PAPER-BASED MICROFLUIDIC DEVICES ON FIBROUS PLATFORMS WITH DESIGNED STRUCTURE

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Paper-based microfluidic devices are emerging as a new technology for high-tech applications in diagnostics or as potential alternative tools for various analytical tasks. This paper demonstrates a novel concept to control fluid transport inside paper-based microfluidic devices, by using paper sheets with designed structure. Fibrous structures with controlled characteristics have been designed and fabricated to be used as a platform for making paper-based microfluidic devices. A series of paper sheets of 80 g/m^2, with different fibrous composition, were fabricated on a lab former in the absence of additives and fillers using two pulps, in different ratios: a softwood bleached chemical pulp and a mercerized pulp (Porosanier J-HP). The mercerized pulp was beaten in a Jokro mill with modified rolls, specially designed for increasing cutting effects. Hydrophobic barriers have been designed inside the paper substrates by the wax printing method. The heating process that follows printing determines wax penetration within paper in both directions, vertically and horizontally, reducing the resolution of printed patterns. The limits of the method are discussed from this point of view. The influence of parameters, such as pulp type, fibrous composition and channel width, on the flow rates through the channel was investigated by monitoring the capillary-driven fluid flow of aqueous solutions.

Keywords: paper-based microfluidics, wax printing, capillary flow, micro-channels design

INTRODUCTION

Microfluidics is the science and technology of systems that process or manipulate small amounts of fluids, usually in the range from microliters (10^{-6}) to picoliters (10^{-12}), using networks of channels with dimensions of tens to hundreds of micrometers.

The development of the microfluidics field was influenced by the achievements in the domain of molecular analysis in the capillary format, by the biodefense research efforts (after the Cold War), by the explosion of genomics in the 1980s, followed by the development of microanalysis in molecular biology and not least by photolithography and associated technologies from microelectronics. Microfluidics has the potential to influence subject areas from chemical synthesis and biological analysis to optics and information technology.[1]

The behaviour of fluids at the microscale differs essentially from that at the macroscale in that factors such as surface tension, energy dissipation and fluidic resistance start to dominate the system. The flow of fluids in narrow channels with sizes of around 100 nm to 500 μm is laminar rather than turbulent (very low values of the Reynolds number). A key consequence is that co-flowing fluids do not necessarily mix in the traditional sense, as the flow becomes laminar; molecular transport between them must often be through diffusion.[2]

The first applications of microfluidic technologies have been in analysis, for which they offer a number of advantages: the ability to decrease sample and reagent consumption and to carry out separations and detections with high resolution and sensitivity, their low cost, the possibility to run multiple analyses simultaneously, short times for analysis, and small footprints for the analytical devices.[3]

Microfluidics has seen rapid development as regards materials, methods of fabrication and the components – microchannels that serve as pipes and other structures that form valves, mixers, microreactors and pumps – which are essential elements of microchemical "factories" or "lab-on-a-chip".

Even if initially the materials of the microfluidic devices consisted only of silicon and glass substrates, as the field advanced, new materials, such as low temperature co-fired ceramics, elastomers and thermoplastics polymers and not least paper, were evaluated.[4] Paper microfluidics is an emerging technology and substantially different from that of devices made of polymeric or inorganic materials.

Paper as a matrix for microfluidic devices offers many advantages compared to other materials, particularly due to its capability to transport liquid by capillary forces. Unlike conventional microfluidic platforms, no external pumps are required for fluid transport inside the paper due to capillary forces. In addition, the paper consists of cellulose, a renewable and very cheap material, with low fabrication cost, which is lightweight, easy to stack, store and transport, disposable and biodegradable. The surface of cellulose fibres can be modified with different chemical functions for the definition of hydrophobic barriers (*i.e.*, small channels inside the paper sheets), or conjugated with sensing elements (*i.e.*, immobilization).[5] Paper-based microfluidic devices provide interesting applications, including clinical "point-of-care" diagnostics, food quality control and environmental monitoring.

