Supporting Information

Self-propelled Carbon Nanotube Based Microrockets for Rapid Capture and Isolation of Circulating Tumor Cell

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1. Experimental Section

Reagents.

CNTs with 4~8 nm i.d., 10~20 nm o.d., 10-30 μm length and purity of >95% were procured from J. K. Impex Company (India). Ferric chloride tetrahydrate, ferrous chloride hexahydrate, transferrin (Tf), and *N*-(3-dimethylaminopropyl)-*Ń*-ethylcarbodiimide hydrochloride (EDC.HCl) were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, MO) was used throughout. All other chemicals were of analytical grade and used without further purification.

Characterization.

TEM analysis was carried out using a Philips (CM 200) TEM machine set at an accelerating voltage of 200 kV. Samples for TEM were prepared by putting a drop of the Tf-CNT-Fe_3O_4 suspension (in DI water) on a Formvar-covered copper grid and subsequently evaporating the water in air at room temperature. The elemental analysis was obtained by Energy Dispersive X-ray fluorescence (EDXRF) using Shimadzu EDX-GP spectrometer. Conjugation of protein (Tf) to CNT-Fe_3O_4 was confirmed by a modified Bradford assay.

Microrocket Tracking.

The autonomous motion of the Tf-CNT-Fe_3O_4 microrocket (500 μg mL^{-1}) in water as well as in DMEM containing varying concentration of H_2O_2 (1, 4 and 8 w/w %), was recorded with Dino-lite digital microscope at 50X magnification, using the DinoCapture 2.0 software. Tf-CNT-Fe_3O_4 particles used in the study were mostly in the form of aggregates as dispersion treatments such as sonication was avoided to prevent denaturation of the Tf protein and also for accurate observation and recording with a microscope of 50X magnification.

Cell culture.

HCT116 cells were procured from ATCC and cultured in DMEM (Invitrogen), supplemented with 10% fetal bovine serum (Invitrogen) and 100 unit mL^{-1} penicillin, 100 μg mL^{-1} streptomycin (Invitrogen). GFP-labeled HCT116 colon cancer cells (TfR^+ cells) were specifically chosen for this particular experiment to facilitate identification and image-based counting of the target cells.

Isolation of human peripheral blood mononuclear cells (hPBMC).

Human peripheral blood mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation. Whole blood from healthy individual volunteers was collected into

sterile heparinized vacutainer tubes. Equal volumes of blood and PBS were mixed gently with a sterile pipette in a 50 mL test tube. This blood–PBS mixture was slowly overlaid on to 15 mL of Ficoll-Hypaque solution. It was then centrifuged at 1500 rpm for 40 minutes at 18^{o}C. The upper layer containing plasma and platelets was removed with a sterile pipette. The buff-coloured layer containing hPBMCs was transferred into a fresh tube, washed twice with 20 ml PBS and resuspended into RPMI complete media. Cells were counted and the viability of the cells was determined by trypan blue exclusion test.

