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SERS Characterization of Lysozyme Aptasensor Prepared for Latent Fingerprint Visualization on Surfaces

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Abstract

Fingerprints are crucial evidence in crime scene investigations, playing a key role in identifying victims and perpetrators. This study focuses on enhancing the visualization of hidden fingerprints using nanoparticles and an aptasensor-based biosensor to improve the secure identification of potential suspects. In this research, gold nanoparticles (AuNPs) were synthesized and modified with 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) and a lysozyme aptamer for fingerprint imaging. After modifying the gold nanoparticle surface, the solution was applied to silicon surfaces containing fingerprints. SERS measurements taken after immobilizing the aptamer-based Raman label on the lysozyme in the fingerprints revealed a peak at 1337 cm^{-1}, attributed to the symmetric stretch of the nitro group in DTNB.

Keywords: Forensic Sciences; Latent Fingerprints; Lysozyme Aptasensor

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Yüzeylerde Gizli Parmak İzi Görselleştirmesi İçin Hazırlanan Lizozim Aptasensörünün SERS Karakterizasyonu

Öz

Parmak izleri, suç mahalli soruşturmalarında kurbanları ve failleri tespit etmede önemli bir rol oynayan önemli kanıtlardır. Bu çalışma, potansiyel şüphelilerin güvenli bir şekilde tespitini iyileştirmek için nanopartiküller ve aptasensör tabanlı bir biyosensör kullanarak gizli parmak izlerinin görselleştirilmesini geliştirmeye odaklanmaktadır. Bu araştırmada, parmak izi görüntüleme için altın nanopartiküller (AuNP'ler) sentezlenerek ve 5,5'-Dithiobis-2-nitrobenzoik asit (DTNB) ve bir lizozim aptameri ile modifiye edilmiştir. Altın nanopartikül yüzeyi modifiye edildikten sonra, çözelti parmak izi içeren silikon yüzeylere uygulanmıştır. Parmak izlerindeki lizozim üzerindeki aptamer tabanlı Raman etiketinin immobilize edilmesinden sonra alınan SERS ölçümleri, 1337 cm^{-1}'de DTNB'deki nitro grubunun simetrik gerilmesine ait bir pik gözlemlenmiştir.

Anahtar Kelimeler: Adli Bilimler; Gizli Parmak İzleri; Lizozim Aptasensörü

1. Introduction

This study investigates the application of aptamers, specifically lysozyme aptamers, attached to gold nanoparticles (AuNPs) to develop aptasensors for the improved detection of latent fingerprints. Latent fingerprints are a critical form of evidence in forensic analysis; however, traditional detection methods often face challenges related to low sensitivity and specificity. By incorporating nanotechnology into forensic science, this research aims to address the limitations of conventional techniques. The use of aptamer-based biosensors that target lysozyme—a protein found in fingerprints—provides a more targeted and environmentally friendly alternative to traditional detection methods such as cyanoacrylate fuming and powder dusting. The conjugation of lysozyme aptamers with AuNPs improves the sensor's ability to visualize latent fingerprints on various surfaces. This innovative approach not only advances forensic science but also introduces a novel biosensor, the lysozyme aptasensor, specifically designed for the detection of latent fingerprints.

Nanotechnology plays a crucial role in forensic sciences, with applications ranging from toxicological assessments and DNA typing to fingerprint detection and the analysis of explosive and firearm residues [1] This branch of science involves the synthesis and study of materials at the atomic level, typically within the size range of 1-100 nm [2]. Nanotechnology has a broad range of applications, including drug development, electronics, textiles, and construction [3].

Gold nanoparticles (AuNPs) are known for their small size, biocompatibility, and stability. Their synthesis is simple, producing particles ranging from 1 to 100 nm. The optical property of AuNPs, known as Surface Plasmon Resonance (SPR), involves the absorption and emission of light by gold electrons in response to incident radiation. AuNPs can be synthesized in various shapes, such as nanospheres, nanorods, nanoshells, and nanoprisms [4]. These nanoparticles exhibit selectivity, long-term stability, and sensitivity in latent fingerprint detection, making them effective on both non-porous and porous surfaces. The amine functional groups in AuNPs bind to the fatty acids present in fingerprints, facilitating their attachment to the surface where the fingerprint is located [5].

