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基于新型智能响应水凝胶材料的便携式定量检测新方法

Target-Responsive Hydrogels for Portable and Quantitative Analysis

闫凌

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Target-Responsive Hydrogels for Portable and Quantitative Analysis

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摘 要

近年来，各种疾病的发病率居高不下，对即时诊断提出了更高的要求。与此同时，科技的进步以及人们生活水平的提高，为即时诊断的发展创造了条件。基于以上背景，各种即时诊断方法应运而生，并迅速成为研究热点。所谓即时诊断（Point of Care Test，POC Test），是指在病人身旁迅速获得检测结果的一项技术手段。该技术具有成本低廉、操作简便、实时快速、定量准确、用户友好等多项优点，在医疗、环境检测、食品安全等领域备受关注。尽管即时检测的各种创新型成功实例百花齐放，但目前成功的商业化产品案例仍十分有限，其中尤以最广泛普及、最具有实用价值的个人用血糖仪为代表。但是，血糖仪的检测靶标仅局限于葡萄糖分子。为拓展血糖仪的检测靶标发展一套通用性强的检测平台，本文将视线投向核酸适体交联智能水凝胶，并构建其与血糖仪联用检测体系。水凝胶是一类具有三维网状结构，能响应多种化学、物理刺激的亲水性高分子聚合物。核酸适体是 20 世纪 90 年代兴起的一类能够特异性地结合靶标分子的新型的分析、诊断分子。特别是将其与分子生物学信号放大技术结合，为解决遗传变异、疾病诊断、治疗等难题提供了新的思路，愈加成为化学、生物医学等相关领域的研究热点，并不断向普及化、便携化等方向发展。基于此，本文通过 DNA 自组装方法和分子识别技术，发展快速、方便、廉价的即时诊断装置，成功地把血糖仪的检测靶标拓展到任何感兴趣的分子。具体开展了如下几部分工作：

（1）合成丙烯酸亚磷酰胺单体，设计、合成、制备了核酸适体交联三维水凝胶，理论计算并分析了凝胶结构交联情况，以金纳米粒子作为可视化信号分子考察了水凝胶成胶-解胶过程。水凝胶工作原理如下：将两条 DNA 短链（链 A 和链 B）与丙烯酰胺单体共聚生成线性聚丙烯酰胺聚合链 A（PS-A）和聚合链 B（PS-B）。其中链 A 和 B 分别能够与 DNA 核酸适体序列 linker aptamer 的两端互补。待 PS-A 和 PS-B 充分混匀后，向其中加入 linker aptamer 链。随着核酸适体的加入，链 A、链 B 与之杂交得到三链复合体的结构，作为交联剂使得线性聚丙烯酰胺 PS-A 和 PS-B 形成三维网状水凝胶结构，并将金纳米粒子包裹于其中。外界刺激，如 DNase I（非特异性）和可卡因（特异性），能够瓦解交联结构，释

放出金纳米粒子，并可借助肉眼观测到凝胶结构的改变。

（2）以可卡因为模型分子，建立了目标响应“sweet”水凝胶手持式传感体系，基于 DNA 碱基对识别和核酸适体-靶分子相互作用之间的相互竞争过程引发的酶促反应，实现了核酸适体交联水凝胶与个人用血糖仪的结合。具体原理同上，以淀粉葡萄糖苷酶替代金纳米粒子作为信号的响应分子。当体系中不存在靶标时，淀粉葡萄糖苷酶稳定地包裹在胶中，导致其与胶外溶液中底物淀粉的物理分离，酶促反应无法发生；加入靶标后，核酸适体特异性的优先与靶标相结合形成靶标-核酸适体复合体，导致水凝胶瓦解并释放出淀粉葡萄糖苷酶。释放出的酶分子催化溶液相中淀粉水解产生大量的葡萄糖，借由个人用血糖仪（PGM）定量读出信号值。该方法具有良好的重现性和稳定性，水凝胶保藏长达 3 个月后仍具有良好的准确度。

