功能化GNPs在流行病快速检测和实体医疗中的应用

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摘要 金纳米粒子(gold nanoparticles, GNPs)与不同分子偶联, 不仅可优化其存储条件, 还可起放大信号的功能。GNPs是种理想的偶联体, 其生物相容性、低毒性、稳定性和表面功能化等特性使其应用具有极强的可塑性。功能化的GNPs为构建体外诊断产品提供了载具基础, 在流行病肆虐前期, 成熟完善的体外检测方法可提供快速实用和操作便捷的医疗检测。该文就功能化GNPs检测在流行病及医疗诊断中的应用进展进行综述, 以期为体外检测和可视化检测等提供借鉴。

关键词 功能化GNPs; 体外诊断产品; 比色型检测; 流行病

Application of Functionalized GNPs in Rapid Epidemic Detection and Physical Medicine

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Abstract GNPs (gold nanoparticles) coupled with different molecules can not only optimize their storage conditions, but also play a role in amplifying signals. GNPs are excellent conjugates with a very flexible applicability due to their biocompatibility, low toxicity, stability and surface functionalization. The functionalized GNPs provide a vehicle basis for the construction of *in vitro* diagnostic products. A sophisticated and flawless *in vitro* diagnostic can offer a quick, useful, and easy medical detection system in the early stages of the pandemic. In order to serve a reference for both visual and *in vitro* detection, this article examines the development of functional GNPs detection in epidemiology and medical diagnosis.

Keywords functionalized GNPs; *in vitro* diagnostic products; colorimetric pattern detection; epidemic disease

近年来人类受流行病胁迫的形势愈加严峻, 新型冠状病毒感染是极具代表性的流行病[1]。流行病学诊断可分为两类: 临床诊断和体外诊断。临床诊断是通过问诊采集病史, 系统了解症状, 并通过体格检查和实验室检测综合分析得出结果。体外诊断是通过检测人体外样本判断是否感染病原体的诊断方法, 体外诊断可在短时间内获得诊断结果。在SARS-CoV-2的全球大流行中, 医院和实验室采用逆转录聚合酶链反应(RT-PCR)的结果作为病原体诊断的金标准[2], 检测需要专门的运输途径、专业的人员[3]及昂贵的分析仪器, 其检测存在成本高、耗时长、操作复杂等无法满足定点监测的问题。

基于功能化GNPs的体外诊断产品[4]对于疾病流行初期的筛查和医疗检测体系的建立都具有实际意义, 其优势在于可提供即时便捷检测、减少样品运输和处理所带来的风险和成本以及缩短医疗诊断的时间。GNPs在纸质生物传感器上可通过特有的机制识别病原体, 从而放大检测信号, 功能化GNPs还可避免生物分子因偶联后活性变化而失效的问题。现今功能化GNPs最普遍产品之一胶体金免疫层析试纸条, 其作为功能化比色型GNPs检测疾病或病毒的实体化载具, 操作简便和应用范围广是其最大优势。本文介绍了基于功能化GNPs检测技术的应用, 重点论述了功能化GNPs比色型检测技术的研究进展。

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1 GNPs的结构与特性

GNPs具有较大的比表面积、生物相容性、医学成像特性、低毒性、药物传递[5]和高反应性等特点，其生物相容性[6]、低毒性和可调稳定性等特性使其在生物传感器[7]、生物成像、医学体外诊断[8]和治疗等方面已成功应用。

功能化GNPs的生物相容性为小分子分析物[9]、抗体[10]、蛋白质[11]、DNA[12]/RNA[13]等提供偶联的途径(表1和图1)。GNPs和配体之间偶联的强弱取决于配体的种类和偶联方法，GNPs表面覆盖一层保护剂分子，可利用其分子与特殊的试剂作用，实现GNPs功能化，如表面活性剂包裹的GNPs可一步盐老化屏蔽电荷排斥以偶联配体；被抗坏血酸包被的GNPs可与氨基发生非共价的静电结合。GNPs偶联配体可稳定GNPs在水或有机溶剂中的形态，实现其功能化，并维持其稳定性，防止聚合沉降。功能化GNPs与配体偶联后，使配体稳定性提高，可在后续应用过程中一定程度上避免非特异性反应的出现。GNPs因其独特性可通过功能化增加纳米结构表面积从而改善电子传递过程，提高检测灵敏度和降低检测限[14]，GNPs功能化过程中金硫键的形成也可使配体更稳固地进行偶联，从而提高其特异性[15]。GNPs易与单核苷酸链或抗体偶联，可被用于检测目的分子，部分功能化GNPs配体已被应用于加速核酸扩增反应方面[16]，可作为诊断疾病的手段。

