Methods 97 (2016) 75–87

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Preparation and characterization of aptamer–polyelectrolyte films and microcapsules for biosensing and delivery applications

Emily Mastronardi [1], Phepafatso K. Tsae [1], Xueru Zhang, Amanda Pach, Yasir Sultan, Maria C. DeRosa *

Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 27 October 2015
Accepted 28 October 2015
Available online 6 November 2015

Keywords:
Aptamer
Polyelectrolyte multilayers
Thin films
Microcapsules
Biosensing
Controlled delivery

ABSTRACT

"Smart" materials are polymer systems that are able to change their physical or chemical properties in response to external stimuli in their environment. By adding a specific molecular recognition probe to a polymer, hybrid materials can be developed that retain the properties of the advanced polymer and gain the ability to respond to a specific molecular target. Aptamers are single-stranded oligonucleotides that are well-suited to serve as molecular recognition probes due to the specificity and affinity of their target recognition as well as their stability and ease of synthesis and labeling. In particular, their negatively charged backbone makes for their facile incorporation into polyelectrolyte-based materials. This article will provide a brief review of the currently reported biosensor and delivery platforms that have been reported employing aptamer–polyelectrolyte materials, as well as a detailed description of the methods used to synthesize and study films and microcapsules containing small-molecule aptamer probes.

1. Introduction

Smart materials are a category of multifunctional materials capable of sensing a change in their environment and reporting it by changing their physical or chemical properties. Examples of these materials respond to many chemical and physical stimuli such as temperature, pH, and electric fields, making them useful in sensing applications [1]. Incorporation of a molecular recognition probe, such as antibodies or aptamers, into these materials could impart the recognition ability from the probe to the material as a whole, creating bioresponsive materials [2]. Aptamers, single-stranded oligonucleotides that can bind targets specifically and selectively, are ideal candidates for incorporation into smart materials. They are synthesized chemically at a relatively low cost, and can be chemically modified at precise locations with a variety of functional groups and reporter molecules, making them highly compatible with polymeric materials and easily adapted to smart material applications. Furthermore, as aptamers are nucleic acids, their structure and binding can also be regulated by the addition of a complementary DNA strand, adding another layer of control to the smart material response. To exploit these characteristics, aptamers have been integrated into a variety of polymeric, dendrimeric, and nanoscale systems, yielding target-responsive materials [3–7].

Polyelectrolyte multilayer (PEM) films and microcapsules belong to a family of multifunctional materials from which many smart materials have been developed. The layer-by-layer (LBL) method for film deposition described by Decher provides a simple technique with which to create PEM films [8]. Nanoscale thin films are created by the alternating adsorption of negatively and positively charged polyelectrolytes onto a charged surface. DNA's negatively charged backbone allows for its simple incorporation into polyelectrolyte films through electrostatic interactions. DNA has been readily incorporated into polyelectrolyte films and capsules for applications such as Hepatitis B DNA detection [9] and gene delivery [10–13]. Thus, the incorporation of DNA aptamers into polyelectrolyte films is a feasible approach to the generation of bio-responsive films for sensor and controlled delivery applications.

The first demonstration that an aptamer incorporated into a PEM film could retain its ability to bind its target came in 2009, where the aptamer's recognition of a dye target was conferred to the whole film with only a minor reduction in binding affinity [14]. Since this initial report, other aptamer-PEM biosensors and smart materials have been reported. The PEM can serve as a simple platform for an aptamer sequence, as was described in a report on an electrochemical aptasensor for the D enantiomer of arginine vasopressin (D-VP) [15]. In this case, aptamer-target binding does not effect a structural or chemical change within the polymer matrix. Alternatively, when the aptamer is embedded within the polyelectrolyte matrix, biosensors can be developed that exploit

* Corresponding author.
E-mail address: maria.derosa@carleton.ca (M.C. DeRosa).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ymeth.2015.10.018
1046-2023/

