Abstract: A novel surface-enhanced Raman scattering (SERS)-based probe to capture heavy metal ion (Zn$^{2+}$) by bovine serum albumin (BSA) using Si-nanowire (SiNW) arrays with silver nanoparticles (AgNPs) was developed. A layer with AgNPs was deposited on the SiNW surface by RF magnetron sputtering for enhancement of SERS signals. Using a high-resolution transmission electron microscope (HRTEM), the observation reveals that the AgNP layer with depths of 30–75 nm was successfully deposited on SiNW arrays. The Ag peaks in EDS and XRD spectra of SiNW arrays confirmed the presence of Ag particles on SiNW arrays. The WCA observations showed a high affinity of the Ag–SiNW arrays immobilized with BSA (water contact angle (WCA) = 87.1°) and ZnSO$_4$ (WCA = 8.8°). The results of FTIR analysis illustrate that the conjugate bonds exist between zinc sulfate (ZnSO$_4$) and –OH groups/–NH groups of BSA. The resulting SiNWs/Ag NPs composite interfaces showed large Raman scattering enhancement for the capture of heavy metal ions by BSA with a detection of 0.1 µM. BSA and ZnSO$_4$ conjugations, illustrating specific SERS spectra with high sensitivity, which suggests great promise in developing label-free biosensors.

Keywords: silicon nanowires (SiNWs); surface-enhanced Raman spectroscopy (SERS); heavy metal ions; BSA

1. Introduction

Heavy metal ions (Zn$^{2+}$, Hg$^{2+}$, and Cd$^{2+}$), which frequently occur in the environment, medicine, and the food industry, are sources of certain chronic and acute visceral syndromes [1,2]. The detection of heavy metal ions is a vital issue due to their extreme toxicity.

Among detection techniques, spectroscopy and fluorescence are usually employed to detect Zn$^{2+}$, Hg$^{2+}$, and Cd$^{2+}$ ions, but these methods require a long time for bio-sample preparation and pre-treatment. Despite the fact that these techniques are precise and accurate in identification of toxic metal ions, the major disadvantages associated with them include high cost, longer execution time, and need of technically sound workers. Therefore, many types of rapid nondestructive measuring methods have been developed for the measurement of heavy metal ions in recent decades.

Raman spectroscopy has been widely used for organic and surface analysis. This technique shows high sensitivity to the disorder of the structure based on the optical measurement of the surface. Owing to the development of a large-scale Raman spectroscopy system, surface-enhanced Raman spectroscopy (SERS) can analyze the vibrational signatures of material associated with its chemical information. SERS signals arise mostly from enhanced electromagnetic fields near the surface, particular vibrational excitation modes [3,4], and specific chemical interactions [5,6]. SERS are capable of nondestructive detection and identification of molecules without exogenous labels due to the inherent vibrational frequency and Raman modes in the surface spectra [7]. In other words, the SERS effect is very fast and accurate in the detection of the biomolecules [8], antibiotics [9].
pesticides [10], food additives [11], sulfate ions [12], and single-molecules [13–15]. Although SERS is widely used in the study of bio molecular detection, there is little literature on detection of BSA-captured heavy metal ions using SERS [16].

Many studies have been demonstrated various SERS substrates based on various substrate materials. Supplementary Table S1 shows the comparison of parameters and sensitivities based on different SERS substrates. It can be seen from Table S1 that SERS substrate can detect a small amount of various substances with high sensitivity, for example using AgNPs/glass SERS substrates to detect paraquat (PQ) up to \(10^{-9}\) M [10]. Moreover, the SERS intensity enhancement depends on size, shape, and others of the materials. Palanisamy and other scholars have shown that when the cluster size increases to more than 100 nm, the SERS intensity shows that there is a very weak or no signal [17]. That is to say, the SERS substrates are smooth, the metal particles are too large, the SERS signal is very weak, or even there is no signal (shown in Table S1).

