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Construction and Estimation of Inorganic-Organic Nanostructured Sensing Plate

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Structural color is well known as one of the system to display various colors. Using this system, we tried to construct novel litmus type colorful sensing tip. We prepared two types of structural color tips using α-helical polypeptide LB film and SiO_2 thin-film on the silicon surface. Depending on the thickness of each thin films, these tips showed various colors based on Bragg's low. After soaking this tip into aqueous solution of *p*-n-butylphenol, distinct color change was confirmed. This color change was originated from swelling of polypeptide film by *p*-n-butyl-phenol sorption. It was also observed that the color change has linear relation with the concentration of *p*-n-butylphenol. We also prepared biotin (antigen) labeled SiO_2 thin-film which showed the structural color. The tip changes its color in aqueous solution of avidin (antibody). This color change was corresponding to the thickness of avidin monolayer formed on biotin site. Developing of these types of structural color tips will lead to construct a novel visual sensing system.

Key words: LB film, structural color, antigen-antibody reaction, visual sensor

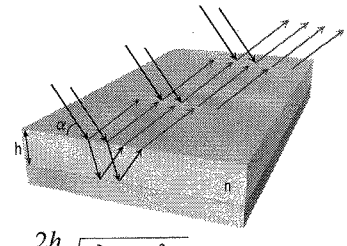
INTRODUCTION

The beautiful color originated from interference of visible light based on highly regulated periodic structure is well known as "structural color". Till now, hundreds of researchers have investigated this structural color system. They constructed thin-film interference system, multilayer interference system, opal structure interference and inverse opal structure interference system etc., and the applications of these systems are in progress. For example, some researchers are trying to apply structural color systems to novel functional optical materials[1~4]. Construction of novel visual sensing tip is one of those applications that we also have investigated [5~6]. In our experiment, we prepared two kinds of thin-film interference tips ; one is a polypeptide film prepared by LB method, and the other is SiO_2 thin-film, which is coated by biotin to sense the antibody (polypeptide thin-film and SiO_2 thin-film). These thin-films showed various color based on interference of visible light. And its color conditions have linear relation with thickness of thin-film. In this paper, we examined the influence of the structure change of the film caused by endocrine disruptor sorption or antigen-antibody reaction upon their color change.

EXPERIMENTAL

Sample preparation (polypeptide thin-film interference tip (polypeptide tip)).

We successfully prepared hydrophobic α -helical polypeptide (PHeLG) by NCA method. And its monolayer at air/water interface was transferred onto Si wafer (Nilaco (100) 0.5mm thickness) to obtain their LB films (40~160 layers) [7~8]. To construct a stable LB film, the Si wafer surface was treated with octadecyl-trimethoxysilane. Originated from PHeLG thin-film formation, reflected visible lights on its



$$\lambda = \frac{2h}{m}\sqrt{n^2 - \sin^2\alpha} \qquad (emphasized)$$

$$\lambda = \frac{4h}{2m-1}\sqrt{n^2 - \sin^2\alpha} \qquad (enfeebled)$$

Fig. 1 Schematic illustration of thin-film interference system and Bragg's equation.

foreground and background are interfered each other, and clear color appeared. Depending on the number of transfer (depending on the thickness of PHeLG thin-film), various colored tips were obtained (Fig. 1)[9~10].

Sample preparation (SiO_2/biotin thin-film interference tip (biotin tip)).

We used Si wafer as a substrate. The wafer was cleaned by acetone and nitric acid. And it was annealed by using electric furnace (950~1120℃). Then, the surface of Si wafer gradually transformed to SiO_2 thin-layer to give bilayer having different refractive indexes. These tips also showed structural color depending on the thickness of configured SiO_2 thin-film. The obtained color tip was deterged by soaking into the solution containing 10% NH_3 and 2.7% H_2O_2 in Milli-Q water for 30 minutes. In order to locate amino group on SiO_2 surface, the tip was soaked into 3-aminopropyl- triethoxysilane (5 v/v % toluene solution) for 24h and annealed at 120℃ for 20 minutes. After that, this amino group reacted with biotin-AC_5sulfo-OSu in PBS buffer (pH=7.4) for 24h[11]. And finally, we obtained a structural color biotin tip (Fig. 2).