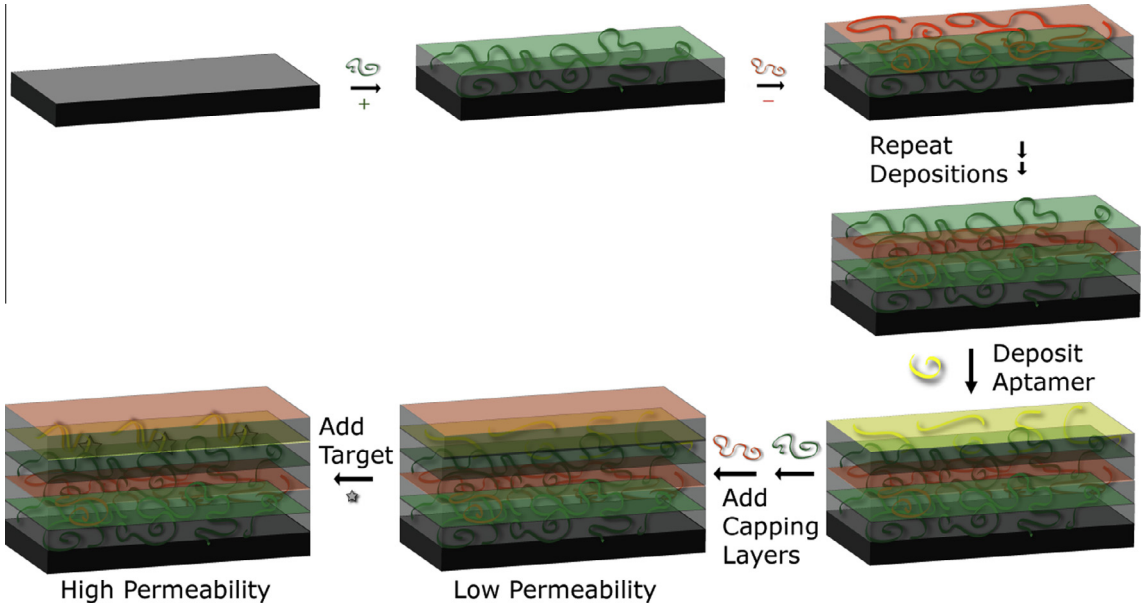

Fig. 1. Preparation of an aptamer-embedded PEM film system. A charged surface (e.g. glass or quartz slide, modified gold electrode, etc.) is sequentially exposed to oppositely charged polyelectrolytes (green and orange ribbons), with alternating rinses, to prepare a base layer. At a desired number of base bilayers, an aptamer sequence (yellow ribbons) is substituted for the negatively charged polyelectrolyte and a fixed number of aptamer-containing bilayers are deposited. The film is capped with a standard polyelectrolyte bilayer. In the absence of the aptamer's cognate target (gray star), the film has limited permeability. Target binding and concomitant structural changes to the aptamer and the PEM lead to an increase in permeability.

the smart material's response. Aptamer-embedded PEM thin films have been used as gatekeepers in a plasmonic nanoparticle visual sensor [16]. A similar mechanism was recently exploited to create an electro-chemiluminescence sensing platform for bisphenol A (BPA) using aptamer-induced permeability change within a polyelectrolyte smart material [17]. While the previously reported PEMs were built from synthetic polyelectrolytes, the generality of aptamer-PEM systems has recently been demonstrated by the successful incorporation of an aptamer into a PEM system comprised of natural polyelectrolytes [18]. Microcapsules based on smart aptamer-PEM systems have also been investigated; the aptamer-target binding event was shown to alter the diffusive properties of the film, with target-binding increasing the flux through the microcapsules [19]. More recently, aptamers have also been employed as collapsible scaffolds for PEMs that undergo target-induced rupture [20].

The ease and utility of aptamer incorporation into PEM systems warrants the investigation of aptamer-PEM smart materials for a variety of applications in medicine, environment, and agriculture [21]. The objective of this paper is to provide the methodology for creating and characterizing aptamer-PEM films and microcapsules for sensing and delivery applications. Three smart material systems will be described: (1) aptamer-embedded polyelectrolyte films (Fig. 1), where the aptamer substitutes for a negatively charged polyelectrolyte within a PEM system (2) aptamer-embedded polyelectrolyte microcapsules (Fig. 2), where the walls of a hollow microcapsule contain aptamers as a recognition agent, and (3) aptamer-loaded polyelectrolyte microcapsules (Fig. 3) where the aptamers are scaffolds within the hollow core of the microcapsule. The aptamers that will be described herein are for small molecule targets such as SB dye for easy visualization.

2. Material and methods

2.1. List of materials

The sulforhodamine B aptamer (SA sequence: 5′-CCG GCC TAG GGT GGG AGG GAG GGG GCC GG-3′), the lysine aptamer (LA sequence: 5′- ATA CCA GCT TAT TCA ATT TGA GGC GGG TGG GTG GGT TGA ATA CGC TGA TTA CCC CAT CGG AGA ACG TTA AGG CGC TTC AGA TAG TAA GTG CAA TCT-3′) and a random oligomer (RO) (RO sequence: 5′-GAC CTA TGA TAG CAT CAG TCG CAT CAG TC-3′) were synthesized on a MerMade 6 oligonucleotide synthesizer using standard phosphoramidite chemistry [22] and DNA synthesis reagents from Glen Research and Controlled Pore Glass (CPG) columns from BioAutomation were used as received. These three sequences were also synthesized with fluorescein phosphoramidite (6-FAM, Glen Research) added to the 5′ ends. Oligonucleotides were purified by polyacrylamide gel electrophoresis (12%) and mass confirmed by ESI-MS (Novatia). Calf thymus DNA (CT, sodium salt, type I) was purchased from Sigma–Aldrich and also used in control films.

Target molecules sulforhodamine B dye (SB) and L-lysine were purchased from Sigma–Aldrich, as well as non-specific control target, L-histidine. Non-specific control molecule tetramethylrosamine (TMR) was purchased from Invitrogen. Polyelectrolytes of varying composition and molecular mass were used for these experiments. Poly(diallyldimethylammonium chloride) (PDDA, MW $\leqslant$ 100,000 Da), poly(sodium 4-styrene-sulfonate) (PSS, MW 100,000 and 70,000 Da), poly(allylamine hydrochloride) (PAH, MW $\approx$ 56,000 Da) were all purchased from Sigma–Aldrich and used without further purification. Hyaluronan (HA, MW 1,580,000 Da), purchased as sodium hyaluronate, and chitosan (CHI, MW 135,000 Da) were purchased from Acros Organics. Sodium carbonate (Na_2CO_3; >99.5%), ethylenediaminetetraacetic acid (EDTA; 99%), anhydrous calcium chloride ($CaCl_2$, $\geqslant$93.0%), and sodium bicarbonate ($NaHCO_3$, $\geqslant$99.5%) used for fabrication of $CaCO_3$ templates, were all purchased from Sigma–Aldrich (Oakville, ON). Potassium ferricyanide ($K_3Fe(CN)_6$; 99%) and hexaammineruthenium chloride ($Ru(NH_3)_6Cl_3$; 98%) for electrochemistry experiments were obtained from Sigma–Aldrich. 2-Mercaptoethylamine hydrochloride (>98%) for electrode modification was obtained from Alfa Aesar.

Deionized water was used for all buffer preparations and buffers filtered through Corning 0.22 μM cellulose acetate filter units before use. All glassware was rinsed in distilled water 5 times, fol-