Parallel Peptide Synthesis on Microfluidic Microarrays for Epitope Mapping and Cell Adhesion Assays

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Abstract

Advances in combinatorial chemistry have enabled the application of small molecule microarrays for study of limitless receptor-ligand interactions. Short synthetic peptides have emerged as popular probes for microarrays due to their stability, diverse range of biomolecular interactions and sequence specific bioactivity. Peptide microarrays are generally made either by spotting presynthesized sequences onto a substrate in array format or by insitu synthesis, wherein the synthesis reagents are delivered in cyclic steps, to build a sequence. Both of these methods have been used successfully for epitope mapping, study of kinase activity, peptide-DNA interactions and detection of hydrolases [1]. However they require either, laborious purification steps and expensive robotic arms for spotting or light masks and photolabile protecting groups which need clean room environment [1, 2, 3]. Of the two methods, in situ synthesis has the advantage of eliminating the need for individual synthesis and purification of a large number of peptides; however the chemistries available for rapid synthesis are limited, with comparatively low efficiencies and high costs. Due to the limitations of these methods peptide microarrays have not yet been able to expedite the target screening process in drug discovery, and there is a need for a rapid, low cost, efficient and flexible synthesis method.

Peptide and peptidomimetic microarrays have been used for various applications, but 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. The main reason being that cell based assays require inert 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 [4,5]. Microfluidic substrates present a bigger challenge since there are additional unwanted interactions between the small channels and the fluid.

This work demonstrates the synthesis and application of in-situ synthetic peptide microarrays for a high-throughput parallel study of peptide-antibody and peptide-cell interactions at amino acid level. The synthesis substrate was a microfluidic microarray of individual reactors connected by channels, converging into a solution inlet and outlet, etched on a flat silicon surface sealed by a glass cover. A combination of solid phase peptide synthesis, photogenerated reagent chemistry and fluorescence imaging techniques was used to generate spatially addressable peptide microarrays with up to 12mer sequences. Coupling of amino acids is achieved by an optimized method using HATU as activator and DIEA for insitu neutralization. In the site specific deprotection step, a photogenerated acid precursor is used for Boc group removal and the resultant step by step synthesis is illustrated in Figure 1.

A constant synthesis efficiency of >98% was observed for sequences of up to 7 amino acids. Peptide sequences from Respiratory Synctial Virus (RSV) were synthesized and used to identify binding sites to the antibody MAb 19 by performing on-chip, high throughput ELISA. Key residues for binding were identified by mutational and deletional analysis on these sequences.

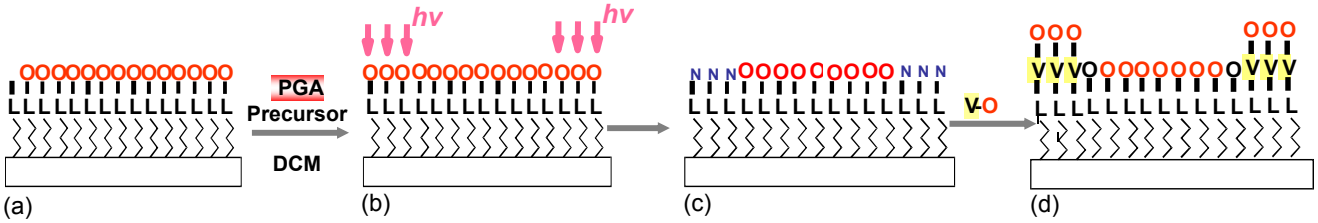

Figure 1: Stepwise synthesis of peptides using photogenerated acid chemistry. The O group indicates acid labile Boc protecting group on amino acids. (a) Boc-protected Leucine (L) is attached to the whole chip surface (b) Selected areas are irradiated with digital light patterns. (c) Boc group is removed in these select areas and terminal amino groups are available for reaction (4) The next protected amino acid Valine (V) is coupled to these amino terminals to build the desrired peptide. This cycle is repeated to generate site specific peptide sequences.

For mapping of cell-peptide interactions, surface of a glass-silicon microchip was modified by a mixed self-assembled monolayer as in Figure 2 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. Variations of peptide sequences that bind to a B lymphoma cell line were synthesized in different chambers of this microchip. 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

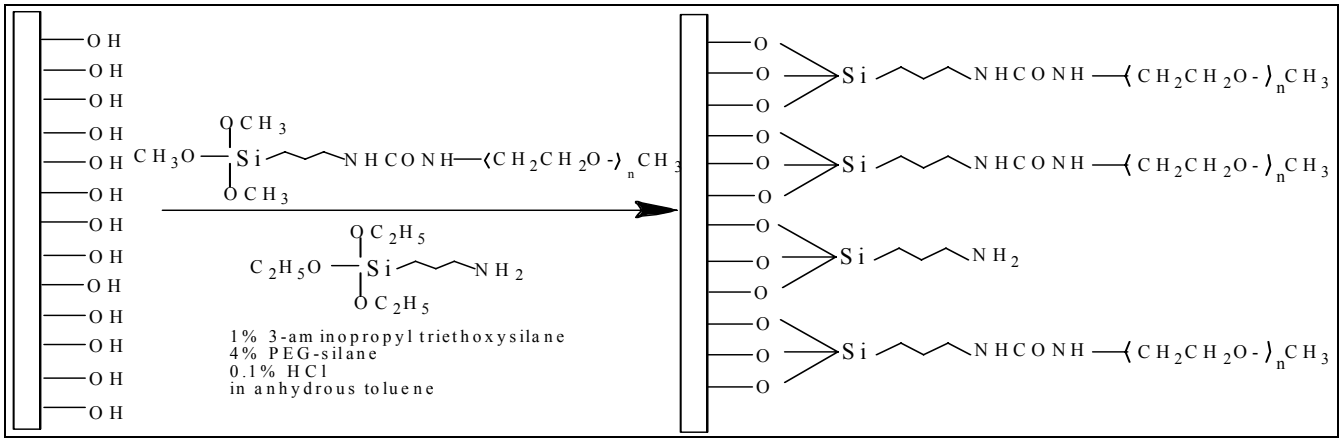

Figure 2: Schematic of surface modification of microchip by a mixed self assembled-monolayer of PEG-silane and aminosilane. The resultant free amino terminals are used for attachment of amino acids to build up cell adhesive peptide sequences.

Abbreviations: **HATU:** O-(7-Azabenzotriazole-1-yl)-N, N,N'N'-tetramethyluronium hexafluorophosphate, **DIEA:** Diisopropylethylamine, **SPPS:** Solid phase peptide synthesis, **PEG:** Polyethylene glycol

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