Appendix A

Modular nucleic acid assembled p/MHC microarrays for multiplexed sorting of antigen-specific T cells

A.1. Introduction

T cells, which play a central role in an adaptive immune response, recognize different antigens via T cell receptor (TCR) generated by gene rearrangement and secondary modification. When a naive T cell triggered through the interaction between TCR and its matching antigenic peptides bound to Major Histocompatibility Complex (p/MHC) on the surface of antigen presenting cells (APC), it begins to proliferate rapidly and form a large population of matured effector cells to defend the host. Even though the pathogen is cleared, some portion of activated cells survives and transforms into memory cell able to give the host a quick immune response toward the previously faced pathogen. As the result, each individual has his/her own T cell repertoire showing immune response toward self and foreign antigens. To detect and characterize these T cell populations, consequently, has fundamental and clinical importance.

In order to detect antigen-specific T cells, soluble p/MHC tetramer has been developed. Four enzymatically biotinylated p/MHC monomers are coupled with a streptavidin molecule primed by chromophore to form a p/MHC tetramer, and the tetramer enables its counter part T cell to be detected via flow cytometry[1]. Because the p/MHC tetramer uses choromophore for its detection, however, this method has inevitable restrictions. At first, each antigen-specificity requires unique optical dye

molecule having little spectral overlap with others. Secondly, the existence of detection limit in flow cytometry, serial flow cytometry detection for multiplexing study is restricted by the sample size. Due to the existence of diverse optical characters in each cell population, moreover, the matter of compensation always follows. Although researchers used polychromatic flow cytometry utilizing quantum dots to resolve those problems[2,3], high cost, long sample preparation time, and complex color compensation still hinder further study.

To avoid such problems, several researchers have reported antigen-specific cell sorting by microarrays, which is contains directly printed p/MHC tetramers on a supporting substrates[4-7]. When a population of cells is applied on the array, only target antigen-specific T cells bind to the region having p/MHC tetramers. After that, the captured cells are observed by a microscopy. Because this method uses the location of p/MHC tetramers to separate a target antigen-specific T cell from the variety of other cells instead of chromophore's emission spectrum, multiplexing is only restricted the number of reagent able to fit on a substrate.

In order to increase the capture efficiency, orientation of p/MHC tetramer should be preserved in a way to react approaching cells and the tetramer must not be denatured through the microarray fabrication. Analogous protein arrays, made via antibody adsorption to unmodified and derivatized surfaces, have shown that the adsorbed antibodies could fully react because of surface-induced effects such as protein denaturation and orientational change to inactive. As the result, amount of functional

antibody is decreased, immobilization occurs heterogeneously, and required concentrations of antibody is increased to compensate for the protein loss[8-10]. To avoid these problems, several mild chemistries have been studied for protein immobilization[11-17], but often the surface that meets the demands of application requires a high level of technical expertise and/or is limited in accessibility. Therefore, the development of a new technology to fabricate p/MHC array in an easy way is important.

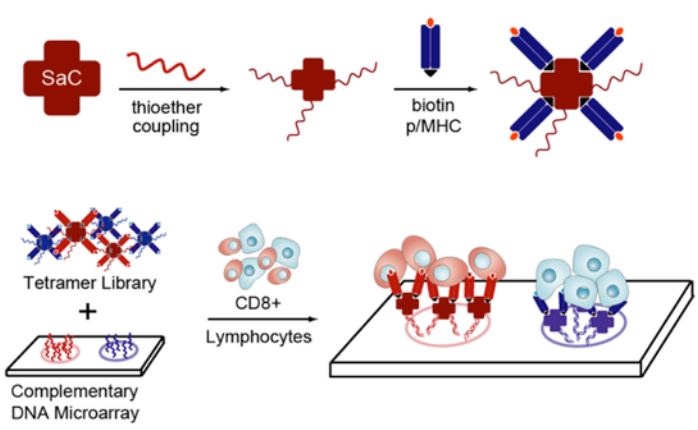

Fig. A.1. Self-assembled ssDNA-p/MHC tetramer arrays for multiplexed sorting of antigen-specific cells. ssDNA-encoded p/MHC tetramers are produced by coupling ssDNA site-specifically to SAC prior to exposure to molar excess of biotinylated p/MHC monomers. p/MHC tetramer arrays are formed by pooling ssDNA-p/MHC tetramers of select specificity and hybridization to a complementary-printed ssDNA microarray. T cells expressing the cognate TCR are detected by binding to the surface.