Microfluidic Paper-based Analytical Devices (μPADs) are fabricated by micro-patterning hydrophobic regions on paper. These regions define paths for liquids that spontaneously wick through the paper. There are several techniques and processes involving chemical modification and/or physical deposition that could be used: (1) photolithography,[6–8] (2) plotting with an analogue plotter,[9] (3) inkjet etching,[10,11] (4) plasma treatment,[12,13] (5) paper cutting,[14,15] (6) wax printing,[16–17] (7) ink jet printing,[18-20] (8) flexography printing,[21] (9) screen printing,[22] and (10) laser treatment.[23]

The photolithography method uses an epoxy-based negative photoresist (SU-8). Paper is impregnated with photoresist, dried and exposed to UV light through a transparency mask, which can be printed. The unexposed photoresist can be washed out of the paper to form the hydrophobic microfluidic channels.

Wax printing is a simple and inexpensive method for patterning microfluidic structures in paper using a commercially available printer and hot plate. The patterns of solid wax are printed on the surface of the paper, and a hot plate melts the wax so that it permeates through the paper.

Current research on paper-based microfluidic devices focuses only on the commercially available filter or chromatography papers. Although filter paper is widely used, it does not always possess the desired physical characteristics, so other types of paper or paper modifications must be explored.

The main objective of this study is to highlight the influence of paper sheet structures on lateral flow in paper-based micro-engineering devices. For this purpose, fibrous structures with controlled characteristics have been designed and fabricated to be used as a platform for making paper-based microfluidic devices.

EXPERIMENTAL

Materials

Two different pulps were used for the preparation of designed paper substrates: bleached softwood chemical pulp (RM-8495 Northern Softwood Bleached Kraft Pulp NSBK – The Procter & Gamble Company, USA) and, respectively, Porosanier™ pulp, available from Rayonier Inc., Jesup, Ga, USA, a "mercerized" fully bleached southern softwood pulp with very high porosity (Frazier porosity – 120 $cm^3/cm^2 \cdot s$) and high content of alpha-cellulose (95.5%).

Whatman qualitative filter paper (grade 1, basis weight: 88 g/m^2, mean pore radius: 5.4 μm, thickness: 180 μm), from Sigma-Aldrich, was also used as the reference for wax spreading in the investigation on paper.

Pulp beating

The Northern Softwood Bleached Kraft Pulp (P&G) was beaten in a Jokro mill (ISO 5264-3:1979) with 1200 revolutions. After the beating process, the softwood pulp had a Schopper-Riegler (°SR) value of 20 (ISO 5267-1).

It is well known that mercerized pulp is a bleached pulp treated with hot diluted alkali to get extra high bulk and high porosity.[24] As the effect of hot alkali treatment, the pulp fibres swell and the hydroxyl groups of the cellulose become cross-linked. The dissolved hemicelluloses are washed out and the pulp will subsequently not respond to refining. To overcome this problem, the mercerized pulp (Porosanier J-HP) was beaten in a Jokro mill with modified rolls, specially designed for increasing cutting effects. The external diameter of the original roll is 89.15 mm. It has 35 bars, each 60 mm long and 2.0 mm wide. The new geometry of the modified roll is presented in Figure 1. The channels are deeper and the bars are only 1.5 mm wide. Using modified rolls, after 2250 revolutions, a similar Schopper-Riegler (°SR) value of only 11 was achieved, in the case of

mercerized pulp beating. The water retention value (WRV) was determined according to ISO 23714:2014.

Preparation of paper handsheets

Five different paper stocks were prepared by proportioning in different ratios (1:0/3:1/1:1/1:3/0:1) the above-mentioned two pulps. A series of isotropic paper sheets with a basis weight of 80 g/m^2 was fabricated on a conventional Rapid-Koethen handsheet former according to ISO 5269/2, without the use of any additives or fillers.

After sheet formation, the paper substrates were equilibrated at 23 °C and 50% relative humidity (climate chamber conditions) for at least 24 h, before further characterization was performed. At this point, the paper sheets contained about 6% moisture on average.

Scanning electron microscopy (SEM) investigations of the internal structure of the paper samples were performed by using a Quanta 200 type scanning electron microscope, operating at 15 kV with secondary electrons, in the low vacuum mode.

