CHARGE-REVERSIBLE SOLID SURFACE AND ITS APPLICATION TO DNA MANIPULATION UNDER MICROFLUIDIC ENVIRONMENTS

Kyu-Youn Hwang[1,2], Joon-Ho Kim[1], Kahp-Yang Suh[2,3], Kak Namgoong[1], Sang-Hyun Paek[1] and Nam Huh[1]

[1]1Bio Lab., Samsung Advanced Institute of Technology (SAIT), Samsung Electronics, Co., Korea
[2]Interdisciplinary Program of Bioengineering, Seoul National University, Korea
[3]School of Mechanical and Aerospace Engineering, Seoul National University, Korea

ABSTRACT

We have fabricated a charge-reversible solid surface based on poly(maleic anhydride) in order to manipulate nucleic acid electrostatically. Its surface charge could be controlled by changing pH of surrounding solution because it has both positively (imidazole) and negatively-charged (carboxyl) ionizable groups at the termination. Measurement of DNA binding efficiency and FT-IR spectra showed that the charge-reversible surface was properly generated on the solid support. It was successfully demonstrated that the charge-reversible surface on beads could be applied to manipulate DNA molecules under microfluidic environments by controlling solution pH.

KEYWORDS: Charge-reversible, DNA manipulation, solution pH

INTRODUCTION

Nucleic acid-based micro total analysis system (μTAS) is a promising approach to fully automate the analytical procedures on a microchip platform. An extraction step to acquire DNA of interest is a prerequisite for successful genetic analysis. Chaotrope-based DNA purification method became widely accepted in μTAS. However, it is desirable to avoid the use of chaotropes or organic solvent in that they are well-known as PCR inhibitors. A different DNA extraction scheme, such as electrostatic interaction [1-2], that would be more compatible with downstream processes is still needed. In this study, we have fabricated the charge-reversible surface (CRS) responding to surrounding solution pH and demonstrated its capability to manipulate DNA under microfluidic environments.

THEORY

CRS is based on a poly(maleic anhydride) backbone. Maleic anhydride ring spontaneously reacts with primary amine with very high yield and results in an amide bond and one free carboxyl group [3]. When 1-(3-aminopropyl)imidazole bearing primary amine group is applied, each repeating unit (RU) yields both imidazole (pKa ~7) and carboxyl group (pKa ~4.5) at its terminations with the ratio of 1 : 1 as shown in Figure 1. At low pH (pH < 3.5), both carboxyl group and imidazole are protonated, thus CRS exhibits one positive charge per RU. At higher pH (pH > 8), both carboxyl group and imidazole are deprotonated, thus CRS exhibits one negative charge per RU. (Figure 2) Therefore, CRS is anticipated to control electrostatic interaction (i.e., attraction and repulsion) with nucleic acids containing negative phosphate group (pKa ~2).

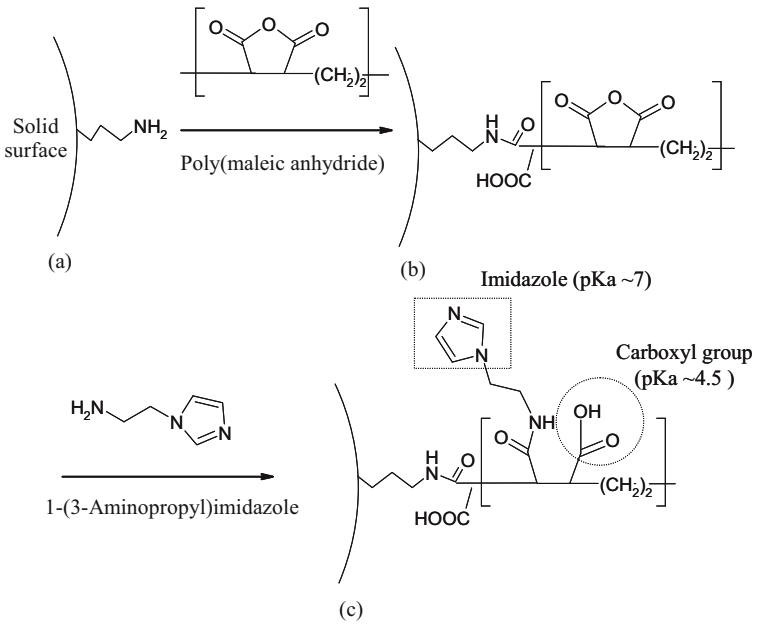

Figure 1: Synthetic scheme for the preparation of CRS. (a) Amine-functionalized silica surface generated by reacting with APTES, (b) Tethering of poly(ethylene-alt-maleic anhydride) onto (a), (c) Ring opening of maleic anhydride with 1-(3-aminopropyl)imidazole.

