Applications of Surface-Enhanced Raman Spectroscopy in Brain Research

Subjects: Neurosciences

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Raman spectroscopy (RS) is a label-free method that provides a molecular signature of any type of biological sample, including tissue, live or fixed cells and biofluids for disease diagnosis. RS allows a sample's biochemical structure to be fingerprinted by analyzing the molecular bond vibrations of its biocomponents and has been employed to detect subtle biomolecular changes, enabling comparisons between a variety of tissues and biofluids.

SERS brain neuropathology Alzheimer's brain cancer biomarkers

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) offers potential in disease screening and diagnosis, with key studies emerging in the field of brain research. SERS has been investigated for use during brain surgery [1][2]. In glioma, which poses challenges in intraoperatively identifying its true margins due to its infiltrative nature, a SERRS probe has been developed [3], while a stimulated Raman scattering (SRS) microscopy method has accurately identified malignant tissue [4]. In the search for blood-based detection of AD biomarkers, a SERS-based sensor has developed for the relative quantitation of tau protein in the plasma of AD patients [5], while the combination of SERS with seed amplification assays (SAAs) offers an intriguing prospect in proteinopathies such as PD and AD [6]. A real-time assay for highly sensitive, label-free, multiplexed electrochemical, and SERS detection of stroke biomarkers has also been developed using a lateral flow device [7].

2. The Use of Surface-Enhanced Raman Spectroscopy in Glioma Research

Glioma accounts for more than 80% of all primary malignant brain tumors [8], and surgical removal is the mainstay of glioma treatment. However, due to the infiltrative nature of gliomas and the textural similarities between normal brain and malignant tissues, neurosurgeons face the challenge of maximizing the resection of the tumor while minimizing neurological deficits [9]. Though various approaches have been employed to identify brain tumor margins, few have truly defined the tumor's infiltrative boundaries. RS has been used for discriminating between glioma grades when combined with machine and deep learning techniques [10][11][12]. Moreover, recent studies suggest SERS has the potential to precisely depict the actual tumor extent with high sensitivity, specificity, and spatial resolution, making it suitable for intraoperative image-guided resection [13].

SERS is emerging as a powerful tool in the realm of intraoperative brain mapping and real-time monitoring of gliomas. Various adjuncts have been proposed to aid neurosurgeons while operating on brain tumors to maximize the extent of resection in the surgical treatment of gliomas, including intraoperative fluorescence-guided microsurgery, intraoperative MRI (iMRI), intraoperative ultrasound (IOUS), intraoperative neuro-navigation, intraoperative frozen section, and intraoperative fluorescence-guided microsurgery [14]. While these methods have their merits, they also have limitations. Intraoperative MRI, for instance, requires specialized operating rooms and has time and environmental constraints. Conversely, intraoperative ultrasound is less expensive but has limitations in sensitivity for identifying residual tumor. RS, on the other hand, offers a non-invasive alternative approach with the ability to provide results in seconds, along with a highly sensitivity and specificity, and without the need for complex environmental requirements.

So far, several animal studies have reported success in delineating glioma margins using a variety of SERS navigation systems [2][15][16][17]. Whole-brain tumor localization has been achieved in mouse models of glioma with a high degree of specificity and resolution using a combination of MRI, SERS and photoacoustic imaging, a technology that overcomes the depth and resolution limits of optical imaging [2][17]. Kircher et al. employed triple-modality imaging, combining MRI photoacoustic imaging, and Raman imaging [2]. NPs were intravenously injected into living mice with an orthotopic brain. This was facilitated through the disrupted blood-brain barrier, and NPs subsequently sequestered and retained by the tumor. This process resulted in accurate delineation of the margins of brain tumors, both preoperatively and intraoperatively [2].

More recently, a ratiometric pH-responsive SERS strategy has been developed for the rapid identification of glioma boundaries using pH-responsive SERS reporters [15][16] exploiting the pH gradient between glioma cells and extracellular fluid. The “Warburg effect” has also been used to characterize the metabolic anaerobic tendency of tumors, which results in significant lactic acid production [18]. SERS has been employed to identify tumor boundaries using a pH-sensitive SERS substrate, 4-mercaptopyridine (4-MPY), which reacts to pH changes. The lactic acid production in glioma cells lowers the local pH, impacting the 4-MPY SERS Raman peaks [16]. This approach has been tested using U87 cells in mice, yielding a surgical navigation system for tumor boundary identification. [15].

