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Silver Nanowires (AgNWs) Growth In-situ on Chitosan Polymer Matrix Film for SERS application[#]

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***Abstract*—Chitosan polymer matrix film is prepared from a mixed solution of chitosan and silver nitrate by drop-casting at different surfaces. The film shows various advantages for handling such as flexibility and mechanical strength. UV-Vis, FTIR, SEM and XRD analysis confirms the reduction of silver nitrate to form silver nanoparticles (AgNPs) and its *in-situ* growth of the silver nanowires (AgNWs) in the film. Surface-enhanced Raman spectroscopy (SERS) analysis demonstrates that the AgNWs/chitosan film displays a robust enhancement of the intrinsic Raman signals of chitosan, which serves as proof-of-concept that the chitosan-AgNW thin film will be unique for SERS verification with the advantage of avoiding the use of fluorophore tags and potentially being an easy-to-fabricate SERS substrate amenable for sensing application.**

I. INTRODUCTION

Surface enhanced Raman spectroscopy (SERS) is a powerful vibrational spectroscopy technique that allows for highly sensitive structural detection of low concentration analytes through the amplification of electromagnetic (EM) fields generated by the excitation of localized surface plasmons[1]. In simple terms, this method enhances the existing Raman signal of the substrate material of interest with help of nanostructured materials over the same substrate. This light concentration occurs preferentially in the gaps, crevices, or sharp features of plasmatic materials, which are traditionally noble and coinage metals (e.g., silver, gold, and copper) with preferably nanoscale features formed on the surfaces[2]. Reproducible and robust structures that strongly enhance the EM field are most desirable for SERS. Depending on the structure of the supporting plasmonic material, EM enhancement for SERS is theoretically calculated to reach factors of ~ 10^{10} – 10^{12}[3]. The success of SERS is highly dependent on the interaction between adsorbed molecules and the surface of plasmonic nanostructures, often the classic SERS substrates of gold (Au), silver (Ag), or copper (Cu)[4]. In general, Au and Ag are most often used as SERS substrates because they are air stable materials, while Cu is more reactive. All three metals have surface plasmon resonance (SPR) that cover most of the visible and near infrared wavelength range, where most Raman measurements occur, also making them convenient to use avoiding the biological emission range which usually hinders the accurate biomolecule detection signal measurements. Novel materials such as graphene[5], semiconductors such as TiO_2 [6], and quantum dots[7] have recently been reported to work with metals for SERS, although they do not fit traditional definitions of SERS substrates.

Chitosan is a known biocompatible polymer, which have its specific Raman vibrational modes, where when laser light of specific wavelength is shined on, due to the molecular bonding vibrations. Raman detector collects specific vibrational peaks which on a whole represents as Raman Spectroscopy. When a specific molecule of detection is employed onto this polymer matrix platform, the existing Raman signal is not enhanced since there are no SERS materials. However, in this research, this polymer matrix will help to reduce the silver nitrate to form silver metal nanoparticles and eventually, as a base, to let the nanoparticle nucleate together to grow Ag nanorods or nanowires (AgNWs) under proper synthesis and growth conditions[8, 9].

Coinage metals like (Ag, Au, Cu), and their nanoparticles and nanostructures have the property of generating SPR, when light of specific energy is shined on them. When these nanostructures are arranged over a detection platform such that they are close enough to each other, localized surface Plasmon resonance (LSPR) will be generated to further enhance the Raman signal existing for the material subject to measure over the platform[10, 11]. Many kinds of silver nanostructures are employed for this need[12-15]. In current research, we use *in-situ* grown AgNWs as SERS enhancement material and chitosan polymer matrix as Raman signal generator, of which the enhancement is measured and monitored due to the presence of mentioned AgNWs. The unique factor and key advantage in current platform are *in-situ* and self-growth of AgNWs in chitosan polymer matrix film and no fluorophore use of the platform for instant verification of SERS.