Identifying the type of surface on which a fingerprint is found is Crucial in forensic research. Each surface has unique characteristics that require different fingerprint detection methods. Surfaces are generally categorized as either porous or non-porous. Fingerprints on non-porous surfaces, such as glass, metal, and plastic, are more prone to damage or degradation [6]. To visualize latent fingerprints, forensic teams must consider the structure, texture, color, and environmental exposure of the surfaces where the fingerprints were found. This analysis helps determine the most appropriate imaging method [7]. Since the 1950s, antibodies—soluble proteins from the glycoprotein family—have been widely used as recognition probes. However, there is an increasing demand for faster, more robust, and cost-effective molecular recognition and detection methods. Aptamers fulfill these requirements and offer several advantages over antibodies. These include precise control during in vitro synthesis, the ability to endure non-physiological conditions such as extreme pH, temperature, and salt concentrations, and enhanced stability due to their phosphodiester backbone. Furthermore, aptamers can be synthesized for targets that do not provoke an immune response in vivo, such as toxic substances [8].

Aptamers interact with their targets by forming specific three-dimensional conformations. Based on the spatial positioning of targets within the complexes formed after binding, they can be categorized into two groups: the embedded group and the external binding group. The embedded group includes small molecules such as ATP (adenosine

triphosphate), cocaine, and K+, while the external binding group comprises larger molecules like thrombin and platelet-derived growth factor-BB (PDGF-BB). There are four main strategies for designing aptamer-based biosensors: (i) the target-induced conformational change (TISS) method, (ii) the sandwich or sandwich-like method, (iii) the target-induced dissociation or displacement (TID) method, and (iv) the competitive displacement method [9].

Lysozyme, also known as muramidase or N-acetylmuramoyl-hydrolase, is an essential protein first identified by Laschtschenko in 1909 and later named by Alexander Fleming in 1922. Often referred to as the body's natural antibiotic, lysozyme plays a vital role in defense mechanisms by breaking down the cell walls of gram-positive bacteria [10].

This enzyme is found in nearly all human body fluids and serves as a biomarker for various pathological conditions, including AIDS and leukemia, where its concentration tends to increase. Furthermore, its antibacterial properties make lysozyme valuable in the food industry [11].

Numerous traditional techniques exist for detecting latent fingerprints, with spectroscopic methods being particularly prominent. Spectroscopic imaging, an advanced technology that integrates digital imaging with molecular spectroscopy, allows for the simultaneous collection of spatial and spectral information. This innovative approach has diverse applications across scientific, industrial, and forensic fields. In forensic science, spectroscopic methods are especially valuable for visualizing latent fingerprints and detecting microscopic particles embedded within them. Williams et al. utilized Fourier transform infrared microspectroscopy to analyze the chemical components of latent fingerprints. As a non-destructive technique, IR enabled the observation of changes in fingerprint composition over time. Their study identified distinct differences between the fingerprint components of adults and children, revealing that the composition of children's fingerprints evolves over time [12].

Ricci and co-workers employed attenuated total reflection Fourier transform infrared spectroscopy for chemical imaging to monitor the effects of temperature and time on fingerprints. Their analysis focused on lipid and amino compounds present in fingerprints collected from various individuals. By examining the spectral data, they identified temperature- and time-dependent changes in the lipid composition, shedding light on the alterations that occur in fingerprints from the moment they are deposited until they are imaged [13].

Raman spectroscopy, a highly sensitive technique widely used in forensic science, involves the scattering of monochromatic, single-wavelength laser beams by molecules [14]. This advanced spectroscopic method enables the observation of molecular vibrations, rotations, and low-frequency modes. Notably, it is non-destructive and requires minimal sample preparation, making it particularly advantageous for forensic applications [15].

Raman spectroscopy, while valuable, is often limited in sensitivity due to its scattering cross-section, making it less suitable for routine applications. To overcome this limitation, Surface Enhanced Raman Spectroscopy (SERS) was developed. Connatser et al. utilized SERS to image fingerprints that were otherwise undetectable under conventional light and heat exposure. The enhanced sensitivity of SERS enabled the detection of a wider range of substances. Their study focused on imaging fingerprints on porous and granular surfaces that had been subjected to heat [16].

