Preferred citation: A. Böhm, M. Gattermayer, F. Carstens, S. Schabel and M. Biesalski. Designing microfabricated paper devices through tailored polymer attachment. In **Advances in Pulp and Paper Research, Cambridge 2013**, *Trans. of the XVth Fund. Res. Symp. Cambridge, 2013*, (S.J. I'Anson, ed.), pp 599–618, FRC, Manchester, 2018. DOI: 10.15376/frc.2013.2.599.

DESIGNING MICROFABRICATED PAPER DEVICES THROUGH TAILORED POLYMER ATTACHMENT

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ABSTRACT

In the present paper we show that polystyrene – based copolymers, which carry a defined amount of photo – reactive benzophenone moities can be transferred and immobilized to paper substrates via a simple dip coating approach and subsequent illumination of the paper substrates with UV-light. Non-bound macromolecules can be removed from the cellulose fibers by solvent extraction. Thereby, the amount of immobilized polymer can be adjusted over a wide range by changing the polymer concentration in the dip coating solution. The resulting polymer-modified paper substrates were characterized using IR spectroscopy, scanning electron microscopy (SEM), fluorescence microscopy and static contact angle measurements. The polymers are attached to cellulose fibers using a photo – chemical approach and stable chemical micro patterns, including paper-defined microchannels, can be designed inside model paper substrates by using conventional UV-lithography. These channels are capable to control the fluid penetration by capillary actions. An engineering of the paper substrate itself allows to modulate the speed of the fluid transport of an aqueous solution inside paper-defined microchannels. The latter will become important for a number of applications.

INTRODUCTION

Throughout the last decade, we have witnessed an increasing amount of studies, addressing the usability of *low-cost* material paper in *high-tech* applications, progressing from substrates for blood-typing,[1] to enzymatic reaction engineering,[2] low-cost DNA-sensors[3] and purification of antibodies.[4] Paper is a particularly attractive substrate for such *low-high-tech* applications due to interesting advantageous properties: (i) no external pumps are needed for the fluid transport due to capillary forces, (ii) paper can be considered as low-cost material consisting of the earths most abundant material: cellulose, and (iii) the lignocellulosic fiber surface can be chemically modified by various chemical means.[5] In order to guide an aqueous solutions inside a paper substrate at confined areas, hydrophobic barriers within the paper sheet need to be designed in a controlled fashion.

This can be achieved e.g. by physical blocking of pores of the hydrophilic paper substrate with hydrophobic substances (e.g. SU-8 Photoresist[6], Polydimethylsiloxane (PDMS) polymers[7] or wax[8]). Citterio and coworkers physically adsorbed a hydrophobic polymer (polystyrene) to filter paper followed by subsequent partly removal of the polymer by multiple ink-jet printing of toluene[9]. Despite the SU-8 photoresist approach the applied hydrophobic material interacts with the cellulose fiber only by physical means. Hence, the created hydrophobic barriers may not withstand mechanical, thermal or chemical stress. Depending on the size and complexity of the created micropattern a large amount of hydrophobic material may have to be applied in order to completely block the pores inside the paper substrate.

A thermally and chemically robust modification of the cellulose fibers may be achieved by chemical hydrophobization of the fiber-surface, i.e. if covalent bonds are established between the cellulosic fibers and the organics used as coating. The latter can be achieved e.g. by using small organic molecules, such as alkyl ketene dimer (AKD), commonly used in paper sizing technology. This technique has been successfully applied by Shen et al.,[10] submerging a filter paper into an

AKD solution followed by thermal reaction of the AKD with corresponding OH-groups present on the fiber surface. Hydrophilic micropatterns could be created by partly removing the AKD coating via plasma treatment. Despite using small organic molecules, functional polymers can be covalently attached to paper substrates by using different grafting techniques, such as “grafting-to” or “grafting-from”[11]. With respect to the grafting-from approach, a large number of polymerization techniques including Atom Transfer Radical Polymerization (ATRP)[12–14], Nitroxide-mediated Radical Polymerization (NMP)[15] and Reversible Addition-Fragmentation Chain Transfer Polymerization (RAFT) [16–17] have been successfully applied for the surface modification of cellulose fibers with different functional polymers. However, by this approach a chemical microstructuring of the paper substrate is not trivial, since most of the used approaches rely on thermally initiated, as well as diffusion-controlled reactions.

An interesting approach for the covalent attachment of functional polymers on solid substrates uses photo-reactive polymers that are surface-immobilized by UV-light illumination. Rühe and coworkers showed that benzophenone-containing polymers can be attached and crosslinked in a one-step photochemical reaction to plastic surfaces and organic tissues[18–19]. The benzophenone-group can react with any organic substances that contain aliphatic C-H groups, because the mechanism follows an excitation of the benzophenone carbonyl into a biradical triplet-state, which abstracts a proton from nearby aliphatic groups and recombines with the residual radicals at the benzophenone, and the aliphatic group, respectively. The latter covalently links the benzophenone onto the aliphatic molecule. Because cellulose contains a large number of aliphatic C-H-groups groups, it is possible to photo-immobilize benzophenone-containing polymers onto paper substrates in a simple, yet very efficient step, as recently reported[20]. Poly(methyl methacrylate, PMMA) based copolymers that contain a defined amount of photo-reactive benzophenone moities were transferred onto cellulose fibers, and UV-lithography was applied in order to generate defined, chemically stable micropatterns inside model filter papers that can be used to guide a fluid driven in such channels by capillary actions[20].

In the present paper, we show that the approach of linking functional, photo-reactive polymers to paper, and designing chemically stable microfluidic channels in paper substrates, is not exclusive to PMMA copolymers, but can also be extended to other types of functional copolymers.

We show, e.g. that polystyrene (PS) – based copolymers that carry photo-reactive benzophenone-groups can be attached to cellulosic fibers in a similar fashion. Benzophenone-containing PS-copolymers are transferred to model paper substrates via simple dip coating processes. Illumination of the polymer-modified paper substrates with UV-light leads to an immobilization of the functional polymers onto the microfibers and non-bound macromolecules are removed by subsequent solvent extraction. Because the copolymers are covalently attached by means of UV-light illumination, illumination through UV-masks and subsequent removal of non-bound polymers by solvent extraction can define chemical micropatterns in paper substrates, suitable for microfluidic applications.

