Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Journal Name

RSCPublishing

ARTICLE

The development of "Fab-Chips" as low-cost, sensitive surface-enhanced Raman spectroscopy (SERS) substrates for analytical applications

Ashley M. Robinson[a], Lili Zhao[a], Marwa Yasmin[a], Paridhi Bhandari[b], Scott G. Harroun[a], Dhananjaya Dendukuri[b], Jonathan Blackburn[c], Christa L. Brosseau[a*]

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

The demand for methods and technologies capable of rapid, inexpensive and continuous monitoring of health status or exposure to environmental pollutants persists. In this work, the development of novel surface enhanced Raman spectroscopy (SERS) substrates from metal-coated silk fabric, known as *zari*, presents the potential for SERS substrates to be incorporated into clothing and other textiles for the routine monitoring of important analytes, such as disease biomarkers or environmental pollutants. Characterization of the *zari* fabric was completed using scanning electron microscopy, energy dispersive X-ray analysis and Raman spectroscopy. Silver nanoparticles (AgNPs) were prepared, characterized by transmission electron microscopy and UV-vis spectroscopy, and used to treat fabric samples by incubation, drop-coating and *in situ* synthesis. The quality of the treated fabric was evaluated by collecting the SERS signal of 4, 4'-bipyridine on these substrates. When AgNPs were drop-coated on the fabric, sensitive and reproducible substrates were obtained. Adenine was selected as a second probe molecule, because it dominates the SERS signal of DNA, which is an important class of disease biomarker, particularly for pathogens such as *Plasmodium spp*. and *Mycobacterium tuberculosis*. Excellent signal enhancement could be achieved on these affordable substrates, suggesting that the developed fabric chips have the potential for expanding the use of SERS as a diagnostic and environmental monitoring tool for application in wearable sensor technologies.

Introduction

Wearable sensors possessing both diagnostic and monitoring capabilities are currently under development and are often made from conductive fabric materials, termed e-textiles.[1-3] Such sensors are mainly used for obtaining physiological information, such as heart rate, blood pressure, body temperature, respiration rate and body posture.[4,5] However, wearable *chemical* sensors that monitor chemical information, such as biomarkers residing on the skin or in the bodily fluids of the wearer, or toxic agents in the environment to which the wearer may be exposed, have also gained much attention in recent years.[3,6,7] Textiles are ideal for the design of wearable biosensors; they are flexible, soft, strong, and light. These favourable properties allow them to be constantly worn without affecting an individual's daily routine.[8] Furthermore, the incorporation of biosensors into textiles simplifies sample extraction and the unique physical and chemical properties of fabrics make them ideal for handling the demands of on-body biosensing.[6] For example, fabrics can act as matrices which immobilize and absorb various substances of interest.[6,8] Wearable chemical sensors that are being explored or suggested include, for example, cloth diapers which contain an integrated sensor to monitor infant exposure to harmful phthalates through their urine[9] or exercise clothes which can monitor a patient's response to medication through their sweat.[10] Clearly many wearable items can be made using fabrics, and a fabric-based sensor would be the easiest sensor platform to integrate into such items. At present however, relatively few fabric based sensing platforms have been developed.

SERS is an analytical technique that has gained much interest for the detection of biomolecules[11] and environmental pollutants[12] due to its rapid, specific, sensitive and non-destructive nature.[13] SERS provides an enhancement of the normal Raman signal of up to nine orders of magnitude[14], by placing the compound of interest on or in very close proximity to a roughened noble metal substrate.[14] The design of the SERS substrate is crucial to providing a strong enhancement of the Raman signal, and has been one of the greatest challenges associated with this analytical technique.[15,16] Ideally, a substrate should provide optimized, predictable and reproducible enhancements and display large-area uniformity.[15,17] There exist various techniques capable of producing uniform SERS substrates with good enhancement factors, such as electron beam lithography

[a]*Department of Chemisty, Saint Mary's University, 923 Robie Street, Halifax, N.S., B3H 3C3.*
[b]*Achira Labs Pvt. Ltd. 57, 1st Main Road, JP Nagar Phase III, Bangalore, 560078, India.*
[c]*Institute of Infectious Disease & Molecular Medicine, and Division of Medical Biochemistry, University of Cape Town, Faculty of Health Sciences, Cape Town, South Africa.*

† Electronic Supplementary Information (ESI) available: See DOI: 10.1039/b000000x/

and deep reactive etching[16], however these methods are expensive, time consuming, and produce substrates with small surface areas.[18]

Paper-based SERS sensors are currently under investigation and are gaining interest as simple substrates because the minimal costs associated with their production would enable high throughput manufacturing. This would in turn facilitate the transition from research laboratories to real-world application for the detection of biomolecules.[19] Inkjet printing of paper-based SERS substrates using a commercial inkjet printer is the most common way for fabricating these inexpensive substrates.[16] Paper quickly draws up water, which allows the metal nanoparticles in solution to be transferred to the substrate in a uniform way, with high density packing.[20] A major issue; however, for the integration of paper-based SERS substrates into wearable sensors is the lack of laundering ability; a paper based sensor would be destroyed during this process. A wearable sensor is something that ideally could be laundered and re-used with little effect on the performance of the sensor. Chakraborty *et al.* [21] have shown that Ag_{152} clusters can be uniformly coated onto paper, as well as cotton and silk. Therefore, it may be noted that textiles, such as cotton and silk, present another affordable material for the creation of SERS substrates. Kim *et al.*[22] have shown that it is possible to use cotton fabrics to observe the exchange reaction between benzenethiol and 4-nitrobenzenethiol by SERS and more recently, it has been reported by Ballerini *et al.*[23] that cotton threads may be used for the detection of various analytes by SERS. These threads have the potential of being incorporated into apparel, such as military uniforms, for the detection of biohazards. By designing SERS-active thread or fabrics, it is possible to circumvent the aforementioned limitations of other SERS substrates, and extend the application of SERS analysis. In particular, modified textiles could be used as SERS substrates for the analysis of various analytes, due to their widespread availability.

