表面增强拉曼光谱技术在肿瘤标志物检测中的研究进展

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摘要 恶性肿瘤已严重威胁我国居民健康, 而肿瘤标志物的灵敏、准确检测对于癌症的早期诊断、治疗及预后评价至关重要。因此, 探究一种灵敏、准确、快速、无创的检测技术, 具有十分重要的临床意义。表面增强拉曼光谱(SERS)是利用光与金、银等纳米结构材料相互作用产生很强的表面等离子激元共振效应, 可显著增强吸附在纳米结构表面上分子的拉曼信号, 以超灵敏获取样品自身或拉曼探针分子丰富的指纹图谱。该技术具有非侵入性和灵敏度高、选择性好、分析速度快、水干扰小等独特优势, 使其在生命科学、临床检验等方面具有良好的应用前景, 成为了一种极具潜力的生物检测技术。本文主要综述了近5年SERS技术在蛋白质类、酶类、核酸类、细胞类、组织类、气体类和其他类肿瘤标志物检测中的研究进展, 分析了该技术在生物检测中亟待解决的问题与挑战, 并对其未来的发展前景进行了展望。

关键词 表面增强拉曼光谱, 肿瘤标志物, 生物检测, 细胞

最新统计数据显示, 恶性肿瘤已成为我国居民疾病死亡的第1位原因^[1], 严重威胁居民健康。肿瘤标志物(tumor markers, TM)是指在肿瘤发生和增殖过程中, 由肿瘤细胞本身合成、释放, 或由机体对肿瘤细胞反应而产生的标志肿瘤存在和生长的一类物质, 主要包括蛋白质、酶、基因产物和代谢物等, 其灵敏检测对于恶性肿瘤的早期发现、诊疗和预后评价至关重要。目前常见的临床检测方法主要包括酶联免疫吸附试验、聚合酶链式反应、荧光法、电化学法、质谱法等^[2,3]。早期肿瘤诊断方面表现出了良好的临床效用, 虽然多数方法具有较高的检测灵敏度和准确性, 但其存在检测周期长、制备繁琐、有创等问题^[4]。因此, 探究一种既能满足灵敏、准确、特异性检测的要求, 又能实现简单、快速、无创诊断的新型技术, 具有十分重

要的临床意义。

拉曼光谱是1928年印度物理学家C.V. Raman等人发现的一种散射光谱^[5], 其拉曼效应来源于分子振动(和点阵振动)与转动, 能够对物质的组成和结构进行无创分析。然而, 传统拉曼光谱信号极弱, 易受荧光干扰, 检测灵敏度低, 限制了其应用范围。而表面增强拉曼光谱(surface-enhanced Raman spectroscopy, SERS)是利用光与金、银等纳米结构材料相互作用产生很强的表面等离子激元共振效应, 可显著增强吸附在纳米结构表面上分子的拉曼信号, 以超灵敏获取样品自身或拉曼探针分子的指纹图谱^[6]。通常情况下, SERS增强因子的平均值约为 10^6 , 但在等离子体间隙和尖锐突起的位置, 局域增强可高达 10^{14} , 弥补了传统拉曼光谱的不足, 成为了一种极具潜力的生物检测技术^[7]。与其他临床检测

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方法相比, SERS具有如下优势^[8]: (1) 具有超高的灵敏度, 可用于单分子的检测; (2) 可提供生物分子特有的“指纹”信息, 且SERS峰带宽通常较窄; (3) 对光漂白和光降解具有抗性, 适合长期监测; (4) 无需样品前处理, 少量样品即可实现快速、非侵入性检测; (5) 水的干扰小, 可在活性状态下研究复杂的生物体系; (6) 可设计不同的SERS活性纳米结构, 用于多重检测. 基于上述独特优势, SERS技术在生物医学领域表现出良好的应用前景. 本文综述了该技术近5年在蛋白质类、酶类、核酸类、细胞类、组织类、气体类和其他类TM检测中的应用研究进展, 分析了该技术在生物检测中存在的主要问题与挑战, 并对今后的研究趋势进行了展望.

1 蛋白质类

蛋白质是生命有机体中至关重要的生物大分子之一, 是能量存储、代谢和细胞功能调节等生命活动的主要承担者. 而异常表达的蛋白质类TM通常出现在肿瘤的早期或晚期阶段, 与多种疾病相关^[9]. 因此, 对其进行灵敏、准确且特异地量化分析在人类生命健康领域具有重要意义. 目前, SERS技术在其检测中的应用, 主要以抗原-抗体间的免疫反应为基础. 首先, 需制备具有高增强、高均一性等优点的SERS基底, 包括基于贵金属的纳米颗粒、纳米阵列、石墨烯材料以及合金复合结构等^[10], 修饰有靶抗体的SERS基底作为捕获元件. 然后, 多数贵金属纳米材料与拉曼探针分子(Raman reporters)、识别抗体相结合, 作为SERS标签(SERS tags)^[11], 以提供更显著的拉曼信号. 当存在TM时, 检测体系会形成夹心结构, 拉近SERS基底与SERS标签之间的距离, 产生“热点”(hot spots)效应, SERS增强因子可高达 $10^{12} \sim 10^{15}$ 倍^[12], 实现超灵敏的定量分析. Yang等人^[13]提出了一种超灵敏的SERS免疫测定法, 其原理如下(图1(a)): 将甲胎蛋白(alpha-fetoprotein, AFP)抗体包被在96孔板上, 以捕获AFP. 通过免疫反应与碱性磷酸酶标记的AFP抗体形成夹心结构, 利用酶诱导沉积反应, 即酶使底物(L-抗坏血酸-2-磷酸三钠盐)去磷酸化形成维生素C, 将 Ag^+ 还原成 Ag^0 并包覆在金纳米颗粒(Au nanoparticles, Au NPs)表面. 增强SERS信号, 线性范围为 $0.5 \sim 100 \text{ pg/mL}$, 检出限为 0.081 pg/mL . 该方法测定临床患者血清样品中的AFP含量与化学发光免疫法相比具有良好的一致性. Li等人^[14]制备了一种新型SERS基底: 银纳米颗粒(Ag nanoparticles, Ag NPs)包覆的 SiO_2 光子晶体珠(SPCBs), 如图1(b)所示, SiO_2 纳米颗粒通过