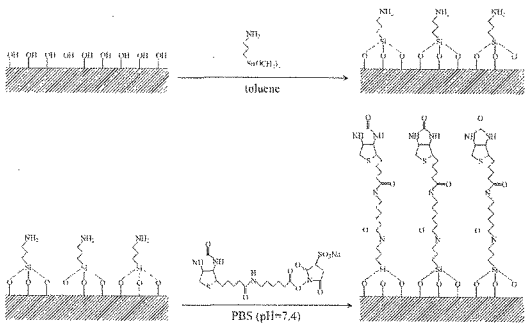


Fig. 2 Schematic illustration of preparation of biotin labeled structural color tip.

Reflection VIS spectrum measurements.

Reflection VIS spectrum measurements were performed by use of a UV/VIS spectrophotometer V-550 (JASCO) together with an attachment, ARV-474 (JASCO) and VIS spectrophotometer LIFES-5501 (MORITEX). All samples were measured with Si wafer spectrum as a background.

RESULTS and DISCUSSION

Polypeptide tip

The polypeptide tip showed various color depending on the thickness of the LB film. These color conditions were much suitable with Bragg's equation. When we put this tip into a vial filled with organic solvent vapor, significant color change was observed. Various kind of organic solvent vapors can induce color change (Fig. 3). From Bragg's equation, h and n (thickness and refractive index of polypeptide film) are the parameters that determine the color condition (λ). Considering refractive index of polypeptide and organic solvents are not so different, these color changes are mainly derived from thickness change. So, the vapor sorption into the polypeptide film induced these color change based on the swelling of film. The tip can recover the original color by drying, thus confirming the reversibility (Fig. 4).

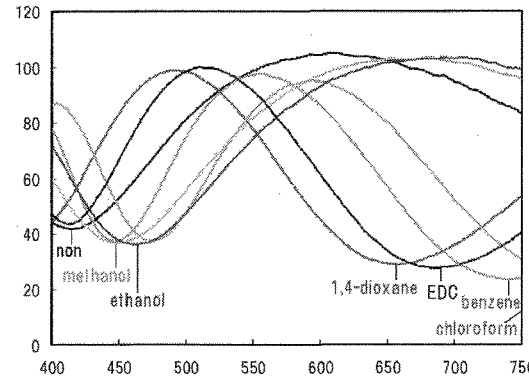


Fig. 3 Reflective VIS spectra of polypeptide tip (119 layers) with various vapor of organic solvents.

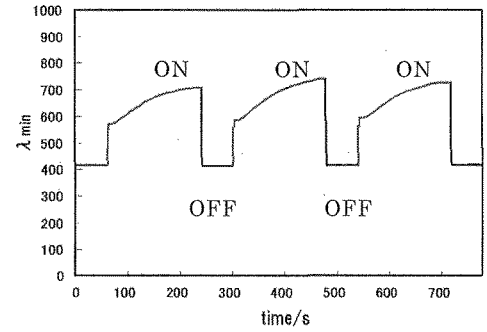


Fig. 4 Reversible color changes respond to sorption and dissociation of benzene.

Using these color changing behavior, it was hopeful to construct novel densitometer. In this experiment, we describe its sensing capability for *p*-n-butylphenol (endocrine disruptor). Then we immersed the polypeptide tips (80 and 120 layers) into several concentrations of *p*-n-butylphenol aqueous solution and checked their color changes by VIS spectrum measurements. After immersing the tip, drastic color changes were confirmed.

And the degree of these color changes, $\Delta\lambda$, estimated from peak position of VIS spectra had a linear relation with the concentration of *p*-n-butylphenol (Fig. 5,6). It was suggested that the concentration of the sample could be quantitatively estimated by the structural color change.

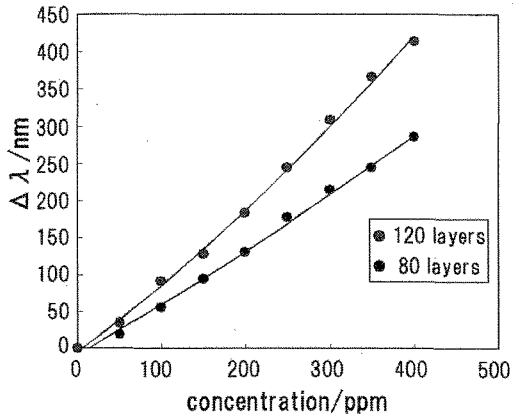


Fig.5 Changes in $\Delta\lambda$ of polypeptide tip (80 and 120 layers) with various concentrations of *p*-n-butyl -phenol in water.

