheres, a shorter DNA oligonucleotide with 22 bases was incorporated into DNA-binding titrations. This short ssDNA was also labeled with fluorescein so that the removal of DNA by CNT-microspheres can be demonstrated by measuring the decrease of fluorescence intensity in the DNA solution. Fig. 4A represents how all DNA (200 pmol) was recovered from the solution after being treated with CNT-microspheres as evidenced by the disappearance of the fluorescence intensity/signal. The resulting titration curve followed a typical one-site binding curve where the amount of bound DNA increased with the increasing number of CNT-microspheres until reaching its saturation point (200 pmol) (Fig. 4B). These results suggested that while CNT-microspheres are effective for removing DNA of different lengths, their efficiency of DNA binding was higher for shorter DNA oligonucleotides (8×10^6 22mer ssDNA molecules on each CNT-microsphere) than for longer oligonucleotides (2×10^6 76mer ssDNA molecules on each CNT-microsphere) under identical conditions.

The binding of ssDNA to CNT-microspheres was further investigated by Raman and FT-IR spectroscopy. Prior to the analyses, CNT-microspheres treated with DNA were washed twice with water to remove any loosely or opportunistically bound DNA. CNT-microspheres treated with DNA exhibited a tangential G-band that was upshifted by 17 cm^{-1} relative to that of naked CNT-microspheres (Fig. 5A). Such a significant shift indicated the presence of DNA wrapped around the CNTs present on the microspheres, as similar Raman upshifts were also previously reported for CNT–polymer composites.[35,36] The

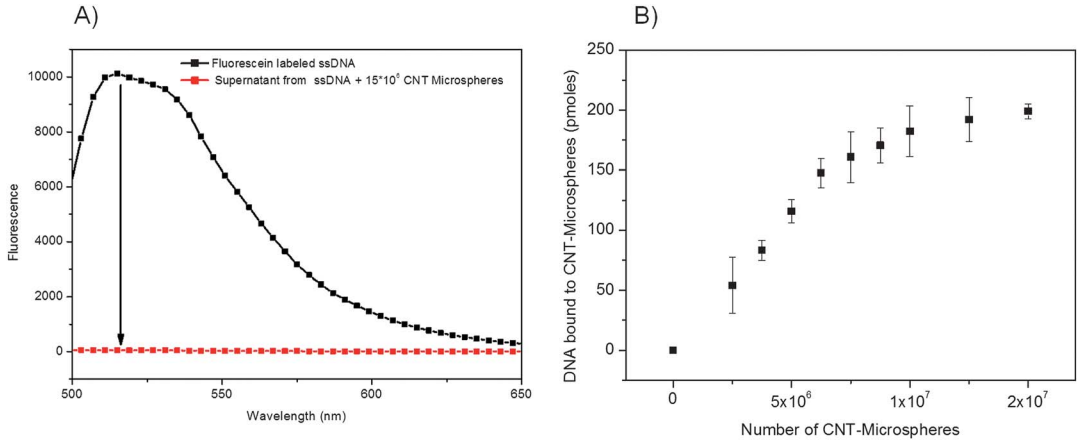


Fig. 4 Binding of 22mer fluorescein labeled ssDNA to CNT-microspheres. (A) The fluorescence of the DNA solution (200 pmol) before (black) and after (red) being treated with 1.5×10^7 CNT-microspheres. (B) Titration curve representing the amount of bound DNA at increasing numbers of CNT-microspheres.

