An automated microfluidic system for selection of aptamer probes against ovarian cancer tissues

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ABSTRACT

Because of the difficulty of treatment in its latest stages, cancer is among the leading causes of death worldwide. Therefore, high-affinity and specificity biomarkers are still in demand for many cancer types, and the utility of aptamers to serve in this regard has been explored recently. Although a process known as “systematic evolution of ligands by exponential enrichment” (SELEX) has been used to generate aptamer-based cancer biomarkers, this approach is complicated, time-consuming, and labor-intensive. An automated microfluidic system was consequently developed herein to screen ovarian cancer-specific aptamers via on-chip SELEX with clinical cancer tissue samples. The integrated microfluidic system consisted of an integrated microfluidic chip, a temperature control module equipped with 12 thermoelectric coolers, and a flow control module for controlling 36 electromagnetic valves such that the entire, tissue-based SELEX process could be fully automated and carried out within 15 h. Highly specific ovarian cancer aptamers with high affinity (dissociation constant of 129 nM) to their cellular targets were screened with this system. Given the comparable specificity to their much more expensive antibody counterparts, these aptamers, when used in conjunction with the developed microfluidic system, may be used to diagnose ovarian cancer in its earliest stages.

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NOMENCLATURE

bp	Base pair	K_d	Dissociation constant
cell-SELEX	Cell-based systematic evolution of ligands by exponential enrichment	NCKUH	National Cheng Kung University Hospital
CNC	Computer-numerical-control	N40	40 random nucleotide bases
ddH ₂ O	Distillation-distillation H ₂ O	OvCa	Ovarian cancer
dNTPs	Deoxynucleotide triphosphates	PCR	Polymerase chain reaction
DPBS	Dulbecco's phosphate-buffered saline	PDMS	Polydimethylsiloxane
dsDNA	Double-strained DNA	PID	Proportional-integral-derivative
EMVs	Electromagnetic valves	PMMA	Polymethylmethacrylate
FAM	Carboxyfluorescein	RT	Room temperature
		SELEX	Systematic evolution of ligands by exponential enrichment
		ssDNA	Single-stranded DNA

TE Thermoelectric
tissue-SELEX Tissue-based systematic evolution of ligands
by exponential enrichment

Greek

ΔG Gibbs free energy

I. INTRODUCTION

Cancer is among the leading causes of non-accident-related death worldwide, and approximately 14 million new cancer diagnoses and 8 million cancer-related deaths are documented each year.^{1,2} Genetically deviant cancer cells not only form tumors but also metastasize and spread to other tissues through the circulatory or lymph systems.³ Among them, ovarian cancer (OvCa) is regarded as a “silent killer” due to its generally moderate symptoms (e.g., abdominal bloating, indigestion, and nausea), which are commonly misdiagnosed as gastrointestinal ailments. Therefore, most OvCa patients are diagnosed at relatively late stages; as such, OvCa has the highest mortality rate of gynecologic cancers, with approximately 14 000 deaths occurring annually in the United States.⁴ Furthermore, the five-year survival rate drops from 90% at stage I to 17%–39% at stage III or IV disease,⁵ suggesting that early diagnosis greatly increases one’s chance of survival. Unfortunately, early-warning diagnostic biomarkers for OvCa are currently lacking.⁶

At present, antibodies that recognize antigens on cancer cells are commonly used as biomarkers for cancer diagnostics.^{7,8} Nevertheless, antibodies are manufactured from animal immune systems and consequently suffer from batch-to-batch variations; furthermore, they degrade readily at room temperature (RT).⁹

Alternatively, aptamer is a single-stranded DNA (ssDNA) molecule which can serve as a substitute for antibodies, and it has been used as probes for proteins,¹⁰ viruses,¹¹ bacteria,¹² cells,¹³ and even tissues¹⁴ in a manner that is contingent upon their three-dimensional structures; they have consequently been used in cancer biomarker screening endeavors.^{15,16}

Currently, systematic evolution of ligands by exponential enrichment (SELEX) is the most common selection method for aptamers.¹⁷ In this well-established technique, target-specific aptamers are selected from a random ssDNA library¹⁸ after undergoing both positive and negative selection. In the former, the target molecule/cell is allowed to bind to aptamers, whereas in the latter, aptamers are incubated with non-target cell types to filter out unspecific oligonucleotides. Although the entire SELEX process can be carried out *in vitro*, it is tedious, labor-intensive, and error-prone, even when performed by experienced technicians.

