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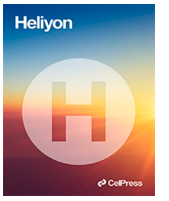

Review article

# SERS analysis of single cells and subcellular components: A review

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ABSTRACT

SERS is a rapidly advancing and non-destructive technique that has been proven to be more reliable and convenient than other traditional analytical methods. Due to its sensitivity and specificity, this technique is earning its place as a routine and powerful tool in biological and medical studies, especially for the analysis of living cells and subcellular components. This paper reviewed the research progress of single-cell SERS that has been made in the last few years and discussed challenges and future perspectives of this technique. The reviewed SERS platforms have been categorized according to their nature into the following types: (1) colloid-based, substrate-based, or hybrid; (2) ligand-based or ligand-free, and (3) label-based or label-free. The advantages and disadvantages of each type and their potential applications in various fields are thoroughly discussed.

## 1. Introduction

Monitoring of various cellular processes such as cell differentiation, division, and apoptosis at the single cell level is a major challenge in systems biology. To address this challenge, various techniques have been proposed so far, including cell fractionation, cytometry, cell culture, microscopy, DNA sequencing and genomics, polymerase chain reaction, blotting methods, micro-array and cytochemistry [1]. However, their widespread use in biomedicine is highly limited due to the following issues. The main issue is the complexity of isolating and culturing single cells in the artificial environment, which can induce unintended cell stress altering the behavior, viability, and profile of the cell. Another issue is a difficulty to perform multiplex analysis of a single cell from various aspects which is essential for understanding complex processes occurring in a living cell. Finally, the conventional techniques are usually very slow and some can be quite expensive. Thus, the development of new approaches to overcome the above mentioned limitations of the conventional techniques is highly needed.

In recent years, it has been proposed to apply surface-enhanced Raman spectroscopy (SERS) for comprehensive analysis of single cells and monitoring the sub-cellular processes [2,3]. This technique provides rapid, reagent-free, and nondestructive analysis of intact cells and subcellular organelles preserved in their native environment. Besides, compared to conventional bioanalysis methods, SERS demonstrates the multiple advantages such as: (1) an ultrahigh sensitivity down to single molecule level (enhancement factor $> 10^7$); (2) an opportunity to obtain an information on the intrinsic molecular fingerprint of biological systems; (3) the resistance to photo-bleaching and photodegradation which is essential for long-term monitoring; (4) an opportunity to acquire data on on multiple molecular functional groups simultaneously which is crucial for analyzing biochemical processes in living cells; (5) a more narrow

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**Abbreviations**

| | *Description* |
|---|---|
| SERS | surface-enhanced Raman spectroscopy |
| NPs | nanoparticles |
| NSs | nanostars |
| PEG | Poly(ethylene glycol) |
| RGD | arginylglycylaspartic acid |
| NLS | nuclear localization signal |
| MLS | mitochondrial localization signal |
| TAT | transactivator of transcription |
| PCR | polymerase chain reaction |
| ASOs | antisense oligonucleotides |
| MBA | 4-Mercaptobenzoic acid |
| PA | poly-L-arginine hydrochloride |
| MBN | 4-mercaptobenzonitrile |
| MPBA | 4-mercaptophenylboronic acid |
| DTTC | 3,3′-diethylthiatricarbocyanine iodide |
| BSA | bovine serum albumin |
| MSCs | mesenchymal stem cells |
| NBs | nanoboxes |
| NBA | Nile blue A |
| RGDFC | arginine-glycine-aspartic acid-phenylalanine-cysteine |
| MPy | 4-mercaptopyridine |
| FITC | fluorescein isothiocyanate |
| FAM | 5-carboxyfluorescein |
| HSeBA | seleno-phenylboronic acid pinacol ester |
| Ab | N-cadherin antibody |
| hERG | human ether-a-go-go related gene |
| AR | alizarin red |
| PD | polydopamine |
| AB | Anti-P-Glycoprotein 1 antibody |
| EGFR | epidermal growth factor receptor |
| MMC | 7-mercapto-4-methylcoumarin |
| DTNB | 5,5′-dithiobis-(2-nitrobenzoic acid) |
| TFMBA | 2,3,5,6-tetrafluoro-4-mercaptobenzoic acid |
| SCA | squamous cell carcinoma antigen |
| ATP | 4-aminothiophenol |
| NTP | 4-nitrothiophenol |
| TN | 2- naphthalenethiol |
| BTFB | 3,5-bis(trifluoromethyl)benzenethiol |
| BPT | biphenyl-4-thiol |
| MIgG | mouse monoclonal antibody |
| CRISPR | clustered regularly interspaced short palindromic repeats |
| MUC | mucin 1 |
| TNC | tenascin C |
| MGITC | malachite green isothiocyanate |
| DTDC | 3,3′-diethylthiadicarbocyanine iodide |
| Cy3 | cyanine 3 |
| ROX | X-Rhodamine |
| cApt | *Salmonella typhimurium*-recognizing aptamer |
| R6G | rhodamine 6G |
| PolyA-DNA | hairpin-structured double-stranded DNAs |
| AFP | α-Fetoprotein |
| IgG | immunoglobulin G |
| DP | 4,4′-Dipyridy |
| LHRH | luteinizing hormone releasing hormone |
| PB | prussian blue |
| DC | dendritic cell |
| Cy5 | cyanine 5 |

| | |
|---|---|
| NWs | nanowires |
| sEVs | small extracellular vesicles |
| BAL | bronchoalveolar lavage |
| NSCLC | non-small cell lung cancer |
| 1NAT | 1-naphtalenethiol |
| DSNB | 5,5-dithiobis(succinimidyl-2-nitrobenzoate) |
| MG | malachite green |
| GOx | glucose oxidase |
| MB | methylene blue |
| TYR | tyrosinase |
| PUVA | psoralen ultraviolet A |
| TDPs | tetrahedral DNA probes |
| EBV | Epstein-Barr virus |
| EF | enhancement factor |

bandwidth of SERS peaks compared to fluorescence emission peaks from organic dyes or quantum dots; (6) an opportunity to adjust sizes, shapes, and coatings of SERS nanostructures to various detection purposes, etc. [3].

The application of SERS for single cell analysis can facilitate early detection of various diseases, such as cancer, diabetes, hypertension, etc. Furthermore, it can promote the development of more efficient and specific drugs. The effectiveness of SERS for single-cell analysis was first demonstrated in the beginning of the 1990s by Nabiev et al. [4–6]. They applied a SERS technique to monitor the amount of antitumor drugs in the nucleus and cytoplasm of living cancer cells. However, the further development of single-cell SERS slowed down until the beginning of the 2000s due to the complexity of the multi-level cell system. Kneipp et al. [7] revived interest in this topic in 2002. They demonstrated the effectiveness of SERS for monitoring of intracellular distributions of native chemicals in living cells. Since then, the research in this field has been constantly growing [8–14], but the greatest progress has been made in the last few years due to the development of novel materials and analytical approaches for SERS analysis [15,16].

In this review, we explore the most recent literature (2019–2023) on application of SERS for single-cell studies and highlight key-findings that demonstrate the potential of this technology for biological research and medical diagnostics. We start our review with a classification and description of SERS-active platforms that can be applied for single-cell diagnostics. Further, we discuss their effectiveness for detection and analysis of various types of cells, followed by a description of particular applications in medicine and biology. Finally, we present the major challenges and opportunities in the development of next-generation SERS substrates for monitoring cellular and subcellular processes in living cells.

## 2. SERS platforms for single-cell diagnostics

Silver (Ag) and gold (Au) are two of the most common materials used for manufacturing bioanalytical SERS platforms [3]. Au and Ag nanoparticles (NPs) of various shapes and sizes (10–100 nm) exhibit unique and tunable plasmonic properties, which makes them highly attractive for multiple SERS applications. The particularly outstanding properties have been demonstrated by non-spherical nanoparticles with nano-gaps, tips, and edges, such as nanostars, nanorods, nanocubes, nanowires, etc. They exhibit superior enhancement factors up to the order of $10^9$-$10^{10}$, as a consequence of both plasmonic near-field enhancements and the so-called lightning rod effect [17,18]. The characteristic absorption peaks of AuNPs and AgNPs of various shapes locate at 500–600 and 400–440, respectively, so they can be detected by the readily available 532 nm and 633 nm lasers. Furthermore, AuNPs have proven to be highly effective for intracellular and in vivo studies due to their excellent biocompatibility. On the other hand, AgNPs are typically used for in vitro ultrasensitive detection since they have a better enhanced performance, but strong toxicity to living systems. To combine the advantages of Au and Ag in bioanalytical SERS diagnostics, it was also proposed to use Au@Ag core-shell nanoparticles and AuAg alloys for SERS fabrication [19,20].