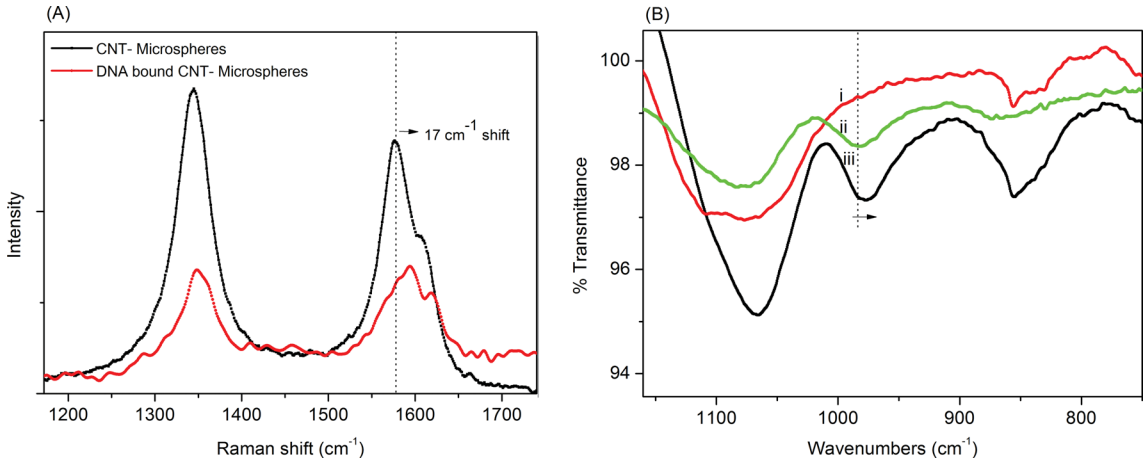


Fig. 5 (A) Raman spectra of CNT-microspheres (black) and DNA bound CNT-microspheres (red). (B) FTIR spectra of CNT-microspheres (i), ssDNA (ii), and DNA bound CNT-microspheres (iii). DNA bound CNT-microspheres were prepared at 200 pmol DNA/10^8 CNT-microspheres.

presence of DNA on CNT-microspheres was further demonstrated by FT-IR spectroscopy (Fig. 5B). A new peak at 980 cm^{-1} emerged relative to naked CNT-microspheres, which is indicative of symmetric stretching of phosphate groups of phosphodiester linkages,[37] thus proving the presence of DNA on CNT-microspheres. Furthermore, this phosphate stretch peak was slightly shifted to a lower frequency (6 cm^{-1} shift) as compared to the peak obtained from ssDNA alone, suggesting that ssDNA molecules were interacting with CNTs in a way that induced a change in the vibrational frequency, probably through π-stacking or van der Waals forces.

SEM images of CNT-microspheres treated with DNA also represented strong binding interactions between DNA and CNTs. The diameter of CNTs adsorbed on microspheres (Fig. 6A) increased when the same microspheres were treated with DNA followed by washing as seen from SEM images obtained at the same magnification (Fig. 6B). The visible fattening of CNTs indicated the wrapping of DNA around CNTs rather than adsorption on the surface of microspheres. It is interesting to note that CNTs remained adsorbed on the surface of magnetic microspheres, and did not dissociate in the presence of DNA, which is actually an effective agent for the dispersion of CNTs. Obviously, the interaction between the magnetic microspheres and CNTs allowed the balance for the simultaneous binding of CNTs to the microspheres and DNA, resulting in a unique functional hybrid material.

Utilization of CNT-microspheres in *in vitro* selection

The strong ability of magnetic CNT-microspheres to remove ssDNA from a solution suggested the possibility of using these novel hybrid structures in *in vitro* selection processes. As a proof of principle, we studied the utilization of CNT-microspheres in Systematic Evolution of Ligands by Exponential Enrichment (SELEX) experiments to select aptamers that can specifically bind to target biomarkers. SELEX requires incubation of the target protein with a pool of random oligonucleotides. Members of the pool that show an affinity towards the immobilized protein are separated from the rest of the pool, eluted, and amplified by PCR, which is then used as the pool for the

