A PLATFORM FOR *IN SITU* SENSING OF BIOMOLECULAR REACTION PRODUCTS USING A CHITOSAN MEDIATED SERS SUBSTRATE FABRICATED IN MICROFLUIDICS

Susan Buckhout-White, Xiaolong Luo, Dean Larios Berlin and Gary W. Rubloff

University of Maryland, College Park

ABSTRACT

Detecting, identifying and quantifying small molecules in bioMEMS are important to metabolic engineering applications, e.g. enzyme reaction pathways. We present a novel method for creating spatially localized sites for surface enhanced Raman spectroscopy (SERS) in completed microfluidic systems to enable *in situ* sensing of reaction products. When adenine, a prototype small molecule, is introduced into the microfluidic channel, its Raman spectrum is detected through SERS interaction at such sites. Furthermore, the chitosan surface is still available for chemical functionalization, thus creating the potential for *in-situ* measurement of enzymatic reaction products at the enzyme site itself.

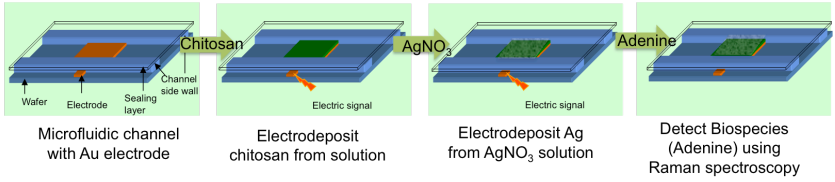
KEYWORDS: SERS, Microfluidics, *In-situ* sensing, Raman spectroscopy

INTRODUCTION

SERS is well known to dramatically enhance Raman sensitivity for identifying molecules without the need for labeling. Within a recreated metabolic pathway it is beneficial to enable *in situ* detection of the enzymatic reaction substrate/products to ensure proper functionality. With pathway recreation now occurring on localized sites in bioMEMS platforms [1], *in situ* detection can be achieved by integrating a SERS substrate into these platforms. We present a sensor site that is an electrode where electrodeposition of a polysaccharide layer (chitosan) and electroreduction of Ag from $AgNO_3$ produce a chitosan matrix with embedded Ag nanoparticles.

EXPERIMENTAL

The 500-µm-wide, 50-µm-high microchannel with 500 µm X 1000 µm electrode sites is fabricated with negative photoresist SU-8, sealed with PDMS layer and packaged within two Plexiglas plates with a view window above the SERS site.



Scheme 1. Creation of SERS site in prefabricated microfluidics.

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Fabrication of the SERS site follows the simple sequential routine depicted in Scheme 1 (Fig. 1). Chitosan is introduced to fill the microchannel, and a current density of 3 A/m^2 is applied for 2 minutes to electrodeposit chitosan on the working in static state. After rinsing with de-ionized water, $AgNO_3$ solution is pumped into the channel at 3 μL/min flow rate. With the flow stopped, Ag is deposited also at 3 A/m^2 current density for 2 min.

To test this SERS platform, a 1 mM adenine solution, purchased from Sigma, was pumped into the 2cm long channel at 3 μL/min flow rate, after which flow was stopped and the Raman spectrum, viewed through PDMS, was obtained using 632nm excitation in a Jobin-Yvon LabRAM HR instrument.

RESULTS AND DISCUSSION

Fig. 1a shows a scanning electron microscope (SEM) of a sequentially fabricated SERS site. The small bright dots clearly show the presence of nanoparticles well dispersed throughout the chitosan matrix. Energy dispersive spectroscopy (EDS) obtained in the SEM shows a spectrum (Fig. 1b) which indicates the Au substrate, C from the chitosan film, and Ag from the silver nanoparticles. Fig. 2-bottom is the Raman spectra measured atop the SERS substrate of 1mmol adenine in packaged microfluidics. This is compared to spectra from adenine solution at a nanostructured Ag SERS site (Fig. 2-top) at the chip level rather than in bioMEMS.

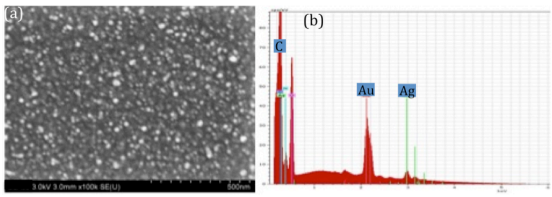

Figure 1. (a) SEM micrograph of the chitosan-nanoparticle film sequentially electrodeposited to form the SERS site, showing Ag nanoparticles as bright spots. (b) analytical SEM by EDS confirms presence of the Ag nanoparticles, showing also the Au electrode and C from the chitosan.

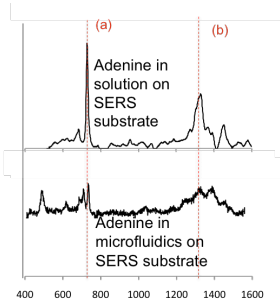

Figure 2. Raman spectra for Ag SERS site (top, no bioMEMS), and adenine at a chitosan/Ag SERS site in bioMEMS, viewed through PDMS (bottom). Peaks a and b, 735 and 1327 cm-1, originate from adenine.

Adenine in bioMEMS shows its signature 735 cm-1 and 1327 cm-1 peaks, with the intensity of the former 100X above a non-enhanced signal. Depending on the identity of the molecule sensed, higher sensitivity may be possible, e.g. molecules which bind to chitosan's amine groups, a question for future work. The availability of chitosan's amine groups was tested by treating a SERS site with a tyrosine-tagged GFP molecule. After 1 hour GFP exposure the surface showed clear fluorescence (Fig. 3), suggesting that the chitosan-nanoparticle SERS site retains its ability to bind biomolecular species. This chitosan-based SERS technique complements our previous work in using chitosan as a platform for enzyme immobilization and catalysis in bioMEMS networks [1].

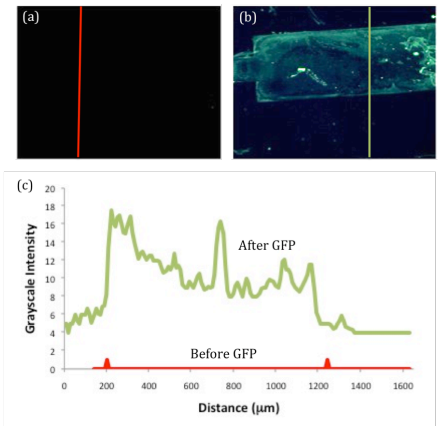

Figure 3. Fluorescence micrographs of sequentially deposited chitosan-nanoparticle SERS(a) before treatment with GFP and (b) after1 hr treatment with GFP. Line scans (c) of the images show substantial fluorescence from the GFP, demonstrating that chitosan remains reactive after Ag nanoparticle deposition.

CONCLUSIONS

Through incorporation of Ag nanoparticles into the already established platform of chitosan electrodeposition we have created a facile means for measurement of enzymatic reaction products within the bioMEMS platform. Furthermore the chitosan allows the opportunity to easily bond species using amine chemistry, thus providing a method to ensure proximity of the measurement target to the signal enhancing particles. Overall we have developed a flexible system that allows for the measurement of biospecies within a microfluidic environment.

ACKNOWLEDGEMENTS

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[1] Luo XL, Lewandowski AT, Yi HM, Payne GF, Ghodssi R, Rubloff GW, Bentley WE. Programmable Assembly of a Metabolic Pathway Enzyme in a Pre-packaged Reusable BioMEMS Device, Lab on a Chip, 8, 420 (2008).