SERS DETECTION USING SILVER NANOCLUSTER-EMBEDDED POROUS POLYMER MONOLITHS

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ABSTRACT

A novel method to prepare sensitive sensors for surface-enhanced Raman scattering (SERS) detection is introduced in this work. The substrate of the SERS sensor is synthesized by photopolymerizing a porous polymer monolith section in a capillary section, in which silver nanoclusters are physically lodged to enhance the Raman scattering of analytes. The SERS activity of the sensor was confirmed by identifying characteristic Raman shifts of rhodamine 6G (R6G). Detection of label-free biomolecules was also demonstrated. The monolith SERS sensor is particularly suitable to be integrated in microfluidic devices as a detector for rapid, sensitive and multiplexed biosensing applications.

KEYWORDS: Monolith, Nanoparticle, SERS, Sensor

INTRODUCTION

Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive detection method relying on the interactions between molecules and noble-metal nanoparticles capable of generating a 10^6-fold increase in Raman scattering efficiency. A wide variety of low-abundance chemical species and even biological entities in complex matrixes can be simultaneously detected and identified according to their unique Raman spectral characteristics without complex sample preparation, thus allowing rapid multiplexed measurements [1].

The development of improved SERS-active materials is an area of active investigation. The simplest approach to create SERS-active materials is to aggregate noble-metal nanoparticles with target analyte in solution to form “hot spots” for Raman signal enhancement. The major issue with this material is that the aggregates are unstable and tend to form large particles, resulting in precipitation and loss of signal. More stable SERS-active materials can be prepared by fabricating nanometer-sized structures on planar substrates; however, these materials are expensive, fragile, and irreproducible in SERS performance [1].

Monoliths are highly porous materials with tortuous fluidic channel networks defined by interconnected micrometer-scale particles [2]. These features allow monoliths to support convective flow with exceptionally rapid mass transfer while offering large surface areas for enhanced analyte-monolith interactions. While monoliths are widely used in analytical separations and sample preparation as stationary phases and solid supports [3], their application as volumetric sensing substrates has received little attention. Recently, we used antibody-immobilized monoliths as flow-through fluorescent immunosensors [4]. As an extension to our previous work, we explore using monolith as SERS sensors for label-free detection of biomolecules. An effective and robust method to prepare SERS-active monoliths is reported in this manuscript.

EXPERIMENTAL

The SERS-active monolith preparation process is depicted in Figure 1. A 5-mm long hydrophilic monolith section was first prepared within a 200 μm silica microchannel. The surface of the channel was pretreated with trimethoxysilyl-propyl methacryalte, enabling covalent attachment of the monolith to the channel wall. A monomer solution containing glycidyl methacrylate (GMA), ethoxylated trimethylolpropane triacrylate (SR454), 2,2’-dimethoxy-2-phenylacetophenone and alcohol solvents was then loaded into the microchannel. A monolith section was formed by exposing a 5 mm section of the channel to UV light through a photomask. The resulting monolith was thoroughly rinsed with methanol before use. To convert the monolith into a SERS-active material, a silver colloid solution was prepared using the reduction technique first developed by Lee and Meisel [5]. The silver nanoparticle solution has an estimated concentration on the order of 1 nM and diluted 3 folds with a 20 mM Tris-HCl solution (pH 8.2). The slightly basic buffer promoted aggregation of the silver nanoparticles to nanoclusters. When loaded to the monolith, silver nanoclusters were observed to be permanently immobilized within the monolith network throughout the length of the section, serving as stable hot spots exhibiting enhanced Raman scattering.

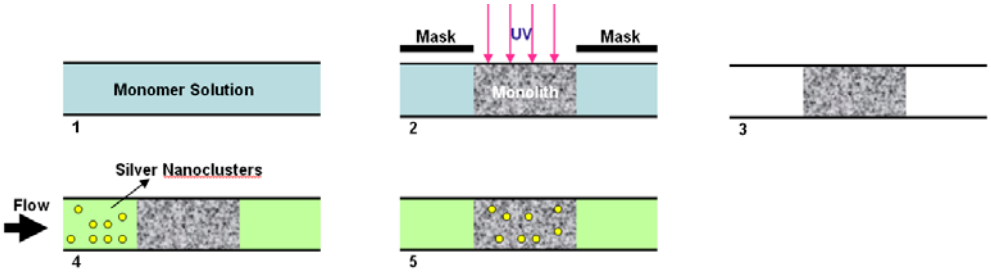

Figure 1: Preparation of SERS-active monolith in a microcapillary or microchannel.