Silk is a fibrous protein, which consists mainly of glycine, alanine and serine, when produced by the silkworm *Bombyx mori*.[24] It has been used as a textile material since antiquity, and has been gaining interest as a protein biomaterial exhibiting many favorable characteristics, including high tensile strength, low inflammability and good biodegradability.[24-26] Also, silk textiles can be produced at low cost on a large scale. In particular, metal-coated fabric, otherwise known as *zari*, has been produced for centuries in India.[27] *Zari* consists of traditional thread or material embroidery that may be incorporated into other textiles, such as sarees, dress material and laces. This type of thread is manufactured by winding a flat and thin metal wire over a base yarn.[27] *Zari* presents a unique flexible metal surface that can easily be incorporated (woven) into clothing, as is tradition, and roughened to create a SERS substrate.

In this work, *zari* fabric was used to design sensitive, reproducible, and cost-effective SERS substrates that could potentially be incorporated into clothing and other textiles for use as wearable sensors. *Zari* yarns can easily be woven into fabrics to create desired shapes and patterns, and has shown promise in the development of fabric-based immunoassay technology.[28] In the current work, we seek to explore the utility of *zari* yarns, woven into fabric, for the creation of SERS-active substrates which would be particularly suited for integration into fabric-based objects. It is the authors' understanding that these *zari* fabric based chips, or "fab-chips" represent the first reported fabric-based SERS substrates. Silver nanoparticles (AgNPs) were used to roughen the *zari* yarns on the nanoscale; three different strategies for this process were evaluated. The SERS signal of 4, 4'-bipyridine (4, 4'-BiPy), a standard SERS probe, on the fab-chip was collected and analysed in order to provide an indication of the uniformity of the substrates and the magnitude of their enhancement. Adenine, a nucleobase which dominates the SERS spectral profile of DNA[29], was employed for proof-of-concept detection of a biomolecule on the fabric substrates.

Materials and Methods

Reagents and Materials

4, 4'-BiPy (98 %) and sodium citrate (≥ 99 %) were purchased from Sigma Aldrich (St. Louis, MO, USA). Silver nitrate ($AgNO_3$, 99.9995 %) and citric acid (> 99 %) were purchased from Alfa Aesar (Wardhill, MA, USA), and sodium borohydride ($NaBH_4$, 99 %) was purchased from Fluka Analytical (Seelze, Germany). All chemicals were used without further purification. All solutions were prepared using Millipore water (solution resistivity ≥18.2 MΩ cm). Glassware was cleaned in a sulfuric acid bath and thoroughly rinsed with Millipore water prior to use. Achira Labs (Bangalore, India) supplied the *zari* fabric samples.

Preparation of AgNPs

Following a method described by Zhao *et al.*[30], solutions of $AgNO_3$ (1.00 mL, 0.10 M), sodium citrate (3.40 mL, 0.17 M) and citric acid (0.60 mL, 0.17 M) were added to 95 mL of water in a three-necked flat bottomed round flask, and stirred under reflux. $NaBH_4$ (0.20 mL, 0.10 mM) was added to this mixture, which was then heated to boiling. The colloidal suspension was removed from the heat after 1 hour of boiling and then allowed to cool to room temperature for 1 hour. To concentrate the AgNPs, 1.0 mL aliquots of the AgNP suspension were added to Eppendorf tubes and each tube was centrifuged at 8000 rpm for 20 minutes. The supernatant was then removed and discarded, and another 1.0 mL aliquot of the colloidal suspension was added. This centrifugation process was repeated five times, and a AgNP "paste" was obtained.

Treatment of Fabric

Fabric samples were modified using the AgNPs by one of three methods: (i) Fabric samples were incubated in the AgNP suspension at room temperature for one hour and then air-dried, (ii) 20 µL of the AgNP paste was deposited on the fabric samples, which were then allowed to air-dry, or (iii) an *in situ* synthesis of the AgNP colloids was conducted directly on the *zari*. The *in situ* preparation involved adding the fabric sample to the initial $AgNO_3$ solution, prior to the addition of $NaBH_4$. At the end of the synthesis, the fabric was removed and allowed to dry. In each case, 40 µL of a 1.0 mM 4, 4'-bipyridine (4, 4'-BiPy) solution was drop-coated on the treated fabric samples and allowed to air-dry prior to measurement. For the adenine studies, 40 µL of the 1.0 mM adenine solution was drop-coated onto the treated fabric chip and allowed to air-dry prior to

measurement.

Characterization of samples

The *zari* fabric was imaged using a LEO 1450 VP scanning electron microscope (SEM), having a maximum resolution of up to 3.5 nm at 30 kV, equipped with an INCA X-max 80 mm^2 energy dispersive X-ray (EDX) system that used Silicon Drift Detector technology to analyze characteristic X-rays emitted from the sample. Characterization of AgNPs was carried out by means of a transmission electron microscope [(TEM) FEI, Tecnai 12; operating at an acceleration voltage of 80 kV]. Size distributions of the colloids were calculated from ImageJ 1.47, a public domain image-processing program (National Institutes of Health, MD, USA).