EXPERIMENTAL

Synthesis of CRS In order to fabricate a CRS, silica beads (SUNJIN Chemical, Korea) with a diameter of *ca.* 15 μm were utilized as a solid support. At first, the silica beads were reacted with 100 mM of 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich) in ethanol at RT with stirring for 1 hr to introduce primary amine groups. They were filtered, washed with fresh ethanol three times, and dried at 100 °C for 1 hr. The amine-modified silica beads were next immersed in a solution of 200 mM poly(ethylene-alt-maleic anhydride) (Mw. 100,000-500,000, Sigma-Aldrich) based on RU (ethylene maleic anhydride) in N-methyl-2-pyrrolidone (NMP) and stirred under N_2 at RT for 1 hr. And then, they were filtered, washed with NMP and ethanol successively, and vacuum dried. Finally, the anhydride-modified silica beads were immersed in a solution of 1-(3-aminopropyl)imidazole (200 mM, Sigma-Aldrich) in NMP and stirred under N_2 at RT for 1 hr. Thereaf-

ter, the CRS-modified silica beads were filtered, washed with NMP and ethanol successively, and vacuum dried. To characterize surface state of silica surface at each synthetic step, infrared spectra (FT-IR spectroscopy, Bio-Rad FTS6000) were collected on KBr crystals.

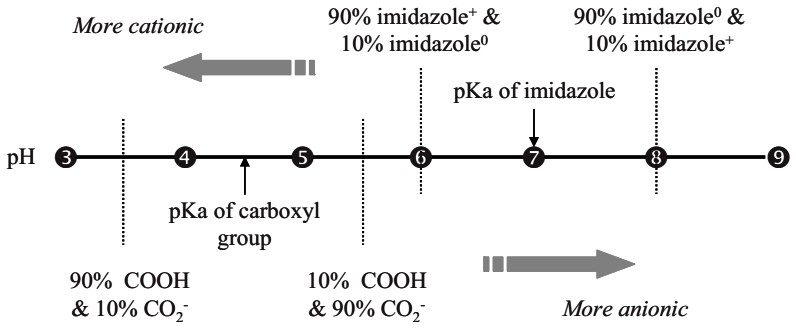

Figure 2: Scheme for the charge change per RU of CRS with varying pH.

Measurement of DNA binding efficiency DNA binding efficiency of CRS was calculated based on UV absorbance (NANODROP™ ND1000, Thermo Fisher Scientific). Three different types of DNA, deoxyribonucleotide triphosphate mixture (dNTP), oligonucleotide with 17 and 48 base pairs (bps) (Bioneer, Korea), were prepared to have the initial concentration of *ca.* 200 ng/μL in various solutions. As 200 μL of DNA solution was used for binding DNA, the applied total DNA amount was *ca.* 40 μg. The CRS-modified beads (0.01 g) were allowed to mix with DNA in a microcentrifuge tube for 3 min. 100 mM of sodium acetate solutions having different pH (3, 4.5, 5, 5.5, 6.5) were employed for this experiment. After mixing with the silica beads, a brief centrifugation was performed, the supernatant was recovered, and its DNA concentration was measured. The bound DNA quantity was calculated by subtracting the residual DNA from the applied DNA amount.

CRS bead-packed PDMS channel PDMS (Sylgard 184, Dow Corning) channel was fabricated to have the internal volume of 10 μL. The channel could be filled with the CRS-modified silica beads by using glass wool as a filter. The microfluidic test module was constructed using a syringe pump (Harvard PHD2000) attached with a custom manufactured jig. It was mounted on fluorescence inverted microscope (Nikon TE300) in order to measure the fluorescence intensity of CRS-modified beads at each DNA manipulation steps such as priming, DNA binding, washing, and DNA releasing. Sodium acetate solution having pH 3 (100 mM) for priming (50 μL), binding (100 μL), washing (100 μL) and Tris-HCl solution having pH 8 (100 mM, 100 μL) were used for elution step, respectively. Each solution was passed through the PDMS channel with the constant flow rate of 50 μL/min. Binding solutions contained two different size of DNA, Cy3-labelled oligonucleotide (50 bps, 1 μM, Bioneer, Korea) and home-made Cy3-end-labelled PCR product (385 bps, 20 nM), respectively.