MIRKIN等[21]是GNPs的比色检测的先驱，最先构建了DNA探针的GNPs结构用于特异性基因序列检测。在应用型GNPs传感检测方面，MIRANDA用荧光团和GNPs非共价偶联物的“化学鼻”方法，制作高灵敏型传感器，其复合物营造“锁钥结构”的特定识别模式，利用一系列可选择受体来识别待分析物，GNPs聚集后产生的颜色变化结果可被用于生物分析物/待检测物的定性分析。功能化比色型GNPs传感器是种具有前瞻性的诊断设备，尤其是在资源有限和医疗手段缺乏的地区[22]。在新型冠状病毒暴发期间，GNPs通过与抗体偶联实现功能化，抗体通过抗原–抗体反应检测抗原，其快检化和便捷性所带来的便利表明功能化GNPs所代表的体外检测对于流行病肆虐前的诊断是可行和必要的。

2 基于功能化GNPs的检测应用

2.1 功能化GNPs比色型检测技术的应用

因GNPs具有独特的距离相关的光学特性(图2)，基于GNPs的比色法检测结果可采用肉眼观察方式进行区分[23]。基于功能化GNPs的比色型检测技术是种受到外部刺激时改变GNPs颜色的检测手段，其机理是非交联式团聚，即GNPs在引入高强度离子后改变表面电荷分布，从而出现团聚现象。其简易性和实用性[24]使其得到广泛应用，尤其是直观比色型检测技术可直接应用于体外诊断，因其可产生可见的信号且无需紫外检测或复杂的检测设备，并可短时间检测多种疾病(表2)。

胶体金免疫层析法是以功能化GNPs的可视化的比色结果来达到检测目的分子的方法，其制作简易

表1 GNPs与不同分子偶联的机制

Table 1 The mechanism of GNPs coupling with different molecules

偶联分子 Coupled molecule	偶联方法 Coupling method	偶联机制 Coupling mechanism	特性 Peculiarity
DNA	The salt-aging method; the freezing method; the low pH method; the rapid dehydration method in butanol	Diminish the repulsion of charges electrostatic adsorption	Protect GNPs against repulsion by charges; enabled the hybridization of DNA; improve signal sensitivity [17]
Dopamine	Hydrogen peroxide method	Modify the surface charge of GNPs to lessen charge repulsion electrostatic adsorption and physical absorption	It can be adjusted to the actual detection and can increase sensitivity and specificity [18]
RNA	The salt-aging method	The sulfhydryl group-modified oligonucleotides can stably bind to the surface of GNPs	Sandwich hybridization improves its specificity [13]
Glycogen	*In vivo* junction	Hydroxyl groups bind GNPs together	The plasma band changes as a result of changes in particle distance [19]
Protein	Chemical coupling	Remote electrostatic action, GNPs and cysteine create gold-sulfur linkages	Increased sensitivity and reduced detection limits [20]