Recently, microfluidic systems have been used to identify cancer cell biomarker probes, allowing for rapid, point-of-care diagnostics.¹⁹ For instance, integrated microfluidic systems have been established to perform cancer cell-SELEX, allowing for the rapid (<1 day) generation of highly specific aptamers.¹³ Aptamers have been screened for various kinds of cancer cell lines, including lung cancer,²⁰

colon cancer,²¹ prostate cancer,²² and OvCa.²³ However, SELEX has not yet been performed with actual tissues from cancer patients in a continuous format. We therefore hypothesized herein that we could build upon a prior work¹⁴ to design a microfluidic, tissue-SELEX-based system capable of screening aptamers targeting OvCa cells in human tissues. Although we were able to identify OvCa-specific aptamers, such as “Tx-02-rc” in our prior work,¹⁴ the screening process required three microfluidic chips (two for positive selection and one for negative selection), and the chip required calibration before each run. In order to streamline and simplify the screening process, a new integrated microfluidic system capable of carrying out six screening rounds on a single chip with OvCa tissues was developed in this work. With this approach, the entire process of tissue-SELEX could be automated without experienced operators. Furthermore, it only took 15 h, which was only one third of operation times when compared with the previous work.¹⁴ Experimental results showed that three screened aptamers exhibited high affinity towards OvCa tissues, and their disassociation constant values were measured to be comparable to existing antibodies. This is the first time that a completely automatic microfluidic system was developed for performing tissue-SELEX in a consecutive format.

II. MATERIALS AND METHODS

A. Experimental procedures and setup

On the microfluidic chip (described below), two negative [Figs. 1(a)–1(c); 1st and 6th] and four positive [Figs. 1(d)–1(g); 2nd–5th] selection rounds were performed in series. For the former approach, which was used to reduce the likelihood of capturing unspecific aptamers, 20 μl of a ssDNA library [0.1 μM; Protech, Taiwan; 5′-GGCAGGAAGACAAACA (forward primer binding site)-N40 (random 40-mer)-TGGTCTGTGGTGTCTGT (reverse primer binding site)-3′] were incubated under the activation of the micromixer (1 Hz frequency) for 30 min with four non-cancerous tissue samples [provided by the National Cheng Kung University Hospital (NCKUH) of Taiwan; approved by the NCKUH institutional review board (A-ER-104-014)] that had been attached to a glass slide (0.7-mm thick; Taiwan Glass) and covered by a layer of wax. Briefly, it was necessary to remove this wax before the initial negative selection step; to do so, 40 μl of “Dewax” solution (Thermo-Fisher Scientific, USA) were heated at 90 °C for 20 min alongside a heat-induced epitope retrieval buffer (TA-100-DHBL, Thermo-Fisher Scientific). After pumping out all melted wax, 40 μl of wash solution [50 ml of DPBS, 250 μl of 1M MgCl₂ [Merck, USA], and 0.225 g of glucose (D(+)-Glucose; Merck)] were used to wash the tissue chambers thrice.

After the first round of negative selection, 20 μl of supernatant containing ssDNA without affinity towards these tissues were transported to a polymerase chain reaction (PCR) chamber [#1 in Fig. 2(b)] to perform on-chip PCR with the following reagents: 0.3 μl of forward primers (5′-GGCAGGAAGACAAACA-3′; 10 μM), 0.3 μl of reverse primers

FIG. 1. On-chip tissue-SELEX featuring both negative and positive selection. The former involved (a) incubation of the ssDNA library with healthy tissue slides, (b) isolation of captured ssDNAs with high affinity towards healthy tissues, and (c) amplification of unbound ssDNA. The positive selection process involved (d) incubation between amplified ssDNAs and cancer tissues (on-slide), (e) isolation of captured ssDNAs with high affinity toward cancer tissues, (f) release of these captured ssDNAs, and (g) amplification of these ssDNAs. (i) A schematic illustration of on-chip workflow with integration of the flow control module and the temperature control module.