（3）为了验证方法的通用性，先后设计了针对可卡因和腺嘌呤核苷三磷酸检测的核酸适体交联水凝胶，分别应用于对可卡因和腺嘌呤核苷三磷酸的定量分析。采用相同的工作机理，将高聚物链按照一定浓度比例制备得到包裹有淀粉葡萄糖苷酶的交联水凝胶。核酸适体-靶标复合物的形成导致水凝胶瓦解，释放出的酶分子催化底物淀粉水解，产生放大的可由血糖仪读出的葡萄糖浓度信号，从而实现对靶标的检测。针对缓冲体系内可卡因的检出限达 3.8 μM，针对缓冲体系内的 ATP，该方法的检出限为 15.5 μM。同时，上述体系成功地实现了复杂体系（尿液、血液）中可卡因的检测。

本论文建立了一种目标响应“sweet”水凝胶手持式传感器，该传感器操作简便、成本低廉，具有快速、灵敏、选择性高、不易受复杂环境干扰等优点，有望应用于重要生物分子的药物监控和临床诊断等过程，并发展成为一种设计简单灵活、成本低廉的通用性检测方法。

关键词：核酸适体　血糖仪　水凝胶　信号放大　定量检测

Abstract

Point-of-care testing (POCT) is defined as the medical testing at or near the site of patient care, which fulfills the requirement of portablity, rapidity, low cost and ease of use for diagnostics in disaster situation, home healthcare settings, and in poorly equipped rural areas. Personal Glucose Meter (PGM) is one of the most practical and portable POCT devices. However, the application of PGM is only limited to monitor the level of glucose molecules. For extending the detecting targets of the PGM to specific molecules of interest and developing a more general detection platform, we combined the PGM with the aptamer crosslinked three-dimensional hydrogels which is very sensitive to the environmental response. Due to the ability to response to many kinds of chemical and physical stimulations, they are also called “smart hydrogels” and widely applied for biosensing. Most existing hydrogel-related biosensors have been designed mainly based on mechanical changes of hydrogel expansion or contration, as well as the optical property changes of during the swelling and shrinking, which often requires sophisticated equipment with laborious and time-consuming process. Recent development of enzyme-based bio-signal amplification technology are highlighted by the feature of robustness, little contamination and portability. The ability of aptamer to recognize molecule target rapidly and sensitively, together with the portable diagnostic equipments has featured nucleic acid amplification technology as a robust diagnostic techniques. In this thesis, we develop a POCT device with high sensitivity and low cost based on DNA assembly and molecule recognition techniques. We successfully extended the dectecting targets of the PGM to any interested molecules.

The main work composed by three parts are listed as follows:

(1) First, acrylic phosphoramidite monomers are synthesized to modify the designed DNA strands at 5’-end for free radical polymerization. A crosslinked three-dimensional hydrogel is prepared by hybridization of poly-DNA strands and

aptamers. The cross-linked structure is analyzed by theoretical calculation. At last, entrapment and release capacity of hydrogels is investigated with gold nanoparticles as visible signal molecules. Two short DNA sequences, strand A and B, are grafted onto linear polyacrylamide polymers to form polymer strand A and B (PS-A and PS-B). Strands A and B are complementary to adjacent areas of a DNA aptamer sequence. Upon the addition of aptamer, strand A and B hybridize with aptamer to yield a three-strand complex, thus crosslinking PS-A and PS-B into hydrogel with gold nanoparticles trapped inside. Cross-linked structure collapses upon external stimulis, such as DNase I (non-specific) and cocaine (specific), releasing gold nanoparticles to obtain visualized results.

