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Surface enhanced Raman scattering of whole human blood on nanostructured ZnO surface

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Surface enhanced Raman spectroscopy (SERS) owing to the greatly enhanced sensitivity is widely utilized to study biological molecules in various states. However, the enhancement in SERS is not uniform throughout the spectra. As a result, the strong enhancement of some transitions in SER overshadows weak Raman peaks that are very important to characterize the molecules. Here we show the SERS investigation for whole human blood on a nanostructured ZnO surface. The result indicates that despite the moderate enhancement (20–30 fold), all spectral components of the blood demonstrated in regular Raman are detected in SERS on ZnO. Moreover, SERS on ZnO identifies some components of the blood that are not easily accessible to regular Raman spectroscopy. Data indicate that SERS on ZnO is a valuable tool to investigate the whole blood for diagnosis of various human diseases.

1 Introduction Human body fluids contain an enormous amount of various functional molecules, some of which are modified in a wide variety of diseases. However, whole blood is the most informative to evaluate human health. Today, many diseases including cancers can be determined by blood analysis. Every disease creates its own molecular signature that can be identified by biochemical, molecular biology, and etc. analysis. Recent years, spectroscopic methods, such as infrared and Raman spectroscopies, are widely utilized for diagnostic proposes [1–4]. The goal is to identify "fingerprint regions" for each disease or abnormality. Spectroscopic methods may also reveal the abnormal components of the blood that are the risk factors for various diseases.

Raman spectroscopy is a valuable tool to investigate blood [1–4]. The method is robust, informative, and does not require any extrinsic labeling. The intensity of regular Raman scattering is very weak that diminishes its application in dilute solutions of biological molecules. Surface enhanced Raman spectroscopy (SERS) has emerged as a valuable complement to Raman techniques. For some transitions, 10^6-10^8 enhancements are readily observed in SERS intensities [3]. This phenomenon significantly increases the applicability of Raman spectroscopy to investigate the structure of biological molecules. Often concentrations of molecules produced by the diseases are very low to be detected by conventional methods. Therefore, greatly enhanced spectral intensities in SERS increase detectability of the target components of the blood. However, application of SERS to molecules of various origins is not straightforward. In some cases, strong enhancement of few bands may mask the Raman transitions that are very important to characterize the molecules.

Previously, Al mirror that contains nano-islands of 10–50 nm heights was used for surface enhanced infrared spectroscopic ellipsometry (SEIRSE) [5] on a protein called silk fibroin. Results have indicated non-uniform enhancement across the spectrum of ε_2. As mentioned above, non-uniform enhancement of the spectral components may limit

the applicability of the surface enhanced techniques on biology and medicine.

In this work, we describe SERS of whole human blood on nanostructured thin ZnO film surface. Despite the moderate enhancement (20–30 fold), all spectral components of the blood observed in regular Raman are detected in SERS on ZnO. Moreover, SERS on ZnO detects some components of the blood that are not accessible to regular Raman spectroscopy.

2 Sample preparation and experimental procedures

2.1 Fabrication of ZnO and ZnO: Al thin films ZnO and ZnO: Al films were fabricated by magnetron sputtering method using an EVOVAC deposition system (Angstrom Engineering Inc., Canada) as described previously [6]. Briefly, ZnO and ZnO: Al films were deposited by magnetron sputtering method on lime glass substrates at 200–400 °C. In addition, substrates were kept under various oxygen/argon mixtures (0–3% O_2). The deposition rate was chosen to be 1 Å s^{-1}. The films were examined by Atomic Force Microscopy (AFM) and X-ray Diffraction techniques. Among of tested films ZnO in argon environment displayed the highest enhancement in Raman scattering. Therefore, below results are shown only for ZnO films deposited at 300 °C.

2.2 Human whole blood Human whole blood samples were collected from the arm vein of healthy voluntaries. To prevent coagulation, the heparin pre-treated vials were used for storage at 4 °C. For the Raman spectroscopy, the blood samples were used at the same day of collection.

2.3 Confocal Raman spectroscopy Raman spectra of the whole blood samples at the surface of a lime glass with and without ZnO film were obtained with a confocal Raman spectrometer (Confocal Raman spectrometer, Nanofinder30, Tokyo Instr., Japan) at a wavelength of 532 nm. The magnification factor of the objective lens was 40. For Confocal Raman spectroscopy, a few μl of blood samples were spread on the desired surface. The laser beam and detection channel were perpendicular to the surface. The fringes observed on the air-glass interface were used to position the focal point of the laser to the specified distance from the surface.