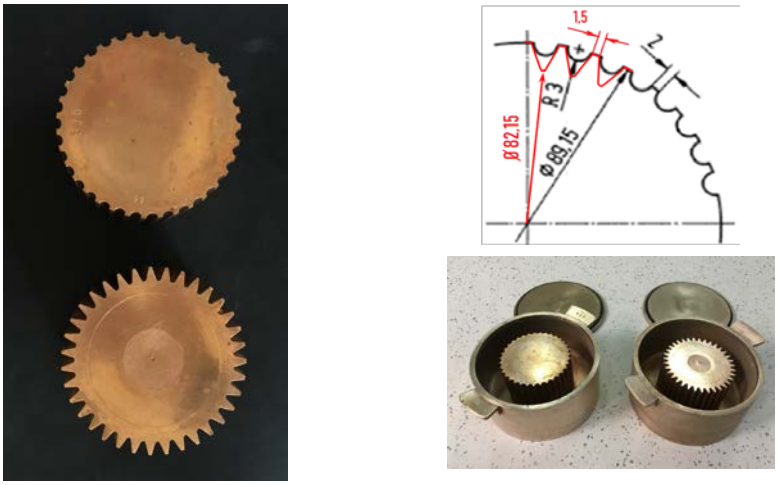
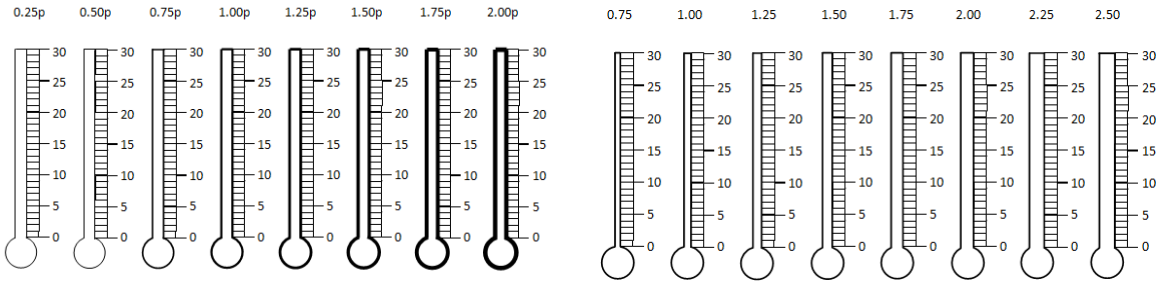


Figure 1: Original and modified geometry of Jokro mill rolls



(a) (b)

Figure 2: Patterns used in the experimental program: a) equal width channels delimited with lines of different weight; b) channels with different width, from 0.75 to 2.50 mm printed with lines of 0.75 points (265 μm)

Fabrication of paper-based microfluidic devices

Hydrophobic barriers have been designed inside the paper substrates by the wax printing method. A Xerox ColorQube 8580 wax printer was used to print microfluidic patterns designed with drawing tools in Microsoft Office software.

Two different patterns were used in the experimental program. The first one (Fig. 2a) is the image of a channel with a reservoir that was delimited with black lines of different weight, from 0.25 to 2.0 points (1 point = 353 μm). The second pattern (Fig. 2b) uses the same image, but the channels have a different width, from 0.75 to 2.50 mm.

The printed paper was heated and the wax melted and spread through the thickness of the paper. Two different methods were tested: heating in the oven and heating on a photo hot plate dryer set at 120 °C for 120 s.

Fluid transport through the paper-defined channels was investigated *in situ via* video streaming. Video streams were recorded and the data points of the fluid front at a given time were decoded after video capture. A solution of “Ferroin” indicator was used as the test fluid. It was prepared in accordance with TAPPI Test Method T 610 om-92: Preparation of indicators and standard solution (1.5 g of 1,10-phenanthroline

monohydrate, $C_{12}H_8N_2 \cdot H_2O$, and 0.7 g ferrous sulphate, $FeSO_4 \cdot 7H_2O$, were dissolved in 100 mL water).

RESULTS AND DISCUSSION

Characterization of designed paper substrates

In this study, the mercerized pulp was beaten in a Jokro mill with modified rolls, specially designed for increasing the refining response of this kind of pulp. The Schopper-Riegler test and WRV were used to evaluate the beating.[25] The beating results are shown in Table 1, which indicates that the Schopper-Riegler beating degree, before and after beating, was almost the same in the case of the mercerized pulp, but the properties of the paper sheets were greatly improved after beating. Even if the beating degree did not change after 2250 revolutions of the Jokro mill, the WRV results for this pulp demonstrated an increase from 6% for the original mercerized pulp to 13% for the same pulp after beating at 2250 revolutions, which was also confirmed by other studies.[26,27]

The properties of the RM-8495 Northern softwood bleached kraft pulp are well correlated with the beating degree.