2. Particle Size Distribution

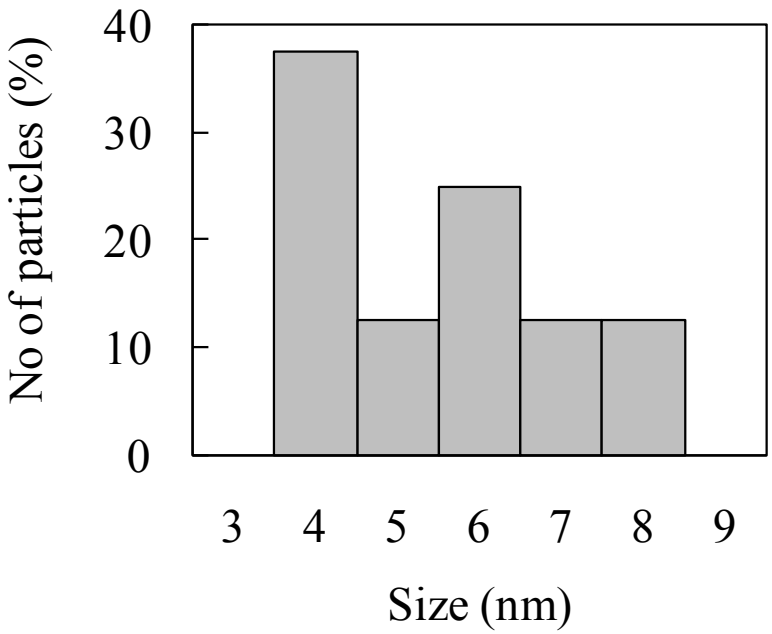


Figure S1. Size distribution of the Fe_3O_4 particles (N = 10) estimated from TEM images.

3. Energy Dispersive X-ray fluorescence Spectroscopy (EDXRF) Analysis

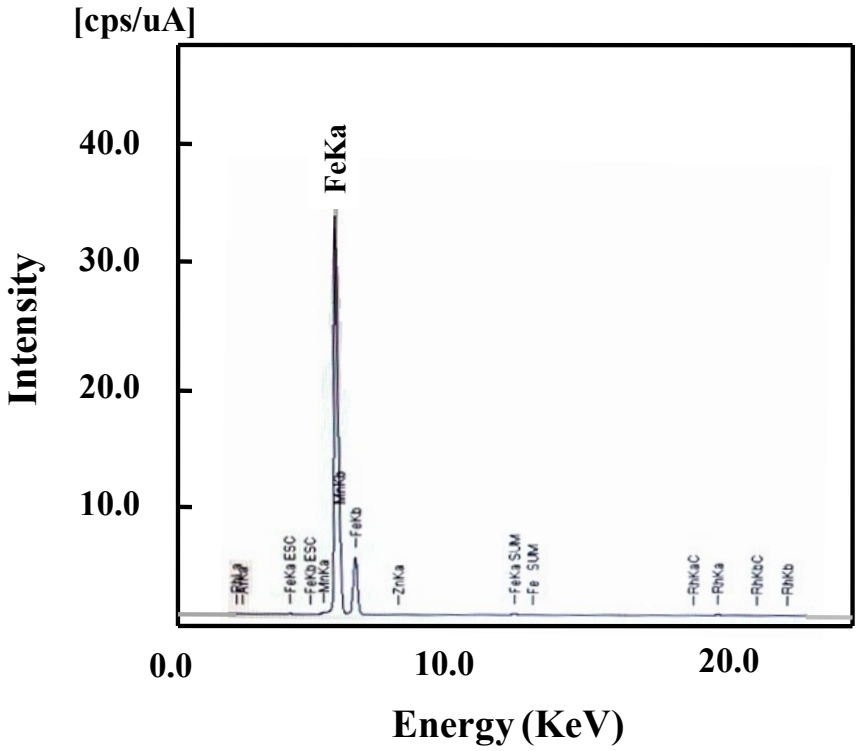


Figure S2. EDS spectrum of Tf-CNT-Fe_3O_4 showing presence of iron as major element.

4. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analysis

In order to confirm the surface functionalization of CNTs with Tf, characterization of Tf-CNT- Fe_3O_4 was done by ATR-FTIR. The spectrum was recorded using a PerkinElmer spectrum One Fourier Transform Infrared (FTIR) spectrometer, USA in the range of 4000 to 500 cm^{-1} with a resolution of 2 cm^{-1}. The IR absorption spectrum of CNT-COOH mainly consist of the asymmetric carboxyl C=O stretch at 1646 cm^{-1}. Tf-CNT-Fe_3O_4 showed new peaks at 1512, 1171, 1082 and 872 cm^{-1} indicating the presence of Tf on CNT-Fe_3O_4.