自组装形成单分散、尺寸可控制的SPCBs, 利用微流控装置使Ag NPs原位沉积在SPCBs上, 即可得到Ag-SPCBs. 通过改变 SiO_2 纳米颗粒的直径, 可获得具有不同反射峰位置的SPCBs, 将不同Ag-SPCBs的反射峰作为编码元素, 并修饰有不同抗体(antibody/Ag-SPCBs), 存在目标物时, 其可与SERS信号放大探针(4-MBA/antibody/Ag NPs)形成夹心结构, 在单个检测管中可同时检测AFP和癌胚抗原(carcinoembryonic antigen, CEA), 其线性范围分别为 $1 \times 10^{-4} \sim 1 \times 10^3$ 、 $1 \times 10^{-5} \sim 1 \times 10^3 \text{ ng/mL}$, 检出限分别为 7.2×10^{-5} 、 $6.6 \times 10^{-6} \text{ ng/mL}$. Li等人^[15]研发了一种基于SERS纳米标签($\text{Ag}@4\text{-MBA@SiO}_2$)的光纤生物传感器, 首次原位免疫测定了未处理全血中的AFP, 线性范围为 $50 \sim 500 \text{ ng/mL}$, 检出限为 17 ng/mL . 此外, 还成功用于体内和原位动态观察Wistar大鼠全血中的AFP, 初步结果表明, 该方法在体内实验中具有良好的应用价值. 随着SERS技术的发展, 其被广泛应用于胚胎抗原、糖蛋白、受体蛋白等蛋白质类TM的灵敏检测分析中, 如表1所示.

2 酶类

端粒酶(telomerase)是一种特殊的核蛋白逆转录酶, 由催化蛋白和RNA模板组成, 可指导染色体末端端粒重复序列的合成, 并保护染色体免于融合、重组和降解^[27]. 而多数肿瘤细胞中会过表达端粒酶, 导致端粒功能障碍且细胞增殖不受控制, 其可作为肿瘤诊断和预后评价的潜在TM. 因此, 建立准确、灵敏的端粒酶活性检测方法十分必要. Zong等人^[28]采用端粒延长受控的SERS效应, 以超灵敏检测端粒酶活性. 首先, 将巯基化端粒酶底物引物(telomerase substrate primer, TSP)和拉曼探针分子DTNB修饰在Au NPs上, 形成SERS活性标签(Au-Tag NPs). 端粒酶可将端粒重复序列(TTAGGG)催化延长至TSP上, 形成富含G的单链, 折叠成G-四联体结构, 其可防止盐诱导而发生Au-Tag NPs聚集, 导致SERS信号强度降低, 检出限是 1 cell/mL . 随后, Zong等人^[29]又提出了一种SERS和比色法双模式的端粒酶活性检测方法, 检出限分别为 1 、 10 cells/mL . Shi等人^[30]通过生物素-链霉亲和素技术将TSP偶联到 SiO_2 微球表面, 端粒酶催化延长TSP, 巯基标记的单链DNA作为捕获探针, 与TSP延长部分杂交形成长双链DNA, 通过Ag-S键与Ag NPs偶联在一起, 作为一次放大. 然后, 将每个偶联的Ag NPs溶解形成 Ag^+ , 其可诱导4-氨基苯硫醇修饰的Au NPs发生聚集, 从而形成SERS

图 1 (网络版彩色)SERS技术在蛋白质类、酶类和核酸类肿瘤标志物检测中的应用. (a) 基于酶诱导Au@Ag纳米结构的SERS免疫分析法用于AFP的超灵敏检测^[13]. (b) Ag-SPCBs的制备和SERS的多重生物检测^[14]. (c) 二次信号放大的SERS方法用于癌细胞和组织中端粒酶活性的检测^[30]. (d) 基于SERS的微滴传感器用于磁性免疫检测PSA^[32]. (e) 原位热点组装的Ag NP二聚体探针用于miRNA的检测^[37]. (f) DNA-rN1-DNA介导的SERS频移法用于ctDNA的超灵敏检测^[38]

Figure 1 (Color online) Application of SERS in tumor markers detection of proteins, enzymes, and nucleic acids. (a) The enzyme-induced Au@Ag nanostructure for ultrasensitive SERS immunoassay of AFP^[13]. (b) The fabrication of silver nanoshells silica photonic crystal beads (Ag-SPCBs) and the multiplex SERS bioassay^[14]. (c) Quadratic amplified SERS detection of telomerase activity in cancer cells and tissues^[30]. (d) The SERS-based microdroplet sensor for magnetic immunoassay of PSA^[32]. (e) The Ag NP dimer probes and the protocol for miRNA detection with the *in-situ* hot-spot assembly strategy^[37]. (f) The DNA-rN1-DNA mediated frequency-shift SERS assay for ultrasensitive detection of ctDNA^[38]

信号的“热点”, 通过二次信号放大(图1(c)), 检出限可达单细胞水平, 线性范围为5~100 cells. Xu等人^[31]构建了Au NPs自组装金字塔DNA结构, 端粒酶活性的线性范

