The Use of a Handheld Raman System for Virus Detection

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ABSTRACT

The combination of surface enhanced Raman spectroscopy (SERS) with a handheld Raman system would lead to a powerful portable device for defense and security applications. The Thermo Scientific FirstDefender RM instrument is a 785-nm handheld Raman spectrometer intended for rapid field identification of unknown solid and liquid samples. Its sensitivity and effectiveness for SERS-based detection was initially confirmed by evaluating detection of 1,2-di(4-pyridyl)ethylene as a reporter molecule on a silver nanorod (AgNR) substrate, and the results are comparable to those from a confocal Bruker Raman system. As avian influenza A viruses (AIV) are recognized as an important emerging threat to public health, this portable handheld Raman spectrometer is used, for the first time, to detect and identify avian influenza A viruses using a multi-well AgNR SERS chip. The SERS spectra obtained had rich peaks which demonstrated that the instrument can be effectively used for SERS-based influenza virus detection. According to the SERS spectra, these different influenza viruses were distinguished from the negative control *via* the principal component analysis and by partial least squares-discriminate analysis. Together, these results show that the combination effective SERS substrates when combined with a portable Raman spectrometer provides a powerful field device for chemical and biological sensing.

Keywords: surface enhanced Raman scattering, handheld Raman system, silver nanorod, influenza virus, principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA)

INTRODUCTION

Influenza viruses are significant human respiratory pathogens that cause both seasonal, endemic infections and periodic, unpredictable pandemics [1, 2]. Rapid and sensitive detection of influenza A viruses is critical to the epidemiology of disease prevention, diagnosis, as well as treatment. Current influenza virus detection methods include ELISA, polymerase chain reaction (PCR), virus isolation, and serologic testing.[3-6]. Although effective, these methods are generally time prohibitive, and their applications are limited by the detection sensitivity, specificity, versatility, and portability.

Surface enhanced Raman scattering (SERS) has been developed as a useful tool for chemical and biological detection, providing molecular structural information together with ultrasensitive detection limits, and even single molecule sensitivity.[7-12] Recent developments in SERS substrate technology have facilitated SERS as a means for virus detection and pathogen detection.[13-16] For example, our laboratories have shown that SERS can be used for rapid and sensitive label-free virus detection and identification using silver (Ag) nanorod (AgNR) arrays fabricated using an oblique angle deposition (OAD) method.[17-21] These studies were performed in a laboratory using bench-top Raman instruments, a feature that limits field use or point-of-care detection. While SERS-based Raman detection is powerful, the requirement of laboratory testing reduces its utility for rapid detection of virus infection. Thus, a portable, compact,

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and versatile Raman instrument that provides timely detection at point of care facilities would significantly advance the prospects of SERS-based detection strategies.

The Thermo Scientific FirstDefender RM instrument is a 785-nm handheld Raman spectrometer designed for use by first responders, homeland security, military, law enforcement, and forensic chemistry personnel.[22] This instrument is compact and lightweight which is intended for rapid field identification of unknown solid and liquid samples. In this report, this portable handheld Raman spectrometer is used for the first time, to detect and distinguish low pathogenic avian influenza A virus, specifically A/Mute Swan/MI/06/451072-2/2006 (MS; H5N1), A/chicken/Pennsylvania/13609/1993 (PA; H5N2), and A/chicken/TX/167280-4/02 (TX; H5N3) from their negative control, i.e. allantoic fluid (AF). The handheld Raman detector was evaluated for its specificity and sensitivity for identification of influenza virus using an OAD AgNR array as highly SERS-active substrate. These studies indicate that the handheld Raman spectrometer can be performed in rapid and effective SERS-based virus detection and imply a potential application of this portable system in human health, defense, homeland security and military.

1. EXPERIMENTAL

1.1 Influenza virus

Three types of avian influenza viruses, i.e. A/Mute Swan/MI/06/451072-2/2006 (MS; H5N1), A/chicken/Pennsylvania/13609/1993 (PA; H5N2), and A/chicken/TX/167280-4/02 (TX; H5N3) were propagated in embryonated chicken eggs. Allantoic fluid (AF) was collected from mock-infected (i.e. PBS) embryonated chicken eggs as a negative control sample. AF is a complex and concentrated background matrix, and when applied without dilution, forms a multilayer on the AgNR substrate which effectively quenches the SERS signal. Therefore, all specimens or analytes were diluted in water 100-fold prior to application to SERS substrate.

Figure 1 Handheld Raman system integrated with AgNR array substrates: Left: with a handheld substrate; Right: with a special substrate holder.

