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Cytophobic surface modification of microfluidic arrays for *in situ* parallel peptide synthesis and cell adhesion assays

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Abstract

A combination of PEG-based surface passivation techniques and spatially addressable SPPS (solid phase peptide synthesis) was used to demonstrate a highly specific cell-peptide adhesion assay on a microfluidic platform. The surface of a silicon-glass microchip was modified to form a mixed self-assembled monolayer that presented PEG moieties interspersed with reactive amino terminals. The PEG provided biomolecular inertness and the reactive amino groups were used for consequent peptide synthesis. The cytophobicity of the surface was characterized by on-chip fluorescent binding assays and was found to be resistant to non-specific attachment of cells and proteins. An integrated system for parallel peptide synthesis on this reactive amino surface was developed, using photogenerated acid chemistry and digital microlithography. A constant synthesis efficiency of >98% was observed for up to 7mer peptides. To demonstrate specific cell adhesion on these synthetic peptide arrays, variations of a 7mer cell binding peptide that binds to murine B lymphoma cells were synthesized. Sequence specific binding was observed on incubation with fluorescently labeled, intact murine B lymphoma cells and key residues for binding were identified by deletional analysis.

Introduction

Combinatorial peptide chemistry has emerged as a powerful tool for mapping limitless receptor-ligand interactions in drug discovery applications[1–4]. The technology has evolved with introduction of various solid supports, synthesis chemistries and has been used for diverse biomolecular assays[5,6]. Peptide microarrays have been used for epitope mapping, study of kinase activity, peptide-DNA interactions and detection of hydrolases[4]. A relatively unexplored area is identification of synthetic peptides that target tumor cells by assaying peptide libraries with purified cell surface receptors or intact cells. Synthetic peptides are smaller than monoclonal antibodies, chemically stable, can include D amino acids which resist proteolytic degradation and are not taken up by the reticuloendothelial system, thereby making them ideal therapeutic agents[7,8]. In a comparative study, Aina put forth the idea of peptides as candidates for targeted cancer therapy over anti tumor monoclonal antibodies[8]. In consensus with this idea, studies showed that synthetic peptides induced *in vitro* programmed cell death in a human B cell lymphoma [9]. The Lam group took this technology a step further and identified a peptide that in addition to binding specifically to the surface receptors of cancer cells was able to trigger signal transduction when synthesized in multimeric forms[10]. These studies enunciate the need for a reliable method for rapid screening of synthetic peptides to identify sequences that are highly specific to target cells.

The benefits of miniaturized assays on microfluidic devices are numerous but have not yet been widely applied to protein and cell based assays. In general, these assays require inert

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substrates that present immobilized binding ligands, which are difficult to create since cells are known to adhere to all man-made materials. The non specific adsorption of cells and proteins hinders the interaction of the ligand with the target cell, besides which adsorbed proteins can introduce different ligands that interact with the cells leading to erroneous data [11,12]. Microfluidic substrates present a bigger challenge since the high surface to volume ratio introduces additional unwanted interactions between the small channels and the fluid. Consequent efforts have been made to control surface chemistries of these channels in various materials by covalent bonding of surface active sites of the substrate with molecules terminated with hydrophilic polymers such as polyethylene glycol[13,14]. These techniques enabled the study of the microenvironment in which cells bind to peptides, wherein the interaction between several mammalian cells and the Arg-Gly-Asp peptide motif found in the extracellular matrix was used as a model. It was shown that the binding of cells to the Arg-Gly-Asp peptide was dependent on its surface density and length of the PEG chain displaying the ligand[15,16]. In addition, PEG-modified surfaces were used to study cell differentiation and proliferation [17, 18], but no attempt was made at high throughput identification of specific cell adhesive sequences.