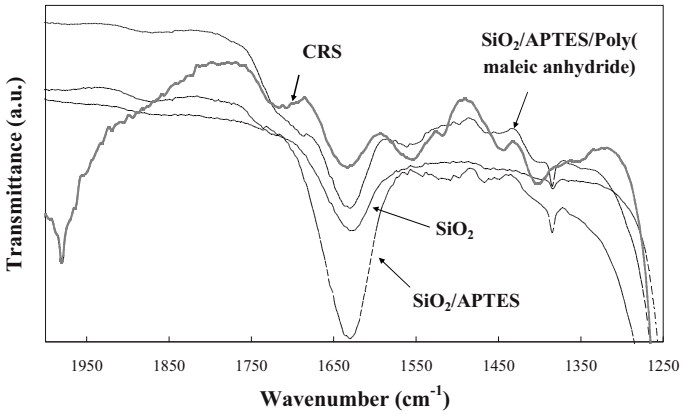

Figure 3: FT-IR spectra of silica beads at each synthetic step.

RESULTS AND DISCUSSION

In order to confirm whether the CRS was generated on silica beads, IR spectra were obtained at each synthetic step. Even though typical peaks for anhydride group (i.e., a doublet in the 1750-1850 cm^{-1}) on SiO_2/APTES/Poly(maleic anhydride) was not detected, possibly due to hydrolysis during the storage, C=O (1720 cm^{-1}) and amide (1560 cm^{-1}) bonds were clearly appeared after ring opening reaction of anhydride with 1-(3-aminopropyl)imidazole on the CRS spectrum. These results indicated that each reaction was properly proceeded, and then two ionizable groups (imidazole and carboxyl) would be produced on silica beads. To further validate charge reversibility of the fabricated solid surface, its DNA binding efficiency was evaluated in comparison with amine-modified silica beads. Different size of DNA were tested in various solution pH as shown in Figure 4. For DNA possessing 48 bps, the amine-modified silica beads showed the constant DNA binding efficiency (~100%) regardless of the applied solution pH. However, DNA binding efficiency decreased on the CRS-modified beads from 100% (at pH 3) to 0% (at pH 6.5) according to solution pH. In particular, it was drastically dropped as the solution pH exceeded the pKa value of carboxyl group (~4.5). Such tendency was more pronounced when DNA

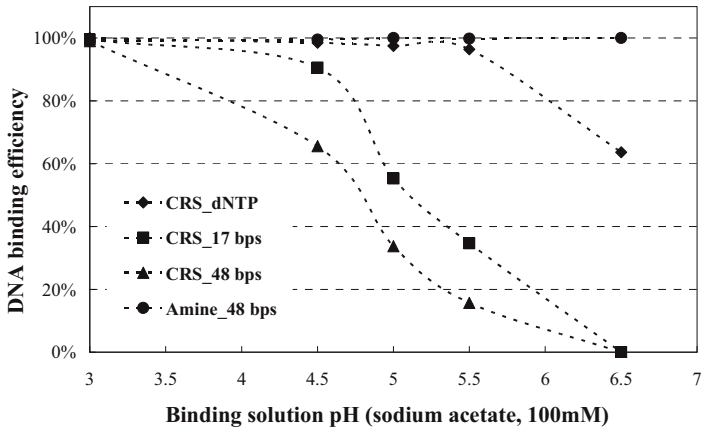

Figure 4: DNA binding efficiency of CRS with varying pH of DNA binding solution and DNA size.

size increased (i.e., number of phosphate group was increased) from dNTP to 48 bps, which suggested that the carboxyl group played an important role in repulsing DNA molecules. Therefore, it was demonstrated that the global surface charge could be switched from positive to negative (or vice versa) by controlling solution pH. Next, DNA attraction and repulsion under microfluidic environments was attempted on the CRS. The CRS-modified beads were packed inside of PDMS channel. For visualization of interaction between DNA and the CRS-modified beads, two types of Cy3-labelled DNA (50 & 385 bps) were applied under fluorescence inverted microscope, respectively. When testing 50 bps of Cy3-labelled DNA, the bare silica beads (SiO_2) in PDMS channel were tested as a control sample. As shown in Figure 5(b) and 6(b), the fluorescence intensity started to increase and reached a certain level while DNA binding solution (pH 3) containing Cy3-labelled DNA was passing through the PDMS channel, which indicated that the injected DNA adsorbed on the CRS-modified beads. The intensity maintained during the washing step (pH 3) and decreased sharply (i.e., DNA eluted) when DNA releasing solution (pH 8) was injected. No fluorescence change was detected on the bare silica beads as shown in Figure 5 (b). Moreover, it was calculated that DNA release efficiency was *ca.* 85% for both DNA types based on the difference of measured fluorescence intensity.