3. The Use of SERS in Alzheimer’s Disease Research

AD is the most prevalent type of dementia and is clinically characterized by significant amnestic cognitive decline, though can occasionally present as non-amnestic cognitive impairment. [19]. The AD brain exhibits microscopic features characterized by the abnormal accumulation of extracellular β-amyloid (Aβ) plaques and intraneuronal neurofibrillary tangles abnormally phosphorylated tau proteins (P-Tau) [20][21]. Using SERS to analyze AD biomarkers holds tremendous potential for accurate and early diagnosis. SERS-based biosensors have been developed, harnessing the optical properties of NPs to enhance detection performance [22]. Yu et al. have developed a sensitive SERS-based method to quantitatively detect serum biomarkers (such as Aβ1-42 and P-Tau-181) for early diagnosis of AD [23]. A SERS-based immunoassay successfully determined Aβ1-42 and P-Tau-181 in human serum, suggesting a promising tool for the early diagnosis of AD [23]. Since the detection AD biomarkers in

blood has proven effective, the simultaneous analysis of multiple AD markers has been explored using SERS-based approaches. A lateral flow assay based on SERS nanotags (SERS-LFA) has been developed, allowing simultaneous quantification of multiple AD biomarkers, including Aβ42, Aβ40, tau protein, and neurofilament light chain, a marker of neuronal damage [24].

To monitor AD progression and rehabilitation treatments, an optimized protocol using SERS analysis of AD and control patient serum samples has been developed. The correlation of RS data with structural MRI demonstrated a direct link between Raman spectra and hippocampal degeneration, suggesting RS as a potential adjunct for monitoring AD diagnosis using scanning technologies [25]. The unique features of SERS, combined with SAAs, have also been shown to successfully improve amyloid β-oligomer detection and characterization of CSF of patients clinically diagnosed with AD. This approach has the potential to provide an early diagnostic test, complementing clinical evaluation and traditional laboratory tests [6]. Such early detection may improve the effectiveness of recently introduced drugs, such as aducanumab and lecanemab [26][27].

4. The Use of Surface-Enhanced Raman Spectroscopy in Parkinson's Disease Research

PD is the most prevalent neurodegenerative movement disorder, characterized by the progressive development of bradykinesia, muscular rigidity, rest tremor, and postural instability [28]. These cardinal motor features stem from the gradual loss of dopaminergic neurons in the substantia nigra pars compacta. Along with other 'synucleinopathies' including dementia with Lewy bodies, multiple systems atrophy and pure autonomic failure, PD is defined by the presence of abnormal intracellular deposits termed 'Lewy bodies' and 'Lewy neurites' [29]. The main constituent of these pathological hallmarks is thought to be the unfolded protein α-synuclein, which is thought to aggregate from its native monomeric α-helical conformation, undergoing a profound conformational transition to a β-sheet-rich structure that form toxic oligomers and amyloid fibrils, accumulating as Lewy deposits [30][31][32].

The pathophysiology of PD and the development of effective treatments depend not only on the successful management of symptoms but also on targeting the underlying disease mechanisms and achieving early diagnosis before symptoms and clinical signs manifest. However, assessment results are often influenced by subjective and objective factors, which pose challenges to clinical diagnosis. Recent diagnostic advancements have harnessed the 'prion-like' properties of α-synuclein to develop SAAs, like the real-time quaking-induced conversion (RT-QuIC) assay, in various tissues and fluids, such as CSF [33] and, more recently, blood samples [34]. The combination of the RT-QuIC method with a targeted SERS-based immunoassay approach using antibodies directed toward specific proteo-forms of α-synuclein may thus offer an intriguing alternative approach in PD diagnostics (**Figure 1**).