围为 $1 \times 10^{-14} \sim 5 \times 10^{-11}$ IU, 检出限为 6.2×10^{-15} IU. 此外, 该探针也可用作细胞内递送载体, 实现细胞内原位检测和成像. 尽管已出现众多端粒酶检测方法, 但是针对

表 1 SERS技术在蛋白质类肿瘤标志物检测中的应用

Table 1 Application of SERS in proteins tumor markers detection

蛋白质类肿瘤标志物	应用材料	检出限	线性范围	文献
甲胎蛋白	银纳米颗粒薄膜	10^{-13} mol/L	$10^{-13}\sim 10^{-10}$ mol/L	[16]
	银芯片和金纳米颗粒	0.5 ng/mL	0.5~1000 ng/mL	[17]
	等离子体多层核壳-卫星纳米结构	0.3 fg/mL	$1\sim 10^6$ fg/mL	[18]
癌胚抗原	包覆金纳米颗粒的磁性镍-铁结构	~ 0.1 pmol/L	0~1 ng/mL	[19]
	包覆银的磁性纳米颗粒和包覆银的金纳米棒	4.75 fg/mL	$10\sim 10^8$ fg/mL	[20]
黏蛋白1	金纳米棒-银纳米颗粒卫星结构	4.3 amol/L	0.005~1 fmol/L	[21]
糖链抗原27~29	金纳米星结构	0.13 U/mL	0.1~500 U/mL	[22]
表皮生长因子受体	金纳米颗粒	10 fg/mL	$10\sim 10^4$ fg/mL	[23]
人表皮生长因子受体2	氧化石墨烯和金纳米颗粒	1 fg/mL	$1\sim 10^4$ fg/mL	[24]
核蛋白	包覆金的纳米尺度玻璃微探针和银纳米颗粒	0.8 nmol/L	1~50 nmol/L	[25]
铁蛋白	金纳米颗粒和磁珠	4 pg/mL	10~200 pg/mL	[26]

复杂病理样品(如组织或体液)中端粒酶活性的检测技术仍需改进和加强,以获得更准确的诊断信息,并应用于临床。

SERS技术也成功用于其他酶类TM的灵敏分析。Gao等人^[32]设计了一种基于SERS的微滴传感器,如图1(d)所示。标记有拉曼探针分子MGITC的Au NPs偶联检测抗体,磁珠偶联捕获抗体,存在PSA时,SERS标签可在一个液滴中形成免疫复合物,而游离的SERS标签则分离在另一个液滴中,该传感器以174滴/min的速度自动测量 1612 cm^{-1} 处的拉曼峰,用于定量测定血清中的PSA,检出限低于0.1 ng/mL。Jiang等人^[33]将抗体修饰的银聚集体和金纳米线作为SERS基底用于检测PSA,检出限为1 fg/mL。Xing等人^[34]报道了一种基于双重分子印迹聚合物的等离激元免疫夹心法,将C端表位印迹的单层AuNPs自组装玻璃板作为等离激元基底,N端表位印迹的Ag/PATP@SiO₂ NPs作为拉曼纳米标签,分别用于高特异性捕获和标记目标物,超灵敏检测了健康人和小细胞肺癌患者血清中神经元特异性烯醇化酶。基于酶与底物之间相互作用的原理,Wen等人^[35]通过修饰有Au NPs的TiO₂纳米管阵列基底,超灵敏测定凋亡细胞中caspase-3的活性,检测范围为 $1\sim 10^4$ pg/mL,检出限为0.25 pg/mL。He等人^[36]将牛血清白蛋白和激酶底物肽功能化的金纳米星作为SERS基底,无标记定量测定蛋白激酶A活性,其检出限为5 mU/mL。

3 核酸类

MicroRNA(miRNA)是一类长度为18~25个核苷酸

的非编码单链RNA,在调控基因表达和细胞增殖、分化、凋亡等过程中发挥着至关重要的作用。研究表明,miRNA的表达水平与多种肿瘤的发生、发展密切相关^[39,40]。因此,对miRNA表达水平的监测,在疾病的早期诊断和预后评价等方面具有十分重要的意义。其所面临的挑战在于:如何开发能精准监测miRNA表达的方法,尤其是在不同的浓度范围内,甚至直接在患者血液中检测出超低含量的miRNA。传统的miRNA分析以扩增技术为主,其虽应用广泛,但仍存在一些不足,如灵敏度较低、动态范围较窄等。近年来,将SERS与扩增技术相结合的研究报道不断涌现,显著提高了miRNA检测的灵敏度和特异性。Liu等人^[37]研发了一种原位热点组装方法(图1(e)):Ag NP二聚体通过级联杂交链式反应(hybridization chain reaction, HCR)引发原位热点组装成大团聚体,产生大量“热点”,实现miRNA-21的检测,检出限为 1×10^{-12} mol/L,同时该方法可定量检测单个癌细胞和正常细胞中的miRNA-21表达水平。He等人^[41]采用磁性SERS基底(Co@C/PEI/Ag)和基于锁式探针的指数滚环扩增技术(padlock probe-based exponential rolling circle amplification, P-ERCA)构建了一种“OFF-ON”型SERS平台,用于超灵敏检测miRNA-155,其线性范围为 $100\sim 10^8$ amol/L,检出限为70.2 amol/L。研究者还将SERS探针和双链特异性核酸酶(DSN)辅助信号放大技术相结合^[42,43],检测灵敏度比基于DSN的传统荧光生物传感器低近3个数量级,甚至可鉴别miRNA家族成员间的单碱基差异^[44]。此外,Zhu等人^[45]结合表面多功能微区印刷技术,设计了一种

