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Supporting Information

Efficient Capture and Release of Circulating Tumor Cells by Temperature-Sensitive Polymer-Graphene Oxide Composite

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Chemicals Acryloyl chloride (Fluka), piperidine (Aldrich), triethylamine (Aldrich), and basic alumina (Aldrich) were used without further purification. AIBN (Aldrich) was recrystallized from methanol before use. Anisole (Aldrich) used was anhydrous grade (99.7%). *N*,*N*-diethylacrylamide was procured from TCI America. Dichloromethane, hexanes, and ethyl acetate used were solvent grade and were used without purification.

Polymer Synthesis

N-acryloyl piperidine was synthesized through reaction between acryloyl chloride and piperidine (Figure 1).[1] In short, 0.11 mol of piperidine and 0.12 mol of triethylamine were dissolved in 100 mL of dichloromethane maintained at 0-5ºC. A solution of acryloyl chloride (0.10 mol) in 15 mL of dichloromethane was added drop-wise to the above solution over 2 hours under constant stirring. After complete addition, the reaction mixture was stirred at room temperature for 24 hours and was extracted with water and purified by column chromatography (hexane:ethyl acetate, 1:1) to yield colorless to light yellow liquid. *N*,*N*-diethylacrylamide was passed through a basic alumina column prior to polymerization. AIBN was recrystallized from

methanol before use. In a typical polymerization reaction, the required amount of monomers was dissolved in anisole and 0.3 mol% (of total monomer content) of AIBN was added to the solution. The reaction flask was completely sealed and the solution was purged with Argon for 20 minutes. The reaction was carried out at 65°C for 20 hours. After the reaction, all the solvent was evaporated at high temperature under vacuum to obtain white solid residue. The residue was re-dissolved in chloroform and then twice precipitated in ethyl acetate to obtain white solid mass. The precipitate was recovered and dried at 60°C under vacuum for 2-3 days.

Polymer Characterization

The synthesized polymers were characterized by gel permeation chromatography (GPC, Waters Inc., 1515 Isocratic HPLC pump and 2414 RI detector) using 3 Styragel columns- HR2, HR3 and HR4 in series maintained at 35ºC with chloroform as eluent (flow rate- 1 mL/min, total elution time- 40 min). The instrument was calibrated with polystyrene standards. LCST was ascertained by measuring UV-vis transmittance (Varian Cary 50 Bio) of a 0.1 wt.% aqueous solution of polymers as a function of temperature. A thermocouple was used for real-time measurement of temperature, with the metal junction dipped in the cuvette during the measurement. For effective measurement, the polymer solution was cooled down to 2-3°C along with the metal cuvette holder to slow down the heating up of sample in ambient condition. $CaCl_2$ was placed inside the UV-vis spectrophotometer chamber to ensure humidity-free environment. This was necessary to prevent atmospheric water vapor from condensing on the cold cuvette walls. UV-vis spectrum was measured from 200-800 nm at every 0.2-0.5°C with more frequent measurements near the transition temperature. Transmittance at 400 nm was

plotted against temperature and the temperature for 50% transmittance was noted as the LCST. Molecular weights and LCSTs of different polymer batches are noted below (Supplemental Table 1).

Table S1. Molecular weights, PDI, and LCST of different batches of synthesized polymers used in the study

Polymer	M_n (kDa)	M_w(kDa)	PDI	LCST (ºC)
P1	209.246	308.086	1.47	13.6
P2	151.332	253.380	1.67	12.7
P3	175.085	255.778	1.46	12.0
P4	173.019	303.009	1.75	11.8

Materials Kapton polyimide tape was purchased from Cole Parmer. Ethanol, acetone, chloroform, and isopropanol were solvent grade and were used without further purification. Surface modifying agents – (Heptadecafluoro-1,1,2,2-tetrahydrodecyl)trichlorsilane (HFTCS) and 2-methoxy(polyethyleneoxy)propyltrimethoxysilane (PEG-silane) – were purchased from Gelest Inc. Microscope glass slides were purchased from Fisher.