Spectroscopic studies

Ultraviolet-visible (UV-vis) spectroscopic studies were completed for the AgNP solutions using a Varian Cary 50 Bio UV-vis spectrophotometer. Raman and SERS measurements were conducted using a Thermo Scientific DXR SmartRaman spectrometer (Thermo Fisher Scientific, Mississauga, ON, Canada) equipped with an air-cooled CCD detector, 532 nm diode laser, as well as a laser line filter. A high-resolution grating was used to collect the Raman signal within the range of 200 to 1800 cm^{-1}. All SERS spectra were collected at laser powers between 1.0 mW and 7.0 mW for acquisition times of 30-40 seconds. The spectrometer resolution was 3 cm^{-1}. Fabric samples were mounted using a support, which compressed the sample, holding it perpendicular to the laser beam. Ten SERS spectra were collected at various spots on each fabric sample. A single normal Raman spectrum was collected for pure powders of the probe molecules and the silk fabric. Data analysis was completed using Origin 9.0 software (OriginLab Corporation, Northampton, MA, USA).

Results and Discussion

Fabric samples were characterized by SEM, EDX and Raman spectroscopy. SEM images (Figure 1) showed that the metal coating on the silk was wrapped in such a way that gaps exposed a small area of the silk fibers. It may also be noted that the metal was very smooth, which is not conducive to SERS enhancement. EDX data revealed that the main constituents of the fabric were carbon (40.04%), nitrogen (7.72%), oxygen (8.55%), aluminum (0.08%), copper (32.65%), zinc (9.68%), and silver (1.17%). The high levels of carbon, nitrogen and oxygen were attributed to the constitution of the silk fibers, and the metal was an alloy of copper and zinc.

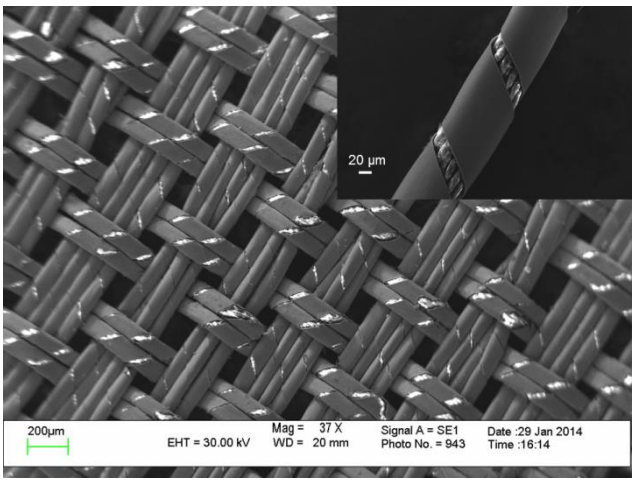


Figure 1. SEM image of metal-coated fabric sample. Inset shows a higher resolution image a single *zari* thread. Gaps in wrapped metal coating indicate areas of exposed silk.

The normal Raman spectrum of the *zari* fabric was collected, as it has been previously reported that silk could be characterized by Raman spectroscopy.[31, 32] Figure 2 shows the Raman signal of the fabric at two different spots. The observed variation in peak position and intensity is most likely due to the conformation and the morphology of the silk fibers. This is consistent with the Raman analysis of *Bombyx mori* silk by Monti *et al.*[31], which showed that two conformations exist within the fibroin; Silk II antiparallel β-sheet and the metastable Silk I modification, which give rise to different signals.

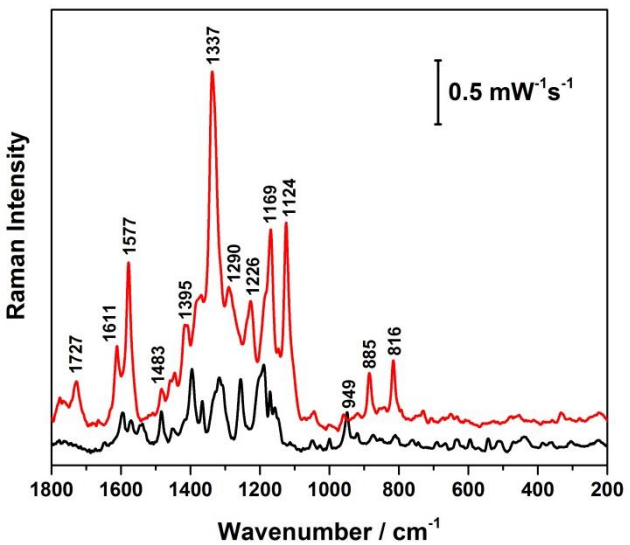


Figure 2. Normal Raman spectra of metal-coated silk, collected at two spots (a,b) using a 532 nm laser, at 10 mW for 40 seconds.

4, 4'-BiPy (molecular structure shown in Figure 3) was selected as a probe molecule for SERS analysis of the *zari* fabric because it displays a strong Raman signal (Figure S1) due to the extensive π-bond conjugated system of the pyridyl rings and the presence of two active nitrogen atoms, which enable electron transport as well as

energy transfer processes.[33] There exist three strong bands in the normal Raman signal at 1606, 1297 and 999 cm^{-1}. The bands at 1606 and 1297 cm^{-1} correspond to C–C and C-N stretching vibrations and in-plane C-H bending, while the band at 999 cm^{-1} corresponds to the ring breathing mode.[33]

Prior to treating the *zari* fabric with AgNPs, the signal of 4, 4'-BiPy drop-coated on its surface was collected. The spectrum of this sample (Figure S2) illustrated that no signal for 4, 4'-BiPy could be observed, and that the Raman signal of silk dominated the spectrum. It is suspected the metal coating on the fabric was too smooth to provide any SERS enhancement, illustrating the necessity of modifying the fabric to generate a SERS-active substrate. Early attempts at electrochemical roughening of the *zari* fabric resulted in poor SERS enhancement and irreproducible results (data not shown). Thus, treatment of the fabric with nanoparticles was attempted instead.