检测多种miRNA的SERS频移方法, 在动态范围内(10^{-6} ~ 10^{-16} mol/L)可测定血清多种miRNA, 检出限为 10^{-15} mol/L. Liang等人^[46]提出了一种基于生物素-链霉亲和素的夹心结构, 以级联放大SERS信号, miRNA-21的检测范围为 $100\sim 1\times 10^8$ fmol/L, 检出限为38.02 fmol/L. Lee等人^[47]通过电化学反应制备了一种内有多银纳米晶体, 且呈周期性排列的金纳米碗结构, miRNA-34a的检出限为50 amol/L. Ye等人^[48]设计了一种基于DNA-linker-DNA的双功能SERS探针, 利用对称信号放大反应同时检测两种miRNA, 测得miRNA-141和miRNA-21的线性范围分别为 1×10^{-14} ~ 1×10^{-9} 和 1×10^{-14} ~ 1×10^{-9} mol/L, 检出限分别为 7.5×10^{-15} 和 5.6×10^{-15} mol/L. Li等人^[49]将miRNA-107作为引发剂, 金纳米球可选择性地自组装到中空的金银合金纳米立方体上, 形成复合等离子体纳米材料, 检测范围为 $1\sim 1\times 10^4$ fmol/L, 检出限为0.7 fmol/L, 并成功用于患者尿液样品的检测, 实现了前列腺癌的诊断.

DNA检测已成为早期癌症诊断的有力工具, 如循环肿瘤DNA(circulating tumor DNA, ctDNA)、甲基化DNA等. Zhang等人^[38]首次设计了一种嵌入RNA位点的DNA-rN1-DNA介导的SERS频移测定法, 用于检测ctDNA, 如图1(f)所示. RNase HII酶可特异性水解DNA-rN1-DNA/ctDNA杂交复合物, 待测物的结合会改变拉曼探针分子的振动频率, 根据其频率偏移的变化量完成检测, 可在 1×10^4 个正常ctDNA(KARS G12D)中区分单个碱基对突变, 肺癌患者血清样品中ctDNA的检出限为 0.9×10^{-14} mol/L. 相比于区分噪声信号以上的拉曼峰强度, 该方法更容易获得拉曼光谱响应信号, 有利于进一步提高检测的灵敏度和准确性. Zhou等人^[50]构建了一种基于单壁碳纳米管和DNA介导的SERS策略, 血液中KRAS G12D的检测含量可低至0.3 fmol/L. Kowalczyk等人^[51]将镀金层的光刻GN作为SERS基底, 并利用烷基硫醇基团连接上单链DNA, 首次根据DNA杂交过程中烷基硫醇的构象变化, 灵敏检测ctDNA突变基因(BRAF c.1799T>A; p. V600E), 检出限为0.17 pg/ μ L, 检测范围为 $6.75\sim 6.75\times 10^4$ pg/ μ L. Ouyang等人^[52]用抗体修饰的AuNPs捕获甲基化DNA, 将石墨烯-Ag阵列作为SERS基底, 可实现快速(<60 min)、灵敏(检出限为0.2 pg/ μ L)的检测. 此外, Lin等人^[53]利用金属羰基化合物的光谱特性, 设计了一种SERS比率测定法, 用于测量鼻咽癌血液中EB病毒的循环无细胞DNA(cell-free circulating DNA, cfDNA), 检测范围为 $5\times 10^2\sim 1\times 10^4$

copies/mL.

4 细胞类

肿瘤细胞检测作为液体活检的代表技术, 已成为一种肿瘤无创诊断和实时疗效监测的有效手段, 在肿瘤患者早期诊断、治疗监测和预后评估等方面具有越来越重要的临床参考价值. 目前, 荧光显微成像技术仍然是研究和诊断肿瘤细胞的重要策略. 但是, 荧光成像中所需的荧光基团存在光漂白、光损伤、组织穿透深度差等问题. 而拉曼成像是一种强大的多组分、高灵敏、高光学稳定性、非破坏性成像技术, 可有效克服上述不足. 通常拉曼散射信号较弱, 因此需要通过表面增强效应来实现拉曼光谱的检测, 其信号强度依赖于金属基底的增强效应. 因此, 摆脱金属纳米材料的限制, 开发自身具有强拉曼信号的纳米材料具有更重要的研究价值. Keisham等人^[54]将脑细胞置于单层石墨烯界面上(图2(a)), 二者发生相互作用, 使石墨烯中电荷重新排列, 改变其原子振动能量, 根据单个细胞在石墨烯界面上引起的2D峰频率变化, 监测癌细胞活性, 并成功区分癌细胞和正常细胞(图2(b)). 然而, 几乎所有的癌细胞都可以通过空穴掺杂机制改变石墨烯的拉曼频移. 因此, 上述工作缺乏特异性, 未能鉴别癌细胞的种类. Jin等人^[55]以炔基、氰基等为原料, 通过聚合的方法得到在生物体内拉曼沉默区($1800\sim 2800$ cm^{-1})具有特征峰的新型拉曼微球探针, 如图2(c)所示, 在其表面修饰不同的靶向基团(AS1411、MUC1和cRGD等), 实现了对多种肿瘤细胞的检测和多色拉曼图像以及对小鼠活体肿瘤的靶向识别(图2(d)), 进一步提高了肿瘤检测的准确性. Zhang等人^[56]将Au NPs组装到三角形金字塔DNA结构(triangular pyramid DNA, TP-Au NPs)中, 作为SERS探针, 并用于高灵敏、选择性检测循环肿瘤细胞和外泌体. Zhang等人^[57]利用微流体芯片筛选出循环肿瘤细胞, 并结合设计的3种光谱正交SERS适配体纳米载体, 成功用于肿瘤细胞膜蛋白的原位分析和肿瘤细胞亚群的高灵敏、高选择性分类. 此外, SERS增强效果依赖于一定尺寸的增强基底, 该尺寸往往大于常见的细胞表面受体, 导致靶向结果不准确. Haldavnekar等人^[58]介绍了一种基于ZnO的3D半导体量子尺寸SERS探针, 进一步提高了生物相容性和靶向性, 将其修饰在纳米枝晶平台上, 用于原位检测DNA、RNA等生物分子, 并成功鉴别了癌细胞和正常细胞, 其检测灵敏度可达单细胞水平.