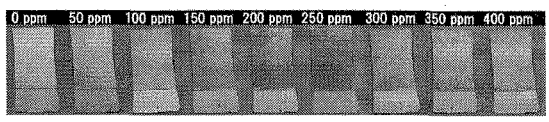


Fig. 6 Polypeptide tips (120 layers) after soaking various concentrations of *p*-n-butylphenol in water.

Biotin tip

Much sensing techniques for specific bio-molecule have been developed. Most of these techniques are using pigments or fluorescent molecules. So we tried to construct novel sensing technique based on visible light interference with no pigments or no fluorescent molecules.

The prepared biotin tip having structural color was immersed into avidin solution (PBS pH=7.4) for 3 hours and well washed with Milli-Q water. After this treatment, definite color change was confirmed from VIS spectrum measurement (Fig. 7). The color change, 8 nm of red-shift in λ_{min}, was saturated above avidin concentration of 1.0×10^{-7}M. Then we observed surface topography change by AFM measurement. The uniform avidin monolayer formed on the tip surface was confirmed and its thickness was about 4 nm (Fig. 8). This thickness corresponds to avidin diameter and consistent with the red-shift of λ_{min} ,8 nm, estimated from Bragg's equation (refractive index, 1.5, of SiO_2 and avidin are used). This showed that the surface structural change based on antigen-antibody reaction induced the structural color change.

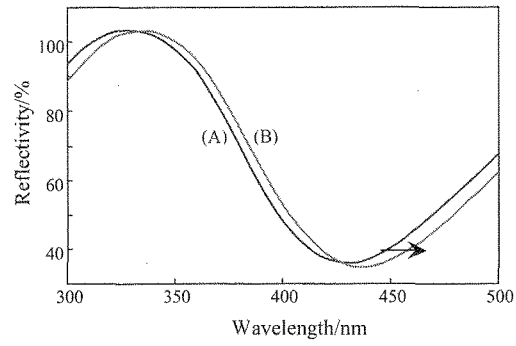


Fig. 7 Reflective VIS spectra of biotin plate before (A) and after (B) soaking in avidin solution (1.0×10^{-5}M, pH=7.4) for 3h.

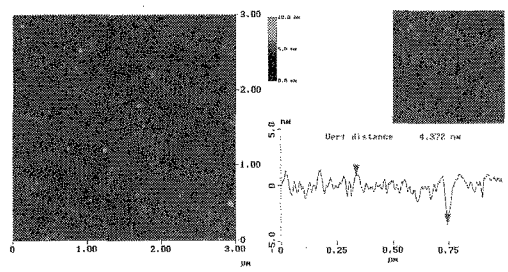


Fig. 8 AFM images of surface of biotin tip after soaking in avidin solution ((1.0×10^{-7}M pH=7.4).

As a complementary experiment, we checked if this reaction is specific or not. Inactive avidin could be made by adding biotin to the avidin solution (avidin: biotin=1:50). Figure 9 shows responsiveness of the biotin tip for the inactive avidin and free avidin. Only in the case of adding active avidin, biotin tip changed its color immediately. So it is clear that this color change is derived from specific avidin-biotin binding.

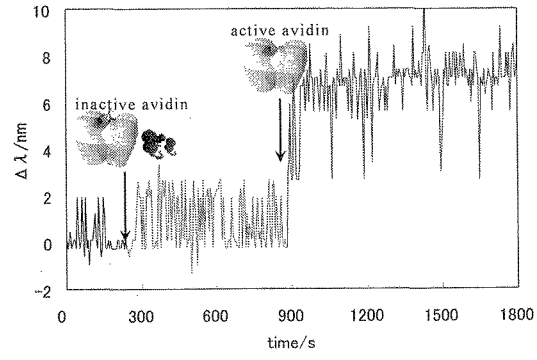


Fig. 9 Changes in $\Delta\lambda$ of biotin tip induced by adding inactive and active avidin (1.0×10^{-6}M).

CONCLUSION

In this study we could construct two types of thin-film interference tips (polypeptide tip and biotin tip). And both of the tips showed structural color based on regulated thin-film structure. The color is dependent on the thickness of thin-films according to Bragg's equation.

Polypeptide tip changed its color originated from sorption and dissociation of low molecular weight molecules. It was showed that the degree of color change had a linear relation with aqueous concentration of *p*-n-butylphenol.

The biotin tip showed a specific binding with avidin; as a result, 8 nm of structural color change was observed. This value corresponds to the thickness of avidin monolayer.

Developing of these types of structural color tip will lead to construct a novel sensing system using visible light.

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