A SERS Substrate for Detection of *E.Coli* on Nanostructured Poly(p-xylylene)

N. Malvadkar*, P. Kao**, H. Wang*, D.L. Allara**, M.C. Demirel*

* Department of Engineering Science and Mechanics, Pennsylvania State University, University Park, PA 16802, mdemriel@engr.psu.edu
** Department of Chemistry, Pennsylvania State Univeristy, University Park, PA 16802

ABSTRACT

Surface enhanced Raman spectroscopy (SERS), as the basis of rapid and reliable biosensing techniques, has significant advantages of minimal sample preparation and ease of operation compared to other culture amplification methods. However, applications to biomedical problems have lagged because current substrates lack highly reproducible SERS responses to biological samples. Recently, we have developed nanostructured polymer substrates that can provide a highly uniform and reproducible nanoscale roughness. In this article, we studied advanced types of SERS substrates that will provide a highly uniform and reproducible "fingerprint" of *E.Coli* on a nanostructured polymer substrate coated with SERS active metals.

Keywords: SERS, nanostructured polymer, poly(p-xylylene), *E.Coli*, Raman spectra

1 INTRODUCTION

Surface enhanced Raman spectroscopy has received much attention due to its capability for single molecule detection[1] and a variety of applications including rapid DNA sequencing[2], pathogen detection[3], nanostructure characterization[4], and food analysis[5]. One of the most promising broad based applications for advanced, high quality SERS substrates would be in the field of diagnostic microbiology with critical applications such as the detection of bacterial and viral pathogens where minimal sample preparation effort, ease of operation, and rapid and reliable detection would offer a great advantage compared to other techniques.[6,7] SERS has remarkable analytical sensitivity but practical diagnostic SERS probes have not been developed. This is due to the difficulty in easily preparing robust and uniform SERS substrates with surface morphologies that can deliver maximum SERS enhancement with high reproducibility.

Here, we present the evidence for a robust, rapid and reliable detection of *E.Coli* on metalized PPX-Cl films. We report the development of a SERS substrate that has high uniformity and spot to spot reproducibility (i.e. individual bacteria) while providing a surface with the capability of sustaining the integral structure of bio-species. The substrates are prepared using a vapor deposition process of poly(p-xylylene) (PPX) based on a recently developed oblique angle polymerization[8-12]. These structures have a high aspect ratio and the production technique does not require any template or lithography method or a surfactant for deposition. The nanostructured PPX films are conformal to any surface and suitable for industrial applications.

2 EXPERIMENTAL

2.1 Materials

All chemicals were A.C.S reagent grade and were used as received. Deionized water of 18.1 MΩ-cm was used for all experiments using Barnstead Nanopure Diamond™ dispenser. Dichloro-[2,2]paracyclophane (DCPC) (PDS, Katy, TX) was used to prepare the poly(o-chloro-p-xylylene) (PPX-Cl) films which were deposited on p-type Si(100) (Wafernet Inc. San Jose, CA) substrates.

2.2 SERS Substrate Preparation

Si (100) wafers were first washed in acetone to remove the loosely bonded contaminants. For the removal of chemisorbed organic contaminants the wafers were treated with a 1:1 (v:v) solution of HCl and methanol for 30 minutes. They were then washed with copious amounts of water and dried using N_2 gas. The wafers were then kept in concentrated H_2SO_4 for 30 minutes for the formation of hydroxyl groups on the surface. The wafers were sonicated in water for 10 minutes. They were dried thoroughly using N_2 gas. Self-assembled monolayer (SAM) solution was prepared by adding 1% allyltrimethoxysilane (Gelest, Morrisville, PA) in toluene containing 0.1% acetic acid. The cleaned wafers were transferred to this solution and left for SAM formation for 60 minutes at 25ºC. The wafers were removed after 60 minutes and sonicated in anhydrous toluene for 10 minutes. The wafers were then dried on a hot plate at 140ºC for 5 minutes for solvent removal.

Nanostructured films of poly(chloro-*p*-xylylene) (PPX-Cl) were deposited on the allyl functionalized silicon wafers using oblique angle vapor deposition polymerization under low-vacuum conditions. The details of the experimental procedure are explained previously[11,13]. Briefly, PPX-Cl films were deposited using 0.3g of DCPC at a vaporizing temperature of 175 ºC while the pyrolysis chamber was maintained at 690ºC. The deposition angle was kept constant at α=10º.

A thin layer of gold (i.e., 60nm) was deposited using thermal evaporation from resistively-heated tungsten and tantalum boats onto PPX-Cl surface. The cryogenically pumped deposition chamber was maintained at a base pressure of ~1x10^{-8} torr. The thickness of the gold films was monitored using a parallel QCM.

2.3 Bacterial Cultivation

E.Coli (Invitrogen, Carlsbad, CA) was cultivated for 16h at 37°C on a LB agar base. Single colonies were collected using sterile plastic inoculating loops after sub-culturing the bacteria. The cells were suspended in water and a 10µL this aliquot solution was placed on the SERS substrate for immediate characterization.

