Two-Dimensional Antifouling Fluidic Channels on Nanopapers for Biosensing

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ABSTRACT: Two-dimensional (hydrophilic) channels were patterned on films prepared from cellulose nanofibrils (CNF) using photolithography and inkjet printing. Such processes included UV-activated thiol-yne click coupling and inkjet-printed designs with polystyrene. The microfluidic channels were characterized (SEM, wetting, and fluid flow) and applied as platforms for biosensing. Compared to results from the click method, a better feature fidelity and flow properties were achieved with the simpler inkjet-printed channels. Human immunoglobulin G (hIgG) was used as target protein after surface modification with either bovine serum albumin (BSA), fibrinogen, or block copolymers of poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) and poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) (PDMAEMA-block-POEGMA copolymers). Surface plasmon resonance (SPR) and AFM imaging were used to determine their antifouling effect to prevent nonspecific hIgG binding. Confocal laser scanning microscopy revealed diffusion and adsorption traces in the channels. The results confirm an effective surface passivation of the microfluidic channels (95% reduction of hIgG adsorption and binding). The inexpensive and disposable systems proposed here allow designs with space-resolved blocking efficiency that offer a great potential in biosensing.

INTRODUCTION

Traditionally, materials employed as support for medical and diagnostic applications are made of nonrenewable sources, including plastics.1–6 Cellulosic-based platforms are sustainable alternatives in these applications. They have beneficial properties such as biocompatibility, controllable morphology, hydrophilicity, and nontoxicity, all of which are desired in the development of new functional substrates for diagnostics.3,4 Moreover, compared to most plastics, cellulose displays better thermal durability and in their nanoscale forms (such as cellulose nanofibrils, CNF), they can be assembled to develop high strength, large surface area, and low density.7 When water is removed from a CNF hydrogel, capillary forces cause fibrils to pack into dense structures, for example, nanopapers or films. The main methods used in their synthesis include vacuum filtration, spraying, pressure filtration, and solvent casting.8 A wide selection of cellulose nanomaterials can form smooth, strong, uniform, and translucent/transparent nanopapers.7,8 They have excellent heat and chemical stability.8

Recently, paper and nanopapers have been considered for microfluidic applications.3–11 The highly porous nature of regular paper facilitates (3D) fluid flow due to capillary effects, where the surface tension and adhesive forces propel the liquid.12 In contrast, liquid does not penetrate into the much denser films produced from CNF. However, surface (2D) flow can still occur under the action of wetting.13,14 However, this demands the application of suitable modifications to control the flow direction on the surface. These include the use of hydrophobic patterning via physical or chemical treatments. Typically, microfluidic channels have been produced by cutting, photolithography, plotting, inkjet printing, and plasma etching, as well as wax printing.15

Inkjet printing has gained interest due to its simplicity and ability to produce tunable, high resolution patterns using a

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one-step process. It allows direct patterning of the substrate’s surface by controlled ejection of ink from the printer nozzle. Previously, inkjet printing has been used to make hydrophobic patterns on porous paper substrates and inks comprising polystyrene (PS) have been utilized in printing microfluidics on filter papers. However, usually the resolution achieved via inkjet printing does not match that from photolithographic methods. In the former, the lateral penetration of the ink in the porous substrate decreases print resolution while photolithography is suited for hydrophobic patterning of paper and nanopapers by using light and photomasks that selectively polymerize patterns on their surfaces. In this context, we recently reported on the use of photochemically induced thiol-yne click coupling to prepare hydrophilic channels and other patterns confined within hydrophobic borders. A drawback of the photolithographic method, however, is the need for expensive chemicals as well as time-consuming processes and purification steps, which may cause changes to the structure of the patterned substrate.