Among all these materials, SiNW arrays are very suitable for SERS substrates due to their high surface areas, high densities, high roughness, and high concentrations of the characteristic tips. The SiNWs show high light-scattering, which increase the interaction between metals and molecules, and thus enhance the SERS signals. It is known that the sizes and shapes of the metal nanoparticles play the major role in the enhancement of the SERS effect [17,18]. Furthermore, it is known that large silver nanoparticles (<100 nm) show strong SERS intensity, due to the increasing intensity of electromagnetic hot spots on silver nanoparticles [17]. Supplementary Table S2 lists the SERS literature comparison based on Ag (or Au)-modified SiNW array substrates. It is confirmed from supplementary Table S2 that the morphology of silicon nanowires and the size of silver (or gold) nanoparticles significantly affect the SERS signal [19,20].

Several methods have been developed to synthesize SiNWs successfully, such as vapor–liquid–solid (VLS) [21], oxide-assisted [22], microwave plasma [23], and chemical vapor deposition (CVD) [24,25]. Promising substrates with metal nanoparticle SiNWs have been grown on silicon wafers using metal-catalyzed CVD [26,27]. However, the SiNW arrays grown by the CVD method are usually in random orientation [28], which affects the results of SERS substrate analysis. Recently, a top-down approach based on silicon wafer processing by wet etching to induce metal particles has been successfully developed to fabricate SiNW arrays with uniform controlled size, crystallographic orientation, and density. The SERS analysis is available for the uniform synthesized large-area SiNW arrays by this approach.

Silver has been reported as one of the SERS substrates. Several Ag-based substrates, such as Ag-coated latex spheres, Ag colloids, and Ag-coated filter membranes, have been analyzed by SERS in the past few decades [29–32]. Large and predictable SiNW arrays have been successfully fabricated, and the decoration of Ag nanoparticles (AgNPs) has confirmative influences on the enhancement of SERS analysis of substrates. For bio-application, serum albumin is an important metal-binding protein in circulation systems. The detection of heavy metals is needed in serum albumin, which plays a role in metal transport, storage, and detoxification in organisms. Nonetheless, there is little literature on the detection of BSA-captured heavy metal ions on SiNW arrays with AgNPs on SERS. In addition, there are few literatures that use Ag modification of SiNW arrays by RF sputtering and its application in SERS. RF sputtering is a powerful technique for the deposition of metal clusters and NPs on surfaces. Moreover, it is good for the SERS environment because not many chemicals are involved in the stabilization of the clusters. It is easy to obtain uniform clusters.

Measuring the interface between nanomaterial and biological molecules is important in biological applications. The interaction of hydrophilic groups is a good and highly sensitive index for antibody immobilization because proteins, ion-binding proteins, and DNA usually have electronic charges on their surfaces. Water contact angle (WCA), introduced by Thomas Young as a way of investigating wetting behavior and surface tension, is now applied in the measurement of single or multi-layer surface treatment
techniques, such as the aging of modified surfaces and the migration of hydrophobic and hydrophilic functional groups in aqueous and non-aqueous environments [33]. Contact angle analysis is sensitive to the chemical composition of the topmost molecular layer. It is an inexpensive and simple technique for the characterization of material surfaces.

The current study aimed to perform heavy metal ion detection using Ag-functionalized SiNW arrays through SERS. The SiNW arrays were fabricated with vertical alignment. SiNW arrays were functionalized with AgNPs deposited by an RF magnetron sputter, and Ag was used as both the catalyst and the oxidant. The immobilization of BSA or ZnSO$_4$ on the AgNP-modified SiNW arrays was investigated. The detection of heavy metal ions on biomolecules was performed using EDS, XRD, WCA, and SERS methods.

2. Materials and Methods

The fabrication of SiNW arrays used 6-inch, p-type (100)-orientated single-crystal silicon wafers with resistivity of 15–25 $\Omega$-cm. The wafers were cleaned to remove chemical impurities and particles using a standard Radio Corporation of American (RCA) cleaning process [34]. A chemical approach was utilized to synthesize SiNW arrays on the wafers by the electroless etching method [35]. Firstly, the Si substrates were cut into 1.5 $\times$ 1.5 cm$^2$ and immersed in an HF/AgNO$_3$ solution at 75 °C for 30 min to obtain SiNW arrays. The as-prepared samples were immersed in HNO$_3$ solution, rinsed with deionized water, and dried in nitrogen. In order to remove oxide substances, the samples were dipped in 5% HF solution. A schematic of Ag modifying SiNW arrays for SERS detection of heavy metal ions is shown in Figure 1.