In general, there are three types of bioanalytical SERS platforms based on Au or/and Ag NPs [9]: 1) colloidal solutions, 2) colloidal/surface hybrid substrates, and 3) surface substrates. Colloidal and surface SERS substrates without any conjugates (tag-free) can detect the intrinsic SERS signals from the cell constituents and can provide dynamic biomacromolecular information about cellular and subcellular processes. However, tag-free SERS substrates have limited specificity due to the high probability of signal interference from different biomolecules in the complex intracellular environment. Therefore, to improve specificity of single-cell diagnostics by SERS platforms, it was proposed to functionalize plasmonic nanoparticles with targeting ligands (such as peptides, antibodies, aptamers, vitamines, polysaccharides, etc.) [21–23]. The targeting ligands facilitate the delivery of plasmonic nanoparticles into specific subcellular compartments which is essential for obtaining SERS signals of high intensity and reliability. Generally, in addition to targeting ligands, SERS tags are functionalized by messenger molecules (Raman reporters) with distinguished signatures and functional coating layers (capping agents) that endow the SERS tags with biocompatibility and stability [24]. However, for some applications, plasmonic nanoparticles can be functionalized with Raman reporters and capping agents without the use of targeting ligands.

Depending on the presence or absence of Raman reporters, SERS detection strategies can be titled as label-free or label-based methods [25,26]. In label-free SERS methods, the spectral bands provide the intrinsic information on molecular vibrations of analytes attached directly to plasmonic nanoparticles. Label-free methods are preferable for most applications because they are compatible with real-time detection [25]. However, for some small molecules and subcellular compartments with low Raman activity,

it is required to use label-based SERS strategies, which are based on a detection of combined optical signal from plasmonic materials (Ag, Au, etc.) and SERS-active Raman reporters, which are resonant with a wide range of available excitation lasers [26].

In the following subsections, we will discuss the use of various types of SERS platforms (Fig. 1) for analysis of cells and subcellular organelles.

### 2.1. Colloid-based/label-free/ligand-free SERS platforms

Colloidal label-free and ligand-free SERS platforms are the most basic platforms for single-cell analysis. They are cost-effective, convenient, and easy-to-implement. Their fabrication process does not require much pre-processing operations and chemical reagents. However, the application of label-free/ligand-free platforms has to face the interference of various impurity signals in biological fluids, so an appropriate design is required to reduce the sensitivity to background signals.

Colloidal label-free and ligand-free SERS platforms based on spherical Au or Ag NPs can be easily synthesized using such methods as chemical reduction, laser ablation, photoreduction, etc. [27,28], but most common techniques for manufacturing bioanalytical colloidal SERS are chemical reduction and seed-mediated methods [29]. Plasmonic nanoparticles of various shapes (nanostars, nanocubes, nanorods, etc.) can be obtained by seed-mediated methods in the presence of reducing agents, stabilizers, and shape inducers [30–32].

Several research groups applied the chemical reduction method for intracellular synthesis of plasmonic nanoparticles within the target cells and studied the obtained colloidal suspension of NPs-containing cells with a SERS technique [33–36]. In particular, Gherman et al. [33] synthesized AgNPs at yeast cell walls and used SERS technique to discriminate various types of yeast cells which has potential in the treatment of yeast infections. Besides, Weiss et al. [34] and Chisanga et al. [35] applied a similar technique for internal synthesis of AgNPs within bacterial cells. They demonstrated the applicability of the obtained SERS platform for pathogenic bacteria detection, single-cell sorting, and as well for three-dimensional visualization of microbial communities. On another hand, Chen et al. [36] synthesized AuNPs within cancer cells and evaluated the feasibility of the intracellularly-grown-AuNPs-based SERS platform for the identification of nasopharyngeal carcinoma cell lines.

The chemical reduction method was also applied by multiple research groups for manufacturing of colloidal SERS platforms based on extracellularly-grown plasmonic nanoparticles [37–47]. For instance, Yan et al. [37] produced AgNPs by fast dropwise addition of $AgNO_3$ into the reductant and simply mixed synthesized AgNPs with bacterial cells. The obtained colloidal SERS platform demonstrated effectiveness for the detection of $^{13}C$ isotopes in bacterial cell membranes and the discrimination of different bacterial species. Zheng et al. [38] applied the same technique to obtain AgNPs-based SERS platform for the analysis of mast cell degranulation which is the indicator of wound healing processes (Fig. 2a). Zhang et al. [39] fabricated colloids of core-shell Au@AgNPs by the chemical reduction method and applied them for non-destructive DNA detection (Fig. 2b).

Several research groups also demonstrated the effectiveness of colloidal SERS platforms obtained by silver nitrate reduction for the detection and staging of the lymphoma cancer cells [40] and oral squamous cancer cells [41], as well as for monitoring cancer cell apoptosis during drug therapy [42,43]. Besides, Darienzo et al. [44] applied the seed-mediated method to produce colloidal SERS platforms based on AuNPs of various shapes (spherical, star-like, and nanocaltrop). They demonstrated that platforms based on spherical and star-like AuNPs can be effectively used for the differentiation of breast epithelial cell lines.

On the other hand, Xu et al. [45] proposed to use a seed-mediated method for the fabrication of a microfluidic SERS platform based

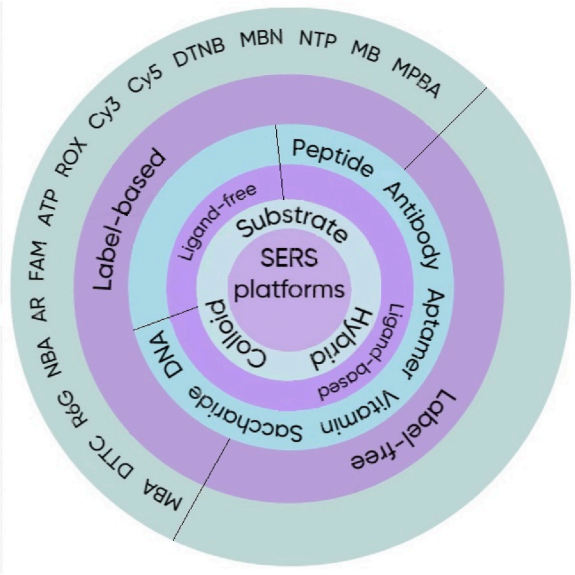


**Fig. 1.** Classification of SERS platforms for analysis of cells and subcellular organelles.

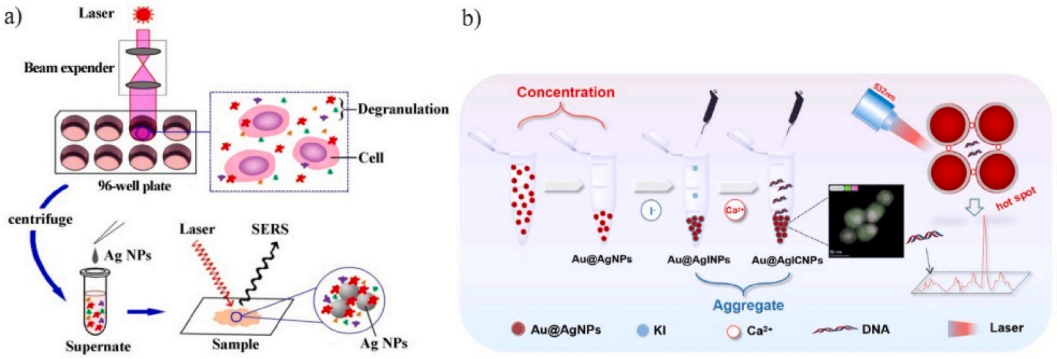


**Fig. 2.** a) Schematic illustration of SERS measurements of degranulation induced by low-intensity laser. Reprinted with the permission from Ref. [38]. Copyright (2023) John Wiley and Sons; b) Schematic diagram for the SERS detection of DNA with Au@AgICNPs. Reprinted with the permission from Ref. [39]. Copyright (2023) MDPI.

on Ag nanostars (NSs) and also proved its effectiveness in detecting various breast cancer cell lines. Seed-mediated and chemical reduction methods were also applied to produce magnetic SERS colloids based on magnetic beads ($Fe_3O_4$ NPs) decorated with Ag or Au NPs [46,47]. These metal-magnetic SERS platforms exhibited several advantages over traditional SERS platforms, such as an efficient adsorption of single cell metabolites, fast separation from complex matrices, and high SERS sensitivity in detection of cancer cells.

