Supplementary Information

Stimuli-Responsive Microfluidic Interface Enables Highly Efficient Capture and Release of Circulating Fetal Cells for Non-Invasive Prenatal Testing

Huimin Zhang[#1], Yuanyuan Yang[#3], Yilong Liu[2], Yidi Wang[2], Weidong Ruan[2], Jia Song[1], Xiyuan Yu[1], Lingling Wu[1], Zhi Zhu[2], Guolin Hong*[3] and Chaoyong Yang*[1,2]

[1] Institute of Molecular Medicine, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, 200127, China

[2] The MOE Key Laboratory of Spectrochemical Analysis and Instrumentation, Key Laboratory for Chemical Biology of Fujian Province, State Key Laboratory of Physical Chemistry of Solid Surfaces, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China

[3] Department of Laboratory Medicine, The First Affiliated Hospital of Xiamen University, Xiamen Key Laboratory of Genetic Testing, Xiamen, 361005, China

*Email: xmhgl9899@sina.com, cyyang@xmu.edu.cn

The Supporting Online Material includes

Experimental Section

Experimental section

Magnetic beads-based cell isolation.

To compare the capture performance of NIPT-Chip with magnetic beads, we carried out a traditional cell capture on magnetic beads. Magnetic beads were pretreated according to user guide. For each capture study, a small quantify of 20 ug/mL biotinylated anti-EpCAM per capture study (the same quantity used with NIPT-Chip) was mixed with 10 uL magnetic beads for 30 min. After PBST washing, anti-EpCAM embedded magnetic beads were mixed with spiked blood sample at room temperature for 45 min. Targeted JEG-3 cell would be prestained by Calcium AM and blood cell would be stained by Hoechst. The captured samples were imaged under the fluorescence microscope to get the number of targeted cell and contaminated blood cells.

Herringbone-patterned PDMS chip-based cell isolation.

To compare the capture performance of NIPT-Chip with herringbone-patterned PDMS chip, we prepared the herringbone chip according to publication. [1] Preparation of anti-EpCAM modified chip was using the method as described in "Device fabrication and antibody immobilization" part.

To decrease the adsorption of blood cells, the chips were pre-blocked by PBS buffer with BSA (3%) and Tween 20 (0.05%) before use. The model cells were stained with Calcein AM and resuspended in whole blood. Spiked sample was injected by Micro Plus Elite syringe pump (Harvard apparatus, MA, USA) through the inlet at flow rate 1.0 mL/h according to reference. Microchip was placed in a fluorescent microscope to record the fluorescent cells in the inlet to help us calculate the actual input cell numbers. After cell capture and PBS washing, fluorescent cells in the chip and inlet were counted. To calculate the non-specific cell binding, after cell capture, Hoechst solution was drained into chip for 10 min and washed away. Blood cell would be calculated manually under microscope.

Results and discussion

Fabrication and Characterization of Chip

The device consists of one main channel with three inlets (blood in the middle and buffer in the other two) and three outlets for waste. The photograph is shown in Figure S1. The depth of the main channel was 50 μm. SEM imaging was used to characterize the structure of micropillars. Figure S2 C showed good consistency as designed. We also employed the PE-labelled second antibody to characterize the modification. The fluorescence intensity of the anti-EpCAM-modified chip was significantly higher than that of the anti-IgG modified chip (Figure S2A&B). This result clearly demonstrated the successful establishment of the antibody-functionalized chip, which ensured CFC capture in an efficient manner.

Figure S1. Photograph of microfluidic chip used in this manuscript. Blue pigment was injected to light the channel.

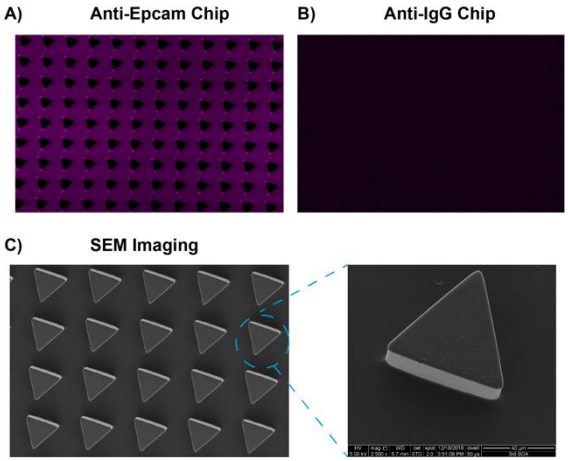

Figure S2. Fluorescence microscopy images of Anti-EpCAM-modified chip (A) and anti-IgG-modified chip (B) incubated with the PE-labelled second antibody. (C) SEM images of stimuli-responsive microfluidic interface (Scale bar: 40 μm).