(5'-TGGTCTGTGGTGTCTGT-3', 10 μ M), 0.6 μ l deoxynucleotide triphosphates (dNTPs; 10 mM), 0.4 DNA polymerase (Super-Therm™ Gold DNA polymerase; Bersing Technology, Taiwan), and ddH₂O to a total volume of 10 μ l. Each cycle of PCR amplification included a 5-min initial denaturation at 90 °C, 30 thermal cycling (denaturation at 87 °C for 30 s, annealing at 60 °C for 20 s, and extension at 70 °C for 15 s), and a 5-min final extension at 70 °C. It took around 90 min to complete one PCR amplification in total. Note that an on-chip cooling method was used for ssDNA regeneration after PCR process. The temperature control module composed of 12

independent thermoelectric (TE) coolers. Not only could these TE coolers be used to maintain the reagents and tissues at a low temperature prior to usage but they were also used to create a locally high temperature area for PCR. For ssDNA regeneration, once the PCR process was finished, the ssDNA product would be transported immediately to the following TE chip, which has been cooled down in advance for the ssDNA regeneration.

For the positive selection round, 20 μ l of the PCR product and 20 μ l of binding solution [50 ml of Dulbecco's phosphate-buffered saline (DPBS; Sigma, USA), 0.225 g of glucose, 250 μ l of

FIG. 2. (a) and (b) The photographs of the microfluidic chip, which consisted of 7 PCR chambers, 7 solution chambers, 6 micro-mixers/micro-pumps, and 24 normally-closed valves. (c) A cross-sectional view of the micropump and micro-mixer. (d) Exposed view of the chip, which featured an air control layer, a liquid channel layer, and a glass tissue slide.

1M MgCl_2 , and 5 mg of yeast transfer RNA (tRNA; Invitrogen, USA) were mixed with cancer tissues (from NCKUH; adhered to the glass slide as for the non-cancerous tissues described above) for 30 min at a mixing frequency of 1 Hz. Afterwards, the supernatant (waste) was transported back to the PCR chamber for a final confirmation by using slab-gel electrophoresis and the mixing chambers were washed thrice with 40 μl of washing solution; the shearing forces associated with the washing process were used to increase the stringency of aptamer selection.²⁴ To release the bound ssDNAs (i.e., those with high affinity for cancer tissues), 40 μl of the washing solution were transported from the solution chamber and heated to 95 $^\circ\text{C}$ for 15 min in the mixing chamber.

Subsequently, three more positive selection rounds and one more negative selection round were performed as described above. A schematic illustration of workflow for the proposed study with integration of the flow control module and the temperature control module was shown in Fig. 1(i). After six rounds of selection, aptamers with high affinity and specificity towards OvCa tissues were screened and identified. The final PCR product was cloned using the TOPA™ TA cloning kit (Invitrogen, USA), and 50 colonies were sequenced to uncover the identity and 3D structure of the isolated aptamers with “mfold” (<http://unafold.rna.albany.edu/?q=mfold/DNA-Folding-Form>). Three aptamers with low Gibbs free energy (ΔG) and multiple-loop structures were

FIG. 3. Illustration of the experimental setup consisting of (a) a laptop, (b) a flow control module, (c) a microfluidic chip, (d) a temperature control module, (e) an air compressor, and (f) a vacuum. (g) Top-down view of the microfluidic chip. The system was capable of performing six rounds of tissue-SELEX using OvCa tissue samples.

synthesized by Protech, labeled with carboxyfluorescein (FAM), and purified to a final concentration of 1 μM . OvCa tissues and normal, healthy ovarian tissues were incubated for 30 min with 50 μl of each aptamer, washed thrice with the washing solution, and optically analyzed with a digital camera (DS-QiMc, 1.5 megapixels) connected to a TE cooling device and a programmable gain amplifier (Nikon, Japan).

To determine the dissociation constant (K_d), cultured OvCa cells (OVCAR-3, National Cancer Institute #0507709, 10^4 cells per test) were incubated for 30 min with 50 μl of each aptamer at each of the following concentrations in 200 μl of binding solution: 0.1, 1, 10, 40, 70, 100, 200, 500, 7500, 1000, and 1500 nM. Cells were washed six times using 200 μl of washing solution, suspended in 200 μl of washing solution, and K_d was determined via flow cytometry (BD Accuri™ C6,

FIG. 4. The transport volume under different applied pressures (10, 30, 50, and 70 kPa). Since the reagent volume to be transported in the tissue-SELEX process was established as 20 μl , 50 kPa was chosen as the applied pressure. All tests were repeated thrice, and error bars represent standard deviations.