**Figure 1.** Procedure for the functionalization of SiNW arrays with Ag by RF magnetron sputtering, the immobilization of bovine serum albumin (BSA), and the detection of heavy metal ion Zn$^{2+}$ on the Ag-modified SiNW arrays. Step description: (1). In order to remove chemical impurities and particles, the wafers were cleaned by a standard Radio Corporation of American (RCA) cleaning process. (2). SiNW arrays were synthesized at the AgNO$_3$/HF solution for 30 min at 75 °C by Ag-assisted electroless etching. (3). Ag nanoparticles were deposited onto p-SiNWs by RF magnetron sputtering for 1 min at 100 °C. (4). The BSA protein was immobilized on the Ag–SiNW arrays for 12 h at room temperature. (5). After washing thoroughly with double-distilled water (ddH$_2$O) three times to remove excess BSA, the samples were dried at room temperature. (6). Then, ZnSO$_4$ was coated onto the treated modified SiNW arrays and incubated for 12 h. (7). Following three washes with washing buffer (0.1% Tween 20), to remove unbounded ZnSO$_4$, the sample was dried again and ZnSO$_4$ immobilized SiNW arrays were obtained.
Ag nanoparticles were deposited on the SiNW arrays by RF magnetron sputtering at substrate temperature of 100 °C for 1 min after the etching process. The sputtering target was a ceramic Ag disc (hot-pressed and sintered as 99.995% purity, Elecmat, North East, PA, USA). The base pressure of the container was vacuumed to $5.0 \times 10^{-6}$ torr during deposition. The distance between the target and substrate (85 mm), and the substrate rotational speed (10 rpm) were kept constant.

For detection of heavy metal ion Zn$^{2+}$ on BSA, BSA proteins were immobilized on the Ag–SiNW arrays. The samples with Ag–SiNW arrays were immersed in BSA solution for 12 h. BSA solution was beforehand prepared by dissolving BSA in PBS buffer solution (pH 7.4). The concentration of BSA solution was 500 µM. Then, they were washed thoroughly with double-distilled water (ddH$_2$O) three times to remove excess BSA and dried at room temperature. To obtain Zn$^{2+}$ immobilized on the BSA–Ag–SiNW arrays, ZnSO$_4$ were then coated onto the treated SiNW arrays and incubated for 12 h at room temperature. The unbounded ZnSO$_4$ was removed with washing buffer (2% Tween 20) three times and then dried again.

Surface morphology of the SiNW arrays was investigated using field-emission scanning electron microscopy (FE-SEM, JEOL JSM-6500F, JEOL, Tokyo, Japan) and transmission electron microscope (TEM, JEM 2010F, JEOL, Tokyo, Japan). An energy dispersive spectrometer (EDS) combined with a mapping method was used to evaluate the distribution of Ag. The crystal structures of the SiNWs and Ag–SiNWs were determined by X-ray diffraction (Rigaku-2000 X-ray Generator, XRD, Rigaku, Tokyo, Japan) using Cu Kα radiation with an angle of incidence of 1°. A contact angle system (CA, KRÜSS GmbH GH-100, Hamburg, Germany) was utilized to analyze the hydrophilicity and hydrophobicity of the SiNW arrays and functionalized SiNW arrays. The chemical bonds and compositions were analyzed by Fourier transform infrared spectroscopy (FTIR, ASTeX PDS-17, Applied Science and Technology, Inc. Wilmington, MA, USA). Raman spectroscopy was utilized to examine the information between the crystallization and atom band of the single-crystal SiNW arrays, Ag–SiNWs, and the detection of heavy metal ions. SERS analyses were accomplished on a Horiba ihr550 Raman equipped (Horiba, Tokyo, Japan) using 488 nm laser excitation of power of 50 mW with 100× objective (~1 µm$^2$ spot size) for an acquisition time of 10 s. The SERS analysis was qualitative in this study. Each test piece was tested by SERS in three places in this study. The SERS spectra shown an average result from three places.

