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Recent advances in aptamer-functionalized materials in sample preparation

Fuyou Du [a,*], Lin Guo [a], Qun Qin [a], Xian Zheng [a], Guihua Ruan [a], Jianping Li [a], Gongke Li [b]

[a] Guangxi Key Laboratory of Electrochemical and Magnetochemical Function Materials, College of Chemistry and Bioengineering, Guilin University of Technology, Guangxi 541004, China
[b] School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, China

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ABSTRACT

Aptamer-functionalized materials (AFMs) are promising specific recognition materials in sample-preparation techniques due to the inherently high affinity and selectivity associated with aptamers, so AFMs attract considerable interest and a wide variety of applications to recognize specific target analytes ranging from small molecules to proteins, cells and even tissues. We briefly introduce sample-preparation techniques and potential applications of aptamers in analytical chemistry, and then briefly discuss the preparation of AFMs. We mainly focus on developments and applications of AFMs in sample-preparation techniques, including AF solid-phase extraction, solid-phase microextraction, and microfluidic sample preparation, and other sample-preparation techniques. We also discuss the challenges and the prospects of AFMs in sample preparation in future developments.

Contents

1. Introduction

With the great development of analytical instruments, especially mass spectrometry (MS), some interesting analytes in complex mixtures can be directly detected [1–4]. However, it still remains a great challenge for detection and quantification of those analytes with trace and ultra-trace amounts due to the restriction of instrumental LODs and interferences of complicated matrixes, particularly in environmental, food, and biological samples [5–8]. It is therefore necessary to introduce suitable sample-preparation techniques to isolate and to enrich interesting analytes from the sample before detection and quantification, while removing uninteresting and interfering substances from the primary matrix as much as possible in order to improve the limit of detection (LOD) and protect analytical instruments from possible damage [9–11].

Sample preparation is still considered the bottleneck of the whole analytical process and impacts on nearly all the later steps in the analytical process, as it is critical for unequivocal identification, confirmation and quantification of analytes [9]. In order to fulfill current requirements in sample preparation for tracing analytes at trace or

* Corresponding author. Tel.: +86 773 5898551; fax : +86 773 5892796.
E-mail address: dufu2005@126.com (F. Du).

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ultra-trace level in complicated samples, many new sample-preparation techniques, including solid-phase extraction (SPE), solid-phase microextraction (SPME), matrix solid-phase dispersion (MSPD), micro-SPE (MSPE), stir-bar sorptive extraction (SBSE), and liquid-phase microextraction (LPME)], have been introduced.

However, all of these techniques suffer from lack of selectivity, so recent years saw increasing interest in the introduction of selective sample-preparation techniques based on restricted access materials (RAMs) [12–14], molecularly-imprinted polymers (MIPs) [6,15–17], antibodies [5,18,19], and aptamers [20–22]. RAM consists of a porous material with a restrictive, hydrophilic outer surface that selectively separates analytes of low molecular mass from large interfering molecules, resulting in very limited applications in sample preparation. MIPs were considered a versatile, highly selective tool in sample preparation [16], but the application to real sample analysis often suffers from problems, including possible template bleeding, tedious searching for optimal conditions for preparation, application to aqueous samples, and limited specific binding sites [17]. Antibodies possess high affinity and specificity against the target analytes, but it is very difficult, time-consuming, and expensive to obtain antibodies, and it is difficult to guarantee the stability and the reusability of the antibody-binding sites in complex samples, so their application in sample preparation is to some extent limited.

In contrast to antibodies, aptamers, a new class of short single-stranded DNA/RNA molecules, possess many advantages, such as high specificity and binding affinity, good stability, low cost, non-toxicity, ease of synthesis, and easy, controllable modification, which make them very promising and dynamic in environmental monitoring, bioanalytical chemistry, and biomedicine [20,23–29]. However, at present, many of these applications, particularly biomedical, are still at the research stage.

Aptamers can be artificially generated by an *in-vitro* method known as systematic evolution of ligands by exponential enrichment (SELEX). However, it is difficult to generate aptamers by the *in-vitro* SELEX method to recognize target proteins, cells and tissues from complex biological samples, so several more efficient selection methods, including cell-SELEX, non-SELEX, automated-SELEX and microfluidic SELEX, were developed in order to improve aptamers to make them more suitable for biological and biomedical applications [29–32]. The research results demonstrated that not only can the aptamers selected by these new SELEX techniques bind to certain molecules with high affinity and specificity, but they can also be chemically modified with relative ease, allowing researchers to improve their binding properties, to help capture a much broader range of target analytes, and to continue pushing their applications further [29,32].