Dynamic changes of fluorescence intensity upon disulfide cleavage by DTT

DTT solution was employed to the main channel for different incubation times. As shown

in Figure S3, the fluorescence intensity of the PE fluorophore decreased rapidly after exposure to 50 mM 1,4-dithiothreitol (DTT) for 7.5 min and plateaued within 15 min. This demonstrated that DTT can efficiently and quickly cleave the disulfide linkers present on the DLD piilars, allowing release of captured cells.

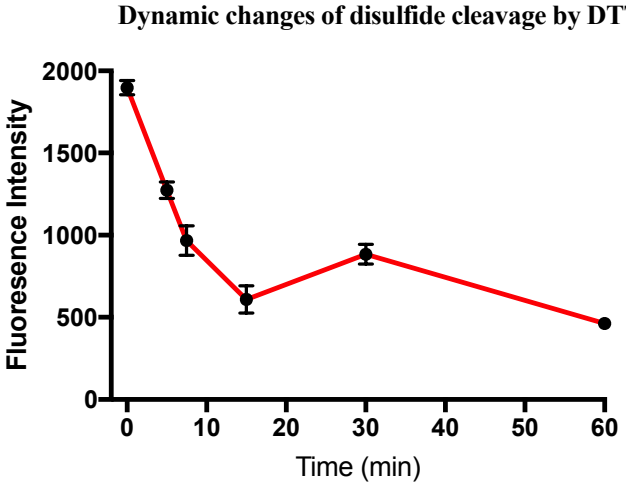

Figure S3. Dynamic changes of fluorescence intensity upon disulfide cleavage by DTT. Average fluorescence intensity vs. DTT reaction time on DLD channel with 50 mM DTT. The surface was treated with DTT (50 mM) in PBS (200 μL) for 0, 5, 7.5, 15, 30, and 60 min, respectively.

Characterization of JEG cell line

JEG-3 was selected as an artificial trophoblast cell line in the main text. Size characteristics based on microscope bright field are shown in Figure S4 A. The JEG-3 cell line has a distribution from 15 μm to 20 μm, which is different from the white blood cell and red blood cell size distributions (red blood cells: 6~8 μm in diameter; white blood cells: 7~15 μm in diameter).

Flow cytometry assay was applied to confirm the expression of JEG-3 cells (**Figure S4 B**). Briefly, approximately 1×10^5 cells of each type were suspended with 200 μL BB buffer (1×PBS, 0.55 mM $MgCl_2$, pH=7.4) in individual test tubes. The mixture of cells and antibodies was incubated at room temperature for 30 min. After incubation, the cells were washed twice by centrifugation with 0.5 mL buffer and resuspended in 0.2 mL buffer. The fluorescence was

determined by counting 10,000 events.