Our approach towards creating a microfluidic platform for high throughput cell-peptide interaction studies was inspired by the potential of cancer targeting peptides and the need for model substrates in cell-based biosensors. To begin with, the surface of a glass-silicon microchip was modified by a mixed SAM as in Figure 1 to create monolayers terminated in PEG moieties and amino groups in a specific ratio. The PEG gives the surface its required biological inertness, while the amino terminals facilitate further attachment of amino acids by SPPS chemistry. The integrity of this surface was characterized by a combination of assays as in Figure 2, where the surface was tested for inertness to non specific binding of intact fluorescent cells and antibodies. Variations of peptide sequences that bind to a B lymphoma cell line were synthesized in different chambers of this microchip using photogenerated acid chemistry as illustrated in Figure 3. The on chip binding assay showed sequence specific binding of the fluorescently labeled cells to the synthetic peptides. These results demonstrate the application of microfluidic microarrays with spatially addressable peptides for screening of target peptides that bind to specific cell lines.

Materials and Methods

Photoresists (PR1813, PR1827) and developer solution (MF319) were from Hoechst Celanese. Resist stripper PRS2000 was from J.T. Baker Inc while photoresist A29260 and resist developer AZ400K were from Clariant Inc. Dimethylformamide (DMF), dichloromethane (DCM), diisopropylethylamine (DIEA), acetic anhydride, trifluoroacetic acid (TFA), trifluoromethanesulfonic acid (TFMSA), ethanedithiol (EDT), thioanisole (TIS), dimethyl sulfoxide (DMSO) were obtained from Fisher Scientific. 3-aminopropyltriethoxysilane and PEG-silane were obtained from Gelest Inc. All BOC protected amino acids and HATU were obtained from Anaspec. Rhodorsil Photoinitiator 2074 was from Secant Chemicals Inc., while thioxanthenone was from Sigma Aldrich. Digital Micromirror Device (DMD) was obtained from Texas Instruments. WEHI-231 B lymphoma cell line, DMEM, PBS and all cell culture material was from ATCC. CellTracker Orange CMTMR was from Cambrex Inc. Carboxyfluorescein (FAM) and Alexa 647 dyes were obtained from Molecular Probes Inc.

Microfluidic device fabrication

All microfluidic chips were fabricated in the Solid State Electronics Laboratory at the University of Michigan, Ann Arbor. This section describes the fabrication of two kinds of chips: the microfluidic array chip with 300 circular chambers and the microfluidic chip with 4 vertical chambers. Both kinds are sandwich devices made of a silicon substrate anodically

bonded to a glass cover. The silicon layer contains three topographic features (microreaction cells, microchannels, and inlet/outlet through-holes) for the microfluidic array chip and two topographic features (microreactor cells and inlet/outlet through-holes) for four chamber microfluidic chip.

For the microfluidic array chip, the fabrication procedure begins with a 4" (100) Si wafer with thermal oxide thickness of 0.6 μm . A layer of photoresist (PR 1813) was applied by spin-coating at 4000 rpm for 30 sec. The wafer was soft-baked at 90 °C in an oven for 30 min and then exposed to UV radiation (404.7 nm, 10 mJ/cm², 10 sec) to define the microreaction cell pattern. After removal of the activated photoresist by developer solution (MF 319) for 60 sec, the wafer was hard-baked at 110 °C for 30 min. The microreaction cell pattern was further transferred to thermal oxide by wet BHF etching. The photoresist was then removed by a resist stripper (PRS 2000). After stripping the photoresist, a new layer of photoresist (PR 1827) was applied by spin-coating at 2000 rpm for 30 sec. The wafer was soft-baked at 90 °C in an oven for 30 min and then exposed to UV radiation (404.7 nm, 10 mJ/cm², 50 sec) to define the microchannel pattern. After removal of the activated photoresist by developer solution (MF 319) for 60 sec, the wafer was hard-baked at 110 °C for 30 min. The microchannel pattern was further transferred to thermal oxide using reactive ion etching and to silicon wafer by deep reactive ion etching to obtain 160 μm deep microchannels. The photoresist was then removed by a resist stripper (PRS 2000) to reveal the underlying microreaction cell patterned oxide used as an etch mask. The wafer was re-etched in the deep reactive ion etcher to obtain 10–20 μm deep microreaction cells. After the microreaction cell was formed on the front side of the wafer, the new layer of photoresist (AZ9260) was applied at the backside of the wafer by spin-coating at 2000 rpm for 30 sec. The wafer was soft-baked at 90 °C in an oven for 30 min and exposed to UV radiation (404.7 nm, 10 mJ/cm², 100 sec) to define the inlet/outlet holes. The activated photoresist was then removed by a resist developer (AZ400K:H₂O, 1:3). The wafer was then loaded into the deep reactive ion etcher to create the through inlet/outlet holes.

