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Electrospin-coating of nitrocellulose membrane enhances sensitivity in nucleic acid-based lateral flow assay

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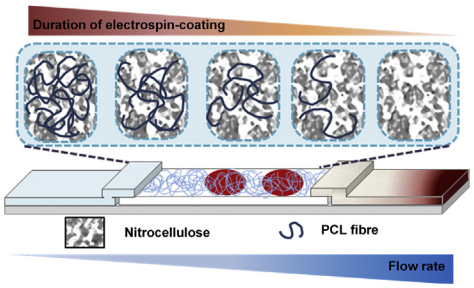
HIGHLIGHTS

- Electrospin-coating PCL onto nitrocellulose membrane of LFA can control the flow rate.
- Electrospin-coating increases sensitivity of nucleic acid-based LFA by 10-fold.
- A proof of concept shows potential in highly sensitive detection of viral nucleic acids.

GRAPHICAL ABSTRACT



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ABSTRACT

Point-of-care biosensors are important tools developed to aid medical diagnosis and testing, food safety and environmental monitoring. Paper-based biosensors, especially nucleic acid-based lateral flow assays (LFA), are affordable, simple to produce and easy to use in remote settings. However, the sensitivity of such assays to infectious diseases has always been a restrictive challenge. Here, we have successfully electrospun polycaprolactone (PCL) on nitrocellulose (NC) membrane to form a hydrophobic coating to reduce the flow rate and increase the interaction rate between the targets and gold nanoparticles-detecting probes conjugates, resulting in the binding of more complexes to the capture probes. With this approach, the sensitivity of the PCL electrospin-coated test strip has been increased by approximately ten-fold as compared to the unmodified test strip. As a proof of concept, this approach holds great potential for sensitive detection of targets at point-of-care testing.

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1. Introduction

Paper-based biosensors, as miniaturized, low-cost and portable platforms at point of care (POC), have found widespread applications in various fields, including medical diagnosis, food safety assurance, and environmental monitoring [1]. For instance, the lateral flow assays (LFAs) have shown great potential as a more affordable, simpler to use and portable diagnostic platform at POC compared to labour-intensive conventional analytical methods [2], providing a powerful tool for pre-clinical diagnosis to be applied by a non-clinician in resource-limited settings [3]. Compared to lateral flow immunoassays, nucleic acid-based LFAs employ nucleic acids, *i.e.*, aptamer and DNA, are more precise and accurate in detection due to their higher stability [4]. However, the sensitivity of these assays has been a perpetual challenge for low concentration targets in samples (*e.g.*, blood) and thus its use at the POC in critical situations has always been limited [5]. In recent years, similar early symptoms and serological cross-reactivity in pandemic diseases, *e.g.*, dengue and zika fevers, have led to many deaths and birth defects due to delayed diagnosis [6]. The number of zika virus infection cases in Brazil alone was reported as 1,673,272 in 2015 and 2016, which includes 1950 cases of infection-related microcephaly [7]. In this sense, there is still an immense demand for nucleic acid-based LFAs with improved sensitivity for early POC diagnosis and timely medical treatment.

Various methods have been developed to enhance sensitivity of LFAs. Some methods are based on physically concentrating the targets in sample solution before assay, including evaporation [8], isotachophoresis [9] and dialysis-based methods [10]. However, these methods may require sample pre-treatment, additional equipment, or a power supply for the assay. Although optimizing temperature and relative humidity could enhance sensitivity, it is still costly to design and build a portable instrument to serve the purpose [11]. Signal amplification of the assay is another alternative, and may be achieved by modifying the reporter particle or reacting probes [12]. However, this may involve additional amplifying probes or proteins to the signal particles, such as silver deposition on gold nanoparticles (AuNPs) [13], gold accumulation facilitated by oligonucleotides [14] or antibodies [15], enzymatic facilitation [16] or combinations of them. Another strategy for enhancing sensitivity is reducing the flow rate of solution in the assay by modifying the physical architecture of the strip [17], or by adding shunts to the LFA (*e.g.*, a cellulose pad [18], modified glass fibres [19] and sponge [20], wax [21] or polydimethylsiloxane (PDMS) [22] shunts). However, the shunts are generally either sensitive to temperature or require complex fabrication [20,23].

