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催化反应增强痕量肿瘤标志物电化学检测性能研究进展*

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摘要：肿瘤标志物的灵敏检测是癌症早期诊断、治疗策略的选择和预后监测的重要保障手段之一。肿瘤标志物在血清中含量低，通常以痕量水平存在，甚至极个别的肿瘤标志物在每毫升血清中仅为飞克或皮克量级，其质量浓度的细微变化对于肿瘤的发生、发展和治疗效果具有一定的指示作用。如何实现痕量水平下肿瘤标志物微小质量浓度变化的灵敏检测具有重要的临床意义，并受到了人们的广泛关注。电化学传感器因具有高灵敏度、快速响应、易于操作及仪器小型化等特点，所以电化学免疫传感器检测痕量水平下肿瘤标志物微小质量浓度变化的研究，受到了人们的普遍关注并取得了巨大进展。本文主要综述了催化反应增强肿瘤标志物电化学检测性能方面取得的研究进展，特别关注了生物酶、纳米酶的应用以及电化学金属催化剂的使用，同时对有待解决的问题进行了展望。

关键词：肿瘤标志物；电化学传感器；催化反应；生物酶；纳米酶；电化学金属催化剂
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Progress in enhancing the electrochemical detection performance of trace tumor markers based on catalytic reactions*

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Abstract：Sensitive detection of tumor markers is one of the most important safeguards for early diagnosis of cancer，selection of therapeutic strategies and prognostic monitoring. The content of tumor markers in serum is low，usually existing at trace level，and even very few other tumor markers are only on the order of femtograms or picograms per millilitre of serum，and subtle changes in their concentrations are indicative of tumor occurrence，progression and therapeutic efficacy. It is of great clinical significance and has received extensive attention how to achieve sensitive detection of small concentration changes of tumor markers at trace level. Because of its high sensitivity，fast response，easy operation，and miniaturization of the instrument，electrochemical immunosensors have received much attention and made great progress in the study of detecting small concentration changes of tumor markers at trace level. This paper reviews the progress made in catalytic reactions to enhance the

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performance of electrochemical detection of tumor markers, with special focus on the application of bioenzymes, nano-enzymes, and the use of electrochemical metal catalysts, and also provides an outlook on the problems to be solved.

Keywords: tumor marker; electrochemical detection; catalytic reactions; bioenzymes; nanoenzymes; electrochemical metal catalysts

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0 引 言

癌症是威胁人类健康的重大疾病和头号杀手,近年来肿瘤发病率呈上升趋势[1-5]。2022年1月,国家癌症中心发布了最新一期的全国癌症统计数据。报告显示,2022年全国恶性肿瘤发病约392.9万人,较2021年增加了12.5万,增长率为3.2%:这意味着,平均每天超过1万人被确诊为癌症,每分钟约有7人被确诊为癌症。肿瘤恶化并被发现时通常已到晚期,限制了治疗的效果[6-8]。肿瘤的早发现早诊断对肿瘤的预防和治疗至关重要。

肿瘤出现的早期,在其周围组织细胞一般会分泌少量的特异性物质,例如特异性蛋白质、核糖核酸(ribonucleic acid,RNA)、脱氧核糖核酸(deoxyribonucleic acid,DNA)等,人们将其称之为肿瘤标志物。肿瘤标志物在血清中含量低,通常以痕量水平存在,甚至极个别的肿瘤标志物在每毫升血清中仅为皮克或飞克量级。肿瘤标志物质量浓度的微小变化对于肿瘤的发生、发展和预后监测具有重要的指示作用,如何实现痕量水平下肿瘤标志物微小质量浓度变化的灵敏检测具有重要的临床意义,并受到了人们的广泛关注。由于肿瘤标志物的含量极低,因此给检测带来了一系列挑战[9-11]。肿瘤标志物临床检测的常用方法中:酶联免疫分析(enzyme-linked immunosorbent assay,ELISA)存在样本的预处理复杂、分析时间长以及检测灵敏度和特异性相对较低等问题;放射性免疫检测存在放射性污染的可能性;电化学传感器因具有灵敏、快速、操作简便和设备小型化等特点,因此在肿瘤标志物检测中广受关注[12-16]。

为了提高电化学传感器的灵敏度,满足在痕量水平下检测肿瘤标志物的质量浓度变化的需求,催化反应作为提高电化学传感器检测灵敏度的有效手段被广泛应用,并取得了巨大进展[17-20]。本综述主要总结催化反应提高肿瘤标志物电化学检测灵敏度方面的研究进展,并对存在的问题进行展望。