EXPERIMENTAL SECTION

Materials

2,2′-Azobis(2-methylpropionitrile) (AIBN, >98%, Fluka), 4-Hydroxybenzophenone (98%, Alfa Aesar), Fluorescein (>98%, Merck), Triethylamine (99%, Grüssing GmbH), Cyclohexane (p.a., Biestefeld), Dichloromethane (p.a., Biesterfeld), Dimethyl sulfoxide (DMSO, 99%, Grüssing GmbH), Ethyl acetate (p.a., Biesterfeld), Methanol (>99.5%, BASF), Tetrahydrofuran (THF, >99.5%, Roth), 1-Pyrenemethyl methacrylate (PyMA) (99%, Sigma Aldrich), Roth Rotilabo 15A filter paper (Fiber source: Cotton linters, Grammage: 84 g m^{-2}, BET area 1.2 m^2 g^{-1}, mean pore radius: 2.5 µm) and lab-engineered paper substrates (Fiber source: Cotton linters, Grammage: 76 g m^{-2}, mean pore radius: 4.3 µm) were prepared using a Rapid Koethen lab sheet former. Methyl methacrylate (99%, Sigma Aldrich), Methacryloylchloride (97%, Sigma Aldrich) and Styrene (99%, Acros) were passed through a basic alumina columm, destilled under reduced pressure and stored under nitrogen prior to use.

Monomer and Polymer Synthesis

The synthesis of the photoreactive monomer 4-methacryloyloxybenzophenone (MABP) was carried-out according to protocols by Berchtold[21] and Freidank[22]. The fluorescent monomer fluoresceine-O-methacrylate was synthesized similar to a protocol by Berchtold et al.[21] and details of the synthesis were reported elsewhere[20]. All copolymers described below, were prepared by statistical free radical copolymerization, and characterized with respect to molar mass and composition by size exclusion chromatography (SEC, PSS SDV linear M was used as column, THF as eluent, and the system was calibrated using narrow dispersed polystyrene standards), and ^{1}H-NMR spectroscopy, respectively. The synthesis of the photo-reactive, fluorescent PMMA-based polymer P(MMA-co-MABP-co-FOMA) has recently been reported[20].

P(S-co-MABP): 4-methacryloyloxybenzophenone (133.0 mg, 0.5 mmol) was added to styrene (5.19 g, 50.0 mmol) in a Schlenk flask under nitrogen atmosphere. The initiator AIBN (10.3 mg, 0.06 mmol) was added and the solution was degassed by three freeze-pump-thaw cycles. The flask was placed in a thermostated bath at 60ºC for 6 h for polymerization.

After this time the solution was diluted with 22 ml THF and the polymer was precipitated in 300 ml methanol and dried in vacuum. The polymer was purified by repeated dissolution in THF and precipitation in methanol solution. The polymer was obtained as a white solid (1.01 g, 19% total yield with respect to monomer consumption). M_n = 89.000 g/mol. ^{1}H-NMR analysis yielded a molar composition of about 1.3 mol% of the photo-reactive monomer in the copolymer.

P(S-co-MABP-co-PyMA): 4-methacryloyloxybenzophenone (266.6 mg, 1.0 mmol) and 1-pyrenemethyl methacrylate (60.06 mg, 0.2 mmol) were added to styrene (3.96 g, 38.02 mmol) in a Schlenk flask under nitrogen atmosphere. The initiator AIBN (19.1 mg, 0.12 mmol) was added and the solution was degassed by four freeze-pump-thaw cycles. The flask was placed in a thermostated bath at 60ºC for 6 h for polymerization. After this time the solution was diluted with 15 ml THF and the polymer was precipitated in 300 ml methanol and dried in vacuum. The polymer was purified by repeated dissolution in THF and precipitation in methanol solution. The polymer was obtained as a slightly yellow solid (0.86 g, 20%), M_n = 82.000 g/mol. ^{1}H-NMR analysis yielded a molar composition of about 4.5 mol% of the photo-reactive benzophenone group and about 3.5 mol% of the fluorescent monomer in the copolymer.

Paper-coating and Photo-chemical Attachment of Polymers to Cellulose Microfibers by UV-irradiation

For modification of the paper substrate, the copolymers were first dissolved in THF at a constant concentration of about 30 mg ml^{-1}. Filter paper was cut into pieces (2.5 × 2.5 cm or in case of the preparation of microchannels 2.5 × 7.5 cm). The functional polymers were adsorbed to the paper by submerging the paper substrates into the polymer solution for approx. 20 sec (dip coating) followed by subsequent air-drying of the samples for at least 30 min. After the polymers had been dip-coated onto the paper-sheets, samples were transferred to a Vilber Lourmat Bio-Link BLX UV exposure chamber ($\lambda = 254$ nm) or Newport 1000 W Oriel Flood Exposure Source ($\lambda = 365$ nm) and illuminated with UV-light. All samples were illuminated with E = 400 mJ cm^{-2} ($\lambda = 254$ nm) or E = 16 J cm^{-2} ($\lambda = 365$ nm) at which approx. 94% of the benzophenone groups have reacted[17]. This corresponds to an illumination time of ~16 min in either cases. In order to generate sufrace micropatterns an appropiate aluminum mask was placed on the samples during the illumination step. Note, in case of the used filter paper which has a thickness of 140 µm it is necessary to illuminate the samples from both sides in order to covalently attach the functional polymers over the entire thickness of the paper[20]. After illumination non-bound polymer was removed by 150 min solvent extraction in a Soxhlet-apparatus using THF as solvent. The samples were air-dried for at least 15 h before they were further characterized.