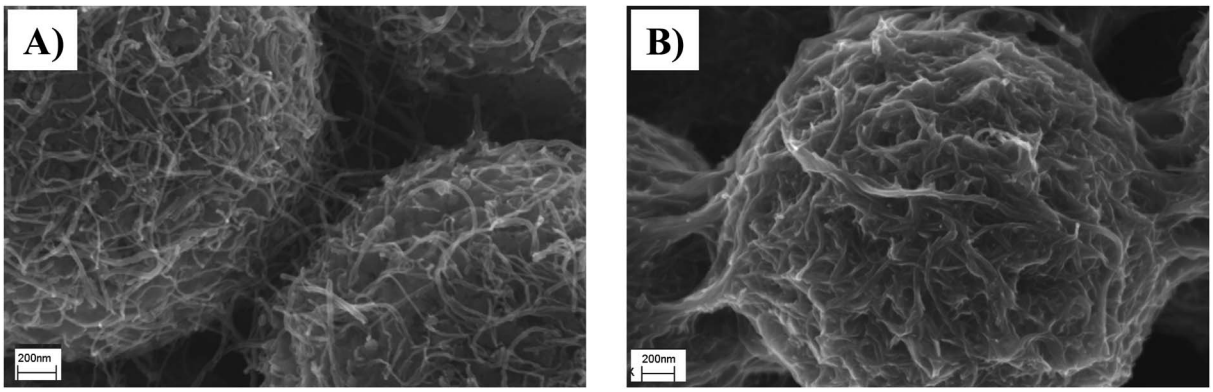
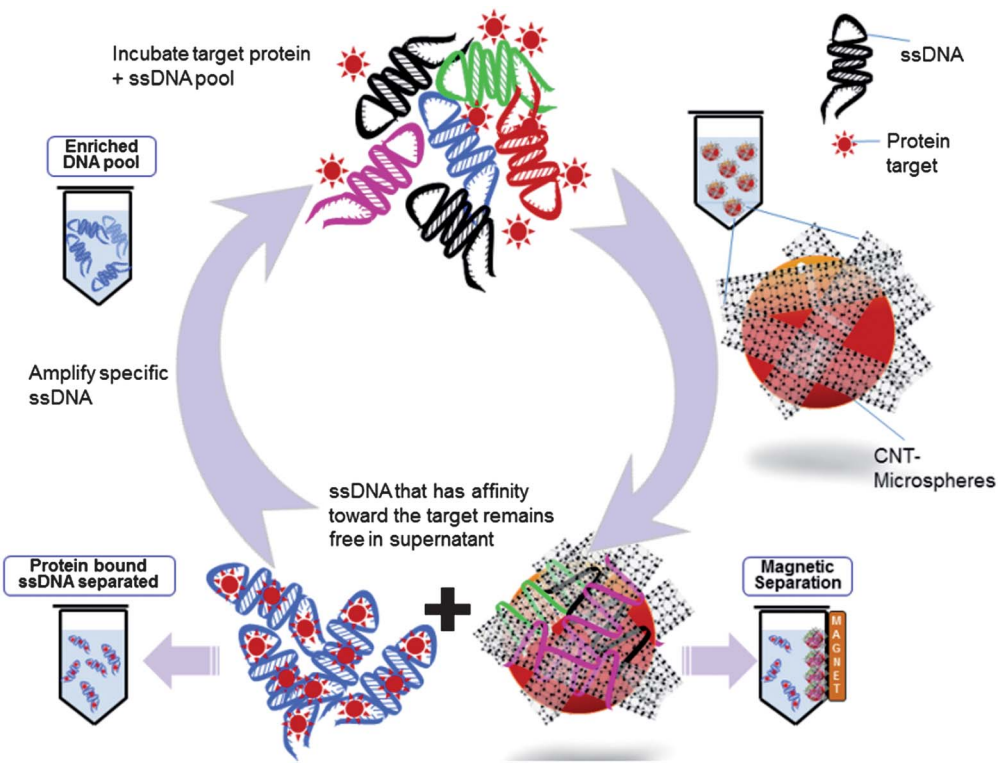


Fig. 6 Scanning electron microscopy images of CNT-microspheres (A) and CNT-microspheres treated with DNA (B), both at 100 K magnification. CNT-microspheres were prepared at 0.1 mg 10^{-7} microspheres concentration.

next round of selection. The selection cycles are repeated until a high affinity aptamer towards the target protein is selected.[38] Separation of the binding and non-binding oligonucleotides is a challenging part of the SELEX process, and usually requires immobilization of the target protein on a surface that enables separation, like microspheres. However, immobilization on a surface can greatly alter the binding interaction of the protein and the oligonucleotides, not to mention the complexity of the experimental procedure. CNT-microspheres described herein are strong alternative tools to improve aptamer selection by SELEX after allowing the incubation of oligonucleotides and target proteins free in solution with simple experimental procedures. We proposed that magnetic CNT microspheres can be used to remove the unbound oligonucleotides after the incubation of the target protein and the pool of oligonucleotides. Oligonucleotides that show strong affinity towards the target protein are not accessible to bind the CNTs on microspheres, while the unbound DNA can be removed from the solution by drawing them using a magnet (Scheme 2). While this approach is beneficial, as it does not require immobilization of the target protein, it also introduces a high level of stringency to the selection because of the competition in terms of binding between CNT–DNA and protein–DNA.

