Fluorescence Microscopy Comparison of Tagged Protein/Antibody on Mesoporous and Nonporous Surfaces

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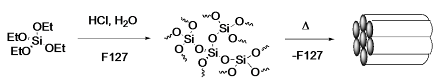
Introduction

Mesoporous (2-50 nm diameter pore) silicates have applications including nanofilters, drug delivery agents, catalysts, and sensors. For example, mesoporous silica has been reported to recognize toxic compounds, small molecules such as VOCs, and proteins/enzymes.[1] This collaborative research involves biosensor design and applications. The transducer is a photonic bandgap multilayer (PBGM). The sensitivity of the sensor is designed to be maximized when the sensing event takes place *inside* the last layer of the PBGM - up to 3 times the field enhancement over a multilayer designed to maximize field at the air-PBGM surface (Figure 2). Therefore, a mesoporous SiO_2 thin film final layer has the potential to make sensors superior to those with merely surface-active substrates. SiO_2 films of reproducible 0.5 µm thickness were made using the synthesis in Scheme 1. Then, the films were derivatized with 3-aminopropyl triethoxysilane to promote chemical binding.[2,3] A model binding protein/antibody system was developed using Bovine serum albumin protein with a Cy5 tag (BSA-Cy5, fluoresces red) and its antibody with a FITC tag (anti-BSA-FITC, fluoresces green). A modified procedure for surface binding of protein[4] was used to attach BSA to the films and the binding shown to occur *specifically* with high concentration of protein/antibody. The first step to determine minimum detection levels of BSA/anti-BSA binding in porous vs. nonporous SiO_2 materials was a comparison of spots of varying concentration of BSA-Cy5 on the two SiO_2 materials using fluorescence microscopy.

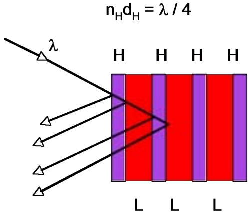


Scheme 1. Sol-gel synthesis of mesoporous silica.

Sensor Design Principle

Ultimately, the transducer/substrate used in detection will be a PBGM. The mode shift of the laser used in an optical sensor device is caused mainly by the binding of material in the final layer of the PBGM (Figure 2).[5]

$$n_H d_H = \lambda / 4$$



$$n_L d_L = \lambda / 4$$

Figure 1. Representation of SiO_2/TiO_2 multilayered PBGM.

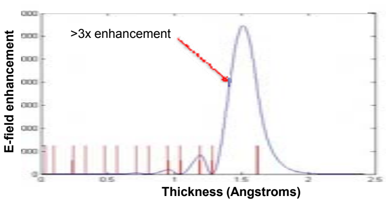
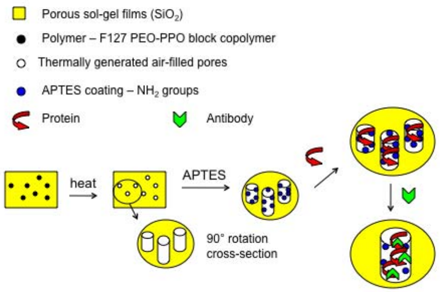


Figure 2. PBGM w/porous SiO_2 final layer.

Design Scheme

The mesoporous film was derivatized with amine, protein, and antibody according to Scheme 2.



Scheme 2. SiO_2 film drying, coating, and binding.

Materials and Methods

Mesoporous Film Synthesis.[6] The sol-gel was prepared from F127 (2.0 g) dissolved in ethanol (12.93 mL) in a round bottom flask. TEOS (4.55 mL) was added, followed by H_2O (0.8 mL) and 2M HCl (0.112 mL) and ethanol (7 mL). The solution was heated at reflux for 1 hour and cooled. The solution was pipetted (40µL) onto clean silica substrates and spun to a film with a SCS Spin-Coater 6204 at 2000 rpm for 30 s. The appropriate rate is 1ºC/min to 400ºC (1 h), followed by 1ºC/min to 600ºC (1 hr).

APTES Coating.[3] A 1% v/v solution of APTES was prepared in toluene in a clean and dry volumetric flask in a drybox. The solution was poured into a staining jar containing films on fused silica 1"x1" slides. The substrates were removed after 1 h then rinsed in toluene twice, dried under a stream of N_2, and heated on a hotplate for 10 minutes at 110º C.

Electron Microscopy. Samples of films were prepared for TEM analysis by scraping the film onto a Formvar-coated Cu grid in water. Samples were examined with a Hitachi Transmission Electron Microscope H-7650. Results in Figure 3 show pores on the order of 10 nm that remain open after APTES coating.