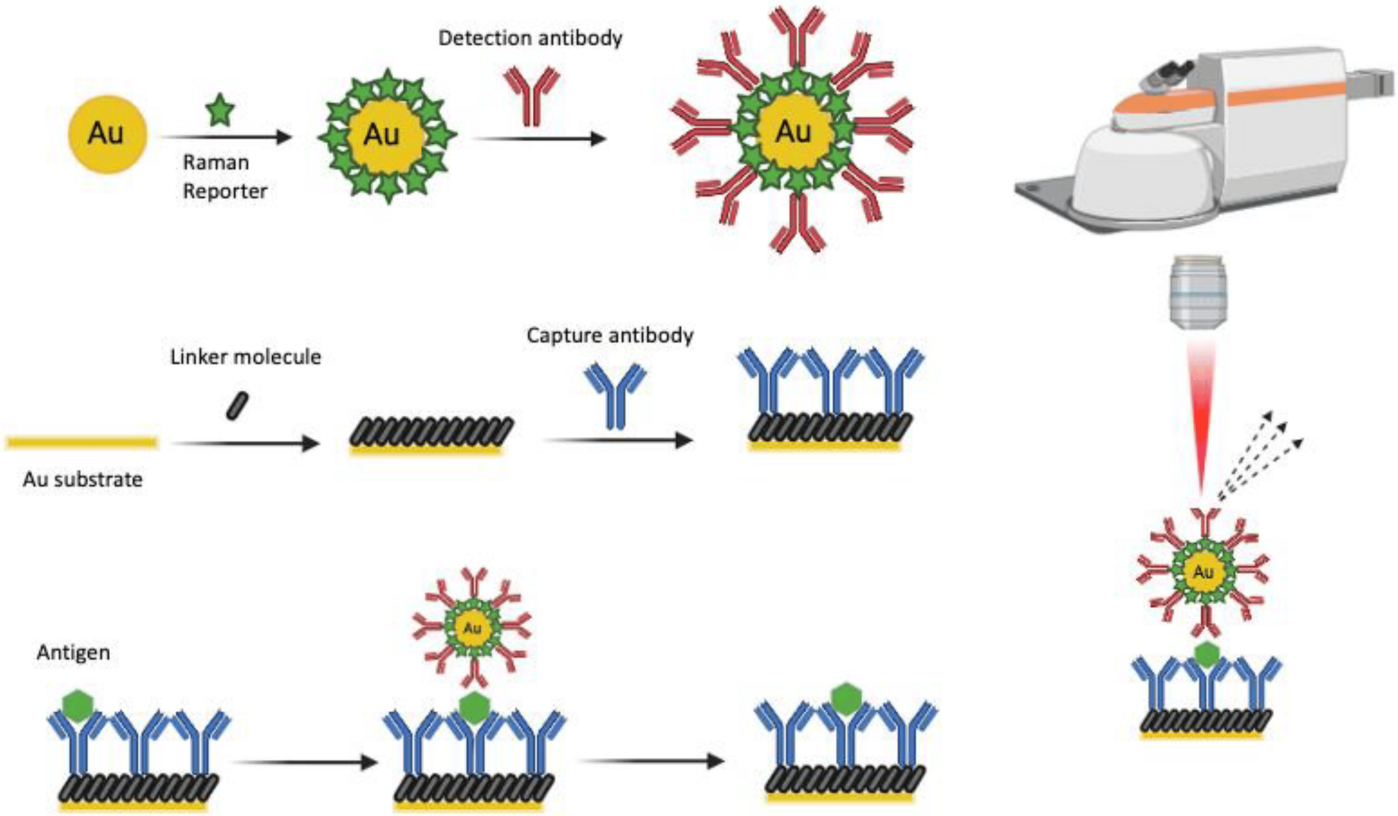

Figure 1. Labeled surface-enhanced Raman scattering (SERS)-based immunoassays for biomarker detection in biofluids for disease diagnostics. SERS-based immunoassay of protein (antigen) carried out by depositing biofluid samples on the Au substrate for immunocapture of the target antigen. SERS measurements performed in the antibody–antigen immunocomplexes. Created with BioRender.com: https://www.biorender.com, accessed on 16 November 2023.

Multiplexed detection of biomarkers could hold huge potential in early diagnosis and personalized treatment of PD. Cao et al. fabricated a robust SERS-enabled lab-on-a-chip (LoC-SERS) platform for the simultaneous quantification of crucial PD-related proteins such as α-synuclein, P-Tau 181, osteopontin, and osteocalcin [35]. A multiplex amplification strategy has been developed to amplify the sensitivity of a lab-on-a-chip SERS system, allowing simultaneous and highly sensitive detection of miR-214 and miR-221, both potential biomarkers for the early-stage diagnosis of PD. [36]. Another study focused on 5-S-cysteinyl-dopamine (CDA), a crucial metabolite with high relevance for the early detection of PD [35]. This research involved assignment of SERS bands for CDA using silver NP substrates in aqueous media, with analysis supported by theoretical calculations and simulated Raman and SERS spectra [37].