Here I introduce a new p/MHC arrays conjugated with nucleic acid for multiplexed antigen-specific lymphocytes sorting. Instead of direct printing, each specific p/MHC tetramer is conjugated to unique sequence of ssDNA designed to be orthogonal to other sequences. On the glass surface where the complementary DNA sequences are printed, the ssDNA-p/MHC tetramer conjugates are self-assembled by DNA hybridization, then used as a p/MHC array to sort mixed population of antigen-specific T

cells (**Fig. A.1.**). This method is called "Nucleic Acid Cell Sorting (NACS)." Because NACS employs DNA as a linkage molecule, fabrication of p/MHC arrays is simple and highly modular[18-21]. Traditional DNA microarray technologies are also available to make cDNA glass substrates. In order to produce ssDNA-p/MHC tetramer conjugates having maximum capacity to immobilize cells, cysteine-engineered SA (SAC) is used a scaffold. NACS arrays have better performance than traditional p/MHC arrays made by direct printing, and the specificity, multiplexing and sensitivity of NACS are studied. Selective detachment after sell sorting by NACS is also illustrated, and its application to primary human T cells is introduced.

A.2. Experimental Methods

A.2.1. Microarray fabrication

All DNAs were acquired from IDT. By the facility at the Institute for Systems Biology (ISB, Seattle), DNA strands were printed on amine coated glass slides (GAPSII, Corning) in the way to form 12x12 arrays having alternative rows of A, B, and C, or A_{EcoRI} and B_{BamHI} spots with a SMPXB15 pin (Arrayit). Sequence of each strand and its counterparts is written at the following **Table A.1.**

Table 2.1. Orthogonal DNA sequences for spatial encoding of p/MHC tetramers

Name	Sequence*
A	5' - AAA AAA AAA AAA AAT CCT GGA GCT AAG TCC GTA AAA AAA AAA AAT CCT GGA GCT AAG TCC GTA AAA AAA AAA AAA A
A'	5' - NH_2- AAA AAA AAA ATA CGG ACT TAG CTC CAG GAT
B	5' - AAA AAA AAA AAA AGC CTC ATT GAA TCA TGC CTA AAA AAA AAA AGC CTC ATT GAA TCA TGC CTA AAA AAA AAA AAA A
B'	5' - NH_2- AAA AAA AAA ATA GGC ATG ATT CAA TGA GGC
C	5' - AAA AAA AAA AAA AGC ACT CGT CTA CTA TCG CTA AAA AAA AAA AGC ACT CGT CTA CTA TCG CTA AAA AAA AAA AAA A
C'	5' - NH_2- AAA AAA AAA ATA GCG ATA GTA GAC GAG TGC
A_{EcoRI}	5' - AAA AAA AAA AAA GAG CTA AGT CCG TAG AAT TCA AAA AAA AAA GAG CTA AGT CCG TAG AAT TCA AAA AAA AAA AAA
A_{EcoRI}'	5' - NH_2 – AAA AAA AAA AGA ATT CTA CGG ACT TAG CTC CAG GAT
B_{BamHI}	5' - AAA AAA AAA AAA TTG AAT CAT GCC TAG GAT CCA AAA AAA AAA TTG AAT CAT GCC TAG GAT CCA AAA AAA AAA AAA
B_{BamHI}'	5'- NH_2 – AAA AAA AAA AGG ATC CTA GGC ATG ATT CAA TGA GGC

* All sequences to be conjugated to SAC (A', B', C', A_{EcoRI}', and B_{BamHI}') were designed with a polyA linker followed by a 20mer hybridization region. The 5' amine is required for the attachment of the hetero-bifunctional maleimide derivative MHPH. Sequences printed on glass substrates (A, B, C, A_{EcoRI}, and B_{BamHI}) were designed with two hybridization regions separated by polyAs. This was designed to facilitate electrostatic adsorption to amine glass substrates.

A.2.2. Synthesis of DNA-SAC conjugates

In order to express SAC, pET-3a plasmid, as a kind gift from Takeshi Sano (Harvard Medical School), was used. SAC was expressed according to the previously published protocol[22], and buffer exchanged to PBS with 5mM Tris(2-carboxyethyl) phosphine hydrochloride (Solulink), using zeba desalting columns (Pierce). MHPH (3-N-

Maleimido-6-hydraziniumpyridine hydrochloride, Solulink) was added to SAC at a molar excess of 300:1. Meanwhile, SFB (Succinimidyl 4-formylbenzamide, Solulink) in DMF was added to 5' aminated DNA oligos at a molar excess of 40:1. In order to finish reactions, these mixtures were incubated at room temperature (RT) for 3-4 hours. Then, both mixture were buffer exchanged to pH 6.0 buffer solution (50mM sodium citrate, 150mM NaCl), and SFB-conjugated DNA oligos were added to MHPH-conjugated SAC at a molar excess of 20:1, and incubated overnight at RT. With Phamacia Superdex 200 gel filtration column at 0.5ml/min isocratic flow of PBS, SAC-DNA oligo conjugates was purified, and concentrated by 10K mwco concentration filters (Millipore).

