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Poly(oligoethylene glycol methacrylate) Dip-Coating: Turning Cellulose Paper into a Protein-Repellent Platform for Biosensors

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Supporting Information

ABSTRACT: The passivation of nonspecific protein adsorption to paper is a major barrier to the use of paper as a platform for microfluidic bioassays. Herein we describe a simple, scalable protocol based on adsorption and cross-linking of poly(oligoethylene glycol methacrylate) (POEGMA) derivatives that reduces nonspecific adsorption of a range of proteins to filter paper by at least 1 order of magnitude without significantly changing the fiber morphology or paper macroporosity. A lateral-flow test strip coated with POEGMA facilitates effective protein transport while also confining the colorimetric reporting signal for easier detection, giving improved performance relative to bovine serum albumin (BSA)-blocked paper. Enzyme-linked immunosorbent assays based on POEGMA-coated paper also achieve lower blank values, higher sensitivities, and lower detection limits relative to ones based on paper blocked with BSA or skim milk. We anticipate that POEGMA-coated paper can function as a platform for the design of portable, disposable, and low-cost paper-based biosensors.

Paper-based devices have attracted widespread interest as portable, low-cost, low-volume, disposable, and simple analytical platforms for bioassays, point-of-care diagnostics, and environmental analysis.[1] Paper-based devices with direct reporting by changes in color,[2] fluorescence,[3] chemiluminescence,[4] or other easily identifiable signals offer particular benefits in resource-limited settings for diagnosis or screening without the need for complex analytical equipment.[2a,5] While several proof-of-concept studies have shown the potential of paper-based devices for meeting these challenges, nonspecific protein adsorption significantly limits both the accuracy and selectivity of such sensors.[1a,6] Typically, this problem is addressed by blocking the nonfunctionalized paper surface with bovine serum albumin (BSA)[2b] or other proteins immediately before use (see Table 1 in ref 1b for other methods used). However, protein blocking is an inconvenient and only partially effective additional step that limits the facile use of paper-based biosensors in the field.

Reducing protein adsorption at interfaces has long been a focus in the biomaterials literature[7] given the oft-cited link between protein adsorption and inflammation.[8] Typically, this is achieved by surface modification of the biomaterial with hydrophilic polymers,[9] with poly(ethylene glycol) (PEG) attracting particular interest.[10] Poly(oligoethylene glycol methacrylate) (POEGMA) exhibits similar non-cytotoxic and protein-repellent properties as PEG[11] while offering the advantage of facile copolymerization or grafting via free radical chemistry.[11c,12] However, the fragility of highly porous papers used for paper-based microfluidics at the typically elevated temperatures and high free radical concentrations required for grafting limits the practical utility of this approach.

Herein we demonstrate a mild and effective approach for passivating protein adsorption on cellulose paper via a simple, scalable sequential dipping method. Smeets and co-workers recently reported the formation of protein-repellent hydrogels based on POEGMA precursor polymers functionalized with hydrazide and aldehyde groups that form gels rapidly by simple mixing at ambient conditions (Figure 1a).[13] Here we apply this chemistry in a sequential dipping strategy to localize hydrogel formation on the fiber surface of filter paper (Figure 1b). Adsorption of aldehyde-functionalized POEGMA (POA) to the paper functionalizes the paper surface with aldehydes, while subsequent dipping into a hydrazide-functionalized POEGMA (POH) solution effectively assembles a thin POEGMA hydrogel layer directly on the fiber surface (Figure 1c).

The efficacy of POEGMA coating for modification of paper interfacial properties was first screened using a model cellulose surface. A cellulose-coated quartz crystal microbalance (QCM) chip was POEGMA-coated by sequentially flowing POA and POH over the chip (Figure 2a). The water contact angle slightly increased from 33 ± 1° (cellulose chip) to 52 ± 1° (with POA) and 53 ± 1° (with POA/POH) (Figure 2b), indicating that the hydrophilicity of the fiber interface was maintained while the potential for steric protein repulsion (characteristic of PEG coatings[10c]) was introduced. Four model proteins with different isoelectric points (pI) and molecular weights (MW) [see Table S1 in the Supporting Information (SI)] were then flowed over the POEGMA-coated chip to assess the capacity of the coating to inhibit protein adsorption (Figure S1 in the SI). A decrease in protein adsorption by at least 1 order of magnitude was observed for all proteins relative to an unmodified cellulose chip, independent of pI or MW (Figure 2c).