图 2 (网络版彩色)SERS技术在细胞类肿瘤标志物检测中的应用. 基于界面石墨烯的拉曼mapping方法用于超灵敏监测癌细胞活性^[54]. (a) 附着多形性胶质母细胞瘤(GBM)细胞的石墨烯掺杂及2D峰声子色散机制; (b) 附着GBM细胞和星形胶质细胞的石墨烯2D峰拉曼图. 多色拉曼微球用于肿瘤细胞和组织的成像及小鼠活体肿瘤的光谱检测^[55]. (c) 三色靶向拉曼微球; (d) AS1411-拉曼微球对小鼠肿瘤的拉曼光谱和成像检测

Figure 2 (Color online) Application of SERS in tumor markers detection of cells. The ultrasensitive detection of the activity of cancer cell via Raman mapping of interfaced graphene^[54]: (a) The doping mechanism of graphene by a well-attached Glioblastoma Multiforme (GBM) cell and the resultant modification of phonon dispersion in 2D peak; (b) Raman spectra of 2D peak positions of graphene interfaced with GBM and astrocyte cell. Multicolor Raman beads for multiplexed tumor cell and tissue imaging and *in vivo* tumor spectral detection^[55]: (c) Three-color targeted Raman beads, m-1 (2121 cm^{-1}), m-3 (2186 cm^{-1}), and m-4 (2236 cm^{-1}), were incubated separately with MCF-7 cells; (d) Raman spectra and imaging detection of mice tumors *in vivo* using AS1411-Raman beads

外泌体(exosomes)是由几乎所有类型的细胞通过质膜融合而分泌的纳米级磷脂双层囊泡, 其内包含了蛋白质、脂质和核酸等生物分子. 研究证明, 肿瘤细胞分泌的外泌体具有特定的分子特征, 并促进肿瘤的生长、转移、侵袭等过程^[59], 因此, 被认为是诊断肿瘤的潜在生物标志物. SERS技术因具有无创、灵敏、快速和所需样品量少等优势, 最近已备受瞩目. Li等人^[60]设计了一种聚多巴胺修饰的免疫捕获基底和一种由超薄聚多巴胺层包覆的抗体-拉曼探针分子-Ag(壳)-Au(核)多层结构组成的SERS标签, 超灵敏和特异性检测了胰腺癌中外泌体, 在2 μL 样品溶液中仅检测到一个外泌体(约 9×10^{-19} mol/L), 检测范围为 $5.44 \times 10^2 \sim 2.72 \times 10^4$

exosomes/mL. Kwizera等人^[61]将镀金显微镜载玻片作为基底, 辅助3D打印技术形成的多孔金阵列免疫捕获外泌体, 修饰QSY21拉曼探针分子的金纳米棒用作SERS标签, 检测范围为 $10^6 \sim 10^8$ exosome/mL, 检出限为 2×10^6 exosomes/mL(3.3 fmol/L), 可在2 h内分析80个以上样品. SERS不仅能快速检测单一外泌体, 还可以灵敏、特异性地同时检测多种外泌体. Wang等人^[62]用CD63适配体修饰的金壳磁珠捕获外泌体, 将修饰不同拉曼探针分子和外泌体适配体的Au NPs作为SERS探针, 同时检测SKBR3、T84和LNCaP三种外泌体, 检出限最低的是SKBR3外泌体, 约为32 exosomes/ μL , 检测范围为4个数量级.

5 组织类

活体组织检查,是病理诊断的主要手段和方法.准确区分病灶区域和正常组织,则需要具有高空间分辨率和高灵敏度的成像工具.然而,现有的成像技术都无法同时满足上述要求.比如荧光成像技术,虽然具有高灵敏度,但其穿透深度和空间分辨率较差^[63].光声成像技术能够提供高空间分辨率和组织穿透能力,但灵敏度却不高^[64].拉曼成像虽然灵敏度不及荧光成像,且存在谱峰纷杂的问题,但在细胞的拉曼沉默区可以实现高信噪比和零背景干扰的检测,十分利于检测活体组织^[65].而灵敏的SERS作为一种新型的拉曼成像技术,则具有非常突出的临床诊断能力.Noonan等人^[66]利用抗体标记的金纳米探针(antibody-functionalized gold nanoprobes, BFNP),对动脉硬化患者的组织样品进行靶向SERS成像检测.研究结果发现,细胞间黏附分子1(intercellular adhesion molecule 1, ICAM-1)和P-selectin存在于动脉硬化和非动脉硬化的组织中,而血管细胞黏附分子1(vascular cell adhesion molecule 1, VCAM-1)只存在于动脉硬化组织中,将动脉组织切开进行拉曼测试可得到增强的检测信号(图3(a)).同时首次靶向成像检测了血管炎症部位的特定生物标志物.此外,该研究还成功用于检测小鼠活体内的炎症分子(图3(b)),结果表明注射BFNP的小鼠中存在ICAM-1、VCAM-1和P-selectin,对动脉硬化的早期诊断意义重大.Harmsen等人^[67]设计了一种表面增强共振拉曼散射(surface-enhanced resonance Raman scattering, SERRS)纳米星结构,其具有星形金核,在近红外光谱中有拉曼共振,且不引入杂质,SERRS纳米星的灵敏度(检出限为1.5 fmol/L)可显示肿瘤组织边缘,做到局部区域肿瘤扩散的精确可视化成像.Qiu等人^[68]报道了一种利用纳米金核壳结构的缝隙增强拉曼探针进行拉曼成像的手段,可明确发现并排除浸润性肿瘤边缘和显微卫星转移灶,并实时监测和根除术中残留微小肿瘤.随后,Zhang等人^[69]开发了一种新型的外壳为花瓣状结构的缝隙增强拉曼探针(P-GERTs).如图3(c)所示,通过将拉曼分子同时嵌入到核壳颗粒内部和外部的花瓣状结构之间的亚纳米缝隙,极大地增强了分子的拉曼信号,其增强因子高达 5×10^9 ,P-GERTs在单颗粒水平上表现出良好的信号均一性和光稳定性.通过调节内嵌拉曼分子的数量,可调节探针的形貌和SERS性能;通过改变外部拉曼分子的种类,可获得多种探针信号以实现多重成像,在6 s