Microfluidic materials have often been used in biosensing to detect pathogens and physiological conditions, for example, by transforming biological responses into a detectable signal produced by specific chemical reactions. Paper-based biosensors provide portable and low-cost analytical platforms that have potential in point-of-care (PoC) and in-field assays. Many reports concerning the development of paper-based microfluidic biosensors such as microfluidic paper-based analytical devices (μPADs) have been reported. In addition, the use of nanocellulose materials in biosensing has raised recent interest.

In general, nonspecific protein adsorption is the biggest challenge in biosensing. It causes false responses and decreases detection sensitivity. To improve the sensing capability, the surface of the biosensor should subdue nonspecific adsorption, for example, by introducing antifouling properties to the substrate via protein-resistant coatings and blocking agents. Typically, noncharged, hydrophilic surfaces are inert to proteins, owing to the presence of hydrogen bond acceptor groups. They are beneficial because of their interactions with water, which create hydration layers.

In this work, two-dimensional fluidic channels were prepared on nanopaper. Photolithography and inkjet printing were used in the preparation of 2D channels (see Scheme 1). The photolithographic method used thiol-yne click coupling, while a polystyrene solution was used as ink in inkjet printing. Adsorption of hIgG on model CNF films was studied after modification with blocking agents that included BSA, fibrinogen, and PDMAEMA-block-POEGMA copolymers. Novel nanopaper-based microfluidic flow channels resistant to nonspecific protein adsorption were successfully produced and demonstrated for biosensing via fast fluid transportation, reduced leakage of the analyte, and improved sensitivity. The developed system could find applications from, for example, disposable rapid diagnostics, where the CNF film based assay reduces the use of fossil-based materials.

**EXPERIMENTAL SECTION**

**Materials.** Nanopaper was produced from bleached birch kraft fibers provided by VTT Technical Research Centre of Finland and prepared as described by Tammelin et al. Toluene (99.9%) and acetone (100%) were purchased from VWR Chemicals, ethanol (99.5%) from Alfa, ethyl acetate (≥99.5%) and dimethyl sulfoxide (DMSO; ≥99.9%) from Merck, fluorescein 5(6)-isothiocyanate (FITC) (≥90%) and p-xylene (98%) from Fluka, poly(ethyleneimine) 30% aqueous solution (PEI; Mw: 5000–100000) from Polysciences Inc., and all other chemicals were purchased from Sigma-Aldrich (Finland): trichlorovinylsilane (TCVS; 97%), 1H,1H,2H,2H-perfluorodecanethiol (97%), cysteamine hydrochloride (≥98%), 1,2-dichloroethane (≥99%), 4-pentynoic acid (95%), 4-(dimethylamino)pyridine (DMAP; ≥99%), N,N’-diisopropylcarbodi-
mide (DIC) (≥98%), Sudan Red G, immunoglobulin G from human serum (hIgG; ≥95%), bovine serum albumin (BSA) (≥98%), fibrinogen from human plasma (50–70% protein), and polystyrene (PS; average Mn, 35 kDa). D33-EGMA-110 and D33-EGMA-137 raft copolymerized poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) and poly(oligoethylene glycol) methyl ether methacrylate) (POEGMA) protein blockers were provided by Dr. Basile Zhu (DWI, Leibniz-Institute for Interactive Materials Research, Germany). All water used in this study was purified with a Millipore Synergy UV unit (Milli-Q).

**Preparation of CNF Model Surfaces.** Ultrathin films of CNF were prepared as described by Ahola et al.14 These films were made as models of the nanopaper surfaces, to study molecular binding. In brief, a CNF suspension of cellulose nanofibers was diluted to 0.148% and defibrillated with a tip sonicator (Digital Sonifier Model 450, Barson Ultrasounds Corp.) for 10 min at 25% amplitude. This CNF suspension was centrifuged at 10400 rpm for 45 min to remove fibril aggregates. Individual nanofibrils were then collected from the supernatant with manual pipetting. The nanofibrils were spin-coated (Model WS-650SX-6NPP, Laurell Technologies) at 3000 rpm and 90 s spinning time onto UV-oxygenated SPR sensors (Oy BioNavis Ltd.) coated with gold and a thin anchor layer of cationic PEL. Finally, spin-coated substrates were cured in an oven at 80 °C for 10 min. The model films were stored in a desiccator and stabilized overnight in water prior to use.