A.2.3. Preparation of T cells

The cDNA from alpha and beta chains of TCR specific for tyrosinase$_{368\text{-}376}$ was obtained from Michael I. Nishimura (Medical University of South Carolina, Charleston, SC). These alpha and beta chain were cloned into a lentiviral vector having transgenes linked by a 2A self-cleaving sequence as written[23]. Supernatant from the lentiviral vector was concentrated and applied to infect Jurkat cells to generate Jurkat$^{a\text{-}Tyro}$ cells. In order to make Jurkat$^{a\text{-}MART\text{-}1}$ cells, MSGV1-F5AfT2AB retroviral vector expressing the F5 MART-1 TCR, obtained from Steven A. Rosenberg and Richard Morgan (Surgery Branch, National Cancer Institute Bethesda, MD), was used to infect Jurkat cells. For generating primary human T lymphocytes expressing the F5 MART-1 TCR, the same vector was applied to PBMCs from leukapheresis. Prior to the infection, PBMCs were activated with 50ng/ml of OKT3 (muromonab anti-human CD3 antibody, Ortho-Biotech, Bridgewater, NJ) and 300 U/ml of IL-2 (adesleukin, Novartis, Emeryville, CA) for 48

hours. Then, on the retronectin-coated wells (Takara Bio Inc., Japan) containing MSGV1-F5AfT2AB retroviral vector, activated PBMC in RPMI with 5% human AB serum supplemented by 300 IU of IL-2 were added and incubated at 37^{o}C for overnight. On the next day, a second set of pre-coated retronectin retroviral vector tissue culture plate was used to transfer the activated PBMC and incubated at 37^{o}C for overnight. Subsequently, with the same media as above, cells were washed and re-suspended. In RPMI supplemented with 10% human AB serum and 1% penicillin, streptomycin, and amphotericin (Omega Scientific), frozen leukapheresis fractions from patients NRA11 and NRA 13 (UCLA IRB#03-12-023) were thawed and incubated overnight. Then using an AutoMACS machine according to the manufacturer's instructions, CD8+ enrichment (anti-CD8 microbeads, Miltenyi Biotech) was done. Following separation, the cells were cultured at in RPMI-humanAB media containing 30 U IL2/mL.

A.2.4. Sorting cells

The HLA-A*0201 restricted MHC class I monomers loaded with tyrosinase369-377 (YMDGTMSQV) and MART-126-35 (ELAGIGILTV) were made in house with previous published protocols[24]. A2.1-restricted EBV BMLF1259-267 (GLCTLVAML), CMV pp65495-503 (NLVPMVATV), murine H-2Kb/-OVA257-264 (SIINFEKL), and murine H-2Db/-gp10025-33 (KVPRNQDWL) as well as all fluorescent HLA-A*0201 tetramers were purchased from Beckman Coulter, and lipophilic cell membrane staining dyes DiO, DiD, and DiL were obtained from Invitrogen.

To prevent non-specific cell binding, 1mg/ml PEG-NHS ester (Sunbio) in PBS was applied on microarray slides for 2 hours at RT. p/MHC monomers were combined in a 4:1 molar excess with ssDNA-SAC at 37^{o}C for 20 min to form ssDNA-p/MHC tetramers. With the tetramers in 200µl media, DNA microarrays was hybridized for 1 hour at 37^{o}C, and washed with 3% FBS in PBS. T cells (10^{6} cells/ml) were incubated on the array for 30 min at 37^{o}C, and washed with the same media.

In the comparative study, SuperEpoxy(covalent) and SuperProtein(hydrophobic) slides were obtained from Arrayit, GAPSII(electrostatic) slide was purchased from Corning, and Hydrogel(hydrophilic) slide was acquired from Xantec. According to manufacturer's protocol, fluorescent MART-1 tetramers were immobilized on each slide, and the result was analyzed with ImageJ and Origin.

Staining p/MHC tetramer after T cell capture was done with adding fluorescent cDNA (Cy5-A' and Cy3-B). For selective T cell release, EcoRI, BamHI or DNase in RPMI media was applied onto the immobilized cells for 1-2 hours at 37^{o}C. Two restrict enzymes were purchased from NEbiolabs, and DNase was obtained from Sigma. All cell capture images were obtained by bright filed (Nikon Eclipse TE2000) and/or confocal microscopy (Nikon E800).

