ChemComm

This article is part of the

Surface Enhanced Raman Spectroscopy web themed issue

Guest editors: Professors Duncan Graham,
Zhongqun Tian and Richard Van Duyne

All articles in this issue will be gathered together online at
www.rsc.org/sers.

COMMUNICATION

Clean and modified substrates for direct detection of living cells by surface-enhanced Raman spectroscopy†‡

Jia-Yi Huang, Cheng Zong, Li-Jia Xu, Yan Cui and Bin Ren*

Iodide adsorption and electrochemical negative potential desorption were proposed and compared to obtain clean SERS substrates. The two methods can effectively eliminate the interference of surface impurities in the SERS detection. SERS signals of membranes of living cells with a good reproducibility have been obtained.

Raman spectroscopy can obtain rich structural information to identify chemicals and biological samples with the vibrational fingerprints, and the Raman signal of water is very weak. So it is well suited for studying living cells in systems containing water. Recently, there has been increasing interest in this area.[1] However, its broader application is still limited by the inherent weak signals of most normal Raman processes.[2,3] This weakness has been overcome at least partially by using surface-enhanced Raman spectroscopy (SERS). SERS can enhance the Raman signals of target species adsorbed on Ag or Au metallic nanostructures by as much as 6 to 14 orders of magnitude, which even allows detection of the signal of single molecules.[4–8] Therefore, SERS is a promising technique highly sensitive for analysis of living cells.

The existing SERS methods developed for studying living cells can be sorted into two categories: indirect detection and direct detection. In the indirect detection, nanoparticles labelled with some molecular tags or markers with extremely strong Raman signals are used to trace the interaction of the nanoparticles with specific components of living cells, which is similar to other probe methods, such as fluorescence spectroscopy.[9–12] It has provided some information complementary to other spectroscopic methods. The biggest advantage of the direct method is the multiplex capability, benefiting from the narrow width of the Raman band, and the excitation with a single wavelength. But the molecular signatures of the target system will be lost. In the direct detection, nanoparticle sols[13–17] or solid SERS substrate[18,19] are used to interact directly with the living cells to obtain the rich molecular and structural information of them. The direct use of well-dispersed nanoparticle sols for SERS study of living cells is quite challenging, because they can only give relatively weak SERS signals, especially before aggregation.[20] Furthermore, the surfactants inherited from the synthesizing process can not be completely removed by centrifugation without sacrificing the stability of the nanoparticles. A common practice in SERS to enhance signal is to use a solid substrate, which can be prepared by dispersing the sols cleaned by centrifugation on a solid substrate. Usually a compact layer of nanoparticles will form on the substrate after several cycles of dispersion and drying, which can provide very high enhancement and a relatively good surface uniformity.[21] However, such a compact layer will reduce the optical transparency of the substrate, hampering the SERS study with an inverted microscope commonly used for studying living cells. Furthermore, the surfactants and reductants will remain on the surface and are difficult to remove, which will severely interfere with the SERS detection of cells.

In this respect, we decided on a trade off between the SERS signal uniformity and optical observation and employed the commonly used self-assembly method to assemble Au nanoparticles[22] on the 3-aminopropyltrimethoxysilane (APTMS)-functionalized surfaces of indium tin oxide (ITO),[23] as shown in Fig. 1. Again, the substrates prepared using this method during the synthesis or the assembly process will still be inevitably covered by reductants or surfactants showing peaks
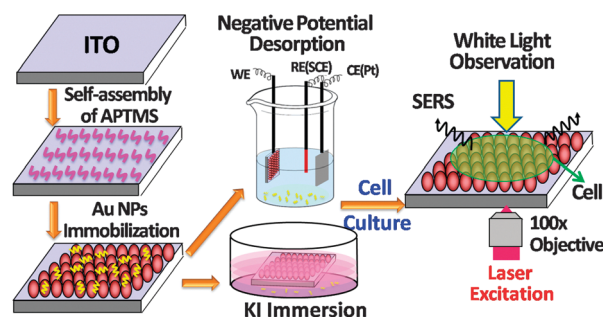
State Key Laboratory of Physical Chemistry of Solid Surfaces, Key Laboratory of Analytical Sciences, Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China. E-mail: bren@xmu.edu.cn; Fax: +86 592-2181906; Tel: +86 592-2186532

Fig. 1 Schematic diagram of the self-assembly processes of Au nanoparticles on an ITO glass substrate and the SERS detection of living cell membrane by using the assembled clean SERS substrate.

in the spectral range of 1400–1500 cm^{-1}, even for adsorbates as strong as pyridine (see Fig. S1, Supporting Information). These species may occupy the hot spot regions of the mostly enhanced electromagnetic field, which will on the one hand block the surface sites for target molecules, especially those with a weak interaction with Au, and on the other hand severely interfere with the analysis of SERS spectra, especially for a complex unknown system like a living cell.