3. Results and Discussion

3.1. Deposition of AgNPs on SiNW Arrays

The SEM image of Ag–SiNW arrays in Figure 2b appears to be more equally distributed than that of the p-SiNW arrays in Figure 2a. The AgNPs, which appear as the brighter area, were uniformly grown onto SiNW arrays (Figure 2b). Owing to the strong galvanic displacement reactions between silicon and the highly reactive HF/AgNO$_3$ solution, the etched SiNWs aligned vertically were easily deposited with silver nanoparticles. Furthermore, the aggregated AgNPs had a higher secondary electron and backscattered electron yield than those of the silicon substrates.

The TEM images in Figure 3a,b illustrate that AgNPs were deposited onto the top and sidewalls of SiNW arrays in a monolayer. AgNPs diameters were in the range of 30–75 nm. As seen in Figure 3c, the Ag peaks in EDS spectra of SiNWs further confirmed the presence of AgNPs on SiNW arrays. However, the as-prepared Ag–SiNW arrays by the Ag-assisted electroless etching method appeared to have rough surfaces, indicating non-uniform nucleation of AgNPs.
The XRD patterns of the unmodified SiNW arrays and the SiNW arrays deposited with AgNPs are shown in Figure 4. The modified SiNW arrays had diffraction peaks at 38.3°, 44.4°, 64.6°, and 77.5° and exhibited the following crystalline peaks of Ag, respectively: (111), (200), (220), and (311) (compared to the database of powder diffraction patterns from the International Centre for Diffraction Data (ICDD) by the Joint Committee on Powder Diffraction Standards (JCPDS) No. 65-1060). This demonstrates that higher Ag crystallinity was successfully deposited on the SiNW arrays and Ag (111) presents as the preferred growth orientation.

3.2. Immobilization of BSA and ZnSO$_4$ on the Ag–SiNW Arrays

The surface morphology of the Ag–SiNW arrays immobilized with BSA and those immobilized with BSA and ZnSO$_4$ are shown in Figure 5. Serum albumin (SA) is the most abundant protein in blood plasma and serves as a depot and transport protein for numerous compounds, such as long chain fatty acids, bilirubin, or heavy metal ions, which are bound with a relatively high affinity to the protein. The peaks of C, N, and O of the treated Ag–SiNWs in the EDS spectrum (Figure 5a) showed the presence of –OH groups and –NH groups, which are the functional groups of BSA. Comparing the surface morphology of Ag–SiNW arrays after BSA immobilization in Figure 5a with that of Ag–SiNW arrays in Figure 2b, the immobilization of BSA created a smoother surface.

The physical absorption of the immobilized BSA onto Ag–SiNW arrays depends on the non-specific interaction between the BSA and the treated Ag–SiNW arrays. These interactions include hydrogen bonds, hydrophilic interactions, and van der Waals forces. Of these interactive forces, the hydrogen bond is the strongest. The extent of these interactions depends mainly on the chemical properties of BSA and the Ag–SiNW arrays, as well as the solvent. On the other hand, the SiNW arrays and the AgNPs play important roles in the retention of the BSA molecules. The SiNW arrays, which have a certain diameter and height, block the movement of large BSA molecules, and the AgNPs have an affinity to BSA because of the strong action between Ag atoms and the mercapto group of the BSA molecules.

The Zn peaks in the EDS spectrum demonstrate the existence of the heavy metal ion Zn$^{2+}$ on the BSA-immobilized Ag–SiNW arrays (Figure 5b). The X-ray diffraction patterns of the Ag–SiNW arrays and the Ag–SiNW arrays after immobilizing BSA and ZnSO$_4$ illustrated in Figure 6 further confirm the existence of Zn$^{2+}$ (JCPDS Data No. 090395). The results reveal that the binding of the heavy metal ion Zn$^{2+}$ onto the BSA-immobilized Ag–SiNW arrays was successfully achieved. The inducing nitrogen-containing functional
groups, that is, –OH groups and –NH groups, by the immobilized BSA were helpful to the heavy metal ion attached onto the surface of the Ag–SiNW arrays for the detection process in this study.

Figure 3. (a) TEM images of the SiNWs deposited with AgNPs, (b) the diameters of AgNPs distributed in a wide range from 30 to 75 nm, and (c) EDS analysis of the SiNWs deposited with AgNPs.
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Figure 4. X-ray diffraction spectra of (a) the p-SiNW arrays and (b) the SiNWs deposited with AgNPs.