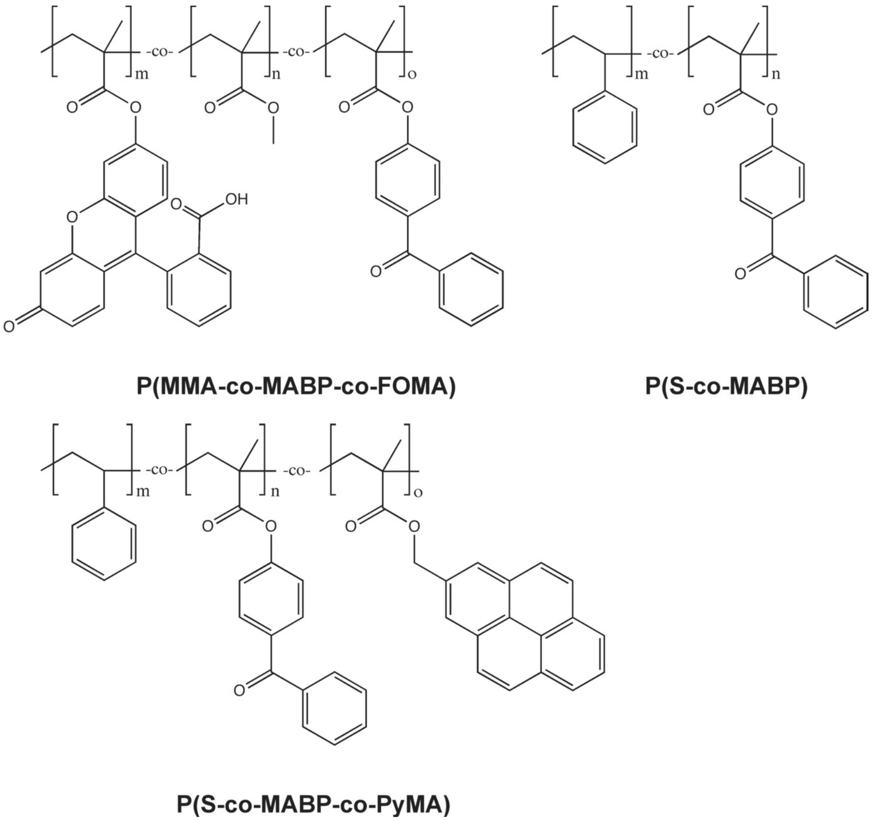
Characterization of Polymer Modified Paper Substrates

Infrared spectra of polymer-modified paper substrates were recorded on a PerkinElmer Spectrum One FT-IR spectrometer equipped with a PerkinElmer single Universal Attenuated Total Reflectance (UATR) unit. 10 scans were accumulated with a resolution of 1 cm^{-1}. Static contact angles were measured, if applicable, using the sessile-drop method with a DataPhysics Contact Angle System OCA instrument using 4 µl droplets of Milli-Q water. The measurements were conducted at ambient temperature.

Fluorescent micrographs were captured on an Olympus BX60 fluorescent microscope equipped with an Olympus XM10 camera. Image analysis was performed using ImageJ software. SEM photomicrographs were captured using a Philips XL30 FEG scanning microscope operated at an acceleration voltage of 10 kV. The specific surface area of the filter paper was determined by BET measurements using a Micromeritics ASAP 2420 Accelerated Surface Area and Porosimetry System. Due to the very small BET surface of paper, krypton instead of nitrogen was used as inert adsorbing gas. The mean pore radius was determined by mercury porosimetry using a Micromeritics AutoPore IV 9500 series system.

RESULTS AND DISCUSSION

In order to chemically modify paper substrates by photo-chemical means, we first synthesized a number of benzophenone-functional polymers by free radical copolymerization. The chemical structures of some of the designed copolymers is illustrated in scheme 1. MMA and PS were chosen as matrix monomers because the corresponding polymers are hydrophobic leading to a change in the interfacial properties of the resulting polymer-modified paper substrates. Furthermore the copolymerizations of these matrix monomers with the methacrylate-based benzophenone and fluorescent monomers show a high reproducibilty with respect to adjust molar mass and composition of the copolymers. Details on the MMA-based copolymers can be found in a recent publication[20]. In case of styrene monomer two different copolymers were designed: (1) a copolymer that contains besides styrene a small amount of the photo reactive benzophenone-group, and (2) a



Scheme 1. Chemical structures of copolymers synthesized and used in this study: (top-left) a photo-reactive, fluorescently labeled MMA-based copolymer P(MMA-co-MABP-co-FOMA); (top right) a photo-reactive styrene-based copolymer P(S-co-MABP); (bottom) a photo-reactive, fluorescently labeled styrene-based copolymer P(S-co-MABP-co-PyMA).

copolymer that consists of styrene, the photo-reactive monomer and a fluorescently labeled monomer (1-Pyrenemethyl methacrylate, PyMA), for analytical means.

The polymers were analyzed with respect to their composition using ^{1}H-NMR and their molecular weight using SEC. The molecular weight of the copolymers is on the order of about 80.000 g mol^{-1} (M_n). ^{1}H-NMR analysis revealed the content of the photo-reactive monomer to be about 1.3 mol% in case of P(S-co-MABP) and 4.5 mol% in case of the fluorescently labeled P(S-co-MABP-co-PyMA). The content of the fluorescent monomer was found to be about

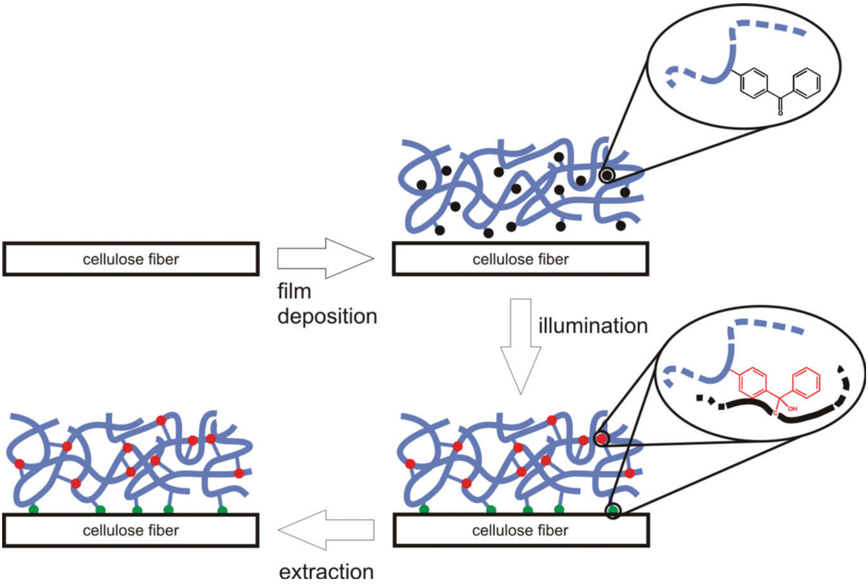
3.5 mol%. Fluorescent labeling was performed in order to analyse the macroscopic homogeneity of the polymer attachment and secondly to prove the successful local immobilization of functional polymers.