The primary distinction between SERS imaging and techniques like FTIR and other fingerprint imaging methods lies in its focus on amino acids present in fingerprint components, rather than the more commonly analyzed hydrocarbon signals from oils. Both Raman and IR-based spectroscopic methods provide valuable information about the functional groups of the analyzed molecules. However, accurate interpretation of this data requires comprehensive and relevant databases. In the absence of such databases, identification becomes significantly more challenging. Consequently, it has been recommended that additional spectral measurements be conducted to enhance the available data and improve analytical accuracy [17].

Zhou et al. developed a lysozyme aptasensor by modifying SERS probes (Au/pNTP/SiO2) with a lysozyme-specific aptamer. In their study, they applied the modified aptasensor to the surface containing latent fingerprints. After allowing the surface to dry, the fingerprints were lifted using adhesive tape and analyzed with a confocal Raman microscope. This method successfully achieved the three levels of fingerprint recognition [18]. The study aimed to analyze latent fingerprints using sweat, an endogenous substance, and lysozyme, a protein found in sweat. The aptasensor, modified with gold nanoparticles (AuNPs), was designed to target lysozyme in fingerprints. Initially, the binding characterization between the gold nanoparticles and the 5’-end thiolated lysozyme aptamer (LBA) was performed using Surface Enhanced Raman Spectroscopy (SERS). A Raman label, 5,5’-Dithiobis(2-nitrobenzoic acid) (DTNB), was employed for imaging.

The DTNB label was first conjugated to the AuNPs, and the LBA was subsequently modified with the DTNB-labeled AuNPs to construct the lysozyme aptasensor.

2. Materials and Methods

The chemicals used in the study were of HPLC grade purity and were sourced from local representatives of chemical suppliers, including Merck, Riedel, and Sigma-Aldrich.

In the initial step, a 2 g PBS tablet was dissolved in 200 ml of distilled water to prepare the stock PBS solution (pH=7.4). Then, 50 µl of Tween-20 was added to this solution. The LBA (5′-SH-C6-ATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3′ (Heliks Biotechnology, Turkey) solution was prepared at a concentration of 0.25 µM in 130.4 ml of PBS buffer. A DTNB solution (50 µM) was prepared by dissolving 19.81 mg of DTNB in 1 ml of ethanol. Additionally, 100 µl of casein blocking agent was mixed with 2 ml of PBS solution. Piranha solution was prepared using a 3:1 ratio of 3 ml of H_2SO_4 and 1 ml of H_2O_2 [19].

Gold nanoparticles (AuNPs) were synthesized using the Turkevich method [20]. Prior to synthesis, 117 mg of tri-sodium citrate was weighed on a precision balance to achieve a 40 mM concentration, as shown in. The tri-sodium citrate was placed in a volumetric flask, and 10 ml of pure water was added. The solution was initially mixed using a vortex device to ensure homogeneous dissolution and then treated in an ultrasonic mixer to ensure complete dissolution of the tri-sodium citrate. Following the dilution processes, AuNP synthesis was carried out according to the Turkevich method. The steps involved in this synthesis method are as follows: 50 ml of pure water was added to a volumetric flask, followed by the addition of 20 µl of auric acid solution. The flask was then placed in an oil bath and heated to 100°C. Once the desired temperature was reached, 5 ml of the prepared 10 ml tri-sodium citrate solution was added. After waiting for 15 minutes, the solution was stirred for an additional 10 minutes without any temperature control and finally stored at 4°C for later use. As a result of these processes, spherical AuNPs were obtained, and the mixture was observed to have a cherry red color, consistent with the Turkevich method.