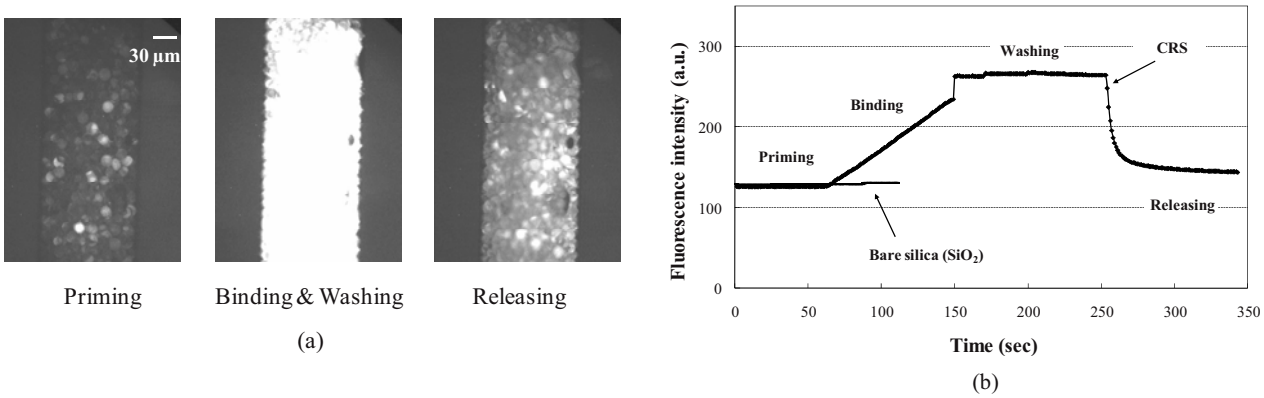

Figure 5: Binding and releasing of Cy3-labelled oligonucleotide (50 bps, 1 μM) on the CRS and the bare silica bead (SiO_2). Fluorescence microscopic pictures (a) and measured intensity at each step (b) were displayed.

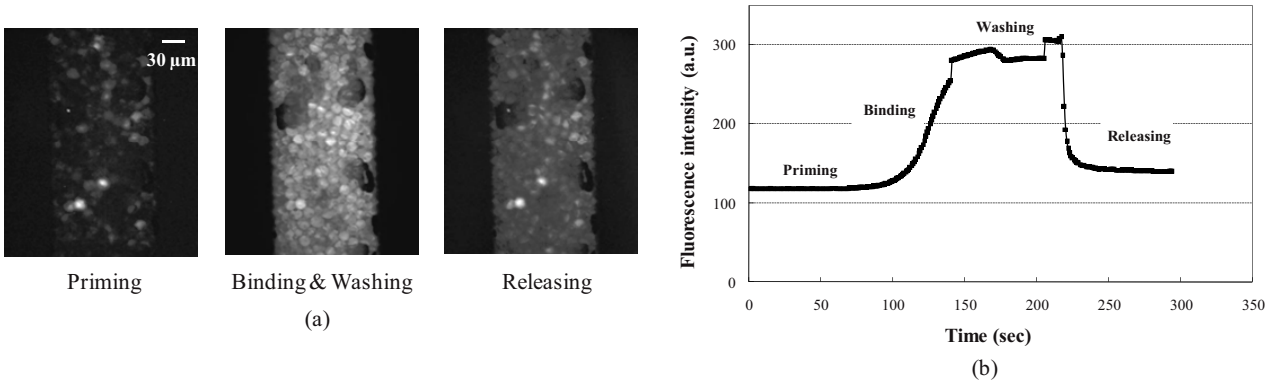

Figure 6: Binding and releasing of Cy3-end-labelled PCR products (385 bps, 20 nM) on the CRS. Fluorescence microscopic pictures (a) and measured intensity at each step (b) were displayed.

CONCLUSION

In conclusion, the charge-reversible surface possessing both positively and negatively-charged groups was successfully fabricated based on maleic anhydride moiety. The fabricated charge-reversible surface demonstrated the potential as an effective DNA manipulation method without chaotrope and organic solvent. Its further application to purification of genomic DNA and smart delivery is in progress.

REFERENCES

[1] K. Y. Hwang and C. E. Yoo, Patent US 74309023

[2] W. Cao, C. J. Easley, J. P. Ferrance and J. P. Landers, *Chitosan as a polymer for pH-induced DNA capture in a totally aqueous system*, Anal. Chem., pp. 7222-7228, 78 (2006).

[3] C-A. J. Lin, R. A. Sperling, J. K. Li, T-Y Yang, P-Y. Li, M. Zanella, W. H. Chang and W. J. Parak, *Design of an amphiphilic polymer for nanoparticle coating and functionalization*, Small, pp. 334-341, 4 (2008).

CONTACT

*K.Y. Hwang, tel: +82-31-2806593; ky4.hwang@samsung.com