A.3. Results and Discussions

A.3.1. Design of ssDNA-p/MHC tetramers

Mostly used reagent to assemble p/MHC monomer into tetramers is SA-phycobiliprotein (PE or APC) conjugates, which is linked via chemical cross-linking. However, we could not use them, because their functional groups for attaching ssDNA are modified during the conjugation. Unmodified SA was not the best choice because its functional groups close to the biotin binding sites prohibit the access of biotin as published[25,26]. We solve the problem with employing a mutant SA, having additional cysteine residue at the carboxy-terminus, which was first introduced by Ramachandiran and co-workers[25]. Because native SA has no cysteine residue, the reacting site can be restricted at the end of carboxy-terminus[27], far from biotin binding pocket, when cysteine-maleimide conjugation was applied.

In order to compare the ability of biotin binding between SA-ssDNA conjugate and SAC-ssDNA conjugate, 2-(4'-Hydroxyazobenzene) benzoic acid (HABA)[28] was used. Because it is a molecular mimic of biotin with distinct optical density coefficients dependent on whether biotin is bound to SA or not, the biotin binding capacity is verified through absorption spectra. The result of HABA absorption spectra showed the binding capacity of SAC-ssDNA conjugate is 3.7 while that of native SA-ssDNA is 2.9. When these conjugates were applied to capture 4 different monoclonal T cells, the difference of binding capacity was signified. NACS p/MHC tetramers assembled by SAC-ssDNA conjugates immobilized T cells with a high efficiency, while SA-ssDNA conjugates coupled with p/MHC showed little cells capture (**Fig. A.2.**). Therefore, all NACS tetramers as follow were assembled by SAC-ssDNA.

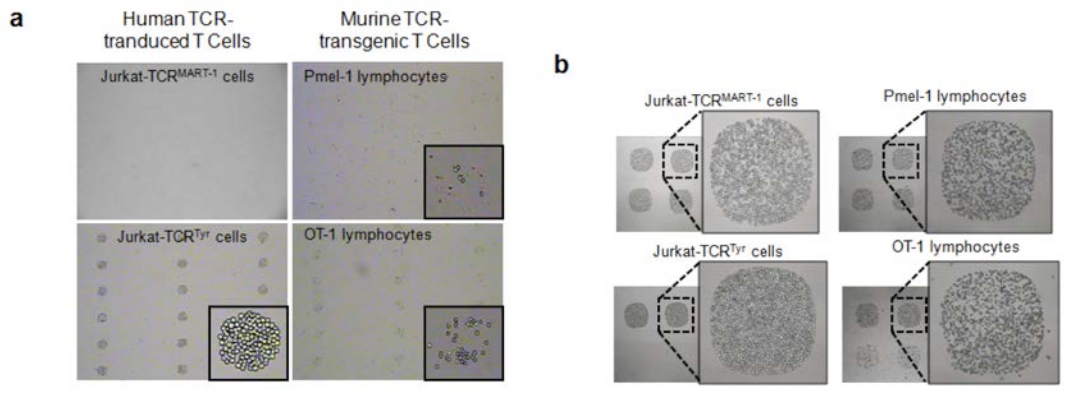

Fig. A.2. T cell capture efficiency is optimal when utilizing ssDNA-SAC conjugates to generate NACS p/MHC tetramers. (**a**) ssDNA-p/MHC constructs derived from native SA were used to sort 4 different human/murine transgenic T cell populations. The T cell capture efficiencies were highly varied amongst the four T cell populations. (**b**) ssDNA-p/MHC tetramers derived from ssDNA-SAC conjugates were used to sort the four T cell populations. The resulting cell capture efficiencies were markedly improved over native SA-oligo conjugates, demonstrating that SAC is necessary for the production of high affinity ssDNA-p/MHC tetramers.

A.3.2. Comparison between NACS and conventional protein microarray

For comparison study between NACS and conventional microarrays based on direct protein spotting method, we chose various substrates to cover different spectrum of commonly used strategies (covalent, electrostatic, hydrophobic, and hydrophilic adsorption) for protein immobilization. According to manufacturer's instruction, serial dilutions of MART-1 SA-PE tetramer (HLA-A2.1 MHC molecules loading melanoma epitope MART-1_{26-35}) were directly printed. Onto these microarray, Jurkat$^{a\text{-MART-1}}$ T cells (Jurkat cells transduced with the F5 MART-1 TCR[29] specific for peptide epitope MART-1_{26-35}) were applied. Collected images of immobilized cells on the various substrates (**Fig. A.3.a**) and quantified analysis **(Fig. A.3.b**) were obtained.