1 催化反应在电化学免疫传感器中的应用

电化学免疫传感器构建过程中,生物识别元件(适配体、抗体或多肽等)的修饰通常会带来传感界面阻抗增大,严重减弱电化学信号,因此研究者们提出各种策略来增强电化学信号,其中引入催化放大策略是较为常用的手段[21-22]。针对电化学免疫传感器产生信号的方式不同,引入不同的催化反应,提出相应的增强方案[23-25]。

电化学免疫传感器主要分为信号增强型和降低型[26-28]。信号增强型电化学传感器主要是利用催化反应或由其组成的级联反应引起电化学活性物质的产生或循环利用,实现电化学信号的增强[29-30]。例如,Feng等[31]设计了一种负载有碱性磷酸酶(alkaline phosphatase,ALP)的新型免疫探针用于检测糖类蛋白抗原72-4(carbohydrate antigen 72-4,CA72-4),沸石咪唑酯骨架-8(zeolitic imidazolate framework-8,ZIF-8)吸附信号分子硫堇(Thionine,Thi),并通过静电作用负载胎牛血清蛋白(bovine serum albumin,BSA),借助化学共价偶联,固定抗体-2(antibody-2,Ab2)和ALP,得到ZIF-8@Thi@ALP@Ab2复合免疫探针;玻碳电极(glassy carbon electrode,GCE)表面修饰多壁碳纳米管-氧化石墨烯(multi-walled carbon nanotube-graphene oxide,MWCNT-GO)复合材料,原位电沉积金纳米颗粒(Au nanoparticles,AuNPs)用于固定抗体-1(antibody-1,Ab1)并用BSA封闭,得到GCE@MWCNT-GO@AuNPs@Ab1传感界面。在抗原存在的条件下,电极表面捕获复合免疫探针,形成GCE@MWCNT-GO@AuNPs@Ab1/CA72-4/Ab2@ALP@Thi@ZIF-8免疫“三明治”结构。在传感界面滴加含有对氨基磷酸酯(para-aminophosphate,p-APP)和Ag^{+}的溶液后,立即触发级联反应,即p-APP在ALP的催化下水解为对氨基酚(para-aminophenol,p-AP),Ag^{+}将p-AP氧化为醌(quinone,QI),同时被还原为银纳米颗粒(Ag nanoparticles,

AgNPs)沉积在电极表面。由于AgNPs具有优异的导电性,Thi氧化产生的电化学信号被显著放大,进而实现了对肿瘤标志物CA72-4的超灵敏检测。

信号降低型电化学传感器主要是基于催化反应或级联反应使得电化学活性物质降解或者在电极表面生成导电性差的物质[32-33],引发电化学信号的降低。例如,Yao等[34]提出了一种基于酶响应的透明质酸水凝胶级联控释体系,用于肿瘤标志物的灵敏检测,该工作涉及2个过程:即免疫识别在试管中进行,信号测定在电极表面进行。在磁珠(magnetic bead,mb)表面,通过化学偶联分别修饰葡萄糖氧化酶(glucose oxidase,GOx)和抗体Ab1,得到mb@GOx@Ab1免疫磁珠(immunomagnetic beads,Imb);以包裹透明质酸酶(HAase)的ZIF-8为载体,在其表面原位制备AuNPs并固定抗体Ab2,得到HAase@ZIF-8@Au@Ab2复合免疫探针。在电极表面,透明质酸聚合生成透明质酸水凝胶并负载亚甲基蓝(methylene blue,MB),作为电化学检测界面。免疫识别在试管中进行,当抗原存在时,通过免疫识别形成HAase@ZIF-8@Au@Ab2/抗原/Imb"三明治"结构;外加葡萄糖溶液,被免疫磁珠中的GOx催化产生H^+,在酸性条件下ZIF-8裂解,释放出HAase。将含有HAase的上清液滴在电极表面,HAase催化透明质酸分解,破坏透明质酸水凝胶,使MB分子泄露,经过水洗去除MB分子,使得电化学信号降低。

催化反应被广泛应用于各种电化学免疫传感器,显著提高了传感器的检测性能。根据催化反应的催化剂类型,重点关注生物酶催化、纳米酶催化以及电化学金属催化3种催化形式,以下介绍催化反应在肿瘤标志物电化学检测中的应用。