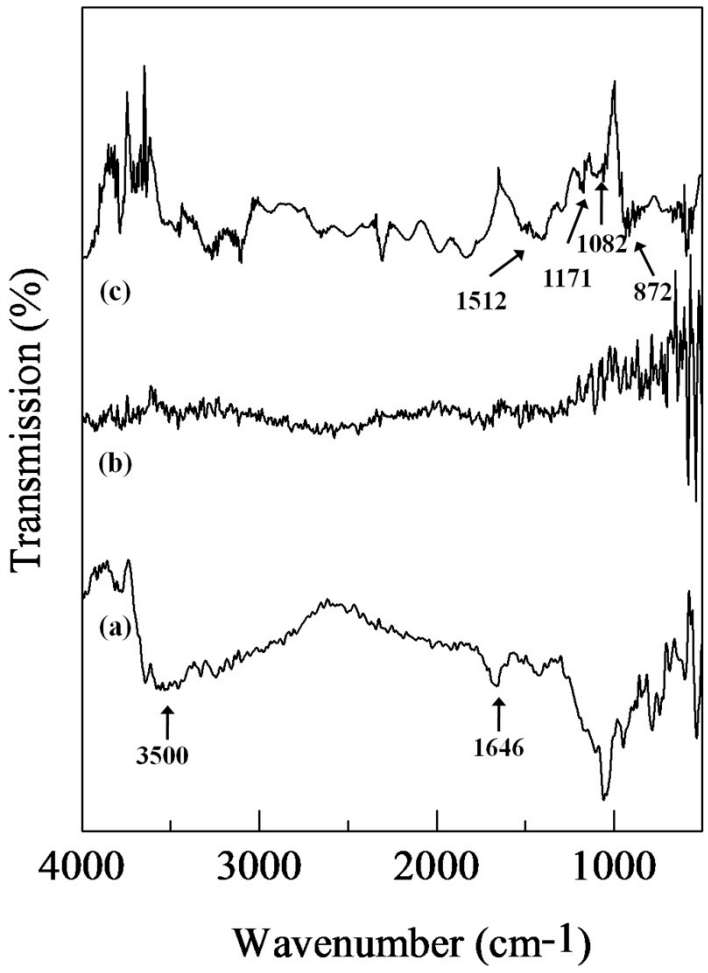


Figure S3. IR spectra of a) CNT-COOH, b) Fe_3O_4, and c) Tf-CNT-Fe_3O_4 microrocket.

5. Magnetic Property

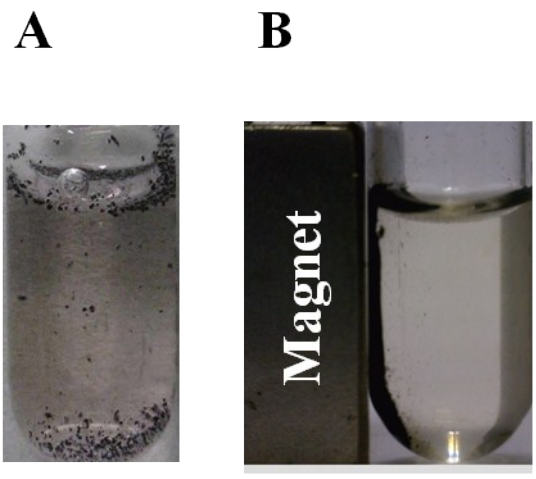


Figure S4. (A) Tf-Fe_3O_4-CNT particles in aqueous solution. (B) Tf-Fe_3O_4-CNT particles are drawn from the solution to the sidewall of the vial when exposed to a magnetic field due to the presence of Fe_3O_4.