II. MATERIALS AND METHODS

Chitosan polymer (molecular wt.: 150,000, 1.5% W/V) and Acetic acid were purchased from Sigma-Aldrich (USA), received without further purification. AgNO3 (>99%) was purchased from Sigma-Aldrich chemicals.

2.1. Synthesis of Silver Using Chitosan

Chitosan (0.5gm) was dissolved in 10 mL of water with 1% v/v acetic acid solution adding to the same. Acetic acid helped chitosan to dissolve in the water. This solution was thoroughly magnetic stirred before using for synthesis of silver. $AgNO_3$ (50mg) was dissolved completely in 5 ml of water (10^{-2}M). The prepared two solutions were taken in 1:5 (by volume) ration and kept for low power ultra-sonication for a few hours till violet color was shown up, confirming the formation of silver in the solution.

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2.2. AgNWs Growth and Thin Film Formation

The synthesized silver/chitosan solution was drop casted (a few µL) onto various substrates like plane glass, cover slip and PET film, and subjected to dry under room temperature (Fig. 1). Due to the adhesion support from substrate, after few hours of leaving the film without disturbing, due to nucleation of formed Ag NPs in the solution tend to nucleate together in the film and growth of AgNWs from the chitosan polymer matrix film was observed. It was observed that PET substrate had low adhesion and so films were easily able to peel off for further characterizations.

2.3. Characterization of Chitosan-AgNWs Composite Film

Number of characterization techniques were used in this research. SEM (Zeiss Auriga FIB/SEM) was used for morphology analysis of the as prepared thin films. XRD (Gemini A single crystal X-Ray diffractometer) was used for crystal structure analysis of Ag-Chitosan thin film. UV-vis (Agilent 600i UV-vis spectrometer) was used for confirming the presence of silver in thin film using silver resonance peak due to UV-vis excitation. FTIR (Agilent 600 IR FTIR) was used for finger print analysis of Ag-Chitosan composite thin film. Raman (XploRA$^{\text{TM}}$, Raman confocal microscope) was used for SERS analysis on the film.

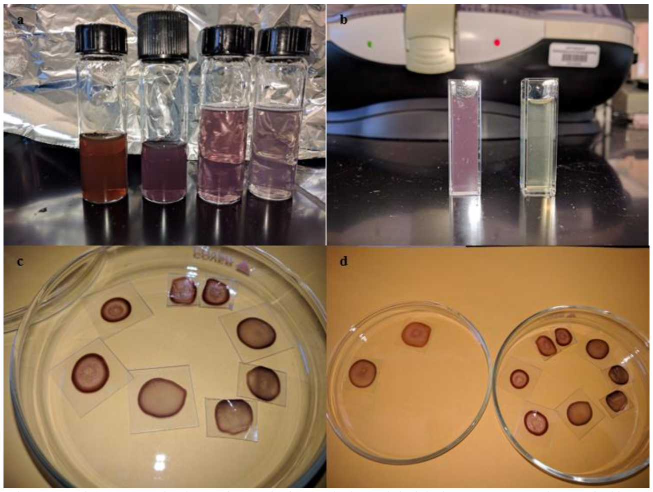

Fig 1. (a-b). Ag solution, from left to right with high concentration of silver to low; (c-d). Ag NWs growth on thin film over various substrates.

III. RESULTS AND DISCUSSION

Chitosan polymer solution was prepared by continuous stirring and dissolving the same in water solvent along with addition of acetic acid, which helped chitosan dissolving in water. Silver Nitrate solution was added to the prepared chitosan polymer solution and was subjected to low power sonication till the color of the solution was changed to purple color as shown in Fig 1, indicating the formation of silver in the chitosan polymer matrix. AgNWs were subjected to self-grown *in-situ* in the chitosan polymer matrix after forming a thin film by simple drop casting the solution over desired platforms. i.e. plane glass, cover slip and PET. The drop casted platform was carefully shifted to hot oven and was heated at 50 °C till the solution gets dries off forming a thin film.