2 生物酶催化

生物酶是由活细胞产生的具有催化作用的物质,大部分为蛋白质,也有部分DNA和RNA,其能够与特定底物相互作用,并催化底物发生反应。生物酶的催化作用被广泛用于电化学传感器[35]。酶催化底物反应,通常会发生氧化还原反应,并伴随电化学信号的变化[36-38]。生物酶催化底物表现出高催化效率,这种高效率的催化反应使得电化学传感器能够产生出数百种生物酶,如GOx、ALP、过氧化物酶(horseradish peroxidase,HRP)、DNA酶和乳酸脱氢酶(lactate dehydrogenase,LDH)等,这为生物酶在肿瘤标志物检测中的应用提供了多样选择[39-44]。

级联反应是一个复杂的反应过程,即前一个反应产物引发下一个反应或前一个反应产物作为下一个反应底物的反应过程。级联反应可以通过以下变化实现电化学信号的增强或降低:级联反应产物可以降解信号物质,或者生成导电性差的物质阻碍电子传输,显著降低电化学信号;级联反应也可通过信号物质的循环生成或者信号物质的响应性释放,显著增强电化学信号。通过这种电化学信号的显著变化进而提高电化学免疫传感器的灵敏度。

Li等[45]利用GOx与血红素(hemin,H)的级联催化反应,设计了信号降低型电化学免疫传感器,用于灵敏检测肿瘤标志物糖类蛋白抗原199(carbohydrate antigen 199,CA199),制备过程如图1所示,$CaCO_3$纳米颗粒表面制备AuNPs并固定GOx和Ab2,得到$CaCO_3$@$AuNP_S$@GOx/Ab2复合免疫探针;制备还原氧化石墨烯-H-$AuNP_S$(reduced graphene oxide-H-$AuNP_S$,rGO-H-$AuNP_S$)、聚亚甲基蓝-$AuNP_S$(polymethylene blue-$AuNP_S$,pMB-AuNPs)和海藻酸钠-铂纳米颗粒(sodium alginate-Pt nanoparticles,SA-PtNPs)混合物,将该混合物在电极表面一步形成复合凝胶,作为电化学传感基底,再共价连接抗体Ab1,得到免疫传感界面。在抗原存在时,通过免疫识别作用,免疫传感界面捕获复合免疫探针,形成免疫"三明治"结构。将电极浸入葡萄糖溶液,复合免疫探针中的GOx催化葡萄糖氧化分解为葡萄糖酸和H_2O_2,H进一步催化H_2O_2产生羟基自由基降解基底上的聚亚甲基蓝,实现电化学信号的显著降低。Zhang等[46]利用酪氨酸酶(tyrosinase,Tyr)和半乳糖苷酶(galactosidase,Gal)的级联催化反应构建了信号增强型电化学免疫传感器检测癌胚抗原(carcinoembryonic antigen,CEA),在多壁碳纳米管(multi-walled carbon nanotubes,MWCNTs)表面将多巴胺原位聚合成聚多巴胺(polydopamine,PDA),再原位制备AuNPs,得到MWCNTs-PDA-AuNPs纳米复合材料,将该复合材料修饰到氧化铟锡电极(indium tin oxides,ITO)表面,共价偶联Ab1并用BSA封闭,得到ITO@MWCNTs-PDA-AuNPs@Ab1免疫传感界面;利用戊二醛化学作为技术手段,制备了Gal/Ab2酶标抗体;在CEA存在时,通过免疫识别,在传感界面形成免疫ITO@MWCNTs/PDA/AuNPs@Ab1@CEA@Ab2/Gal"三明治"结构;将电极浸入到含有β-D-半乳糖和Tyr的混合

溶液中,Gal催化β-D-半乳糖分解产生苯酚,Tyr催化苯酚转化为邻苯二酚并转化为1,2-苯醌,即1,2-苯醌被电化学还原为邻苯二酚,邻苯二酚又被Tyr催化转化为1,2-苯醌。通过信号物质的循环产生,电化学信号得到了显著增强,检测灵敏度大幅提高。