1.2 SERS measurement

SERS spectra were collected on uniform AgNR array substrates fabricated by OAD which was described in our previous works [23, 24]. Before adding analytes, the SERS-active AgNR array substrates were patterned by polydimethylsilox-ane (PDMS) with a well-array patterning mold (with 40 wells in 3 mm diameter) first to provide a uniform well-array for high throughput multiplexing. The detailed fabrication process also can be found in our previous work [19].

Bruker Senterra Raman confocal microscope system (OPUS, Bruker Optics, Inc., Billerica, MA) and a handheld Raman system (FirstDefender RM, Thermo Fisher Scientific Inc., Wilmington, MA, Fig. 1) were used to collect the SERS spectra of BPE or/and viruses. For Bruker Raman system, a 785 nm near-IR diode laser was used as the excitation source and the light was focused into 10 μm diameter spot using a 10× objective, the laser power at the sample surface was set at 48 mW. SERS spectra were collected using the high resolution (3-5 cm^{-1}) grating (1200 lines/mm) and 10 s exposure. Instrumental settings for the handheld Raman system were: excitation wavelength 785 nm, power 250 mW, exposure time 50 ms. The key to obtain a good scan is to accurately position the laser focal point on the detected substance. The focal point is permanently positioned at about 3/4 inch (18 mm) from the laser aperture and the beam diameter at the plane of the focal lens is approximately 9.6 mm.[22] For performing a best practice for SERS scanning on the patterned AgNR array substrate, a custom-designed special substrate holder was fixed to the nose cone of the handheld Raman system and used to underprop the chip. This holder has a hole with the same size as the well pattern,

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which can allow the excitation light to pass through the holder and approximately cover the whole sample well, and focus the beam on the sample.

1,2-di(4-pyridyl)ethylene (BPE, ≥98%, Fluka) methanol (≥99.8%, Sigma-Aldrich) solution of 10^{-5} M in 2 μL volumes were added into 5 wells respectively, and the SERS spectra of BPE measured by Bruker and handheld Raman systems were obtained from all the wells. For influenza virus detection, each diluted sample was spotted into 10 patterned wells (10 μL/well) and allowed to evaporate at room temperature prior to spectrum acquisition. The individual SERS signal spectrum was recorded from each well.

1.3 Data analysis

Raw SERS spectra of positive influenza viruses and negative control samples were baseline corrected by using a concave rubber band algorithm (OPUS, Bruker Optics, Inc., Billerica, MA). Classification and identification of the virus was achieved by principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) using PLS Toolbox version 6.2.1 (Eigen Vector Research Inc., Wenatchee, WA). Briefly, raw SERS spectra were first processed by taking the first derivative of each spectrum and then normalizing to unit vector length followed by mean-centered preprocess.[25] PCA and PLS-DA were performed using above preprocessed spectra for the classification of each virus type.[26, 27]

2. RESULTS

Figure 2 shows the average SERS spectra (n = 5) of BPE recorded from the AgNR array substrate by both the Bruker Raman system (black curve) and the Handheld Raman system (red curve). Both the spectra show the characteristic peaks of BPE at around 1198, 1606, and 1637 cm^{-1}, corresponding to C-C stretching mode, aromatic ring stretching mode, and in-plane ring mode, respectively.[28, 29] However, comparing the SERS intensity at different wave numbers we can find that these two instruments show different response. For example, the SERS signal detected by Bruker Raman system shows a strongest peak at 1637 cm^{-1} and the peak at 1198 cm^{-1} has the lowest intensity among these three characteristic peaks. But the spectrum obtained by Handheld Raman system has a strongest SERS peak at 1198 cm^{-1} and a lowest peak at 1637 cm^{-1}. This is mainly due to the difference of instrumental response (CCD sensitivity calibration) to the signals located at short or long wavenumbers. Though there are some response differences between these two instruments, according to the coherence of the characteristic peaks of BPE shown in both spectra, we can still claim that the handheld Raman system can perform effective SERS-based detection.

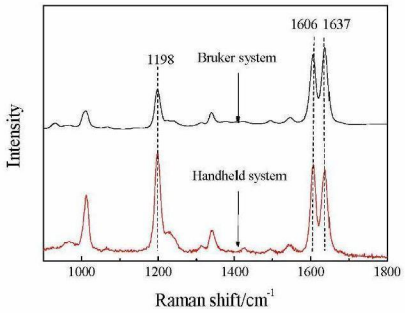

Figure 2 SERS spectra of BPE recorded from the AgNR array substrate by Bruker system (black curve) and Handheld Raman system (red curve).