6. Dynamics of Microrockets

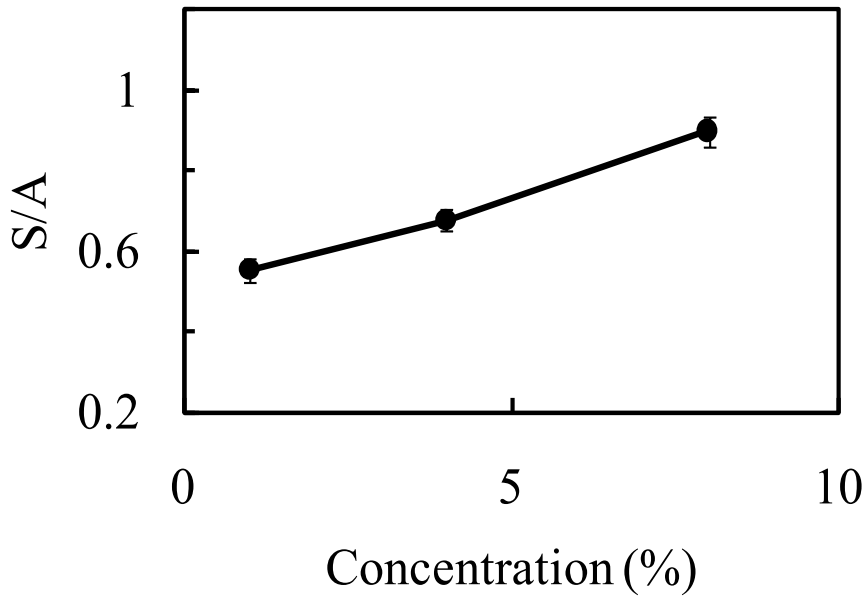


Figure S5. Speed per unit area of Tf-CNT-Fe_3O_4microrockets in upward direction in aqueous solutions with different H_2O_2 concentrations (1-8%). Error bars: standard deviation for n = 5.

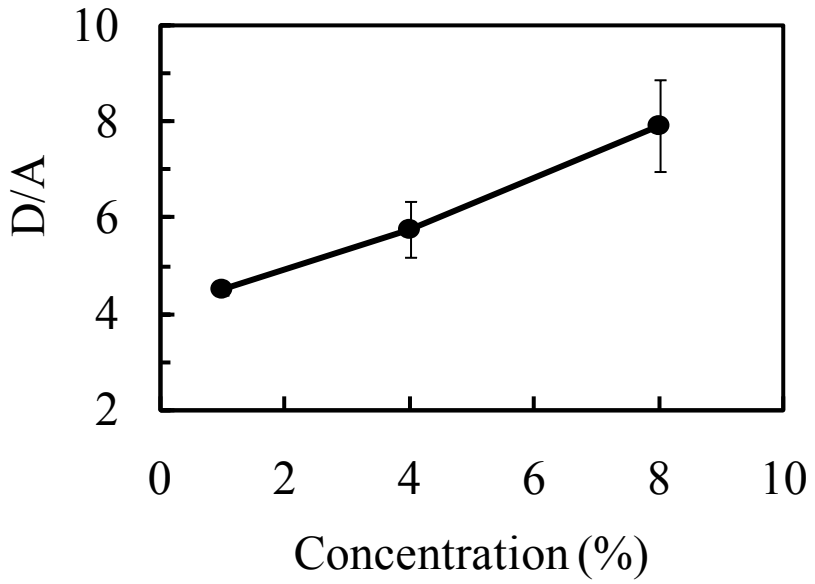
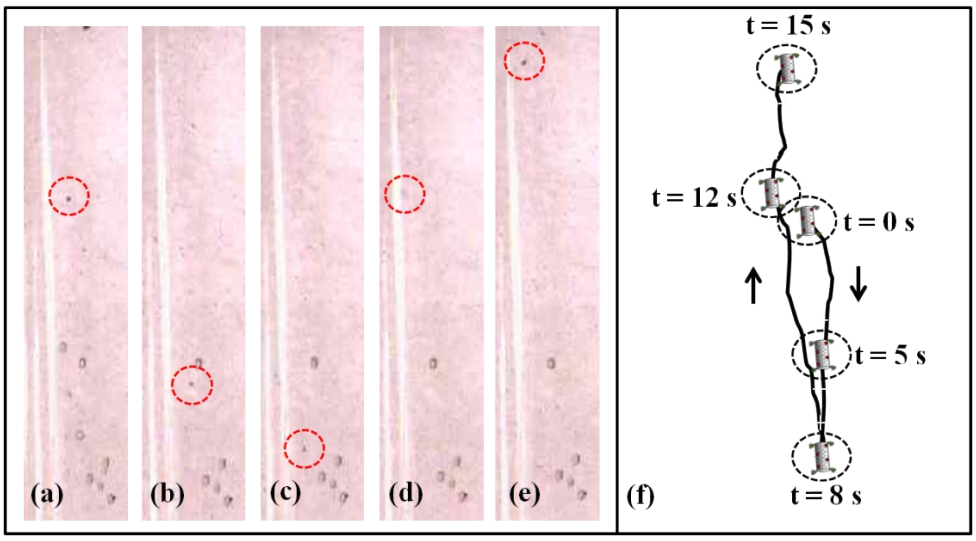