Much effort has been devoted to cleaning SERS substrates.[24–26] There is still no effective method that can avoid re-adsorption of impurities, reduction of surface adsorption sites and decrease of SERS enhancement. On the other hand, some biomolecules on the membrane of a living cell are dynamic and one would expect a dynamic changing signal in SERS. Therefore, to minimize the signals of impurities on the SERS substrate while retaining the SERS activity is crucially important to the applicability of SERS to the study of living cells. To this end, two methods were developed to obtain SERS substrates with minimal surface impurities and to improve the affinity of the substrate with some special biological molecules on the surface of cell membranes.

The first method utilizes the electrochemical desorption of reductants and surfactants at an appropriate negative potential. The potential dependent SERS study of the substrate in the absence of any adsorbates reveals that the signal of impurities approaches the minimum at −0.7 V in 0.1 mol L^{-1} NaClO$_4$ solution (Fig. S2a). The time dependent study of the same system at −0.7 V reveals that an immersion time of 10 min gives a clean spectral background. The potential and the immersion time were then chosen as the optimal condition for cleaning (Fig. S2b). To prevent the re-adsorption of the contaminants, the substrate was taken out of the solution with the potential control on while being rinsed with a fresh 0.1 mol L^{-1} NaClO$_4$ solution until it lost contact with the solution.

The second method is simply to dip the SERS substrate into a solution containing 0.1 mol L^{-1} KI solution for 10 min followed by rinsing with water. Iodide can be strongly adsorbed on Au surfaces forming the strong Au–I bond at 158 cm^{-1} and easily replace the existing impurities and prevent re-adsorption of impurities or those species with a weak interaction with Au, showing a clean background in SERS spectra (see Fig. S3). Iodide has no effect on the physiological activity of cells when the concentration is lower than 3 × 10^{-2} mol L^{-1}.[27] In fact, even when the whole surface is covered by iodide, iodide concentration is still much lower than the threshold value. Thus, iodide protected substrates can be conveniently used for study of living cells.

The SERS spectra of living cells obtained on the surface of an unclean substrate and two substrates cleaned by negative potential and iodide replacement are shown in Fig. 2 (see supporting information for experimental procedures). Compared with the unclean substrate, the SERS spectra from the clean substrates show a better reproducibility with time. On the cleaned substrates, the bands located at 1000 cm^{-1}, 1034 cm^{-1} and 1200 cm^{-1} (or 1204 cm^{-1}) are from phenylalanine (Phe) and tyrosine (Tyr), see Fig. 2b and c,[14,15,28] which could not be easily detected on the untreated substrate. Phe and Tyr are aromatic amino acids and can form peptides
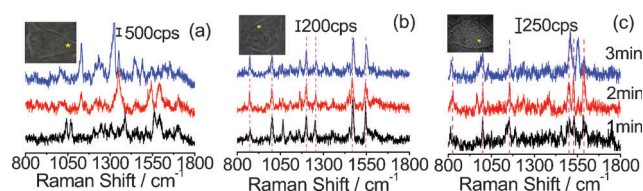

Fig. 2 Time-dependent SERS of cell membranes from (a) the unclean substrate, (b) the substrate cleaned at −0.7 V for 10 min in 0.1 mol L^{-1} NaClO$_4$ solution, and (c) the substrate cleaned by immersing in 1 × 10^{-3} mol L^{-1} KI solution for 10 min. The acquisition time for each point was 10 s, and the laser power at the sample was 1 mW. Yellow star marks the measurement spot.

with cysteine that has a thiol group and can be strongly adsorbed on Au surface by forming the strong Au–S to replace Au–I, producing a strong SERS signal.[29]

It should be noted that even on the cleaned surfaces, we still observed quite different SERS signals at different positions of the cell membrane, as shown in Fig. 3. Three representative positions on a membrane of each cell (marked with yellow numbers) were selected for both negative-potential and KI treated substrates. Spectra Blank in Fig. 3 are from the same SERS substrate but at a position in the absence of cells. From the figure, we are surprised to find that although two substrates were treated by different methods, the bands of Phe and Tyr (1000 cm^{-1} and 1034 cm^{-1}) were both obtained. But, it should be pointed out that the frequencies of most other bands from a cell with a high reproducibility in Fig. 3a are quite different from that in Fig. 3b. Clearly, the cell membrane is chemically inhomogeneous and some of the biomolecules in the cell membrane may move around. Therefore, it is understandable considering the inherent inhomogeneity of a cell membrane and differences in each cell and the treatments of the substrates.