In order to coat filter papers (Roth 15A), the copolymers at first were dissolved in THF at a concentration of about 30 mg ml^{-1}. We used THF as the solvent because both types of copolymers show a good solubility in THF, and the used filter paper exhibits a high stability towards THF treatments[20]. In a second step, the used model paper substrates were cut into pieces (2.5 × 2.5 cm) and the copolymers were transferred onto the filter paper using dip coating Note, we also tested spray coating techniques (data not shown), however dip coating yielded the best results with respect to control the transferred amount of polymer mass onto the paper substrates[20]. Typically the paper substrates were submerged into the polymer solution for about 20 s, pulled out of the solution and finally air-dried. After the polymers had been adsorbed to the cellulose fibers the samples were transferred to an UV-light exposure chamber and illuminated with UV-light ($\lambda = 254$ nm). The illumination of the polymer – modified paper substrates with UV-irradition leads to a π, π^* – transition in the carbonyl group of the benzophenone group resulting in a biradical species that can abstract a hydrogen from an aliphatic C-H-group in close proximity. Recombination of the two resulting C-radicals leads to the formation of a new C-C bond and thus to a photochemical attachment of polymer chains to the cellulose fibers, as well as within the polymer layer (Scheme 2).

The half-life energy dose of the benzophenone group (i.e. the dose at which 50% of the benzophenone group have been excited) is $E_{1/2} = 100$ mJ cm^{-1} at $\lambda = 254$ nm[21]. In our present experiments, all samples were illuminated with an energy dose of $E = 400$ mJ cm^{-1},hence, about 94% of the benzophenone groups of the paper-adsorbed copolymers had been excited. The latter ensures a complete cross-linking and efficient coupling of the copolymers to the cellulose fibers inside the model filter paper. Unbound copolymer was removed form the paper substrates by subsequent solvent extraction in a Soxhlet apparatus under dark conditions using THF as the solvent.

In order to prove the chemical identity of the paper-attached copolymer, UATR FT-IR spectra of the samples were recorded after coating, illumination and extraction. A comparison of the FT-IR spectra of polymer – modified paper substrates with the IR spectra of similar polymers in bulk indicates that all signals which can be attributed to the polymer could be found in the IR spectrum of the copolymer – modified paper substrate demonstrating the chemical identity of the copolymers on top of the model filter paper[20]

In a next step, we investigated the surface morphology of the paper substrates before and after polymer modification by using scanning electron microscopy. Representative SEM micrographs of unmodified and polymer – modified filter papers are shown in Figure 1.



Scheme 2. Schematic illustration of the photo-chemical attachment of functional polymers bearing benzophenone moities (black dots) to cellulose fibers. During the illumination with UV-light a surface – attached polymer network is formed through a photochemical reaction of the benzophenone group with aliphatic C-H – groups of the cellulose fibers (green dots) as well as other polymer segments in close proximity (red dots) (For details of the reaction see text).

At a first sight, SEM micrographs captured before and after polymer modification seem to be very similar. A comparison of the cross – sectional micrographs for an untreated and P(S-co-MABP-co-PyMA) – modified filter paper clearly indicates that the polymer modification does not lead to a complete blocking of the pores inside the model filter paper (Figure 1B). A closer look on the micrographs that were captured on the surface before and after the polymer modification reveals a higher concentration of cellulose-attached polymer at the outer surface compared to the inside of the filter paper (Figure 1A). This result can be explained by mass transfer during the air-drying process after dip coating of the filter paper. Finally, the structural integrity of the filter paper is not changed upon UV-light illumination with $\lambda = 254$ nm.

Next, we investigated whether the polymer modification with PMMA – and PS – based copolymers leads to a change of the interfacial properties, in particular, wetting properties of the modified paper substrates. For this, we measured

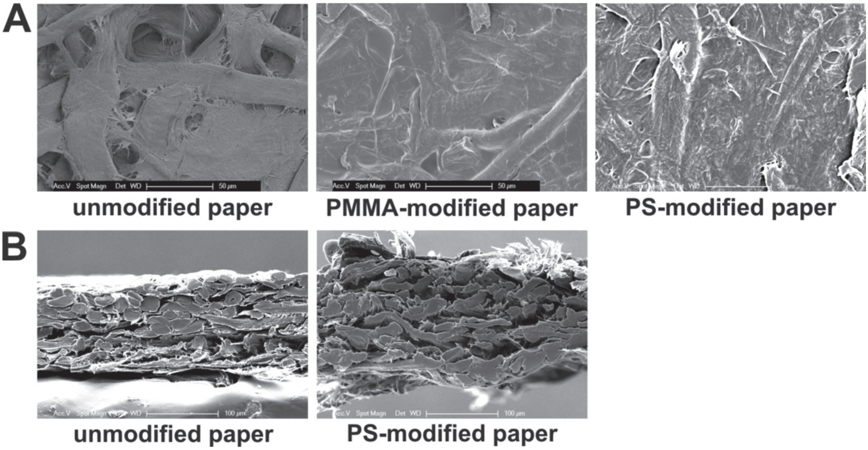


Figure 1. **A** SEM micrographs captured on the surface of untreated filter paper (left), P(MMA-co-MABP-co-FOMA) – modified filter paper (middle) and P(S-co-MABP-co-PyMA) – modified filter paper (right). **B** SEM images of the cross-section of an unmodified (left) and P(S-co-MABP-co-PyMA) – modified filter paper. The copolymers were attached to the filter paper by dip coating of the paper substrate into a solution of 30 mg ml^{-1} of the photoreactive P(MMA-co-MABP-co-FOMA) and P(S-co-MABP-co-PyMA) copolymer in THF, respectively, illumination with UV-light followed by subsequent solvent extraction to remove non-bound copolymers.

changes in the static water contact angle before and after the polymer modification. Polymer – modified paper substrates were prepared as outlined above using P(S-co-MABP) copolymers and a water droplet was placed onto the model filter paper before and after the copolymer modification. Pictures of the surface were taken directly after application of the water droplet (t = 0 sec) and at t = 30 sec (at a later stage changes in the contact angle can be observed due to water evaporation).

In case of an untreated filter paper the applied water droplet is rapidly penetrating into the surface at a fraction of a second due to the hydrophilic character of the cellulose fibers, making the measurement of an contact angle impossible (Figure 2 a,b). Due to the polymer coat on top of the cellulose fibers, the paper substrates are now more hydrophobic and a contact angle of about 115º was observed.

