Investigation of Functional Electrospun Bionanofibers in Microfluidic Channels

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Abstract:

In this study, we investigated the incorporation of poly(vinyl alcohol) (PVA) nanofibers within poly(methyl methacrylate) (PMMA) microchannels in order to allow for sample preparation and concentration within microfluidic detection systems. Nanofibers were electrospun onto gold microelectrodes and integrated into polymer microfluidic systems using ultraviolet (UV)-assisted thermal bonding. We determined that nanofibers spun across microchannels maintain their morphology during fluid flow at linear velocities of 3.4 and 13.6 mm/s. In addition, we showed the ability of positively charged nanofibers to serve as bioseparators for negatively charged nanovesicles using hexadimethrine bromide (polybrene) modified PVA fibers.

Summary of Research:

Sample purification and concentration are essential to the success of miniaturized detection assays that use small feature sizes and sample volumes in the nL-µL range [1]. Lab-on-a-chip devices aim to incorporate sample preparation and analyte detection within the same device, however this has proven difficult for many detection systems [2]. We aim to address this need for sample preparation within microfluidic systems by incorporating functionalized electrospun nanofibers within polymer microchannels.

Gold microelectrodes were fabricated on PMMA using standard microfabrication techniques. A 10 nm layer of chrome and a 200 nm layer of gold were evaporated onto the PMMA using a CHA evaporator. After evaporation, the PMMA was coated with positive photoresist (Shipley 1813) at 3000 rpm for 30 seconds. The PMMA was then exposed for 11 seconds using an ABM contact aligner and developed for one minute in MF 321. The PMMA was then etched for one minute in gold etchant and 15 seconds in chrome etchant.

We studied the effects of electrode spacing and width on nanofiber orientation using a basic five-fingered electrode design with a large square electrode pad. Electrodes were fabricated with various gap sizes (0.1 mm to 10 mm), square sizes (50 µm to 500 µm), and electrode widths (1 mm to 2.5 mm). We determined that electrodes with a gap of 5 mm and width of 1 mm produced well aligned nanofiber mats between the electrode fingers (Figure 1).

The PMMA microchannels were formed using hot embossing with a copper template [3]. The copper masters were fabricated using negative photoresist (SU-8) photolithography and copper electroplating to produce raised copper channels on the copper plate. The microchannels were embossed onto PMMA using a Carver Laboratory Hot Press. Completed microfluidic devices were fabricated by bonding a piece of PMMA containing channels and a piece of PMMA patterned with a microelectrode and nanofibers using UV-assisted thermal bonding.

Nanofibers spun across microfluidic channels were subjected to fluid flow and analyzed to confirm that the fibers were not damaged during fluid flow. DI water was injected into the microchannels at 3.4 and

Figure 1: Five fingered electrode design with 5 mm spacing.
13.6 mm/s for 400 and 100 minutes respectively. The effluent was collected and analyzed using Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). There are two FTIR peaks that indicate the presence of PVA in a solution: one for CH₂ (2930 cm⁻¹) and one for CH (2850 cm⁻¹). These peaks were not observed in the effluent samples, confirming that the fibers were retained within the channels during prolonged fluid flow. NMR analysis was also used to determine fiber stability during fluid flow. There are two NMR peaks characteristic of CH₂ in PVA, neither of which was observed in the effluent samples, once again confirming the stability of the fibers during fluid flow. We therefore concluded that the nanofibers are sufficiently stable for use in microfluidic devices for bioanalysis.

We fabricated positively and negatively charged nanofibers by incorporating hexadimethrine bromide (polybrene) and poly(methyl vinyl ether-alt-maleic anhydride) (poly(MVE/MA)) into a PVA spinning dope. Nanofiber composition was examined using FTIR and x-ray photoelectron spectroscopy (XPS) to confirm the incorporation of polybrene and poly(MVE/MA) within the fibers. Thermally stimulated current (TSC) measurements in pH 7 buffer solution confirmed the positive surface charge on fibers containing polybrene and negative surface charge on fibers containing poly(MVE/MA) [4].

Liposomes were used as a model analyte, and were modified with sulforhodamine B to allow for fluorescence imaging. Microchannels containing positively or negatively charged nanofibers were filled with HEPES-Sucrose-Saline (HSS) solution (pH 7) and a 1:1000 dilution of fluorescent liposomes at a flow rate of 1 µL/min. The liposome solution flowed through the channels for 30 minutes and was then washed out of the channels using a HSS solution at 1 µL/min for 60 minutes. The concentration of the liposomes within the channels was determined by measuring the fluorescence within the channels over time. As expected, channels containing positively charged nanofibers attracted and retained the liposomes, while channels containing negatively charged nanofibers did not retain a significant number of liposomes (Figure 3). The binding of liposomes to the positively charged fibers was quantifiable already during liposome flow and pre-wash buffer addition as peak fluorescence signals were significantly higher.

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