A common strategy to overcome this problem is to average the SERS spectra from the same cell to obtain the representative signature of the cell.[7] To this end, we chose four cells for each substrate and three representative positions on each cell similar to that in Fig. 3. Three to five spectra were acquired at each position. Each spectrum in Fig. 4 is an average of 10–15 spectra. For both substrates, the number of peaks with good reproducibility decreases after average. The 1000 cm^{-1} and 1034 cm^{-1} bands can be observed. Furthermore, the bands at 1145 cm^{-1} and 1358 cm^{-1}, which may be assigned to deoxyribose-phosphate and proteins,[14,15] were also detected. It is interesting to find that the average SERS spectra
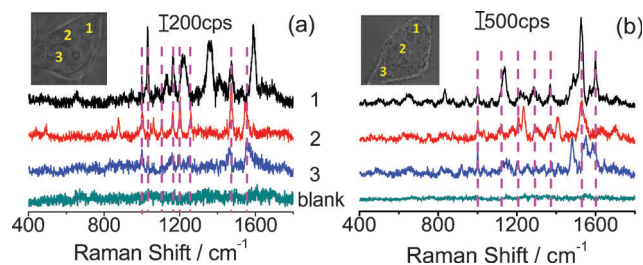

Fig. 3 SERS spectra of cell membrane obtained at different spots of the same cell from substrates cleaned (a) at a negative potential and (b) in 1 × 10^{-3} mol L^{-1} KI. All the conditions are the same as Fig. 2.
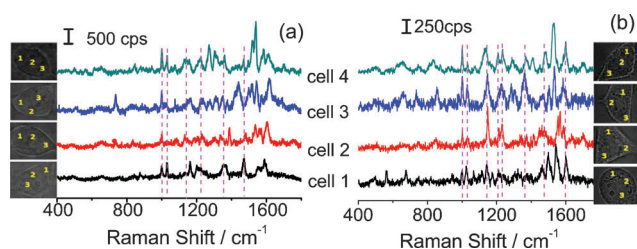
Fig. 4 The average SERS spectra of cell membrane obtained at different living cells from substrates cleaned (a) at a negative potential and (b) in 1×10^{-3} mol L^{-1} KI. All the conditions are the same as Fig. 2. Yellow numbers mark the spots for acquiring the SERS spectra to obtain the average SERS spectra.

of different cells cultured on the KI treatment substrate are quite similar to each other (Fig. 4a and b) and the reproducibility is apparently better than for the negative desorption method. The time dependent SERS spectra obtained at the same spot of the same cell on the two types of substrates also reveal a slightly better reproducibility of the KI immersion method compared with the negative potential one.

To understand the different behaviors of the two types of substrate, it will be worthwhile to compare the SERS spectra of the KI treated substrate before and after the cell adhesion (Fig. S3 and S5). After the adhesion of cells, the band at 158 cm^{-1} of Au–I vibration becomes weaker (see Fig. S5), and a strong and broad band appears at 286 cm^{-1} coming from Au–S vibration, indicating that part of the iodide has been replaced by the cellular components via the stronger Au–S interaction. Therefore, the advantage of the present cleaning method lies in the fact that we can selectively detect the SERS signal coming from the cellular components that can compete with iodide and be directly attached to the Au surface. Meanwhile, the other regions of the substrate are still covered by iodide. In this way, it can prevent the direct adsorption of those weakly and dynamically adsorbed species from the solution on the Au substrates. Thereby, it can minimize the signal variation and the interference in the SERS detection of the living cell. On the other hand, the negative potential desorption method can provide a substrate with open surface sites for adsorption. Therefore, all species can be attached to the surface, which may produce much rich information, but also more dynamic spectral response. The combination of the two methods may provide more complete information on the living cell systems.

In summary, we propose an electrochemical negative potential desorption method and an iodide immersion method to obtain a clean SERS substrate to allow reliable SERS measurement of living cells from a methodological point of view. On both substrates, nice signals of Phe and Tyr can be obtained for different cells and at different times. Compared with the iodide immersion method, negative potential desorption is suitable for gaining the SERS of both weak and strong binding molecules with Au in cell membranes, but can only be applied to conductive substrates. The iodide immersion method is convenient to work with and can be applied to both conductive and non-conductive SERS substrates. More importantly, the iodide treated substrates can selectively enhance the

Raman signals of molecules forming a stronger bond with Au than Au–I, which significantly improves the reproducibility of SERS, and avoids the interference of the species having a weaker interaction with Au in the cell solution.