Besides, some research groups proposed alternative methods for the fabrication of label-free colloidal SERS platforms [48,49]. In particular, Qi et al. [48] fabricated colloids of microscale metal-organic beads covered with AuNPs via electrostatic assembly and demonstrated the effectiveness of the obtained SERS platform for monitoring of cancer cell apoptosis during electrical stimulation. Another research group [49] produced colloidal SERS platforms based on NiNPs (cubicles with sharp and round edges) by laser ablation and utilized them for the prediction of cancer dissemination and monitoring behavior of cancer stem cells.

### 2.2. *Colloid-based/label-free/ligand-based SERS platforms*

Colloidal label-free and ligand-based SERS platforms allow specific sensing of targets with Raman signals and can be used for accurate detection of the physiologically relevant analytes in complex biological fluids. They are characterized by increased signal intensity and stability, but have a short shelf life since the commonly used ligands are susceptible to oxidative degradation. Typically, to fabricate ligand-based SERS platforms, AuNPs or AgNPs are functionalized with capping agents and targeting ligand molecules (peptides, antibodies, aptamers, vitamines, saccharides, etc.) by chemical reactions. For instance, Qu et al. [50], Wang et al. [51], Qi et al. [52], Yue et al. [53,54], and Zhang et al. [55] modified AuNPs of various shapes (nanospheres, nanosunflowers, nanorods, and nanostars) with Poly(ethylene glycol) (PEG) derivatives and grafted cancer-cell-specific targeted peptide (arginylglycylaspartic acid, RGD), nuclear localization signal (NLS) peptide, mitochondrial localization signal (MLS) peptide, or cell-penetrating transactivator of transcription (TAT) peptide on the surface of PEGylated AuNPs via the covalent linking between gold and thiol group of cysteine (Fig. 3). The obtained colloids were proposed to be used for highly-sensitive monitoring of dental pulp stem cell differentiation [51] and detection of changes in cells and subcellular organelles under the external stimuli such as electro stimulation [52], photodynamic therapy [53], drug therapy (metformin hydrochloride) [50], and toxin exposure [55].

Chia et al. [56] and Murali et al. [57] fabricated colloidal SERS platforms based on AuNPs modified with saccharides (glucosamine

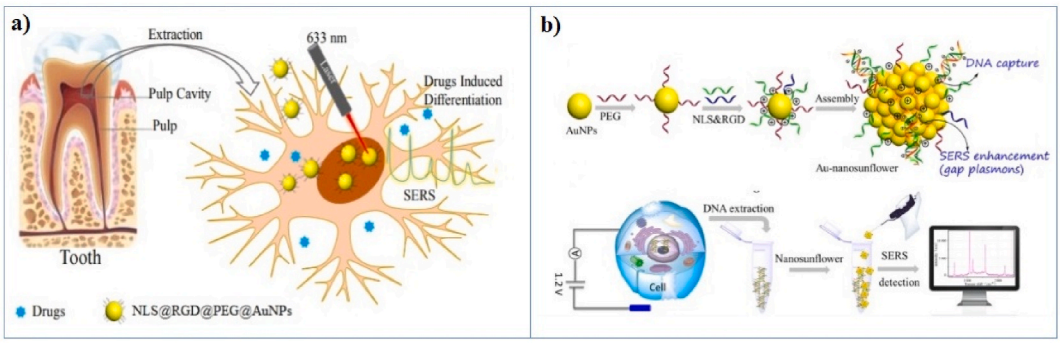


**Fig. 3.** a) Schematics of dental pulp stem cells extraction from adult teeth as well as monitoring their differentiation processes stimulated by drugs using SERS Spectra. Reprinted with the permission from Ref. [51]. Copyright (2023) American Chemical Society; b) Schematic preparation of Au nanosunflowers and their use for in vitro SERS tracing of cellular DNA structural changes after electrical stimulation. Reprinted with the permission from Ref. [52]. Copyright (2023) American Chemical Society.

and galactoside) as targeting ligands. The obtained SERS platforms demonstrated effectiveness for monitoring of metastatic potential in cancer cells [56] and detection of bacterial cells [57]. Other research groups [58,59] proposed to use phosphine-based targeting ligands for modification of plasmonic nanoparticles. In particular, they grafted triphenylphosphine on the surface of Au-Pt NPs and AuNPs in order to apply the obtained SERS platforms for detection of mitochondrial reactive oxygen species in living cells [58] and monitoring of the changes in mitochondrial membrane potential [59].

### 2.3. Colloid-based/label-based/ligand-free SERS platforms

Label-based colloidal SERS platforms are suitable for subcellular compartments with low Raman activity that can't be detected by label-free methods. Label-based platforms lose the advantage of providing material intrinsic information, but possess high accuracy and ultra-high detection sensitivity for quantitative measurements [11]. In label-based approach, plasmonic nanoparticles are usually functionalized by both Raman reporters with distinguished signatures and the capping agents that provide nanoparticles with high stability and biocompatibility. To achieve the maximum efficiency in the use of Raman reporters, a number of requirements are imposed on them: (1) a high stability under laser irradiation; (2) a small number of Raman peaks without overlap with analyte signals; (3) the ability to bind to plasmonic nanoparticles through chemical bonds or physical interactions; (4) a relatively large Raman scattering cross section to produce strong SERS signals [3,21].

A detailed review of Raman reporters for biological applications has been published by Matschulat and co-workers in 2010 [60]. However, new developments have been made in this area in recent years. In particular, Xie et al. [61], Zhang et al. [62], and Yang et al. [63] modified AuNPs (nanoflowers and nanostars) with a thiol-based Raman reporter molecule 4-Mercaptobenzoic acid (MBA) whose SERS spectrum changes as a function of pH. The last two teams also functionalized AuNPs with capping agents (poly-L-arginine hydrochloride (PA) [62] and azido-polyethylene glycol [63]) in order to achieve a more pronounced long-term stability and biocompatibility. The obtained colloidal SERS platforms demonstrated a high efficiency for 3D visualization of intracellular pH [61], detection of cancer cells through Ph mapping [62], and monitoring the repairing process in cancer cell membranes [63].

Martinez Pancorbo et al. [64] also applied MBA for functionalization of tunable core-shell Au-$SiO_2$-$WO_3$ nanoparticles and proposed them to be used for 3D imaging of live cancer cells (Fig. 4a). Another pH-sensitive Raman reporter (cysteine-hydroxyl merocyanine, CyHMC) was applied by Wen et al. [65] to detect cancer cells by monitoring the pH fluctuation in subcellular organelles (lysosomes) (Fig. 4b).

He et al. [66] and Cong et al. [67] fabricated colloidal label-based SERS platforms using 4-mercaptobenzonitrile (MBN) and 4-mercaptophenylboronic acid (MPBA) as Raman reporters. MBN and MPBA can be effectively applied for sialic acid detection whose expression level is closely connected with various biological and pathological processes. In particular, it was recommended to use MBN@AgNPs, MPBA@AgNPs, or MBN@MPBA@AgNPs for monitoring of surface glycosylation in membranes of living cells and cancer cell detection.

Another Raman reporter with a high potential for single-cell detection was used by Hua et al. [68]. They modified Au nanostars (AuNSs) with 3,3′-diethylthiatricarbocyanine iodide (DTTC) whose emission and absorption peaks are located in the infrared spectral region. It is helpful in avoiding the adverse influence of the background of organic compounds and biological systems. DTTC@AuNSs modified with a capping agent (bovine serum albumin, BSA) and silver sulfide nanoparticles ($Ag_2S$) have been effectively applied for dynamic observation of the in vivo functionality of mesenchymal stem cells (MSCs) which is essential for a wide range of regenerative medicine applications.