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The average raw and baseline corrected SERS spectra (n = 10) for each virus and their negative control (AF) collected with the handheld Raman spectrometer are shown in Figure 3a and 3b, respectively. Similar characteristic nucleic acid bands between 400 and 1800 cm^{-1} are observed in all spectra. Notable differences are in the relative intensities of some bands, but the spectra appear very similar to one another in terms of the number and location of peaks. It is difficult to visually differentiate their unique vibrational fingerprints and classify these analytes. To make an effective classification of these viruses and negative control, PLS-DA and PCA were used to establish statistically significant differences between these SERS spectra. The PLS-DA model generated was used to classify the 40 spectra (3 influenza viruses and 1 negative control by 10 replicates). Class prediction calculated by the PLS-DA model for the viruses and negative control is shown in Figure 4, in which the first 30 data points represent the spectra of three influenza viruses (black square for TX virus, red diamond for PA virus, and green up-triangle for MS virus) . The last ten data points (blow down-triangle) correspond to the ten spectra of negative control AF. The plots reveal that the positive virus samples and negative control are only assigned membership to its correct class. Sample points which lie above the dashed threshold line are identified as positive virus while those samples which fall below the threshold are identified as negative control.

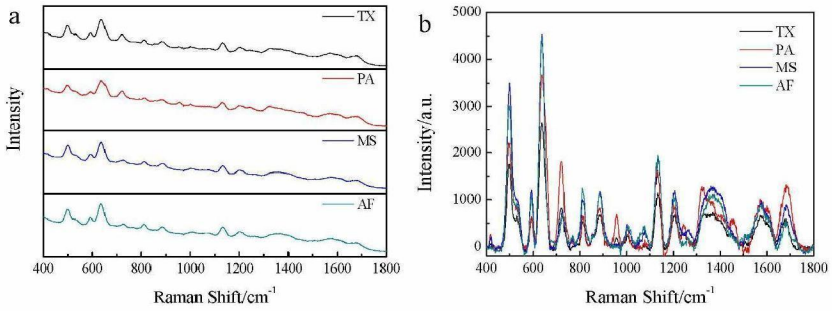

Figure 3 Average raw (a) and baseline corrected (b) SERS spectra (n=10) of the viruses, TX, PA, MS, and their negative control AF collected with the handheld Raman spectrometer.

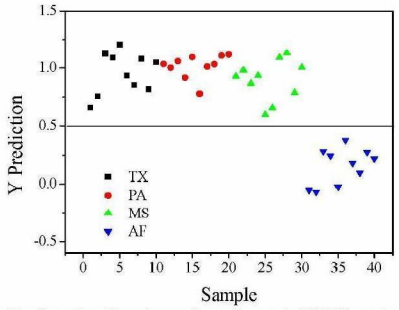

Figure 4 Classification of positive virus and negative control SERS spectra by PLS-DA.

For further classify different types of viruses, PCA has been applied to visualize the clustering of the data according to classes (e.g., TX, PA, MS, AF). Figure 5 shows the PCA score plots of the first versus second principal components (PC1 versus PC2) for the 40 preprocessed spectral data. The plot reveals four distinct clusters with 10 sample points

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belonging to each cluster. The PCA classification result allows a direct visualization of spectral differences of these positive specimens as well as their negative control. Classification results are direct evidence that the Handheld Raman system intended for rapid field identification of unknown samples can be employed as a rapid, direct virus detector.

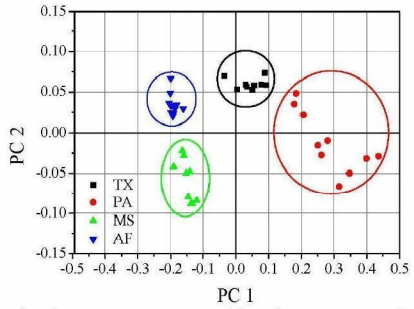

Figure 5 PCA scores plot of PC2 versus PC1 computed from the SERS spectra of TX, PA, MS and AF.

3. CONCLUSIONS

Herein, we show that a handheld Raman spectrometer (FirstDefender RM instrument) can be used to rapidly and accurately detect and identify three types of avian influenza viruses applied to effective AgNR SERS substrates. The efficacy and sensitivity of SERS-based Raman detection was initially confirmed with BPE, a SERS reporter, using AgNR array substrates, and compared to the results obtained by a confocal Bruker Raman system. The following results from this study show that SERS virus detection is effective using a hand held Raman instrument, a feature which allows for field and point-of-care SERS biosensing and detection of pathogens.

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