

論文の内容の要旨

Thesis Summary

論文題目

Fabrication and Design of Electrospun Polymeric Microfibers for Rapid Immunoassays

(高速イムノアッセイデバイスを目指したエレクトロスピンニングによるマイクロファイバーの設計と作製)

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本文

1. Introduction

Immunoassays have been leveraged for a number of years for the detection of antibodies and antigens a top a variety of platforms and systems. A major example of its use is diagnosing highly transmissible diseases that affecting millions worldwide, effectively costing the medical treatment industry billions of dollars every year [1], [2]. To overcome this many it has been suggested that immunoassays can be leveraged in methods such as point-of-care (POC) diagnostics to which patients are diagnosed of target diseases or analytes bedside. However, a number of hurdles are still in place which have limited the widespread commercial use of immunoassays for rapid and reliable diagnostics. In a consolidated review, Chin et al. details the development of POC diagnostics, noting that immunoassay tests can be more economical than nucleic acid tests at the current state and future direction [1]. In particular, it is important to emphasize the need for low-cost, simple and robust immunoassay detection methods, particularly for developing regions of the world, in order to improve global healthcare.

As noted by the World Health Organization (WHO) the “Affordable, Sensitive, Specific, User-Friendly, Rapid and Robust, Equipment-free and Deliverable to End Users” (ASSURED) method has been an integral focus for development [3], [4]. The desire is for the

development of rapid immunoassay-based tests with the development of new diagnostics which falls under three groups: 1. Improve existing tools, 2. Adapt available platforms, 3. Develop new platforms. The desire is a product with the combination of all three which are able to meet speed, sensitivity, and specificity requirements while also being simple, low tech and inexpensive [4].

The key objectives thusly are listed as follows:

1. To fabricate and design a new inexpensive, simple and low tech platform which can be implemented for rapid analyte capture
2. Leverage this platform and design a protocol for rapid immunoassay directly upon the platform surface
3. Introduce the system into a portable device and showcase a clinical application to which it can be applied to

As a whole, the novelty of this work is to introduce a platform, the polymeric electrospun microfibers to a new application of rapid immunoassay testing. Firstly, the microfibers will be fabricated to have tailored characteristics beneficial to rapid immunoassay testing such as a large surface area to volume ratio and layering to further increase this surface area while adding physical support to the membrane. Secondly, the introduction of a new testing protocol which utilizes this designed microfiber such that rapid immunoassay testing can be achieved was

completed. The capability of this system both with controlled vacuum pump pressures as well as a device-based hand pump environment can illustrate both how this system can benefit from bulk flow as well as that it can be applied to a handheld device type environment. Lastly, within the aforementioned handheld device, a proof of concept with clinical testing in mind, multiplexed rapid immunoassay spots atop the design fiber mats were also illustrated. The concept behind allowing for a rapid immunoassay which can detect multiple analytes within a handheld device can be considered to be applied to preventative healthcare measures for patient self-diagnosis for the need for vaccinations / immunizations. All in all, the low cost, low tech, simple to apply to a variety of instrument electrospun polymeric microfiber membrane for rapid immunoassay testing is the new concept being introduced within this paper, together with protocol designs which can match its goal of rapid testing capabilities.

2. Fabrication and Design of Electrospun Fibers for Immunoassay Applications

The first study, three-dimension membrane fabrication, involves leveraging a microfiber fabrication technique in the form of electrospinning, Polystyrene (PS) microfibers are fabricated in a number of varieties and measured for immunoassay relevant properties including pore size and surface area. Further, this is tested against the final goal of this section, to create a large surface area platform capable of high density antibody immobilization. Generally it was found that electrospun PS (ESPS) 10wt% was successful in creating a high antibody immobilization density platform in comparison to flat plate surfaces. In particular, layering said fibers was able to further increase the surface area and thus immobilization density.