The scanning electron microscopy (SEM) micrographs obtained for the paper samples fabricated from the pulp suspensions with different composition (Fig. 3) reveal that the most porous structure is obtained for the paper formed from 100% mercerized pulp, and the more compacted is the one obtained from 100% NSBK pulp, similar to that of Whatman No.1 filter paper.[26]

Effects of wax reflow

After wax printing, the heating process allows the wax to penetrate both vertically and horizontally within the paper matrix. The vertical spreading creates the hydrophobic barrier across the thickness of the paper. The lateral spreading decreases the resolution of the printed pattern and results in hydrophobic barriers that are wider than the original printed patterns.

The first part of our experimental program tries to give an answer concerning the minimum amount of wax that should be deposited and spread inside the structure of the paper sample in order to obtain functional hydrophobic barriers. To this end, the first pattern (Fig. 2a) was printed on Whatman qualitative filter paper, grade 1, using the solid ink printer set to the default parameters for photo-quality printing. As can be seen in Figure 3, the channels drawn with the line weight under 0.75 p (265 μm) are not able to develop real hydrophobic barriers after the heating step (test liquid spread over the edge of the channel).

The second part of our experimental program investigates the resolution of the wax printing method for fabricating paper-based microfluidic devices. For this purpose, microscopic examination was carried out to reveal details of wax spreading, which are too small to be seen with the unaided eye. A microscope for measuring (MM1-IOR) was used to determine the width of the microfluidic channels, prior and after the heat treatment. Horizontal wax diffusion inevitably reduces the resolution of printed patterns (Fig. 5). If the channel width before heating is of 750 μm (Fig. 5a) or lower, after wax spreading, it is possible for the channel to be practically completely closed (Fig. 5b). The smallest functional hydrophilic channel with an average width of 380 ± 45 μm was fabricated from two printed lines of 265 μm (0.75 p) separated by a nominal width of 950.

In a similar experiment, Carrilho *et al.*[28] reported very close values. Even if two different methods of heating were tested, in the oven or on the photo hot plate dryer, no significant differences between them were recorded.

Table 1
Properties of beaten and unbeaten pulps

Properties	Porosanier™ mercerized pulp		RM-8495 Northern softwood bleached kraft pulp	
	Unbeaten	Beaten at 2250 rev.*	Unbeaten	Beaten at 1200 rev.
Schopper-Riegler degree, SR°	11	11	17	20
Water retention value, %	6	13	129	136
Tensile index, Nm/g	15.8	22.7	32.6	63.4
Burst index, kPa m^2/g	0.25	0.34	1.9	4.4
Density, g/cm^3	0.41	0.42	0.57	0.60