Fabric samples were treated with AgNPs as outlined in Materials and Methods. A TEM image (Figure S3) revealed the approximate size of the nanoparticles was 28 $\pm$ 6 nm. The uniformity in shape and size of these nanoparticles was further emphasized by the narrow absorbance band (FWHM = 52 nm) in their UV-vis spectrum (Figure S3).

Three different methods were explored for treating the fabric with the silver colloids. The uniformity of each substrate was examined by collecting ten SERS spectra at different spots on the same sample, after drop-coating 4, 4'-BiPy solution on their surface. Firstly, fabric samples were treated by a simple incubation in the AgNPs. Figures 3a and b show SEM images of this substrate at low and high resolution. It may be noted the nanoparticles are dispersed on the fabric, leaving much of the underlying metal exposed. As shown in Figure 4a, the enhancement of the 4, 4'-BiPy signal varied significantly across the surface of the substrate. Additionally, it may be noted that not all of the bands in the normal Raman signal of 4, 4'-BiPy were observed in the SERS signal, and the characteristic bands for 4, 4'-BiPy that were present were shifted due to the interaction of the analyte with the silver substrate.[33] Such differences between normal Raman and SERS spectra are commonplace.[34,35]

A second treatment involved drop-coating the *zari* fabric with the concentrated colloidal silver paste. It was suspected that this would greatly increase surface coverage of the AgNPs on the fabric, potentially eliminating the silk signal interference and increasing the SERS enhancement. The high resolution SEM image of this substrate (Figure 3d) revealed a thick, nanoporous structure with grain size ranging from approximately 80 to 350 nm. At lower resolution (Figure 3c), showing a wider scanning area, cracks in the AgNP coating can be observed. Nevertheless, the substrate is homogenous, suggesting it would produce analogous SERS spectra for a given analyte at any location. Figure 4b shows that the signal of 4, 4'-BiPy collected on these substrates was relatively uniform, and the average signal enhancement was greater than the average signal obtained on the fabric treated by incubation in the AgNPs (Figure 4a).

The third treatment was an *in situ* synthesis of the AgNPs directly onto the fabric sample. From the SERS signal of 4, 4'-BiPy collected on this substrate (Figure 4c), it was noted that the spectra contained background and the peak intensities varied significantly depending on where the laser was focused. This result was strikingly similar to the signal obtained on the fabric treated by incubation in the AgNPs. SEM images of this sample (Figure 3f) revealed large silver crystals on the fabric, which were formed as a result of the spontaneous redox reaction of silver cations in solution and solid copper in the metal coating of the *zari* fabric. These crystals were too large in size to give rise to a SERS enhancement, and may have been the cause for significant background in the spectra. Additionally, the nanoparticles were disperse on the fabric sample, leaving much of the underlying metal bare (Figure 3e). It is important to note that the silver colloids prepared during the *in situ* synthesis were altered in size and shape, due to the addition of the fabric to the reaction vessel. Many long silver rods formed in solution (Figure S4), shifting the size distribution to 53 $\pm$ 14 nm and the UV-vis absorbance maximum to 415 nm (FWHM = 67 nm).

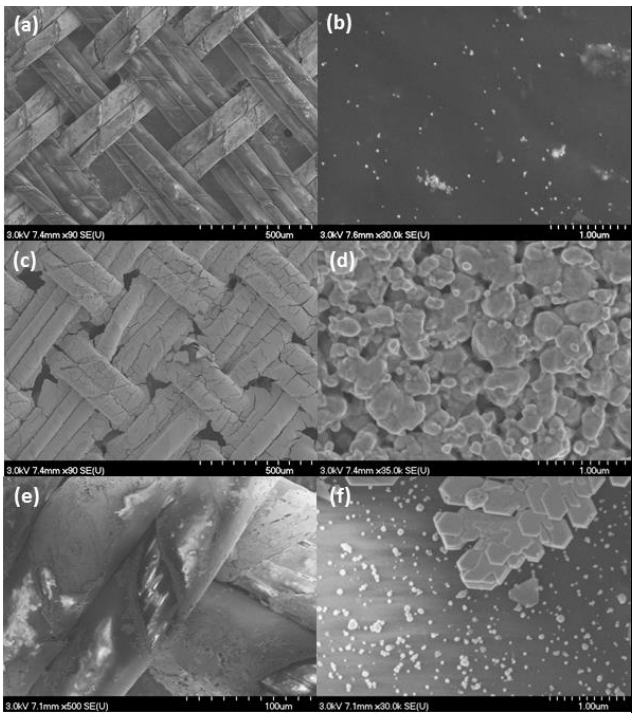


Figure 3. SEM images of fabric treated by (a,b) incubation in AgNPs, (c,d) drop-coating of AgNPs, and (e,f) *in situ* synthesis of AgNPs, at low and high resolution.