RESULTS AND DISCUSSION

An series of monolith recipes have been tested in the preparation of SERS-active monolith sensors. It was found that most common hydrophobic or hydrophilic monoliths were easily clogged by silver nanoclusters at monolith heads during loading. An abrupt improvement was achieved when GMA and a tri-vinyl crosslinker, SR454, were co-polymerized to prepare SERS-active monoliths. Owing to its favorable morphology, silver nanoclusters can be pumped through the macropores and then captured by the fused polymer microglobules of GMA-SR454 monolith, thus providing "hot-spots" for SERS detection of analytes along the length of a monolith section. SEM images shown in Figure 2 reveal both silver nanocluster distribution and monolith morphology. As shown in Figure 2A, silver nanoclusters (bright spots) are trapped throughout the monolith. Figure 2B indicates a typical silver nanocluster "hot-spot" nesting in the GMA-SR454 monolith network. The individual nanoparticles have a diameter of approximately 50 nm and the number of aggregated nanoparticles in the clusters in the monolith ranges from 5 to approximately 200 according to SEM image analysis.

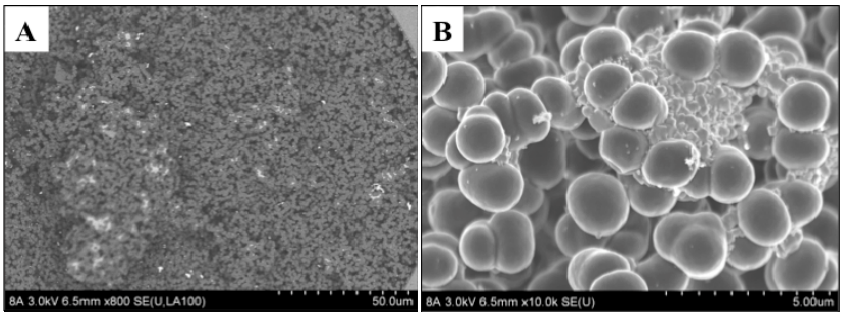

Figure 2: (A) SEM image of the silver-nanocluster-embedded monolith. The bright spots denote the embedded silver-nanoclusters; (B) a magnified view of a typical silver-nanocluster embedded in the monolith.

The electromagnetic enhancement effect of these silver-nanocluster "hot-spots" was verified in the detection of R6G dye. A series of characteristic bands of R6G can be recognized from the SERS spectrum (Figure 3A) even at a total loading level below 400 fmol, and no signal degradation was observed on the same monolith over 24 hours. The ability of the SERS-active monolith to detect label-free biomolecules was demonstrated by identifying native cytochrome c from its aqueous solutions. Presented in Figure 3B is a typical SERS spectrum of cytochrome c obtained using a SERS-active monolith, with spectral features similar to those observed using metal colloid solutions [6]. The larger peak width and lower resolution in the protein spectrum indicate that relative large biomolecules may adopt multiple orientations when interact with the silver nanoclusters lodged in the monolith matrix.

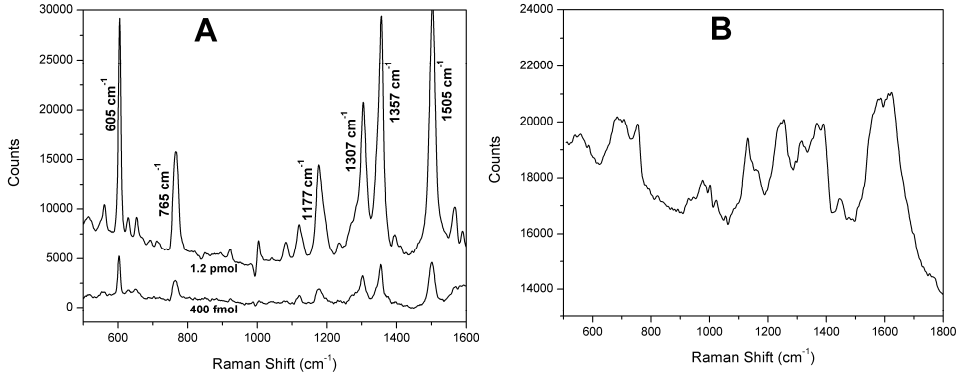

Figure 3: SERS detection of (A) R6G and (B) 1 μM label-free cytochrome c with a SERS-active monolith section. Spectrum integration time is 2 sec.

CONCLUSION

A novel SERS-active sensor was fabricated by physically trapping silver nanclusters in the matrix of porous polymer monoliths. The hydrophilic GMA-SR454 monolith used in this work provides large flow-through pores and irregular trapping structures to capture silver nanoclusters, thus forming a stable 3D "hot-spot" network in the polymer matrix. SERS activity of the silver-nanocluster-trapped monolith was demonstrated by detection of R6G dye. The label-free detection capability of the sensor was further evaluated by detecting Raman signals of native cytocrome c in an aqueous environment. In comparison to common SERS sensing systems using noble metal colloids, monolith SERS sensors offer much higher signal stability while providing similar sensitivity. The unique in-situ preparation method of polymer monoliths facilitates the integration of SERS detection element in any microfluidic device or microcapillary section, an essential functional unit in a spectrum of highly sensitive label-free biosensing platforms designed for applications in clinical diagnosis, environmental analysis, forensic analysis and homeland security.

ACKNOWLEDGEMENTS

This research was supported by the National Institutes of Health grant R01GM072512.

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