DTNB was used as a label to obtain a signal for the characterization of AuNP-LBAs by Raman spectroscopy [21]. First, 5 ml of the AuNP solution was placed in a 10 ml tube and centrifuged at 10.000 rpm for 10 minutes. The supernatant was discarded, and 1 ml of pure water was added to the pellet. The solution was centrifuged again at 10.000 rpm for 10 minutes, and the supernatant was discarded. Next, 1 ml of DTNB solution was added to the pellet, and the DTNB-labeled AuNP solution was left overnight. After incubation, the solution was centrifuged at 10.000 rpm for 10 minutes to remove any unbound particles. The supernatant was discarded, and the DTNB-labeled AuNP solution was washed with 1 ml of PBS. This centrifugation and PBS washing process was repeated twice. Finally, the solution was centrifuged again at 10.000 rpm for 10 minutes. After discarding the supernatant, 2 ml of LBA solution was added, and the mixture was incubated overnight at 4°C to allow the LBAs to bind to the AuNPs.

The DTNB-labeled AuNP-modified LBA solution (DTNB-AuNP-LBA) was centrifuged at 10.000 rpm for 10 minutes to remove unbound particles. After discarding the supernatant, 1 ml of casein blocking agent was added to prevent non-specific protein binding to the AuNPs and to ensure homogeneous 3D binding. The resulting solution was incubated at room temperature for 30 minutes and centrifuged at 10.000 rpm for 10 minutes. The supernatant, which contained non-specific proteins and casein, was discarded, and the solution was washed with 1 ml of PBS. The DTNB-AuNP-LBA solution was then centrifuged again at 10.000 rpm for 10 minutes, and the supernatant was discarded. Final solution was resuspended in 1 ml of PBS.

Piranha solution was used to wash the silicon wafers prior to SERS measurement. The wafers were immersed in the prepared Piranha solution for 20 minutes. Afterward, the silicon wafers were transferred to 98% ethanol for 20 minutes to remove any residual Piranha solution. Fingerprints were then applied to the cleaned wafers. Following the fingerprinting process, the silicon wafers were placed in a Petri dish, and 20 µl of the DTNB-labeled AuNP-modified LBA solution was applied onto the wafers.

In the final stage, the Petri dish was surrounded with paraffin and stored in the refrigerator at 4°C for two hours to allow the solution to bind to the surface. After 2 hours, one of the silicon wafers was removed from the Petri dish and washed with PBS solution. This wafer was then placed in a separate Petri dish and stored at room temperature. The second silicon wafer was kept in the refrigerator overnight for the binding time measurement.

Recognizing the DTNB signal is crucial for visualizing the binding on silicon wafers that have been fingerprinted and washed with PBS solution. In fact, the signal from an unwashed silicon wafer without fingerprints and from washed, fingerprinted wafers will be the same if the binding to LBA is successful. If no signal is observed, or if the signal differs, this would suggest the binding of non-specific, unwanted proteins.

To confirm this, the DTNB-AuNP-LBA solution was applied to the silicon wafer without fingerprints, and ten scans were performed on the SERS device to characterize the DTNB signal. Following this, the DTNB-AuNP-LBA solution, which was bound to lysozymes in the fingerprints, was characterized by SERS on the silicon wafers that had been stored in the refrigerator at 4°C for 2 hours and for one day.

During the experiments, the following equipment was used: a Sartorius Entris® precision scale for accurate measurements, a SPECORD® 50 PLUS UV-VIS device for characterizing gold nanoparticles (AuNPs), a Firlabo vortex mixer and a Bandelin (Germany) ultrasonic mixer for thoroughly mixing solutions, a Hermle Z 326 K and an Eppendorf Centrifuge 5418 for separating unbound particles from all solutions, an N-BIOTEK NB-101MC Combination Shaker mixer for mixing solutions during the binding period, a water/oil bath for synthesizing AuNPs, and an ExamineR™ High-Performance Raman Microscope from DeltaNu, equipped with a 785 nm laser source, for characterizing the binding of the lysozyme aptamer.

3. Results and Discussions

To obtain baseline values using the Ultraviolet-Visible Spectroscopy (UV-VIS) device, ultrapure water was first added to the UV-VIS cuvette, and a measurement was recorded. Next, 100 µL of gold nanoparticles (AuNPs) were added to the pure water in the cuvette, mixed thoroughly, and measured. The UV-VIS absorption spectrum of the synthesized AuNPs was then obtained, showing a peak at 524 nm, as illustrated in Figure 1.