AgNWs growth was observed in the thin film of the chitosan polymer matrix due to the available energy consumption and the nucleation of the synthesized AgNPs by the presence of chitosan. Further characterization of the thin film with various analytical tools confirmed the presence of AgNWs along with Ag NPs still on the surface of the chitosan polymer matrix.

3.1. SEM analysis for Morphology of the AgNWs Film

After forming the AgNWs integrated with chitosan polymer matrix thin film by above mentioned method, SEM analysis were performed on the film. From Fig 2a, it can be observed that chitosan polymer film thickness (~500 nm) with the deposition of 300 µl of as prepared Ag/chitosan solution. The film is quite strong, flexible and can be easily peeled off from the deposited surface. Fig 2b shows the spread of the synthesized AgNPs with various size distribution in the range of approximately 4nm to 100nm in the chitosan film. This image was taken immediately after the deposition of the Ag solution on to the substrate, where it was observed no nucleation of Ag NPs and no formation of Ag NWs yet. Fig 2c-d shows Ag NWs growth at different scales of SEM with thin film being one-day old compared to film in Fig 2b. This is quite a proof for claiming Ag NWs self-growth from inside the chitosan polymer matrix. Further experiments are in progress for increasing the aspect ratio of the Ag NWs.

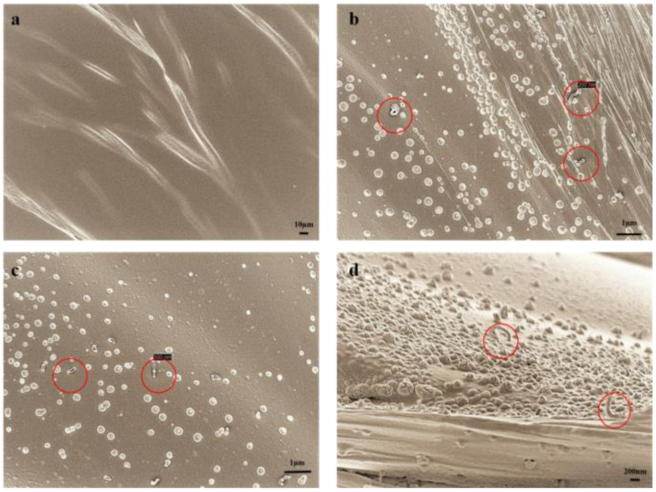

Fig 2. SEM morphology of Ag embedded chitosan thin film at various magnifications. (a). Ag/chitosan film showing the top view in the beginning; (b, c). Ag NWs growth on film surface with visible NPs nucleation at 1µm scale; (d). Ag NWs growth image at 200 nm scale.

3.2. XRD Analysis

The XRD pattern of the AgNWs-Chitosan thin film is shown in Fig 3 A. Five standard X-ray characteristic peaks (111), (200), (220), (311), and (222) confirmed that Ag in the thin film is in the FCC structure. Dominant peak (200) plane is more intense than typical (111) plane, suggesting that the (200) plane is the predominant orientation, demonstrating the film is highly crystalline[16]. The time resolved growth of the Ag NW study is underway.

3.3. FTIR Analysis

Infrared analysis of as such chitosan polymer matrix film was performed with the help of standard IR laser and ZnSe source. In a typical case of pure chitosan, similar to various research articles the standard peaks observed are: peaks in broad bands between 3360 cm^{-1} and 3295 cm^{-1} correspond to amine (N-H) and hydroxyl (O-H) stretching vibrations; further dominant peak at 2870 cm^{-1} corresponds to asymmetric stretching vibrations of CH_3 and CH_2 of chitosan polymer. Absorption peak at 1560 cm^{-1} corresponds to bending vibration of amine N-H (N acetylated amide II band). Peak at 1420 cm^{-1} typically corresponds to amide III (N-H) stretching vibrations. The peaks observed at 1060 cm^{-1} and 1030 cm^{-1} are corresponding to the vibrations of secondary hydroxyl group and primary hydroxyl group, which also correspond to carbonyl stretch. Significant stretch in these two peaks with respect to pure chitosan peaks were observed in Ag-Chitosan composite film IR peak, however with different intensities, this is because silver particles are bonded by chitosan, which served as a stabilizing agent that helps in reducing $AgNO_3$ ions to Silver NPs. Clear depiction of this stretch is seen in the inset figure of Fig. 3 B.