For four chamber microfluidic chip, a layer of photoresist (PR 1813) was applied by spin-coating at 2000 rpm for 30 sec on a silicon wafer. The wafer was soft-baked at 90 °C in an oven for 30 min and then exposed to UV radiation (404.7 nm, 10 mJ/cm², 30 sec) to define the chamber pattern. After removal of the activated photoresist by developer solution (MF 319) for 60 sec, the wafer was hard-baked at 110 °C for 30 min. The pattern was transferred to the silicon wafer by deep reactive ion etching to obtain 160 μm deep chambers. The photoresist was then removed by a resist stripper (PRS 2000). The new layer of photoresist (AZ9260) was applied at the backside of the wafer by spin-coating at 2000 rpm for 30 sec. The wafer was soft-baked at 90 °C in an oven for 30 min and exposed to UV radiation (404.7 nm, 10 mJ/cm², 100 sec) to define the inlet/outlet holes. The activated photoresist was then removed by a resist developer (AZ400K:H₂O, 1:3). The wafer was then loaded into the deep reactive ion etcher to create the through inlet/outlet holes.

The silicon wafers containing etched structures were cleaned and a 0.2 micron thick thermal oxide was then grown on the wafers to provide the necessary surface for in-situ peptide synthesis. Finally, the silicon wafer was anodically bonded with the glass wafer.

Derivatization

The microchip surface was cleaned by circulating ethanol for 20 minutes. Surface OH groups were generated by circulating a solution of ammonium hydroxide, hydrogen peroxide and DI water in a ratio of 1:1:5. This was followed by washing with DI water and drying. A derivatization solution of 1% 3-aminopropyltriethoxysilane and 4% PEG-silane and 0.1% hydrochloric acid in toluene was prepared. This solution was circulated through the microchip for 4 hours and it was then washed with anhydrous toluene and ethanol consecutively. A

peristaltic pump was used for circulating all solutions at a flowrate of 0.1 ml/min. Finally, the microchip was cured at 100 °C for 30 minutes.

Peptide synthesis using in situ neutralization and HATU chemistry

The integrated peptide synthesis system includes an Expedite 8909 PNA synthesizer modified for peptide synthesis and an optical setup consisting of a UV light source, focusing system and a digital micromirror device (DMD). All reagents were delivered to the chip through a PEEK holder with Luer-lok fittings. The two systems communicate with each other via a in-house software called Chipmaker which allows predesigned patterns of light to be projected on the chip during the deprotection step of the synthesis cycle. Each amino acid addition to the growing peptide chain goes through the following steps:

1. **Coupling:** A **modified *in situ*** neutralization protocol was used for coupling[19,20]. The chip was treated with a solution of 0.4 mmoles of BOC protected amino acid, 0.4 mmoles of 0.6 M solution of HATU and 0.5 mmoles of DIEA in DMF. An activation time of 3 minutes and coupling time of 10 minutes was allowed for completion of coupling.
2. **Capping:** Unsuccessful couplings were capped by treatment with a 20% solution of acetic anhydride in DMF for 2 minutes.
3. **Deprotection:** The chip was filled with a 10% solution of Rhodorsil 2074 (a photogenerated acid precursor) and a thioxanthenone photosensitizer in a 1:2 ratio in dichloromethane. Using the DMD and a collimated beam from a Hg-Xe UV lamp, a light pattern was projected onto the chip to irradiate specific chambers of the chip during each deprotection cycle. Each deprotection cycle required an exposure time of 6 minutes for successful acid generation and BOC group removal.