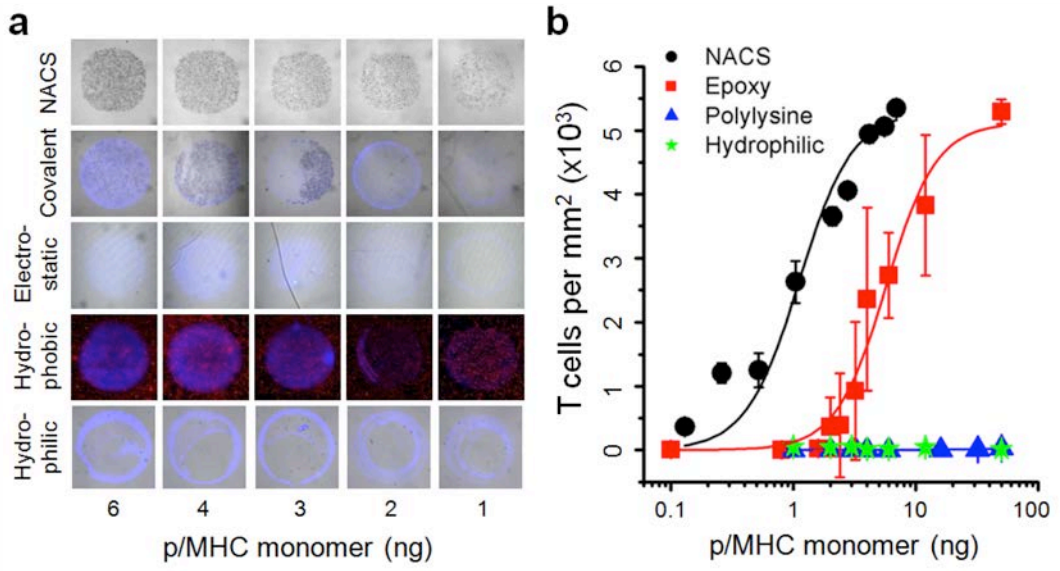

Fig. A.3. Comparison of NACS versus spotted p/MHC arrays. (**a**) Bright field and fluorescent images of Jurkat$^{a\text{-MART-1}}$ T cell capture on various model substrates. (**b**) Quantification of T cell capture efficiencies. (Hydrophobic surface was excluded because signal:noise ≤ 1.)

Compared to conventional microarrays with identical concentration of p/MHC tetramers, NACS was superior. Electrostatic and hydrophilic immobilization have little/no captured T cells, and hydrophobic immobilization has huge noise. Even though covalent immobilization succeeded to capture T cells, it had intra-spot and inter-spot heterogeneity while NACS gave homogeneous result at the same concentration of tetramers. Furthermore, as shown in the quantified analysis, only with a fifth of materials required in covalent immobilization, NACS could capture equivalent T cell. (p/MHC monomer at half max ≡ $K_{1/2}$ = 1.1ng for NACS and 5.7ng for covalent immobilization).

Two factors can be reasons of superior capturing efficiency in NACS. At first, linked by DNA-cDNA hybridization, p/MHC tetramers of NACS have great orientational freedom while those of conventional microarrays hardly have other options except just absorbed on the surface. This may increase the density of functional p/MHC tetramers

and result in reduced $K_{1/2}$. Secondly, NACS does not disrupt hydration state of arrayed proteins, which can be modified during production and storage process of conventional protein microarray to decrease reproducibility[11,16,21]. Because p/MHC tetramer arrays are self-assembled in solution immediately prior to an experiment and only stable DNA oligos are printed on the surface when the chip is stored, NACS may shows intra-spot and inter-spot homogeneity and guarantee the array reproducibility.

A.3.3. Specificity of NACS and its detection limit

In order to study the specificity of NACS, SAC-ssDNA p/MHC tetramer (human HLA-A*0201 MHC molecules loaded with melanoma antigen peptide epitope tyrosinase368-376 with pendant DNA sequence A') was applied to a DNA microarray made with the complementary strand A and two other orthogonal strand B and C. Then, Jurkat$^{\alpha\text{-Tyr}}$ cells (human T leukemia cell line transduced with a TCR specific for tyrosinase368-376)[30] were applied to the array. T cells were immobilized only on A spots hybridized appropriate p/MHC, not B or C spots (**Fig. A.4.a**), and the mean binding number of Jurkat$^{\alpha\text{-Tyr}}$ cells calculated from three spots was ~1486 ± 62. When a 1:1 mixture of Jurkat$^{\alpha\text{-MART-1}}$ and Jurkat$^{\alpha\text{-Tyr}}$ cells pre-stained with lipophilic dyes (green and red respectively) was applied to the array, those cells were captured into alternating columns and few cells were localized to spot C. The average number of captured cell was a factor of two less than that of homogeneous sorting. (661 ± 19 T cells/spot) (**Fig. A.4.b**). In order to find the detection limit of NACS, populations of Jurkat$^{\alpha\text{-Tyr}}$ were spiked at 10%, 1%, and 0.1% into wild Jurkat T cells and sorted. The number of captured cells per spot was analyzed, which shows linear correlation with the fractional

composition of Jurkat$^{\alpha\text{-Tyr}}$ cells in the mixture (**Fig A.4.c**). The limit of detection is around 1 in 1000 cells, and non-specific binding between wild Jurkat T cells and the array was always constant.