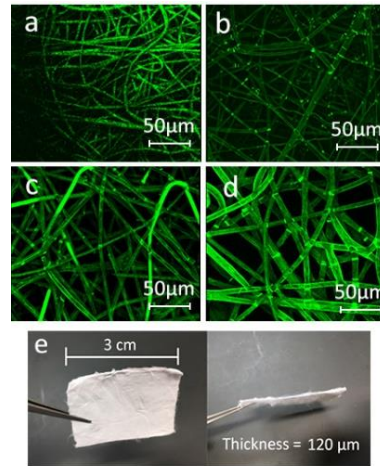


Figure 1. Confocal microscopic 3D projection compiled images of ESPS at: (a) 10 wt%, (b) 15 wt%, (c) 20 wt% and (d) 25 wt%. (e) A photographical representation of the measured ESPS fiber (10 wt% 4-layer).

From the aforementioned ESPS 10 wt% with high antibody immobilization, a rapid antigen capture protocol was designed. Rapid antigen capture was encouraged by combining the ESPS fiber mats with a bulk flow system (vacuum pump). This ESPS-bulk flow system displayed a capability for a greater ratio of antigen captured (captured antigen concentration to initial antigen concentration) in 5 seconds compared to that of the flat plate-diffusion conventional immunoassay in 60 minutes.

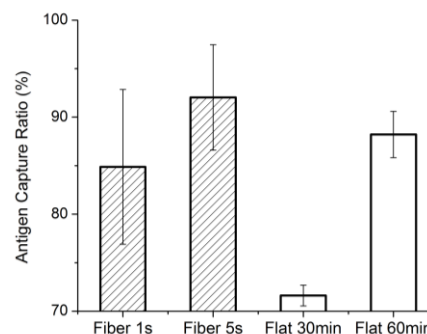


Figure 2. Capture percentage of antigen with varying incubation times. Initial concentration = 10 μg/mL.

Error bars illustrated are in standard error. Background signals from samples without antibodies nor antigens were subtracted for all conditions.

3. Design and Assessment of a Rapid Immunoassay Protocol Utilizing Electrospun Microfibers

To further the applicability of the previous study, the ESPS-bulk flow system's capability for rapid immunoassay testing directly upon the fiber mat was also assessed. Firstly it was found that within 2 minutes (including washing) human serum albumin (HSA) was able to be detected utilizing a fully rapid immunoassay protocol similar to that of the previous test's ESPS-bulk flow system. A range of HSA from 10 to 1000 ng/mL was able to be differentiated by fluorescence intensity signal as long as the labeled Ab (FITC anti-HSA) dilution was less than 1:100 dilution ratio.

In a similar study, Middle-East Respiratory Syndrome (MERS) was also tested utilizing the same fully rapid immunoassay protocol as the HSA testing method. It was found similarly that at labeled Ab (FITC anti-MERS) dilutions 1:200 or less, a difference between fluorescence intensity from 200 to 1600 ng/mL could be discerned.

As a conclusion to both of these tests, it can be first noted that detection of an antigen agent through the designed rapid immunoassay protocol can be completed within 2 minutes for detection.

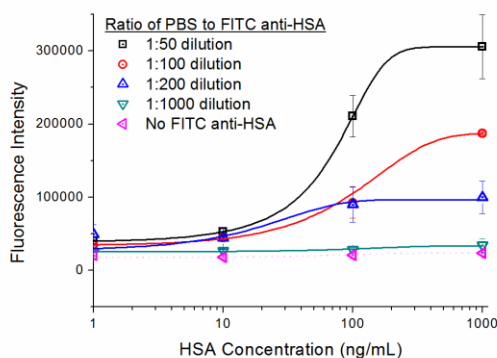


Figure 3. Rapid HSA and labeled Ab capture upon ESPS 10 wt% 4-layer samples. Each point indicates the average of 4 samples while error bars are standard error.