Device Fabrication

The glass slides were sequentially washed with chloroform, acetone, and isopropanol via sonication for 5 minutes each. The glass slides were then air dried and treated in a UV-ozone generator for 30 minutes to remove any carbon contamination and to obtain a high density of surface hydroxyl groups. The cleaned substrates were patterned using Kapton tape by masking the active device area. Kapton tape was chosen for its impermeability to silane vapors and good stability at high temperatures. The patterned substrates were then cleaned with wipes dipped in

ethanol to remove any adhesive residue and treated with HFTCS via vapor phase surface modification at 100°C for 30 minutes. HFTCS treatment results in hydrophobic fluoroalkyl groups on the unmasked peripheral regions of the substrates which prevent the use of any physical confining barrier to pattern the device with polymer-GO film by drop-casting method. After HFTCS treatment, the Kapton tape mask was removed and the glass slides were washed with copious amounts of ethanol to remove any physisorbed silane as well as any adhesive residue. The second surface modification was done in liquid phase by immersing the glass slides in 3.35mM-of PEG-silane in ethanol for 12-15 hours. Subsequently, the glass slides were again washed with ethanol to remove any physisorbed silane. A polymer-GO blend solution containing 10 mg/mL of polymer in 975 µL DMF and 25 µL of GO-PEG solution was then drop-casted in requisite amount on the surface modified glass substrates and allowed to dry at 60°C in an oven. The PDMS chamber was assembled on the glass substrate with polymer-GO composite film through corona discharge to produce a microfluidic device (Figure S1).

Fluorescent biotin assay

To verify the ability to immobilize biotinylated antibody to the polymer-GO film surface, surface coverage by a fluorescently labeled biotin (Biotin (5-fluorescein) conjugate, Sigma Aldrich) was assessed (Figure S4a). Three polymer-GO films underwent the entirety of the conjugation chemistry (i.e. treatment with the GMBS cross-linker and NeutrAvidin; termed "Condition") with fluorescent biotin addition as the terminal step. To account for non-specific binding, three polymer-GO films were treated only with the fluorescent biotin to serve as a control in an analogous fashion to an isotype control (termed "Control"). ImageJ was used to

quantify the fluorescence. This technique showed a statistically significant increase in fluorescence intensity relative to the control (Figure S4b).

Cell labeling for optimization experiments

Cells were stained with CellTracker™ Green CMFDA Dye (Life Technologies/ThermoFisher Scientific) according to the manufacturer's protocol. The staining process takes approximately two hours and was performed in parallel with device preparation.