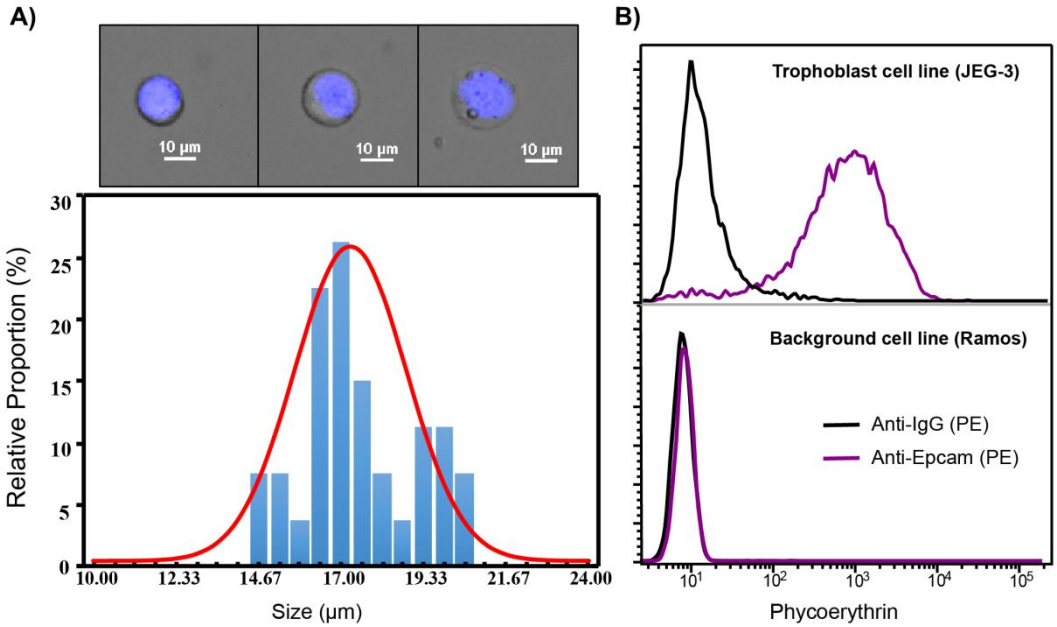

Figure S4. (A) Graph of single cell area, imaging with bright field and nuclei stained with Hoechst (upper panel). Size distribution of JEG-3 cell line measured by microscope (lower panel). (B) Flow cytometry analysis of JEG-3 cell line with anti-EpCAM for confirmation of EpCAM expression.

Characterization of viability of cells treated with 50 mM DTT

Cell viability assay (calcium AM and PI solution) was applied to determine the damage due to DTT treatment. As shown in **Figure S5**, there was only a small difference between untreated and DTT-treated samples, indicating that after DTT release, cell maintain viability.

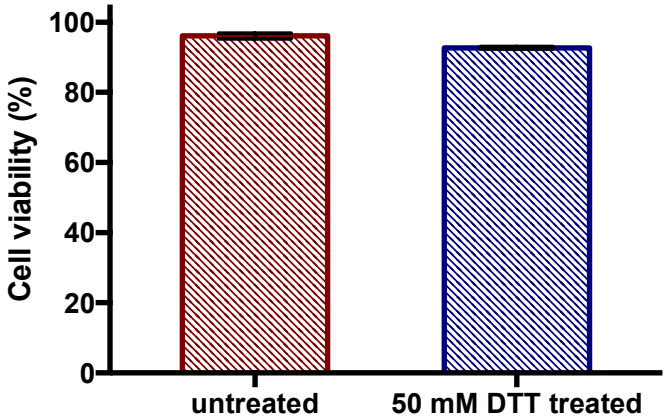

Figure S5. Cell viability of untreated (red) and DTT treated (blue) cells for 30 min at 37 °C

STR profiles and whole genome sequencing of fetal DNA amplified from CFCs.

STR fingerprints of isolated trophoblasts and their matching maternal cells from a pregnancy sample were showed in Figure S6A. Whole genome amplification product was used as template to amplify genetic loci in single fetal cells. Maternal genome obtained from lysis of whole blood was used as maternal fingerprints. We found seven informative STR markers inherited from mother and father. In particular, loci with three or more alleles were rare detected suggesting the purity of fetal cells.

We also sequenced the amplified fetal gene to analyze the chromosomal copy numbers (Figure S6B). Mapping rate of 77.48% and average coverage of 19.84% were obtained according to the bioinformatics analysis. Some chromosomal deletion observed from the data may resulted from the amplification bias of single cell whole genome amplification.

