Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2012

Electronic Supplementary Information

Targeted Isolation and Analysis of Single Tumor Cells with Aptamer-Encoded Microwell Array on Microfluidic Device

Qiushui Chen, Jing Wu, Yandong Zhang, Zhen Lin, Jin-Ming Lin*

Beijing Key Laboratory of Microanalytical Method and Instrumentation, Department of Chemistry, Tsinghua University, Beijing 100084, P.R. China

* To whom correspondence should be addressed. E-mail: jmlin@mail.tsinghua.edu.cn. Fax/Tel: +86 10 62792343

This journal is © The Royal Society of Chemistry 2012

1. Fabrication of PDMS chip

This device consists of two components: PDMS layer with two microchannels and glass substrate with microwell array. The PDMS chip was fabricated by using traditional standard soft lithography techniques.[1] In brief, a sillcon wafer was pre-cleaned by H_2SO_4 : 30% H_2O_2 (3:1, v/v) and then coated with the negative photoresists SU-8 2050 (2000 rpm, 60 μm thick film). This sillcon wafer was prebaked at 85 ℃ for 10 min, and then exposed by UV light under the photomask. Further, the patterned microstructure can be generated by developing solution. After hard-baked for 10 min, the master was silanized over night by tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane vapor. PDMS prepolymer and curing agent (10:1) was premixed and poured onto the master. After degassed in a vacuum chamber, the PDMS replicas were cured in an 85 °C oven for 3 h. Finally, the PDMS was peeled from the master and connection holes were made by using a needle. The microfluidic chamber is 60 μm depth, 2.3 mm width and 10 mm length. The volume is about 1.38 μL. The PDMS layer was cleaned with CH_3OH and H_2O for further application.

2. Operation of single-cell capture platform

Before experiments, the glass substrate was treated by piranha solution to keep its interface hydrophilic. Specially, the device was fabricated by non-covalent bonding between PDMS layer and glass substrate. As shown in Fig. S1, the two components were immobilized by using two clamps. Then, PBS buffer was used to rinse and wash the microchannels. Further, 5.0 μL of 1.0 mg/ml avidin was incubated into the microchannels for 5.0 min and washed with 5.0 μL PBS buffer 3 times. Finally, 4.5 μL of 100 μM biotin-aptamer in

tris-EDTA (TE) buffer was incubated with microwells for 5.0 min and rinsed with 5.0 μL PBS buffer 3 times. For single-cell capture experiments, a cell suspension of 5×10^7 cells/ml was injected into microwells. After 1.0 min, the residue cells were gently washed away by 5.0 μL PBS buffer. Finally, the microfluidic device was put on the object stage of fluorescence microscope for acquiring the images.

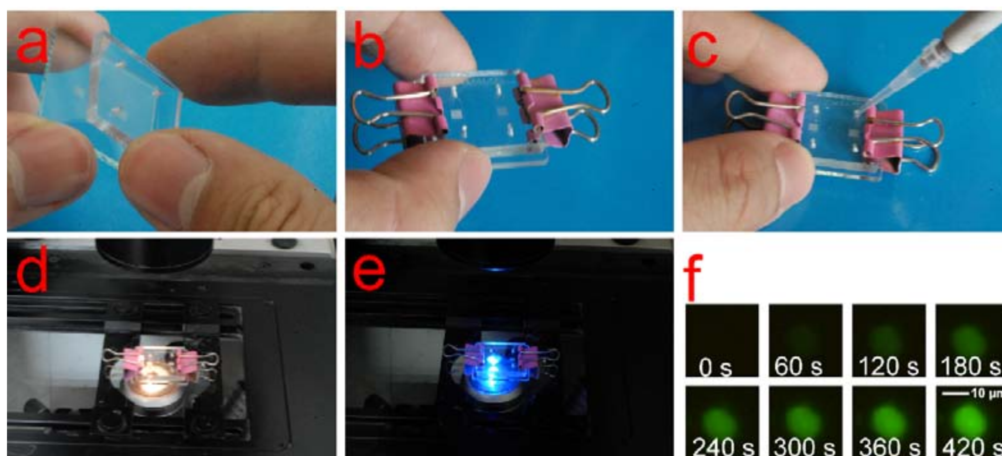


Figure S1. Operating procedures of microfluidic single cells isolation by using aptmer-encoded microwells. a, b) The PDMS layer and microwell array was aligned, and then non-covalent bonding was conducted by using two clamps. c) Injections of PBS buffer, avidin, biontin-aptamer and cells was performed by using a liquid-transfering gun. d, e, f) The microfluidic system was placed on microscope, and the images were acquired.

3. Characterization of aptamer immobilization on microwells by AFM

The immobilization of aptamer on glass substrate was conducted by previous methods.[2] The glass substrate with microwell array, as well as that coated with avidin and biotin-aptamer was rinsed in deionized water for 3 times. Then, these three pieces of glass were dried in a cleaned dish at room temperature. The AFM images were conducted by a tapping mode atomic force microscopy (Fig. S2).