In a next step we investigated the amount of photo-attached polymer as a function of the concentration of the copolymers in the dip coating solution. In case of PMMA – based copolymers a linear relationship between the immobilized amount of polymer and the polymer concentration in the dip coating solution was

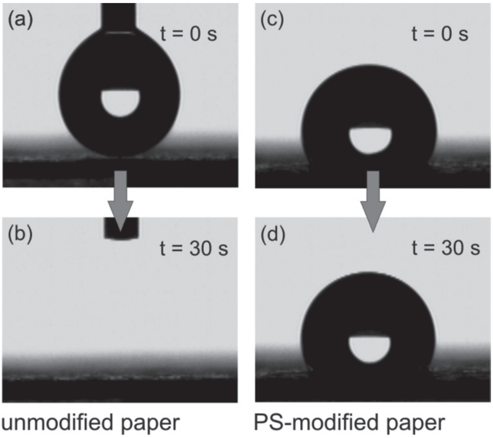
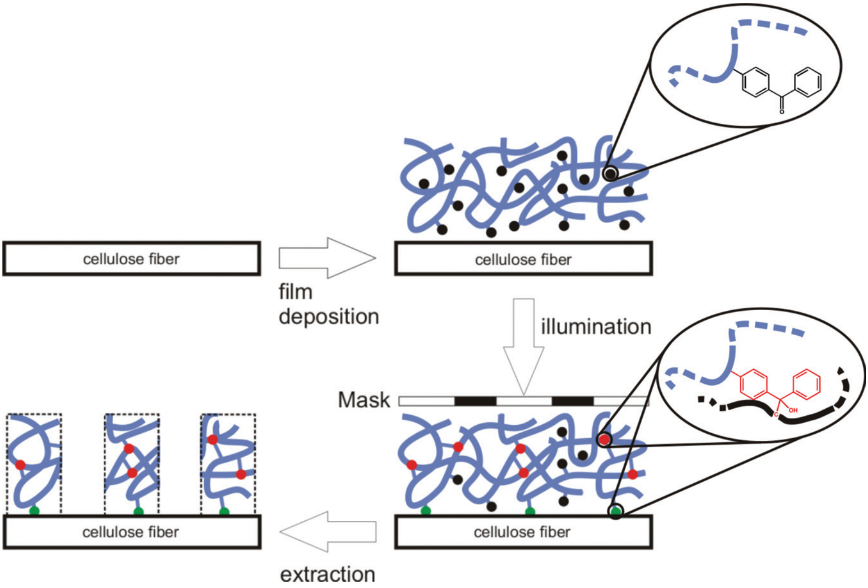


Figure 2. Comparison of static water contact angles between untreated and polymer – modified filter paper. Left: water droplet on an untreated filter paper: (a) was captured when the droplet was just brought into contact with the paper substrate; (b) was captured 30 sec after deposition of the droplet. Right: water contact angle on a P(S-co-MABP-co-PyMA) – modified paper substrate: (c) after 0 sec, (d) after 30 sec of deposition, respectively.

observed. These data show that the amount of photo-attached copolymer can be adjusted over a wide range from few mg m^{-2} up to several tenth of mg m^{-2} by adjusting the copolymer concentration in the dip coating solution[16].

Because all copolymers are attached to cellulose fibers by photochemical means, we were next interested whether chemical micropatterns on filter paper can be fabricated using simple UV-lithography (Scheme 3). In order to generate a surface micropattern, filter paper was modified in a first step with P(S-co-MABP) and P(S-co-MABP-PyMA) copolymers, respectively as outlined above (Note, in case of P(S-co-MABP-co-PyMA) illumination was performed at $\lambda = 365$ nm). During the illumination step a lithographical mask, compromising spots of 3 mm diameter, was placed on top of the paper substrates. Macromolecules that are adsorbed to cellulose fibres in shaded areas are not covalently attached to the paper substrate during the illumination with UV-light and therefore can be removed by solvent extraction, yielding a chemical micropattern.

A representative fluorescence micrograph of a lithographically microstructured paper substrates is shown in Figure 3 A. Here, hydrophobic, fluorescent polystyrene copolymer is surrounded by extracted filter paper. As can be inferred from the micrograph, a simple one-step photo chemical reaction combined with conventional lithography can be used to obtain defined, chemical stable micropatterns on filter paper. First simple wetting experiments using an aqueous



Scheme 3: Schematic illustration of the lithographic process for creating chemical micropatterns. During the illumination step a mask is brought into contact with the substrate. No light passes through sections blocked by the mask. Non-bound macromolecules in shades areas can be removed by solvent extraction. Thus, this approach leads to spatially-defined immobilization of the functional polymers on the paper substrate.

ink solution were performed in order to test the performance of the chemical micropattern with respect to guide an aqueous fluid. A photograph of a P(S-co-MABP) modified filter paper, taken after the sample has been submerged into an aqueous ink solution is shown in Figure 3 B. The aqueous ink solution homogeneously wets all areas of the filter paper from which the polymer has been extracted, i.e. where hydrophilic, non-modified cellulose fibers are present. In contrast, the ink solution did not wet areas, which had been exposed to UV-light and which consist of cellulose fibers coated with the hydrophobic copolymer. The latter illustrates that chemical dewetting of aqueous solutions inside the paper substrate is possible by attaching polystyrene-based copolymers, and hence, it demonstrates that similar to PMMA-copolymers[20] these copolymers can easily used to define hydrophobic micropatterns in paper. Scheme 4 depicts the pathway that was followed to prepare chemical micro patterns inside paper substrates.

Next, we determined the minimum amount of immobilized hydrophobic PS-copolymer that is necessary to cause a change of the interfacial properties

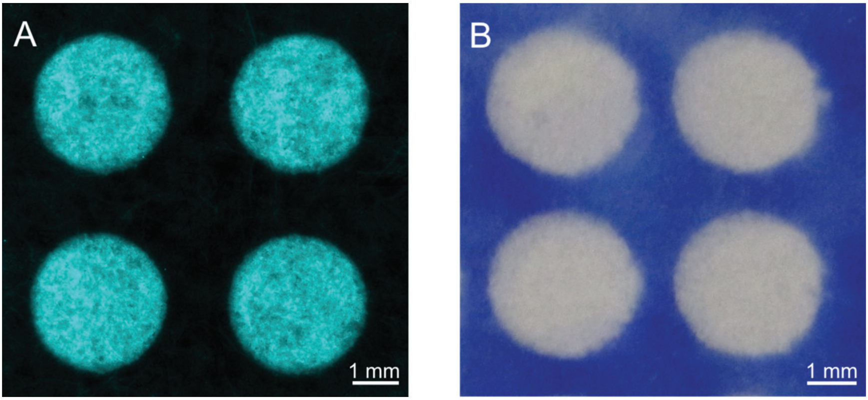
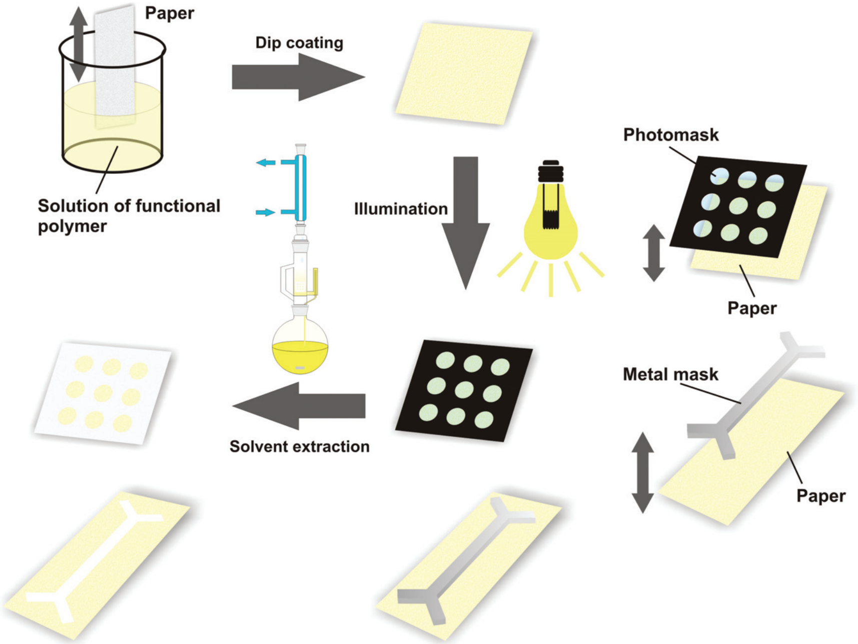