**Surface Plasmon Resonance (SPR).** The SPR experiments were performed with a multiparametric Surface Plasmon Resonance instrument (MP-SPR Model Navi 200, Oy BioNavis Ltd.). SPR was performed with a multiparametric Surface Plasmon Resonance instrument with a sensitivity factor for the sensor (109.94 ± 20 nm, 10 mW).

The thickness of the adsorbed layer was determined with eq 1:14

\[
d = \frac{l_2 \Delta_{\text{angle}}}{2m(n_r - n_0)}
\]

where \(d\) is the thickness, \(l_2\) is the characteristic evanescent electromagnetic field decay length (estimated as 0.37 of the light wavelength 240 nm), \(\Delta_{\text{angle}}\) is the change in the SPR angle, \(m\) is the sensitivity factor for the sensor (109.94 ± 20 nm, 10 mW), \(n_r\) is the refractive index of the bulk solution (1.334 ± 0.005), and \(n_0\) is the refractive index of the adsorbed substance (for proteins 1.57±0.02).

The mass of the adsorbed layer per unit area was determined with eq 2:14

\[
\Delta m = d \times \rho
\]

where \(\Delta m\) is the adsorbed mass, \(d\) is the thickness, and \(\rho\) is the packing density of the proteins (estimated to be 1.3 g/cm³).12 All SPR measurements were run in duplicate at 20 °C under constant flow of 100 μL/min.

**Adsorption of Protein Blocking Agents on CNF Surfaces.** Prior to the measurement, samples were stabilized in a continuous flow of phosphate buffer (pH 7.4, 10 mM) until a stable baseline was observed. BSA (0.1 g/L), fibrinogen (0.1 g/L), and PDMAEMA-block-POEGMA copolymers (D33-EGMA-110 and D33-EGMA-137; 0.5 g/L) were allowed to adsorb on the CNF surfaces at pH 7.4. After adsorption plateau was reached, the system was rinsed with phosphate buffer to remove loosely bound blocking agents.

**Adsorption of hIgG on Blocked CNF Surfaces.** Adsorption of hIgG (0.1 g/L) on bare and blocked CNF surfaces was determined. After adsorption plateau, phosphate buffer (pH 7.4, 10 mM) was used to remove loosely adhered proteins.

**Atomic Force Microscopy (AFM).** AFM (MultiMode 8 Scanning Probe Microscope, Bruker AXS Inc.) was used to analyze the surface topographical changes on the unmodified and blocked CNF substrates after hIgG adsorptions. Tapping mode in air and silicon cantilevers (NSC15/AIBS, MicroMasch) were used to scan 5 × 5 μm² surface areas. Three different spots on each sample were imaged and flattening was used in the image processing.

**Patterning by Photoinduced Click Reactions.** Fluidic channels (3 × 20 mm) were prepared by producing hydrophobic–hydrophilic patterns on nanopaper. Patterns were prepared via click coupling following Guo et al.15,16 except for minor modifications. In brief, before patterning, thiol–xylene reactive alkyl groups were introduced to the surface of the nanopaper. First, vacuum oven-dried substrates were immersed in 50 mL of dichloroethane. Then, 150 mg 4-pentynoic acid, 20 mg DMAP, and 500 μL of DIC were added. The reaction was left to happen for 1.5–2 days under stirring. The substrates were then washed with acetone and dried with nitrogen gas. After functionalization, patterning of the films was performed. A substrate with the alkyl groups was placed on a glass slide, wetted with cysteamine hydrochloride solution (20 wt%, in ethanol) and covered with a photomask. Exposure to UV light (254 nm, 10 mW-cm⁻²) was done for 20 min. Then, the substrate was extracted completely with ethanol in the dark and dried with nitrogen gas.