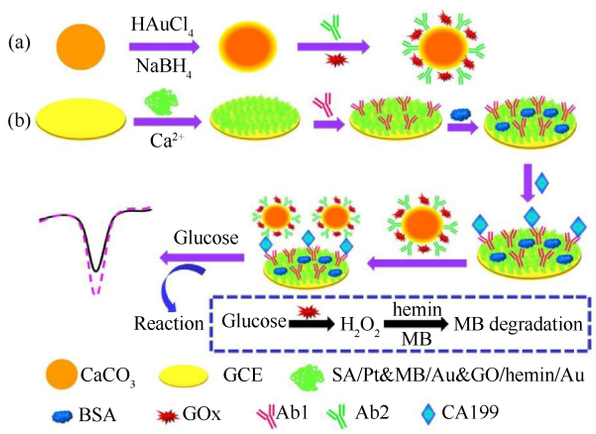


图1　电化学免疫传感器的制备[45]

(a)复合免疫探针的制备;(b)糖类抗原蛋白199(CA199)信号放大过程及检测原理

3　纳米酶催化

纳米酶是一类具有催化活性的纳米材料,具有类生物酶的催化功能[47-49]。纳米酶通常比生物酶的稳定性和环境耐受性更强[50-55],能够在常温下保存,并且酸性或碱性等苛刻环境下仍可保持催化活性。纳米酶易于制备且成本相对较低,具有可控性和可设计性,其催化活性可调控[56-59]。这些特性使得纳米酶在生物传感领域受到了广泛关注。

3.1　金属有机框架(metal organic framework,MOFs)基纳米酶催化增强

MOFs是一类以金属离子或金属团簇为连接点通过配体连接的多孔材料。由于MOFs结构的多孔性、孔道类型的多样性和纳米孔微环境的特殊性,使其催化活性位点具有高密度性、分布的均匀性,因此MOFs的类酶活性可通过孔结构和组成进行调控[60]。MOFs本身的过渡金属节点具有低配位的特点,具有分子或原子级别的催化中心,可以充当高效的仿生催化。常见的过渡金属节点,如Fe、Cu、Co、Ag、Zr和Zn,与羧基、胺基、磷酸基和磺酸基等有机配体的结合,可产生丰富的活性中心,进而形成优异的催化能力;与其他相同尺寸的纳米颗粒相比,MOFs具备开放的孔结构,可使催化反应进入MOFs的孔道内发生,提高活性中心的使用效率[61-62]。MOFs基纳米酶的种类众多,可以模拟多种酶的活性,如氧化酶、过氧化物酶、过氧化氢酶、超氧化物歧化酶和水解酶等,目前已成功用于催化增强肿瘤标志物电化学传感器检测性能[63-66]。

Zhang等[67]利用铜基MOFs纳米颗粒(Cu-MOFs)的类过氧化物酶催化活性,设计级联反应催化自由基聚合构建了阻抗型电化学免疫传感器,用于糖类蛋白抗原15-3(carbohydrate antigen15-3,CA15-3)的超灵敏检测(图2),通过戊二醛偶联,氨基化的Cu-MOFs表面固定抗体Ab2和GOx,得到Cu-MOFs@GOx@Ab2复合免疫探针;GCE表面电沉积AuNPs,固定抗体Ab1并用BSA封闭,得到GCE@AuNPs@Ab1免疫传感界面;当CA15-3存在时,通过抗体抗原特异识别,传感界面捕获复合免疫探针,形成Cu-MOFs@GOx@Ab2/CA15-3/Ab1@AuNPs@GCE免疫"三明治"结构;将含有葡萄糖、乙酰丙酮(acetylacetone,ACAC)、N-异丙基丙烯酰胺(N-isopropylacrylamide,NIPAM)和2-巯基苯甲酸(2-mercaptobenzoic acid,MBA)的混合溶液滴到传感表面,通过复合免疫探针中的GOx触发级联催化引发自由基聚合,即GOx催化葡萄糖氧化生成H_2O_2,Cu-MOFs具有过氧化物酶活性,催化H_2O_2生成羟基自由基并与ACAC反应生成ACAC自由基,进而引发NIPAM聚合生成聚异丙基丙烯酰胺;该聚合物作为电阻增强剂覆盖在电极表面,使得电流显著降低(电流变化值显著增大),进而实现检测灵敏度的显著提高。