Figure S6. Total distance (mm) covered per unit area (μm^2) of Tf-CNT-Fe_3O_4microrockets in solutions of cell media with different H_2O_2 concentrations (1-8%). Error bars: standard deviation for n = 5.

7. Calculation of motion parameters.

The microrocket motion videos recorded by Dino-lite microscope were processed by VirtualDub software (GPL) to collect individual frames in JPEG format from videos. The collected images were imported with image sequence option in ImageJ software (NIH) where the option of desired image numbers along with starting image can be selected. The image scale was set by correlating pixels with scale set besides the sample test tube. MTrackJ, an ImageJ plugin for particle tracking was used to monitor the motion of the particles in the cell media containing Tf-CNT-Fe_3O_4. Moving particles were manually tracked with the difference of five frames. The distance travelled by particle and time required by the particle to travel the distance was determined by using MTrackJ plugin. The area, perimeter and the shape parameters (circularity and aspect ratio) of the individual particles were then measured from these images using an inbuilt ImageJ plugin. We analyzed five particles from 1%, 4% and 8% H_2O_2 concentration in cell media and calculated the average distance, time and velocity of the particle. The size of the Tf-CNT-Fe_3O_4 aggregates determined were ~ 50 μm and the circularity value ~ 0.89 indicating highly rounded shape.

A



B

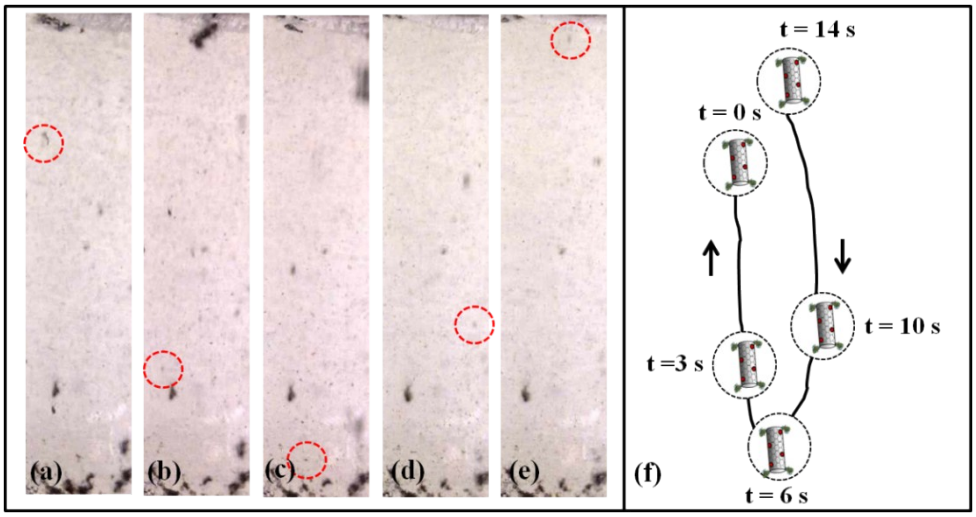
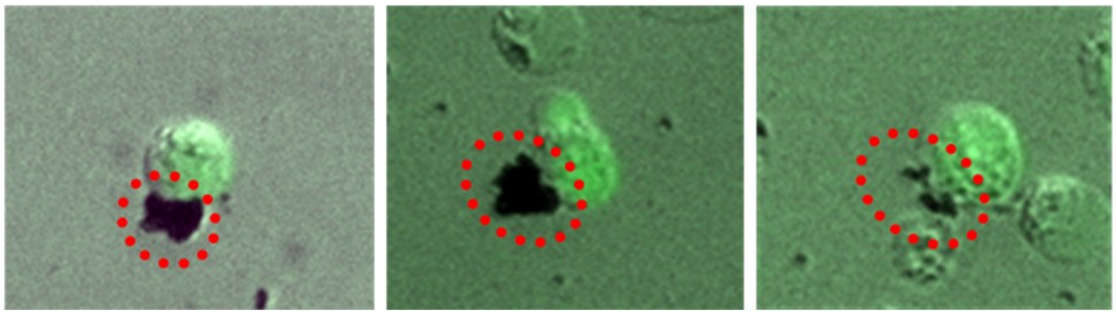