In recent years, aryl diazonium salts have emerged as a new generation of surface modifiers for plasmonic nanoparticles [69,70]. In particular, Li et al. [71] functionalized AgNPs with aryl diazonium salts (D-$NO_2$, D-CCH, and D-CN) as Raman reporters. This strategy results in robust covalent bonds between a Raman reporter and silver cores, thus ensuring a high stability of the obtained colloidal

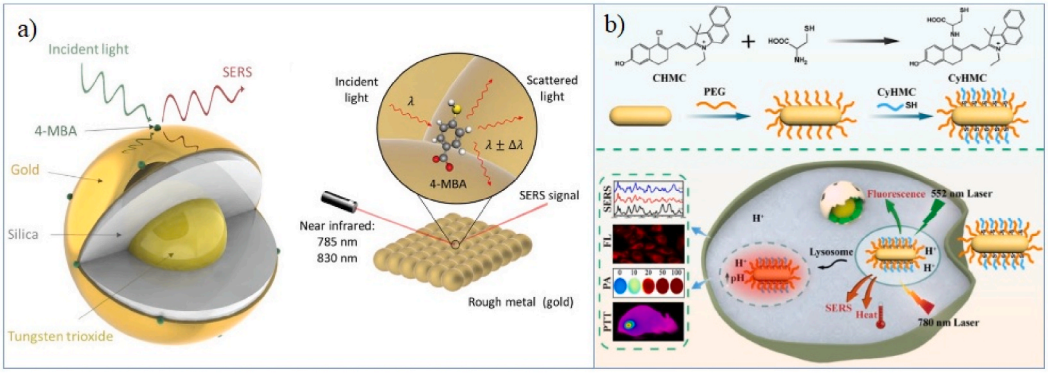


**Fig. 4.** a) Schematic of the composite NP design and SERS effect over the gold nanoshell, respectively. Reprinted with the permission from Ref. [64]. Copyright (2023) John Wiley and Sons; b) Schematic diagram of the preparation of AuNRs@CyHMC and the principles of gold nanotheranostics for *in situ* SERS monitoring pH and multimodal imaging-guided near-infrared photothermal therapy. Reprinted with the permission from Ref. [65]. Copyright (2023) American Chemical Society.

SERS platforms. Besides, the experimental studies demonstrated high efficiency of them for sensitive and selective cancer cell detection.

### 2.4. Colloid-based/label-based/ligand-based SERS platforms

Colloidal label-based/ligand-based SERS platforms are the most common platforms for real-time in vivo imaging of single cells. The modification of plasmonic nanoparticles with specific Raman reporters and their corresponding targeting ligands allow highly advanced imaging of multiple molecular targets in the desired part of living cells. The targeting ligands, such as peptides, antibodies, aptamers, etc., bind to specific target analytes and their highly enhanced characteristic SERS signals are measured indirectly through the Raman reporters. However, their activity might be impaired by conjugation to a bulky nanoparticle. Besides, high quality SERS tags are not currently commercially available, and must be fabricated in-house. Robust calibration and standardization procedures do not yet exist, making it difficult to compare results across labs or measurement platforms.

#### 2.4.1. Peptide-based SERS platforms

In SERS studies of single cells, peptides are the most commonly used targeting ligands due to their chemical versatility for covalent conjugation and biodegradability. In particular, Sun et al. [72] modified Au nanoboxes (NBs) with a caspase3-specified peptide which binds to the surfaces of the AuNBs by the sulfhydryl group of cysteine. They also labeled AuNBs with Nile blue A (NBA) as a Raman reporter. The obtained colloidal SERS platform is designed for detection of enzymatic activities in the physiological processes of cells and monitoring caspase-3-related cervical cancer. Another team of researchers (Song et al. [73]) functionalized AuNSs with a RGD peptide labeled with MBA as a Raman reporter molecule. RGD molecules acted as ligands selectively targeted to $\alpha_v\beta_3$ integrin (a membranous receptor that was overexpressed in A549 human lung adenocarcinoma cells) on the cell membrane and thus succeeded in achieving active targeting of cancer cells. The modified AuNSs demonstrated a great potential for a wide spectrum of light-mediated applications, such as optical imaging and image-guided phototherapy in both the NIR-I and NIR-II biological windows. Sloan-Dennison et al. [74] also designed a colloidal SERS platform for the detection of $\alpha_v\beta_3$ integrin receptors of living cells. They modified spherical AuNPs with an arginine-glycine-aspartic acid-phenylalanine-cysteine (RGDFC) peptide and MBN as a Raman reporter. The obtained AuNPs were effectively used for monitoring metastatic colon cancer cell viability.

A colloidal SERS platform based on TAT peptide and pH-sensitive 4-mercaptopyridine (MPy) Raman reporter was proposed by Zheng et al. [75] for monitoring the intracellular pH during the cell cycle at the single cell level. It was demonstrated that cell-penetrating TAT peptides can dramatically improve cellular internalization efficiency of AuNPs without sacrificing the pH response. Thus, the proposed SERS platform has a great potential for revealing the intracellular pH-related biological and pathological processes.

A colloidal SERS platform for the detection of pathogenic bacteria was proposed by Franco et al. [76]. They functionalized AuNPs with a P9b phage clone, displaying the specific peptide, able to bind *Pseudomonas aeruginosa*, and fluorescein isothiocyanate (FITC) as a Raman reporter.

Another team of researchers (Li et al. [77]) modified AuNPs with seleno-mitochondria location peptides (MLS) for mitochondrial targeting, 5-carboxyfluorescein (FAM) Raman reporter, and seleno-phenylboronic acid pinacol ester (HSeBA) organic small molecules for specific $H_2O_2$ response. The obtained AuNPs exhibited good resistance to abundant thiol under biological conditions and superior performance for mitochondria $H_2O_2$ monitoring in living cells, which is essential for the early diagnosis of various diseases such as cancer, inflammatory disease, cardiovascular diseases, diabetes, neurodegenerative Alzheimer's disease, and Parkinson's disease.

#### 2.4.2. Antibody-based SERS platforms

SERS platforms based on antibodies can be used for capture, isolation, and detection of multiple biomarkers. In particular, Qu et al.

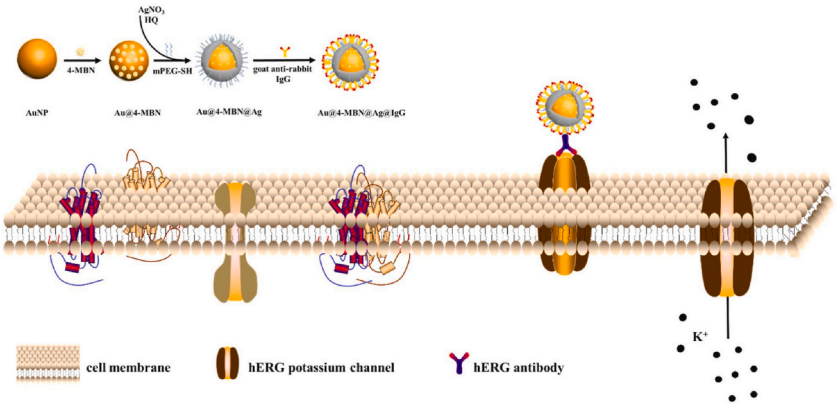


**Fig. 5.** Schematic representation of Au@4-MBN@Ag@IgG nanoparticle formation and the SERS-immunoassay for the detection of hERG ion channels on the surface of living cells. Reprinted with the permission from Ref. [78]. Copyright (2023) Elsevier.

[50] employed N-cadherin antibody (Ab) to trace the dynamic expression of the tumor metastasis-related N-cadherin. The SERS platform based on AgNPs, N-cadherin antibody, and a Raman reporter (MBA) demonstrated a high efficiency for monitoring of cancer cells during drug therapy by metformin hydrochloride. Zhang et al. [78] functionalized core-shell Au@MBN@Ag nanoparticles with goat anti-rabbit immunoglobulin G and applied the obtained SERS platform for investigating the human ether-a-go-go related gene (hERG) potassium ion channel in cell membranes (Fig. 5).

