Electrospun Polymeric Microfiber Substrates for Rapid Protein and Cell-based Assays

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Biosensing devices such as assays require both speed and sensitivity as two integral components for its effectiveness. Within this review, developed three-dimensional (3D) microfiber polystyrene (PS) platforms are leveraged to introduce a means for effectively rapid and sensitive assays. Considered broadly, the 3D structure contributes to an increased surface area for antibody immobilization while the natural hydrophobic nature of PS promotes this immobilization for immunoassay diagnostics. More specifically, rapid antigen capture can be realized by combining this platform with vacuum pressure to mitigate diffusion limitations for antibody-antigen interactions. Alternatively, the increased antibody immobilization density can realize the selective capture and release of circulating tumor cells (CTC). Through both low concentration CTC capture and effective release of said cells, patient-specific cancer care can be achieved.

Keywords: Electrospinning, Microfiber, Polystyrene, Assay, Circulating tumor cells

1. Introduction

Recently, there has been an influx and large interest in clinical research involving bedside patient care in the form of “point of care testing” (POCT). To realize the full potential of POCT systems, both the speed and convenience of biosensing systems or device must be optimized [1–3]. Clinical diagnostics, for example, typically require long assay times and would benefit from immediately testing and diagnostics at the patient’s site [1,4–6]. Furthermore, in the case of cancer metastasis, it has been reported that circulating tumor cells (CTC) and excreted vesicles (50-150 nm in size) known as exosomes which have infiltrated the blood vessels can be measured and correlated to the level of metastasis [7–12].

By leveraging microfiber technology as the platform basis, this review illustrates the capability of rapid and convenient detection of blood sample viral and cancer markers. More specifically, this review examines the use of polystyrene (PS) as the base material for fabricated fiber mats. PS fibers offer the benefit of, first, the hydrophobic nature of the polymer promoting antibody immobilization through hydrophobic interactions, and secondly, the three dimensional large surface area of a fiber mat which can offer a high antibody immobilization density [13].

To promote the desired rapid antigen detection, convection through vacuum pressure was introduced to promote the antibody-antigen interaction while also effectively washing the fiber sample internal structure to prevent any background noise during diagnostics. In this case, in contrast to a conventional biopsy, a liquid biopsy is conducted where bodily fluids containing cancer cells and its components are targeted. In terms of cancer treatment, the treatment must be patient specific with thus the CTCs acting as markers [14]. By successfully capturing CTCs to the fiber mats and further being able to release these cells, they can be thoroughly examined to determine patient specific cancer care [15,16].

2. Rapid immunoassay development [13]

The following study illustrates the utilization of microfiber PS platforms in combination with vacuum-driven bulk flow to induce a rapid and
Fig. 1. SEM images of ESPS at (a) 5 wt%, (b) 10 wt%, (c) 15 wt%, (d) 20 wt%, and (e) 25 wt%.

highly sensitive immunoassay. This study utilized model proteins in the form of human serum albumin (HSA) as the antigen unit. The overall immunoassay was conducted as a sandwich assay with initially immobilized primary anti-HSA to capture the HSA antigen followed by a FITC labelled secondary anti-HSA antibody for detection.

The fabrication of PS microfibers was conducted through electrospinning technology. PS pellets ($M_w = 9.0 \times 10^5$) were dissolved in a 1:1 ratio solution of tetrahydrofuran (THF) and $N,N$-dimethylformamide (DMA). PS pellets were introduced varying from 5 to 25 w/v%. Furthermore, Triton X was added as a surfactant to each solution at 0.1 w/v%.

The fabricated microfiber PS samples were qualitatively and quantitatively assessed under imaging microscopy to determine its morphological characteristics. Scanning electron microscopy (SEM) images (Fig. 1) qualitatively illustrated the overall increasing fiber thickness with increasing weight percent with overall uniformity excluding 5 w/v%. Confocal microscopy was then utilized to quantitatively determine the ideal microfiber PS for antibody immobilization. The overall goal is to achieve a platform with a high antibody immobilization density without increasing the overall size. As such, the surface area / volume ratio was measured to which PS 10 w/v% showed the highest value thus indicating the capability for the highest immobilization density (Table 1).

Table 1. Surface area / volume and average pore size values measured from ESPS fiber mat substrates.

<table>
<thead>
<tr>
<th>ESPS wt%</th>
<th>Surface Area / Volume</th>
<th>Average Pore Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.700</td>
<td>2.54 ± 1.46</td>
</tr>
<tr>
<td>15</td>
<td>0.521</td>
<td>3.44 ± 1.72</td>
</tr>
<tr>
<td>20</td>
<td>0.408</td>
<td>7.76 ± 3.84</td>
</tr>
<tr>
<td>25</td>
<td>0.288</td>
<td>13.18 ± 9.89</td>
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Following the selection of PS 10 w/v% for further testing, an increase in surface area for antibody immobilization was desired for comparison. Multiple layers of the same PS 10 w/v were stacked upon one another following the ‘wet-press’ technique introduced by Wu et al. [17]. 1 to 4 layers of PS 10 w/v% were stacked and then compared in terms of antibody immobilization amount. Additionally, a flat PS plate typically suggested in enzyme-linked immunosorbent assay (ELISA) kits was also used as a comparison. All of the microfiber PS platforms showed a higher antibody immobilization than the flat PS plate with increasing immobilization with an increasing number of layers. Furthermore, the 4-layer microfiber PS indicated a 6-fold increase in antibody immobilization compared to that of the flat PS plate (Fig. 2).