Figure 3. **A** Fluorescence micrograph of a P(S-co-MABP-co-PyMA) – modified paper substrate taken after illumination through a lithographical mask and solvent extraction. Fluorescence microscopy verifies the successful local immobilization of the functional polymer. Note, in order to have a comprehensive view of the microstructured surface, individual captured images were stitched together. **B** Photograph of a chemical microstructured paper substrate (modified with P(S-co-MABP)) taken after absorption of blue ink due to capillary forces. A spatial control of fluids can be achieved with chemical micropatterns.

from hydrophilic to hydrophobic, i.e. a change from a wetting to a dewetting state. For this, P(S-co-MABP) copolymers were dissolved in THF at various concentrations (c = 5, 10, 15, 20 and 30 mg ml^{-1}) and micro-patterned filter papers were prepared as described previously. Similar wetting experiments were performed using an aqueous ink solution (Figure 4) and it was observed that a polymer concentration of 20 mg ml^{-1} in the dip coating solution is sufficient to achieve quantitative dewetting of the copolymer spots. The same series of experiments was carried out using P(MMA-co-MABP) copolymers. In case of PMMA copolymers a similar mass of fiber-attached copolymer is needed for a complete change of the interfacial properties for the polymer-modified paper substrates, although PMMA – modified paper substrates exhibit a smaller static water contact angle (around 102°)[20].

The latter suggests that at this particular concentration of polymer in the paper substrate, the entire surface of the cellulose fibers is covered. At present we are investigating this finding in more detail.

Since in our approach UV-lithography is used to generate chemical micropatterns on paper substrates, the structure of the micropattern can be easily changed from e.g. circular spots to lines or other structures by using defined lithographical masks. Figure 5 (top) shows a part of a fluorescent micrograph of a paper-defined



Scheme 4. Schematic illustration of the strategy, which was followed to create chemical micro patterns inside paper substrates. In a first step, functional polymers are adsorbed to cellulose fibers from solution via dip coating. During the illumination step with UV-light a photomask is placed on top of the paper substrate and the photo-immobilization only takes place in non-shaded areas. The chemical micro pattern is developed in a final step by removing non-bound macromolecules in shaded areas via solvent extraction.

microchannel, using P(MMA-co-MABP-co-FOMA) as photoreactive polymer and lithographical mask that illuminates areas outside of the channel itself. The hydrophobic areas exhibit a strong fluorescence, whereas polymer-free areas do not exhibit fluorescence. The chemical micropattern was further analyzed using imaging-FTIR and scanning electron microscopy, respectively[20]. Again a simple wetting experiment using an aqueous blue ink was carried out in order to test the paper-defined microchannel. A photograph of a part of the channel, captured after the blue ink has been transported through the channel by capillary action, is shown in Figure 5 (bottom). As can be inferred from the photograph the fluid is transported exclusively within areas that have not been modified with the hydrophobic PMMA copolymer.

In a first proof-of-concept study we showed that these channels can be used to (1) mix two different analytes applied through seperate inlets and (2) to detect

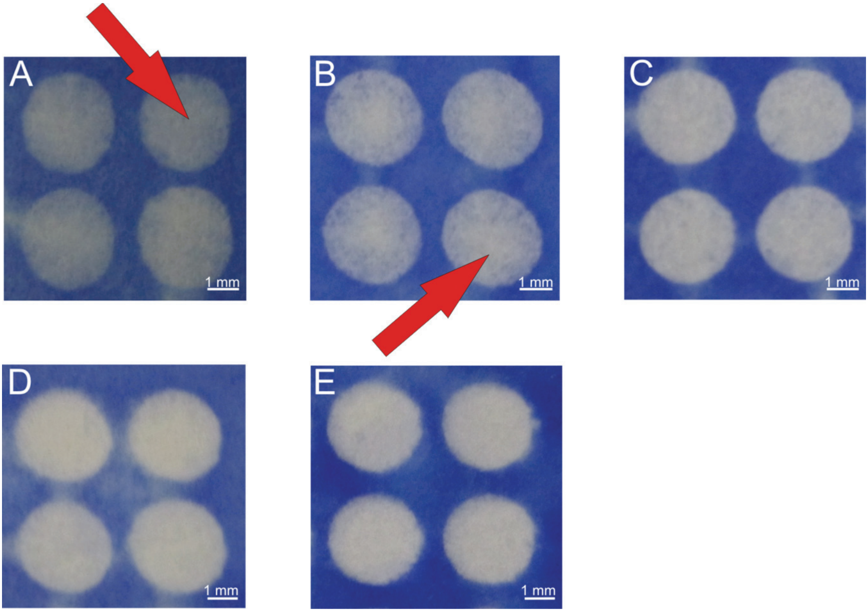


Figure 4. Photographs of chemical microstructured paper substrates [modified with P(S-co-MABP)] captured after absorption of a blue ink aqueous solution due to capillary forces. In case of A,B and C (the corresponding polymer concentrations are c = 5, 10 and 15 mg ml^{-1} THF, respectively) the blue ink penetrates into the polymer spots. At a polymer concentration of 20 mg ml^{-1} and 30 mg ml^{-1} no ink penetrates into the polymer spots indicating a sufficient high dewetting (D+E).

both analytes at the same time after mixing using specific binding assays. For this, ammonium thiocyanate and potassium ferrocyanide were spotted on either end of the Y-channel. Those substances can be used to detect specifically Fe^{3+} and Fe^{2+}, respectively. Aqueous solutions of Fe^{3+} and Fe^{2+} were brought into contact with the inlet of the paper device through silicon tubings. The solutions were guided through the channel, driven by capillary forces and at the same time mixed accordingly. A chromatic change of the spots inside the channel outlet was used to qualitatively observe the sensing event (Figure 6B). The appearance of a red and blue color indicated the successful sensing of the respective iron species ($Fe^{III}(SCN)_3$ exhibits a red color, $Fe_3^{II}[Fe^{III}(CN)_6]_2$ exhibits a blue color). Details on that experiment can be found elsewhere[20].

