CONTINUOUS TISSUE-SELEX UTILIZING A PRE-SCREENING PROCESS FOR MEMBRANE TARGETING APTAMERS ON AN INTEGRATED MICROFLUIDIC SYSTEM

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ABSTRACT

Clinical tests of liquid and tissue biopsy are critical for cancer diagnosis and prognosis. Therefore, biomarkers capable of be applicable on both tests are of great importance. In this work, on-chip cell culture and a continuous cell-based systematic evolution of ligands by exponential enrichment (cell-SELEX) process were carried out on a single chip for optimizing final aptamer candidates screened from tissue-SELEX that could specifically bind to membrane molecules. Aptamers were successfully screened through this optimized SELEX conditions and could be applied both on the liquid and tissue biopsy in the near future.

EXPERIMENTAL PROCESS

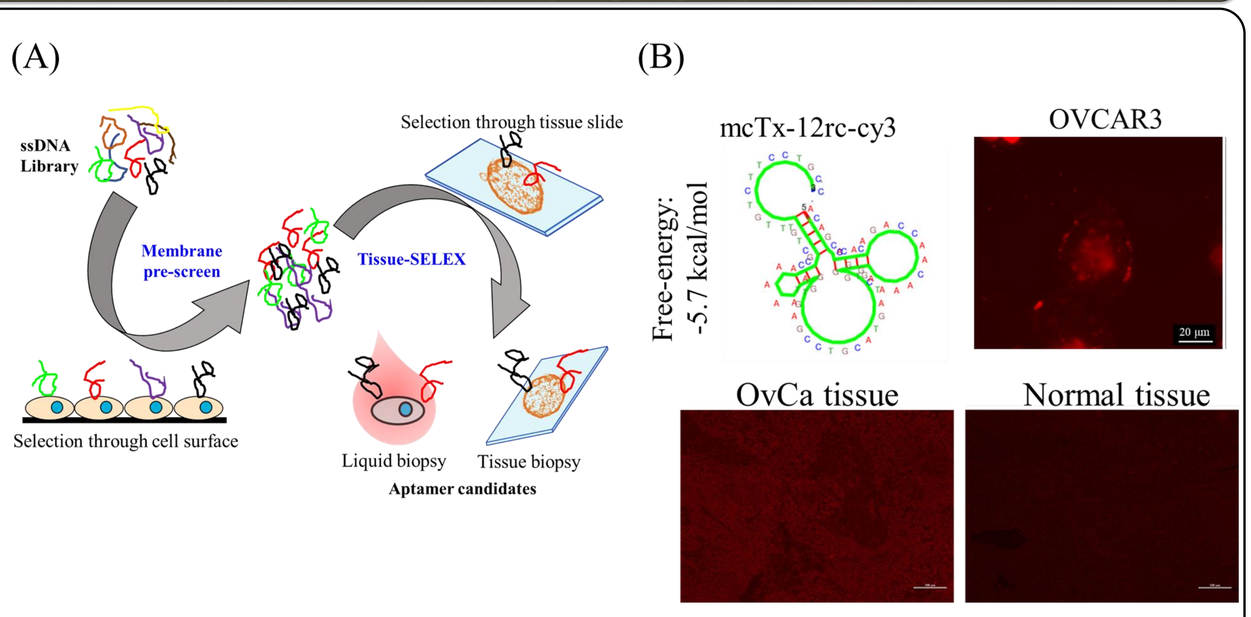

Figure 1: (A) Illustration of the screening strategies for dual-function aptamers targeting membrane binding sites. (B) On-bench screened aptamer which binds to cell membrane and was found to be specific to ovarian cancer (OvCa) tissue.