**Fluorescein Labeling of hIgG.** To modify hIgG antibodies with a fluorescent probe, a protocol for immunoglobulin modification by Hermanson17 was used after few alterations. First, 2 mg/mL protein solution was prepared in 0.1 M sodium carbonate buffer (pH 9.0).

In a dark room, fluorescein 5(6)-isothiocyanate (FITC) was dissolved in dry DMSO in a glass bottle to obtain a 1 mg/mL FITC solution. This solution was protected from light by wrapping the glass bottle in aluminum foil. Next, 100 μL of the FITC solution was slowly
added to each ml of hlgG solution and gently mixed. The reaction was left to occur at 4 °C in the dark for over 8 h. To purify the obtained FITC-stained human lgG (hlgG-FITC) from unreacted FITC molecules, the solution was centrifuged at 4000 rpm for 30 min by using centrifugal filter units (Amicon Ultra-15, MWCO 30 kDa). The centrifugation was repeated four times.

hlgG Detection Tests in Fluidic Channels. The adsorption of fluorescent hlgG was studied on the unmodified, BSA-blocked and D33-EGMA-137 copolymer-blocked channels (twice-printed channels with 40 μm drop spacing and 2 mm polystyrene edges). Blocked channels were prepared by adsorbing BSA (0.1 g/L pH 7.4) and D33-EGMA-137 copolymer (0.5 g/L, pH 7.4) onto channels for 20 min and washing with a phosphate buffer (pH 7.4, 10 mM) for 10 min hlgG was introduced to channels by dropping 30 μL of hlgG-FITC solution (100 μg/mL) onto the channels. The drop was applied few millimeters from the edge of the channels. The samples were washed with the phosphate buffer after 15 min adsorption.

Confocal Laser Scanning Microscopy (CLSM). The hlgG-FITC exposed channels were imaged with CLSM in order to detect the adsorbed hlgG. Images were taken with a laser scanning spectral confocal microscope (Leica TCS SP2, Leica microsystems CMS GmbH) by using 488 nm excitation wavelength and 500–540 nm detection wavelength range. Images were acquired with 727 V laser power and under constant imaging conditions. The images were taken of unmodified and blocked channels, which were exposed to hlgG-FITC. In addition, reference samples without adsorbed hlgG-FITC were imaged.

■ RESULTS AND DISCUSSION

Passivation of CNF Surfaces by Adsorbing Protein Blockers and Detection (SPR) of Adsorbed hlgG. Protein adsorption was studied by using SPR for CNF surfaces after modification with the two block copolymers as well as fibrinogen and BSA. Figure 1a includes the SPR sensograms for the blocking agent adsorbing from aqueous solution on the CNF films. Adsorption of copolymers caused a large increase in SPR angle, corresponding to an adsorbed amount of ~2.8 mg/m², slightly lower than the previously reported value of 3.6 mg/m² for CNF thin films at pH 7.4. However, the adsorption of the copolymers was fast and a good covering layer was produced. The adsorption occurred mainly through electrostatic interactions owing to the cationic character of the PDMAEMA-blocks.

The introduction of BSA on the CNF film caused a very small increase in the SPR angle (Figure 1a); therefore, only a small amount of BSA (0.2 mg/m²) was adsorbed to the surface. In addition, the adsorption of fibrinogen caused a small change in SPR angle (equivalent to 0.8 mg/m² adsorption). BSA has a isoelectric point (pI) of 5.8 while that for fibrinogen is 5.8, which makes them negatively charged under physiological pH of 7.4. As a result, adsorption of both proteins onto CNF is electrostatically unfavorable. Nevertheless, some adsorption occurred because of the effect of other interactions, such as hydrophobic and van der Waals interactions. Orelma et al. reported adsorbed amounts of 0.46 mg/m² for BSA on cellulose at a pH of 7.4 and, to our knowledge, no reports are available for fibrinogen adsorption on CNF.