Side chain protecting group removal

The side chain protecting groups were removed by adding a mixture of ethanedithiol, thioanisole and water in TFA (94:2:2:2) for 2 hours at 4°C. Bzl and Trt were removed by slow addition of TFMSA, allowing the deprotection to occur over 1.5 hours.

Cell culture

Murine B cell lymphoma cell line WEHI-231 was cultured in DMEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 units/ml penicillin, 50 mg/ml streptomycin and 50 μ M 2-mercaptoethanol. The cells were cultured in 25 cm² tissue culture flasks and fed every 2–3 days with fresh medium and maintained at 37 °C in a humidified 5% CO₂ atmosphere. The culture was subdivided every week to maintain a constant seeding density of 1×10^5 cells/ml.

Cell viability and fluorescent labeling

Cell viability and concentration of cells was determined by diluting the cell suspension in a 1:2 solution of Trypan blue and counting them at 100X magnification under an inverted microscope using a hemacytometer. The cells were fluorescently labeled with CellTracker Orange CMTMR (5-(and 6-)-(((4-chloromethyl)benzoyl)amino)tetramethylrhodamine (541/565 nm). The lyophilized product was dissolved in DMSO to a concentration of 10 mM to make a stock solution. For cell staining, the stock solution was diluted to a working concentration of 10 μ M in pre-warmed serum free medium. The cell suspension was centrifuged and the supernatant aspirated to obtain a pellet. This pellet was re-suspended in the 10 μ M working solution and incubated at growth conditions for 45 minutes. The stained cells were centrifuged again and suspended in full growth medium for 30 minutes at growth

conditions. They were then washed extensively with PBS and the resultant suspension of a concentration of 2×10^6 cells/ml in PBS was used for the cell adhesion assay.

Binding assay

The peptide chip was flushed with a suspension of CellTracker labeled intact B lymphoma cell line WEHI-231 at a concentration of 2×10^6 cells/ml. It was then incubated with cells for 1 hour at 37°C. After thorough washing with a 0.5% solution of Tween20 in PBS, the chip was scanned using an array scanner, Genepix 4000B at an excitation wavelength of 532 nm.

Fluorescent labeling of peptide with Alexa 647 and FAM

The free amino terminals after removal of terminal BOC group were labeled with Alexa 647 or FAM with emission wavelengths of 668 and 520 respectively. Alexa 647 was used for peptide labeling during cell adhesion experiments to be able to distinguish between fluorescence from cells at 532 nm and fluorescence from amino terminals at 635 nm.

Imaging instrumentation

All chips were imaged using Genepix 4000B, a microarray scanner used for fluorescence studies in microarrays. The resolution of all scanned images is 5 pixels and data was acquired by simultaneous excitation at two wavelengths of 532 nm in the green channel and 635 nm in the red channel.

Results and Discussions

Surface modification of microfluidic chip with mixed SAM of PEG and 3-aminopropylsilane

