Label Free Identification of Glucose Using Flexible SERS Platforms

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Abstract—**Metal assisted chemical etched Si nanostructures have been transferred onflexible sheets (adhesive tape) followed by deposition of Au/Ag nanoparticles to make SERS active platform. It shows significant enhancement of Raman signal of glucose in the vicinity of metal nanoparticles due to its coupling with the surface plasmon of nanostructures. SERS detection of glucose is extremely difficult because of its weak Raman cross section and poor adsorption to metal NPs. Fabricated SERS platform was utilized to detect glucose without any label at low concentration 10 micro molar (μM). The developed cost-effective SERS platform has potential applications as a platform device for non-invasive, label free detection of glucose in human fluid for point of care applications.**

Keywords—Si nanostructures, SERS, Glucose

I. Introduction

Since the first attempt of Clark and Lyons in 1962 for the glucose detection, many analytical methods have been reported based on reduction of glucose on enzymetic, non-enzymetic electrodes biosensor viz. viz., electrochemistry [1], colorimetry [2] and fluorescence [3] methods. Till date, electrochemical methods are considered to be best and are having higher sensitivity. Electrochemical methods areadopted for regular monitoring of blood glucose in patient for clinicalpurpose, but major disadvantage such systems is the need of regular calibrationand it is invasive in nature in the sense that one need to prick small samples of blood using a "finger-stick". Other methods also experience several limitation as they based on same principle of proteinmediated binding of glucose [4]. Additionally, fluorescence biomolecules similar to glucose can interfere with actual signal and giving false positive results. Though, fluorescence technique have physiologically relevant detection range and is promising technique [5].

Consequently, it is essential to develop a faster, easiermethod for monitoring of glucose levels at high sensitivity and selectively. Developments of such methods are important for societal applications.Since the discovery of Surface Enhanced Raman Spectroscopy (SERS) in 1970, SERS has emerged as powerful tool for various applications including single molecule detection, forensic, medical diagnosis, analytical chemistry and bio-sensing [5-7]. Several research studies have been demonstrated for the detection of glucose using SERS [9-12]. However, sensitivity and reproducibility of glucose sensing via SERS still is challenging as glucose shows a very weak Raman signal and poor adsorption of glucose at metal nanoparticles (NPs). Therefore, surface functionalization mechanism on the surface of metal NPs using self-assembled monolayer is used to capture the signal of glucose. G. Qi et al. demonstrated glucose sensing using the turn off mechanism of SERS, and metal NPs were labeled with 4-mercaptopyridine [13].

SERS substrates show significant enhancement of Raman Signal of molecule in the vicinity of metal NPs due to chemical and electromagnetic enhancement [6-7]. The main contribution of Raman intensity enhancement is electromagnetic phenomenon by the local electromagnetic field in the vicinity metallic surfaces due to the excitation of localized surface plasmon [8-9]. To achieve high sensitivity of SERS, many research groups have explored SERS substrates by fabricating rough metal surface, nanowires or by nanomaterials [14]. Most of the SERS substrates are realized on the top of fragile and stiff base substrates (silicon, glass and alumina) [14-16]. Although hard SERS platforms are satisfactorily useful in many sensing applications, traditional rigid SERS platforms are not suitable for direct adsorption of molecule from curved surfaces during sample collection.

Recently, flexible platforms such as paper, cotton, and polymer material emerged as an alternative forflexible SERS substrate to overcome limitations of conventional rigid platforms. Flexible SERS platform can be enfoldedand swabbed onto curved, irregular and non-planner surface to collect the analytes by simply covering the targeted samples surface[15].Additionally, flexible SERSsubstrateoffers non-destructive and non-invasive detection of analytes. Various types of flexible SERS substrates have been described in literature based on the basis of process of plasmonic nanoparticles on the surface of flexible platforms such as polydimethylsiloxane (PDMS), flexible polymer film, paper, adhesive tapeand etc. J Chen et al. [16] have demonstrated the flexible SERS based on adhesive tape with activity of Au nanoparticles which is limited to 2D structures. Adhesive tape is widely used in daily life as well as in research.

Here, we have demonstrated the 3D Si nanostructures (NSs) on flexible tape utilizing sticky feature of adhesive tape. These Si NSs were coated with Au/Ag nanoparticles (NPs) for SERS operation. Metal NPs on 3D structures provide high density of hot spots in compare to 2D structures. Further, glucose sensing at low concentrationwas demonstrated on the developed flexible SERS platform.

