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Visual Sensing System from Polypeptide Nano-Assembly

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It was found that the artificial polypeptide has the ability for the structural color display system based on hybrid with silica substrate. The silicon wafer has been sintered at high temperature, resulting in the formation of pre-colored silica. Silane terminated poly (ε-benzyloxycarbonyl-L-lysine) was synthesized and it was deposited as monolayer on the pre-colored silica substrate using Langmuir -Blodgett (LB) technique. The surface of silica with the peptide monolayer was analyzed with infrared spectroscopy and atomic force microscope (AFM). The secondary structure of polymer, α-helix was also confirmed by circular dichroism spectroscopy. The ε–benzyloxycarbonyl group was removed from the monolayer of polypeptide to obtain poly (L-lysine) system. Finally, a bio-molecular sensing unit can be immobilized on to the poly (L-lysine) color plate for the preparation of biosensor i.e. a visual sensing of biological materials.

Key words: structural color, biosensor, polypeptide, LB technique, AFM

1. INTRODUCTION

Research on biosensors increased attention being given to the development of new synthetic materials that truly mimics the different colors of butterfly wings. This form of color is usually known as structural color. It is formed due to the reflection and interference of scattering light. It is also fundamentally differs from the usual coloring by dyes and pigments. The structural color will change with the angle of incident light. The development of chameleon-type display system would be an impressive accomplishment in our group [1, 2]. Based on this principle, the RNA aptamer are immobilized on to the peptide monolayer deposited color plate and it was developed for the identification of bacteria which is called as biochips. The biochips are one of the key technologies in biology [3]. Here, the biological materials are hybrid with pre-colored silica (SiO_2) substrate which is used for the entire studies. In order to bind the RNA aptamer on the silica substrate, the suitable polypeptide has been selected i.e.poly (L-lysine) (PLL). In this approach, the pre-colored silica surface is first derivatized with PLL in order to have a stable surface charge.

2. EXPERIMENTAL

The silane-terminated poly (ε-bezyloxycarbonyl-L-lysine) (PBCL) was synthesized from its NCA (BCL) in DMF solvent medium at 25°C for 24 hr with the presence of 3-aminopropyltrimethoxysilane as an initiator. The silicon wafer has been sintered at high temperature, resulting in the formation of pre-colored silica.

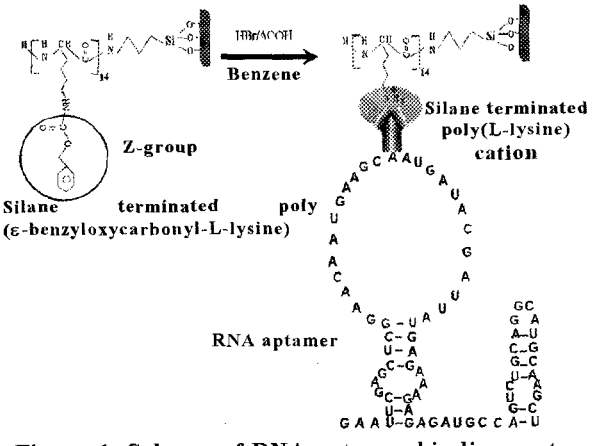

Figure 1. Scheme of RNA aptamer binding on to cationic surface of poly (L-lysine) $_{14}$

The polymer dissolved in DMF: Benzene (3:7) mixture and it was spread over the pure water subphase at 25°C and it was allowed for few minutes for the evaporation of solvent mixture and finally the monolayer was transferred by upward drawing LB method to the pre-colored silica substrate at a constant surface pressure (~5mN/m). Further, the monolayer deposited silica substrate annealed at 110°C, 20min. for the fixation and then treated with HBr/AcOH/Benzene in a sonicator at room

temperature for 120 min to the protonation and removal of ε-benzyloxycarbonyl group (`Z` group) from the monolayer of polypeptide to obtain cationic poly (L-lysine). The monolayer layer of substrate was rinsed with toluene, acetone, ethanol and distilled water, and dried in room temperature. Finally, a RNA aptamer (stem-loop structure) which shows in Figure 1, could be immobilized on to the poly (L-lysine) cationic plates with the appropriate concentration for the identification of bacteria.

3. RESULTS AND DISCUSSION

The polymerization was confirmed by FTIR and the degree of polymerization was evaluated from ^{1}H NMR. The secondary structure of α-helical content was conformed and estimated from Circular Dichroism spectroscopy. From the FTIR analysis, the amide I and amide II adsorption peaks at 1652 cm^{-1} and 1543 cm^{-1}, respectively, indicate the α-helical conformation. The helical content of PBCL is 60% when dissolved in trifluoroethanol.