On the basis of this result, coatings were subsequently applied to cellulose filter paper (Whatman no. 40 ashless). The dipping procedure induced no significant changes in the fiber or pore morphology of the paper in either the dry state (scanning

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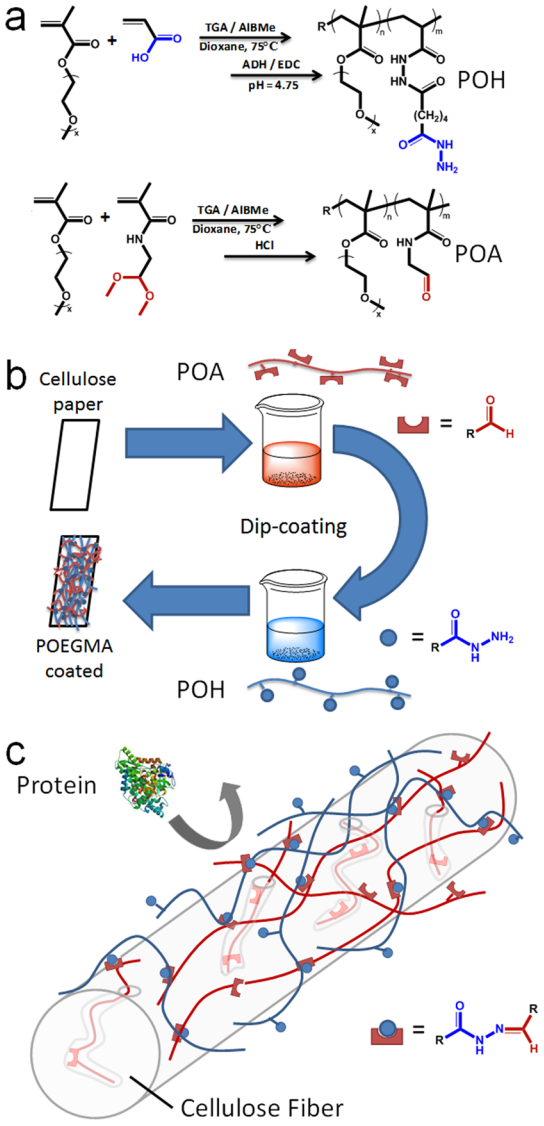

Figure 1. (a) Synthesis of hydrazide-functionalized POEGMA (POH) and aldehyde-functionalized POEGMA (POA). (b) Dip-coating procedure for modifying filter paper. (c) Hypothesized structure of surface-modified cellulose fibers and resulting mechanism of protein repellency.

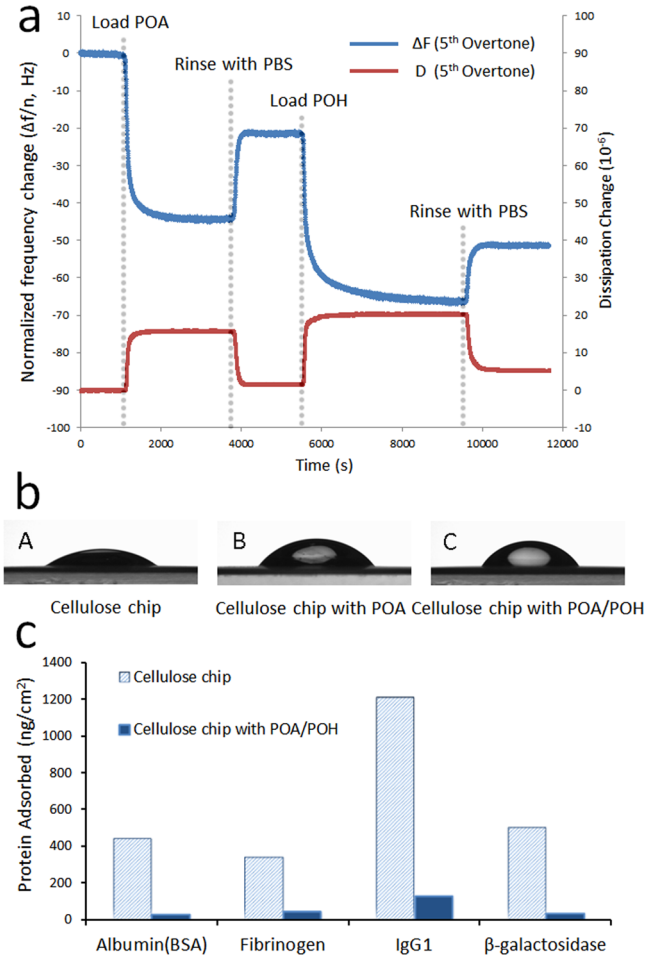

Figure 2. Interfacial properties of POA/POH-modified cellulose-coated QCM chips. (a) QCM-D monitoring of POA and POH adsorption (both 4% w/v) on a cellulose QCM chip. (b) Contact angle of water on a cellulose QCM chip before and after POA/POH coating. (c) QCM-D monitoring of protein adsorption on a cellulose QCM chip before and after POA/POH coating (100 μg/mL protein).