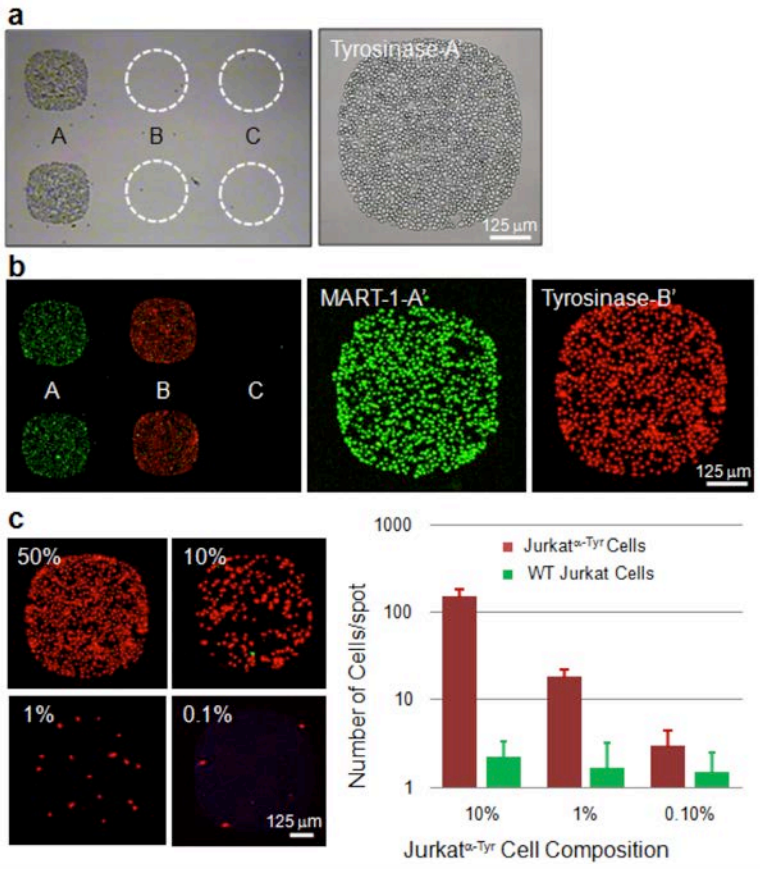

Fig. A.4. Nucleic acid cell sorting of antigen-specific T cells. (**a**) Tyrosinase p/MHC tetramer conjugated to ssDNA sequence A' was hybridized to an array printed with DNA complement strand A and non-complement strands B and C (dashed circles). Jurkat$^{a\text{-Tyr}}$ cells were localized to spot A only. (**b**) A prestained 1:1 mixture of Jurkat$^{a\text{-MART-1}}$ (green) and Jurkat$^{a\text{-Tyr}}$ (red) cells was selectively sorted to MART-1 and tyrosinase p/MHC spots. No T cells were detected on spot C. The right two panels are representative images of spots A and B. (**c**) Jurkat$^{a\text{-Tyr}}$ cells (red) were serially diluted in wild-type (WT) Jurkat cells (green) and sorted by NACS (left panels). The average number of Jurkat$^{a\text{-Tyr}}$ cells and WT Jurkat cells per dilution per spot was plotted in a histogram (right panel).

A.3.4. Selective release of immobilized T cells with restriction endonucleases

After immobilization of cells, immunohistochemistry(IHC), fluorescent in situ hybridization(FISH), and cytokine secretion assays were traditionally performed[4,6]. In order to study functional status of captured cells through mRNA or TCR, however, selective release of cell is required for eliminating the interference of back ground noise. Contrast to the conventional protein arrays made by direct printing, NACS can employ DNA sequence having unique cleavage site by restriction enzyme to selectively release captured cells (**Fig. A.5.a**). Before the specific release by restriction enzyme, Jurkat$^{a\text{-}MART\text{-}1}$ and Jurkat$^{a\text{-}Tyr}$ cells prestained by lipophilic dyes (red and green, respectively) were immobilized on a NACS array employed by DNA stands A_{EcoRI} and B_{BamHI} (**Fig. A.5.bi**). Then BamHI was applied on the array for 1 hour to cleave B_{BamHI} strands. Meanwhile, on a separate but identical array, EcoRI was applied to cleave A_{EcoRI} strands at the same condition. In each case, selective release was observed (**Fig. A.5.bii and biii**). When a complementary restrict endonuclease was applied on those arrays, remaining cells were released (**Fig. A.5.biv**). Of course, non-selective release was achieved in a single step with an addition of DNase (data not shown).