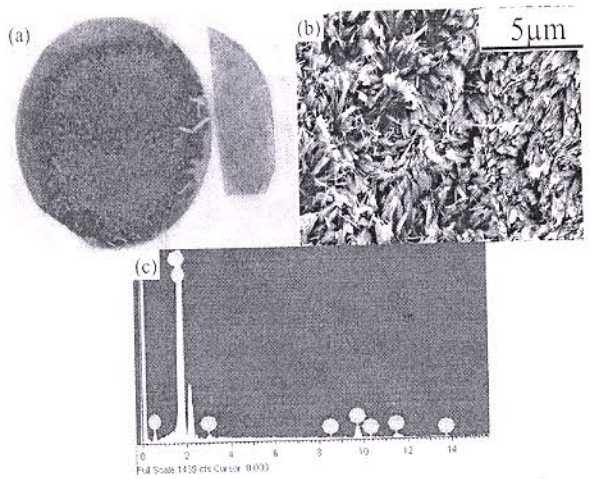

Fig. 1 (a) Developed Si NSs on wafer and their transfer on flexible substrate (b) SEM image of Si NSs on flexible substrate (c) EDS spectra of transferred Si NSs and Au/Ag NPs on SERS active substrate

II. EXPERTIMENTAL DETAIL

Silicon nanostructures (Si-NSs) were developed using electroless metal deposition methods based on galvanic cell process and obtained on the sticky surface of scotch tape. To develop Si NSs, p-type Si wafer were dipped in solution containing HF & $AgNO_3$ for 5 minutes at room temperature to deposit Ag nanoparticles (Ag NPs) on Si surface. Further, Ag NPs coated Si wafers were kept for etching in an etching solution of HF and H_2O_2. Etched wafers were transferred in HNO_3 acid to dissolve Ag metal present on the etched sample. Further, Si NSs were obtained on scotch tape; using sticky nature of the tape.The detail understanding can be obtained from our previous publication [17]. At last, Au and Ag NPs were deposited using DC sputtering on transferred Si NSsto make SERS active platform. To examine the SERS functionality of developed SERS substrate for glucose sensing aqueous solutions of D-glucose in concentrations of (10μM, 20μM, 30μM, 40μM and 50 μM) were prepared in DI water.

Surface morphology of all samples was studied by field scanning electron microscope (Carl Zeiss Sigma series supra-55). Raman measurements were carried out on a Renishaw Raman spectroscopy system at room temperature, using 532 nm LASER as an excitation source with 15mWpower. Accumulation time was kept 10 seconds during Raman measurement.

III. RESULTS AND DISCUSSION

Fig. 1 (a) shows the developed of Si NSs on entire wafer. After the development of Si NSs on wafer, wafer looks black in color because of negligible reflection of light from the surface of wafer. To obtain the Si NSs on flexible sheet, sticky tape i.e. scotch tape is pressed over the developed Si NSs on wafer and then slowly removed from the surface. Si NSs break from the wafer and stick to the flexible scotch tape as shown in Fig. 1(a). The FESEM image in Fig. 1 (b) shows presence of 3D SiNSs on scotch tape. The length of the NSs was the

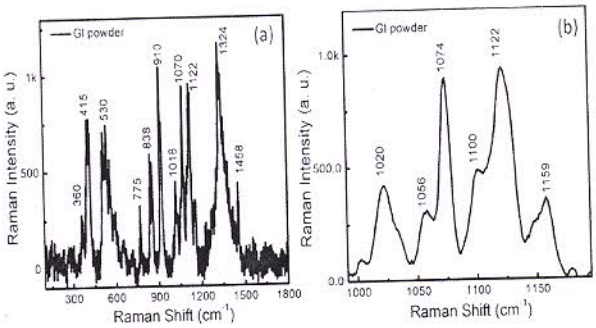

Fig. 2 (a) Raman spectra of powder D-glucose (0.01 mg) (b) in-large view of Raman spectra of glucose in the rage of 1000-1200 cm-1

approximate ~10 μm. The scanning electron microscope image is shown in Fig. 1 (b) and clearly reveals long Si NSs at the top of flexible scotch tape. Energy dispersive spectroscopic (EDS) analysis of Si NSs in Fig. 1 (c) exposed the presence of Au/Ag NPs.

Prior to examine the SERS functionality of developed SERS substrate for low concentration of glucose in aqueous solution (DI water); it is necessary to understand the various mode of glucose to interpret the results. Therefore first of all, Raman spectra of powder glucose (0.01 mg) were studied. Understanding of various vibration mode of powder glucose molecule could provide a reference point and help us to correlate Raman signal of glucose in aqueous solution by monitoring wave number i.e. Raman shift and change inintensity counts corresponding to various vibration mode of glucose molecules.