5. The Use of Surface-Enhanced Raman Spectroscopy in Stroke Research

Globally, stroke continues to be the second most prevalent cause of mortality and the third most common cause of disability [38]. The rapid and reliable analysis of stroke is a primary goal for relevant therapeutic intervention. CT

results may appear normal in the early stages of ischemic stroke or in patients with minor symptoms and MR imaging is often not feasible [39]. Although many blood biomarkers have been suggested for stroke diagnosis [39], the need for more sensitive and specific biomarkers remains a priority.

SERS detection of stroke biomarkers is considered a robust approach to overcoming these limitations. In a recent study by Sun et al., a real-time assay for highly sensitive, label-free, multiplexed electrochemical SERS identification of stroke biomarkers, specifically neuron-specific enolase (NSE) and S100-β protein, was developed using a lateral flow device [7]. Another study used a novel gold–silver alloy nanobox (AuAgNB)@SiO2-gold nanosphere nanoassembly based on a core–shell–satellite structure for the SERS detection of S100 calcium-binding protein B protein (S100B) [40]. Zhang et al. developed a novel lateral flow assay based on Raman encoded core–shell SERS nanotags for the rapid relative quantification of three cardiac biomarkers in the early diagnosis of acute myocardial infarction [41].

6. The Use of Surface-Enhanced Raman Spectroscopy in Neurotransmitter Detection

Neurotransmitters are endogenous signaling molecules secreted by neurons affecting a receptor on a target cell. Precise and proportional neurotransmitter release is vital for normal brain function and imbalances in neurotransmission has long been proposed to underlie many psychiatric and neurological conditions, including major depressive disorder [42], schizophrenia [43], epilepsy [44], PD [45] and AD [46]. Therefore, the monitoring of neurotransmitter concentrations could offer an exciting prospect in the diagnosis, prognosis, and treatment monitoring of brain disorders.

Lussier et al (2017) introduced a dynamic SERS nanosensor (D-SERS), by modifying a patch clamp nanopipette with gold nano-raspberries [47]. The nanosensor can be precisely positioned within specific regions containing analytes under a microscope, enabling concurrent measurements of ATP, glutamate, acetylcholine, gamma-aminobutyric acid (GABA), and dopamine. The acquired SERS spectra of these neurotransmitters were subsequently subjected to barcode data processing techniques. This D-SERS nanosensor represents a versatile and reliable tool for investigating the secretion profiles of neurons. Dopamine measurement in human serum was achieved through SERS detection using a gold nanostructure fabricated on a silicon wafer to enhance plasmon resonance. To enable this detection, 4-mercaptophenylboronic acid (4-MPBA) was employed as a reporter molecule capable of forming covalent bonds with dopamine. The constrained Raman mode of dopamine-bound 4-MPBA exhibited a directly proportional variation to dopamine concentration, suggesting the sensor possesses high sensitivity and selectivity when applied to human serum for the purpose of dopamine detection [48].

Zheng et al (2023) recently developed a SERS-based method for neurotransmitters detection using gold-nanoislands, decorated tapered optical fibers with sub-10 nm gaps, enabling molecular fingerprint identification. The nonplanar repeated dewetting approach amplifies the high-density layer's broadband near-field amplification, allowing the detection of neurotransmitters without the use of exogenous reporters [49]. A SERS-active neural probe

with gold nanoislands platform can detect neurotransmitters in the micromolar range, with a limit of detection of 10^{-7} M for rhodamine 6G and 10^{-5} M for serotonin and dopamine [50].

Quantitative SERS-based multiplexed detection of dopamine, serotonin and noradrenaline in human urine has been developed through chemometric analysis [51]. The consistent SERS signal intensities were a direct result of the precise sub-nanometer gaps between neighboring NPs. These findings indicate that this sensor has the potential for monoamine neurotransmitter detection in human urine at clinically relevant levels [51]. For serotonin, GABA, and glutamate, it was found that the lowest limits of detection were achieved using AgNPs SERS enhancing substrate at an excitation wavelength of 633 nm. In contrast, for indolic molecules like melatonin, dopamine, epinephrine and norepinephrine, the lowest limit of detection (LOD) was obtained with AuNPs at an excitation wavelength of 785 nm. This discrepancy is primarily attributed to the strong affinity of AuNPs to the indole ring [52].

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