Figure 1b shows SPR sensograms for hlgG adsorption from aqueous solution on CNF surfaces treated with proteins or block copolymers used as antifouling (blocking) agents. Additionally, hlgG was also adsorbed on neat, untreated CNF surfaces in order to determine the blocking efficiencies. It can be seen that the adsorption of hlgG on CNF was fast in the beginning but, after a short time, the surface became covered with the antibody molecules, causing adsorption to slow down and level off. The change in the SPR angle corresponds to the 3.1 mg/m² hlgG on CNF. To some extent, the adsorption was driven by electrostatic interactions. The pl of hlgG is ~8.82 Therefore, given its positive charge at pH of 7.4, the hlgG adsorbed electrostatically to the anionic CNF. A small decrease in hlgG adsorption (17%) was obtained when CNF was treated with fibrinogen, Figure 1b. BSA blocking efficiency was even lower. Most likely, the adsorbed fibrinogen layer was partially hydrated due to its large, elongated conformation, which created some steric repulsion between hlgG and fibrinogen-blocked CNF.

The copolymers limited hlgG adsorption quite extensively. The POEGMA-block of the copolymers is highly hydrophilic and has an extended conformation in aqueous media. Hydration of the copolymer layer on CNF caused repulsive interactions between the antibody and the swollen polymer, decreasing significantly hlgG adsorption. Modification with D33-EGMA-137 produced 95% lower adsorption (0.17 mg/m²) compared to that on bare CNF (3.1 mg/m²). Vuoriluoto et al. reported complete passivation of TOCNF with D33-EGMA-137 at pH 7.4. However, in this latter work the copolymer layer adsorbed on TOCNF was relatively more extensive (6.1 mg/m²) compared to that on CNF (2.8 mg/m²).
The topography of the unmodified and blocked CNF surfaces, before and after adsorption of hlgG, was followed by AFM imaging, Figure 2. It can be seen that the pure CNF surface (Figure 2a) contained only nanofibrils (RMS roughness of 4.27 nm), but some globular features appeared after introduction of hlgG (RMS roughness of 3.55 nm), indicating its adsorption (Figure 2b). It is important to note that AFM imaging was carried out in air (dry), which may cause aggregation of proteins upon water removal. The D33-EGMA-137 copolymer can be observed in the form of small globular structures around the CNF fibrils (RMS roughness of 3.21 nm), Figure 2c. An extensive copolymer surface coverage was noted. After introduction of hlgG (Figure 2d), the AFM image was blurred and darker structures could be seen (RMS roughness of 3.63 nm). This indicates that some hlgG was adsorbed to the surface, despite preadsorbed blocking agent. In addition, the BSA-blocked sample adsorbed hlgG. A clear difference in the AFM images can be seen. The BSA-blocked samples, with and without hlgG (RMS roughness of 3.21 and 3.72 nm, respectively; Figure 2e,f). The BSA protein can be seen as globular features. Likewise, the adsorbed hlgG proteins was revealed as globular structures around the fibrils. It is not possible to estimate the hlgG adsorbed amount from AFM images, but it is clear that all samples, including the blocked ones, adsorbed some hlgG.

**Fluidic Channels and Surface Flow.** Figure 3 shows the images of the prepared fluidic channels made by photo-

![Figure 3](image-url)

Figure 3. Flow tests on fluidic channels on nanopapers produced via (a) thiol-yne click coupling and (b) inkjet printing of polystyrene with 1 mm drop spacing; (c) 2 mm edges polystyrene printed by using 30 μm drop spacing; (d) handmade channel; (e) polystyrene channel with 2 mm edges printed by using 40 μm drop spacing and (f) 2 mm edges printed by using 40 μm drop spacing and two layers. Channel size: 3 × 20 mm.
analyte concentration may decrease due to solution spreading. To our knowledge, there are no previous reports on the nanopaper-based surface flow channels. This is despite the possible benefit of using surface flow microfluidic materials in biosensors, which can be fast for fluid transportation, reduce leakage of the analyte and improve sensitivity.

