Supporting Information

Nanostructures Embedded Microchips for Detection, Isolation, and Characterization of Circulating Tumor Cells

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Genotyping for single pancreatic CTC (also see Figure 6f)

1. Surface functionalization of PLGA NanoVelcro Chip

We prepare and characterize electrospun PLGA NanoVelcro Chip using our previous published method. [1] To obtain stable streptavidin-coated PLGA NanoVelcro Chip used in our studies, the NHS chemistry were used to create a covalent bond between the carboxylic acid end groups and free amines on the streptavidin molecules. PLGA nanofibers were first reacted in 0.5 mL 1xPBS with EDC (8.0 mg mL^{-1}) and sulfo-NHS (2.0 mg mL^{-1}) to convert the terminal carboxyl group to an amine-reactive sulfo-NHS ester. Streptavidin of 250 mg mL^{-1} was then reacted with the NHS-functionalized carboxyl group in 1.0 mL 1xPBS at room temperature. Modified PLGA NanoVelcro Chips were then washed carefully with PBS for three times to remove excess reactants. Prior to the cell-capture studies, biotinylated anti-EpCAM (R&D, 8 μg mL^{-1}, in 500-µL PBS with 1% (w/v) BSA) was freshly grafted onto the PLGA NanoVelcro substrates.

2. General procedure for sample preparation and pancreatic CTCs capture

The PLGA NanoVelcro Chip was first washed by PBS for three times to eliminate unconjugated biotinylated anti-EpCAM. Subsequently, the PDMS component with herringbone was carefully assembled on the chip freshly functionalized in previous step. The chip holder was applied to tight the microfluidic chip in a sandwich fashion. After complete the set-up of the microfluidic chip, 1 mL patient whole blood (or artificial) sample (collected or prepared freshly within 2 h) was injected into the system at the flow rate of 0.5 mL h^{-1}. As the completion of sample processing in the microfluidic chip, 0.5 mL ethanol was injected into the microfluidic chip to fix the captured cells. After slightly wash with 0.1 mL PBS, 0.5 mL of fluorophore labeled anti-Cytokeratin (CK)-FITC (Life technologies) and anti-CD45-TRITC (Life technologies) mixing solution was injected to flow through the chip for immunostaining at flow rate of 0.5 mL h^{-1}. The chip was first washed gently with 0.1 mL PBS. Then the membrane slide was taken out from the disassembled microfluidic device for on-site Fluorescent scanning under microscope

(Nikon Eclipse 90i). The fluorescent imaging of whole slide was processed and analyzed by a custom designed program to screen out pancreatic CTCs with clear identification and accurate location, which will further lead to precise isolation of pancreatic CTCs by laser microdissection. Generally, the whole procedure for capturing, imaging and isolating of pancreatic CTCs in 1 ml patient blood sample can be finished around 6h. The scanned chip was then maintained under -80 ℃ before single pancreatic CTCs isolation.

3. General procedure for laser microdissection

Owing to the transparency of PLGA NanoVelcro Chip, the captured pancreatic CTCs can be specifically isolated by Laser Microdissection (Leica, LMD7000) without contamination from other cells in the whole blood sample. After being captured, pancreatic CTCs on the PLGA-nanofiber layer were fixed by ethanol, frozen and air-dried. Further manipulation was achieved on the pancreatic CTCs captured substrate under the microscope. By examining cells at two different fluorescent wavelengths (521 nm and 575 nm) and comparing with pre-scanned fluorescent image, the CK positive pancreatic CTCs can be precisely identified and isolated from CD45 positive WBCs conveniently. The dissected pancreatic CTCs were collected in PCR-tube (200 μL) and maintained in 2 μL of lysis buffer (WGA4, Sigma-Aldrich) for subsequent gDNA extraction and amplification.

4. Results of molecular analysis for single pancreatic CTCs

The single pancreatic CTC collected by laser microdissection was then proceeded to extract gDNA for amplification using the GenomePlex® Single Cell Whole Genome Amplification Kit (WGA4, Sigma-Aldrich). After further purification using QIAquick PCR Purification Kit (QIAGEN, Valencia, CA), 5 μL of the whole-genome amplification (WGA) product was used for quality control by Gel Electrophoresis. Another 1 μL WGA product was applied for KRAS exon 2 (containing the $KRAS^{G12V}$ codon) amplification by PCR using the primers listed in **Table S1**. The purified samples were sent to The UCLA Genotyping and Sequencing Core for Sanger sequencing (Biosystems 3730 Capillary DNA Analyzers).

The sequence reads were aligned to the human reference genome using Novoalign V2.07.13 from Novocraft (http://www.novocraft.com).

Table S1. KRAS exon 2 (containing the $KRAS^{G12V}$ codon)-specific primers

Sequence	Forward	Backward
KRAS	AAGGTACTGGTGGAGTATTTG	GTACTCATGAAAATGGTCAGAG

Reference

(1) Hou, S.; Zhao, L.; Shen, Q.; Yu, J.; Ng, C.; Kong, X.; Wu, D.; Song, M.; Shi, X.; Xu, X.; OuYang, W. H.; He, R.; Zhao, X. Z.; Lee, T.; Brunicardi, F. C.; Garcia, M. A.; Ribas, A.; Lo, R. S.; Tseng, H. R. Polymer nanofiber-embedded microchips for detection, isolation, and molecular analysis of single circulating melanoma cells. *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 3379-3383.