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Microintaglio Printing (μ-IP) of Biomolecules using APTES Functionalized Substrate.

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Microarrays are tools used in the studies of expression profiles, analyses of interactions between biomolecules, and so on. Previously, we have demonstrated the usability of microintaglio printing (μ-IP) as a technique to create mRNA and protein microarrays[1-2]. The μ-IP method is more advantageous over conventional methods such as robotic spotters as it creates a very high density array with uniform patterns[3]. In the process optimization of μ-IP of mRNA molecules using PDMS micromold, a gold-coated glass was used as a substrate[3]. For fluorescence based analysis of microarrays, the use of gold as a substrate is disadvantageous as quenching of fluorescence intensity by gold hampers accurate quantitation. Furthermore, light-based manipulation of printed biomolecules during imaging is restricted as only one side of the substrate is transparent. Replacing the gold-coated substrate with a glass substrate is one way to overcome these setbacks. Here, we report the efficiency of μ-IP using (3-aminopropyl)triethxoysilane (APTES) functionalized glass substrate. APTES functionalized glass substrate is prepared using a water based procedure that we had previously studied. The desired biomolecules can then be immobilized onto this substrate using chemical linkers. For immobilization of mRNA onto APTES functionalized glass, it is first reacted with N-(6-Maleimidocaproyloxy)succinimide (EMCS) linker, after which thiolated-linker DNA molecules are joined to the EMCS linkers. mRNA molecules can then be microintaglio printed onto the surface by hybridizing it to the linker DNA. The formation of silane layer on glass is pH dependent and figure 1 shows the results of μ-IP of mRNA using APTES functionalized glass substrates prepared at two different pH. A higher signal-to-noise ratio is obtained using APTES functionalized substrate fabricated at pH 4 as compared to pH 10. A preliminary examination on the possibility of fluorescence based quantification of printed biomolecules using μ-IP and APTES functionalized substrate would also be discussed and compared to that of gold-coated substrate.

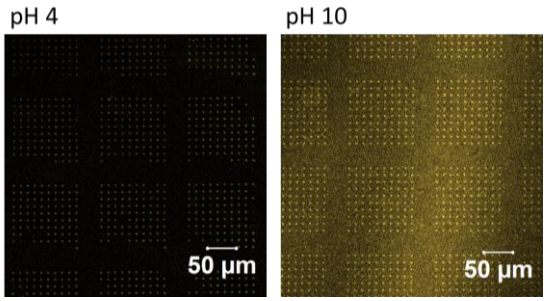

Figure 1: μ-IP of Cy3-labeled mRNA using APTES functionalized glass substrate fabricated at pH 4 and pH 10.

Reference:
(1) Biyani et al., Appl. Phys. Express, 4 ,047001 (2011)
(2) Biyani et al., Appl. Phys. Express, 6, 087001 (2013)
(3) Kobayashi et al., Biosensors and Bioelectronics (2014).