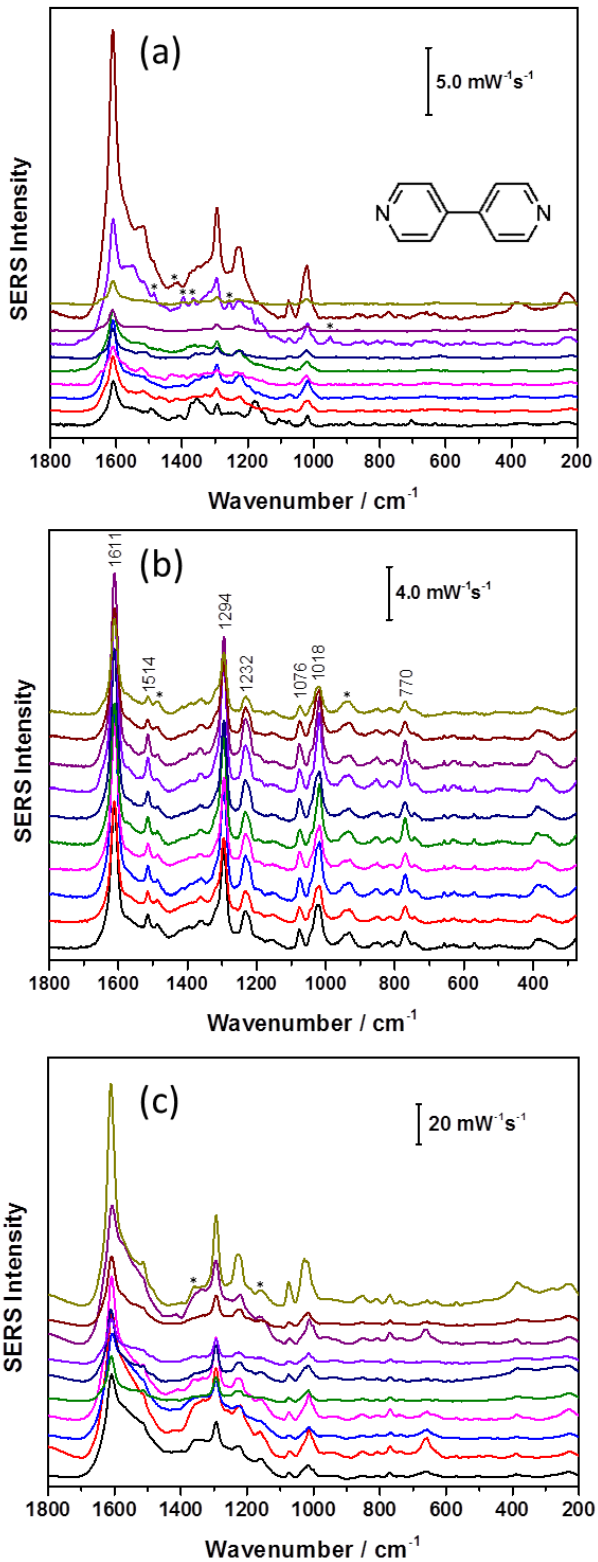


Figure 4. SERS signal of 4, 4'-BiPy on *zari* fabric treated by (a) incubation in AgNPs, (b) deposition of concentrated AgNPs and (c) *in situ* synthesis of AgNPs.

Based on these results, it was determined that the highest quality SERS substrate was the *zari* fabric drop-coated with concentrated AgNPs. On this substrate, good enhancement of the 4, 4'-BiPy signal was achieved, the signal was uniform, and more spectral features were observed, in comparison to the other substrates.

A statistical analysis of the signal reproducibility was completed for the three types of substrates illustrated above. In each case, the peak height of the 1020 cm^{-1} band for 4, 4'-bipyridine was determined for 10 different spots, and the % relative standard deviation was calculated for each case. The results of this analysis (average peak height and % RSD) were as follows: for the fab-chip incubated in AgNPs: 1.1 (±97.6%), for the fab-chip with deposited AgNPs: 3.7 (±28.7%), for the in-situ synthesis fab-chip samples: 15.9 (±55.1%). Clearly the signal reproducibility is best for the sample which has the AgNPs deposited directly onto it. It should be noted however, that this level of signal reproducibility is not adequate for doing quantitative analysis with such a substrate; however qualitative analysis is definitely possible. For the sample with the deposited AgNPs, the enhancement factor EF (details provided in the supplementary information) was determined to be ~10^8.

To further illustrate the quality of this fabric substrate, the SERS signal of 4, 4'-Bipy collected on the treated fabric samples was compared to the 4, 4'-BiPy signal obtained for concentrated AgNPs deposited on a glassy carbon substrate. This comparison is shown in Figure 5.

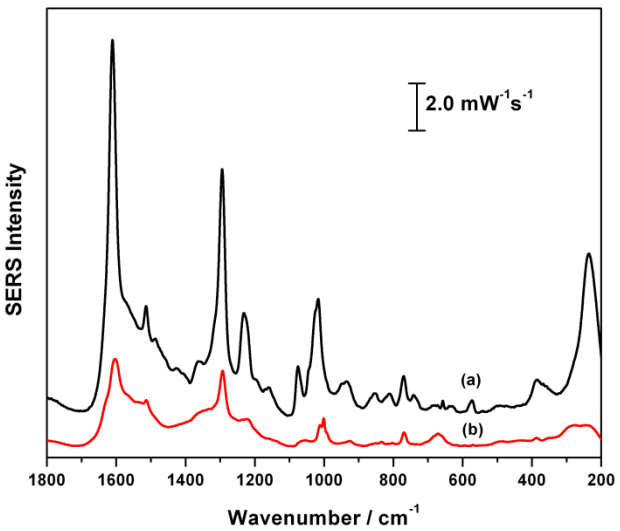


Figure 5. SERS signal of 4, 4'-BiPy on (a) metal coated fabric drop-coated with 20µL of AgNPs and on (b) carbon drop-coated with 15 µL of AgNPs. Each spectrum represents the average of 10 spectra collected at different spots on each substrate.