Figure 5. SEM images and EDS analysis of (a) the Ag–SiNW arrays immobilized with BSA and (b) the Ag–SiNW arrays after immobilizing BSA and ZnSO$_4$.
The WCA observations of the Ag–SiNW arrays using a CCD before and after the immobilization process of BSA and ZnSO₄ are depicted in Figure 7. WCA analysis shows a correlation between immobilization and hydrophilicity. The Ag–SiNW arrays before immobilization only had a contact angle of 130.3°, which depicted hydrophobic characteristics (Figure 7a). The WCA of the Ag–SiNW arrays immobilized with BSA was reduced to 87.1° (Figure 7b). This result indicated that the bulk surface, while immobilizing BSA, induced –OH and –NH functional groups. Thus, the Ag–SiNW arrays became hydrophilic. In addition, when the Ag–SiNW arrays were immobilized with ZnSO₄, the WCA was further reduced to 8.8° (Figure 7c). The immobilization of ZnSO₄ made the Ag–SiNW arrays more hydrophilic than BSA immobilization. Obviously, one common feature of the nitrogen-containing functional groups and the heavy metal ions is their hydrophilicity. Since the duration of BSA immobilization increases the number of hydrophilic ions, the longer duration of immobilization resulted in higher hydrophilicity on the surface of the Ag–SiNW arrays.

FTIR spectrum analysis was performed to evaluate the functional groups on the surfaces of the immobilized Ag–SiNW arrays. The chemical bonds among the functional groups of BSA and ZnSO₄, the immobilized Ag–SiNW arrays, and their compositions

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**Table 1:** X-ray diffraction data for ZnSO₄

| In this study | JCPDS Data |
|--------------|------------|
| 29.7         | 29.9       |
| 33.6         | 33.3       |
| 36.2         | 36.3       |
| 37.7         | 37.5       |
| 39.7         | 39.8       |
| 47.8         | 47.7       |
| 48.7         | 48.1       |

**Figure 6.** Comparison of X-ray diffraction spectra of (a) the Ag–SiNW arrays and (b) the Ag–SiNW arrays immobilized with BSA and ZnSO₄ for the detection of Zn²⁺ ions.

**Figure 7.** WCA observations of the Ag–SiNW arrays (a) before the immobilization process (WCA = 130.3°), (b) after the immobilization of BSA (WCA = 87.1°), and (c) after the immobilization of ZnSO₄ (WCA = 8.8°), using a CCD.
are shown in Figures 8 and 9. The FTIR spectrum of the Ag–SiNW arrays without immobilization of BSA only shows the existence of Si–Si and Si–O–Si in Figure 8. The FTIR peaks of the Ag–SiNW arrays after immobilizing BSA at 1531 and 1652 cm\(^{-1}\) correspond to the stretching vibrations of \(-\text{OH}\), amide A (mainly \(-\text{NH}\)) and amide I (mainly C=O), respectively. The peaks at 3062 and 3430 cm\(^{-1}\) correspond to amide II, that is, the coupling of bending vibration of N–H and the stretching vibration of C–N. These peaks depict nitrogen content from the BSA. Figure 9 demonstrates the differences between the FTIR spectrum of Ag–SiNW arrays only immobilized with BSA and those immobilized with BSA and ZnSO\(_4\). The characteristic peaks of \(-\text{NH}\) groups disappeared for the Ag–SiNW arrays immobilized with BSA and ZnSO\(_4\) (black line), suggesting that there might have been interactions between Zn and \(-\text{NH}\) groups of BSA. The results indicate that the conjugate bonds exist between the ZnSO\(_4\) and \(-\text{NH}\) groups through physical adsorption, which provides further information concerning the immobilization effects of BSA and ZnSO\(_4\) on the surface of Ag–SiNW arrays.