Flow tests show that the channel patterned via click coupling (Figure 3a) displayed poor and delayed flow. Even after 2 min, the propagation of the fluid was minimal. The handmade polystyrene channel (Figure 3d) held the fluid well and fluid flowed into the channel. However, 30 μL of the water did not flow to the end of the channel. Additionally, water flow was slow on the channel printed with 1 mm edges and 50 μm drop spacing (Figure 3b), and the fluid flowed slightly over the edges. The printed channel with 2 mm edges and 30 μm drop spacing (Figure 3c) did not hold the fluid well, but the flow was observed before spreading. The printed channel with 2 mm edges and 40 μm drop spacing (Figure 3e) held fluid quite well and the flow of the fluid was fast. The channel with twice-printed 2 mm edges and 40 μm drop spacing (Figure 3f) held the fluid well and showed the fastest flow. Figure 4 shows the position of the fluid front line in a doubled-printed channel after 0, 1, 3, 9, and 30 s after contact with water droplet. The water flowed fast into the printed channel and the applied fluid reached the end of the channel. Already, after 9 s from placing the water droplet, the fluid in the printed channel had almost reached the end. Therefore, the channels bearing two

Figure 4. Photos of rectangular, twice-printed PS flow channels showing water flow with elapsed time, as indicated (40 μm drop spacing).

polystyrene layers were chosen for further analysis. Furthermore, the regeneration of the fluidic channels was not studied in this work. However, the simplicity of the developed system and renewability of substrate material indicate good disposability with low environmental impact on disposal.

Nanopaper Topography and Water Contact Angle.

SEM images of the unmodified nanopaper and section of polystyrene-coated and thiol-yne modified nanopapers are shown in Figure 5. The surface of the unmodified nanopaper was smooth, fibrils were packed tightly with no apparent void spaces (Figure 5a). Polystyrene uniformly covered the fibrils of the nanopaper after printing from organic solvent (Figure 5b). Cellulose fibrils are still visible in the click-modified nanopaper, after hydrophilic modification with the cysteamine hydrochloride (Figure 5c). There are some dark features around the fibrils and aggregation can be seen. Figure 5d, shows the SEM image of the hydrophobic layer after click reaction with 1H,1H,2H,2H-perfluorodecanethiol. The hydrophobic thiol compound covered the surface and the roughness of the substrate increased.

The wetting properties of unmodified and patterned nanopapers were studied by water contact angle measurements. The measurements were carried out so that both the edge and the flow channel were measured, showing the possible distribution of unwanted hydrophobic materials to the areas subjected to fluid flow. The unmodified nanopaper was hydrophilic (Figure 5a) with a 22° water contact angle (WCA), which is similar to previously reported values.21,52 1H,1H,2H,2H-Perfluorodecanethiol was used as the hydrophobic thiol compound in the photolithographic click modification. The hydrophobization with this fluoralkyl molecule by thiol-yne reactions increased the WCA to >120° (Figure 5d). Hydrophobicity is known to be enhanced with surface roughness and low surface energy.3–55

Before hydrophobization, a hydrophilic thiol compound was used to treat the nanopaper. The WCA of nanopaper treated with cysteamine hydrochloride was higher than that of the unmodified sample (Figure 5c). It is conceivable that the introduction of the alkyl groups created nanoscale roughness that affected the hydrophilicity. After esterification, the RMS
and (d) copolymer-blocked channels.

adsorbed FITC-stained human IgG (b) unmodified, (c) BSA-blocked, and (d) copolymer-blocked channels.

Figure 6. (a) Schematic illustration of the channel, in which green circle and arrow indicate the drop point and flow direction, respectively, and yellow arrows indicate the areas shown in images (b, c). CLSM images of the inkjet-printed fluidic channels with adsorbed FITC-stained human IgG (b) unmodified, (c) BSA-blocked, and (d) copolymer-blocked channels.