Notes and references

1 H. O. Hamaguchi, *Curr. Sci.*, 2009, **97**, 186–191.
2 B. Pettinger, *in Adsorption at Electrode Surface*, ed. J. Lipkowski and P. N. Ross, VCH, New York, 1992, pp. 285–345.
3 R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, Wiley-Interscience, New York, 2000.
4 M. G. Albrecht and J. A. Creighton, *J. Am. Chem. Soc.*, 1977, **99**, 5215–5217.
5 D. L. Jeanmaire and R. P. Van Duyne, *J. Electroanal. Chem.*, 1977, **84**, 1–20.
6 S. M. Nie and S. R. Emory, *Science*, 1997, **275**, 1102–1106.
7 K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari and M. S. Feld, *Chem. Rev.*, 1999, **99**, 2957–2975.
8 K. Kneipp, H. Kneipp, I. Itzkan, R. R. Dasari and M. S. Feld, *Chem. Phys.*, 1999, **247**, 155–162.
9 C. Y. Song, Z. Y. Wang, R. H. Zhang, J. Yang, X. B. Tan and Y. P. Cui, *Biosens. Bioelectron.*, 2009, **25**, 826–831.
10 Y. Cui, B. Ren, J. L. Yao, R. A. Gu and Z. Q. Tian, *J. Raman Spectrosc.*, 2007, **38**, 896–902.
11 D. Du, H. X. Ju, X. J. Zhang, J. Chen, J. Cai and H. Y. Chen, *Biochemistry*, 2005, **44**, 11539–11545.
12 B. H. Jun, M. S. Noh, J. Kim, G. Kim, H. Kang, M. S. Kim, Y. T. Seo, J. Baek, J. H. Kim, J. Park, S. Kim, Y. K. Kim, T. Hyeon, M. H. Cho, D. H. Jeong and Y. S. Lee, *Small*, 2010, **6**, 119–125.
13 K. Kneipp, A. S. Haka, H. Kneipp, K. Badizadegan, N. Yoshizawa, C. Boone, K. E. Shafer-Peltier, J. T. Motz, R. R. Dasari and M. S. Feld, *Appl. Spectrosc.*, 2002, **56**, 150–154.
14 J. Kneipp, H. Kneipp, M. McLaughlin, D. Brown and K. Kneipp, *Nano Lett.*, 2006, **6**, 2225–2231.
15 W. Xie, L. Wang, Y. Y. Zhang, L. Su, A. G. Shen, J. Q. Tan and J. M. Hu, *Bioconjugate Chem.*, 2009, **20**, 768–773.
16 R. J. Dijkstra, W. Scheenen, N. Dam, E. W. Roubos and J. J. ter Meulen, *J. Neurosci. Methods*, 2007, **159**, 43–50.
17 I. A. Larmour, K. Faulds and D. Graham, *Chem. Sci.*, 2010, **1**, 151–160.
18 E. A. Vitol, E. Brailoiu, Z. Orynbayeva, N. J. Dun, G. Friedman and Y. Gogotsi, *Anal. Chem.*, 2010, **82**, 6770–6774.
19 S. Shanmukh, L. Jones, J. Driskell, Y. P. Zhao, R. Dluhy and R. A. Tripp, *Nano Lett.*, 2006, **6**, 2630–2636.
20 X. M. Lin, Y. Cui, Y. H. Xu, B. Ren and Z. Q. Tian, *Anal. Bioanal. Chem.*, 2009, **394**, 1729–1745.
21 D. Y. Wu, J. F. Li, B. Ren and Z. Q. Tian, *Chem. Soc. Rev.*, 2008, **37**, 1025–1041.
22 P. P. Fang, J. F. Li, Z. L. Yang, L. M. Li, B. Ren and Z. Q. Tian, *J. Raman Spectrosc.*, 2008, **39**, 1679–1687.
23 L. Wang and E. K. Wang, *Electrochem. Commun.*, 2004, **6**, 225–229.
24 K. L. Norrod and K. L. Rowlen, *J. Am. Chem. Soc.*, 1998, **120**, 2656–2657.
25 V. M. Bermudez and L. M. Ericson, *Langmuir*, 2006, **22**, 2258–2263.
26 M. D. Li, Y. Cui, M. X. Gao, J. Luo, B. Ren and Z. Q. Tian, *Anal. Chem.*, 2008, **80**, 5118–5125.
27 M. Vitale, T. Di Matola, F. D'Ascoli, S. Salzano, F. Bogazzi, G. Fenzi, E. Martino and G. Rossi, *Endocrinology*, 2000, **141**, 598–605.
28 J. De Gelder, K. De Gussem, P. Vandenabeele and L. Moens, *J. Raman Spectrosc.*, 2007, **38**, 1133–1147.
29 F. Wei, D. M. Zhang, N. J. Halas and J. D. Hartgerink, *J. Phys. Chem. B*, 2008, **112**, 9158–9164.