内可获得高分辨(2500 pixels)的单细胞拉曼成像(图3(d)),在52 s内可获得高对比度、大范围($3.2 \text{ cm} \times 2.8 \text{ cm}$)的小鼠活体前哨淋巴结成像(图3(e)),为构建超快速、超灵敏的生物传感技术提供了新思路.Qi等人^[70]结合荧光、光声和拉曼3种成像技术的优点,首次报道了one-for-all型有机分子探针(OTPA-TQ3),该探针能够同时产生很强的荧光-光声-拉曼信号,具有用于精准肿瘤成像以及手术不同阶段的精确导航的潜能.术前,荧光和光声成像确定肿瘤的位置和尺寸信息;术中,荧光和拉曼成像精确区分微小的残余肿瘤、肿瘤和正常组织的边界,极大提高了肿瘤切除手术的成功率和存活率.Wei等人^[71]开发了一种结合组织透明化技术和受激拉曼散射显微镜的三维化学成像技术,提升了超过10倍的成像深度,并结合三维相量分析法,实现了三维组织结构的化学聚类 and 分割,以用于研究组织的三维结构、组成和功能.

6 气体类

肺癌患者中42种挥发性有机化合物(volatile organic compounds, VOCs)的含量可升高至10~100 ppb(parts per billion),已被确定为肺癌生物标志物^[72].其主要是拉曼惰性分子,可大致分为7类^[73]:碳氢化合物类、醇类、醛类、酮类、酯类、腈类和芳香族化合物类.其中,呼出气中的某些醛类因具有致癌性、致畸性、诱变性和遗传毒性,可作为诊断肺癌的重要指标^[74].理论上,SERS技术可通过贵金属纳米结构的SPR现象,实现单分子的检测.但气态分析物具有极低拉曼截面以及在SERS基底上吸附性差等因素,限制了该技术在气体检测中应用.因此,Wang课题组^[75]展示了一种基于树突状银纳米晶体的SERS传感器,可超灵敏检测肺癌患者中ppb水平的气态醛,检测苯甲醛的线性范围为 $2 \sim 2 \times 10^4$ ppb.随后,该课题组^[76]设计了一种基于gold superparticles(GSPs)@MOF核壳结构的SERS传感器,用于检测气态肺癌标志物,如图4(a)所示.在GSPs内部,纳米粒子有序、均一的间距可增强等离子体耦合作用,增强检测信号.气体待测物通过MOF多孔结构时流动性减弱,延长了气体滞留时间,呼出物中的气态醛通过MOF通道到GSPs基底上,与其表面的4-ATP发生希夫碱反应,被有效吸附及捕获.同时MOF层的高介电常数可有效阻碍等离子体波的衰减,极大提高了检测灵敏度,该传感器对气态醛检测有良好的选择性和线性响应,检出限为10 ppb(图4(b), (c)).通过结

图 3 (网络版彩色)SERS技术在组织类肿瘤标志物检测中的应用。金纳米探针用于多种血管炎症分子的SERS成像^[66]。(a)完整和切口的动脉粥样硬化组织和非动脉粥样硬化组织的SERS光谱;(b) HA-NSG 小鼠体内炎症分子的非侵入性SERS光谱。花瓣状缝隙增强拉曼探针(P-GERTs)用于生物成像^[69]。(c)P-GERTs示意图和透射电子显微镜图;(d)单个H1299细胞的拉曼图像及其3个不同位置的SERS光谱;(e)注射P-GERTs后,小鼠腋窝淋巴结的高对比度、大范围活体拉曼成像

Figure 3 (Color online) Application of SERS in tumor markers detection of tissues. Multiplex molecular SERS imaging of vascular inflammation using gold nanoprobes^[66]. (a) SERS spectra of the intact (left) and opened (right) artery on atherosclerotic and non-atherosclerotic tissues; (b) non-invasive *in vivo* SERS spectra of adhesion molecules on HA-NSG mice. Gap-enhanced Raman tags with a petal-like shell (P-GERTs)^[69]. (c) Schematic diagram and TEM image of P-GERTs; (d) Raman image of a single H1299 cell and three representative SERS spectra obtained from different points in panel b; (e) high-contrast and wide-area *in vivo* Raman image of the hind-limb popliteal lymph node after injection of P-GERTs