INTEGRATED MICROFLUIDIC SYSTEM

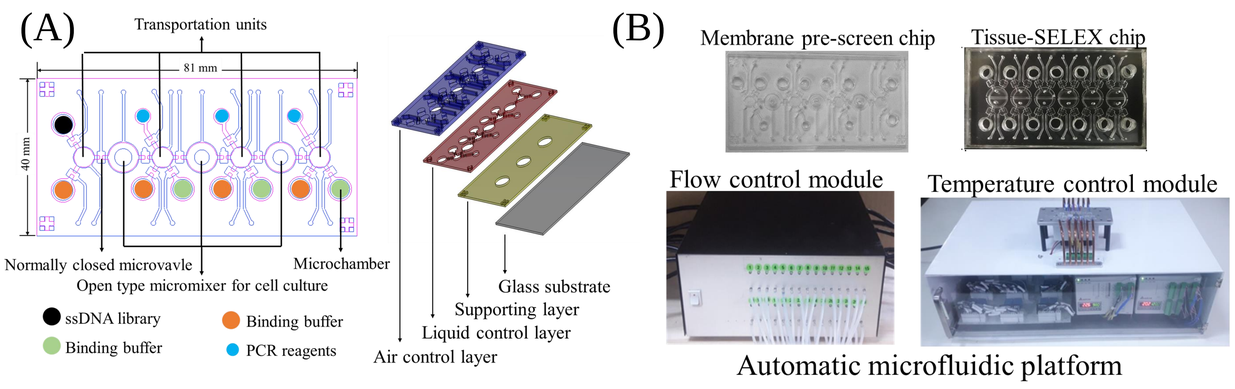

Figure 2. Illustration of the integrated microfluidic system; (A) a photograph of the microfluidic chip equipped with micropumps, microvalves and micromixers to automate the entire process. (B) Home-made flow control and temperature control modules for a pre-screen chip and a tissue-SELEX chip.

CONCLUSIONS

1. A ssDNA library pre-screen protocol was established that allowed the aptamer to be screened from tissue-SELEX specific to cell membrane of the ovarian cancer tissue.
2. The integrated microfluidic system was also able to perform on-chip cell culture for the continuous cell-SELEX.

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RESULTS

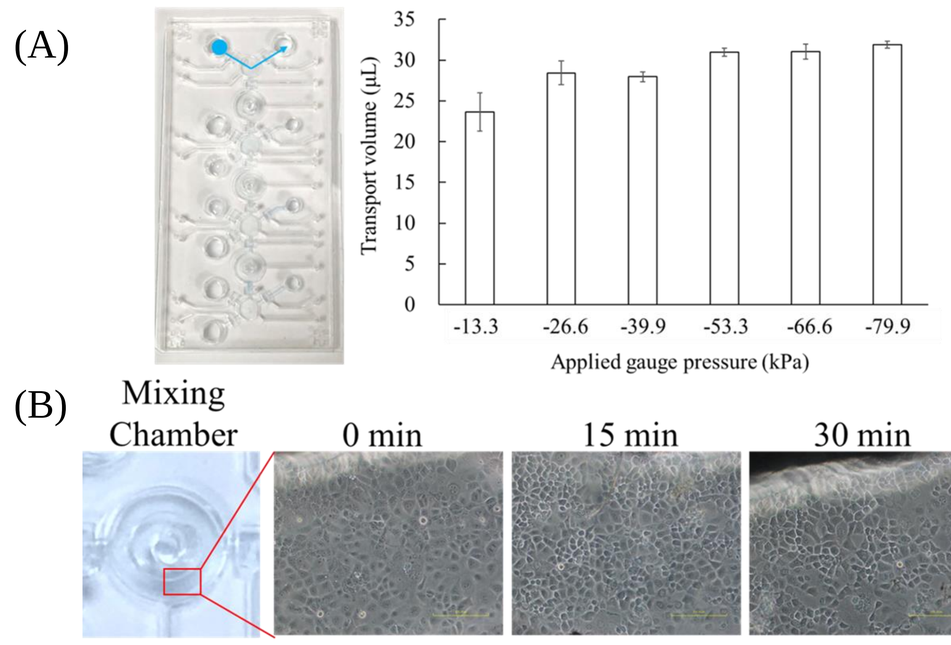

Figure 3. (A) Transport volumes of the micro-pump under different gauge pressures. (B) Cell attachment tests during the micro-mixing process. The attached OvCa cells were mixed with ssDNA at a mixing freauency of 0.5 Hz for 30 min.

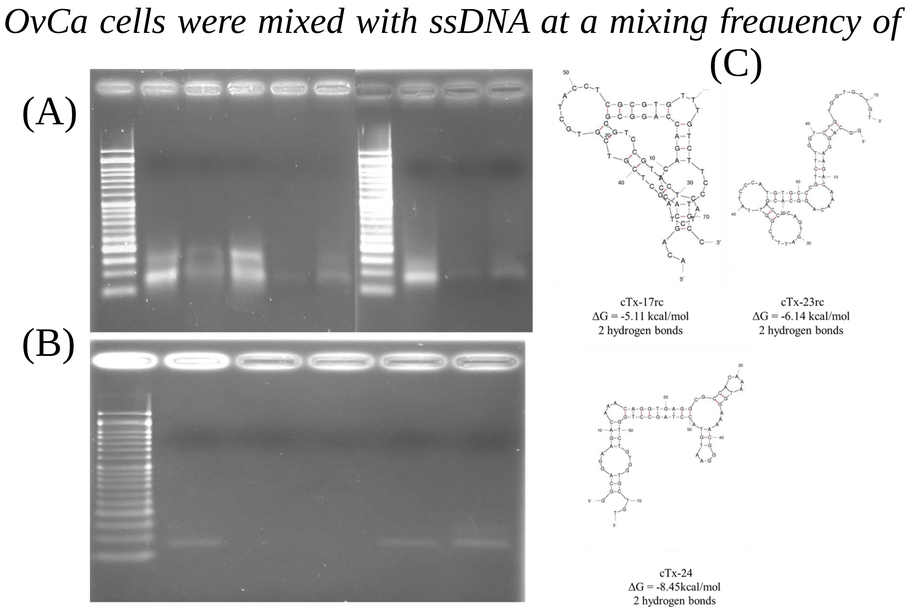

Figure 4. Electropherograms of PCR products for the continuous on-chip pre-screen process (A) and the following continuous tissue-SELEX (B). (C) 2D structures of aptamer candidates were predicted.