![Figure 8](image.png)

**Figure 8.** Comparison of FTIR spectra of the Ag–SiNW arrays (a) before the immobilization of BSA (black line) and (b) after immobilizing BSA (red line).

| Wavenumber (cm\(^{-1}\)) | Assignment |
|---------------------------|------------|
| 810                       | Si–Si      |
| 1110                      | Si–O–Si    |
| 1390,1450                 | COO\(^{-}\) |
| 1531                      | C–N        |
| 1652                      | C=O        |
| 3062                      | C–H        |
| 3430                      | N–H        |

3.3. SERS Biodetection and Detection of Heavy Metal Ions

It has been known that SERS signals, while interacting with targeted molecules, enable the detection and identification of molecules, without the need of exogenous labels, due to the inherent vibrational signatures and the specific spectral of species [4–6]. The SERS signals enhanced by silver nanoparticles have also been investigated in the literature [7,13,14]. The SERS spectra, that is, the Raman analysis of BSA protein on Ag–SiNW arrays and BSA protein conjugation, are shown in Figure 10. When BSA proteins were adsorbed onto the Ag–SiNW arrays, the SERS spectra exhibited the characteristics of amide bands and aromatic chain vibrations. The maximum peak around 1550 cm\(^{-1}\) corresponded to amide II, and the peak at 1380 cm\(^{-1}\) corresponded to amide I, as shown in Figure 10a. The peaks observed near 2960 cm\(^{-1}\) in the SERS spectra of Ag–SiNW arrays immobilized with BSA corresponded to the stretching vibrations of NH\(_2\). These SERS peaks deviated from results reported in previous literature [24,28,30–32], possibly due to the effects of the protein and the substrate.
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After the immobilization of ZnSO₄, three vibration peaks at 980, 1120, and 3400 cm⁻¹ were observed for specific protein conjugation (Figure 10b), possibly due to the conformational change after immune reaction. The inherent “fingerprint” of the SERS spectra allows for the differentiation of closely related species, which is essential for immunoassays [14]. The resulting SiNWs/Ag NPs composite interfaces showed large Raman scattering enhancement for the capture of heavy metal ions by BSA with a detection of 0.1 µM.

The effects of AgNPs on the features of the SERS spectra of the BSA-immobilized SiNWs are shown in Figure 11. It was observed that the SERS spectrum of the SiNWs immobilized with BSA, without AgNPs, displayed only two peaks at 520 and 920 cm⁻¹, which corresponded to the silicon–oxygen symmetric stretching vibration mode. The Raman intensities at peaks 1550, 1650, and 2924 cm⁻¹ of the SiNW arrays covered with
Ag NPs became strong, owing to the strong intensity of the electromagnetic hot spot on the Ag NPs, implying that the Ag NPs may enhance the Raman signals of the functional groups of the amide II, amide I, and CH$_3$ band. Compared with the synthesized SiNW arrays, the resulting SiNWs/Ag NPs composite interfaces showed large Raman scattering enhancement for BSA with a detection of 500 µM and an enhancement factor of $2.0 \times 10^3$. This large enhancement factor was attributed to the presence of “hot” spots on the SiNWs/Ag NPs substrate. In addition, the diameters of the prepared Ag NPs (30–75 nm) were less than 100 nm in this study, which is beneficial for the enhancement of the SERS effect in the Raman spectra [17].

![Figure 10. Comparison of SERS spectra of (a) the Ag–SiNW arrays immobilized with BSA (red line) and (b) the Ag–SiNW arrays immobilized with BSA and ZnSO$_4$ (green line).](image)

![Figure 11. Comparison of SERS spectra of BSA immobilized onto (a) Ag–SiNW arrays (red line) and (b) SiNW arrays (black line).](image)

The SERS spectrum of BSA adsorbed on AgNPs (Figure 11) strongly differed from its corresponding Raman spectrum in both the band positions and relative intensities. As the SERS spectrum of a protein is sensitive to its orientation to the plasmonic surface, changes in intensities of various modes as well as appearance of modes are expected [36,37]. The peaks connected to C–N stretching vibrations (at ~1105 cm$^{-1}$), to the amide III band (at ~1250 cm$^{-1}$), to the amide II band (at ~1550 cm$^{-1}$), and to the amide I band (at ~1650 cm$^{-1}$), to the sidechain stretching mode CH$_3$ band (at ~2924 cm$^{-1}$) were easily recognized. In contrast, at the same protein dilution and in the absence of AgNPs, no BSA Raman features were observed. This is due to the enhancement of surface plasmon resonance (SPR) and SERS of SiNWs array after Ag modification. When conductive electrons were concentrated at the Ag–SiNW arrays vertices, corners, or tips, the charge density considerably increased. In other words, the Ag–SiNW arrays effects resulted in tremendously enhanced local electromagnetic field near sharp corners/tips and inter-/intra-nanogaps, i.e., plasmonic hotspots. The electromagnetic field significantly enhanced the Raman signals of target molecules in the hotspots, which made morphology-dependent SERS behaviors available for analysis and detection. The induced aggregation of AgNPs was effective in SERS substrate generation of BSA detection, indicating that the AgNPs modified SiNWs are feasible for SERS bio-detection.