FIG. 5. Mixing index over time for each of three applied negative gauge pressures at frequencies of 0.5 (a), 1.0 (b), and (c) 2.5 Hz. Although 2.5 Hz mixing frequency resulted in rapid and efficient mixing, it was associated with high shearing forces (data not shown), which may be unsuitable for tissue-SELEX. Instead, we used a frequency of 1 Hz at 50 kPa for all experiments discussed in the main text. Note that the measurements were made thrice, and error bars represent standard error.

Becton-Dickinson, USA) by plotting fluorescence intensities [i.e., specific binding intensity (Y)] versus aptamer concentration (X): $Y = B_{\max}X/(K_d + X)$.²⁵ Note that $B_{\max}$ is the concentration of cancer cells in this formula. Prism software (GraphPad, USA) was used for this analysis. Unless noted otherwise, three replicates were used for all experiments.

B. Construction of the integrated microfluidic system

The integrated microfluidic system (Fig. 3) capable of automatically carrying out tissue-SELEX was constructed from a temperature control module (Super Solution & Services, Taiwan), a flow control module [featuring 36 electromagnetic valves (EMVs; SMC S070B-SBG-05; Wei-Chia Electro Materials, Taiwan) that were connected to the microfluidic chip to regulate compressed air and vacuum such that the micropumps, microvalves, and micromixers could be activated pneumatically], an air compressor (S-301, Sharpgun Dental Instrument, Taiwan), a vacuum pump (UN-90V, Uni-Crown, Taiwan), a laptop computer, and a $40 \times 75 \times 8$ mm (width $\times$ length $\times$ height) microfluidic chip [Fig. 2(a)] featuring 3 layers [an air control layer, a liquid control layer, and a glass tissue slide; Fig. 2(d)], 7 on-chip PCR chambers, 7 solution chambers, 6 micropumps/micromixers, and 24 microvalves [Figs. 2(a) and 2(b)]. The chip (whose design is outlined below) was placed on top of the temperature control module, which consisted of 12 independent $8 \times 8 \times 2$ mm TE chips (9502/031/012M; FerroTec, Taiwan) aligned in a 2×6 layout to match the positions of the six PCR chambers and the six micro-mixers/micropumps [Fig. 1(d) in the supplementary material]; these TE chips controlled the temperature in the PCR, ssDNA thermal lysis steps, and ssDNA generation process. Note that TE chips were used to cool down the neighboring area such that reagents and tissues could be protected (Fig. 4 in the supplementary material). The microfluidic devices were pneumatically controlled by a flow control module with a new, membrane-type micromixer containing two semi-circular micropumps [Fig. 2(c)]. By alternating the

FIG. 6. (a) A comparison of the normalized concentration distribution before and after the mixing process, where C_+ is the normalized concentration profile and D_+ is the normalized location (r/D) measured across the center of the mixing chamber (b).

FIG. 7. Thermal cycles of six TE chips (Nos. 1, 3, 5, 7, 9, and 11). (a)–(f) show a five-minute period of thermal cycling performed by the TE chips. Each of the TE chips had its own PID control values to stabilize the heating and cooling efficiency. It took no more than 3 min to carry out one PCR cycle, and the whole 20-cycle PCR amplification spent around 60 min.

deformation of semi-circular polydimethylsiloxane (PDMS) membranes of the micropumps, ssDNA aptamer libraries could be efficiently mixed with tissues mounted on a glass (Fig. 3 in the [supplementary material](#)).

The air control and liquid channel layers of the chip [Figs. 1(a) and 1(b) in the [supplementary material](#)] were made of PDMS (Sil-More Industrial, USA), and standard PDMS soft lithography approaches (Fig. 2 in the [supplementary material](#)) were used to replicate mold templates made of polymethylmethacrylate (PMMA; acrylic 5 mm, Da-de-hang, Taiwan) via a computer-numerical-control (CNC; EGX-400, Roland, USA) engraving machine.²⁶ Briefly, to replicate the inverse structures of the PDMS layers, a pre-mixed PDMS solution was poured onto the PMMA molds [Fig. 2(a) in the [supplementary material](#)] and cured at 80 °C for 8 h; the PDMS layers were then mechanically peeled from the molds, and inlet/out holes for air control were drilled. The two PDMS layers were then bonded by oxygen plasma treatment (Cute MP/R; Atlas Technology, Taiwan) for 1 min at 90 W followed by a 4-h hot pressing process.¹³ Then, the PDMS layers and the tissue glass slide were bound together with oxygen plasma (4-h at RT). A hard mask was used to cover the tissue samples on the glass to prevent them from being damaged by the plasma treatment.