3.2　贵金属颗粒类酶催化增强

贵金属纳米颗粒,如Pd、Pt、Au纳米颗粒等,具有高催化反应活性,是一类重要的纳米酶[68]。纳米尺寸的贵金属颗粒具有高催化反应活性,其主要原因有:(1)表面效应。小尺寸和高比表面积,使得更多的低配位原子暴露出来,活性表面位点增加,因此反应机会更多,催化反应速率增加。(2)尺寸效应。纳米尺寸改变了纳米颗粒的电子结构和晶格结构,影响催化材料与反应物之间的轨道杂化和电荷转移,进而可以有效调控催化活性与选择性。(3)表面增强效应。其表面有较高的表面能级和结构缺陷,有助于吸附反应物,调控化学反应路径,进而提高催化反应速率。通过精准调控贵金属颗粒的尺寸、形状和组成,实现不同的催化效果[69-70]。贵金属颗粒纳米酶被用于肿瘤标志物的电化学检测中并取得了进展。Yin等[71]利用Au-Pd双金属纳米颗粒具

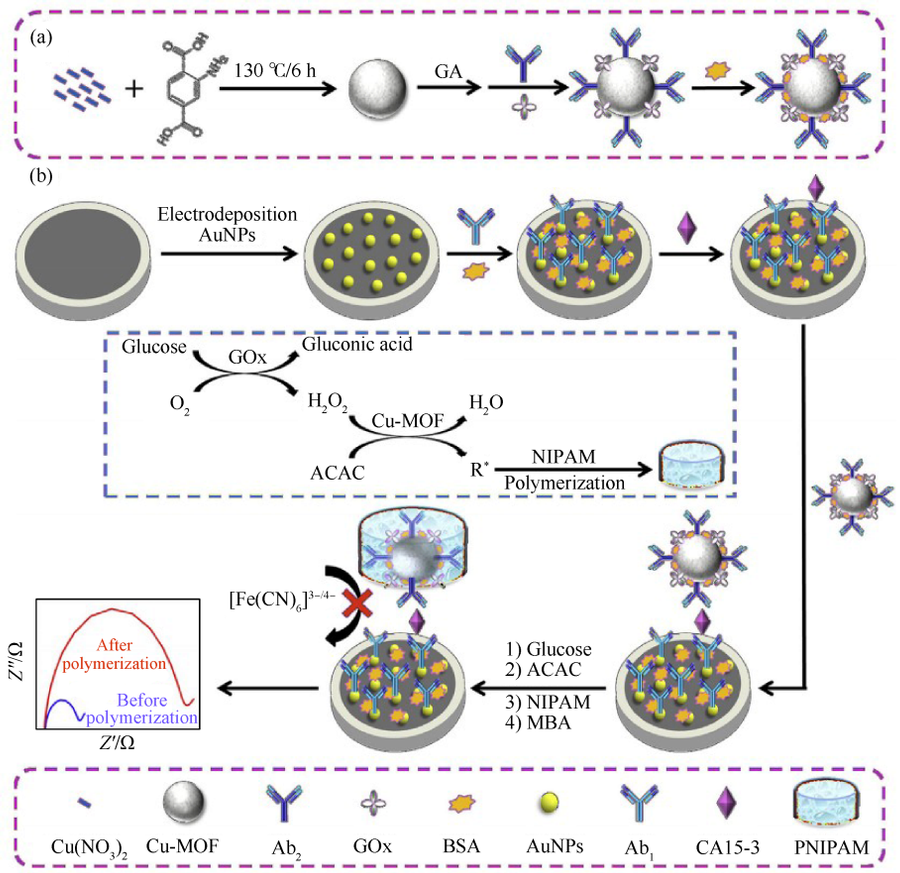


图2　级联催化自由基聚合扩增阻抗免疫传感器[67]

(a)复合免疫探针制备过程;(b)电化学免疫传感界面构建过程

有的类过氧化物酶活性,开发了一种无生物酶参与的非标记电化学免疫传感器检测肿瘤标志物神经元特异性烯醇化酶(neuron-specific enolase,NSE)(图3),通过电极表面一步法制备AuPd-MWCNT复合材料、二茂铁甲醛(Fc-CHO)和壳聚糖组成的复合水凝胶。电极表面修饰抗体Ab1,得到免疫传感界面;当检测液中存在H_2O_2时,AuPd纳米颗粒催化H_2O_2分解,同时将Fc^+还原为Fc,Fc产生电化学信号后,被氧化成Fc^+;Fc/Fc^+的循环变化,使得电子转移过程循环进行,电流显著增强,这为提高电流变化即提高检测灵敏度提供了先决条件;当NSE检测液滴加到电极表面时,通过免疫识别,NSE结合到电极表面,由于NSE导电性差,引起电流信号的降低,实现了NSE的超灵敏检测,检出限达到了0.483 pg/mL。贵金属颗粒类酶催化的引入,显著提升了非标记电化学免疫传感器的检测性能。

