# Bioanalytical Applications of Microfluidic Devices: From Patterning to Genotyping

Huaibin Zhang Final Seminar May 24, 2010

Microfluidics is the science and technology of manipulating small volumes of liquid (< $10^{-6}$ litres) using sub-millimeter channels.[1] In the past 15 years, microfluidics has demonstrated its potential as a powerful new tool for biology,[2] medicine,[3] and chemical analysis.[4] In this talk, two bioanlytical applications of microfluidic devices are highlighted: patterning biomolecules[5] and analyzing DNAs.[6]

Micro-patterning techniques, such as microcontact printing[7] and microfluidic patterning,[8] have been widely used to pattern substrate surfaces with various substances in a precisely controlled manner. In typical microcontact printing schemes, a patterned polydimethylsiloxane (PDMS) stamp is used to transfer 'ink' (e.g., thiol) through contact with the substrate (e.g., gold surface).[9] A challenge for this technique is to print multiple solutions in one printing attempt without cumbersome registration steps. Microfluidic patterning could solve this problem by locally delivering different substances through an individual channel system sealed to the substrate. The major drawback of this technique, however, is its lack of reusability and inability to form complex patterns. In addition, both techniques are usually limited to patterning rigid substrates. The capability to pattern soft materials, such as a hydrogel, is critical for biological studies because cells behave differently on rigid versus soft surfaces.[10]

By combining the best aspects of each patterning technique, we developed a novel microfluidic contact printing technique which can be used to pattern biomolecules on hydrogel substrates (Figure 1).[11] A microfluidic printing device was fabricated by sealing a PDMS channel system with a porous polycarbonate membrane. A streptavidin-incorporated hydrogel was placed on the sealed device where a biotinylated biomolecular solution flowing through the underlying channels, could diffuse up through the membrane to be captured by the hydrogel. After printing was complete, the hydrogel was removed and a new gel was put in place to commence the next printing cycle. We illustrate the device fabrication, the reproducibility and reusability of the device, as well as the many possible PDMS channel designs that can be used with this system. We also highlight the system's potential to pattern various biomolecules (e. g., polysaccharide and protein) on hydrogel substrate for biological studies.

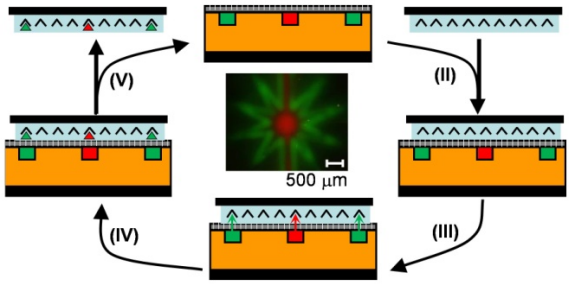


**Figure 1**: Microfluidic Contact printing

Single Nucleotide Polymorphism (SNP) is a DNA sequence variation that involves one change in a single nucleotide. As the most common type of human genetic variation,[12] SNPs have been associated with genetic diseas[13] and used as gene marker.[14] Point-of-Care (POC) diagnostic devices that discriminate SNP genotypes have potential applications in global healthcare, epidemic control, and forensic analysis.[15] An ideal POC device should be portable, inexpensive to fabricate, easy to operate, and capable of rapid discrimination of multiple analytes. Miniaturization of existing bench-top discrimination methods and assay formats has yet to adequately meet these requirements. Enzyme-assisted genotyping methods generally require expensive, and often chemically sensitive, reagents which may not be ideal for POC applications. Recent studies, however, have shown impressive progress in developing novel non-enzymatic analysis methods.[16]

The second part of this presentation describes a novel non-enzymatic, isothermal method to discriminate SNPs. SNP discrimination using alkaline dehybridization has long been neglected because the pH range in which thermodynamic discrimination can be performed is quite narrow ($\Delta pH < 0.3$). We found, however, that SNPs can be discriminated by the kinetic differences exhibited in the dehybridization of perfectly matched (PM) and single-base mismatched (MM) DNA duplexes in an alkaline solution using fluorescence microscopy.[17] We combined this method with multifunctional encoded hydrogel particle array (fabricated by stop-flow lithography)[18] to achieve fast kinetics and high versatility (Figure 2). This approach may serve as an effective alternative to temperature-based method for analyzing unamplified genomic DNA in point-of-care diagnostic.

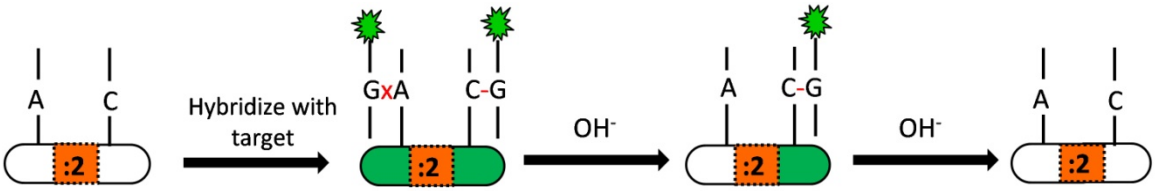


**Figure 2**: Kinetic difference of PM and MM duplexes (formed between allele-specific probes and a target) in alkaline dehybridization is used to discriminate SNP.

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