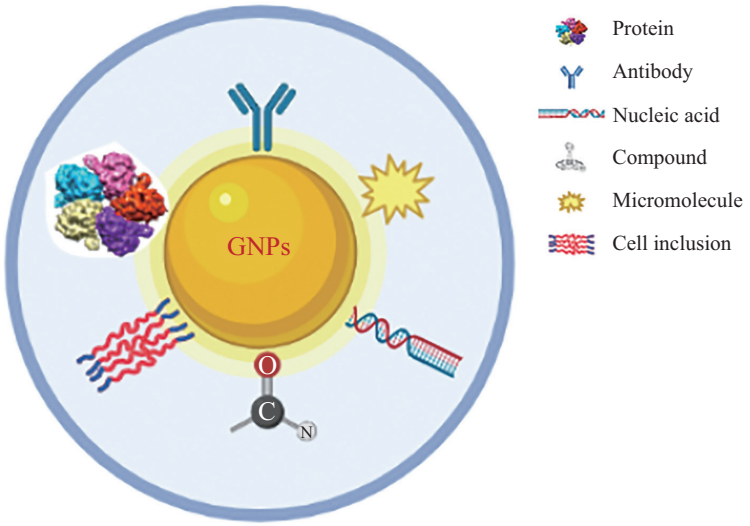

图1 GNPs与不同分子偶联的示意图

Fig.1 Diagram of GNPs coupling with different molecules

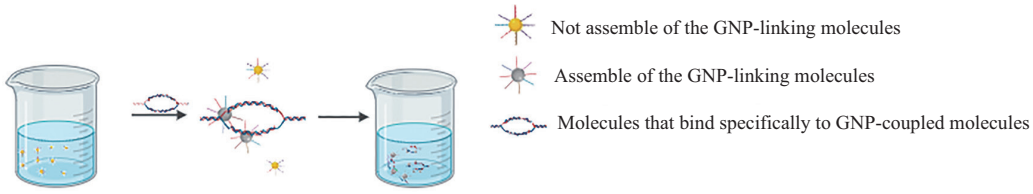

图2 GNPs的距离依赖性的光学特性示意图

Fig.2 Schematic diagram of distance-dependent optical properties of GNPs

表2 功能化GNPs比色型检测在流行病检测中的应用

Table 2 Functional colorimetric GNPs detection is used in epidemic detection

联合策略 Joint strategy	检测时长 Detection duration	结果呈现 Presenting the result	检测限 Limit of detection
Development of colorimetric sensors based on gold nanoparticles for SARS-CoV-2 *RdRp, E* and *S* genes detection [25]	2.5 h	Visual settlement	1.62×10^{-12}-1.62×10^{-11} ng
A novel dengue virus detection method that couples DNAzyme and gold nanoparticle approaches [26]	Within 15 min	GNPs gathers signals	10 TCID50
Side-flow immunization strip (ImmuView *Streptococcus pneumoniae* and *Lactobacillus pneumophilus* tests; BinaxNOW *Legionella* antigen card) [27]	Within 15 min	Visual strip	99.4% (specificity)
Colorimetric test for fast detection of SARS-CoV-2 in nasal and throat swabs [28]	Within 15 min	Universal microplate reader	90% (sensitivity and specificity)
Introduction of multilayered dual-signal nanotags into acolorimetric-fluorescent coenhanced Immunochromatographic assay for ultrasensitive and flexible monitoring of SARS-CoV-2 [29]	Within 30 min	Double visual banding	50 pg/mL and 2.2 pg/mL

1 TCID50病毒包含约500个病毒拷贝。TCID50: 半数组织培养感染剂量。

1 TCID50 virus contains about 500 copies of the virus. TCID50: median tissue culture infective dose.

且可较长时间保留功能化GNPs的大部分活性。胶体金免疫层析法可分为夹心法和竞争法。胶体金免疫层析法试纸条由五部分组成: 样品垫、滤血膜、结合垫、硝化纤维素膜和吸收垫, 它们依次堆叠在聚氯乙烯底板上。胶体金免疫层析检测试纸条的检测信号是样品中的抗原或抗体在试纸条上层析的结果, 通过抗原–抗体反应将吸附在金标垫孔径中的GNPs-抗体或GNPs-抗原偶联物带动, 最后使其停留在硝化纤维

素(NC)膜相对应划线处, 划线处包埋有可与待测物另一结合位点结合的抗体或抗原, 其机制类似于“双锁联动式结构”, 试纸条的检测信号以可视化的结果呈现(图3)。

2.2 其他基于功能化GNPs的检测的应用

GNPs具有的较大的比表面积等特性, 有助于增加其表面共轭受体的数量来识别检测对象, 提高检测性能[30]。GNPs在与生物分子等结合后, 产生特定结构, 表面电子受到影响, 从而产生表面增强拉曼散射(surface enhanced Raman scattering, SERS)效应[31], GNPs分子结构的任何微小变化都会呈现在拉曼散射光谱中, 在纳米层面上产生增强电磁场效应, 从而放大检测物信号。功能化GNPs还可与其他纳米颗粒、小分子标记物、电化学芯片[32]和其他诊断手段联合应用于疾病筛查、病毒检测、细菌感染预防[33]、食品监控[34]等领域(表3)。