4　电化学金属催化

金属催化材料,如泡沫镍、硼化镍、碳化钨、双金属催化剂和金属磷化物,能够显著提高电化学传感器的性能[72-76],其通常具有高催化活性,可加速底物的氧化或还原反应,产生明显的电流响应[77-78]。

电化学传感器通常需要电化学活性物质,如有机染料分子、Pb^{2+}、Cu^{2+}、Ni^{2+}等,提供电化学信号。电化学活性物质通常借助非共价作用,如静电吸附、框架材料包覆、主客体识别等,负载到复合免疫探针或电极表面,这些固定方式可能会导致复合免疫探针或电极表面的电化学活性物质的脱附,造成电化学信号稳定性差和环境污染的问题。因此,迫切需要不依赖电化学活性物质的新电化学信号读出模式。Li等[79]通过测量Ag-Cu双金属催化剂的析氢反应(hydrogen evolution reaction,HER)过电位,设计了一种基于HER的新型读出模式的电化学免疫传感器(图4),用于肿瘤标志物基质金属蛋白酶-3(matrix metalloproteinases-3,MMP-3)的灵敏检测,PDA纳米球表面原位还原制备Ag颗粒并固定抗体Ab2后用BSA封闭,得到PDA@AgNPs@Ab2复合免疫探针;MB表面通过化学共价偶联的方式固定

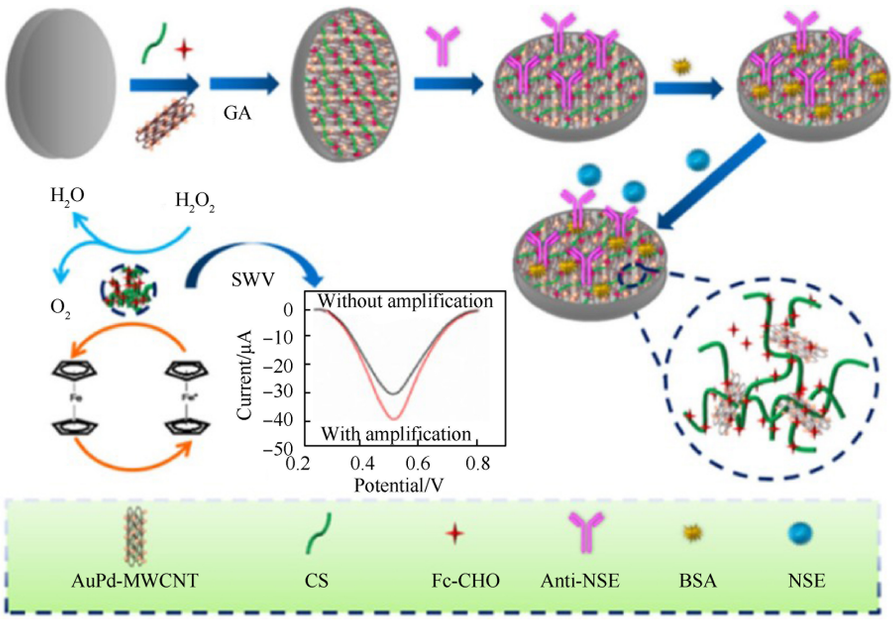


图 3　基于无酶催化扩增的无标记免疫传感器灵敏监测神经元特异性烯醇化酶(NSE)[71]

抗体 Ab1 并用氨基聚乙二醇封闭,得到 MB@Ab1;泡沫镍表面电沉积 Cu 颗粒,得到镀 Cu 的泡沫镍作为电极材料;在试管中进行免疫识别,当抗原存在时,通过抗原与抗体的特异性识别形成 PDA@AgNPs@Ab2/抗原/MB@Ab1 免疫“三明治”结构,外加一定浓度的 H_2O_2 溶液,AgNPs 被消解,得到含 Ag^+ 的上清液;取上清液滴在电极材料表面,Cu 颗粒将 Ag^+ 还原,形成不同比例 Cu 与 Ag 的双金属催化析氢材料,双金属比单金属具有更高的催化活性,因此泡沫 Ni-Cu 表面附着 AgNPs 可以显著降低析氢过电位,进而达到灵敏检测抗原的目的;该方法避免了电化学活性物质的使用,为传感器的设计提供了新思路。