*Modified Jokro roll

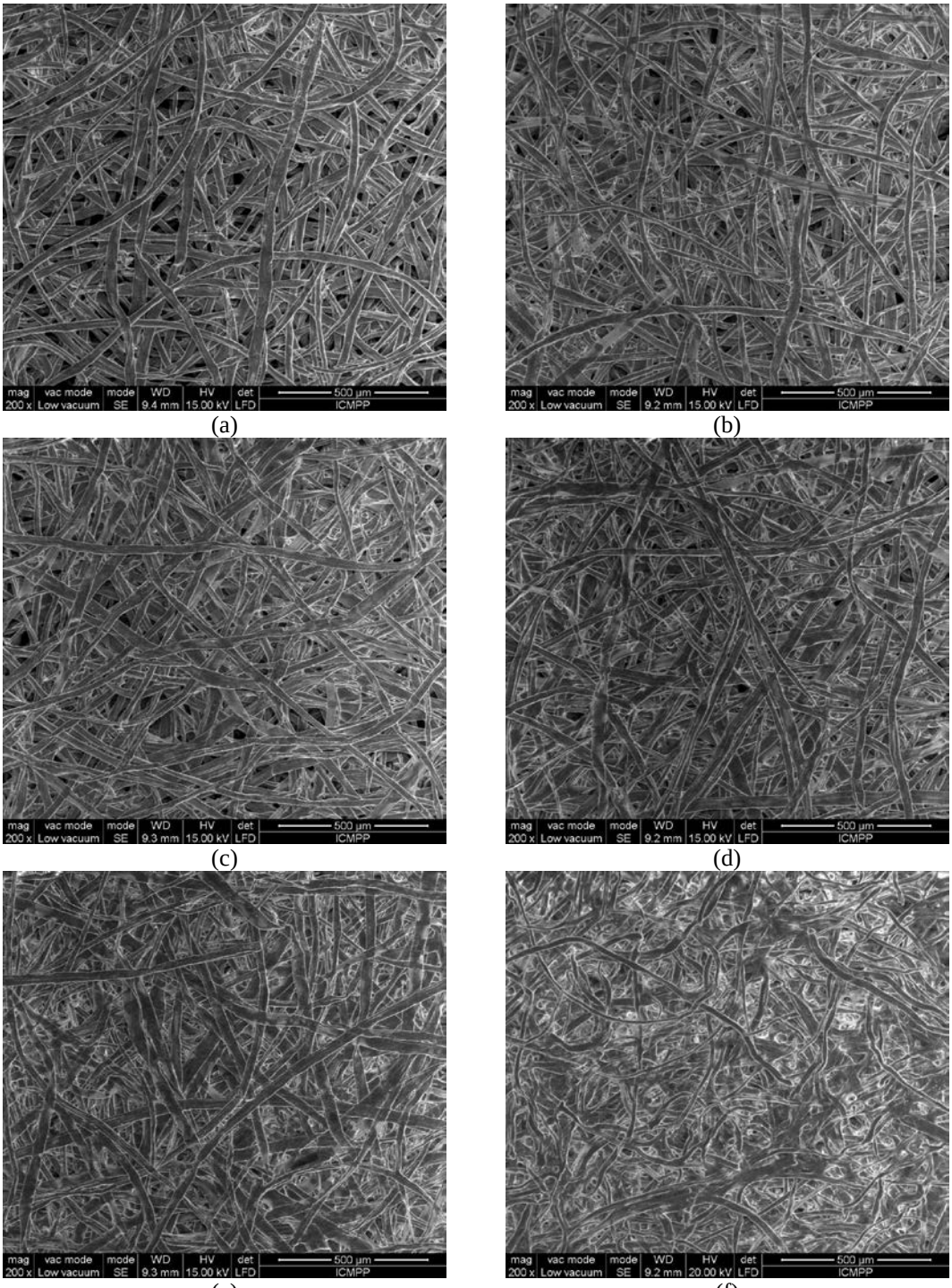


Figure 3: SEM micrographs of paper samples formed from pulp suspensions with different fibrous compositions (a) 100% Porosanier, (b) 75% Porosanier+25% NSBK, (c) 50% Porosanier+50% NSBK, (d) 25% Porosanier+75% NSBK, (e) 100% NSBK, (f) Whatman No.1 filter paper

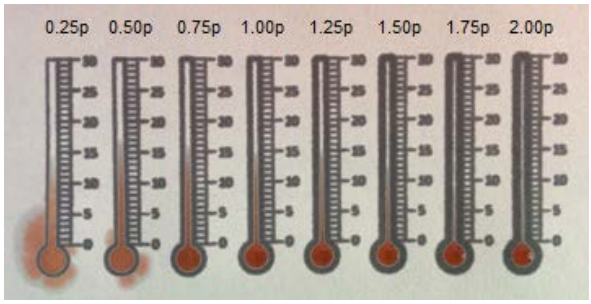
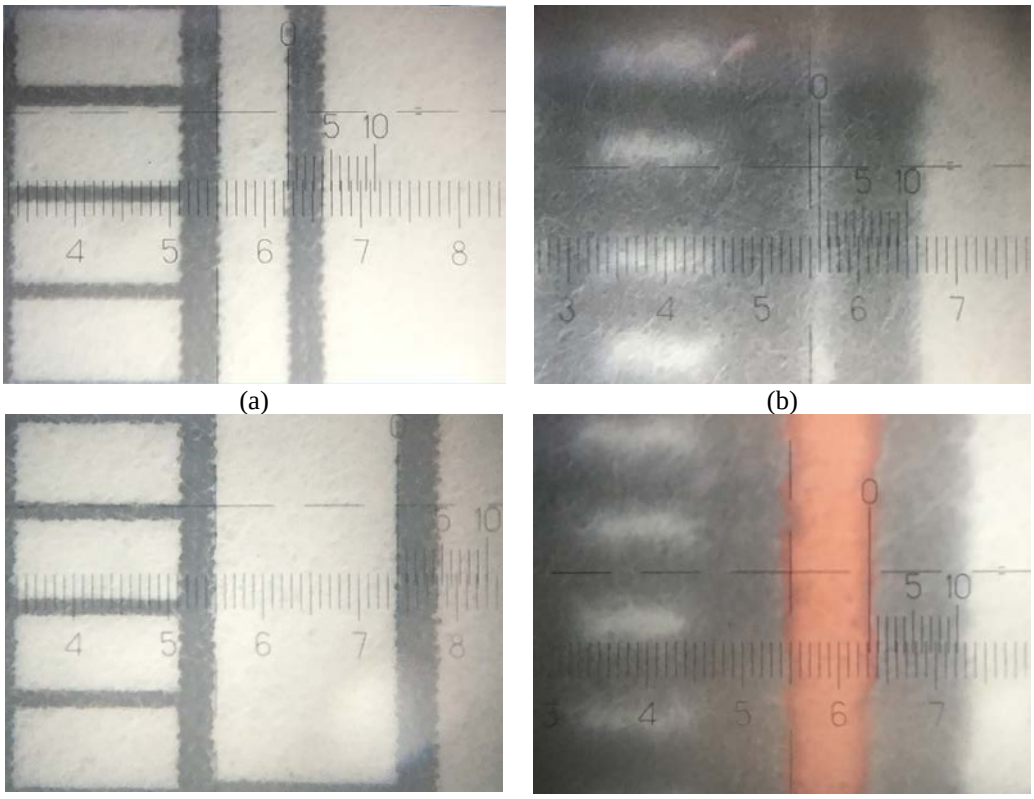