基于功能化GNPs的生物传感器还可代替RT-PCR检测病毒[40], 因其传感器机制可检测病毒侵入早期阶段的RNA, 且无需核酸扩增和逆转录。凭借功能化GNPs的检测体系可建立高效、低成本、便携式的即时检测机制, 实现生物的条形码检测, 在公共场合实现通行检测, 将其日常检测常态化和多领域化。

以功能化GNPs为基础的监察型生物传感器, 可实现多种技术联合使用。GNPs的生物相容性可兼并多种快速检测技术, BI等[41]结合CRISPR/Cas12a核酸识别系统, 开发了一种被称为手持纳米离心装置辅助CRISPR/Cas12a(Hand-CRISPR)的比色平台。YANG等[42]建立一种采用多重环介导等温扩增联合GNPs的快速检测结核分支杆菌的侧流生物传感器, 多重联合型检测机制可提高特异性反应率, 该方法扩展了便捷化医疗检测体系。以GNPs适配体为基

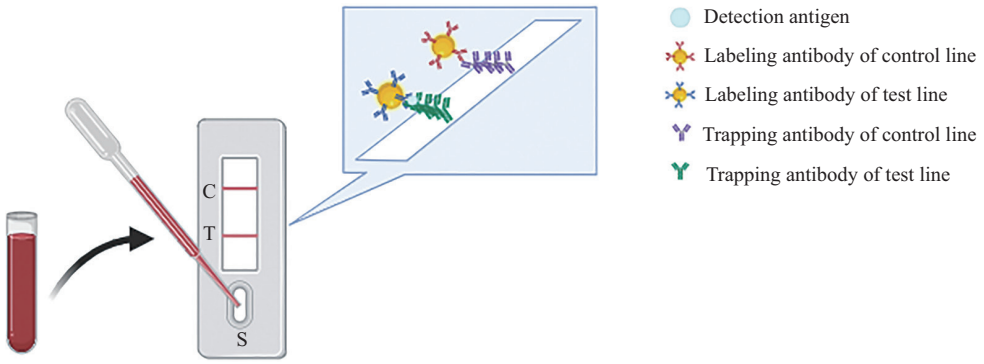

图3 胶体金免疫层析法示意图

Fig.3 Colloidal gold immunochromatography

表3 联合功能化GNPs检测的应用

Table 3 Application of joint functional GNPs detection

联合策略 Joint strategy	应用领域 Application area	结果呈现 Presenting the result	检测限 Limit of detection
Label-free detection of SARS-CoV-2 spike S1 antigen triggered by electroactive gold nanoparticles on antibody coated FTO (fluorine-doped tin oxide) electrode [35]	SARS-CoV-2 spike S1 antigen dectection	Voltammetry	6.3×10^{-16} mol/L (standard buffer solution)/1.2×10^{-14} mol/L (tagged saliva sample)
Bacterial growth monitoring and antigenic TB detection technology (magnetic nano-Au@Pd NZ sandwich ELISA) [36]	Serological identification of tuberculosis in the lungs	Universal microplate reader	5.0×10^{-13}-5.0×10^{-4} g/mL
Using GNPs coated with bifunctional polyvinylimide, four tumor indicators were measured concurrently [37]	Detection of tumor biomarkers	Electrochemical signal	1.7, 1.6, 0.9 and 1.0 fg/mL
A novel and simple cell-based electrochemical impedance biosensor for evaluating the combined toxicity of DON (deoxynivalenol) and ZEN (zealenone) [38]	Detection of mycotoxin toxicity	Electrochemical impedance spectroscopy	0.03 and 0.05 μg/mL
Electrochemically renewable SERS sensor: a new platform for the detection of metabolites involved in peroxide production [39]	Detection of hydrogen peroxide metabolites	SERS signal	1.59×10^{-7} mol/L and 8.57×10^{-8} mol/L