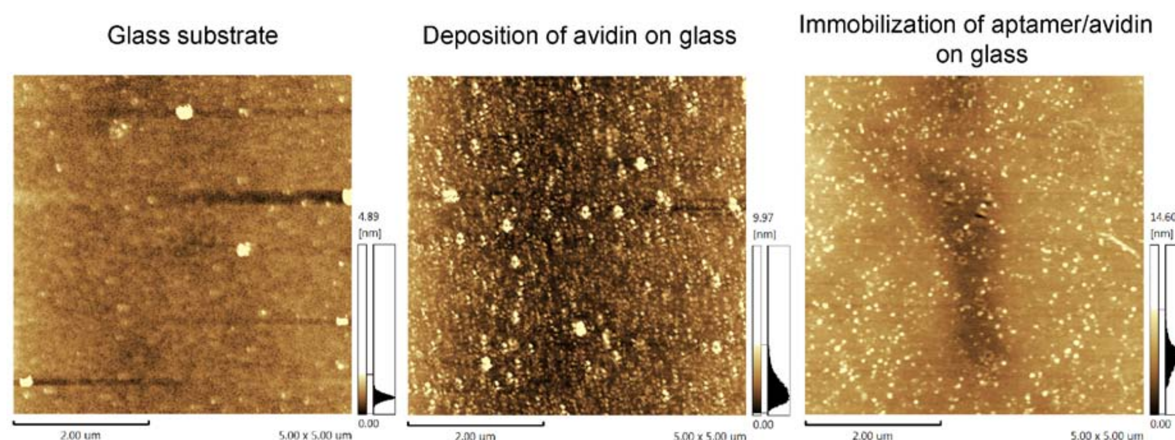
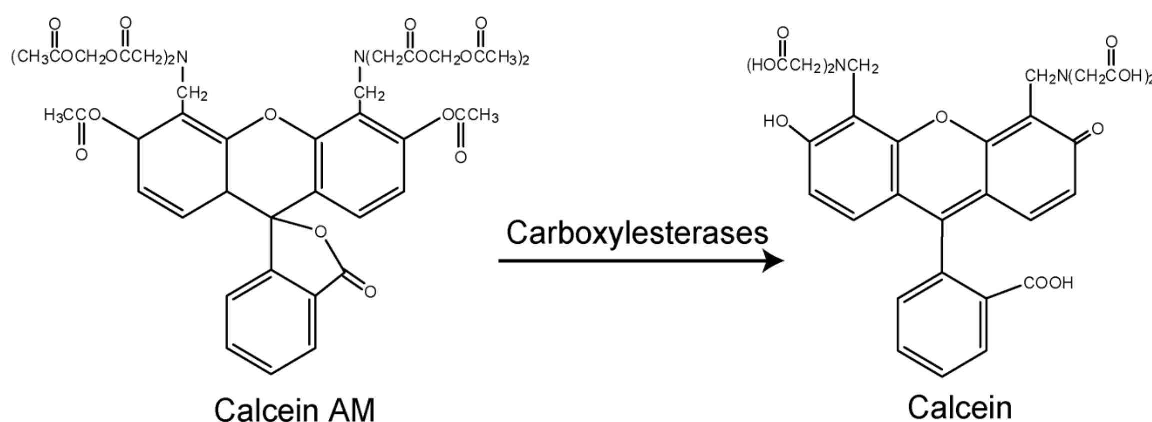


Figure S2. AFM imagesof glass substrate, deposition of avidin on glass and immobilization of biotin-aptamer.

4. Enzyme dynamic analysis by using Calcein AM as a substrate

The cellular hydrolysis of aster is very important in drug-related reaction, thus will be responsible for multidrug resistance of cancer cells.[3] In this work, calcein AM was uses as a substrate to study the cellular carboxylesterase activity at single-cell level. The reaction process was shown in scheme 1.



Scheme 1. The cellular carboxylesterases reaction between calcein AM and calcein.

4. Single-cell occupancy under different concentrations

This journal is © The Royal Society of Chemistry 2012

The single-cell occupancy experiments with different cell concentrations are performed at 5×10^4, 5×10^5, 5×10^6 and 5×10^7 cells/mL, respectively. As shown in figure S3, the results show that the single-cell occupancy is increased by the increased concentration of injected cells. In our experiments, we used a high cell concentrations of 5×10^7 per ml for high single cell occupancy.

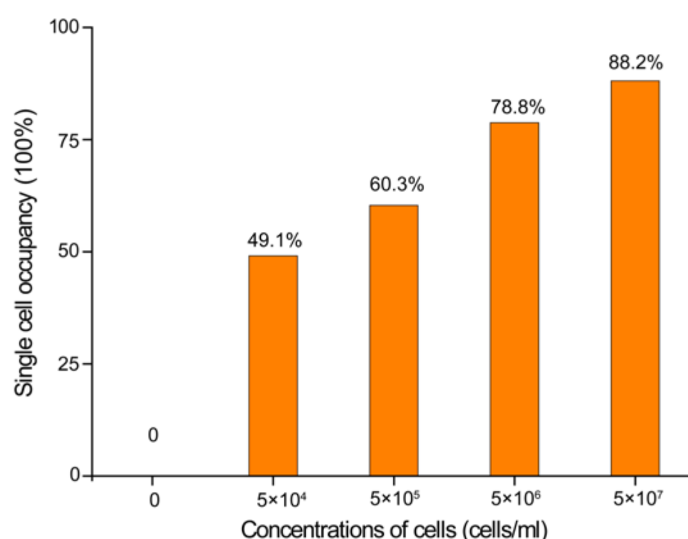


Figure S3. Single-cell occupancy in different concentrations at 5×10^4, 5×10^5, 5×10^6 and 5×10^7 cells/mL, respectively. Avidin: 1.0 mg/ml, Biotin-aptamer: 100 µM.

REFERENCES

(1) Chen, Q..; Wu, J.; Zhang, Y.; Lin, J.-M. *Anal. Chem.* **2012**, *84*, 1695-1701.

(2) Phillips, J. A.; Xu, Y.; Xia, Z.; Fan, Z. H.; Tan, W. *Anal. Chem.* **2009**, *81*, 1033-1039.

(3) Essodaigui, M.; Broxterman, H. J.; Garnier-Suillerot, A. *Biochemistry* **1998**, *37*, 2243-2250.