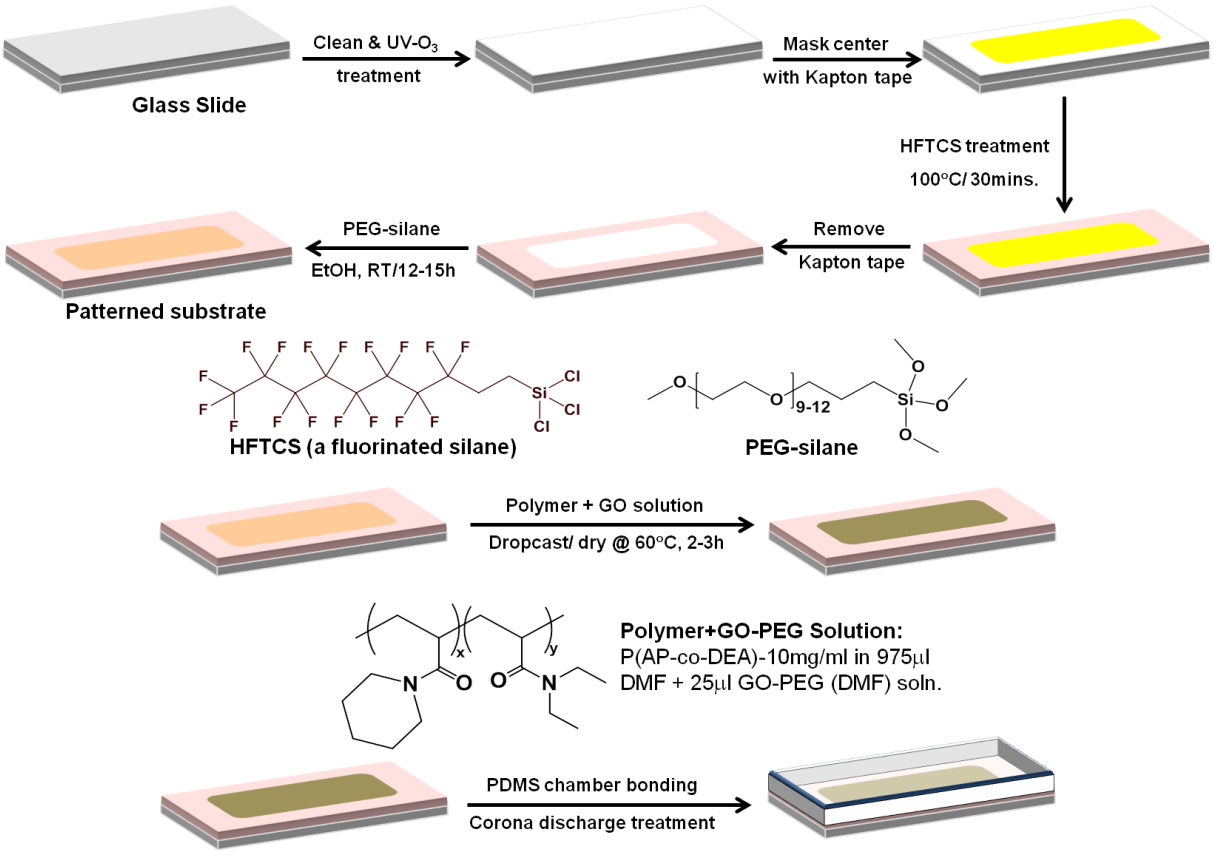

Figure S1. Schematic for CTC device fabrication.

