Protein Functionalization of Nanostructured Polymer Surfaces

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Abstract

Immobilization of proteins on polymer surfaces is of great interest for applications in biosensing, cell and tissue culturing, and medical device coating. This research studied the functionalization of a fluorescent protein on a structured polymer surface. Copolymerization of 4-trifluoroacetyl-[2.2]paracyclophane and 4-amino-[2.2]paracyclophane by a vapor deposition technique result in the formation of slanted, columnar, porous structures of the copolymer poly(*o*-trifluoroacetyl-*p*-xylylene-*co*-o-amino-*p*-xylylene-*co*-*p*-xylylene), (PPX-$COCF_3$-NH_2). The coupling of green fluorescent protein (GFP) to the structured and planar (control) polymer surfaces was studied by chemisorption (i.e. using a linking reagent, hexamethylene diisocyanate (HMDI)) and physisorption (i.e. without any linker). The fluorescence intensity of GFP on the surfaces was measured by an optical microscope and the data was analyzed using imaging software. The fluorescence intensity on the structured surfaces was higher than planar surfaces. This method will open a new wealth of applications to functionalize proteins that have desired functional groups for biomedical applications.

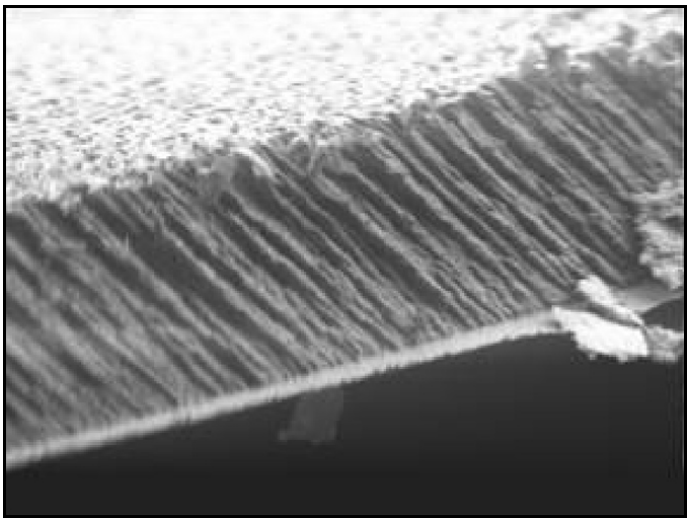

Figure 1: Cross sectional SEM image of a structured PPX film [2].

Introduction

Nanostructured poly(p-xylylene) (PPX) films are of great interest because of their unusual physical and chemical properties such as surface chemistry, morphology, and topology [1]. Nanostructured PPX films have increased surface area which enhances the efficiency of functionalization, and this is the significant difference between a planar and structured surface (Figure 1) [2]. Planar surfaces have functional groups only on the top surface whereas structured surfaces have spaces between the columns of porous polymer strands, which provide more functional groups available for attachment to proteins.

Experimental Procedure

Surface functionalization was achieved by placing the films into a flame dried 50 mL round-bottom flask. First 5 mL of anhydrous toluene, 30 μL of HMDI, and a small amount of catalyst (di-n-butyl tin dilaurate) were added to the flask and the top was capped. The reaction was let go for 4 hours. The film was removed from the flask, washed subsequently with toluene and placed in the desiccator to dry for 20 minutes.

GFP coupling to the surfaces was achieved by measuring 5 mL of GFP solution into a 50 mL round-bottom flask. The film in the desiccator was removed, placed into the flask, submerged, and the flask was capped. The reaction was let go overnight. The film was removed from the flask and placed in the desiccator to dry for 20 minutes. The film was removed from the desiccator, washed subsequently with deionized water and placed in the desiccator to dry. The schematic of protein coupling onto a functionalized structured PPX surface is shown in Figure 2.

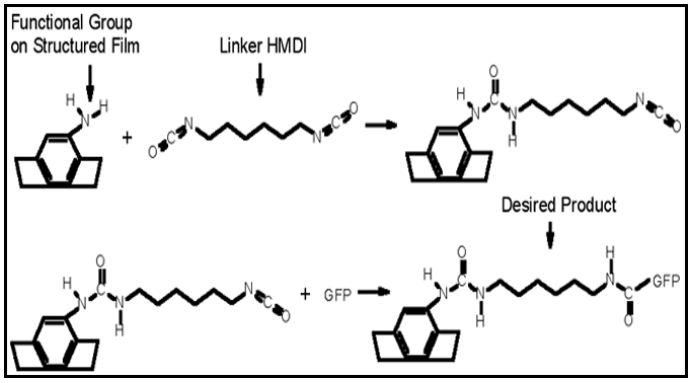

Figure 2: Schematic of protein coupling to PPX surfaces.

GFP imaging was performed using the Olympus Fluoview 300 confocal laser scanning microscope with a single-line 488 nm blue laser using the 40X oil objective. The intensity per field analysis was performed using ImagePro Plus 5.0 [3].