electron microscopy; Figure S2A–F) or the wet/swollen state (optical profilometry; Figure S2G–I), and no significant difference in surface roughness was observed for unmodified paper and POA or POA/POH dip-coated paper ($p > 0.05$). In addition, tensile testing of unmodified and POA/POH-modified paper strips indicated that the dipping procedure either maintained or slightly increased all of the key indicators of paper mechanics (Table S2). These results suggest that the dip modification procedure impacts neither the physical structure of the fiber nor the macroporous network morphology of the paper, unlike other techniques for polymer surface modification that cause significant fiber damage and/or clog the pore network and thus impact the paper's functionality as a microfluidic device.[14]

Confocal microscopy of dip-coated paper prepared by sequential dipping in rhodamine-labeled POA and fluorescein-labeled POH confirms adsorption of POA on the fiber surface throughout the paper cross section and suggests that dipping fills small pores inside the fibers (see Figure 3a-i). Subsequent colocalization and immobilization of POH on the fiber surface following the second dipping step are also confirmed (see Figure 3a-ii). Attenuated total reflectance FTIR spectroscopy shows an ester peak at 1727–1735 cm^{-1} (characteristic of POEGMA) that increases in intensity after each dipping step (Figures S3 and S4), and the dry weight of the treated papers increases significantly following each dipping step performed (Figure S5); both of these observations confirm deposition of the POEGMA polymers on the paper. Interestingly, following soaking of the papers over 24 h in phosphate-buffered saline (PBS), the POA/POH dip-modified paper shows a lower wet mass than unmodified paper (Figure S5). We hypothesize that this result is attributable to the filling of smaller pores inside and between cellulose fibers with polymers during the dipping procedure and restriction of the swelling of those polymers by the rigid cellulose network around those pores. This hypothesis is supported by the enhanced fluorescence observed at the edge and junction points between fibers (arrowheads in Figure S6A) and mercury porosimetery results that indicate a decrease in the total free pore volume upon POA/POH dipping accompanied by the effective disappearance of smaller pores (<0.1 μm) in the fiber network (Figure S7). Thus, while the dipping treatment does influence the fiber

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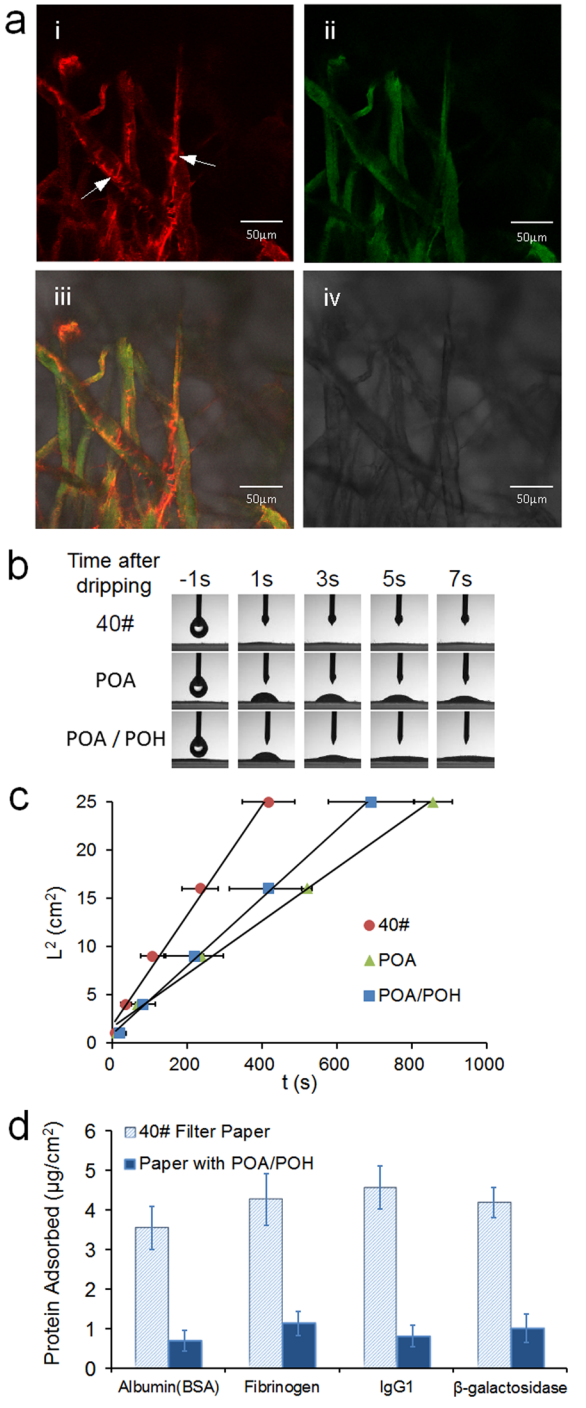