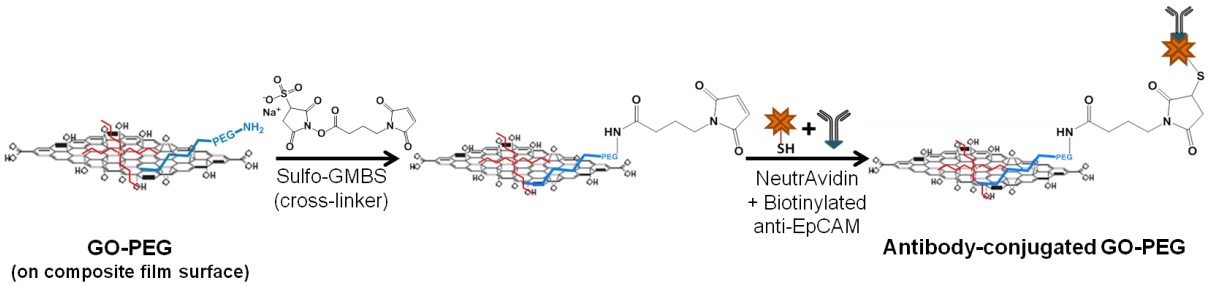

Figure S2. Schematic of graphene oxide functionalization chemistry

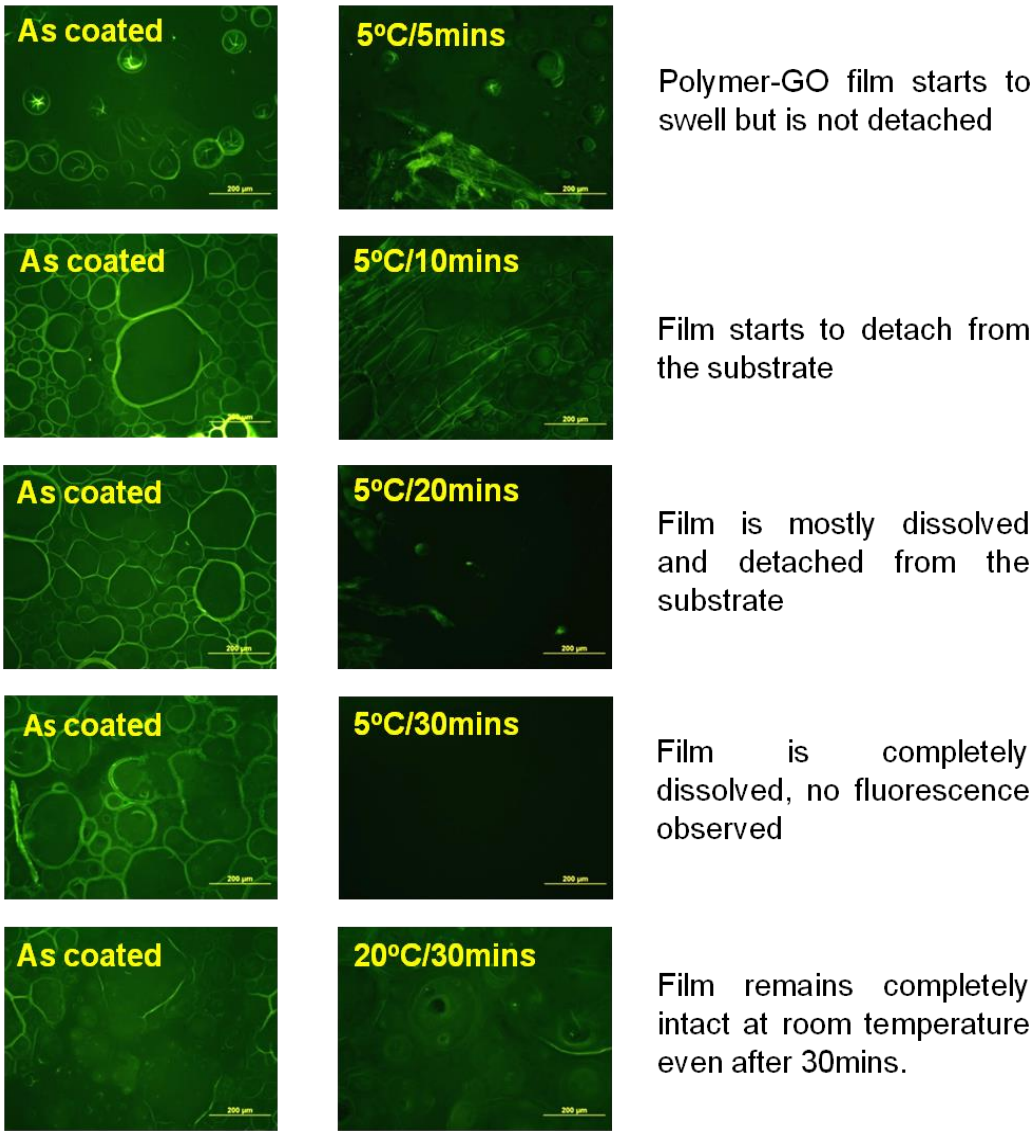

Figure S3. Fluorescence microscopy images of polymer-GO films incubated with FSE dye before and after being dipped in either cold (5°C) or room temperature (20°C) water for the specified time durations. Scale bar: 200 µm.