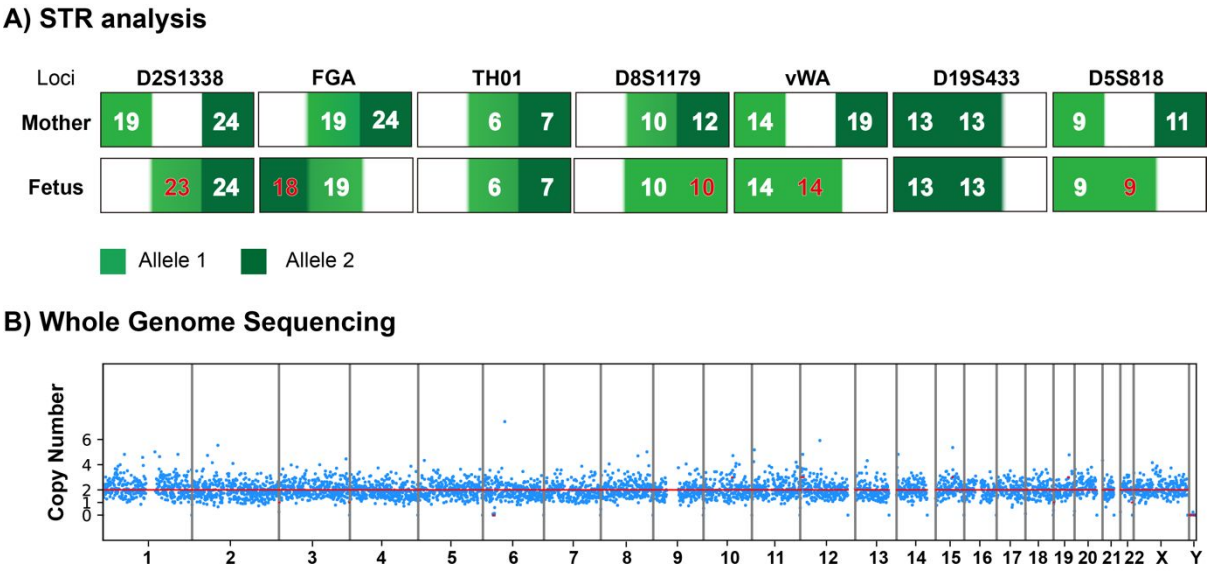

Figure S6. A) STR analysis of fetus-mother relationship and B) sequencing information of isolated trophoblasts cells.

Table S1. Clinical characteristics of pregnant, postpartum and healthy donors (HD) enrolled in our study.

ID Number	Age	Gestational age (Week)	Trophoblast cell number (/2 mL)
Pregnant-1	34	7w	5

Pregnant-2	27	7w+6d	3
Pregnant-3	28	7w+6d	6
Pregnant-4	31	9w+1	2
Pregnant-5	28	10w+3	9
Pregnant-6	28	10w	8
Pregnant-7	35	14w	10
Pregnant-8	27	13w	5
Pregnant-9	34	19w	8
Pregnant-10	36	19w	3
Pregnant-11	28	21w	10
Pregnant-12	26	18w	10
Pregnant-13	26	17w	12
Pregnant-14	25	19w	11
Pregnant-15	34	18w+2d	13
Pregnant-16	40	14w+5d	12
Pregnant-17	33	19w+1d	20
Pregnant-18	26	21w+3d	14
Postpartum-1	25	3d	0
Postpartum-2	42	3d	0
Postpartum-3	26	2d	0
Postpartum-4	25	5d	1
Postpartum-5	35	3d	1
Healthy-1#	26	-	0
Healthy-2#	30	-	0
Healthy-3#	35	-	0
Healthy-4#	40	-	1
Healthy-5#	28	-	0
Healthy-6#	22	-	0
Healthy-7#	34	-	0
Healthy-8#	36	-	1

Author Contributions. Huimin Zhang and Yuanyuan Yang contributed equally to this work. Huimin Zhang designed and performed experiments, analyzed data and wrote the manuscript.

Yuanyuan Yang performed experiments and collected data. Yilong Liu and Yidi Wang analyzed data and edited the manuscript. Weidong Ruan and Jia Song analyzed the sequencing data. Xiyuan Yu and Zhi Zhu analyzed data and edited the manuscript. Chaoyong Yang and Guolin Hong designed the research and wrote the manuscript.

References

1. Park, M.-H.; Reátegui, E.; Li, W.; Tessier, S. N.; Wong, K. H. K.; Jensen, A. E.; Thapar, V.; Ting, D.; Toner, M.; Stott, S. L.; Hammond, P. T., Enhanced Isolation and Release of Circulating Tumor Cells Using Nanoparticle Binding and Ligand Exchange in a Microfluidic Chip. *J Am Chem Soc* **2017,** *139* (7), 2741-2749.