It is evident from Figure 5 that the enhancement of 4, 4'-BiPy is greater on the fabric substrate than on carbon. This enhanced signal may be due to the underlying periodic microstructure of the metal coated fibers, or could also be a result of an enhancement brought about by the presence of the copper-zinc alloy. According to the charge-transfer (C-T) model of SERS enhancement, photon-driven charge transfer occurs between the Fermi level of the metal and an unoccupied level of the adsorbed molecule.[36] The presence of the Cu-Zn alloy in close proximity to the Ag surface may influence the electrochemical environment of the Ag, and in turn its Fermi level, thereby resulting in an enhanced signal in this case.

Keeping in mind a long-term objective of this project– using *zari* fabric substrates to detect biologically relevant molecules – the detection of adenine (molecular structure shown in Figure 6) was pursued. Adenine, a fundamental component of RNA and DNA, displays interesting adsorption properties in the presence of gold and silver, and has been extensively studied by SERS in the literature for this reason.[37, 38] DNA is a urinary biomarker for various illnesses, including urinary tract infections[39] and cancer.[40] DNA fragments from pathogens, such as *Mycobacterium tuberculosis*, *Plasmodium spp.* and *Schistoma mansoni*, may also be detected in urine.[41, 42] For these reasons, urinary DNA (when coupled with DNA hybridization strategies) can be used as a disease activity indicator. It may also be noted that urinalysis provides a noninvasive alternative to blood collection for disease detection and monitoring, which is highly favorable for routine analysis.[41,42] SERS is an attractive technique for the analysis of DNA in urine because it does not require the sample to be transparent, water does not interfere significantly with the signal, and it has been shown that the noise from the sugar and phosphate groups of the DNA backbone do not interfere with the SERS signal of nucleotide bases.[43-45] Detection of DNA in urine using "fab-chip" based sensing would be straightforward, particularly in the case of infants (as a cloth diaper insert) and for adults experiencing incontinence (adult diaper). It should be noted that a truly useful SERS-based sensor for DNA would employ a DNA hybridization detection strategy, to ensure adequate sensitivity and specificity.

The normal Raman spectrum of adenine is displayed in Figure 6. Since the bands at 1331 and 721 cm^{-1} are the most intense features in the Raman signal, they were selected as the marker bands for the SERS detection of this analyte. These two bands correspond to in plane C5-N7, N1-C2, and C2-H, C8-H bending, and ring breathing of the whole molecule, respectively.[46, 47]

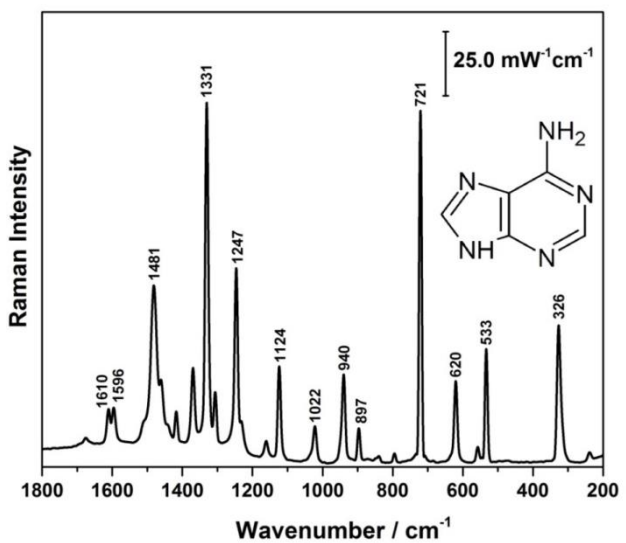


Figure 6. Normal Raman signal of pure adenine powder (532 nm, 7 mW, 30 s) and molecular structure of adenine.

The SERS signal of adenine at 1.0 mM was collected on *zari* that had been drop-coated with concentrated AgNPs, as shown in Figure 7b. Prior to collecting the SERS signal of adenine, a spectrum was collected for the bare substrate (Figure 7a). This spectrum shows no characteristic bands, however background from approximately 1500-1700 cm^{-1} is present.

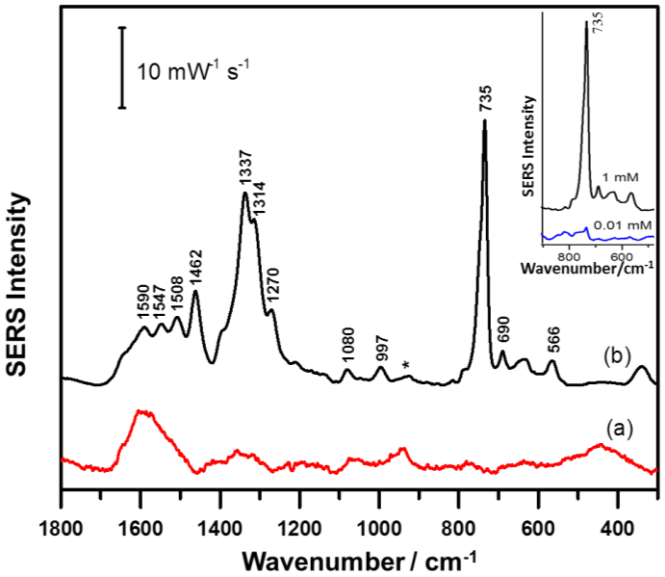


Figure 7. SERS spectra of (a) *zari* fabric drop-coated with concentrated AgNPs, i.e. the bare substrate, (b) 1.0 mM adenine on the substrate. Asterisks indicate signal of citrate. Inset: SERS spectra of 1 mM and 0.01 mM adenine on the substrate (blue line: magnified by a factor of 5 for easy comparison).