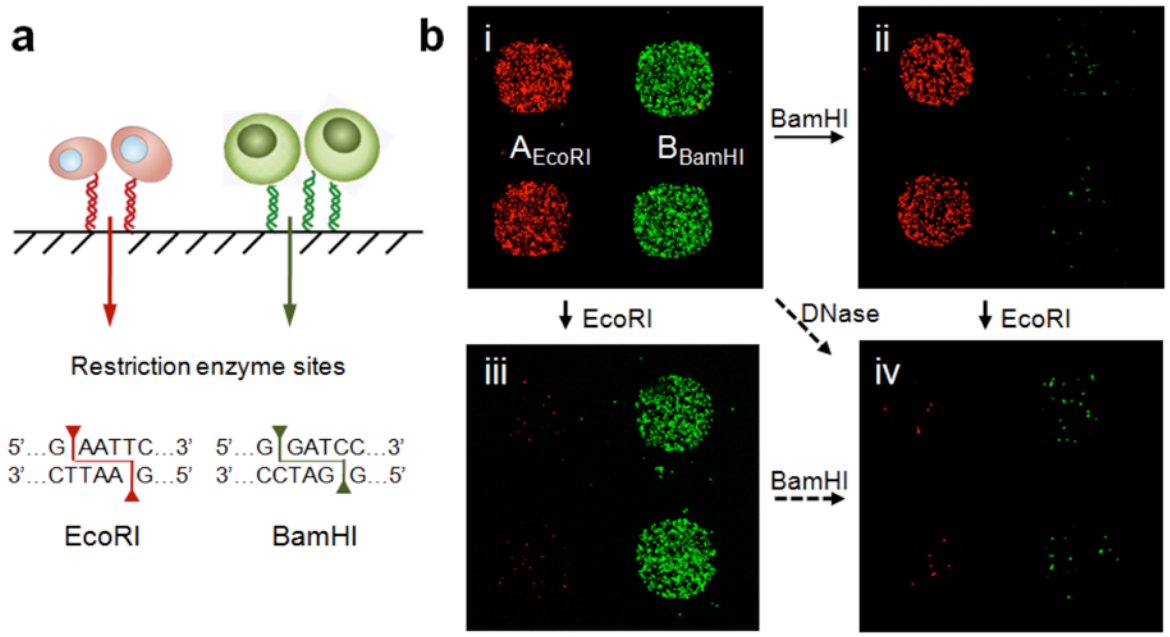

Fig. A.5. Programmed release of captured T cells by endonuclease cleavage. (**a**) DNA microarrays were printed with orthogonal sequences containing EcoRI and BamHI restriction sites. (b) Fluorescent images of Jurkat$^{a\text{-}MART\text{-}1}$ (red) and Jurkat$^{a\text{-}Tyro}$ (green) cells captured on p/MHC array (*i*) and after treatment with BamHI (*ii*) or EcoRI (*iii*). Only cells localized to DNA spots containing the target restriction sequence were released. A second round of enzymatic treatment released the remaining bound cells (*iv,* image representative of BamHI to EcoRI cleavage).

A.3.4. Sorting of TCR-engineered and endogenous primary human T cells by NACS

Recently, generating a huge number of tumor antigen-specific T cells and developing a therapy using those cells in patients with melanoma and other cancers have been reported[31,32]. In the study, T cells of patients was collected and engineered with a TCR against a target cancer antigen to get rid of tumor cells. In order to verify its clinical relevance, detecting TCR-engineered human lymphocytes is important, and it is achieved by NACS in this study. Via leukapheresis, human peripheral blood mononuclear cells (PMBCs) containing CD8+ cells of patients were extracted, and those cells were expanded and transduced with a retrovirus vector containing the F5 MART-1 TCR. Then, the cells were applied on a NACS array containing MART-1 and Cytomegalovirus (CMV)

pp65$_{495\text{-}503}$/HLA-A2.1 p/MHC tetramers. Only on the MART-1 spots, transduced T cells were captured. On the array, the antigen-specific sorting was validated again via antigen-specific staining with fluorescent MART-1 and CMV p/MHC tetramers (red and blue, respectively) (**Fig. A.6.a**)