Fig. 2. Antibody concentration captured to both the flat plate surfaces and varied ESPS fiber mats. Initial concentration = 10 µg/mL. Error bars illustrated are in standard error. Background signals from samples without antibodies were subtracted for all conditions.

Lastly, the capability of antigen capture and overall immunoassay upon the microfiber PS platforms were assessed. Again, in comparison to the flat PS plate samples, a 5 second bulk flow antigen capture system with the microfiber PS platforms were able to exceed the sensitivity to that of the 60 minute diffusion based flat PS plate antigen capture protocol (Fig. 3). Additionally,
completing a fluorescence based immunoassay upon both the microfiber and flat plate PS samples, the microfiber sample was not only able to capture the antigen within 5 seconds but also increase the linear range of detection from 5 to 1000 ng/mL in comparison to the flat plate PS at 5 to 100 ng/mL (Fig. 4).

Fig. 3. 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. Development of a specific cell capture and release system [9,10]

The next study introduced the utilization of microfiber PS platforms in combination with vacuum-driven bulk flow for the application to the device of CTC capture and release from patient’s blood. In this case, the ESPS fiber with a pore size of about 10 µm were used for the specific capture of CTCs [10]. Therefore, the 20 wt% PS concentration of ES was used. The antibody was immobilized using functional peptides, not physical adsorption, which has a cleavable site for collagenase type IV (Fig. 5) [18–20]. This peptide conjugated to anti-EpCAM antibodies enables gentle detachment of CTCs captured on antibody-immobilized ESPS fiber.

Fig. 4. Fluorescence based immunoassay results for both PS flat and ESPS fiber systems with changing antigen (HSA) concentrations. Best fit curves are included based on a 4 parameter fit.

Fig. 5. (a) Schematic images of cancer cell capture and detachment using the functional peptide and collagenase type IV. (b) Structure of the peptide and cross-linkers for cell capture and detachment. The antibody and peptide were combined using a cross-linker containing polyethylene glycol (PEG).

The peptide and antibody were combined using polyethylene glycol (PEG) cross-linkers, and then the peptide-conjugated antibody was immobilized onto ESPS fiber. Before conducting the cell experiments, model cells, MCF-7 cells as CTCs, and CCRF-CEM cells as blood cells, were fluorescently labeled with Cell Tracker Green and Cell Tracker Orange respectively. The mixed suspension of MCF-7 and CCRF-CEM cells was passed through the ESPS fiber with vacuum aspiration and the microfiber mats were observed by confocal laser scanning microscopy (Fig. 6). Furthermore, the correlation between the fiber thickness and capture efficiency was examined. The microfiber fabrics of 0.7 mm, 1.3 mm and 2 mm thickness were prepared by vertically stacking and modified with peptide-conjugated antibody. The MCF-7 cells were passed through the prepared ESPS fiber, and then the cells captured on the microfibers were counted (Fig. 7).

These results demonstrated that peptide-conjugated antibody-immobilized ESPS fiber could specifically capture the $10^5$ MCF-7 cells in $10^6$ non-targeted cells. CCRF-CEM cells were not observed on the microfibers because the pore size would be appropriate for non-targeted cells passing through and non-specific capture was suppressed by vacuum
system. Additionally, ESPS fiber mats with 2 mm thickness could achieve about 80% capture efficiency.

Finally, the cell-release experiment was conducted. After cell suspension passed through the ESPS fiber mats immobilized with the peptide-conjugated antibody, the ESPS fiber mats were treated with collagenase type IV solution for 30 min and rinsed with vacuum aspiration. Fig. 8 shows the fluorescence images before and after collagenase treatment. From this result, it was found that captured cells could be released from the ESPS fiber using collagenase type IV.

In conclusion, this study illustrated the appropriate development of the functional peptide which has a cleavable sequence and demonstrated that the vacuum system with this peptide enables specific cell-capture and -release.

4. Conclusion

Through the development and control of electrospun PS microfibers, protein and cell-based assays were designed. The overall increase in surface area first allowed for an increased density of capture antibodies immobilized to the surface. In particular, the combination of the fabricated microfibers with a vacuum-pressured bulk flow system allowed for a highly rapid protein assay,
within seconds, while not only maintaining sensitivity but also an increase range of detection. Furthermore, when the same microfiber mat system was specifically considered for CTC capture, it could successfully not only capture, but also release the cells in a timely and effective manner.

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