础的横向流动检测条采用GNPs互补DNA(cDNA)纳米探针[43]之间的靶标诱导解离竞争的结合原理，在小分子检测上具有广阔的实用化前景。功能化GNPs代表体外诊断产品在执行其检测诊断功能的基础上，还可结合人工智能和计算机处理实现信息化快速检测。

3 基于功能化GNPs的检测的影响因素和改良

3.1 影响功能化GNPs检测信号的因素

3.1.1 pH值 偶联配体主要通过静电吸附到GNPs，减少电荷排斥是其偶联关键。免疫球蛋白G抗体很容易偶联到抗坏血酸覆盖的GNPs上，通过静电作用与半胱氨酸的金硫键进行偶联，但蛋白质表面带负电的碱性氨基酸会和GNPs的负电荷产生排斥现象，因此可通过调节pH值使GNPs从质子化转变为携带正电荷的状态[20]。MOULAHOUM[44]发现在纸基生物传感器上pH和NaCl会影响检测的特异性；SEELE等[45]通过比较14 nm GNPs在不同pH下的稳定性从而确定检测探针的偶联条件；PANIKAR等[46]基于SERS的GNPs纳米探针在高pH范围内较稳定，使其信号在复杂介质中更具可信度。

3.1.2 配体类型 不同配体偶联GNPs实现功能化的途径不同，RUIZ等[20]发现部分蛋白配体吸附亲和度不受pH影响，蛋白在GNPs表面接触的局部区域可能出现重排或展开的过程，上述过程用于促进半胱氨酸残基与GNPs的化学吸附。配体所用的偶联时间和样品浓度都需达到最佳适配条件，未达到最佳条件可能会在偶联过程中使GNPs出现非交联团聚或功能化失败现象，其偶联现象可通过肉眼观察或者吸光度值检测反映。部分配体会在表面进行修饰以提高检测信号，如GNPs以金硫键的形式相连使寡核苷酸探针牢固地连接在GNPs的表面；寡核苷酸探针修饰可在5′或者3′端加一段腺嘌呤碱基(polyA)然后再进行巯基化，polyA片段不仅可以将寡核苷酸探针固定在AuNPs表面，还可以减少其上的其他DNA碱基与GNPs之间的非特异性位点结合，从而提高GNPs探针对靶序列的识别能力。WANG等[47]将四个具有互补对的功能性寡核苷酸组装成DNA-GNNA，而DNA-GNNA可偶联更多的GNPs以提高检测灵敏度。

3.1.3 稳定剂和GNPs的形态 抗坏血酸是合成GNPs的稳定剂，抗坏血酸的室温反应与热还原反应相似，偶联过程中紫外光下的氧化反应可能会造成小批量的功能化GNPs检测结果出现误差；抗坏血酸pH值变化也可能导致检测信号不稳定，稳定剂状态[48]在制备GNPs的过程中可直接影响后期检测信号，抗坏血酸包裹的GNPs在高盐或者电荷差值小等条件下会不稳定，这限制了其应用；为避免GNPs自我发生聚集现象，可使用稳定性比抗坏血酸更强的包覆稳定剂(如十二烷基苯磺酸钠、Tween-20、聚乙二醇和非离子氟表面活性剂等)来稳定GNPs的形态。

ZHAO等[49]以超分子环糊精作为稳定剂和还原剂，以温和的方法合成了环糊精修饰的GNPs，超分子环糊精作为催化剂可提高免疫传感器的稳定性。不同粒径GNPs与生物分子偶联时可能会出现差异性，即不同粒径的GNPs偶联同种分子会出现截然不同的结果。胶体金免疫试纸条制备过程会出现颜色灰暗且分布不均、GNPs流动堵塞等问题，这些问题可能与功能化GNPs-生物分子偶联程度、两者发生聚合、试纸条干燥温度过高和保护剂等有关。