Figure 3. Interfacial and transport properties of POA/POH dip-coated Whatman no. 40 filter paper. (a) Filter paper fiber network following dip-coating of fluorescently labeled POA and POH viewed by confocal laser scanning microscopy (CLSM): (i) rhodamine 123-labeled POA; (ii) fluorescein-labeled POH; (iii) merged CLSM image; (iv) bright-field image. Arrowheads indicate the distribution of POA inside the cellulose fibers. (b, c) Hygroscopicity and surface properties of no. 40 filter paper before and after dip-coating with POA and POA/POH: (b) screenshots of sessile drop testing of the contact angle of filter paper samples; (c) plot of the square of the capillary rise distance (L^2) against capillary time (t) for a 0.8 cm × 8 cm paper strip ($n = 4$). (d) Adsorption of proteins on paper samples before and after POA/POH coating (protein concentration of 100 μg/mL, $n = 6$).

nanostructure, it does not significantly impact the paper macroporosity or topology.

To confirm that POA and POH chemically cross-link on the paper surface, residual aldehyde and hydrazide groups were reacted with fluorescein-5-thiosemicarbazide (5-FTSC) and 5-fluorescein isothiocyanate (5-FITC) respectively. POA-treated paper exhibits the same 5-FITC intensity but twice the 5-FTSC intensity compared with untreated paper, indicating adsorption of POA. POA/POH-treated paper exhibits the same 5-FTSC intensity of native paper (indicating consumption of POA aldehyde groups) but double the 5-FITC intensity, suggesting the presence of residual hydrazide groups (Figure S8). This result, coupled with the fluorescence result in Figure 3a, indicates that POA and POH chemically cross-link on the fiber surface to create a thin interfacial hydrogel layer.

The impact of POA/POH dip-coating on paper interfacial properties was assessed by measuring the penetration speed of water into and through the paper. Screenshots of sessile drop tests on filter paper samples before and after POA/POH dipping are shown in Figure 3b. The penetration speed of water into the paper varies as unmodified paper > POA/POH paper > POA paper. A similar result was observed via capillary rise experiments tracking the lateral flow of water through the filter paper before and after POEGMA dip-coating (Figure 3c). The speed of capillary rise obeys the Washburn equation, which in the case of zero applied pressure can be expressed as $L^2 = tD\gamma \cos\theta/4\eta$,[15] where L is the height of water penetration up the strip, t is the time, η is the viscosity, γ is the surface tension, θ is the contact angle, and D is the pore diameter. The slope of a plot L^2 versus t (representing differences in $D \cos\theta$ at constant fluid properties) varies as unmodified paper > POA/POH paper > POA paper, mirroring the sequence of water drop penetration speeds. Both results are consistent with the dipping procedure filling small pores inside cellulose fibers (lower D; Figure 3a-i) and slightly decreasing $\cos\theta$ (Figure 2b) while still preserving sufficiently fast water transport for microfluidic biosensor applications.

Consistent with the cellulose-coated QCM chip results, POA/POH coating on filter paper facilitated at least a 4-fold decrease in adsorption for all tested proteins (Figure 3d). No further reduction was noted following layer-by-layer deposition of additional POA/POH layers (Figure S9). On the basis of this result, the utility of POA/POH dip-coated paper as a platform for paper-based microfluidic or lateral flow devices was assessed. A model reaction in which β-galactosidase (β-GAL, enzyme) converts chlorophenol red β-galactopyranoside (CPRG, yellow, substrate) to chlorophenol red (red-magenta, product) was selected; capture of the chlorophenol red product by poly(L-arginine) in the sensing area generates a purple reporting signal indicative of capillary transport of the enzyme solution up the paper test strip (Figure 4a). Untreated paper cannot support the transport of β-GAL, and therefore, no purple band appeared (a1). BSA-blocked paper supported protein transport, but the signal was broadly dispersed up the test strip, suggesting that BSA blocking interferes with the activity of the color-capturing agent (a2). In contrast, POA/POH dip-coated paper successfully facilitated protein transport (signal generation) and confined the signal to the detection area (a3) at the cost of slightly increasing the test time due to the decreased rate of capillary rise through the POA/POH-coated paper (5.3 ± 0.7 min vs 3.7 ± 0.6 min for BSA-blocked paper, 4 cm transport length).

