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Research paper

Feasibility of a simple microsieve-based immunoassay platform

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ABSTRACT

The intrinsic properties of silicon microsieves, such as an optically flat surface, high overall porosity, and low flow resistance have led to an increasing number of biotechnology applications. In this report, the feasibility of creating a microsieve-based immunoassay platform was explored. Microsieves containing 5 μm pores were coupled with poly-acrylic acid chains, and then mounted into a plastic holder to enable rapid reagent exchanges via a wicking mechanism. The mounted microsieves were coated with infectious disease-related antigens at [2.5 and 25 μg/mL], [20 and 50 μg/mL], and [20 and 100 μg/mL] to facilitate detection of serum-derived human antibodies against Rubella (3-day measles), *B. burgdorferi* (Lyme disease), or *T. pallidum* (syphilis), respectively. The prototype microsieve-based immunoassay platform was able to distinguish positive control sera containing antibodies against Rubella, *T. pallidum*, and *B. burgdorferi* from negative control sera with similar qualitative results as FDA-approved ELISA tests. Testing of a WHO IgG syphilitic standard at 0.3, 0.15, 0.075, 0.0375, and 0.01875 IU/mL demonstrated that the *T. pallidum* microsieve assay is able to distinguish disease-specific IgG signal from background signal at similar, and possibly lower, levels than the corresponding ELISA. The *T. pallidum* microsieve assay prototype also differentiated positive clinical serum samples from negative donor samples, and the results were in good agreement with ELISA ($R^2 = 0.9046$). These feasibility studies demonstrate the potential for utilizing microsieves, along with a reagent wicking device, as a simple diagnostic immunoassay platform.

1. Introduction

Membrane-based filtration of cells and smaller biomolecules is a commonly utilized technique throughout various fields of biotechnology (Rathore and Shrike, 2011; Guo and Ngo, 2012). Specialized microfiltration membranes termed microsieves have been described and consist of a thin silicon nitride membrane layer harboring uniform pores, resting atop of a mono-crystalline silicon support (Kuiper et al., 1998). In addition to silicon nitride microsieves, more recently described polymeric microsieves have been fabricated to contain pores with a wide range of diameters, at varying densities (Kuiper et al., 1998; Gironès et al., 2006; van Rijn, 2004), thus representing promising tools for various biomedical applications. For example, several studies have demonstrated that microsieves are more effective at capturing and imaging circulating tumor cells derived from matrices such as whole blood, relative to other filter types (Lim et al., 2012; Coumans et al., 2013a, 2013b). Microsieves have also been developed to contain specialized pores that facilitate isolation, fluorescent imaging, and subsequent nucleic acid-based analysis of single tumor cells (Swennenhuis et al., 2015). In addition to eukaryotic cell capture, microsieves are beginning to show utility within the field of microbiology. In one such study, microsieves were used as a novel microbial co-cultivation and microscopic analysis platform (Hesselman et al., 2012). Furthermore, covalent coupling of antibodies to the microsieve surface enabled efficient capture and imaging of *Salmonella enterica* from different sample matrices (Nguyen et al., 2015). Considering the usages of microsieves such as blood filtration and microbial enrichment, numerous infectious disease-related diagnostic applications can be envisioned for microsieves that are based upon direct capture and analysis of cells from biological matrices. However, in addition to directly capturing or visualizing a disease-causing agent, confirmation of a current or past infection can also be made by indirect detection of biomolecules that are elevated within a patient's serum due to a pathogen-induced immunological or physiological response. Such serologic detection of bacterial and viral-specific biomolecules is a mainstay of clinical practice; however, newer rapid diagnostic devices offer the potential to improve patient care and improve workflow (Andreotti et al., 2003). Herein we describe

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a simple microsieve-based immunoassay platform and present feasibility experiments aimed at demonstrating its potential for infectious disease serology applications.

2. Materials and methods

2.1. Microsieves and reagent wicking apparatus

The microsieves utilized in these studies were 5 × 5 mm square, with a silicon nitride filter membrane thickness of 1 μm. The pores within the microsieve were circular, with a diameter of 5 μm. Further details describing the fabrication of such microsieves have been previously described (Kuiper et al., 1998; Gironès et al., 2006; van Rijn, 2004). Polyacrylic acid (PAA) chains were covalently attached to the microsieve surface by first coating with 3-aminopropyltrimethoxysilane, followed by PAA (average Mw ~ 250 kDa). Each 5 × 5 mm PAA-coated microsieve was subsequently mounted into a plastic holder, which was fabricated to slide in and out of a custom wicking device. The wicking device was designed to facilitate buffer exchanges during the microsieve immunoassay procedures via contacting the bottom of the microsieve with an absorbent cube.