3.4. UV-Vis Analysis

Further analytical analysis for the presence of silver in the prepared Ag-Chitosan polymer matrix thin film was performed by using UV-Vis spectroscopy. Fig. 3 C-D present the UV-vis spectra of Ag-chitosan composited solution and its thin film. It is observed clearly that, in Fig C, the intensity of dominant peaks at 390 nm and over 550nm which typically denotes the presence of Ag NPs below 10nm and distribution of different structured Ag respectively in the solution. The peaks of solution can be compared with film peaks in Fig 3 D, where it is clearly seen the intensity reduction of AgNPs peak at 390 nm probably due to the less concentration of Ag composite and embedment of the Ag NPs in chitosan, as well as the reduced AgNPs amount since they come together to nucleate and grow as AgNWs. This growth of AgNWs is also supported by the narrowing of peak over 550nm compared to peak at the same wavelength in solution, suggesting some other nanostructured shape other than AgNPs, i.e. Ag NWs.

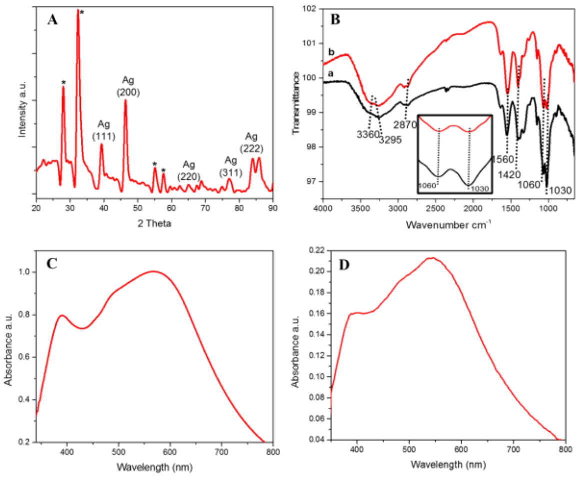

Fig 3. A. XRD spectra of the AgNWs-Chitosan film, peaks marked with stars arising from the crystalline organic phase; B. FTIR spectra of AgNWs-chitosan film (a) and pure Chitosan film (b), Inset figure shows the key stretch of the peaks at 1060 cm^{-1}, and 1030 cm^{-1} due to presence of silver. C. UV-vis spectrum of Ag-Chitosan solution. D. UV-vis spectrum of Ag NWs-Chitosan film.

3.5. SERS Analysis

SERS analysis on the film platform is presented. Fig. 4 displays pure chitosan polymer matrix film with basic Raman signals of peaks at 1300 cm^{-1}, 1500 cm^{-1} and a main peak at 2800 cm^{-1}. In this analysis, pure chitosan polymer matrix film at varying laser powers i.e. using different filters (1%, 10%, 25%, 50%, 100%) for allowing laser to shine on the sample; the spectra show ascending order increase in the intensities associated to the peak 2800 cm^{-1} and less significant enhancement on rest of peaks at 1300 cm^{-1}, 1500 cm^{-1}. There is good enhancement in the dominant peak signal with the increase of laser power, however it is known that the strong light intensity of the laser power shining on the molecules will possible spoil the system of detection, especially if there is silver metal for SERS enhancement in detecting biomolecules.