On the basis of this successful initial assay, POA/POH dip-coated papers were subsequently assessed as supports for paper-based enzyme-linked immunosorbent assays (ELISAs) (Figure 4b) using goat anti-rabbit IgG as the antigen and horseradish peroxidase (HRP)-conjugated rabbit IgG as the enzyme-linked

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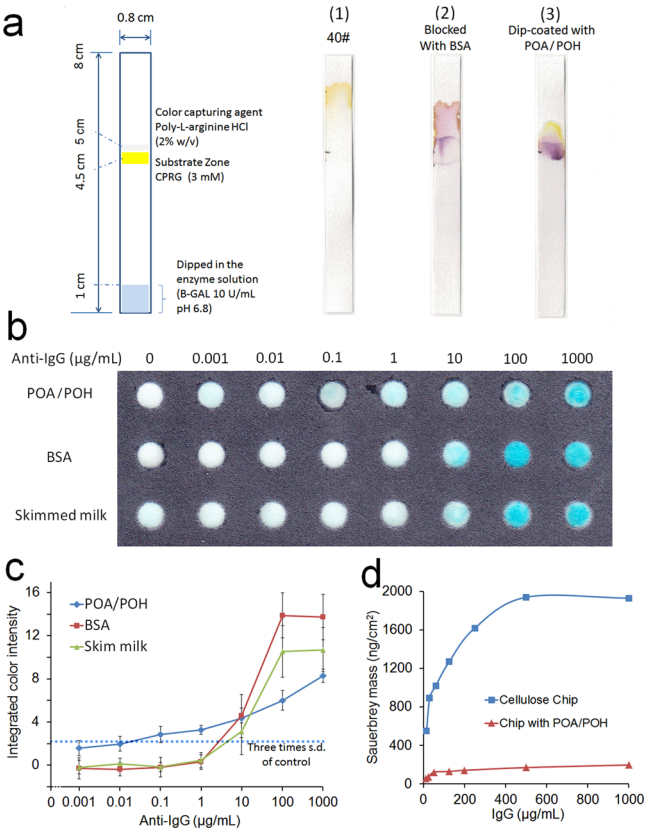

Figure 4. Model paper-based diagnostics using POA/POH dip-coated paper. (a) Schematic of β-galactosidase (β-GAL) test strip design and experimental mobility of β-GAL on 0.8 cm × 8 cm paper strips: (a1) pipetted control paper; (a2) BSA-blocked paper (0.1% w/v); (a3) POA/POH dip-coated paper. (b) Paper-based ELISA on a wax-printed microzone plate using dip-coated POA/POH as the blocking reagent, compared with BSA and skimmed milk. (c) Color intensity of paper-based ELISA using different blocking reagents (the blue dashed line represents the LOD, 3× the standard deviation of the nonblocked control). (d) Isothermal adsorption of rabbit immunoglobin (IgG) on a cellulose-coated QCM chip with or without POA/POH coating from QCM-D analysis.

antibody. The use of POA/POH dip-coating to block the filter paper resulted in a lower blank signal and a lower limit of detection (LOD) of between 0.1 and 1 μg/mL antigen relative to the use of BSA or skim milk, both of which result in significantly higher LODs (between 1 and 10 μg/mL) and lower dynamic ranges (Figure 4c). Given that POEGMA-coated cellulose can maintain nonspecific IgG adsorption at very low levels (<150 ng/cm^2), ~3-fold lower than for BSA-blocked paper (Figure S11), even at high concentrations (>1 mg/mL; Figure 4d), we hypothesize that POA/POH dip-coated papers are ideal platforms for performing ELISAs with a wide range of antibodies.

In summary, we have demonstrated the effective surface modification of paper via a simple, scalable, and mild dip-coating procedure that significantly suppresses nonspecific protein adsorption to paper without impacting the fiber morphology or paper macroporosity. We anticipate that POA/POH-coated paper has potential as a platform for the design and fabrication of complex biosensors, bioarrays, and other high-throughput tests in which protein transport is essential. Such modified papers may also have applications in the design of nonfouling filter papers for protein separation or protein chromatographic supports.

■ ASSOCIATED CONTENT

Supporting Information

Materials and methods, full polymer characterization, paper mechanical data, mercury intrusion results, surface topologies, dry and wet weights, FTIR data, and raw QCM results. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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