The construction of aptamer-functionalized materials (AFMs) results in structures capable of precise molecular recognition and enhanced target specificity, so AFMs attract considerable interest and are used for a wide variety of applications to recognize specific target analytes ranging from metal ions [33] and small organic molecules [20,21] to proteins [22,34], whole cells [23,26,29,35,36], and even tissues [36,37]. Recently, several excellent review papers on AFMs were published with different emphases [21–24,27].

Herein, we attempt to trace the developments and the applications of AFMs in sample-preparation techniques, including AF-SPE, AF-SPME, and microfluidic sample preparation, and other sample-preparation techniques. Furthermore, we discuss remaining challenges and future perspectives to develop novel AFMs and to apply them further in sample preparation.

2. Preparation of AFMs

The key reagent for preparation of AFMs is the aptamer. A detailed description of aptamer production, purification and assessment is outside the scope of this review but some general comments are relevant. Generally, aptamers with desired affinities are isolated from large libraries containing 10^{13}–10^{16} random combinatorial nucleic-acid sequences through SELEX [25,30]. The SELEX or *in-vitro* process includes:

(1) the incubation of target molecules with random sequence pools;
(2) the subsequent separation of unbound oligonucleotides and the elution of bound oligonucleotides; and, then,
(3) PCR amplification of bound aptamers.

The whole procedure can take from weeks to months to obtain an ideal aptamer with high affinity and specificity against the targets by the conventional SELEX method, so several novel modified SELEX techniques, including CE-SELEX, non-SELEX, automated-SELEX and microfluidic SELEX, have been developed and applied in order to improve the efficiency of the SELEX, and several highly selective SELEX techniques (blended, counter, negative, and subtractive SELEX) have also been explored to produce aptamers with desired features [26,29–32]. In addition, the modification of aptamers is necessary before immobilization on a support material, and, in most cases, the chemical modification of the oligonucleotide is at its 3′-end or its 5′-end to incorporate functional groups or molecules that facilitate linkage of the surface. The most common functional groups include amino, carboxyl and thiol, and the most functional molecule is biotin [38].

The other key reagent for preparation of AFMs is the solid support. In addition to the desired properties, such as chemical and biochemical inertness, good chemical and mechanical stability, uniform particle size and morphology, the support materials should be easily activated to allow aptamer attachment, and should possess an appropriate hydrophilic surface in order to minimize non-specific interactions. Typical supports for immobilization of aptamers included silica [39–43], sepharose [43–47], synthetic polymers [34,48–50], magnetic particles and beads [24,27,51–54], gold particles [55–57], organic-inorganic hybrid monolithic materials [58–60], carbon nanotubes (CNTs) [61,62], and graphene oxide (GO) [63–65].

Another important reagent is the linker (i.e., spacer arm) between the aptamer and the support surface. Linkers are used to provide a “chemical” spacer between the solid-support surface and the aptamer that is anchored to the support surface by an appropriate functional group, so both the length and the chemical structure of the spacer arm affected aptamer performance [43,66–68]. The study results of Balamurugan et al. [67] indicated that there are two factors affecting target-capture efficiency – the immobilized aptamer density on the support surface and the distance from the immobilization surface of the aptamers. With the increase of linker length, the aptamer surface density decreased, and, at the same time, the accessibility of the target to the aptamer increased, resulting in an inconsistent effect on both target-capture efficiency and binding capacity [67,68]. In addition, Wang et al. [57] demonstrated that poly(oligo(ethylene glycol) methacrylate) (POEGMA) as an anti-fouling spacer enhanced the specificity and the selectivity of the AF gold-nanoparticle layers for capture of Ramos cells from serum-containing samples compared with that of the capture without the spacer arm. The linker length should therefore be carefully considered in order to improve the capture efficiency of AFMs. Moreover, the capture efficiency was affected by the linker chemical structure. For example, the protein-binding efficiency was different for AF monolayers formed with different thymidine linkers, and the incorporation of a hexa(ethylene glycol) moiety into the linker increased the amount of thrombin bound, though it did not affect the immobilized aptamer density on support surface [67]. The common linkers include 3-aminopropyltriethoxysilane [39–41,54,57],