Fig. 2 (a) shows various Raman peaks corresponding to different functional group and mode of d-glucose. In the Raman spectra of glucose several peaks below 1500 cm^{-1} are distinct explicitly 1324 cm^{-1}, 1122 cm^{-1}, 1070 cm^{-1}, 910 cm^{-1}, 530 cm^{-1} and 415 cm^{-1} attributed to various vibration mode of

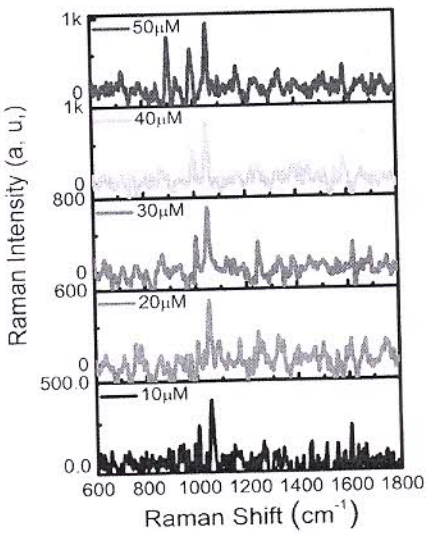

Fig. 3.Raman spectra of various glucose concentrations (10μM to 50μM) in DI water on SERS substrate

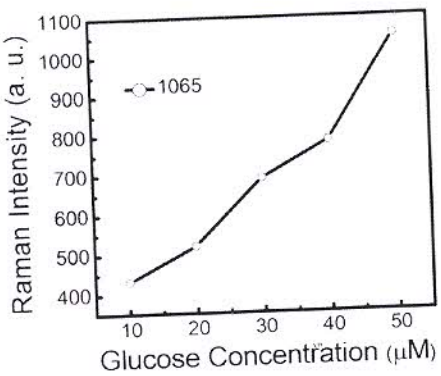

Fig.4.Intensity variation of prominent SERS peak 1065 cm^{-1} of glucose as a function of glucose concentration

C-H and C-C and C-O atom in glucose [18]. The peak 1324 cm^{-1} could be assigned to wagging mode of CH_2 in d-glucose [9, 18-19]. The most study and identify peaks 1020 cm^{-1}, 1060 cm^{-1} and 1122 cm^{-1} are used by various groups to detect and calculate the glucose concentration in human fluid and consider as reference peaks for bio-sample analysis. Therefore, these peaks are shown enlarge view of Raman Shift from 1000 cm^{-1} to 1200 cm^{-1}in the Fig. 2 (b). Raman peak 1122 is attributed to the bending of C-O-H vibration mode while peaks 1020cm^{-1} and 1070 cm^{-1} could be assign to the C-O stretching mode of d-glucose.

Now developed SERS substrates were incubated with glucose solution for 1 minute followed by the drying of sample at room temperature. Raman measurement was performed on dry sample to suppress the Raman signature corresponding to water molecules. The recorded Raman spectra of various glucose concentrations on the SERS platform are shown in Fig. 3. The most prominent peaks of glucose on SERS platform is corresponding to 1065 cm^{-1} was observed for all concentration and might correspond to 1074 cm^{-1} as recorded in powder glucose signature as represented in Fig. 2(b). As the concentration of glucose increases from 10 μM to 50 μM, other peaks corresponds to various vibrations mode of powder glucose sample were also observed in the Raman spectra (see Fig. 3). One notable discrepancy in the spectra is the absence of sharpness in the peaks and their shifting rather intense Raman spectra of powder glucose. This can be assign to formation of hydrogen bond which in turn reduces the frequency shift of CH_2 vibrations. Shifting of glucose peaks was also observed with increasing concentration of glucose in the aqueous solution [18]. The notable peaks 910 cm^{-1}, 1000 cm^{-1} and 1065 cm^{-1} were clearly observed in glucose sample at concentration of 50 μM.

The variation in intensity counts of prominent peak 1065 cm^{-1} under SERS mode is shown in Fig. 4 as a function of glucose concentration. It can be noted that the intensity value of the peak follows almost linear relation to the concentration of the glucose. The experimental conditions were kept very straight forward without any labeling to capture the glucose on the metal NPs. Developed SERS platform is sensitive to low concentration of glucose and respond to change in intensity of Raman peaks ofglucoseas a function of concentration which can be directly monitored.

IV. Conclusion

This work demonstrates a novel approach to realize flexible SERS substrate. Fabrication process is comparatively cheap and feasiblechoice for mass production of SERS platform. Further, a label free direct detection of glucose at low concentration usingflexible SERS platform is very significant as glucose has modest Raman cross section. This method could be significant to develop invasive point-of-care monitoring of glucose in diabetic patients.

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References

[1] P.A. Palod,S. S. Pandey,S. Hayase,V. and V. Singh,"Template-assisted electrochemical growth of polypyrrole nanotubes for development of high sensitivity glucose biosensor" Applied biochemistry and biotechnology, 174(3), pp.1059-1072, 2014.