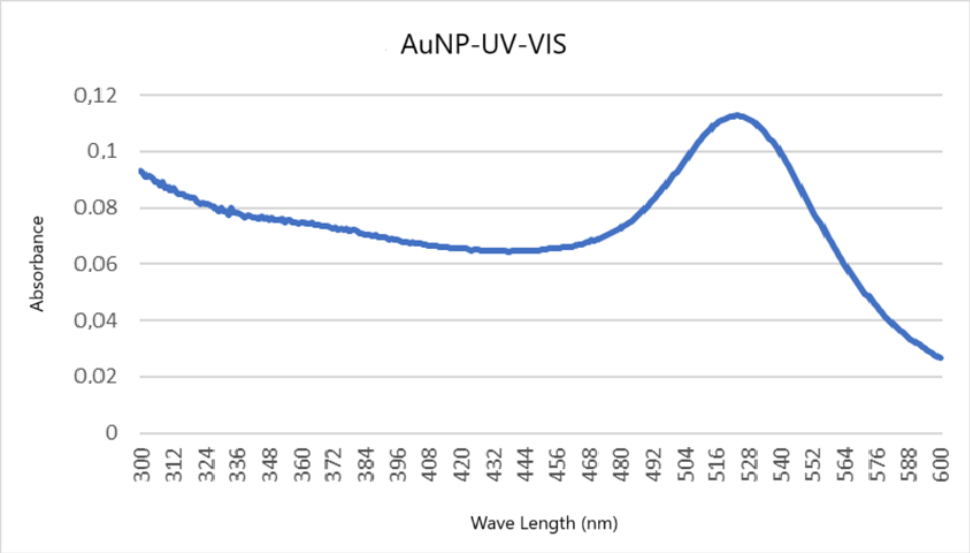

Figure 1. Absorption spectrum of AuNP

The UV-Vis measurement of the AuNP suspension showed a maximum absorbance wavelength of 534 nm. According to the literature, nanoparticles with this absorbance wavelength have an approximate size of 20 nm [22].

The DTNB-AuNP-LBA solution, prepared with a 0.25 µM LBA concentration, was applied to bare silicon wafers, and SERS measurements were performed with ten scans (Figure 2). The results indicated successful binding between DTNB-AuNP and LBA, with a Raman vibration at 1337 cm^{-1}, which was attributed to the symmetric stretch of the nitro group in DTNB [23].

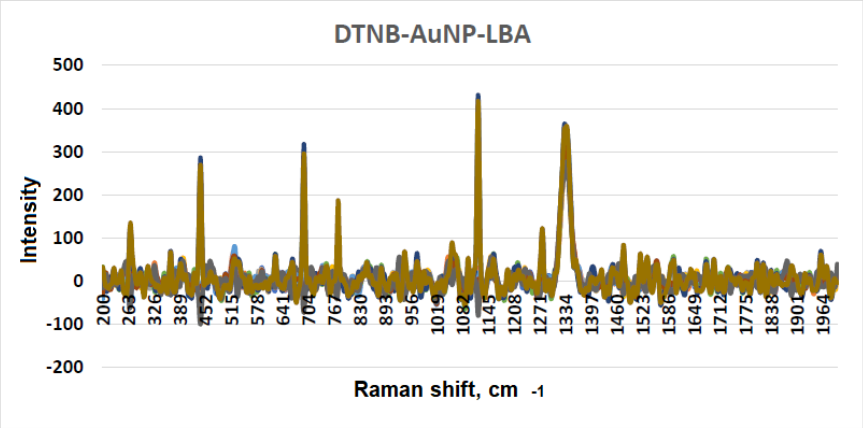

Figure 2. Raman spectra of DTNB-AuNP-LBA solution prepared with 0.25µM LBA Solution

The DTNB-AuNP-LBA (5 µM LBA) solution containing the Raman label was dropped onto fingerprints left on silicon surfaces. After a 2h incubation period, the surfaces were washed, and spectra obtained from SERS scans with ten scans are given in Figure 3. The nitro peak observed at 1337 cm^{-1} indicated the binding of the aptamer-modified Raman label to the fingerprints. SERS measurements were repeated 1 day later, and the same spectra were obtained (Figure 4).

