

Supporting information

Aptamer-Mediated Transparent-Biocompatible Nanostructured Surfaces For Hepotocellular Circulating Tumor Cells Enrichment

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1. SELEX process applied for Sialyl Lewis X aptamer screen

The size of ssDNA library was about $10^{14}\sim 10^{15}$. ssDNA library were synthesized by Invitrogen (China) contained a central randomized sequence of 30 nucleotides (nt) (ssDNA:5'-ATG ACC ATG ACC CTC CAC AC—N₃₀—TC AGA CTG TGG CAG GGA AAC-3'(70nt). 5'-primer (5'- ATG ACC ATG ACC CTC CAC AC -3') and a biotinylated (Biotin) labeled 3'-primer (5'-Biotin- GTT TCC CTG CCA CAG TCT GA -3') were used in the polymerase chain reaction (PCR) reactions for the synthesis of double-stranded DNA sequences. The PCR mixtures contained 100 nM each primer, 2.5 mM dNTPs, and 1U Easy-Taq DNA polymerase in a total volume of 25 μ L. The condition of PCR were as follows: 95 °C for 4 min (initial denaturation), in order to prevent amplify of skewed population, PCR was limited to 8 cycles: 94°C for 30s, 58°C for 30s, and 72°C for 30s, followed by a single final extension at 72 °C for 3 min. After denaturing in alkaline conditions (0.2M NaOH, 3 min), the sense ssDNA strand was separated from the biotinylated antisense ssDNA strand by Streptavidin Agarose from Invitrogen (China) and used for the next round of selection. The cyanogen bromide activated agarose beads (CNBr-beads) functionalized with Sialyl Lewis X (SLeX) (SLeX-beads) were kept at 4 °C in PBS buffer until use.

For the first round of selection, 4nmol of initial ssDNA library was reconstituted thoroughly with 400 μ L of binding buffer (Tris-Cl pH7.4 200mmol/L, NaCl 1370mmol/L, MgCl₂ 25mmol/L, DTT 100mmol/L), and the resulting solution was heated at 95 °C for 5 min and cooled immediately on ice for 10 min. SLeX-beads (about 400 pmol of protein) were incubated with the initial ssDNA library at 37 °C on a rotary shaker for 80 min for positive selection. After incubation, beads were washed three times with binding buffer, eluting buffer (EDTA 50mmol/L) was added sequentially to elute bond ssDNA. The primary purified ssDNA collected were added to the PCR cocktail for subsequent amplification by PCR to increase the number of copies of individual sequences. The selected sense ssDNA strands were separated from the biotinylated antisense ssDNA and used as a new library to perform the second round of selection using the same procedure as described for first selection.

Negative selection was introduced after the second round. Before incubation with sLeX -beads, the DNA library was incubated with CNBr -beads for 10 min to remove the sequences that may bind to CNBr-beads. The nonbinding supernatant DNA was collected and used in the positive selection by incubation with sLeX-beads. In order to acquire aptamers with high affinity and specificity, the wash strength was enhanced gradually by increasing the volume of washing buffer (from 200 to 1000 μ L) and the number of washes (from 3 to 6 times). Additionally, the incubation time with CNBr-beads as the negative control was also increased from 10 to 40 min gradually to reduce the nonspecific binding of the selected pools. After 12 rounds of selection, the resulting ssDNA pool was PCR-amplified using unmodified primers for cloning and sequencing by Invitrogen (China).

2. DNA aptamer synthesis

sLeX-AP: biotin-5'- ATG ACC ATG ACC CTC CAC ACG TTT TTG TGT GCA TGT GAC GCT TGT ATG ATT CAG ACT GTG GCA GGG AAA C-3'

Random DNA: biotin-5'- AAT ACC GCG CCT TTC TCC CAC TAG CTT CCA CTG GTC TCA TCC TTC ATT GGT GAG TCG GAG AGC CTA GAA G-3'

All the DNA aptamers were synthesized by Invitrogen (China).

3 Dimension of CTC-BioTChip

Table S1 Dimension of CTC-BioTChip

Sample	Composites[wt]	Efficient area	Nanoroughness
CTC-BioTChip	HA/CTS 5:2	1CM*1CM	120-170nm

4. Different capture performance of anti-EpCAM coated CTC-BioTChip with slex-AP coated CTC-BioTChip

During our experiment, we also compared the capture performance of anti-EpCAM coated CTC-BioTChip with slex-AP coated CTC-BioTChip using artificial blood. (Figure S1). We respectively spiked 50,100,250,500,1000 HepG2 cells into 1 ml DMEM medium or 1 ml blood sample from health donor. From two kinds of artificial blood, slex-AP coated CTC-BioTChip captured more artificial CTCs than anti-EpCAM coated CTC-BioTChip captured. These initial results showed that slex-AP coated CTC-BioTChip

could realize hepatocellular cancer CTCs capture.

Figure S1

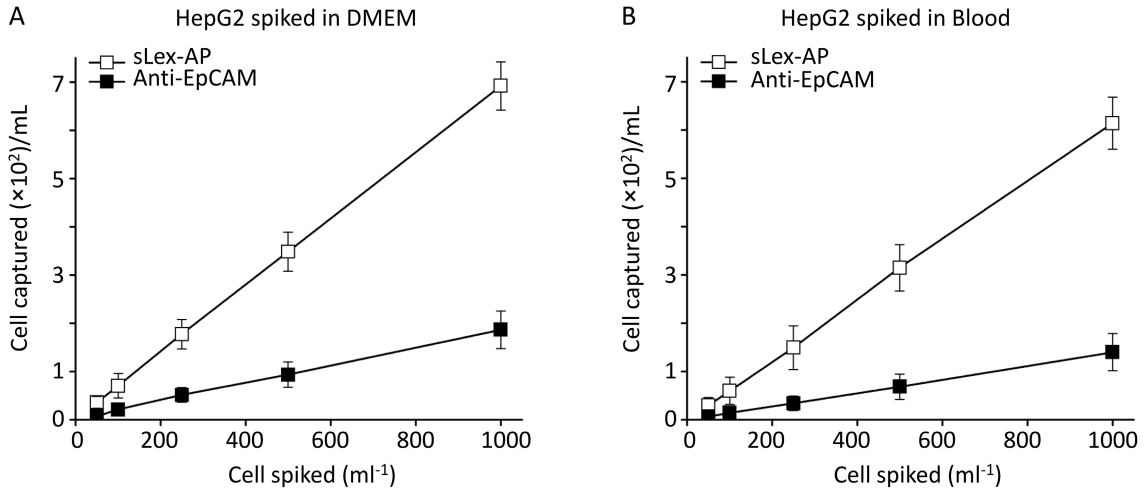


Figure S1 (A) Capture efficiencies of sLex-AP coated and anti-EpCAM coated CTC-^{BioT}Chip at the optimal cell capture conditions (HepG2 cell line spiked DMEM). (B) Capture efficiencies of sLex-AP coated and anti-EpCAM coated CTC-^{BioT}Chip at the optimal cell capture conditions (HepG2 cell line spiked in Health donor's blood). Error bars show standard deviations (n = 5).