(2) Second, a target-responsive “sweet” hydrogel handheld sensor was developed for cocaine detection. It is based on the competition of complementary base pairing and aptamer-target interactions to trigger the enzymetic reaction, realizing the integration of target-responsive DNA cross-linked hydrogel with personal gluocose meter （PGM）. The hydrogel preparation is similar as the above, just replacing gold nanoparticles with glucoamylase. Without target, the glucoamylase is stably trapped inside the gel and physically separated from its substrate amylose, which is in the solution outside the gel. No enzymatic reaction would happen. When target molecules are introduced, aptamers specifically and preferentially bind the targets to form target-aptamer complexes, leading to the breakdown of the hydrogel and releasing glucoamylase, which catalyzes the hydrolysis of amylose to produce a large amount of glucose for quantitative readout by PGM. This system shows good stability and repeatability. Even after refrigerated storage for 3 months, the hydrogel still has similar performance as the freshly prepared material.

(3) To test the versatility, a “sweet” hydrogel-PGM system has been designed for the quantitative detection of both cocaine and adenine nucleotide triphosphate (ATP) using the same mechanism. Polymer chains and linker strands are mixed in stoichiometric concentrations to form the glucoamylase trapped target-responsive hydrogel. The formation of aptamer-target complexes leads to the collapse of hydrogels and the releasing of enzymes, enzyme released. Amylose, as substrates, was

hydrolyzed to produce a huge number of glucose under the catalysis of glucoamylase. The concentration of glucose can be read by a PGM, achieving the quantitative detection of targets. For SH-PGM buffer, the detection limits of cocaine and ATP are 3.8 μM and 15.5 μM, respectively. Moreover, this system can successfully detect cocaine in complex body fluids such as urine and plasma.

In summary, a target-responsive “sweet” hydrogel handheld sensor is fabricated . It has the features of ease of use, low-cost, rapidity, sensitivity, selectivity and stability. The sensor can be potentially applied on drug monitoring and clinical diagnosis of certain important biological molecules. They could be further developed into a highly universal detection protocol with the advantages of simple to design, flexible to use and low-cost.

Keywords: aptamers; PGM; hydrogel; signal amplification; quantitative detection

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第一章 绪 论

自从 1988 年，英国 Unipath 公司将家庭测孕试纸推向市场[1]，家庭检测装置凭借其成本低廉、操作简便等优势，在过去的二十五年中，应用领域迅速扩展。其中，尤以 POC test，即床边诊断技术的兴起、应用和发展最具代表性[2]，该技术的进步极大地推动了国际范围内医疗卫生、环境监测等诸多领域的研究工作的深入开展。与此同时，智能水凝胶作为一种靶标及外界刺激响应的新型材料，在药物的可控传递、组织工程骨架的构建、微装置的动力源及生物传感器的应用等方面已取得广泛应用[3]。特别是 DNA 水凝胶的出现，为检测提供了更多可能的机理与手段。核酸适体是一类能够高亲和性和高特异性地与靶标分子结合的单链寡核苷酸，作为一类新型的分析、诊断分子，自出现以来，备受科研工作者的关注。利用核酸适体特异性识别靶标，经由智能水凝胶将信号加以转导，再通过酶分子放大获得高灵敏信号，最终应用于即时诊断设备快速、方便地读出结果，这一方法已成为现代医疗检测的新途径。本章分别介绍了手持式传感技术、水凝胶在生物传感中的最新研究进展、核酸适体、酶放大技术以及淀粉酶的主要分类和应用，并在此基础上提出本文的工作构思。

1.1 手持式传感技术的发展及应用

1.1.1 即时检测的定义及意义

所谓即时诊断（Point of Care Test，POC Test），是指在病人身旁迅速获得检测结果的一项技术手段。该技术方便、快捷，不依赖昂贵的实验仪器设备或者专业的技术人员，亦不需借助复杂的样品处理步骤，依靠简单的手持式设备读出信号或者通过观测试纸上条纹、斑点等的颜色变化来反映检测结果。除应用于医院、诊所中迅速获取病人信息外，即时诊断还使疫情早期预警、家庭保健防护、贫困地区医疗乃至食物、水、环境等的现场监测等成为可能。

如图 1.1 所示是一种理想化的 POC 设备[4]，它藉由廉价的一次性芯片提供小型的微流控通道，集成样品与试剂的混合、流速控制、反应时间控制、样品分离