[2] P. A. Palod, and V. Singh, "Improvement in glucose biosensing response of electrochemically grown polypyrrole nanotubes by incorporating crosslinked glucose oxidase". Materials Science and Engineering: C, 55, pp.420-430, 2015..

[3] Y. Song,K. Qu,C. Zhao, J. Ren, and X. Qu, Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection. Advanced Materials, 22(19), pp.2206-2210, 2010.

[4] P. Wu, Y. He,H.F. Wang, and X. P. Yan"Conjugation of glucose oxidase onto Mn-doped ZnS quantum dots for phosphorescent sensing of glucose in biological fluids". Analytical chemistry, 82(4), pp.1427-1433, 2010.

[5] D. A. Stuart,C. Yonzon,X. Zhang, O. Lyandres, N. C. Shah, M. R. Glucksberg, J. T. Walsh, and R. P. Van Duyne, "Glucose sensing using near-infrared surface-enhanced Raman spectroscopy: gold surfaces, 10-day stability, and improved accuracy". Analytical chemistry, 77(13), pp.4013-4019, 2005.

[6] C. Zhang, P. Yi, L. Peng, X. Lai, J. Chen, M. Huang M, Ni J. Continuous fabrication of nanostructure arrays for flexible surface enhanced Raman scattering substrate. Scientific reports, 7, pp. 39814, 2017.

[7] G. McNay, D. Eustace, W. E. Smith, K. Faulds, D. Graham,"Surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS): a review of applications". Applied spectroscopy, 65 (8), pp. 825-37, 2011.

[8] S. Park, J. Lee, H. Ko, "Transparent and flexible surface-enhanced Raman scattering (SERS) sensors based on gold nanostar arrays embedded in silicon rubber film" ACS applied materials & interfaces, 9, pp. 44088-95, 2017.

[9] N. C. Lindquist, P. Nagpal, K. M. McPeak, D. J. Norris, and S. H. Oh, "Engineering metallic nanostructures for plasmonics and nanophotonics", Reports on Progress in Physics, 75, pp.036501, 2012.

[10] K. E. Shafer-Peltier,C. L. Haynes, M. R. Glucksberg,R. P. Van Duyne,"Toward a glucose biosensor based on surface-enhanced Raman scattering", Journal of the American Chemical Society, 125, pp.588, 2003.

[11] Y. Yi, J. Deng,Y. Zhang, H Li, and S. Yao"Label-free Si quantum dots as photoluminescence probes for glucose detection". Chemical Communications, 49, pp. 612, 2013.

[12] A. Ceja-Fdez, T. López-Luke, A. Torres-Castro, D. A. Wheeler, J. Z. Zhang, E. De la Rosa"Glucose detection using SERS with multi-branched gold nanostructures in aqueous medium" RSC Advances, 103, pp. 59233, 2014.

[13] G.Qi, K. Jia, C. Fu, S. Xu, W. Xu. "A highly sensitive SERS sensor for quantitative analysis of glucose based on the chemical etching of silver nanoparticles". Journal of Optics,17,pp. 114020, 2015.

[14] G. J. Zhang, L. Zhang, M. J. Huang,Z. H. H. Luo,G. K. I. Tay, E. J. A. Lim,T. G. Kang, and Y. Chen, "Silicon nanowire biosensor for highly sensitive and rapid detection of Dengue virus". Sensors and Actuators B: Chemical, 146, pp.138-144 2010.

[15] H. Qiu, M. Wang, S. Jiang, L. Zhang, Z. Yang, L. Li, J. Li, M. Cao, J. Huang, "Reliable molecular trace-detection based on flexible SERS substrate of graphene/Ag-nanoflowers/PMMA". Sensors and Actuators B: Chemical, 249, pp. 439-50, 2017.

[16] J. Chen, Y. Huang, P. Kannan, L. Zhang, Z Lin, J. Zhang, T. Chen, L.Guo. "Flexible and adhesive surface enhance Raman scattering active tape for rapid detection of pesticide residues in fruits and vegetables". Analytical chemistry, 88 pp. 2149, 2016.

[17] A. Kumar, J. Agrawal,A. K. Sharma, V. Singh, and A. Agarwal,"A cost-effective and facile approach for realization of black silicon nanostructures on flexible substrate". Journal of Materials Science: Materials in Electronics, pp.1-82019.

[18] M.Mathlouthi, D. V. Luu. "Laser-raman spectra of d-glucose and sucrose in aqueous solution". Carbohydrate Research.81, pp. 203, 1980.

[19] E. Wiercigroch, E. Szafraniec, K. Czamara, M. Z. Pacia, K.Majzner, K. Kochan, A. Kaczor, M. Baranska, andK. Malek,"Raman and infrared spectroscopy of carbohydrates: A review". Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.185, pp. 317, 2017.