2.2. Covalent coupling of proteins to PAA-microsieves

A 30 μL drop of deionized water was placed onto the microsieve surface, incubated for 15 min, and removed by wicking. Next, 4.6 mg of *N*-hydroxysuccinimide (NHS) and 25 mg of Ethyl-(dimethylaminopropyl) carbodiimide (EDC) were each dissolved in 800 μL of 50 mM MES buffer (pH 4.5). A 30 μL drop of freshly made EDC/NHS solution was placed onto the microsieve surface, incubated for 30 min, and the microsieve surface was rinsed with 5 mM acetic acid (pH 5.5). Next, the protein of interest was diluted to the desired coating concentration in 5 mM acetate buffer (pH 5). A 30 μL drop of the diluted protein was then added to the microsieve surface, incubated for 1 h, and the microsieve surface was rinsed with deionized water. Next, a 30 μL drop of 100 mM ethanolamine (pH 8.5) was added to the microsieve surface, incubated for 30 min, and rinsed with phosphate buffered saline (pH 7.4). To provide additional blocking, a 30 μL drop of Stabilguard reagent (Surmodics, Eden Prairie MN) was added to the microsieve surface, incubated for 15 min, and removed by wicking through the bottom. Another 30 μL drop of Stabilguard reagent was added to the microsieve surface, incubated for 15 min, and removed by wicking through the bottom just prior to initiating the microsieve immunoassay procedure.

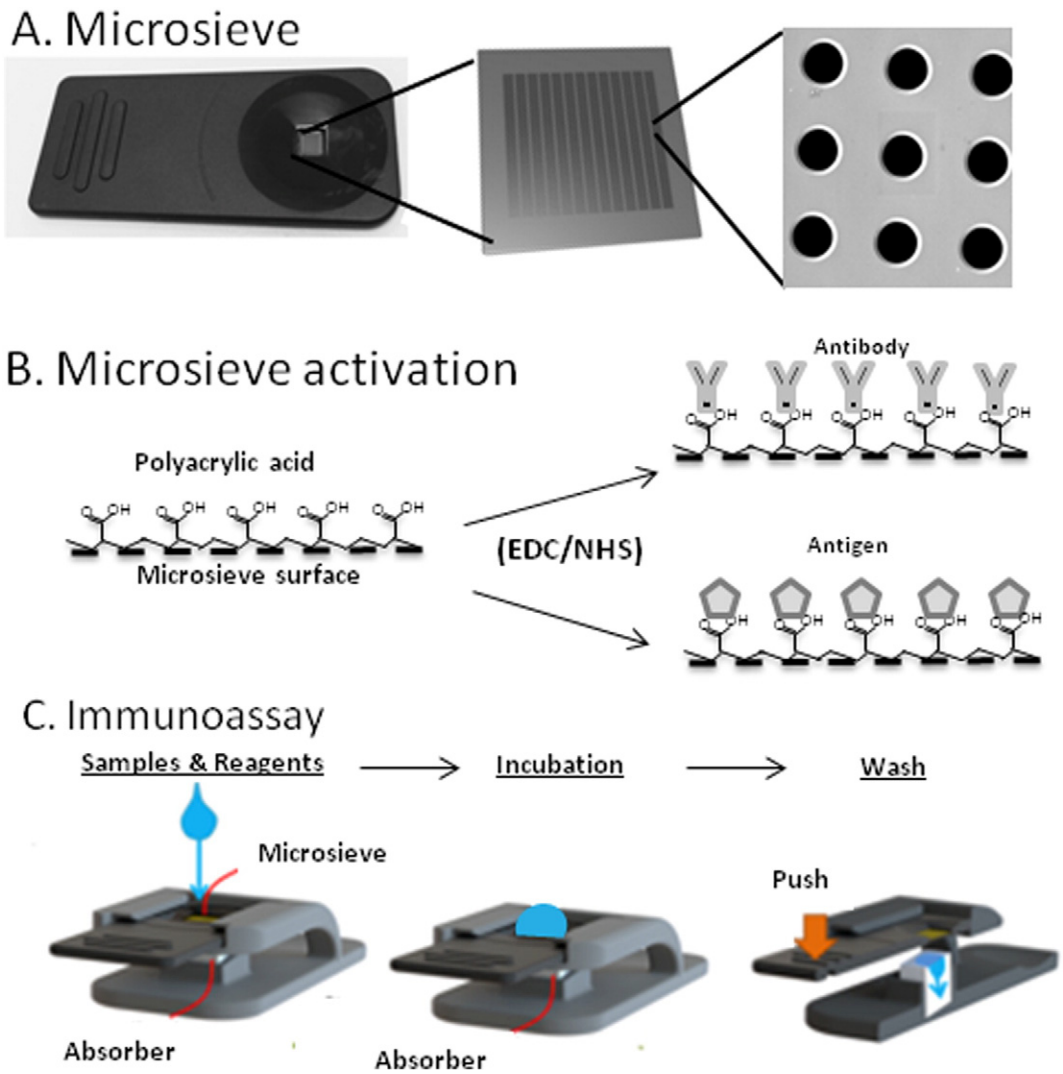

Fig. 1. (A) Images depicting a poly-acrylic acid coated 5 × 5 mm microsieve mounted within a plastic holder. The scanning electron micrograph of the microsieve surface highlights the shape and spacing of the 5 μm pores. (B) Schematic presentation depicting a general overview of how disease-specific capture biomolecules are covalently coated to the microsieve surface. (C) Simple wicking apparatus that enables reagent transfer during the microsieve-based immunoassay.