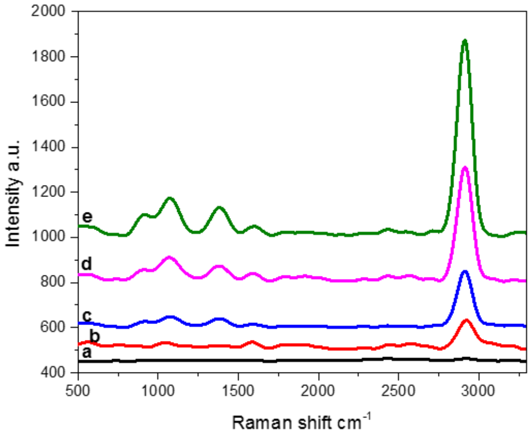

Fig 4. Raman spectra of pure chitosan with varying power (a-e, 1%, 10%, 25%, 50%, 100%) of used laser for measurements, with clear display of dominant peak intensity changes.

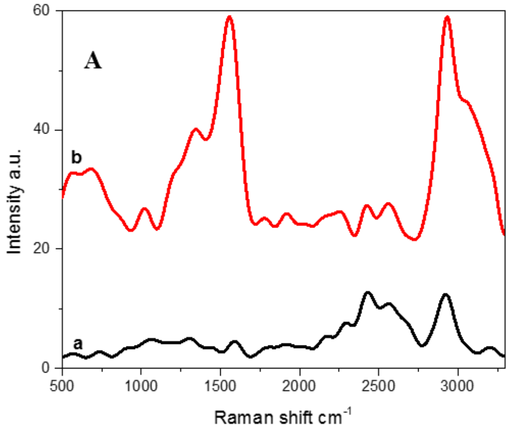

Fig 5. Raman spectra at 1% filter of laser intensity for (a) pure chitosan film and (b) AgNWs-chitosan film.

With the presence of silver in the chitosan polymer matrix, the Raman signals of chitosan were enhanced significantly even at the very low power laser measurements, which would be highly helpful for the non-damage detection of biomolecules. Fig. 5 depicts the comparison of the SERS enhancement due to the presence of AgNWs with respect to pure chitosan film Raman signals at low power laser (1% filter of the Raman equipment). With the presence of AgNWs, as SERS theory suggests, existing Raman signals of chitosan were greatly enhanced and hidden Raman signals were captured. This is clearly seen in Fig 5, where chitosan Raman peaks at 1100 cm^{-1} and 1550 cm^{-1}, which are not visible in pure chitosan Raman measurements in Fig 4a, are clearly visible in AgNWs-chitosan composite film (>40x increase at 1550 cm^{-1}). SERS enhancement (~5x increase of the AgNWs-chitosan film) is seen at the dominant 2800 cm^{-1} peak with 1% filter of light intensity illustration. It was observed that higher laser power itself damages the sensing platform (data not shown). The AgNWs-chitosan platform demonstrates advantages of the SERS enhancement at low power laser source, and the intrinsic Raman signals may be used for biomolecule detection by avoiding using additional fluorophore Raman tags.

3.6. Enhancement Factor (EF) calculation:

Analytical Enhancement Factor(*AEF*) calculation approach is followed for analyzing the SERS enhancement efficacy of the designed platform. The related equation is given below:

$$AEF = \frac{I_{SERS}/C_{SERS}}{I_{RS}/C_{RS}} \qquad (1)$$

where I_{SERS} is intensity of Raman peak obtained on the SERS platform and C_{SERS} is the concentration of solution used for SERS measurement on the platform. I_{RS} is the standard Raman peak intensity of the analyte used for SERS analysis on plane glass and C_{RS} is the concentration of the same subjected to measurement on plane glass.

In the Experimental conditions, Rhodamine 6G (R6G) analyte C_{RS} is of 20 µM and C_{SERS} is of 500 nM. Fig. 7 are the plotted Raman signals of R6G on plane glass, SERS enhancement of R6G on the Ag NWs platform, respectively.