Another research team (Lin et al. [79]) modified core-shell B-$TiO_2$ NPs with a Raman reporter (alizarin red, AR), capping agents (polydopamine (PD) and PEG), and a Anti-P-Glycoprotein 1 antibody (AB). The modified NPs was proposed as a promising SERS platform to quickly and accurately identify MCF-7/ADR cancer cells.

### 2.4.3. Aptamer-based SERS platforms

Plasmonic nanoparticles functionalized with aptamers can bind with high specificity and affinity to a wide range of target molecules including nucleic acids, proteins, peptides, and small molecules. Aptamers, i.e., so-called "chemical antibodies," offer several advantages over traditional antibodies as targeting ligands. They have lower synthesis cost, higher pH and thermal stability, minor immunogenicity, and better biocompatibility.

Lv et al. [80] functionalized AuNPs with thiol-modified aptamers to target mucin 1 (MUC-1) and tenascin C (TNC) proteins, both of which are cancer markers overexpressed in tumor cells. They also applied MPy and ATP as Raman reporters to label SERS probes for MUC-1 and TNC proteins, respectively. The proposed SERS platform can be used for detailed analysis of cell surface saccharide profiles, which is essential for the early diagnosis of various cancer diseases.

Other research teams (Gao et al. [81] and Zhang et al. [82]) proposed to use aptamer-based colloidal SERS platforms for phenotyping of various cancer cells. In particular, Gao et al. applied AuNPs functionalized with thiolated DNA aptamers and specific Raman reporters (malachite green isothiocyanate (MGITC) and 3,3′-diethylthiadicarbocyanine iodide (DTDC)) for the phenotype analysis of hepatocellular carcinoma cells. Zhang et al. applied a SERS platform based on Ag@AuNPs, DNA aptamers targeting cellular surface proteins (HER2, EpCAM, and EGFR), and various Raman reporters (MBA, MMC, DTNB, and cyanine 3 (Cy3)) for phenotyping of breast cancer cells.

### 2.4.4. Vitamin, hormone, saccharide-based SERS platforms

Several research teams proposed to use vitamins as targeting ligands. In particular, Xu et al. [83] conjugated core-shell B-$TiO_2$NPs with folic acid (FA), capping agent (PEG), and AR as a Raman reporter. The functionalized NPs demonstrated a high efficiency for the *in situ* isolation and direct detection of circulating tumor cells in peripheral blood. Similarly, Chen et al. [84] and Xue et al. [85] used folic acid for the fabrication of colloidal SERS platforms based on AuNPs for the detection of cervical cancer cells. They also used capping agents (PEG and BSA) and Raman reporters (MBN and MBA) to conjugate plasmonic nanoparticles (Fig. 6).

Another research team (Tang et al. [86]) applied hormone molecules for modification of plasmonic nanoparticles with targeting ligands. They fabricated a SERS platform based on AuNPs, a Raman reporter (MBA), a capping agent (BSA), and luteinizing hormone releasing hormone (LHRH). The obtained platform could rapidly detect circulating tumor cells expressing LHRH receptors in blood and prove to be an effective tool for point-of-care testing in cancer patients. Besides, Zhang et al. [87] proposed to use ovalbumin as a saccharidic ligand for functionalization of Gd-AuNPs. They also functionalized NPs with prussian blue (PB) molecules as a Raman reporter and applied them for monitoring of dendritic cell (DC) migration in the lymphatic system, which is essential for evaluating the

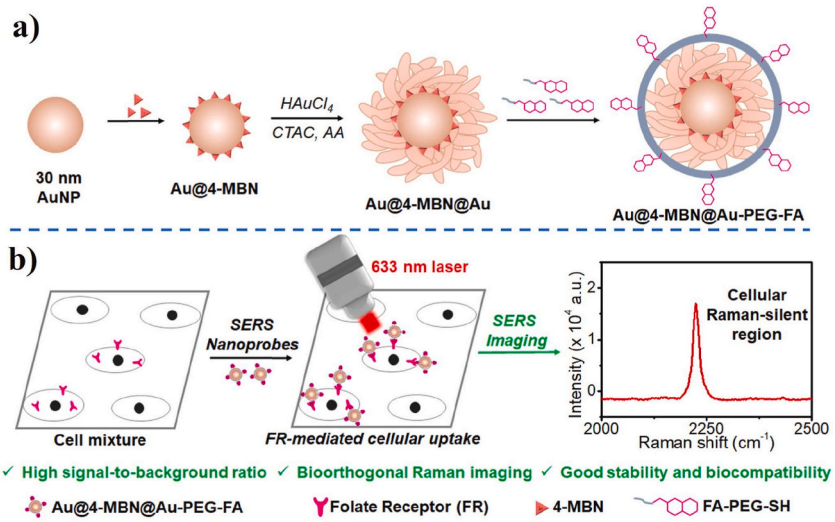


**Fig. 6.** a) Schematic illustration for the synthesis of bioorthogonal SERS nanoprobe Au@4-MBN@Au-PEG-FA, b) SERS imaging in the cellular Raman-silent region for identifying FR-positive cancer cells from co-cultured cells by Au@4-MBN@Au-PEG-FA. Reprinted with the permission from Ref. [84]. Copyright (2023) Elsevier.

outcome of DC-based immunotherapies.

### 2.5. Substrate-based/label-free/ligand-free SERS platforms

Generally, colloidal SERS platforms are preferred for single-cell studies due to a Raman signal of higher intensity compared to substrate SERS platforms [88,89]. However, their widespread use is limited due to a hypoosmolar effect of a colloidal solution on cells, the presence of toxic byproducts remaining after chemical synthesis, uncontrolled aggregation of colloidal nanoparticles, and low reproducibility of the obtained signal [90]. Furthermore, various factors, such as surface chemistry, charge, adsorbed molecules, external environment, and dynamic interaction between target and colloidal nanoparticles, can compromise the stability of SERS colloids, resulting in constant fluctuations of a detected SERS signal [91]. Currently, there is a trend towards using substrate-based SERS platforms, which are more stable and convenient for transportation and storage. Notably, most substrate-based platforms proposed in recent years are label-free and ligand-free which contributes to their longer shelf life and cost-efficiency.

For instance, Shvalya et al. [92] and Elena Dina et al. [93] designed label-free/ligand-free SERS substrates for the studies of bacterial cells (Fig. 7). In particular, Shvalya et al. applied a plasma reduction technique to cover a Si substrate with dodecahedral crystals of gold and demonstrated the effectiveness of the obtained SERS platform for the detection of bacterial DNA. Besides, Elena Dina et al. deposited AgNPs on a plexiglas substrate via a laser-induced photochemical synthesis and proved the applicability of the fabricated platform for the characterization of bacterial species.

An alternative approach was proposed by Das et al. [94] and Nikelshparg et al. [95]. They fabricated SERS substrates for single-cell studies by precipitation of AuNPs aggregates on polymer thin films. The obtained SERS platforms have been effectively applied for the detection of cancer cells [94] and monitoring of subcellular organelles [95]. Also for the detection of cancer cell organelles, Wu et al. [96] proposed SERS substrates based on core-shell Au@AgNPs transferred on a Si substrate via PVP-assisted deposition. A high sensitivity of the obtained SERS platform has been demonstrated in the detection of exosomes related to nasopharyngeal cancer cells.

Other research groups proposed to use label-free/ligand-free SERS substrates based on semiconductor nanowires (NWs) and plasmonic materials for DNA detection and monitoring. For instance, Mussi e al [97]. and Gaidi et al. [98] fabricated SERS substrates based on SiNWs and an Ag thin film (or AgNPs). Similarly, Han et al. [99] and Pal et al. [100] produced SERS platforms based on ZnO NWs decorated with Ag or AuNPs (Fig. 8). Besides, Salammal Sheena et al. [101] also designed SERS substrates without any conjugates for in-situ cellular DNA detection. They deposited AgNPs on a Si substrate and covered them with a thin layer of biocompatible $SiO_2$, which protects the NPs from oxidation and helps to avoid contact with culture medium.