除此之外,Cao 等[80]利用 MWCNTs-磷化钴(MWCNTs-CoP)作为电化学催化材料催化 HER 和

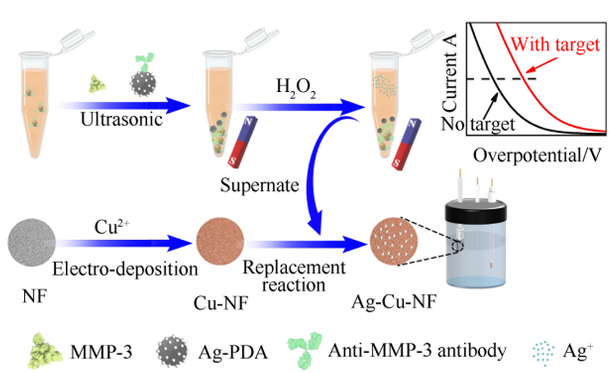


图 4　基于银铜双金属催化析氢的肿瘤标记物电化学免疫传感器示意[79]

析氧反应(oxygen evolution reaction,OER),构建了电化学免疫传感器检测肿瘤标志物 CEA(图 5),MWCNTs-CoP 表面化学偶联 Ab2 并用 BSA 封闭,得到 MWCNTs-CoP@Ab2 复合免疫探针;在 GCE 表面,电沉积 AuNPs 并固定 Ab1,用 BSA 封闭,得到 GCE@AuNPs@Ab1 免疫传感界面;在抗原 CEA 存在的情况下,通过免疫识别,在电极表面形成 MWCNTs-CoP@Ab2/CEA/Ab1@AuNPs@GCE 免疫“三明治”结构;将电极浸入电解液中,分别进行 HER 和 OER 的线性伏安扫描法(linear sweep voltammetry,LSV)测试,MWCNTs-CoP 对 HER 和 OER 均表现出优异的电催化活性,不同浓度 CEA 结

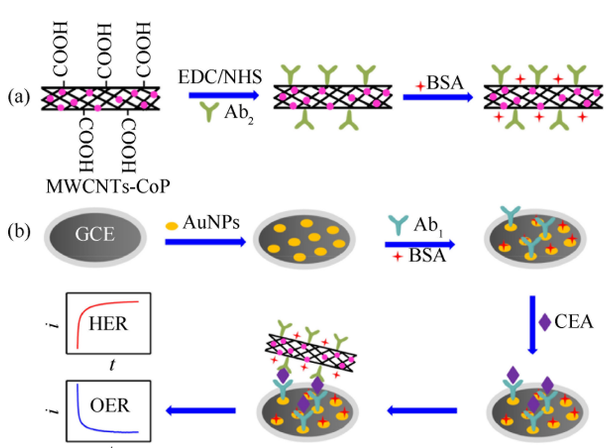


图 5　电化学免疫传感器检测肿瘤标志物癌胚抗原示意[80]
(a)MWCNTs-CoP-Ab2 复合免疫探针的制备;(b)电化学免疫传感器的构建

合的复合免疫探针的数量不同,导致HER和OER过电位不同,实现了CEA的灵敏检测。

5 总结与展望

催化反应是显著提高电化学传感器检测灵敏度的有效方法,可实现痕量水平的肿瘤标志物质量浓度及其含量变化的灵敏检测,为癌症的早期诊断和治疗提供支持。这方面的研究尽管取得了巨大进展,但仍存在一些有待解决的问题:(1)生物酶的环境耐受性差。如需在4 ℃冰箱中保存,需在模拟生理条件下使用,酸碱的耐受性差。纳米技术为改善生物酶的环境耐受性提供了解决方案,如可利用MOFs将酶包裹起来,保持酶的三维结构,有效提高生物酶的环境耐受性。(2)纳米酶的催化专一性差,催化效率低。通过调控MOFs的内部孔道结构模拟生物酶的三维结构是解决其催化专一性问题的可能路径;利用造缺陷技术,在纳米颗粒或MOFs结构中形成更多的催化反应的活性位点,可提高其催化效率;通过单原子催化剂的开发也是解决催化效率低的有效途径。(3)贵金属催化材料成本高。这一问题可通过掺杂非贵金属元素,研发新的非贵金属合金,设计非贵金属化合物等方式解决。

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