### 3.4. SERS Mechanism for the Detection of Heavy Metal Ions

Raman spectroscopy relies on inelastic scattering or Raman scattering of monochromatic lights, usually excited from a laser in the visible, near infrared, or near ultraviolet range. The laser lights interacting with phonons result in shifting the patterns of the photons, which gives the information of the modes. The Raman effects occur when light impinges upon a molecule and interacts with the molecular bonds of the electron clouds.
This incident photon excites one of the electrons into a virtual state. For the spontaneous Raman effects, the molecule will be excited from its ground state to a virtual energy state and relaxed to a vibrational excited state, which generates Stokes Raman scattering. If the molecule is already in an elevated vibrational energy state, the Raman scattering is then called anti-Stokes Raman scattering.

In the current study, two major effects are involved in the enhancement of Raman signals: (1) electromagnetic effects associated with dipolar resonance occurring on the metal surface, and (2) chemical effects from the scattering process induced by chemical interaction between molecules and metal surfaces. For the mechanism, there are possibly two kinds of surface plasmon resonance: the local resonance from individual AgNP, and the surface electromagnetic wave on the substrate. The former is similar to that in the colloidal system. The AgNPs are periodically distributed on the whole surface of the SiNW arrays, and the Si can effectively transmit the electromagnetic wave through the substrate. Therefore, each electromagnetic wave produced from individual AgNP may be spread on the whole surface of the Ag–SiNW arrays and results in a resonance effect. The strength of the surface plasmon resonance on each AgNP is thus coherently enhanced. The surface electromagnetic waves from all AgNPs may provide significant contributions to the enhancement of Raman scattering. On the other hand, the AgNPs are grown via redox reaction at room temperature, and their surfaces possess several active sites that can effectively bond analytic molecules. Furthermore, the BSA and ZnSO$_4$ conjugations have a high affinity for the Ag surface and enhance the interaction between the biomolecules and AgNPs. All these effects contribute to chemical enhancement.

4. Conclusions

The purpose of the current study is to develop a SERS-based probe to detect SA and heavy metal ion (Zn$^{2+}$) using SiNW arrays deposited with AgNPs. The AgNPs are deposited on SiNW surfaces by RF magnetron sputtering for enhancement of SERS signals. The SERS active substrates for the detection of BSA and Zn$^{2+}$ using Ag–SiNWs were prepared. Observations through HRTEM revealed that AgNP layers with a depth range of 30–75 nm were successfully deposited on SiNW arrays. The peaks in EDS and XRD spectra of SiNW arrays confirm the presence of Ag particles, BSA, and Zn$^{2+}$ on SiNW arrays. After BSA immobilization, the bulk surface induces hydrophilic functional groups. The WCA observations show the affinity of the Ag–SiNW arrays immobilized with BSA (WCA = 87.1°) and ZnSO$_4$ (WCA = 8.8°), so the Ag–SiNW arrays become more hydrophilic after the ZnSO$_4$ immobilization process than after BSA immobilization. The results of FTIR analysis illustrate that conjugate bonds exist between zinc sulfate and –OH groups and –NH groups of BSA. The AgNP-modified SiNWs have demonstrated feasibility for SERS detection of biomolecules and heavy metal ions. BSA and ZnSO$_4$ conjugations show specific SERS spectra with high sensitivity, which depicts great promise in developing label-free biosensors.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/coatings11060685/s1, Table S1: shows the comparison of parameters and sensitivities based on different SERS substrates, Table S2: lists of the SERS literature comparison based on Ag (or Au) modified SiNW arrays substrates.

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