The WCA of 94° formed a hydrophobic layer on the surface of nanopaper with a roughness of 39 to 51 nm. It is also possible that some of the unreacted 1H,1H,2H,2H-perfluorodecanethiol remained on the film even after purification, increasing the WCA of the hydrophilic areas or channels. The WCA of hydrophobized and hydrophilized nanopaper are similar to the values reported earlier by Guo et al. For comparison, the WCA of the inkjet-printed polystyrene patterns are shown in Figure 5b. Polystyrene formed a hydrophobic layer on the surface of nanopaper with a WCA of 94°, which is similar to previously reported values. The benefit of inkjet printing, compared to the click-modified nanopaper, is that it does not alter the substrate in the channel area.

Noticeably, the polystyrene channels held fluid better than those produced by click coupling (Figure 3). This is despite the relatively higher WCA difference between the channel and the hydrophobic edges in the click pattern, compared to that in the polystyrene channel. The reason for this is that polystyrene formed a three-dimensional barrier, which contained the fluid. The channel patterned with click chemistry had a surface tension gradient but otherwise prevented the fluid from flowing over the edges.

**HlgG Detection in Fluidic Channels.** Flow tests with fluorescent-stained hIgG antibodies were performed to investigate adsorption of hIgG on the channels and whether the blocking agents in fact reduced nonspecific adsorption. Figure 6 shows the CLSM images taken along the length of the channels after washing and drying. The green color indicates hIgG adsorption. Figure 6 shows that the unmodified channel had the highest hIgG adsorption. Compared to the unmodified channel, the blocked one displayed significantly less green areas. However, the BSA-blocked channel had some aggregations of adsorbed hIgG. The observed aggregation occurred upon drying, when the residual antibody solution precipitated due to capillary forces. The lowest hIgG amount was adsorbed on the copolymer-blocked channel, in which only pale spots of green color could be seen in the front end of the channel. Thus, the blocking agents passivated efficiently the surface from hIgG binding. Furthermore, the hIgG adsorption was higher at the front-end of the channel, which may be due to the effects of external pressure upon deposition. This can be seen particularly for the BSA-blocked channel, where green areas are reduced along the length of the channel. The bright green lines at the end of the channels indicate the presence of hIgG. Especially, the hydrophobic and rough borders of the unmodified channel adsorbed antibodies. It is known that proteins tend to adsorb more on hydrophobic and rough surfaces. A particularly interesting finding is that the introduced antifouling effect reduced the overall adsorption, including the adsorption on the edges. In the copolymer-blocked channel, the adsorbed amount decreased significantly, only few parts of the edges adsorbed hIgG. This indicates the possible accumulation of the antifouling agent by capillary effects (coffee-ring) upon its introduction developing a stronger effect at the edges. Therefore, no separate procedure was needed to prevent protein adsorption to the edges.

**CONCLUSIONS**

Two-dimensional fluidic channels with enhanced sensitivity were produced on nanopaper substrates. Thiol-yne click coupling and polymer patterning via inkjet printing were utilized. The thiol-yne click procedure did not produce accurate fluidic channel whereas the inkjet-printed system (with two layers of polystyrene and 40 μm drop spacing) was most efficient. These latter channels displayed fast flow and the fluid did not flow over the channel edges.

The antifouling properties were introduced to the channels successfully. PDMAEMA-block-POEGMA copolymers decreased adsorption of hIgG onto CNF up to 95% owing to the POEGMA block that formed a hydrated layer on the CNF surface, which was anchored with the cationic PDMAEMA block. The successful blocking of the channels and efficient fluid flow indicate that the proposed surface patterns could be developed further for biosensing. Compared to microfluidics using porous substrates, the proposed surface flow microfluidic materials have better pattern resolution and show no leaking nor lateral flow. The use of surface flow in microfluidic materials for biosensing offer advantage of fast fluid transport and improved sensitivity.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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