At a concentration of 1.0 mM, the two marker bands for adenine were easily identified in the SERS spectrum, although they were shifted to higher wavenumbers (1331 to 1337 and 721 to 735 cm^{-1}) due to interactions between the analyte and the AgNPs. This result is consistent with the literature.[38] Bands at 1462 and 566 cm^{-1} in Figure 7b were also characteristic of adenine[45], while many others (1590, 1547, 1314, 1080, 997 and 690 cm^{-1}) were characteristic of silk. Other spectral features are band characteristic of the citrate, used in the synthesis of the AgNPs. This is indicated by an asterisk in Figure 7. For lower concentration of adenine, such as 0.01 mM, the characteristic band at 735 cm^{-1} can also be detected, although weaker, indicating that good signal enhancement could be achieved on these affordable substrates.

Conclusions

This study presents the first use of metal-coated *zari* fabric as a low-cost SERS substrate that provides strong enhancement of analyte signal after minimal treatment with AgNPs. These substrates have the potential for expanding the applications of SERS through their incorporation into other textiles, as wearable fabric-based sensors. Optimization of the *zari* substrates for SERS analysis was achieved by varying the method of treatment with AgNPs; by incubation, deposition, and *in situ* synthesis. 4, 4'-BiPy served as a probe for evaluating the uniformity and sensitivity of the substrates, while adenine provided proof-of-concept results for SERS detection of biomolecules on treated *zari* fabric. Future investigations concerning

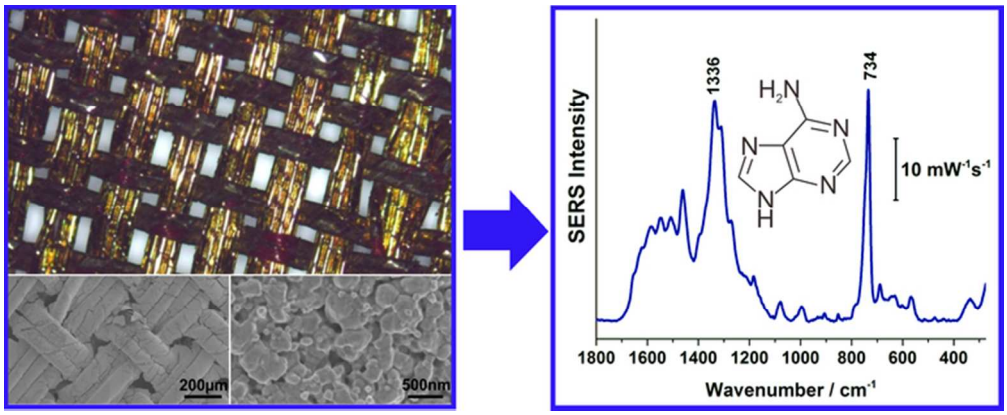
the weave style and method of coating metal on silk fibers are planned. In addition, optimization of the AgNP synthesis will be crucial for improving the signal reproducibility of these substrates for any eventual quantitative analysis.

Acknowledgements

This work was financially supported by Grand Challenges Canada and the Natural Sciences and Engineering Research Council of Canada. The authors would also like to recognize Xiang Yang from Saint Mary's University, the Department of Biology and the SEM-FIB Facility of the Institute for Research in Materials at Dalhousie University, Halifax, Canada, for their assistance with imaging the samples presented in this work.