III. RESULTS AND DISCUSSION

A. Characterization of micropump and micromixer

The pumping test is demonstrated in Fig. 4. Distilled water was transported from chambers to chamber of the chip ($n = 3$). At a positive gauge pressure of 50 kPa, the transported

volume was measured to be 19.4 μ l, near the target value of 20 μ l.

The relationship between the mixing index and mixing time was also tested and shown in Fig. 5 at different driving frequencies and different applied positive gauge pressures. Within a short period of time, the micromixer could efficiently mix distilled water and blue ink by using pneumatic-driven force. A comparison of the normalized concentration distribution before and after the mixing process was shown in

FIG. 8. Electropherograms of the 10 samples collected from six consecutive rounds of tissue-SELEX. L: 50-bp ladder; lane 1: PCR product from 1st selection, lane 2: wastes from 2nd selection, lane 3: PCR product from 2nd selection, lane 4: wastes from 3rd selection, lane 5: PCR product from 3rd selection, lane 6: wastes from 4th selection, lane 7: PCR product from 4th selection, lane 8: wastes from 5th selection, lane 9: PCR product from 5th selection, and lane 10: PCR product from 6th selection. Since the 1st and 6th selections were negative selections, we did not collect wastes afterwards.

FIG. 9. The two-dimensional structures of three aptamers (cTX-24, cTX-36, and cTX-45) selected after six rounds of tissue-SELEX, as well as an aptamer, 02rc, identified in a prior work.¹⁴

Fig. 6, which indicated that a mixing index as high as 93% had been achieved after mixing for 1 s at a driving frequency of 2.5 Hz. The mixing index under the unmixed condition was found to be only 13%. As expected, the concentration profile showed that the normalized intensity could almost approach 0.5 that represented complete mixing of liquids in the liquid chamber. At 2.5 and 1 Hz, the mixing indices were measured to be 90% and 93%, respectively, within 2 s at gauge pressures of 20, 50, and 100 kPa. At the latter frequency, tissues were unlikely to have been damaged and therefore were chosen for the subsequent experiments. When compared to a previous micromixer,¹³ the one developed herein exhibited an improved mixing performance. This could be because the two membranes acted in an alternating fashion, thereby reducing the “dead” volume and resulting in rapid and efficient yet gentle mixing.

B. Characterization of temperature control module

In order to amplify the selected aptamers using PCR reaction, stable thermal cycling is a pivotal issue in this system. Six of the 12 TE modules were used to perform 20-cycle PCR amplification. We used K-type thermal couples to measure the temperature in six PCR chambers, and the temperature was recorded one time per second. Every PCR

chamber contained 30 μl of distilled water to simulate the PCR reagent and 20 μl of mineral oil to prevent reagent from evaporation. Figure 7 shows 5-min PCR thermal cycling curves, indicating that six TE modules have stable thermal cycling performance. Comparing with the benchtop PCR machine, our temperature control module is able to adjust the heating rate and cooling rate by applying appropriate proportional-integral-derivative (PID) value. Since the TE chips may not be able to have the comparable heating and cooling efficiency under the same controlling parameters, it is useful to apply a set of exclusive PID values for each TE chip. Therefore, the temperature curve could be much more stable to achieve the PCR thermal demands.

C. Tissue-SELEX on-chip

The tissue-SELEX herein only took 15 h to finish the entire screening process [Fig. 1(i)], and 36–48 h were normally required for benchtop protocols.⁶ This also represents an improvement of our prior work,¹⁴ in which three separate chips were necessary for aptamer screening, and the microfluidic system required calibration prior to use. Furthermore, the new temperature and flow control modules developed in this work permitted consecutive screening without human intervention.

TABLE I. Aptamer candidate sequences and their ΔG (kcal/mol). Please note that the last aptamer was identified in a prior work¹⁴ and differed from CTX_24 at only the two bases underlined in bold font.