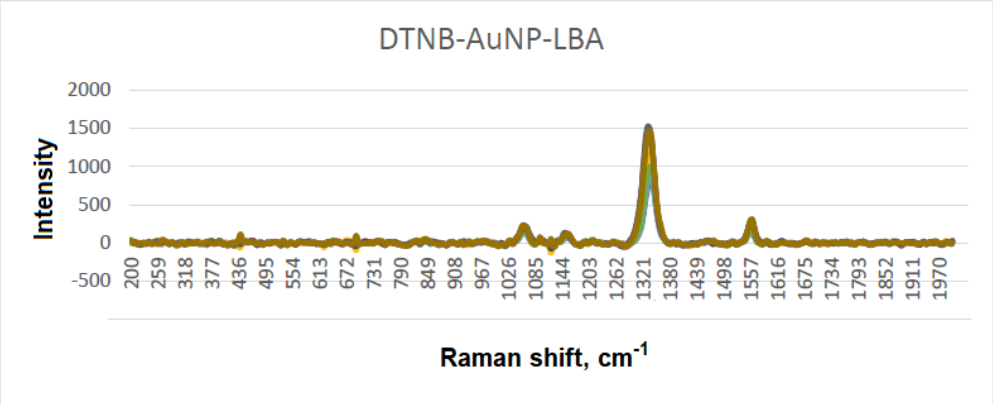

Figure 3. Raman Spectra of the DTNB-AuNP-LBA dropped onto a Silicon Surface Containing a Fingerprint

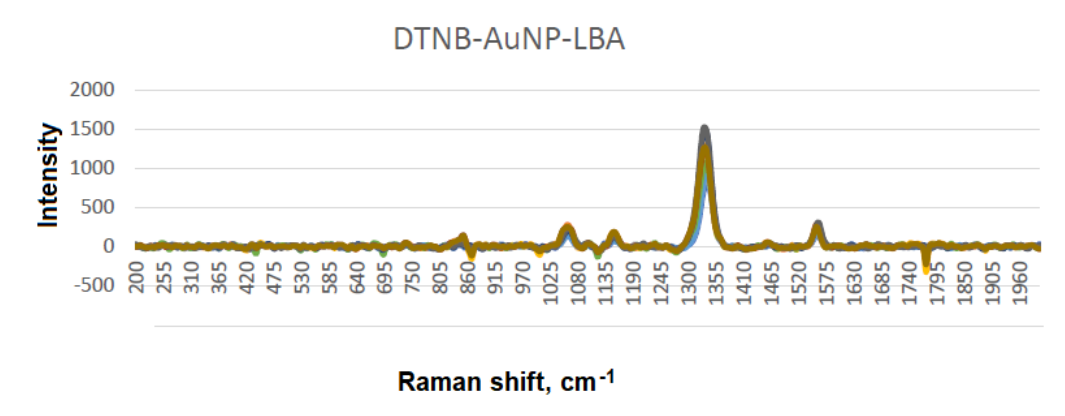

Figure 4. Raman Spectra of the DTNB-AuNP-LBA dropped onto a Silicon Surface Containing a Fingerprint (after 1 day)

4. Conclusion

In this study, the presence of fingerprint residues on surfaces was identified using a lysozyme aptamer-based Raman label. To enhance the SERS intensity, gold nanoparticles were synthesized. The particle size of the AuNPs was found to be approximately 20 nm, as characterized by ultraviolet-visible spectroscopy. After modifying the gold nanoparticle surface with DTNB and LBA, the solution was dropped onto silicon surfaces containing fingerprints. SERS measurements were taken after the immobilization of the aptamer-based Raman label onto the lysozyme in the fingerprints. A peak at 1337 cm^{-1} corresponding to the symmetric stretch of the nitro group in DTNB was observed. The same peaks were observed in the control after one day. After cleaning the fingerprint surface, the nitro peak disappeared from the surface where the aptamer-based Raman label had been applied. As a result of these experiments, it was concluded that fingerprints can be identified through mapping measurements using the SERS technique with an aptamer-based Raman label.

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