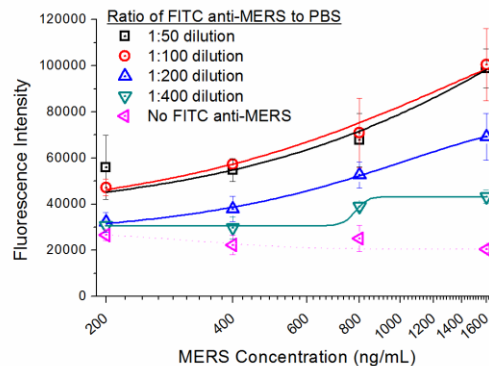


Figure 4. Rapid MERS immunoassay conducted on 4-layer ESPS 10 wt% at varied antigen and labeled antibody concentrations. Each point indicates the average of 4 samples while error bars are standard error.

4. Spot Detection Multiplex Immunoassay Design & Application

The prior chapters introduced the successes of the fully rapid FLISA system in introducing a methodology for rapid immunoassay testing. The next step to closing the gap in applying this system to a commercial setting would be introducing a means to get rapid immunoassay testing without the use of a vacuum pump (no external power source), utilization in a handheld device, as well as a proof of concept clinical application.

The handheld device was a de-constructible filtration device setup in such a way that a lateral-flow assay can be conducted in a manner using bulk flow induced upon the ESPS fiber mats fabricated. In addition, the ESPS fiber mats were pre-treated with O₂ plasma to create individual hydrophilic spots for spot detection of desired analytes. Follow this, a new rapid immunoassay protocol was designed such that antibodies could be detected upon the ESPS fiber mat in a multiplexed manner. It was found that with both HSA and MERS antigens immobilized to separated spots as well as spots with no antigen immobilized, a clear distinguish of anti-HSA and anti-MERS captured to their respectively

immobilized antigen is noted. Further, when a mixed solution of anti-HSA and anti-MERS was flushed through the fiber mat, a multiplex detection of each upon individual spots could be noted while the negative spots showed no signal intensity as expected. Finally, all tests were conducted with a detection time of <4 minutes. However on top of this, the setup of the immunoassay itself (antigen immobilization and blocking) together with the detection time, from start to finish only required <10 minutes.

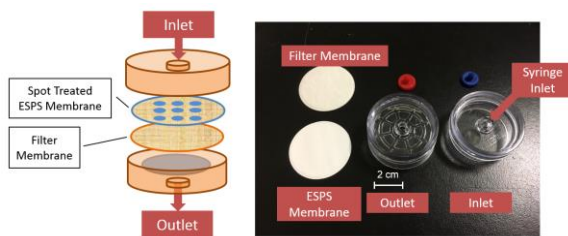


Figure 5. Schematic and photographical representation of the housing environment and membrane setup.

Figure 6. a. Bar graph representation of multiplex testing, b. schematic of the Ag immobilization setup, c. FITC anti-MERS only solution, d. FITC anti-HSA only solution, e. multiplex. Each bar indicates the average of 3 samples while error bars are standard error. 1:100 and 1:50 dilutions of FITC anti-HSA and FITC anti-MERS were used respectively in all solutions.

5. Conclusion & Future Work

The study objective within this thesis was to introduce a method to conduct rapid immunoassay testing to add to the wealth of information that currently exists within the field of immunoassay type devices. Rapid immunoassays have currently been limited in terms of simplicity, low tech and low cost construction while maintaining performance. The overall goal was to fabricate a platform, introduce a device with the platform and create a protocol which overcomes all of these barriers such that rapid diagnostic speeds can be achieved whilst maintaining sensitivity.

This work was able to introduce a design for ESPS to be applied together with a bulk flow system for rapid immunoassay capabilities. A protocol was created that could rapidly conduct immunoassays with sensitivity levels comparable to a conventionally used flat plate-diffusion based assay. Finally, with multiplexed preventative diagnostics in mind as a clinical application, this portable device ESPS-bulk flow system was able to conduct rapid multiplex immunoassay testing upon directly upon its surface.

The introduced platform, protocol and methodology in this thesis can act as a starting point to encourage research guided towards utilizing an ESPS-bulk flow system for rapid immunoassay testing. An immunoassay capable of rapid detection with a wide range of picogram to milligram, semi-quantitative detection could possibly be achieved by a device which leverages concepts and ideas from this thesis.

Work Cited

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