Furthermore, several research groups demonstrated that label-free and ligand-free SERS substrates patterned with microscale or submicron features possess increased efficiency for analysis of various macromolecular objects such as DNA, bacterial cells, mammal cells, and subcellular components. In particular, Jabbar et al. [102], Xiang et al. [103], Luo et al. [104], and Hubarevich et al. [105] developed SERS substrates made of plasmonic materials (Au, Au@Ag, or Ag@Pd) and patterned with nanopores of various sizes (10–500 nm in diameter) via etching or anodic oxidation techniques (Fig. 9). They demonstrated that such nanopores promote the immobilization of DNAs or bacterial cells on their inner surfaces and provide a significant enhancement of SERS signal intensity due to the increase in the effective contact area of an analyte with plasmonic nanoparticles on the substrate surface [106]. Besides, Rigo et al. [107], and Šakalys et al. [108] applied alkaline etching and 3D printing, respectively, to fabricate SERS substrates covered with microscale pores (0.5–2.2 μm) of pyramidal or hemispherical shapes. The obtained substrates can be potentially used for immobilization and analysis of various biological microobjects, including bacterial cells, cell organelles, and DNAs.

Jonak et al. [109] and Milewska et al. [110] also patterned SERS substrates with arrays of nanostructures, but convex ones. In particular, Jonak et al. fabricated SERS substrates covered with an array of Au nanopyramids (400 nm) via E-beam lithography and applied them for detection of small extracellular vesicles (sEVs) or exosomes (30–150 nm) isolated from bronchoalveolar lavage (BAL)

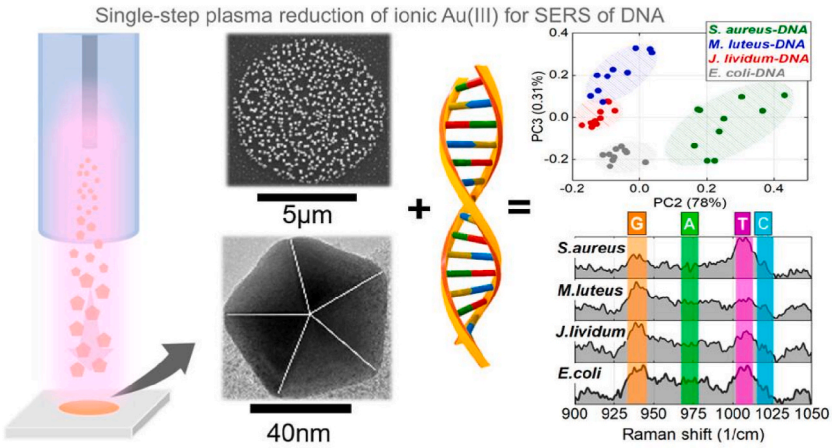


**Fig. 7.** Research methodology for SERS bacterial identification. Reprinted with the permission from Ref. [92]. Copyright (2023) American Chemical Society.

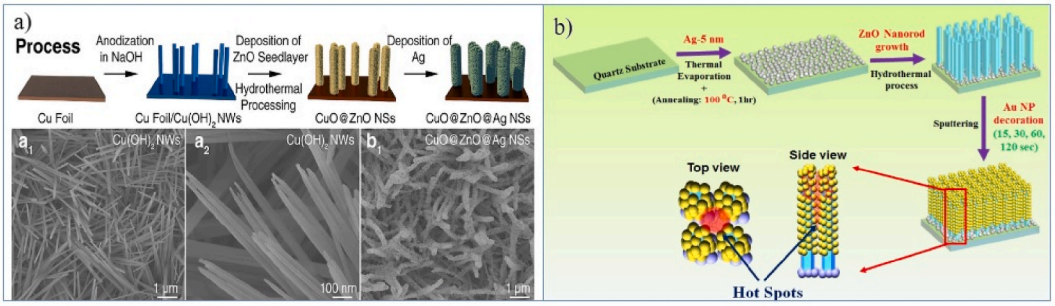


**Fig. 8.** a) Preparation process of CuO@ZnO@Ag NSs, SEM images of (a1, a2) $Cu(OH)_2$ NWs and (b1) CuO@ZnO@Ag NSs. Reprinted with the permission from Ref. [99]. Copyright (2023) De Gruyter; b) A hypothetical model representing the flow chart of the fabrication of Ag/ZnO/Au hybrid structures. Reprinted with the permission from Ref. [100]. Copyright (2023) Elsevier.

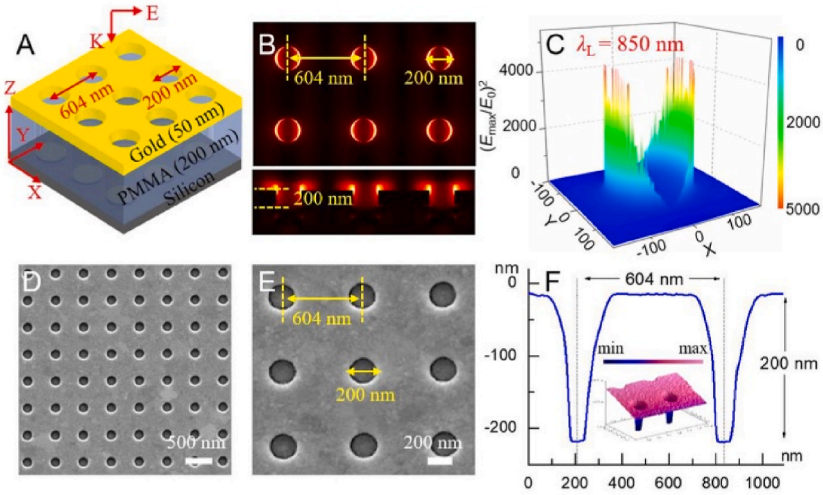


**Fig. 9.** a) 3D schematic illustration of the plasmonic gold nanohole array (PGNA) nanostructure, b) finite difference time-domain simulated electric field distribution at the x−y (top) and x−z (bottom) plane of PGNA, c) 3D electric field distribution displayed at the top Au/water interface of a nanohole, d), e) SEM images at low and high magnification of the as-fabricated PGNA, f) a line profile shows the depth as 200 nm, pitch as 604 nm, and radius as 100 nm of the as-fabricated PGNA. Inset is a 1.5 μm × 1 μm 3D AFM image. Reprinted with the permission from Ref. [104]. Copyright (2023) American Chemical Society.

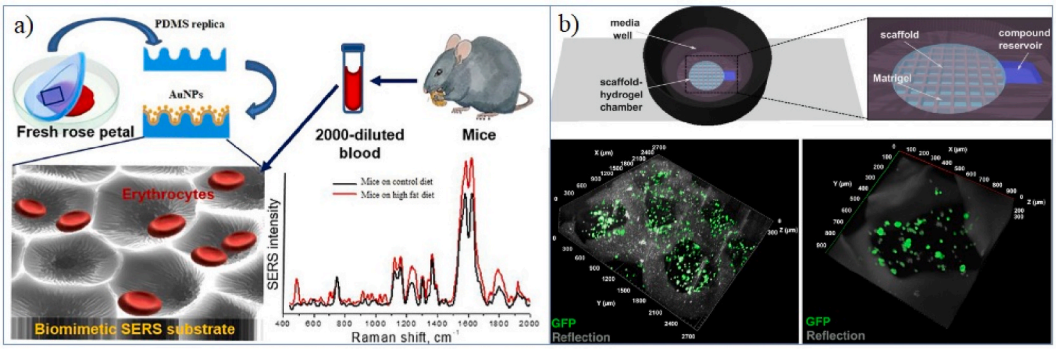


**Fig. 10.** a) SERS substrates based on rose petal replicas for the oxidative stress detection. Reprinted with the permission from Ref. [118]. Copyright (2023) Elsevier; b) Scheme of a custom-made device to integrate nanocomposite scaffolds within a 3D tumor cell environment, comprising MCF-7 cells and Matrigel. Reprinted with the permission from Ref. [119]. Copyright (2023) American Chemical Society.

and related to non-small cell lung cancer (NSCLC). Milewska et al. produced SERS substrates patterned with an array of Au nanoislands (7–37 nm) by an E-beam deposition technique and demonstrated their effectiveness for screening of osteogenic differentiation of mesenchymal stromal cells.