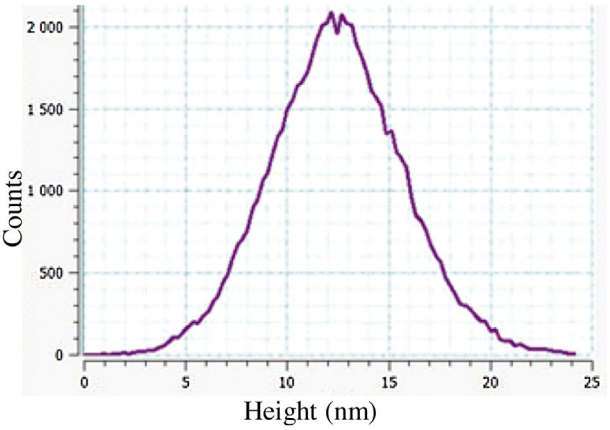

Figure 2 The distribution of the height values for the projections on the surface of the ZnO thin film.

2.4 Atomic force microscopy The surface morphology of the ZnO films were assessed using AFM (SmartSPM 1000, AIST-NT, Japan). The AFM images were analyzed by using the software provided by the company.

3 Experimental results and discussion

AFM image of the ZnO film deposited on the lime glass at 300 °C is illustrated in the Fig. 1. The surface roughness of the ZnO films reaches up to 20 nm.

The histogram in the Fig. 2 shows the distribution of the heights of the ZnO projections.

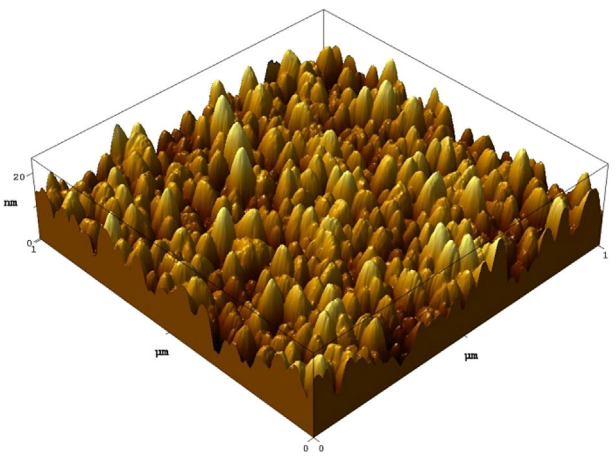

Figure 1 AFM image of the ZnO surface used in the SERS application.

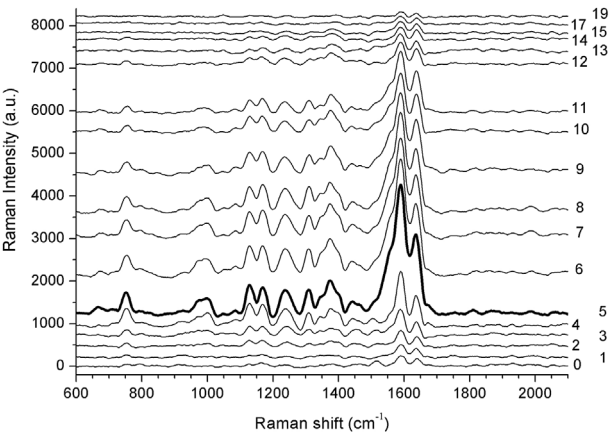

Figure 3 Confocal SER spectra of the whole blood at various distances from the surface of the ZnO thin film. Numbers on the right side indicate distance (in μm) from the surface.

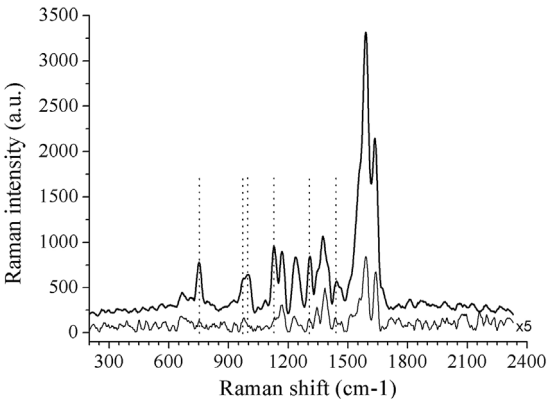

Figure 4 Confocal SER spectrum (bold line) of the whole blood at the distance of 5 μm from the surface of the ZnO thin film versus the regular Raman spectrum (thin line). Vertical dotted lines denote the positions where regular Raman peaks are not distinguishable from the noise.

As can be seeing from the Fig. 2, the ZnO projections span from about 5 nm to 20 nm with the peak value around 12.5 nm. To check mechanism of enhancement, confocal SERS measurements of the whole human blood were performed at various distances from the ZnO surface (Fig. 3). The Raman intensities of the whole blood are distance dependent and reach to the highest values for the major component around 5 μm from the surface.