Light directed combinatorial synthesis of peptides on a glass or silicon microarrays derivatized with aminopropyl silane has been reported and applied to screening of antibody-peptide and metal-peptide interactions[21–23]. The antibody-peptide binding assay involves passivating the surface by physical adsorption of a layer of BSA and this successfully prevents the non specific binding of the target antibody. We attempted to apply BSA based passivation to cell-peptide adhesion assays but were unable to prevent non specific attachment of cells, even at high concentrations of BSA. To eliminate this, we passivated the surface by covalently bonding a mixed monolayer of PEG-silane and 3-aminopropylsilane as seen in Figure 1. By varying the ratio of PEG-silane to aminosilane we were able to get an optimized ratio of 4:1, where the resultant surface was inert to attachment of cells. To ensure the successful formation of a PEG-aminosilane mixed monolayer on the silicon surface, a scheme of experiments as in Figure 2 was designed. Here, the same surface-modified chip was assayed with fluorescently labeled WEHI-231 B lymphoma cells, a fluorescent antibody and an amine reactive fluorophore Alexa Red 647. The chips were scanned simultaneously at two different wavelengths, to view CellTracker labeled cells at 532nm and fluorescence from Alexa coupled amino terminals at 635 nm respectively. The scanned images indicated cell and protein resistant surfaces while the amine terminals could be detected by fluorescence from the attached Alexa. Our next step was to ensure that the monolayer was resistant to the harsh chemical conditions used during peptide synthesis. The same scheme of experiments was used, but a tri-Glycine oligomer was synthesized in 2 out of the 4 chambers using photogenerated acid as shown in Figure 3. The amine protecting BOC group on the tripeptide was removed in chambers 2 and 4, and the whole chip was labeled with Alexa647. Chambers 1 and 3 had a protected monomer which was not reactive to Alexa. The resultant scanned image at 635 nm is seen in Figure 4, where fluorescence from Alexa is observed in chambers 2 and 4 only. This chip was also resistant to cell binding when scanned at 532 nm as seen in Figure 5. In Figure 5 (a) the chip is flushed with fluorescent intact cells and incubated for 1 hour while Figure 5 (b) is an image after extensive washing, indicating no non-specific attachment of cells on the surface.

These images confirm the successful formation of a chemically reactive amino layer for subsequent peptide synthesis on a surface that is otherwise biologically inert. Surfaces that allow patterning of cells are currently being researched comprehensively for study of cell differentiation and proliferation. This work is focused on synthesis of cell adhesive peptides; however our surface modified chips can be used for these studies by allowing certain chambers to be cell adhesive while the others to be cell repellent. By using photogenerated acid chemistry to alter cytophobicity of different chambers, the use of expensive UV masks and clean-room conditions can be eliminated.

Synthesis scheme and stepwise yields of upto 7mer

Conventional peptide synthesis on a glass surface derivatized with aminosilane involves coupling, capping and deprotection steps in a cyclic manner till the desired peptide is synthesized. The amino acid monomers are protected with an acid labile BOC group, which is removed in the deprotection step to allow the next amino acid to be coupled. To be able to synthesize a different peptide sequence in each chamber of the chip we use a photogenerated acid (PGA) precursor which selectively forms acid in areas when irradiated with light at 405 nm. PGA allows localized deprotection as seen in Figure 3, thereby enabling addition of different monomers at each location. All reaction steps were optimized for microscale conditions and a standard coupling time of 10 minutes for all monomers was achieved by using HATU as activator and a **modified *in situ*** neutralization protocol[19,20]. This eliminated the need for the neutralization step after acid generation during deprotection therefore reducing synthesis cycle time. Addition of each amino acid to the growing peptide chain had a cycle time of 40 minutes. Synthesis efficiencies were calculated by comparing a count of free amino termini obtained after deprotection and labeling with amine reactive 4,5 carboxyfluorescein (FAM). Normalized fluorescence intensity was used as a count of free amino terminals obtained after coupling and deprotection on addition of each monomer till a 7mer was completed. Glycine was used as standard monomer to avoid variations in fluorescence intensity due to different coupling efficiencies of amino acids. A microfluidic chip with 300 chambers of 600 micron diameter is used as seen in Figure 6, for the stepwise synthesis efficiency experiment. Starting from the top right, 4 reactors with Gly, Gly-Gly and so on were synthesized till a 7mer was completed in the bottom right set. Each smaller chain was capped upon completion of synthesis and deprotected only after the final 7 mer was synthesized. It was observed as seen in Figure 6, that the fluorescence from the chambers with 7 mers was uneven. This could be attributed to a combination of non-uniform flow of reagents to the chip and improper alignment of UV lamp during synthesis. These factors led to uneven exposure of the 7 mer chambers as compared to the 1 mer to 6 mers causing incomplete deprotection reactions in certain parts of these chambers. Data analysis for all the synthesized peptides was done using normalized fluorescence intensities obtained from the Genepix software and a step wise synthesis efficiency of >98% for 7mer sequences was obtained as in Figure 7.