Results and Conclusions

Using the crosslinking agent (HMDI) surface coupling between the reagent and the amino groups on the film was achieved. To characterize the surface of the film, it was run on the FT-IR spectrometer and the resulting spectrum showed 2 peaks that were indicative that the surface coupling reaction linked the amino surface to the coupling reagent (HMDI). The spectra of the unreacted film and HDMI coupled film is shown in Figure 3. The IR spectrum shows an amide bond peak (between 1630-1695 cm^{-1} for the C = O stretching and between 3300-3500 cm^{-1} for a secondary amine). An isocyanate peak at 2200 cm^{-1} is also observed.

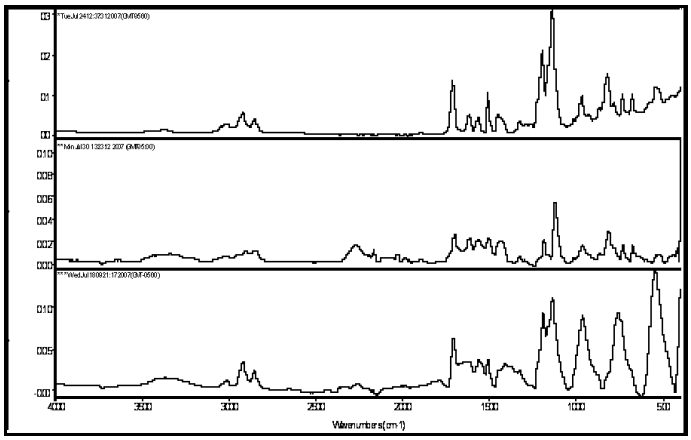

Figure 3: IR spectra: amino film (bottom line), amino film with HMDI (middle line), amino film with GFP (top line) [3].

Next, GFP was attached to the free isocyanate group on the surface. In addition to the fluorescence imaging of PPX surfaces, an IR spectrum confirmed the coupling of GFP to the surface (Figure 3). The spectrum shows that the free isocyanate group has reacted with the GFP.

The GFP intensity was measured in four different conditions using the confocal microscope: (i) a planar PPX-$COCF_3$-NH_2 surface where the GFP is linked with HMDI (planar-chemisorption); (ii) a structured PPX-$COCF_3$-NH_2 surface where the GFP is linked with HMDI (structured-chemisorption); (iii) a planar PPX-$COCF_3$-NH_2 surface where the GFP is adsorbed to the surface without a linker (planar-physisorption); and (iv) a structured PPX-$COCF_3$-NH_2 surface where the GFP is adsorbed to the surface without a linker (structured-physisorption). The fluorescence intensity results are shown in Figure 4 [3].

Figure 4 shows that nanostructured PPX films have higher intensities compared to planar PPX films. It was also observed

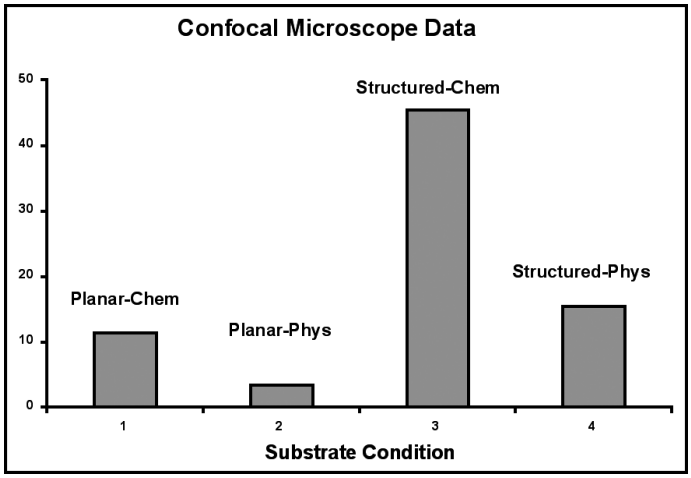

Figure 4: Fluorescence intensity results.

that GFP physisorption on nanostructured PPX films is higher than planar PPX films. This is due to the increased surface area and the porosity of nanostructured PPX films. However, the overall GFP attachment is highest when the GFP is chemisorbed to the nanostructured PPX surface. Novel surface properties can be obtained by coupling reagents attached to the structured PPX film. Therefore, the surface may be incorporated into medical devices or other engineering device applications [3].

Future Work

Another proposal would be to functionalize nanostructured PPX surfaces with RGD peptides, seed cells onto the film and monitor the cell growth. The amino acid sequence of RGD interacts with integrin receptor sites and is found in the extracellular matrix; therefore, it is suggested that cells would be highly attracted to the RGD peptides on the surface and increase cell growth.

Acknowledgments

I would like to thank Dr. Melik C. Demirel, Dr. Serhan Boduroglu, the National Nanotechnology Infrastructure Network REU Program, the NSF, and the Penn State Nanofabrication Facility staff and coordinators.

References

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