3.2 基于功能化GNPs的检测应用的改良

3.2.1 GNPs稳定性和检测改良 功能化GNPs可提供简便、低成本、即时的快速诊断方法，但GNPs有自我形成聚集体的倾向，导致了GNPs表面互作位点的减少，并降低了每个原子的比表面积，增强了GNPs间的强范德华力[50]，导致了GNPs的催化活性损失、电容率和化学稳定性的降低。减少功能化GNPs的聚沉、维持其稳定性是GNPs与生物分子结合并发挥其功能化的关键。大多数功能化GNPs会形成网络状复合物，短时高速离心是打开网络状复合物相对有效的方法[51]，从而提高功能化GNPs特异性。花青素可作为螯合剂帮助GNPs在溶液中产生复杂的结构，并可作为还原剂减少在紫外线照射下GNPs的用量，其耐盐性还可帮助合成稳定的GNPs，并防止GNPs的聚集。提高GNPs在溶剂中的分散性可避免其聚沉，部分表面活性剂如Tween-20可使GNPs保持球形，避免GNPs纳米棒的形成[52]，还可保护柠檬酸离子不受干扰，使GNPs免受高离子强度条件的影响。CAVALERA等[53]观察到在进行功能化GNPs-抗体检测过程中溶液中的抗原在遇到检测抗体前可能已经饱和，抗原上表面位点被捕获抗体占据，该现象被称为“抗原HOOK效应”，可通过降低检测抗体浓度来提高检测的敏感性。CHUNG等[54]开发出基于功能化GNPs的立体调节式的“Signal-ON”

表4 核酸适配体与mAb对比

Table 4 Nucleic acid aptamer compared with mAb

类别 Categories	核酸适配体 Nucleic acid aptamer	单克隆抗体 mAb
Screening mode	Artificial *in vitro* screening	Innate immunity
Affinity	High	High
Tissue penetration	Fast and easily internalized by cells	Low penetration and internalization efficiency
Thermal/chemical stability	High	Instability
Ease of modifying	Easily modified or coupled functional groups	Modification difficulty
Mode of production	Solid phase synthesis technology	Mammalian cell system
Batch variance	Little variation between batches and the product is highly homogenous	Large variation between batches
Time	Several hours	Days to months
Cost	Low	High
Target/receptor	Broad range; capable of recognizing viruses, bacteria, cells, tissues, ions, peptides, tiny molecular compounds and nucleic acids	Identification of immunogenic targets

小分子竞争分析机制，其优点是可进一步提高小分子分析灵敏度和分析速度。

3.2.2 功能化GNPs偶联配体改良 功能化比色型GNPs检测手段基于颜色变化得出检测结果，无需额外分析仪器来读取结果[55]。例如在新型冠状病毒感染的抗原检测中，患者可用肉眼观察结果并进行自我诊断。功能化GNPs的优良程度可直接决定体外诊断产品质量，其中胶体金免疫层析法需要稳定效果的批量单克隆抗体原料[56](monoclonal antibody, mAb)，但不同批次的mAb的差异性和大小尺寸[57]都可能影响试纸条结果。由于mAb的稳定性较差，何晓婷等[58]尝试用纳米抗体新型原料代替mAb，实验后期验证试纸条同样具有灵敏和快捷的特点；和病原体特异性结合的细胞表面受体[59]也可作为即时检测点，表面受体的恰当选择可缩短筛选mAb的时间，加快病原体快检技术的研发；具有mAb相同效果核酸适配体[60]也逐渐被用于病原体检测[61-62]上，适配体是ssDNA或ssRNA链通过折叠形成3D构象的产物，其与检测分子的特异性结合原理与mAb相似，但适配体更稳定更便宜(表4)。

4 展望

本文介绍了GNPs的独特特性及其在开发特异和敏感的生物传感器方面的广泛应用。最近的研究表明，功能化GNPs的特异性和敏感性是医学诊断和预后处理的基本要求，其本身的操作简捷、应用灵活等特点使其在实际应用中具有良好的前景。研发检测快速的基于功能化GNPs的体外诊断体系不仅可以缓解公共医疗体系的运行压力，也可减少流行病初期人员流动过快所带来的传染压力。凭借GNPs的生物相容性、距离依赖性的光学特性、低毒性等生物学特点，可将GNPs代表的检测体系嵌入社区的医疗中，使其成为一种类似于“二维码”的普遍检测方法。虽然GNPs具有低毒性特性，但在全民覆盖化的预先初筛检测机制下，应思考如何回收处理已使用的携带病毒的体外检测产品，并在生物安全和环保的情况下进行处理。

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