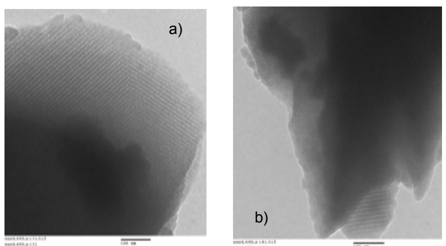


Figure 3. SiO_2 film a) before APTES b) after APTES, scale bar = 100 nm.

Protein Processing.[4] After slides were coated with APTES and dried completely, protein was applied to each slide's surface. BSA-Cy5 was used at a concentration of 0.2 µg/µL. After drying, slides were heated on a hot plate at 95ºC for 25 min. Surfactant (sodium dodecylsulfate, 0.1%) was washed over the slides for 5 min. Then, they were placed in DI water for 3 min. ArrayIt blocker was applied at 1 µL/mm² of coverslip. The blocker reacted for 1h.

Antibody Binding.[4] Antii-BSA-FITC was added at a concentration of 100 µg/mL prepared in ½ volume DI water and ½ volume ArrayIt blocker using 1µL/mm² cover slip. The slide was then placed in the dessicator for 30 minutes. The cover slip was then removed by dipping in a wash station with 0.1% SDS.

Fluorescence Images

Binding of BSA-Cy5 and anti-BSA-FITC was tested using fluorescence microscopy. The results in Figure 4 show that the binding here is specific and isolated to the spot region. Also, the lack of fluorescence beyond the spot edge indicated that the antibody bound specifically to the protein instead of nonspecifically to the slide.

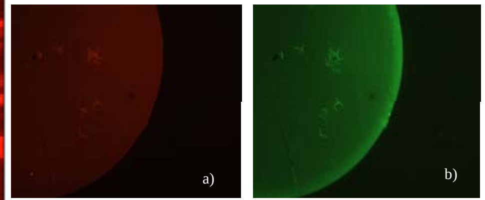
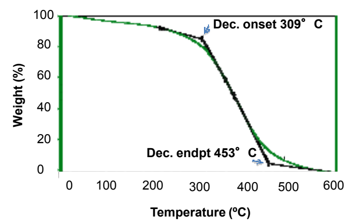


Figure 4. Fluorescence images of: a) BSA-Cy5 (red) and b) anti-BSA-FITC (green).

Physical Characterization

The calcining rates were determined through thermogravimetric analysis. The slow process minimized macroscopic cracks.



Thicknesses were measured with a KLA Tencor 7 profilometer. Water contact angle goniometry (Rame-Hart 100 Goniometer with tilt stage) was used to determine hydrophobicity.

Table 1. Results of Goniometry and Profilometry

Sample	Contact Angle After Calcification		Contact Angle After APTES		Profilometry (Å)
	${}^a\theta_r$ (º)	${}^b\theta_a$ (º)	θ_r (º)	θ_a (º)	
F127 SiO_2	15 ± 2	17 ± 2	39 ± 2	46 ± 2	4338 ± 30

[a]Receding angle, [b]advancing angle

Sensor Device

Ultimately, the biosensing event will be detected with label-free sensing using a surface optical wave device shown in Figure 5.

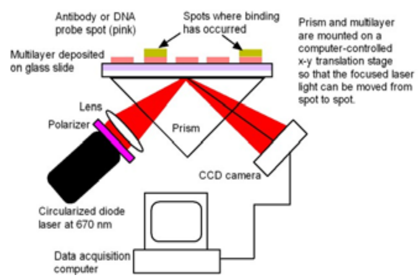


Figure 5. Sensor array device design (Dr. Robertson, MTSU Physics).

Selectivity Limitations

In order to create an array of BSA-Cy5 concentrations for microscopic comparison, 1 µL spots of BSA were applied to a 1" x 3" commercially amine-coated slide (ArrayIt SuperAmine). The spots varied in concentration from 0.2 µg/µL to 0.01 µg/µL. Figure 6 is a schematic of the slide. This experiment is in progress.

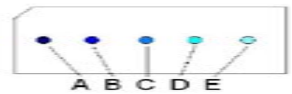


Figure 6. amine-coated slide with spots of varying BSA-Cy5 concentration. A=0.2 µg/µL, B=0.1 µg/µL, C=0.04 µg/µL, D=0.02 µg/µL, E=0.01 µg/µL

Conclusions

- Sol-gel synthesis and spin-coating of films was reproducible and gave profilometric film thicknesses average of 0.5 µm after calcining.
- Drying at room temperature, followed by controlled heating to 400 ºC then final calcining at 600 ºC prevented excessive microscopic cracking.
- Goniometry analysis shows that APTES coating changes the advancing water contact angle by +30º. Hysteresis is 7º.
- Films coated with APTES for one hour showed properties indistinguishable from those coated overnight: thickness, TEM, and contact angle.
- Protein showed evidence of specific binding to amine-coated mesoporous silica by fluorescence microscopy.
- Antibody bound specifically to protein.

References

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