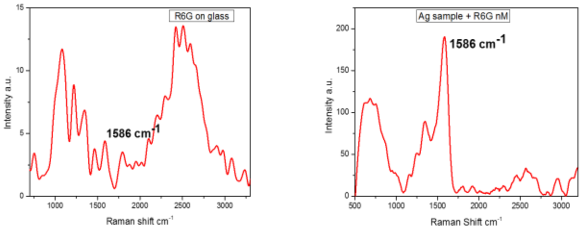

Fig 7. (a). R6G of 20 µM on plane glass with indicated 1586 cm^{-1} peak; (b). R6G of 500 nM on Ag sample with indicated enhanced 1586 cm^{-1} peak.

Using equation 1, plot intensity data of the signature 1586 cm^{-1} Raman peak of R6G and employed R6G concentrations. Obtained AEF is of 0.175×10^4. The obtained signal enhancement is reasonably good compared to the ease of design of the detection platform.

3.7. Finite-difference time-domain (FDTD) Analysis:

Theoretical simulation for field enhancement of the proposed experimental platform which had shown surface plasmon enhancement with the presence of AgNWs was verified using FDTD simulation software[17].

Simulation was performed on single NWs with similar dimensions as shown in the SEM images, with diameter of 150 nm and length of 500 nm in average. The single NW is directly placed on the Chitosan film with material properties defined form reference [18] and of dimensions X = 1 µm, Y = 1µm and Z = 200 nm. The Simulations setup is shown in below Fig. 8a. Subjected simulation conditions include, the electrical field enhancement monitor placed along the boundaries of the Ag NW and optical source used for simulation defined as Total-field scattered-field (TFSF) source of wavelength range from 300 nm to 400 nm.

Electrical or E-field enhancement is shown in the Fig. 8b, where the field intensity distribution is in ascending order form the center of the Ag NW towards the sides of the same. This theoretical results represent the field enhancement of a single NW grown on the chitosan film. This setup is in close relation with the experimental growth of AgNWs on chitosan film, where it is assumed that the SERS enhancement measurement is obtained from the individual AgNWs.

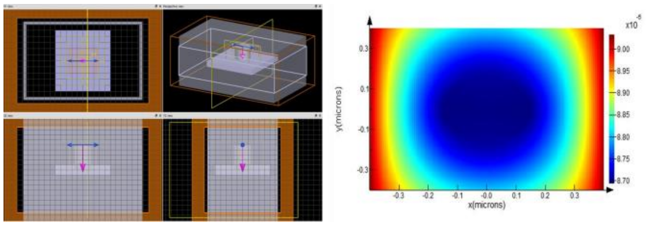

Fig 8. (a). Simulation setup viewed in various planes (XY, YZ). (b). E-field enhancement distribution along the single AgNW at the chitosan film.

IV. CONCLUSION

Portable devices for biomolecules detection is need of current day. They are many proposed methods for this need. SERS is one of the simplest and easy method in designing the platform in operational sense. Our proposed platform has various unique advantages than other platforms of the same league. Since this design method is a simple in-situ growth of the SERS silver NWs in chitosan polymer-silver nitrate matrix rather than previous works where silver NPs or NWs or any other nanostructures formed as composite. This silver in-situ growth platform shows clear difference in the range of Raman spectral enhancement. In addition, there is one key advantage in operational side with the chitosan film by using the intrinsic Raman signals while, in most SERS measurements a fluorophore tag is needed in order to verify the enhancement of the designed platforms. The AgNWs-chitosan platform provide a non-fluorophore based enhancement verification method, in which, as presented in the paper, the AgNWs are embedded in the chitosan polymer film, and near 2000× enhanced chitosan Raman signal is obtained with good agreement with the electrical field enhancement obtained by FDTD simulation. Since this platform can be made into flexible thin films, it can potentially be used as an easy-to fabricate, low-cost SERS substrate amenable for sensing many analytes and portable device integration.

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