The intensity values of the major components of the SER spectra increased about 20–30 folds. Positions of the peaks are very consistent throughout the distance depended measurements. The besides that, all components of the spectra are discernable in the surface enhanced measurements. To check the quality of the SERS on ZnO, the surface enhanced spectrum of the whole blood with the highest intensity was compared to that of obtained with regular Raman scattering (Fig. 4). It is evident that peak positions of the both spectra are well agreed. Furthermore, some peaks that are not apparent or within the noise level (dotted vertical lines) in regular Raman transition are well identified in the SER spectrum. Potentially each peak in Raman spectra carries the information that helpful for diagnosis of human diseases.

It should be noted that qualitatively the whole blood samples showed the same kind of enhanced Raman spectra on the ZnO: Al surfaces (data not shown). However, the degree of the enhancement was noticeably less compared to those obtained on the ZnO described above. One may speculate that less enhancement may be the result of changes in structural as well as chemical properties of the surface. More experimental data are needed to properly describe these findings.

In the SERS of the whole human blood, the peak positions and their assignment are shown in the Table 1. It is evident that the SER spectrum on ZnO surface reveals the vibrational bands of the molecules with different origin. Both oxy- and metRBC cells are detected.

Table 1 Positions (in cm^{-1}) and assignments of the Raman bands of whole human blood.

ν (cm^{-1})	assignments	ref.
668	unknown origin	
624	C–C twisting mode of phenylalanine	[2]
710	ν_{11} in both oxy- and metRBC in SERS	[3]
752	heme group (ν_{15})	[3]
824	ring breathing, Tyr	[2, 7, 8]
975	unknown origin	
1000	symmetric ring breathing mode of Phe	[2, 9]
1050	C–H in-plane bending mode of Phe	[10]
1085	unknown origin	
1129	ν_5 of oxyRBC in non-SERS application	[3]
1145	C–C/C–N stretching mode	[2]
1170	ν_{30} in SERS application	[3]
1237	Trp and Phe n(C–C6H5) mode, amide III	[10]
1308	collagen, and ν_{21} (the same in both SERS and non-SERS application	[3, 7]
1347	Glu, Asp, Asn, Gln: CH2 sciss, Ala, Leu, Val, Ile: CH3 deformation	[10]
1374	ν_4 oxyRBC in non-SERS application	[3]
1401	ν_{20} oxyRBC in non-SERS application	[3]
1443	δ (CH_2) collagen; phospholipids; hypoxanthine	[7, 10, 11]
1464	C–H deformation	[3]
1510	amid II	[1]
1557	tryptophan	[2]
1589	heam (CC stretching (conjugated)	[9]
1635	amide I (C=O stretching mode of proteins, beta-structure conformation)	
1672	amide I (C=O stretching mode of proteins, alpha-helix conformation)	[1, 2]

Assignments of the peaks indicate that not all peaks are positioned for SERS application. The position of some peaks, such as ν_4, ν_5, and ν_{20} are consistent with regular Raman scattering.

In SER scattering, the enhancement of the transitions originated at least by two mechanisms, the electromagnetic (EM) and chemical [10]. EM enhancement comes from surface localized plasmon resonance excitation of nanostructure metallic surfaces. The local EM field enhancement gives a rise Raman transition up to 10^4 fold. The enhancement factor from chemical mechanism is about 10^2. For this mechanism formation of a charge-transfer state between a nanostructured surface atoms and investigated molecules is necessary.

The data, enhancement factor, and the peak positions of SERS on ZnO, indicate that both factors play an active role in the enhancement. Low enhancement factor can partially be contributed to the adsorption properties of the molecules on the substrate. A contribution from multiple reflections to the SER can be excluded. Mainly heterogeneous enhancement is observed when the enhancement phase is located at the surface, as in this work. However, relatively homogeneous SERS were observed for whole blood on the ZnO

surface. Further work should be conducted to elucidate the detailed mechanism of the Raman scattering enhancement on the ZnO surface.

4 Conclusions The whole human blood samples on nano-structured ZnO surface show enhanced Raman scattering where the enhancement factors of about 20–30 fold are comparable across the spectral range. Therefore, the enhanced Raman spectra are similar to that of regular Raman. Furthermore, SERS on ZnO reveals some spectral components of the whole blood that are not easily noticeable in the regular Raman spectra. These features of SERS on ZnO increase its diagnostic value. Data indicate that SERS on ZnO is a valuable tool to investigate the whole blood for diagnosis of various human diseases.

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