Commonly, methods used for nano/micropatterning of SERS substrates (X-ray/UV lithography, etching, electroplating, molding, etc.) require complex and costly fabrication processes with highly sophisticated templates, which is crucial for their transfer from laboratory prototyping to mass production [111,112]. To address these issues, a number of research teams proposed to use a soft lithography technique and various biological objects covered with complex hierarchical microstructures as templates for cheap and scalable SERS substrate manufacturing [113–118]. Most of these biomimetic substrates have been applied for the detection of low molecular weight compounds such as paraquat [113], pesticides [115], herbicides [117], and various dyes [114,116]. However, Barshutina et al. [118] used rose petals as soft-lithography templates to produce SERS substrates for the analysis of macromolecular objects such as living cells (Fig. 10a). The obtained substrates patterned with microcavities (30 μm in diameter) and covered with AuNPs (30–60 nm) demonstrated a high effectiveness for monitoring individual erythrocytes (6–8 μm) and detecting first signs of their membrane oxidation, which can be used for early diagnostics of various blood and metabolic diseases. Another alternative approach for the fabrication of label-free/ligand-free SERS substrates has been proposed by Plou et al. [119]. They 3D-printed gelatin@alginate hydrogel@AuNRs scaffolds (500 μm) mimicking a specific cell niche and applied them for the monitoring of drug transport in cancer cells with high spatiotemporal resolution (Fig. 10b).

### 2.6. Substrate-based/label-based/ligand-free SERS platforms

A number of research groups applied Raman reporters to produce SERS substrates for the detection of subcellular compartments with low Raman activity which can't be detected by label-free methods. In particular, Han et al. [120] fabricated a 3D SERS platform based on octahedral AuNPs (with a side length of 30–100 nm) conjugated with a Raman reporter (MBA). The obtained SERS platform was used to differentiate between living liver normal and cancer cells with a prediction accuracy of 86 %. Besides, Abalde-Cela [121] designed label-free SERS substrates for continuous multiplex monitoring of the single cell metabolism. They used gellan gum "spongy-like" hydrogels as a matrix for two different types of anisotropic metallic nanostructures, AuNSs and Au@AgNRs, functionalized with Raman reporters such as 1-naphtalenethiol (1NAT) and DTNB.

### 2.7. Substrate-based/label-free/ligand-based SERS platforms

Several research teams designed ligand-based SERS platforms for the selective detection of the physiologically relevant analytes in complex biological fluids. In particular, the research team of Guselnikova et al. [122–124] fabricated ligand-based SERS substrates for the detection of DNA damages and melanoma-associated fibroblasts. They fabricated nanopatterned substrates via laser ablation, covered them with Au thin films, and functionalized the obtained surfaces with folic acid [122] or DNA molecules [123,124] as targeting ligands (Fig. 11).

### 2.8. Substrate-based/label-based/ligand-based SERS platforms

As in the case of label-based/ligand-based colloidal SERS platforms, label-based/ligand-based SERS substrates offer advantages in high selectivity and intensity of a SERS signal. The presence of targeting ligands and specific Raman reporters facilitates the detection of multiple molecular targets in the desired part of living cells. A number of research groups [125–127] designed

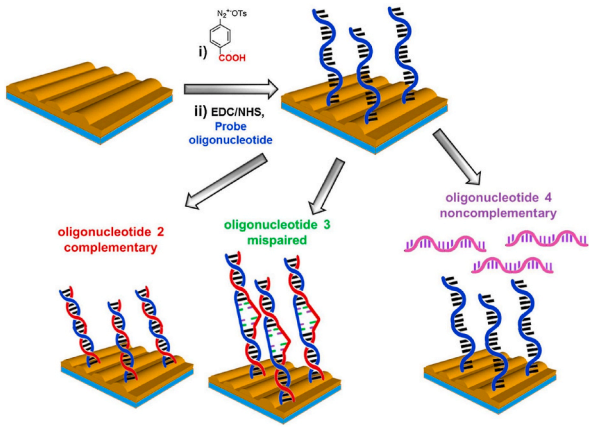


**Fig. 11.** Schematic representation of proposed DNA detection concept: two step grafting of Au grating with single stranded DNA, followed by the interaction with the solutions of complementary, mispaired and non complementary ODNs. Reprinted with the permission from Ref. [123]. Copyright (2023) Elsevier.

label-based/ligand-based SERS substrates covered with AgNPs of various shapes (nanospheres, nanocubes, nanorods, etc.). They labeled AgNPs with specific Raman reporters (ROX, 5,5-dithiobis(succinimidyl-2-nitrobenzoate) (DSNB), and MBA) and modified them with hairpin DNA or signal DNA (sDNA) as targeting ligands. The obtained SERS platforms have been effectively applied for the detection of cancer cell DNA. Other research groups also fabricated label-based/ligand-based SERS substrates based on the conjugated AuNPs and applied them for the medical diagnostics. For instance, Cao et al. [128]) proposed SERS substrates formed with an array of Au nanobowls covered with $Cu_2O$ octahedral NPs (($Cu_2O$)NPs). They conjugated ($Cu_2O$)NPs with a Raman reporter (DTNB) and hairpin DNA as a targeting ligand. The fabricated SERS substrate demonstrated a high efficiency for the detection of circulating cancer DNA.

Besides, Ge et al. [129] manufactured a SERS substrate covered with an array of $SiO_2$@AuNPs and functionalized them with a Raman reporter (FAM) and DNA probe molecules as targeting ligands. The obtained SERS platform has been proposed as an effective diagnostic tool for the detection of cancer cell DNA. Choi et al. [130] also used an array of AuNPs to fabricate label-based/ligand-based SERS substrates (Fig. 12). They synthesized tooth-like AuNPs (250 nm), covered them with GO and modified with a dopamine-specific aptamer labeled with a malachite green (MG) as a Raman reporter. The obtained SERS platform has been applied for monitoring of neuronal stem cell differentiation.

### 2.9. *Colloid-based + substrate-based hybrid SERS platforms*

In order to ensure high SERS signal intensity and measurement reliability, several research groups proposed to combine colloidal and substrate approaches in SERS analysis of single cells and subcellular components. In particular, Liu et al. [131] synthesized core-shell Au@AgNPs functionalized with MBA and placed the obtained colloids with a cell-containing analyte on a substrate with an array of square-cavities (7 μm x 7 μm). This label-based SERS platform has been effectively applied for capturing of single cells and monitoring of tyrosinase (TYR) activity associated with melanoma in living cells at psoralen ultraviolet A (PUVA) therapy.

A ligand-based hybrid colloidal/substrate SERS platform was designed by Reokrungruang et al. [132]. They impregnated a filter paper with AuNRs and used it as a SERS substrate for analysis of target cells separated from the initial colloidal samples by magnetic nanoparticles conjugated with a EpCAM-specific antibody (Fig. 13). The designed approach demonstrated a high efficiency for cancer cell detection and screening.

A number of research groups [133,134] fabricated hybrid colloidal/substrate SERS platforms using ligand-based and label-based approaches. In particular, Wang et al. [133] manufactured a SERS substrate based on an array of self-assembled AuNR conjugated with a Raman reporter (Cy5) and an EpCAM aptamer as a targeting ligand. They also modified target organelles in analyte samples with a Raman reporter (R6G) and HER2/CD63 aptamers to achieve a better selectivity of measurements. The obtained SERS platform demonstrated high accuracy in the specific detection of exosomes secreted by cancer cells, which is promising for early cancer diagnosis.

Lee et al. [134] also fabricated a hybrid colloidal/substrate SERS platform using label-based and ligand-based approaches. They covered Si micro pyramids with AgNPs functionalized with a coupling agent (MPTMS-GMBS), an binding agent (streptavidin), and anti-EpCAM antibodies and used them as a substrate for colloids of analyte samples modified with AgNPs, a Raman reporter (MBA), and Epstein-Barr virus (EBV) target DNA. The obtained SERS platform exhibits superior cell capturing ability and can sensitively detect EBV DNA at very low concentrations. This platform has great potential to become a promising diagnostic tool for monitoring disease status in nasopharyngeal carcinoma patients.

