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### Patterning and Immobilization of Cytochrome C Using Conducting Sulfonated Polyaniline Micro Arrays

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## Patterning and Immobilization of Cytochrome C Using Conducting Sulfonated Polyaniline Micro Arrays

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A cytochrome c onto photochemically patterned conducting sulfonated polyaniline (SPAN) was immobilized by using a self-assembly technique. The SPAN polymer was employed as a specific binding site for cytochrome c. The electrical conductivity of SPAN was allowed by doping of HCl. Physical and electrochemical properties of the self-assembled cytochrome c monolayer were investigated from the measurements of cyclic voltammetry and atomic force microscopy (AFM). Especially, the electrochemical activity of self-assembled cytochrome c immobilized on the SPAN with a high electrical conductivity was superior.

**Keywords:** cytochrome c; SPAN; pattern; self-assembly;  
electrochemical activity

### INTRODUCTION

The use of conducting polymers in the development of analytically useful biosensors or biodevices has been received much attention for

detection of electro active ion or protein. Especially, pattern formation and recognition by a self-assembled monolayer technique have currently studied. The self-assembled monolayer serve not only to create bioelectrochemical interface but also to shield protein from the high energy metal surface and prevent structural unfolding and nonspecific adsorption of protein. In this work, we have immobilized a cytochrome c onto photochemically patterned conducting SPAN using a self-assembly technique and then investigated the dependency of electrical conductivity of SPAN on the electrochemical activity of cytochrome c.

## EXPERIMENTAL

SPAN was synthesized by the polymerization of aniline-2-sulfonic acid with an ammonium peroxydisulfate as oxidant agent in pyridine aqueous solution. Pattern formation of SPAN polymer was carried out using a conventional photolithographic technique. SPAN was spin coated on glass substrate. After UV resist was spread on the SPAN film, it was exposed by using a 500 W high-pressure mercury lamp in conjunction with a narrow band pass filter for 254 nm (Spectral Energy Co.), developed and removed (Figure 1). Finally, cytochrome c was immobilized on the conducting SPAN by using a self-assembly technique.

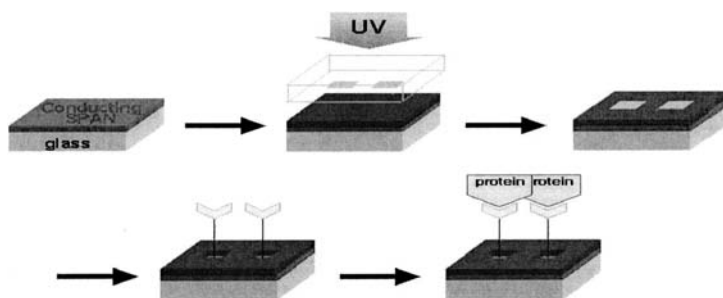


Figure 1. Patterning procedure of self-assembled cytochrome c monolayer using a conducting SPAN.

Doping of SPAN polymer was carried out with HCl aqueous solution. The electrical conductivity of SPAN at room temperature was measured using a conventional four-probe method. The surface morphology of self-assembled cytochrome c was observed with an AFM (Autoprobe cp, PSI USA). The redox activity of cytochrome c was investigated through the measurement of cyclic voltammetry (Zahner Elektrik, IM6 system).

## RESULTS AND DISCUSSION

The prepared SPAN polymer was soluble in water and exhibited a low electrical conductivity of  $8 \times 10^{-6}$  S/cm. The electrical conductivity of SPAN increased to  $2 \times 10^{-3}$  S/cm upon HCl doping. Figure 2 showed the positive tone image of SPAN observed with a Nikon optical microscope. Micro array patterns with square of  $2 \mu\text{m}$  side length were obtained using  $40 \text{ mJ/cm}^2$  for polyimide resist material. Figure 3 showed the changes in cyclic voltammograms of self-assembled cytochrome c monolayers immobilized on the patterned SPAN before and after HCl doping. Cytochrome c immobilized on HCl fully doped SPAN exhibited a high current, which is mainly due to the enhancement of electron transfer between cytochrome c molecular interface.

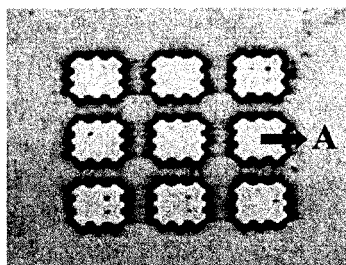


Figure 2. Positive tone image of SPAN.

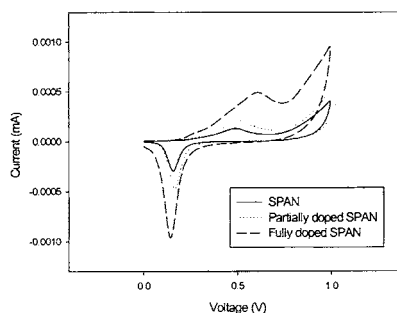


Figure 3. Cyclic voltammograms of cytochrome c immobilized on SPAN before and after HCl doping.

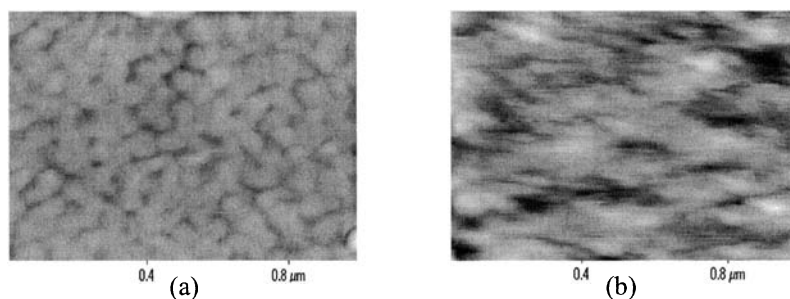


Figure 4. AFM images of patterned conducting SPAN (a) and self-assembled cytochrome c (b).

Figure 4 showed the AFM images of the point A in figure 2 which are the patterned conducting SPAN and cytochrome c monolayer immobilized on the micro array by a self-assembly technique. A uniform and dense self-assembled cytochrome c monolayer was observed. As a consequence, it can be concluded that the sulfonic acid group of conducting SPAN plays a role in specific binding site for orienting the cytochrome c molecule and enabling electron transfer of biomolecule interface.

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