Synthesis of 7mer cell binding peptide and sequence specific adhesion studies

Having successfully synthesized 7mer peptides on cell/protein resistant microfluidic devices, our next step was to study the sequence specificity of the cell-peptide interaction. The peptide sequence DLWYDAV is known to bind specifically to a B lymphoma cell line [10]. We used this binding interaction as a model to test the cell binding ability of the synthetic peptides on our microarrays. The individual B lymphoma cells are 8 μ m in size, but tend to form clusters which clogged the narrow inlet and outlet channels and chambers of our microfluidic chips. To overcome this problem, we fabricated chips with wider channels and only four chambers. All chips used in these assays were first derivatized with a mixed SAM of amino and PEG silane and then a positive control of GGG-DLWYDAV and negative control GGG were synthesized in 2 out of the 4 chambers. Sequences with deletions in the binding sequence were synthesized in the other two chambers to be able to identify the key residues involved in

binding. All side-chain protecting groups of amino acids were removed after synthesis. The chips were then flushed with the cell suspension in PBS and scanned to ensure equal distribution of cells in all chambers. The inlet and outlet of the chip was then sealed to prevent drying out and cell adhesion assay was carried out at 37°C for 60 minutes. After extensive washing with 1% Tween in PBS buffer, they were scanned at 532 nm to detect binding of cells.

A scanned image of chip A is seen in Figure 8, wherein the positive control is synthesized in chambers 1 and 3, negative control in chamber 4, while chamber 2 has the deletion sequence GGG-DLWYV. Successful cell binding is seen in chambers 1 and 3, while chamber 2 has reduced binding and chamber 4 has no binding as expected. Chip B was then synthesized with the same layout and protocol, but with a different deletion in chamber 2, namely GGG-DLDAV. The same incubation protocol was followed but with a higher concentration of cells. The results as seen in Figure 9 show reduced binding for the deletional sequence. On increasing the initial number of cells, we observed a corresponding increase in number of attached cells. Four different deletions were introduced in the binding sequence on different chips. To quantify the sequence specific attachment, a 25% section in the centre of the chip was chosen and the attached cells for each sequence were counted with results tabulated in Table 1 and Figure 10. The number of bound cells to each sequence has been normalized with respect to the initial number of cells used for incubation. On an average, 70% of the initial number of cells during incubation was found to bind to the positive control sequence.

Analysis of the data in Table 1 and Figure 10 indicates that the residues Tryptophan (W), Tyrosine (Y) and Aspartic Acid (D) are the necessary residues for binding. These results are in consensus with the reported findings of the Lam group, where several binding motifs for the surface idiotypes of this particular cell line of WEHI-231 were identified[10]. They screened random libraries on beads for binding with these cells and identified the consensus dipeptide motif of WY and three residues (V, I and T) that could replace the D residue[10]. These results elucidate the sequence specificity of this assay and its potential to be used as a screening method for identification of peptides that target specific cell receptors.

Conclusion

This study enabled the advantages of miniaturization of assays in microfluidic devices and molecular tailoring of surfaces to be applied to cell based assays. Binding ligands that are specific to cell type can be identified with possible use in targeted cancer therapy. The ease and short time scale of synthesis along with ability to incorporate artificial amino acids gives this technology an edge over other phage display libraries. The mask-less *in situ* synthesis system allows easy introduction of mutations and deletions in the binding sequence to identify key residues involved in the cell-peptide interaction. Although the experimental trials were conducted on chips with only 4 chambers, a higher throughput platform can be created simply by fabricating chips with more number of chambers. Concentration of discrete ligands displayed on the surface can be altered by varying concentration of SAM on surface. This method also has the potential to create whole cell arrays by selectively deprotecting the cell adhesive Arg-Gly-Asp peptide in different chambers of the device and allowing a specific cell line to attach to a specific chamber. Attachment of cells to the immobilized peptide can be reversed by treating with a higher concentration of Arg-Gly-Asp peptide in solution. Finally, we have created a flexible and cost effective microfluidic platform for application of synthetic peptide microarrays to identification of sequence specific cell binding ligands.