Because there are huge number of T cells expressing different monoclonal or polyclonal TCR, detection of specific primary human T cell isolated from peripheral blood is much more difficult than cultured cell lines. Also, these T cells have endogenous expression level of TCR. In order to show that the specificity, multiplexing, and sensitivity of NACS can be equally applied to endogenous primary human T cells as like to cultured cell lines, frozen leukapheresis samples form patient NRA13 was studied. These cells were CD8+ enriched and applied to a CMV and Epstein-Barr virus (EBV BMLR1$_{259\text{-}267}$/HLA-A2.1) p/MHC array. Only on the EBV spots, T cells were immobilized, and the result of flow cytometry showed ~5% of NRA13 CD8+ cells were EBV-specific, but ~0% of them were CMV-specific.

Multiplexed detection was also available. When a 1:1 mixture of EBV-specific and CMV-specific CD8+ T cells, produced by combining NRA 13 lymphocytes with CMV-specific T cells from NRA11, was applied on a NACS array and stained with fluorescent p/MHC tetramer, immobilized cells showed appropriate color of fluorescence (**Fig. A.6.b**). In order to verify the detection limit, serial dilutions of EBV-specific T cell mixtures (~0.4%, ~0.2% and ~0.1% by FACS) were applied on NACS array, and the result demonstrated the resolution can be lowered at 0.1% (**Fig. A.6.c**).

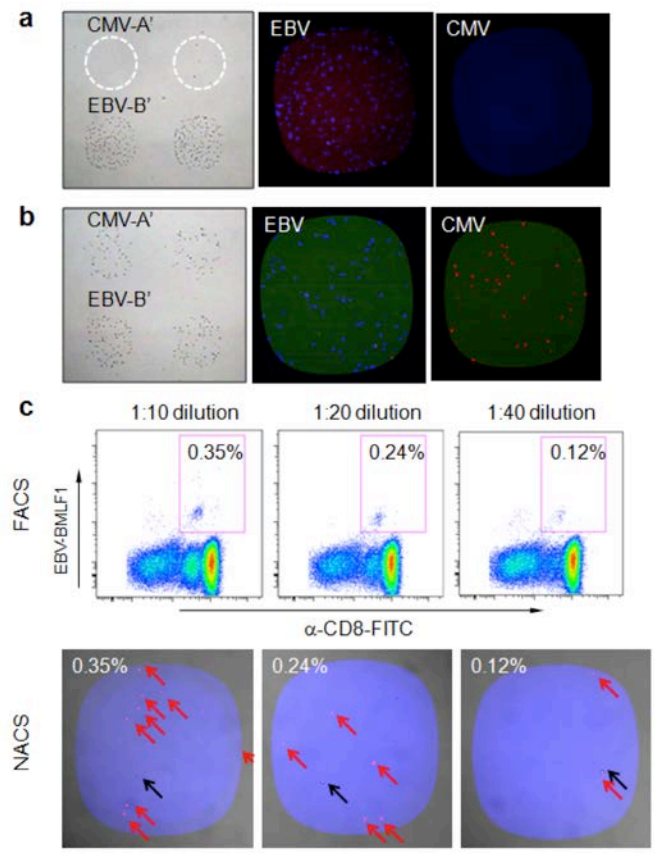

Fig. A.6. NACS sorting of endogenous primary human T cells specific for Epstein-Barr virus and Cytomegalovirus. (**a**) CD8+ T cells from patient NRA 13 were captured on EBV BMLF1 p/MHC spots and no T cells were captured on CMV pp65 spots (left panel). The right two panels are representative images after the cells were stained with fluorescent EBV BMLF1 (blue) and CMV pp65 p/MHC tetramers (red). (**b**) T cells detected from a 1:1 mixture of NRA11 and NRA 13 (left panel) were verified to be specific for EBV BMLF1 and CMV pp65 (right panels). (**c**) Mixtures of ~0.4%, 0.2% and 0.1% EBV BMLF1-specific T cell populations (upper panels) were detected via NACS (bottom panels). Populations of EBV BMLF1-specific T cells (red arrow) were detected that decreased in proportion to the dilution ratio. The number of non-specific cells (black arrow) was constant throughout all dilutions.

A.4. Conclusions

We showed the NACS platform is efficient, facile, and modular on-chip strategy for the immobilization of antigen-specific T cells. Because the DNA-printed glass substrate can be easily made by traditional DNA printing technologies, NACS is also inexpensive. Streptavidin-cysteine conjugated with single stranded-DNA is rationally designed to bind any family of proteins or small molecules labeled with biotin, therefore, the application of NACS can be extended to other capture agents, such as biotinylated antibodies.

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