Aptamer	Sequence (5' to 3')	ΔG (kcal/mol)
cTX_24	GGCAGGAAGACAAACACCCCGCGGTTCCATCCCGTCATGATTTCCGTAATTTGATGGTCTGTGGTGCTGT	−4.84
cTX_36	GGCAGGAAGACAAACACACCATGTCTTTCGATGCGAAGGCCCTTCTGCTCTCGCTTGTGGTCTGTGGTGCTGT	−10.93
cTX_45	GGCAGGAAGACAAACACGGGGCGCCATTGAACCGATCAAGGCGGTATGGTCAGGTGTGGTCTGTGGTGCTGT	−11.21
02rc ¹⁴	GGCAGGAAGACAAACACCCCGCGGTTCCACCCCGTCATGATTTCCGTAATTTGATGGTCTGTGGTGCTGT	−10.60

FIG. 10. The fluorescence images of ovarian cancer (OvCa) and non-cancerous tissues stained with FAM-labeled cTX-24, cTX-36, and cTX-45 aptamers.

To verify the efficacy of tissue-SELEX (Fig. 8), gel electrophoresis of PCR products was undertaken. Since the negative selection process does not involve washing, screened ssDNAs were only obtained in the 1st and 6th rounds. Since the multiple TE chips permitted ssDNA coalescing into their native, 3D structures upon cooling, 144-base pairs (bp) double-strained DNA (dsDNA) fragments in lanes 1, 3, and 10 may be caused by dimerization of the 72-bp aptamers.²⁷ Nevertheless, lane 10 reveals that OvCa specific aptamers were successfully screened after six rounds of tissue-SELEX, and this PCR product was cloned, sequenced, and analyzed by mFold to estimate ΔG . Among the 50 sequenced aptamers, 2, cTX-36 (consecutive tissue-SELEX-36) and cTX-45, possessed stable

($\Delta G < -10$ kcal/mol) secondary loop structures (at least four loops; Fig. 9). Since these features were similar to those of high-specificity aptamers screened in our previous work,¹⁴ they were considered to be promising candidates. In fact, another aptamer (cTX-24) differed by only 2 bp from an aptamer identified in our previous work;¹⁴ it was considered to also represent a promising candidate (Table I). These three aptamers were labeled with FAM such that their binding to OvCa tissues could be visualized (Fig. 10); all three aptamers successfully stained cancerous tissues, and no staining of normal ovarian tissues was documented.

The K_d values of cTX-24, cTX-36, and cTX-45 were 1348 ± 518.1 (std. dev.), 129.2 ± 24.81 , and 178.0 ± 43.5 nM,

FIG. 11. The binding affinity analysis of cTX-12 (a), cTX-24 (b), and cTX-45 (c). Their K_d values were measured to be 1348.0 nM, 129.2 nM and 178.0 nM, respectively. All samples were analysed in triplicate, and error bars represent standard deviations.

respectively (Fig. 11). The K_d values of the latter two aptamers are comparable to those of screened aptamer identified in our prior work ($K_d = 2.9 \pm 0.8$ nM) and are indicative of a strong affinity toward OvCa tissues.

IV. CONCLUSIONS

In this work, an automatic microfluidic system was developed and successfully carried out 6 rounds of consecutive tissue-SELEX for the first time. In the integrated microfluidic chip, all of the microdevices were optimized. Micromixers were characterized to exhibit a mixing index over 90% with an applied gauge pressure of 50 kPa at 1 Hz of the mixing frequency within 4 s. Micropumps were experimentally optimized to transport 19.4 μ l reagents at an applied gauge pressure of 50 kPa, which is able to satisfy the transportation process for the developed tissue-SELEX process. A flow control module consisting of 36 independent EMVs and a temperature control module composed of 12 TE coolers were integrated to automate the tissue-SELEX on the microfluidic chip automatically. After 6 rounds of tissue-SELEX, three aptamers were successfully screened. According to specificity tests, cTX-24, cTX-36, and cTX-45 were able to distinguish OvCa and normal ovarian tissues by showing significantly different fluorescent intensities. Their K_d values were measured to be 1348.0 nM, 129.2 nM, and 178.0 nM, respectively, using flow cytometric approach, indicating that they exhibited high affinity toward OvCa tissues. Automated, microfluidic, tissue-SELEX system developed herein is a promising tool for rapid screening cancer tissue-specific aptamers.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for the photographs of the air control and liquid channel layers as well as 12 thermoelectric (TE) chips on the temperature control module (Fig. 1), microfabrication process of the microfluidic chip (Fig. 2), a photograph of the six tissue samples mounted on a glass (Fig. 3), and 12 infrared photographs of the relative TE chips (Fig. 4).

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