图 4 (网络版彩色)SERS技术在气体类和其他类肿瘤标志物检测中的应用。基于GSPs@ZIF-8核壳结构的SERS传感器用于VOCs的定量检测^[76]。(a) 自组装GSPs@ZIF-8核壳结构及SERS检测VOCs的示意图；(b) 检测不同浓度4-乙基苯甲醛的SERS光谱及标准曲线；(c) SERS基底的选择性；(d) 健康和肺癌患者模拟呼出的主成分分析。基于SERS和荧光双模式的纳米传感器用于定量检测Cyt c^[80]。(e) 检测Cyt c的示意图；(f) SERS和荧光双模式检测Cyt c；(g) 经不同浓度的AFB1和孵育时间处理后，活细胞的共聚焦和SERS图像

Figure 4 (Color online) Application of SERS in tumor markers detection of gases and others. The SERS sensor for quantitative detection of volatile organic compounds (VOCs) in GSPs@ZIF-8 core-shell structure^[76]. (a) Diagrammatic sketch of a self-assembly GSP@ZIF-8 core-shell structure and VOCs detection via SERS spectroscopy; (b) SERS spectra of different concentrations of 4-ethylbenzaldehyde and the detection standard curve; (c) selectivity of the SERS substrate; (d) PCA of set of simulated breath samples of healthy and lung cancer. The SERS-fluorescence dual-mode nanosensors for cytochrome c (Cyt c) quantitative detection^[80]. (e) Schematic illustration of Cyt c detection; (f) SERS and fluorescence detection of Cyt c; (g) confocal and SERS images of living cells with different aflatoxin B1, corresponding to real time

合主成分分析, 该传感器可甄别模拟呼出气中特征分子浓度是否达到阈值(图4(d)). 此外, Chen等人^[77]利用石墨烯和Au NPs, 根据呼出气中14种VOCs拉曼谱带提供的指纹图谱, 区分了不同模拟呼气样本和200个临床患者的呼气样本, 其灵敏度高于83%, 特异性高于92%. Koh等人^[78]设计了一种“等离子体鼻”结构, 其由MOF

包覆的Ag纳米立方体(Ag@ZIF)组成, 以将气体分子和电磁场集中在该结构三维空间的纳米尺度上, 检测低至ppm(parts per million)级的VOCs. Yang等人^[79]提出了一种新型阵列辅助SERS微流控芯片(ArraySERS芯片), 用于气体传感分析, 三角形阵列结构可增加通道中气体分子与传感界面之间的碰撞概率, 以提高检

测灵敏度, 将包覆MOF和半胱胺的银纳米立方体(AgNC@CA@ZIF)作为SERS探针, 其中MOF和半胱胺用于捕获气体。研究表明, MOF对气体的吸附性更好, 而半胱胺的SERS背景值较低, 因此该探针可进一步提高传感性能, 成功检测了多种痕量醛类气体, 检出限低至1 ppb。

7 其他

SERS技术也广泛用于其他生物标志物的量化分析中。Reza等人^[81]研发了一种基于SERS和微流控技术的免疫检查点检测平台, 其利用纳米酵母单链可变区片段(NY-scFv)捕获目标物, 特异地检测了多种免疫检查点生物标志物(PD-1、PD-L1和LAG-3), PD-1和LAG-3的检出限均为100 fg/mL, 检测范围为100~1×10⁶ fg/mL, PD-L1检出限均为50 fg/mL, 检测范围为50~1×10⁵ fg/mL。Jiang等人^[82]研发了一种内嵌拉曼分子的金银核壳隙增强拉曼探针, 以内嵌拉曼分子信号作为校准信号(内标), 结合经典最小二乘法的机器学习方法, 成功地定量检测了细胞内的胆固醇含量。Zhang等人^[80]通过合成修饰适配体的金纳米三角形结构(gold nanotriangles, AuNTs), 构建了一种基于SERS和荧光能量共振转移的双模式纳米传感器, 如图4(e)~(g)所示。用于定量测定活细胞中的细胞色素c(cytochrome c, Cyt c), AuNTs具有增强SERS信号和猝灭荧光的效应, 细胞被外界刺激(如毒素)可诱导释放Cyt c, 与该适配体特异性结合, 并释放Cy5修饰的互补链, 可导致SERS信号降低和荧光信号恢复, 双模式检测的线性范围为0.044~9.95 μmol/L, 活细胞中的最低检出限为0.02 μmol/L。Wen等人^[83]设计了一种基于碱性艳蓝4R的纳米探针, 用于检测痕量人绒毛膜促性腺激素(hCG), 该探针可增强hCG在1615 cm⁻¹处的信号强度, 其线性范围为0.05~20 ng/mL。Liang等人^[84]研发了一种基于4-巯基苯硼酸(4-mercaptophenylboronic acid, MPBA)的SERS纳米传感器, 其由MPBA修饰的Ag NPs组成, 该复合物可作为SERS基底(Ag NPs)、拉曼探针分子(MPBA)和基于苯基硼酸和唾液酸聚糖识别的目标受体, 特异性定量检测HepG2细胞膜上唾液酸(sialic acid, SA)的表达水平, 检出限约为8.9 μg/mL。而Hernández-Arteaga等人^[85]则将柠檬酸盐还原的Ag NPs作为SERS基底, 用于检测唾液中SA, 100名乳腺癌患者唾液中SA平均浓度(18.3 ± 9.4 mg/dL)显著高于106名健康人(3.5 ± 1.0 mg/dL), 该方法的灵敏度为94%, 特异度