2.4 SERS Measurements

Renishaw inVia microRaman with a 35mW HeNe (632.8nm) laser source, a motorized microscope stage sample holder and a CCD detector was used to study the bacterial samples on the SERS substrate. Typical instrument parameters were: 50x objective, 1µm laser diameter, and 10 second acquisition time. For normalizing the variation of power in different scans, a fixed silicon wafer is used as a reference. Uniformity data were collected using 25 random spots over a 1mm^2 area.

3 RESULTS

We introduced a novel method to template the deposition of nanoporous materials onto nanostructured PPX films by an oblique angle polymerization technique. Poly(chloro-p-xylylene) (PPX-Cl) films are deposited using oblique angle vapor polymerization. The nanostructure consists of parallel assembly of nanowire arrays having a diameter of 150nm. These nanostructured films of PPX-Cl are then subjected to conformal metallization of gold. Figure 1A and 1B show the AFM and SEM micrographs of gold layer deposited on the nanostructured PPX-Cl film. Alternately, metallization can be carried out using electroless deposition[8].

The oblique angle polymerization makes it possible to prepare films with uniform nanostructures on a large area (~2cm^2). Thus, uniform metalized nanostructured PPX-Cl films are ideal candidates for detecting bacterial cell wall structures. As Gram-negative organisms, the cell wall of *E.Coli* shows the presence of lipopolysaccarides (LPS) layer in addition to the peptidoglycan layer. Typical Raman bands of proteins, phospholipids[14] and polysaccharides[15] can be observed on the spectra. For example, guanine, tyrosine at 653 cm^{-1}, adenine 724 cm^{-1}, C=C deformation at 958 cm^{-1}, O-P-O symmetric stretching 1091 cm^{-1}, amide III at 1242 cm^{-1}, CH deformation at 1330 cm^{-1}, COO- stretching at 1372 cm^{-1}, CH_2 deformation at 1456 cm^{-1}, adenine ring stretching at 1593 cm^{-1}, and amide I at 1710 cm^{-1} are observed. The peak positions of the SERS spectra match well with the literature[16].

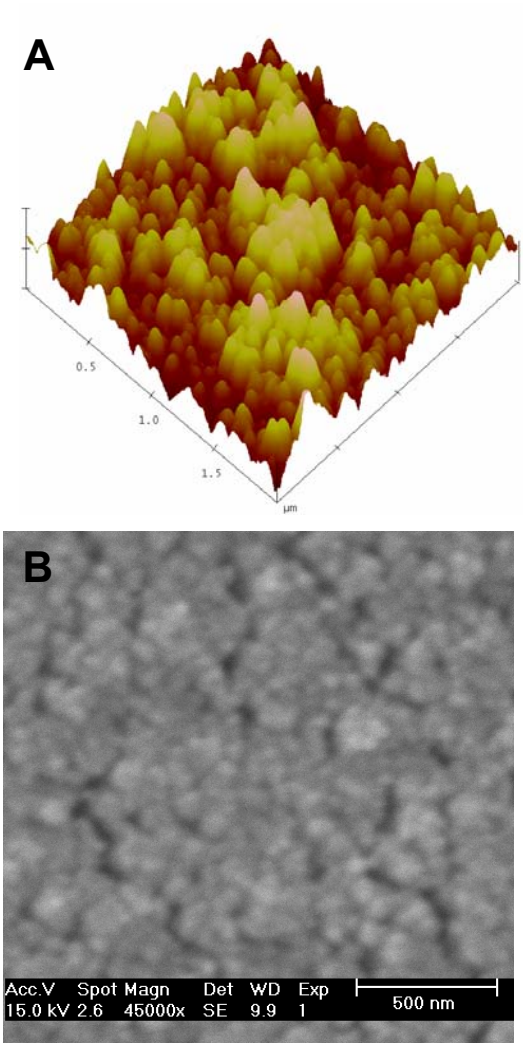

Figure 1. A. AFM image of nanostructured PPX-Cl film deposited with 60nm Au film. B. SEM image for the same substrate.

SERS signal uniformity is characterized by the peak intensity variation for the *E.Coli* sample at 1372 cm^{-1}. Figure 2 shows the variation of the *E.Coli* spectra on the SERS substrate obtained from 25 different spots in an area of 1mm^2. The peak at the 1372 cm^{-1} shows a relative deviation of 15%. In addition, the substrate remained stable even after 2 months in storage under ambient conditions with little or no reduction in the enhancement or the structural and SERS uniformity.

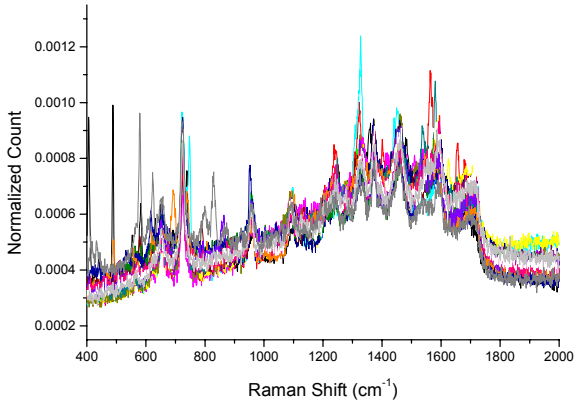

Figure 2: SERS spectra collected for 25 random cells on 1mm^2 area show highly reproducible fingerprint for *E.Coli*.

