Electronic Supplementary Information

Circulating tumor cell-based digital assay for detection of EGFR T790M mutation in advanced non-small cell lung cancer

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SUPPLEMENTARY METHODS

NanoVelcro Chip fabrication

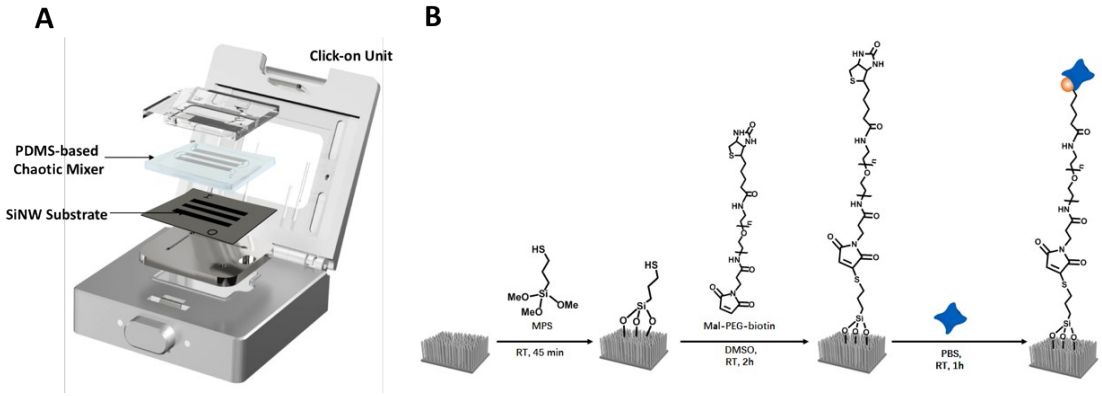
The NanoVelcro CTC chip is composed of three parts: (i) a serpentine chaotic mixer chip made of polydimethylsiloxane (PDMS), (ii) a patterned SiNW substrate, and (iii) a home-machined holder set to sandwich a well-aligned PDMS mixer chip with the SiNW substrate (Fig. S1A). The PDMS mixer chips were fabricated using a standard soft-lithography method. In order to minimize system error caused by inconsistency of PDMS mixer chips, a silicon mold with a desired configuration was made by dry etching, and then framed in a metal container. Well-mixed PDMS was poured onto this mold and cured in an oven at 80 °C. After 48 h of baking, the cured PDMS layer was peeled off and punched with two through holes at both ends of the channel for tubing connection. Consistent channel structure and PDMS thickness were achieved using this method.

The fabrication method for the SiNW substrate was reported earlier [1]. Briefly, a patterned layer of photoresist (AZ 5214, AZ Electronic Materials USA Corp.) was deposited on the silicon wafers using standard photolithography technique. Then a chemical etching solution composed of deionized water, HF (4.6 M), and silver nitrate (0.02 M) was applied to the wafer to construct SiNWs with an average length of 15 μm at the uncovered surface area defined by the photoresist pattern. After that, boiling aqua regia (3:1 (v/v) HCl/HNO_3) was added to the nanostructured substrate for 15 min to remove the silver film. The remaining photoresist was then removed by repeatedly

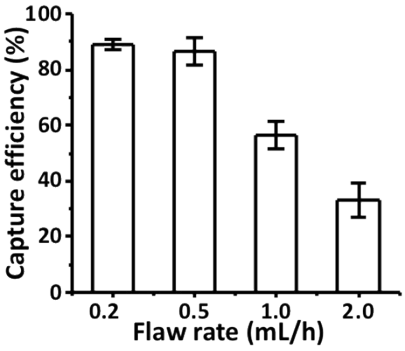
rinsing with acetone and ethanol. After DI water rinsing and nitrogen blow-dry, the substrate was ready for subsequent streptavidin coating.

Streptavidin coating was also conducted following a previously established protocol. Basically, the substrate was first treated with 4% (v/v) 3-mercaptopropyl trimethoxysilane in ethanol for 45 min at room temperature. Then a work solution was prepared using Biotin-PEG-Maleimide (MPB, Nanocs, PG2-BNML-1k, 5.3mg MPB in 500 ml DMSO). Put chips in MPB working solution and seal/cover the beaker with plastic sheet; agitate 10 minutes and then keep at room temperature for 2 h. After incubation with streptavidin (SA, 10 μg/ml in 1x PBS) for another 1 h, the streptavidin-coated substrate was stored at 4 °C to avoid activity decay before usage.

Prior to each test, the PDMS chaotic mixer chip and streptavidin-coated substrate were sandwiched together using a home-machined holder set consisting of (i) one stainless steel bottom plate, (ii) one PMMA (poly(methyl methacrylate)) clapboard, (iii) one stainless steel top frame, and (iv) a screw at each corner (Figure S1A). To form a complete device, the PDMS chip and SiNW substrate in accurate alignment were placed on the center of the bottom plate. Then PMMA clapboard was carefully laid on top to ensure the two through holes on PDMS chip were located right in the middle of their corresponding bores pre-machined on the clapboard. Finally, the top frame was anchored to the PMMA clapboard and bottom plate by screws to form a constant pressure seal to prevent leakage.



Supplementary Figure 1. (A) Schematic diagram of the components of the NanoVelcro Chips. The chip holder compresses the PDMS-based chaotic mixer against the channels of the silicon nanowire chip to create a microfluidic channel which optimizes cellular interactions with the chip surface. (B) Illustration of the modification procedures of the NanoVelcro Chips before capture of biotinylated anti-EpCAM labeled tumor cells.



Supplementary Figure 2. CTC-capture efficiency of the NanoVelcro Chips was studied at flow rates of 0.2, 0.5, 1.0 and 2.0 mL h^{-1}.

Table S1. Clinical characteristics of healthy donors enrolled in this study

Sample ID	Gender	Age (y)	Smoking (y)	Copy numbers of T790M transcripts in CTCs[a]
1	F	50	0	-
2	M	43	0	-
3	M	38	0	-
4	M	45	20	-
5	M	47	0	-
6	F	28	0	-
7	M	57	30	-

[a]per 2 mL blood..

Reference

1. S. Wang, K. Liu, J. Liu, Z. T. F. Yu, X. Xu, L. Zhao, T. Lee, E. K. Lee, J. Reiss and Y. K. Lee, *Angewandte Chemie International Edition*, 2011, **50**, 3084-3088.