References

1 S. J. Choi and Z. W. Jiang. Sensor. Actuat. A-Phys 2006, **128**, 317-326.
2 G. E. Collins and L. J. Buckley, *Synthetic Met.*, 1996, **78**, 93-101.
3 S. Patel, H. Park, P. Bonato, L. Chan and M. Rodgers, *J. NeuroEng. Rehabil.*, 2012, **9**, 1-17.
4 O. Atalay and W. R. Kennon, *Sensors*, 2014, **14**, 4712-4730.
5 Y. D. Lee and W. Y. Chung, *Sensor. Actuat. B-Chem.*, 2009, **140**, 390-395.
6 J. R. Windmiller and J. Wang, *Electroanalysis*, 2013, **25**, 29-46.
7 S. Coyle, Y. Wu, K. T. Lau, S. Brady, G. Wallace and D. Diamond, *4th International Workshop on Wearable and Implantable BSN*. 2007, **13**, 35-39.
8 D. Staneva, I. Grabchev and R. Betcheva, *Dyes. Pigments*, 2013, **98**, 64-70.
9 A. M. Calafat, J. Weuve, X. Ye, L. T. Jia, H. Hu, S. Ringer, K. Huttner and R. Hauser, *Environ. Health Perspect.*, 2009, **117**, 639-644.
10 M. Aladjem, B. P. Fine, N. Lasker, J. D. Bogden, J. P. Gardner, F. Kemp, M. Miller and A. Aviv, *J. Hypertens.*, 1992, **10**, 69-76.
11 Y. S. Yamamoto, M. Ishikawa, Y. Ozaki and T. Itoh, *Front. Phys.*, 2014, **9**, 31–46.
12 D. W. Li, W. L. Zhai, Y. T. Li and Y. T. Long, *Microchim. Acta*, 2014, **181**, 23-43.
13 R. W. Browne and J. J. McGarvey, *Coord. Chem. Rev.*, 2007, **251**, 454–473.
14 K. A. Willets, *Chem. Soc. Rev.*, 2014, **43**, 3854-3864.
15 B. Sharma, M. F. Cardinal, S. L. Kleinman, N. G. Greeneltch, R. R. Frontiera, M. G. Blaber, G.C. Schatx and R. P. Van Duyne, *MRS Bull.*, 2013, **38**, 615-624.
16 J. F. Betz, W. W. Yu, Y. Cheng, I. M. White and G. W. Rubloff, *Phys. Chem. Chem. Phys.*, 2014, **16**, 2224-2239.
17 M. J. Banholzer, J. E. Millstone, L. Qin and C. A. Mirkin, *Chem. Soc. Rev.*, 2008, **37**, 885-897.
18 X. Zhang, C. R. Yonzon, M. A. Young, D. A. Stuart and R. P. Van Duyne, *IEE Proc. Nanobiotechnol.*, 2005, **152**, 195-206.
19 S. Z. Oo, R. Y. Chen, S. Siitonen, V. Kontturi, D. A. Eustace, J. Tuominen, S. Aikio and M. D. B. Charlton, *Opt. Express*, 2013, **21**, 18484-18491.
20 E. W. Nery and L. T. Kubota, *Anal Bioanal Chem.*, 2013, **405**, 7573–7595.
21 I. Chakraborty, S. Bag, U. Landman and T. Pradeep, *J. Phys. Chem. Lett.*, 2013, **4**, 2769−2773.
22 K. Kim, H. B. Lee, J. W. Lee and K. S. Shin, *J Colloid Interf. Sci.*, 2010, **345**, 103-108.
23 D. R. Ballernini, Y. H. Ngo, G. Garnier, B. P. Ladewig and W. Shen, *AIChE J.*, 2014, **60**, 1598-1605.
24 P. Monti, G. Freddi, M. Tsukada, A. Bertoluzza and T. Asakura, *J. Raman Spectrosc.*, 2001, **32**, 103–107.
25 Z. Zhu, Y. Kikuchi, K. Kojma, T. Tamura, N. Kuwabara, T. Nakamura and T. Asakura, *J. Biomater. Sci. Polym. Ed.*, 2010, **21**, 395-411.
26 E. Wenk, H. P. Merkle and L. Meinel, *J. Control. Release*, 2011, **150**, 128-141.
27 R. Garg and M. Sidhpuria, *AI & Soc.* 2013, DOI 10.1007/s00146-013-0506-7
28 P. Bhandari, T. Narahari and D. Dendukuri, *Lab Chip*, 2011, **11**, 2493-2499.
29 R. Huang, H. T. Yang, L. Cui, D. Y. Wu, B. Ren and Z. Q. Tian, *J. Phys. Chem. C.*, 2013, **117**, 23730-23737.
30 L. Zhao, K. Ding, X. Ji, J. Li, H. Wang and W. Yang, *Colloid Surf. A-Physicochem. Eng. Asp.*, 2011, **386**, 172–178.
31 P. Monti, P. Taddei, G. Freddi, T. Asakura and M. Tsukada, *J. Raman Spectrosc.*, 2001, **32**, 103-107.
32 T. Aguayo, C. Garrido, R. E. Clavijo, J. S. Gómez-Jeria, C. Araya Monasterio, M. Icaza, F. Espinoza Moraga and M. M. Campos Vallette, *J. Raman Spectrosc.*, 2013, **44**, 1238–1245.
33 Z. Zhuang, J. Cheng, X. Wang, B. Zhao, X. Han and Y. Luo, *Spectrochimica Acta Part A.*, 2007, **67**, 509–516.
34 T. Aguayo, C. Garrido, R. E. Clavijo, J. S. Gómez-Jeria, C. Araya Monasterio, M. Icaza, F. Espinoza Moraga and M. M. Campos Vallette, *J. Raman Spectrosc.*, 2013, **44**,1238-1245.
35 C. Blum, T. Schmid, L. Opilik, S. Weidmann, S. R. Fagerer and R. Zenobi, *J. Raman Spectrosc.*, 2012, **43**, 1895-1904.
36 A. Kudelski, J. Bukowska, M. Dolata, W. Grochala, A. Szummer and M. Janik-Czachor, *Mater. Sci. Eng.*, 1999, **A267**, 235-239.
37 E. Papadopoulou and S. E. J. Bell, *J. Phys. Chem. C.*, 2010, **114**, 22644-22651.
38 J. Kundu, O. Neumann, B. G. Janesko, D. Zhang, S. Lal, A. Barhoumi, G. E. Scuseria and N. J. Halas, *J. Phys. Chem. C.*, 2009, **113**, 14390-14397.
39 V. G. Moreira, B. P. Garcia, T. de la Cera Martinez and F. V. A. Menéndez, *Clin. Biochem.*, 2009, **42**, 729-731.
40 Y. H. Su, M. Wang, D. E. Brenner, P. A. Nortonm and T. M. Block, *Ann. N. Y. Acad. Sci.*, 2008, **1137**, 197-206.
41 H. Bordelon, P. K. Russ, D. W. Wright and F. R. Haselton, *PLOS ONE*, 2013, **8**, e68369.
42 Z. G. Najafabadi, H. Oormazdi, L. Akhlaghi, A. R. Meamar, M. Nateghpour, L. Farivar and E. Razmjou, *Acta Trop.*, 2014, **136**, 44-49.
43 Browne, R. W.; McGarvey, J. J. Coord. Chem. Rev. 2007, 251, 454–473.
44 A. Kudelski, *Talanta*. 2008, **76**, 1-8.
45 F. Feng, G. Zhi, H. S. Jia, L. Cheng, Y. T. Tian and X. J. Li, *Nanotechnology*, 2009, **20**, 1-6.
46 B. Giese and D. McNaughton, *J. Phys. Chem. B.*, 2002, **106**, 101-112.
47 J. Li and Y. Fang, *Spectrochimica Acta Part A.*, 2007, **66**, 994-1000.



64x26mm (300 x 300 DPI)