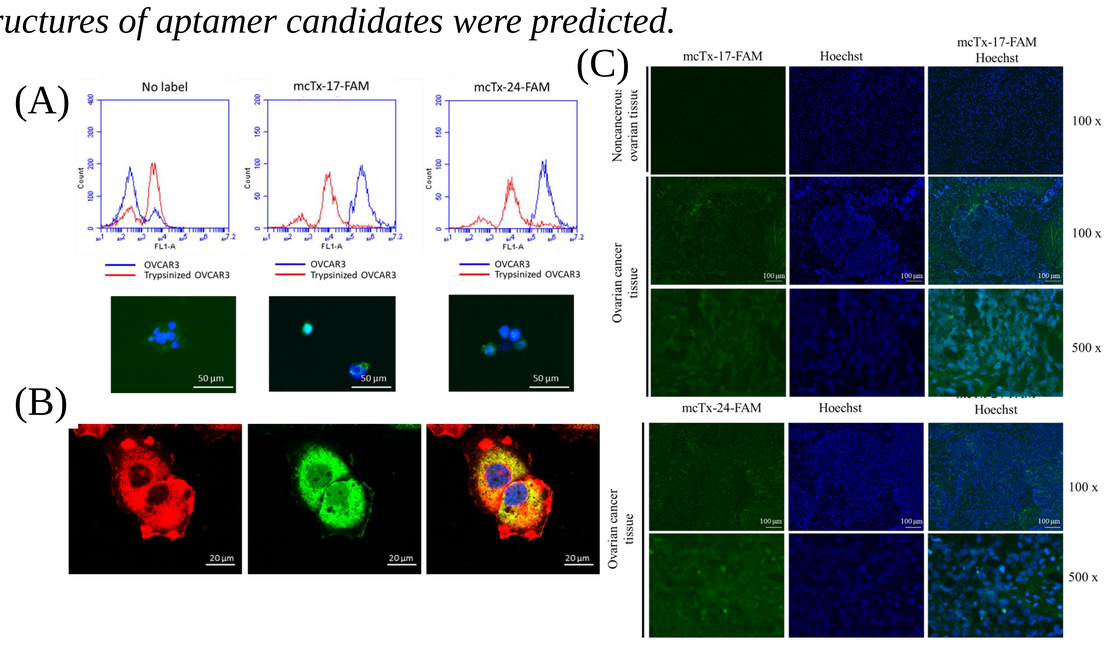

Figure 5. Tests of the screened aptamer mcTx-17 and -24 on the membrane specificity by measuring the staining intensity on the cell membrane (A) and confocal analysis of staining images (B). (C) The fluorescent staining images of mcTx aptamers on normal cells and cancerous tissues.

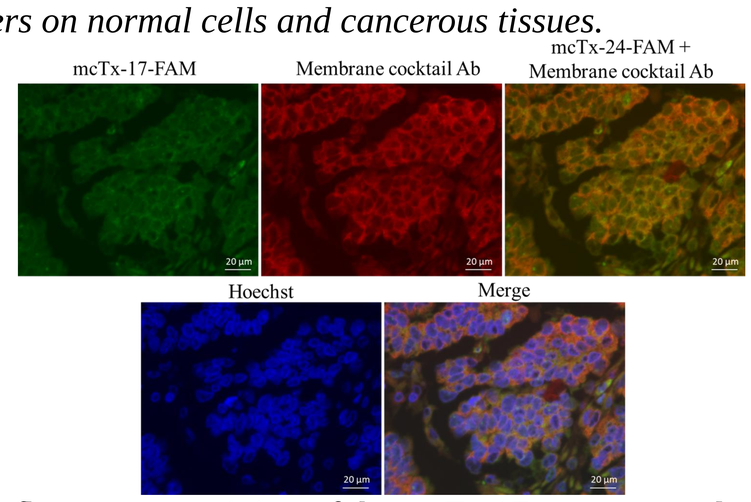

Figure 6. The fluorescent staining of the mcTx-17 aptamer and membrane cocktail antibodies on the ovarian cancer tissue, indicating that mcTx-17 could bind to the cell membrane of the cancer tissue.