4 CONCLUSION

A SERS-active metal deposited onto the nanostructured PPX result in SERS active film with high sensitivity and reproducibility to form a biosensing surface for detection of *E.Coli*. The new biosensor substrate provides a significant advantage over traditional SERS surfaces because this technique does not require templating or lithography; thus making it simple and quick method to prepare uniform nanostructured SERS active films.

This technique based on SERS is a non-invasive and non-destructive method for *E.Coli* detection without amplification of cultures using an inexpensive, re-usable SERS substrate, thus has significant practical advantage over conventional PCR based techniques[17]. The large area uniformity property of the PPX-Cl films is exploited to build the SERS substrate for *E.Coli* detection. Bacterial species differentiation (Gram-positive vs. Gram-negative) based on differences in the cell wall structure is another potential application which is left for future work.

REFERENCES

1. Moskovits, M., Tay, L. L., Yang, J. & Haslett, T. in *Optical Properties of Nanostructured Random Media* 215-226 (2002).
2. Kneipp, K. et al. Detection and identification of a single DNA base molecule using surface-enhanced Raman scattering (SERS). *Physical Review E* **57**, R6281-R6284 (1998).
3. Farquharson, S., Smith, W. W., Lee, V. Y., Elliott, S. & Sperry, J. F. in *Chemical and Biological Early Warning Monitoring for Water, Food, and Ground* (eds. Jensen, J. L. & Burggraf, L. W.) 62-72 (SPIE, Boston, MA, USA, 2002).
4. Lefrant, S., Baltog, I. & Baibarac, M. Surface-enhanced Raman scattering studies on chemically transformed carbon nanotube thin films. *Journal of Raman Spectroscopy* **36**, 676-698 (2005).
5. Peica, N., Pavel, I., Pinzaru, S. C., Rastogi, V. K. & Kiefer, W. Vibrational characterization of E102 food additive by Raman and surface-enhanced Raman spectroscopy and theoretical studies. *Journal of Raman Spectroscopy* **36**, 657-666 (2005).
6. Zeiri, L., Bronk, B. V., Shabtai, Y., Eichler, J. & Efrima, S. Surface-enhanced Raman spectroscopy as a tool for probing specific biochemical components in bacteria. *Applied Spectroscopy* **58**, 33-40 (2004).
7. Vo-Dinh, T., Yan, F. & Wabuyele, M. B. Surface-enhanced Raman scattering for medical diagnostics and biological imaging. *Journal of Raman Spectroscopy* **36**, 640-647 (2005).
8. Demirel, M. C., Cetinkaya, M., Singh, A., Dressick W.J. A Non-Covalent Method for Depositing Nanoporous Metals via Spatially Organized Poly(P-xylylene) Films. *Advanced Materials* **19**, 4495-4499 (2007).
9. Demirel, M. C., Boduroglu, S., Cetinkaya, M. & Lakhtakia, A. Spatially organized free-standing poly(p-xylylene) nanowires fabricated by vapor deposition. *Langmuir* **23**, 5861-5863 (2007).
10. Demirel, M. C., So, E., Ritty, T. M., Naidu, S. H. & Lakhtakia, A. Fibroblast cell attachment and growth on nanoengineered sculptured thin films. *Journal of Biomedical Materials Research Part B-Applied Biomaterials* **81B**, 219-223 (2007).
11. Cetinkaya, A., Boduroglu, S. & Demirel, M. C. Growth of nanostructured thin films of poly (p-xytylene) derivatives by vapor deposition. *Polymer* **48**, 4130-4134 (2007).
12. Boduroglu, S., Cetinkaya, M., Dressick, W. J., Singh, A. & Demirel, M. C. Controlling the Wettability and adhesion of nanostructured poly-(p-xylylene) films. *Langmuir* **23**, 11391-11395 (2007).
13. Cetinkaya, M., Malvadkar, N., Demirel, M. Power-law scaling of structured poly(p-xylylene) films deposited by oblique angle. *Journal of Polymer Science Part B: Polymer Physics* **46**, 640-648 (2008).
14. Susi, H., Sampugna, J., Hampson, J. W. & Ard, J. S. Laser-Raman Investigation of Phospholipid-Polypeptide Interactions in Model Membranes. *Biochemistry* **18**, 297-301 (1979).
15. Jarvis, R. M. & Goodacre, R. Discrimination of bacteria using surface-enhanced Raman spectroscopy. *Analytical Chemistry* **76**, 40-47 (2004).
16. Premasiri, W. R. et al. Characterization of the Surface Enhanced Raman Scattering (SERS) of bacteria. *Journal of Physical Chemistry B* **109**, 312-320 (2005).
17. Belgrader, P. et al. Infectious disease - PCR detection of bacteria in seven minutes. *Science* **284**, 449-450 (1999).