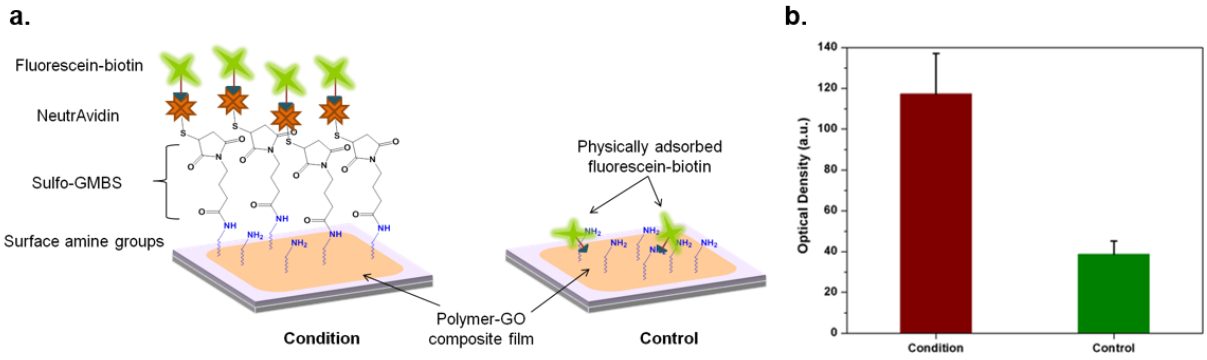

Figure S4. a) Schematic represents fluorescent biotin assay and negative control. b) The full conjugation chemistry features statistically higher fluorescence than the negative control as assessed via optical density ($p = 0.019$).

Table S2. Experimental results from Live/Dead assay (MCF-7 cell line).

Device	Live cells after release	Dead cells after release	Live/Dead (%)
D1	264	22	92.31
D2	353	7	98.06
D3	174	10	94.57
D4	270	35	88.52
D5	152	27	84.92
Average			91.68

Table S3. Comparison of CTC isolation technologies. Both commercially available and recently developed CTC isolation technologies are compared across multiple relevant metrics and abilities, showing the high versatility and performance of the technology put forth in this work. [2-13]

Table S3. Comparison of CTC Isolation Technologies							
Technology	**Approach**	**Flow rate mL/hr**	**Cell line capture efficiency**	**CTC detection in patient samples**	**Whole blood**	**Live cells**	**Release capability**
CellSearch	Anti-EpCAM coated magnetic beads	NA	>80%	34 out of 92 patients had ≥5 CTCs/7.5 mL in metastatic breast cancer[2], 21 out of 101 patients had ≥2 CTCs/7.5 mL in NSCLC[3]	No	No	NA
Epic Sciences	No enrichment, RBCs lysed blood deposited on slides	NA	NA	57 out of 78 in NSCLC[4], 22 out of 40 in melanoma[5]	No	No	NA
Mag Sweeper	Immunomagnetic capture	NA	62%	17 out of 17 in metastatic breast cancer[6]	Yes, need dilution	Yes	Yes
ISET	Size based filtration	NA	As low as 1 CTC per 1 mL of blood[7]	32 out of 40 in NSCLC[8]	No	No	NA
CTC iChip	Size based separation +ve or –ve selection with mag beads	9.6	>95% for –ve 78%-98% for +ve	37 out of 41 in CRPC[9]	Yes, not a single step	Yes	Yes
FACS Sorting	Surface marker based selection	Very Low	NA	3 out of 8 brain metastatic breast cancer[10]	Yes	Yes	Yes
RosetteSep kit	Depletion of WBCs	NA	NA	NA[11]	multiple steps	Yes	NA
CTC Chip	Positive selection	1	>87%	14 out of 19 in lung cancer[12]	Yes	Yes	No
GO Chip	Nano pillars with Graphene Oxide	1-3	>82.3%	7 out of 7 in breast cancer 8 out of 9 in pancreatic cancer[13]	Yes	Yes	No
Polymer-GO Release Chip	**Thermosensitive polymer-graphene oxide composite**	**1-3**	**>88.2%**	**8 out of 10 in breast cancer** **2 out of 3 in pancreatic cancer**	**Yes**	**Yes**	**Yes**

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