Nanofibrous materials (mostly fabricated by electrospinning) feature high surface-area-to-volume ratio, high porosity, interconnected porous network, and flexible functionality due to the modifiable nature of the polymeric materials [24], which have been widely utilized in biomedical applications [25,26]. Nanofibrous materials have also recently been integrated in biosensors for gaseous [27] and chemical detection [28]. For instance, chemicals and biomolecules have been incorporated in the electrospinning solution or electrospun membrane to produce functionalized fibres that can bind with target molecules like microorganisms, proteins and nucleotides [29–31]. These have been used in the development of biological optical sensors [32], electrochemical sensors [33], enzyme-linked immunosorbent assays (ELISA) [34], and other sensors [35]. Electrospun materials have also been integrated into LFAs to provide the functionalities of binding, absorption and wicking [31,36–38]. For instance, an electrospun polylactic acid (PLA)-biotin nanofiber membrane has been also used as a lateral flow sensing platform for *E. coli* DNA detection via biotin-streptavidin specific binding [36]. A co-polymer electrospinning material has been integrated into LFA, which provides a higher specificity of liposome binding as compared to nitrocellulose (NC) [37]. A bio-resorbable scaffold made of electrospun poly(glycolic acid) has been integrated as a part of a theranostic system combined with LFA to detect wound biomarkers [38]. However, the possibility of incorporating hydrophobic electrospun material into LFAs to control fluid flow for signal enhancement has not been explored yet.

The present study showcases the application of electrospun fibres to a nucleic acid-based LFA as a proof of concept to improve sensitivity. Polycaprolactone (PCL) nanofibers were directly electrospun onto the NC membrane at different duration of time to explore the conditions for improved sensitivity. The results showed that electrospin-coating of NC membrane for 60 s enhances the sensitivity of the nucleic acid based assay by approximately tenfold, compared to the unmodified test strip. This electrospin-coating strategy holds great promise for sensitive detection of various targets at point of care testing.

2. Experimental methods

2.1. Materials

PCL ($M_n = 80{,}000$), gold(III) chloride hydrate, trisodium citrate, sodium dodecyl sulphate (SDS), trisodium phosphate, phosphate buffer saline (PBS) and Tween-20 were all purchased from Sigma Aldrich (USA). N,N-dimethylformamide (DMF) and sodium chloride (NaCl) were procured from Merck, USA. Chloroform was purchased from Friedemann Schmidt. Sucrose and sodium acetate were obtained from Ajax Finechem Pty. Ltd, Australia. Bovine serum albumin (BSA) was acquired from AMRESCO, USA. Streptavidin was purchased from Promega, USA. Saline sodium citrate buffer (SSC × 20) was obtained from Roche Diagnostics, USA. 95% ethanol was acquired from Systerm, Classic Chemical Sdn Bhd, Malaysia. Glacial acetic acid was procured from Fisher Scientific, Malaysia. All solutions were prepared using ultrapure water from Aquinity P30 LS ultrapurification water system (membraPure GmbH, Germany). All the oligonucleotide probes and target were procured from SBS Genetech Co., Ltd (Beijing, PRC). Designation of the sequences for each oligonucleotide can be referred to Table S1 in Supplementary Material. Paper materials included HFB 13500 nitrocellulose (NC) membrane (Millipore, USA), H-1 cellulose pad (JNBio Co., Ltd, Shanghai, PRC) as absorbent pad, Pall 8964 glass fibre (Pall Corporation, Saint Germain-en-Laye) as sample pad and J-B6 backing card (JNBio Co., Ltd).

2.2. Far-field electrospin-coating of NC membrane

A 10% (w/v) PCL solution was prepared in a co-solvent system consisting of 9 parts chloroform and 1 part DMF (9:1 v/v) in a condensed flask under stirring conditions at room temperature to obtain the spinning dope. The electrospinning was carried out using an electric field of 12 kV (Gamma High Voltage Research, Ormond Beach, FL, USA), a blunt 20 G needle (Terumo), needle to aluminium collector distance of 18 cm, while the feeding rate was kept constant at 3 ml/h with a syringe pump (KD Scientific, Inc., Holliston, MA, USA). The NC membranes were taped on the aluminium collector using double-sided tape and electrospun fibres were directly deposited on them at different time duration. The electrospin-coated NC membrane was then left to dry at 37 °C at least overnight before assembly into the LFA test strip. The setup for the electrospinning and the resultant electrospin-coated NC membrane is depicted in Fig. 1A.

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