In case of the used model filter paper the fluid was transported along the complete channel over a length of about 5 cm within 60 min[20]. With respect to absolute time scales the latter appears to be rather long. However, the time for

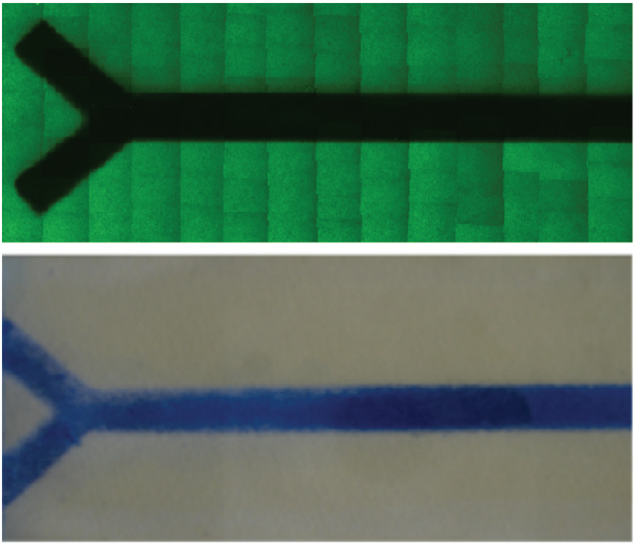


Figure 5. Top: Fluorescent micrograph of a paper substrate modified with a hydrophobic, fluorescent polymer. The polymer carries photoreactive groups, hence, adsorption of the polymer onto the fibers, with subsequent UV-illumination through a photo-mask chemically links the polymer exclusively in non-shaded areas, chemical microstructures, such as the microchannel (width: 4 mm) shown can be easily formed. Bottom: optical micrograph of fluid-transport (aqueous ink test fluid) inside the same channel. The fluid is transported by capillary actions exclusively within areas that have not been modified with the hydrophobic barrier polymer.

fluid penetration inside a porous medium is a function of the porosity and surface chemistry of the paper substrate taking a simple Lucas Washburn kinetics into account (Eq. 1)[23–24]:

$$x(t) = \sqrt{\frac{\sigma \cdot t \cdot r \cdot cos\theta}{2\eta}} \qquad \text{Eq. 1}$$

Here, x(t) is the position of the fluid front at a time t, σ is the surface tension, r is the average pore radius, θ is the contact angle and η the viscosity. The model filter paper used in the present studies had rather small pore radii (about 2.5 µm, determined by Hg-porosimetry), hence a fast water penetration was not expected a priori. Taking into account Lucas Washburn kinetics (Eq. 1) one can expect that changes in the porosity of the paper substrates can lead to a significant change of absolute times for fluid penetration inside the paper – defined microchannels.

Furthermore, commercial filter papers normally contain a wet-strengthening additive. Typically hydrophobic polymers, that covalently cross link cellulose fibres are used. Those polymers can change the surface chemistry to some extend and cannot be removed by a simple solvent extraction. Finally, different filter papers may contain different amounts of wet-strength additives. Therefore, a series of lab-engineered paper materials were prepared in our labs. Among those we prepared model paper lab sheets that consist of 100% cellulose using cotton linters as fiber source and having a grammage similar to our used model filter paper (about 80 g m^{-2}). Paper-defined microchannels were prepared as outlined above using P(S-co-MABP) and the capillary rise of the aqueous solution inside the microchannel was captured by video streaming. An image sequence of the fluid flow inside the channel is shown in Figure 6A.

As can be inferred from Figure 6A the fluid is driven along the complete channel having a length of about 5 cm within about 23 min indicating a significant higher flow rate inside the paper – defined microchannel. The paper substrate used in this study had a pore radius of about 4 μm. Thus a significant change in the absolute time for fluid penetration inside the channel was expected. This simple experiment already shows that changes in the porosity of the used paper material

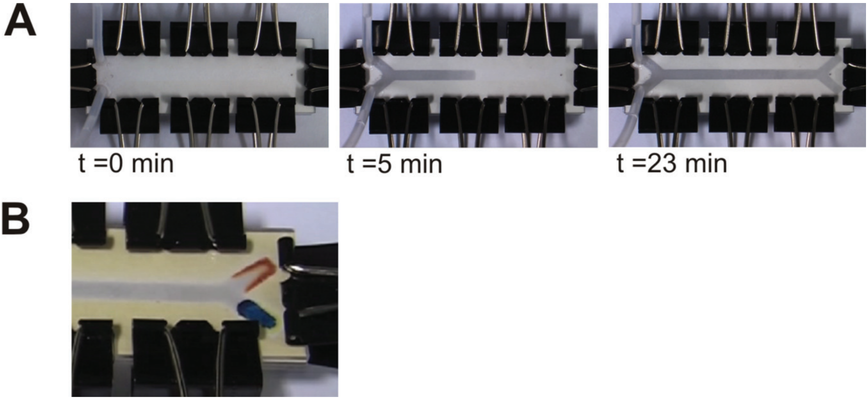


Figure 6. A Image sequence of flow inside the paper defined microchannel. Images were taken at 0, 5 and 23 min after the fluid was brought into contact with the channel. **B** For the duplex analysis ammonium thiocyanate and pottasium ferricyanate were spotted at either end of the Y-channel. The filter paper containing the microchannel was clamped between two PMMA slides and the inlets of the Y-channel were brought into contact with two fluid reservoirs containing aqueous solutions of Fe^{2+} and Fe^{3+} ions using silicon tubings. The appearance of the red and blue color, in respective spots, indicades the successful sensing of the respective iron species.

leads to a significant change of the fluid flow. The results of an in-depth study where different types of commercial filter papers as well as lab-engineered paper substrates have been used will be reported in future communications.