为98%。

8 总结与展望

SERS技术在水环境下可以超灵敏、快速、无创地提供生物分子固有的“指纹”信息, 因此, 该技术已成功用于灵敏和特异检测蛋白质类、酶类、核酸类、细胞类、组织类和气体类等TM, 使得该技术在生命科学、临床检验等方面具有良好的发展前景。然而, 从其1974年发现至今的40多年中, SERS技术仍未能在生物医学检测领域得到广泛应用。SERS生物检测技术涉及光、表面等离激元纳米材料和生物检测体系三者之间非常复杂的相互作用^[8], 会导致一系列亟待解决的问题。例如, 在生物检测体系中, 复杂的环境与组成成分是否会影响SERS探针的结构以及其传感性能? 在SERS检测时, 光谱信号波动现象十分常见, 如何提高该检测信号的重现性? 在活体检测时, SERS探针是否会影响生物体的正常生命活动? 检测信号能否真实地反映生物体系自身的性质? 这些问题均会导致难以获得重现且可靠的拉曼光谱数据。因此, 拉曼检测的重现性和可靠性仍然是限制SERS技术在生物检测领域发展的主要挑战, 使其不易实现广泛的临床化和标准化应用。因此, 研究者在该领域仍有许多工作要做, 才能在实践中充分解决生物检测中的瓶颈问题。SERS生物检测技术重现性、可靠性和灵敏性, 取决于SERS基底的合理设计, 生物样品的适当处理, 检测条件的精确控制以及光谱数据的充分分析。今后应着力控制其潜在因素: (1) 制备高均一性、高增强效应且表面洁净的SERS基底, 可进行适当的修饰; (2) 选择合适的拉曼探针分子, 以提高其生物相容性, 避免非特异性吸附, 并将待测物捕获至检测“热点”区域; (3) 保存和预处理生物样品(细胞、组织等)时, 尽可能将其保持在接近正常生理状态的环境中, 以免发生不可逆的改变; (4) 选择恰当的SERS检测条件, 如激发波长、曝光时间、激光功率等, 避免热效应和光催化反应, 并加以精确控制, 以获得可靠的检测信息; (5) 建立生物分子的标准SERS数据库以准确指认光谱信息, 必要时可将探针在检测体系中的响应进行校正, 或者选择适当的数据处理和统计分析方法。近期, SERS技术用于多重检测和实时监测生物体液、细胞、组织等环境中生物分子的报道已不断涌现^[86-90]。可以预见, 未来SERS技术在肿瘤生物标志物早期检测领域的前景, 在于开发一个超灵敏、高特异、高通量和通用型的检测平台, 以满足

生命科学和临床诊断的需求。相信通过医学、化学、材料学等多方研究者的共同努力与合作, SERS技术将在重现性和灵敏性等方面不断改进与完善, 使其在生物医学检测中得到更精准、更广泛的应用。

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Summary for “表面增强拉曼光谱技术在肿瘤标志物检测中的研究进展”

Recent advances in surface-enhanced Raman spectroscopy for the detection of tumor markers

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Sensitive and accurate detection of tumor markers is critically essential for the early clinical diagnosis, treatment and prognosis of cancers. Surface-enhanced Raman spectroscopy (SERS) utilizes plasmonic nanomaterials to generate a strong surface plasmon resonance (SPR) effect in the visible to near-infrared region, such as silver and gold nanostructures. The SPR effect can significantly enhance the Raman signals of adsorbed molecules on the surface of nanostructures, allowing to gain the sensitive fingerprint information for the analyte itself or the Raman reporter. Benefitted from the superior properties of high sensitivity, good selectivity, fast and noninvasive analysis, SERS has attracted increasing interest and become a very promising technique for biological analysis. In comparison with other bioanalytical techniques, such as enzyme-linked immunosorbent assay, fluorescence and electrochemical methods, SERS possesses the following advantages: (1) SERS has an ultrahigh sensitivity, even down to single molecule level; (2) SERS can provide the intrinsic molecular fingerprint information, and the bandwidths of SERS peaks are usually very narrow; (3) SERS is suitable for long-term monitoring, because of the resistance of photobleaching and photodegradation; (4) non-invasive detection can be achieved with a low amount of sample and no preparation; (5) the interference of water is very weak, so biological samples can conveniently be detected in aqueous solutions; (6) various SERS-active nanostructures can be designed for multiplex detection. In this review, we provide an overview of recent advances in SERS technology for the detection of different tumor markers, including proteins, enzymes, nucleic acids, cells, tissues, gases and others. Great successes have been achieved by utilizing SERS, which can facilitate the fabrication of assays for tumor biomarkers. However, SERS has not been widely applied in clinical bioanalysis and biodiagnosis in the past over 40 years. SERS-based bioassay involves complex interactions between light, surface plasmon nanomaterials, and bioassay systems. It is difficult to obtain reproducible and reliable Raman spectroscopy data, which is one of the main bottlenecks in SERS bioassay, resulting in the deficiency of clinical and standardized applications. Therefore, there are still many efforts to be done to control the potential factors: (1) It is necessary to prepare a SERS substrate with high uniformity, high enhancement, clean, or/and to properly modify its surface; (2) the suitable Raman reporters and tags are selected to improve the biocompatibility and avoid unspecific adsorption, which can trap analytes in the “hot spots”; (3) biological samples are retained in their native state during the storage and pretreatment procedure to avoid irreversible degradation; (4) appropriate SERS detection conditions are performed, such as excitation wavelength, exposure time, and laser power, to obtain real detection information; (5) accurate biomolecule database are established for spectral assignments. By further improving these challenges, we anticipate the more widespread application of SERS-based assay in biomedicine and bioanalysis fields.

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