We tested the possibility of using CNT-microspheres in SELEX by using a pool of 76-nucleotide-long DNA with a 40-base-long random region, and CEA was used as a target cancer



Scheme 2 Immobilization free SELEX using CNT-microspheres.

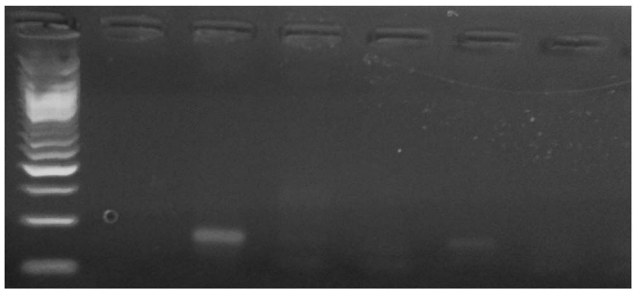


Fig. 7 Agarose gel electrophoresis image (3%). 100 bp DNA marker (lane 1), negative PCR control (lane 2), positive PCR control (lane 3), amplified product of the supernatant remaining after CNT-microspheres were removed from the solution containing DNA and 6 pmol CEA (lane 4), 17 pmol CEA (lane 5), 28 pmol CEA (lane 6), and 0 pmol CEA (lane 7).

biomarker for the selection of aptamers. Incubation of CEA with the DNA pool was followed by pulling the CNT-microspheres to the walls of the tube by using a magnet and the removal of the supernatant solution. Oligonucleotides of the pool that did not bind to CEA were predicted to bind to CNT-microspheres, whereas only oligonucleotides expressing a stronger affinity towards CEA than CNTs remained in the supernatant solution. The agarose gel electrophoresis image in Fig. 7 shows the amplified product from the supernatant along with controls.

When the pool of oligonucleotides was incubated with varying concentrations of CEA followed by incubation with CNT-microspheres, some DNA remained in the solution which could be amplified by PCR as indicated by the visible bands on gel electrophoresis (Fig. 7, lanes 4–6). However, in the absence of CEA, all DNA was bound to CNT-microspheres and was removed from the solution, and thus no PCR product was amplified (lane 7). Using CNT-microspheres for the partitioning step, as opposed to classical systems where the target protein is immobilized, brings the issue of the competition between CNT molecules and the target protein for binding to the DNA oligonucleotides. When for certain targets the nonspecific CNT–DNA interaction is stronger than the specific DNA-target protein binding interaction, only a few copies of the DNA will be free which can make it difficult to amplify the binders. While this issue seems to be a disadvantage for the use of CNT-microspheres for the isolation of aptamers, it also provides a very stringent system that only allows the amplification of oligonucleotides with very strong affinity. These results demonstrated that CNT-microspheres can be powerful tools for the separation of oligonucleotides from those that are bound to a protein and can be utilized in various affinity separation applications, which is a crucial element of *in vitro* selection procedures.

Conclusions

A facile preparation and characterization method for magnetic CNT-microspheres was reported. These microspheres uniformly decorated with CNTs through non-covalent immobilization interacted strongly with DNA. The potential applicability in the selection of aptamers through SELEX was demonstrated. They have been shown to be an alternative tool for the partitioning step of SELEX which is the separation of ssDNA from ssDNA bound to the target molecule. The magnetic character of CNT-microspheres combined with DNA binding ability renders these microspheres as novel functional hybrid materials for affinity selection applications in general. The strong interaction with DNA suggests the ability of CNT-microspheres to bind different types of polyaromatic molecules. Therefore, CNT-microspheres represent a general platform that allows the investigation of interactions of CNTs with other molecules. They can be used as affinity ligands that allow the analysis of how various molecules like peptides, proteins, dyes, and drugs interact with CNTs and the exploitation of these interactions. Furthermore, from a materials science point of view, CNT decorated microspheres reported herein can find further applications. The uniform CNT network formed on the surface of the microspheres can be investigated for potential applications in energy storage including rechargeable Li-ion batteries and supercapacitors, or water purification systems.

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Grant no: 110E287. We thank Dr M. Sezen for helping us with scanning electron microscopy.

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