CONCLUSIONS

Polystyrene (PS) – based copolymers that contain defined amounts of photo – reactive benzophenone groups can be transferred and immobilized to cellulose fibers by dip coating and subsequent illumination of the paper substrates with UV-light. The amount of transferred polymer can be adjusted by changing the polymer concentration in the dip coating solution. Unbound copolymer can be removed from paper substrates by using a simple solvent extraction. SEM analysis revealed that paper substrates are chemically modified under mild conditions, i.e. the morphology of the paper substrate is not altered and the porous structure of the polymer – modified filter paper stays intact.

Static contact angle measurements showed that the polymer modification leads to a significant increase in the hydrophobicity of the resulting polymer modified paper substrates and therefore to a change in the interfacial properties. Because a photo chemical approach was used to attach the polymers to cellulose fibres, UV-lithography can be used to obtain defined, stable chemical micropatterns, including millimeter-sized microchannels on paper substrates. A polymer concentration of about 20 mg ml^{-1} in the dip coating solution is sufficient high to cause dewetting on the paper surface. With our approach chemically stable, paper – defined microchannels can be designed, which are capable to guide a capillary driven, i.e. pump-less fluid flow inside paper defined micropatterns. An engineering of the paper sheet itself, in particular, adjusting the pore-size and the grammage of lab-sheets allows to control and modulate the speed of fluid penetration of an aqueous fluid inside paper defined micro channels. The latter will become important to control a number of *low-high-tech* applications of paper materials, where fluids need to be driven at defined speeds through capillary actions. With respect to the latter, it is important to investigate the fluid transport of more complex fluids, e.g. used for applications in bioanalytical chemistry. An in-depth study on this will be done in the future and we will report on this in future communications.

ACKNOWLEDGEMENTS

We thank Martina Ewald and Heike Herbert for various technical support. A. Boehm likes to thank the Excellency Cluster ‘’Center of Smart Interfaces, CSI” for a research fellowship. Financial support by the Hessian excellence initiative LOEWE within the cluster SOFT CONTROL, and from the Verband der Papierfabriken (VDP), grant No. INFOR137, and INFOR154, is gratefully acknowledged.

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Transcription of Discussion

DESIGNING MICROFABRICATED PAPER DEVICES THROUGH TAILORED POLYMER ATTACHMENT

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Bob Pelton McMaster University

We have done quite a lot of this sort of thing in Canada. You pretty quickly come to situations where I think you need to fill the pores. So to give you a simple example, if you are designing a biosensor that involves breaking cells, one of the standard solutions for breaking cells is a commercial product called Viper™. This solution contains some alcohol and surfactants that will break through most coatings where the pores are not filled. So in our experience, at least, just coating the filter paper is not enough, you need to really plug up the pores.

Alexander Böhm

That's right, especially if you use solutions or solvents other than aqueous solutions; of course, they will penetrate inside the hydrophobic areas. One thing we want to do in order to be able to transport other solvents and water, for example, would be to use oleophobic polymers that are not only hydrophobic to water, but also to other solvents or to oil. For example, so that you can separate oil and aqueous solutions in the channels.

Discussion

Stephan Kleemann Munich University of Applied Sciences (from the chair)

How small are the channels that you have been able to produce?

Alexander Böhm

It depends of course also on the paper substrates that we use. If we go down to very low grammages in our paper substrates, we are able to produce channels or polymer spots in the range of 200 to 300 µm. At the moment we are analyzing this in more detail by using a confocal microscope, and we can see that, even these very fine patterns, or very low size polymer spots, are quite precisely transferred to the paper.

Roger Bollström Åbo Akademi University

Actually, my comment is more related to Robert Pelton's previous comment, when he mentioned that the pores need to be filled up. Why do you not use coated paper where you first, for example, have a barrier layer and on top of that you have a top coating where you can exactly control the porosity with mineral pigments?

Alexander Böhm

Of course this is also a possible approach; a process where you also chemically modify the paper substrate itself before you further, for example, immobilise polymers. For this application, maybe it makes sense also to first modify the paper, that's right.

Pierre Caulet Munksjö

I might have a suggestion for you. You might use some techniques that we have tried out using double ply paper. If you have two different furnishes, you can make a bottom ply which has very closed pores, by boosting up refining or that sort of thing, and including sizing agents. The top ply you can make very thin, if you have got, for example, a cylinder mould or similar machine, and thus have a top ply which will act as a channel for your liquid. You can then have a barrier underneath which is fully paper, and a carrier layer which is also fully paper. I do not know if it could work, but you could try it out even with a handsheet former.

Ramin Farnood University of Toronto

If I understood correctly, by applying a polymer patch, you can control how rapid the flow is, even downstream of that polymer patch. Based on the continuity equation, how do you explain this? Maybe I am misunderstanding the phenomenon.

Alexander Böhm

We still need to investigate. At the moment, we are performing some experiments on the confocal microscope in order to understand if our theory about what is happening is right. We know already from the confocal microscope that not the whole fibre surface is homogeneously attached to the polymer, we have got some kind of polymer agglomerate on the fibre surface. We believe the reason is that if we adhere a polymer patch, but the pressure is not high enough to change the contact angle of water to our polymer, in a way that its contact angle is smaller than for the fibre surface, then the pore will be blocked for the liquid. If we have pores that are not accessible anymore to water, and we think about the remaining pores that are still accessible for the water, we have some kind of apparent decrease in the pore diameter. As we have learned from our other experiments, the decrease in the pore diameter would lead to a decrease in the speed of fluid as well. So in other words, what we are thinking is if we do not use a polymer that is not water soluble, but go to polymers like PMA, for example, that are water soluble polymer networks, then we would expect totally different behaviour. We would expect that, once the polymer network has swollen, and so is again accessible for water molecules to pass through it, then we would expect to get an increase in the slope again after the polymer patch. So this should be different to what we saw here. This is what we are doing at the moment to find out if our theory could explain this behaviour, why the slope is not changing anymore after the polymer patch.

Asaf Oko SP Technical Research Institute of Sweden

Very nice piece of work, and I am jealous of what you have accomplished, being a PhD student myself. In the explanation you just gave, you were saying that the pores are either blocked or open, but it could also be that we simply have a change in the contact angle and therefore capillary pressure. Second which is a minor comment. It is very convenient to use the Lucas-Washburn equation, it gives you a lot of insight. One of the problems is that you cannot really relate average pore size to paper. You can do it qualitatively, which is maybe enough, but the point is that you should also maybe consider effects like permeability.