A single-step Microfluidic Synthesis of Microspheres Immobilized with ssDNA Probes

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Abstract

Aptamers are short and single strand DNA (ssDNA) oligonucleotides capable of ligand binding in variety of biological assays[1]. They can be obtained by in vitro selection according to the SELEX procedure. The generation and isolation of ssDNA from the amplified sub-DNA pools are essential to make DNA aptamer. Asymmetric PCR method has been used to generate ssDNA in SELEX. However, since both ssDNA and double strand DNA (dsDNA) are usually generated, additional separation experiments are necessarily required such as a strepatavidin-biotin separation. In this study, we fabricated PDMS microfluidic device that includes microsphere generator (X-junction). Polyacrylamide solution was applied to microfluidic device with acrydite modified oligonucleotides DNA probe [Figure 1][2]. The main challenge of the use of polyacrylamide is in the covalent immobilization of ssDNA probes onto the microbead surfaces. In addition, the three-dimensional configuration of the microspheres can improve the complementary binding between DNA-DNA to the surface. DNA probe immobilization and their extension were confirmed by using fluorescent labeled complement partner [Figures 2]. Our microspheres are available to use ssDNA generation, DNA fishing and DNA microarray analysis.

References

[1] R. Stoltenburg, C. Reinemann, B. Strehlitx, Biomelecular Engineering, 4(2007) pp. 381-403

[2] Farah N. Rehman et al., Nucleic Acids Research, 2(1999) pp.649-655

Figures

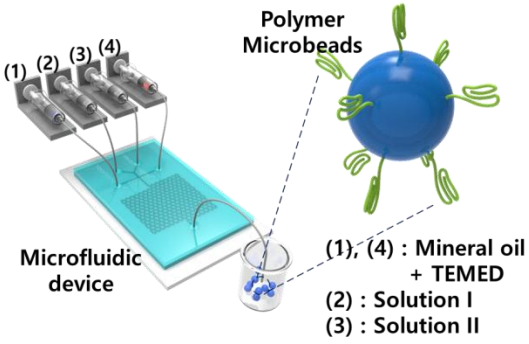

Figure 1. Schematic diagram of PDMS microfluidic device and Polyacrylamide Microbeads

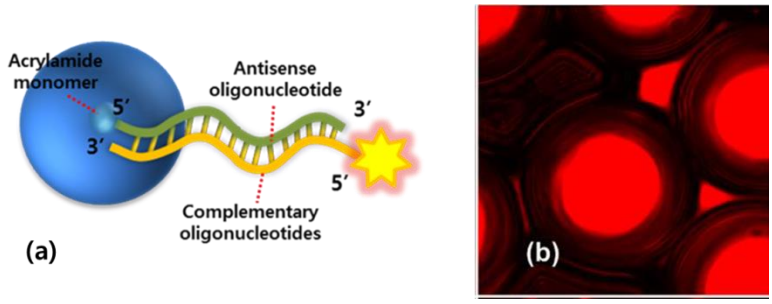

Figure 2. 3-Dimensional configuration of the microspheres and ssDNA probe

(a) To confirm immobilization and extension of ssDNA probes onto microbeads, antisense oligonucleotide was used. The fluorescence signal was observed by using confocal laser scanning microscope (b). These result showed that oligonucleotides were exposed to surface of microbeads generated by microfluidic device.