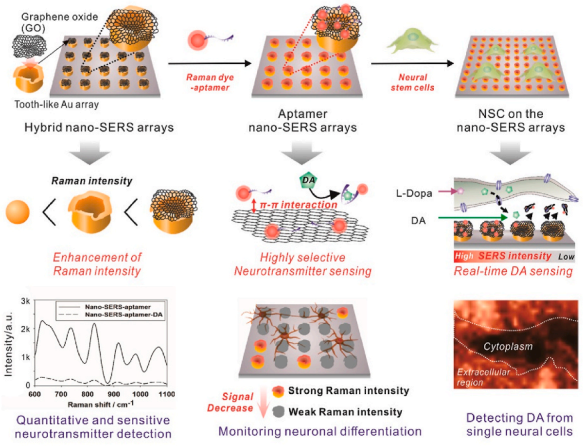


**Fig. 12.** Schematic diagram illustrating the method to detect dopamine (DA) releasing from single live cells using graphene oxide (GO)-hybrid nano-SERS. Reprinted with the permission from Ref. [130]. Copyright (2023) American Chemical Society.

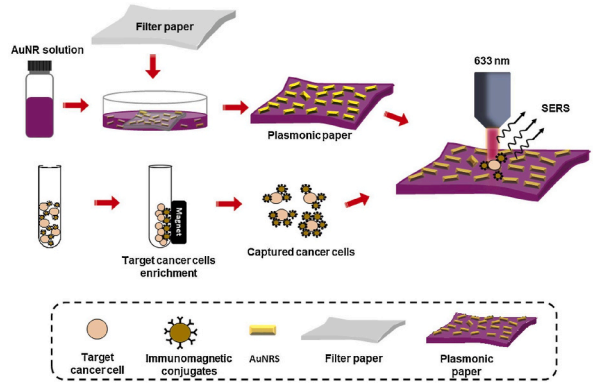


**Fig. 13.** Schematic diagram illustrating the plasmonic paper fabrication and cancer cell detection with the assistance of magnetic enrichment. Reprinted with the permission from Ref. [132]. Copyright (2023) Elsevier.

## 3. Challenges and perspectives

Despite significant breakthroughs in the field of single-cell SERS in recent years, there are some challenges and limitations that still need to be considered and addressed. For substrate-based SERS platforms, the main challenge is to achieve a high level of substrate homogeneity which is fundamental for biological analytes producing an intrinsically variable SERS response [135]. To ensure that the signal variations are due to the target analyte and not to the SERS substrate, it is essential to fabricate substrates with a highly homogeneous distribution of uniform plasmonic nanoparticles over the substrate surface. To achieve this objective, the development of effective techniques for size-controlled synthesis of uniform plasmonic NPs and their homogeneous dispersing over the substrate is highly required. Besides, shape-controlled synthesis of plasmonic NPs can also be beneficial for manufacturing of single-cell SERS platforms. The shape NPs can be designed according to the structural characteristics of target analytes in order to capture analyte molecules and specifically enhance their SERS signals [136].

The main issue for colloid-based SERS platforms is their poor stability in complex real samples, since the colloidal stability of plasmonic NPs is highly compromised by the addition of hydrophobic and macromolecular species. Besides, the colloids of NPs are optically inefficient because of the lack of plasmonic coupling between them and thus, the lack of hot spots. To address these issues, it was proposed to use various surfactants as capping agents [137]. However, their application can hinder the interaction between analytes and NPs, which results in the decrease of SERS signal intensity. Thus, the development of novel techniques for manufacturing of stable and sensitive colloid-based SERS platforms for single-cell analysis is further needed. One of the promising strategies to overcome the current limitations of colloidal SERS platforms is the integration of SERS platforms with microfluidic systems which enable the precise control of the aggregation time, make more efficient mixing with the analyte, decrease the likely contamination, and reduce the mechanical and optical variations of the colloids [138]. Another promising strategy is the development of new plasmonic materials based on semiconductors, nanocarbons, MXenes, conjugated polymers, etc. [139,140]. Due to the low cost, simple preparation, and outstanding optical properties, these materials could bring transformative changes to plasmonic devices.

However, in the development of SERS platforms based on new plasmonic materials, it is essential to perform thorough evaluation of their biocompatibility and applicability for biological studies of living cells and subcellular organelles. Biocompatibility of medical devices is usually assessed through irritation, sensitization, and toxicity tests according to the ISO 10993 standard family. Irritation tests are usually used to evaluate the device potential to cause an immediate irritation reaction following exposure to the body. Sensitization and toxicity tests are performed to ensure that medical devices do not cause severe allergic and toxic effects on living tissues during use.

The adverse effects of SERS platforms on cells could stem from (1) direct cell damage by the penetration of plasmonic NPs across the cell membrane (2) or indirect cell damage due to the generation of toxic ions and radical oxygen species. These effects can be minimized by controlling the parameters of plasmonic NPs, such as size, shape, structure, agglomeration state, surface charge, material type, etc. For instance, NPs with smaller sizes (1–100 nm) can be more toxic due to their ability to overcome the cellular barrier. On the other hand, NPs with a spherical shape exhibit less toxicity in comparison to nanotubes, nanofibers, nanocubes, etc. [141]. Hence, all aspects should be considered simultaneously in order to improve biocompatibility of SERS platforms designed for biomedical applications.

Another issue associated with single-cell SERS platforms is the background interference which can seriously affect the quantification of trace analytes in the physiological environment. The development of SERS platforms with anti-interference capabilities is a key challenge in bringing the SERS technique to the practical applications in single-cell analysis. Several approaches have been employed to eliminate the influence of background interference on SERS spectra, such as surface modification of plasmonic NPs with specific nanotags (labels and targeting ligands), however, there is a great scope for further improvement. In particular, more stable and repeatable SERS nanotags are much needed since the existing ones have an insufficient thermal stability and high sensitivity to the certain ions or pH values in the biological medium [142].

The problem of background interference and random noise becomes even more relevant for cellular components with a weak

spectral expression. In recent years, to extract valuable information from a raw SERS signal, it was proposed to use additional data processing techniques based on artificial intelligence (AI) and machine learning (ML) algorithms [143,144]. The combination of Raman spectroscopy, AI, and MI offers unprecedented opportunities to achieve fast identification of previously weak feature signals and specific biochemical responses in cells. Considering that AL and ML are able to analyze large amounts of Raman spectroscopy datasets, identify relationships, patterns, and connections in the datasets, as well as perform classifications, their application for the analysis of complex single-cell data is highly promising.

Finally, the development of low cost and easy to implement techniques for large-scale and standardized manufacturing of highly sensitive and reliable single-cell SERS platforms is a major challenge standing in the way of their widespread use in medical, pharmaceutical, biological, environmental, and other related fields. The potential techniques to explore include nanoscale glancing deposition [145,146], dip-pen lithography [147], and innovative self-assembly methods [148–150].

## 4. Conclusions

This paper reviewed the recent progress of SERS technology in the field of single cell studies. Due to its high sensitivity and specificity, SERS is one of the most efficient techniques for non-destructive analysis of biological objects. In this work, we summarized recent literature on SERS platforms designed for analysis of living cells and subcellular organelles. According to their physical state, single-cell SERS platforms can be categorized as colloid-based or substrate-based. Colloid-based platforms offer an advantage of high enhancement factor (EF), however, their widespread use is limited due to a hypoosmolar effect on cells, uncontrolled aggregation of plasmonic NPs, and low reproducibility of a SERS signal. Substrate-based SERS platforms are more stable and convenient for transportation and storage, but their EF is much lower. To improve the detection abilities of colloid-based and substrate-based SERS platforms, various conjugates (Raman reporters and targeting ligands) can also be applied. In this work, we analyzed the main advantages/disadvantages of conjugated and non-conjugated SERS platforms, and discussed their potential applications for single cell analysis. In particular, they demonstrated high efficiency for the detection of cancer cells, bacterial cells, viruses, and DNA mutations. Besides, they have also been proposed for the use in monitoring of cell metabolism and stem cell differentiation. Despite great progress in the field of single-cell SERS, some challenges and limitations in this field still remain, including high-cost manufacturing, poor stability of SERS signal, background interference, low biocompatibility, etc. Focusing on these issues can accelerate the progress of single-cell SERS and promote its practical use not only in research fields, but also in biomedical, and healthcare industries.

## Data availability

No data was used for the research described in the article.

## CRediT authorship contribution statement

**M. Barshutina:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Arsenin:** Supervision, Resources, Project administration, Funding acquisition. **V. Volkov:** Supervision, Resources, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37396.

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