The silane-coupling agent at the terminal of PLL was anchored by a covalent bond on the surface of pre-colored silica substrate by LB method which shown in Figure 1[4]. α-helix rod of PLL in self-assembled monolayer was arranged in well manner at surface pressure

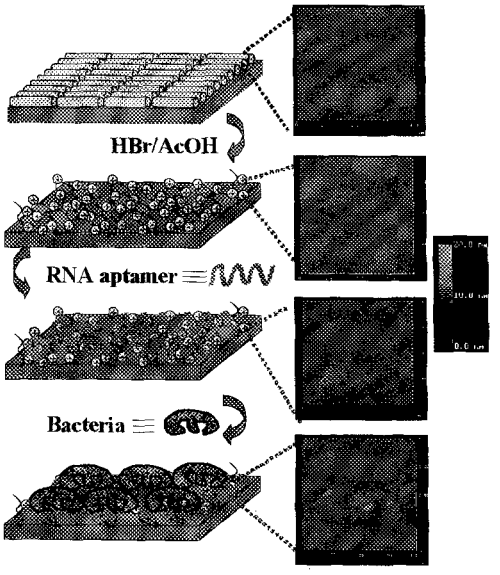

Fig.2 Schematic representation of biochip preparation and its application for an identification of bacteria

5mN/m and also confirmed by AFM topology (Figure 2). The typical surface-area (π-A) isotherm has been found in air-water interface for silane-terminated PBCL. Further, the α-helix

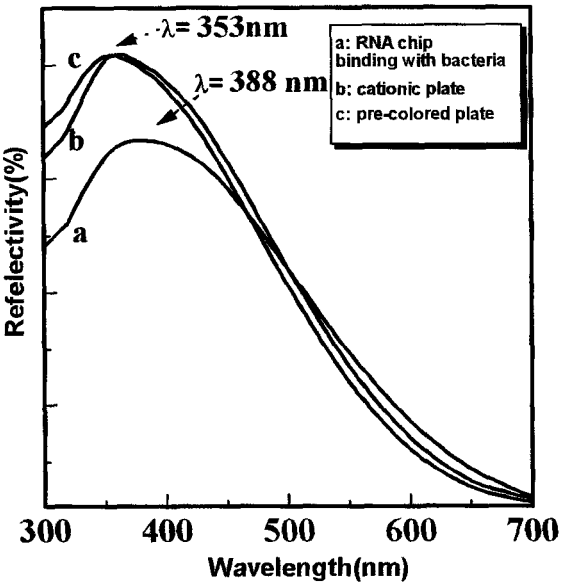

Fig.3. UV-Visible reflective spectra profiles of biochip at 10° incident angle.

structure becomes random coil after removing the Z-group as well as the formation of cationic charges on the surface. The surface film properties including thickness and wettability were changed significantly when PBCL converted to PLL cationic surface [5].The stability of the poly(L-lysine) monolayer was exist very well when the chip was rinsed with different solvents during the cleaning process of debenzylation method. It was also monitored and conformed by AFM topology (Figure-2).

Further, we have selected suitable RNA aptamer which can bind with high specificity and affinity to respective target bacteria. The RNA aptamer immobilization carried out based on the electrostatic interaction between Poly (L-lysine) and RNA aptamer which contains cationic and anionic charges respectively. Figure 2 shows a surface morphology of the plate after successful immobilization of RNA aptamer on PLL cationic surface, hereafter which is called as RNA chip.

The RNA chips are tested with Sphingobium yaoikuyae bacteria solution, the AFM topology also certainly distinguished from the original RNA chips which also shown in Figure 2. The surface strains were observed in the morphology when bacteria were identified with RNA aptamer. In addition, the RNA chips showed distinct color spots after immersion into Sphingobium yanoikuyae bacteria solution which is also confirmed by UV-reflection spectroscopy analysis. In Fig.3, a small shift on the spectrum peak is shown in between the UV and visible region when compared the biochips with before

and after dipping into the bacteria solution. This is a good characteristic evidence for the change of biochip color after binding with bacteria.

4. CONCLUSION

The results are proved that biologically hybrid pre-colored silica substrates are sensitive and longer-lasting probe and its detection process extremely well and worked out as a biosensor. The self-assembled monolayer of polypeptide is playing a key role in addition with oxidized layer of pre-colored silica. It was demonstrated as a new category of stimulus-responsive system after immobilization of RNA aptamer. Even though, we have a big challenge to suitable linkage between the sensing element and surface tethered substance where one may control the surface organization, orientation, density, spatial distribution of the elements and structural color change to optimize biosensing capabilities.

5. ACKNOWLEDGEMENT

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