Figure S7. Time-lapse images of a microrocket driven by oxygen bubble propulsion in DMEM cell media containing (A) 1% and (B) 8% H_2O_2.

8. Viability of Captured Cancer Cells

A



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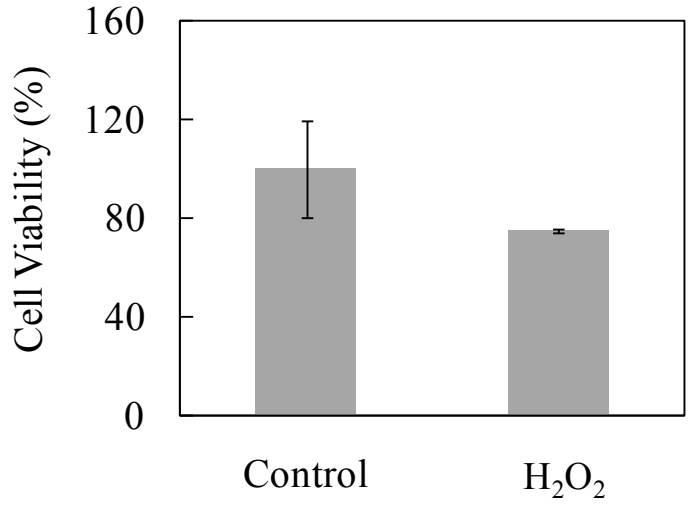

Figure S8. (A) Images of HCT116 cells after 30 min of captured by Tf-CNT-Fe_3O_4 microrocket. (B) Plot showing cell viability of HCT116 cell in DMEM containing 4% H_2O_2 after 1 h.

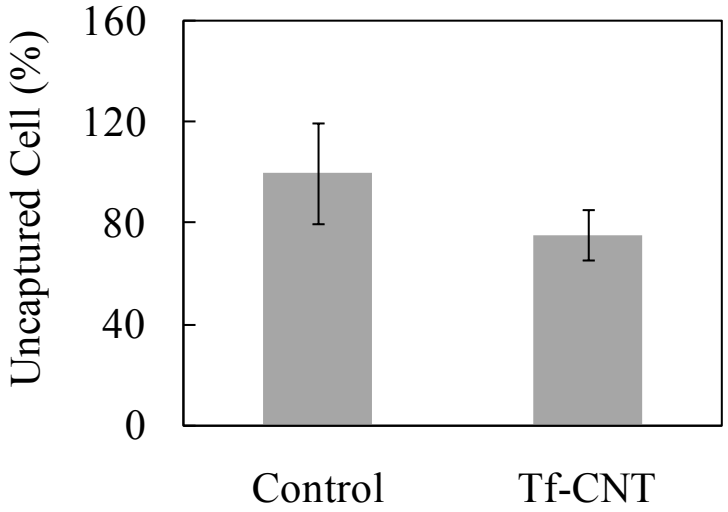

***Figure S9*.** Plot showing cell capture by Tf-CNT in DMEM containing 4% H_2O_2.