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Notation

SAM	Self Assembled Monolayer
PEG	Polyethylene glycol
PEG-silane	2-[methoxypoly(ethyleneoxy)propyl]trimethoxysilane
HATU	O-(7-Azabenzotriazole-1-yl)-N,N,N'-tetramethyluronium hexafluorophosphate
Arg-Gly-Asp	Arginine-Glycine-Aspartic Acid
DMF	N,N Dimethylformamide
DIEA	N,N Diisopropylethylamine
BOC	<i>tert</i> -butoxycarbonyl
DCM	Dichloromethane
PGA	Photogenerated acid
TFA	Trifluoroacetic acid
TFMSA	Trifluoromethanesulfonic acid
PBS	Phosphate Buffer Saline
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide

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Figure 1.
 Schematic of surface modification of microchip by mixed SAM of PEG-silane and aminosilane

Figure 2.
Schematic of experiments to characterize surface passivity and chemical reactivity of microchip surface

Figure 3.

Stepwise synthesis of peptides using photogenerated acid chemistry. The O group indicates acid labile BOC protecting group on amino acids. (a) BOC-protected Leucine (L) is attached to the whole chip surface (b) Selected areas are irradiated with digital light patterns. (c) BOC group is removed in these select areas and terminal amino groups (N) are available for reaction (4) The next protected amino acid Valine (V) is coupled to these amino terminals to build the desired peptide. This cycle is repeated to generate site specific peptide sequences.

Figure 4. Scanned image of microchip at 635 nm where chambers 1 and 3 have acetyl capped Glycine, chambers 2 and 4 have deprotected Gly-Gly-Gly-NH₂, labeled with amine reactive Alexa 647.

Figure 5. Scanned image of microchip at 532 nm, derivatized with mixed SAM of PEG and amino silane. Trimer peptide synthesized in chambers 2 and 4. (a) incubated with fluorescently labeled cells. (b) After extensive washing with 1% Tween solution in PBS.

Figure 6. Scanned image of chip with 1mer to 7mer synthesized in sets of 4 spots. The top right set has a 1mer and a growing chain is synthesized step wise with a 7mer in the bottom right.

Figure 7.

Plot of relative fluorescence intensities, where Ac = Acetyl capped and FR = fluorescein labeled. (1) G-FR (2) G-G-Ac (3) G-G-FR (4) G-G-G-Ac (5) G-G-G-FR (6) G-G-G-G-Ac (7) G-G-G-G-FR (8) G-G-G-G-G-Ac (9) G-G-G-G-G-FR (10) G-G-G-G-G-G-Ac (11) G-G-G-G-G-G-FR (12) G-G-G-G-G-G-G-Ac (13) G-G-G-G-G-G-G-FR

Figure 8. Scanned image of Chip A at 532 nm: Positive control DLWYDAV in chambers 1 and 3, deletion DLWYV in chamber 2, negative control GGG in chamber 4. Incubated with 1×10^5 cells/ml and washed extensively.

Figure 9. Scanned image of Chip B at 532 nm: Positive control DLWYDAV in chambers 1 and 3, deletion DLDVA in chamber 2, negative control GGG in chamber 4. Incubated with 2×10^6 cells/ml and washed extensively.

Figure 10.

Plot indicating sequence specific binding of murine B lymphoma cells to various deletional sequences of DLWYDAV. All data is from the same selected area in the chips and normalized to initial concentration of cells.

Table 1

Amino acid sequence of synthesized peptides and the corresponding number of bound cells in selected area. Initial number of cells in area before incubation is 480.

Name	Amino acid sequence	Number of cells attached per selected area
C	GGG-DLWVDAV	280
N	GGG	9
V1	GGG-DLWYV	180
V2	GGG-DLDAV	161
V3	GGG-DLAV	25
V4	GGG-DLWAV	28