Figure 4: Test results regarding the minimum line weight to develop a hydrophobic barrier



(a) (b)

(c) (d)

Figure 5: Images of microfluidic channels of different width before (a, c) and after (b, d) the heating process

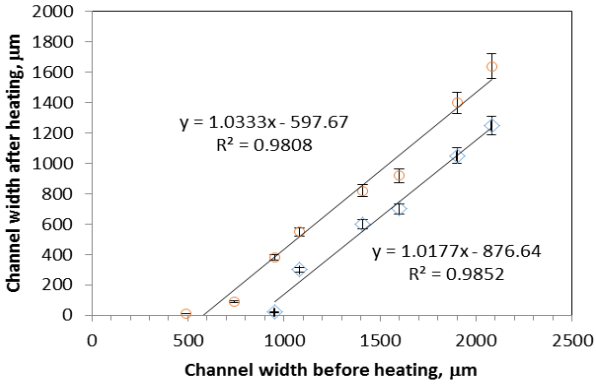


Figure 6: Effect of wax reflow on channel width for microfluidic devices printed on papers with different porosity (circles – Whatman No.1, squares – Porosanier 100%)

Decreasing horizontal wax diffusion by an optimum thermal treatment will result in wider hydrophilic channels for patterns printed with equal line distances. Measuring the channel width modification after wax reflow for different patterns permitted us to establish a correlation and

a model for wax spreading for each fibrous composition (Fig. 6).

Due to the nature of the fibrous matrix, paper tends to align the wax in a horizontal, rather than vertical direction. As a result, the wax spreads faster in the horizontal direction, causing a wider line, compared to the original width of the applied wax. Therefore, the reproducibility of the fabrication method is highly dependent on the width of the wax line and the heating temperature.

In the last part of our study, the fluid transport through the defined channels on different papers with designed structure was investigated *in situ via* video streaming. The data collected and decoded from the captured video were used to fit the profile of the flow according to the Washburn model (Eq. 1). The flow in the paper is a process of capillary flow in porous materials that is described by Washburn's equation:[29]

$$x(t) = (\sigma r t \cos\theta / 2\eta)^{0.5} \quad (1)$$

where x is the distance that a liquid of viscosity η, surface tension σ and contact angle θ penetrates into a porous material with an average pore radius r in time t.

The position of the fluid front (x) as a function of time (t), in non-linearized and linearized dependence (x as a function of $t^{0.5}$), is shown in Figure 7a and b, respectively. The effect of channel width is evident and by varying the width of the channel, the distance covered by the fluid front within a given time interval can be controlled over a wide range.

Depending on the porosity of individual substrates and on the channel width, significant differences in the slope of the studied microfluidic papers were observed (Fig. 7b, d). A slope of 6.73 was obtained for the paper sheets featuring with the bigger pore radius and porosity (72.96%),[30] whereas a slope of 1.064 was obtained for the paper substrates featuring the smaller pore radius and porosity (67.41%).

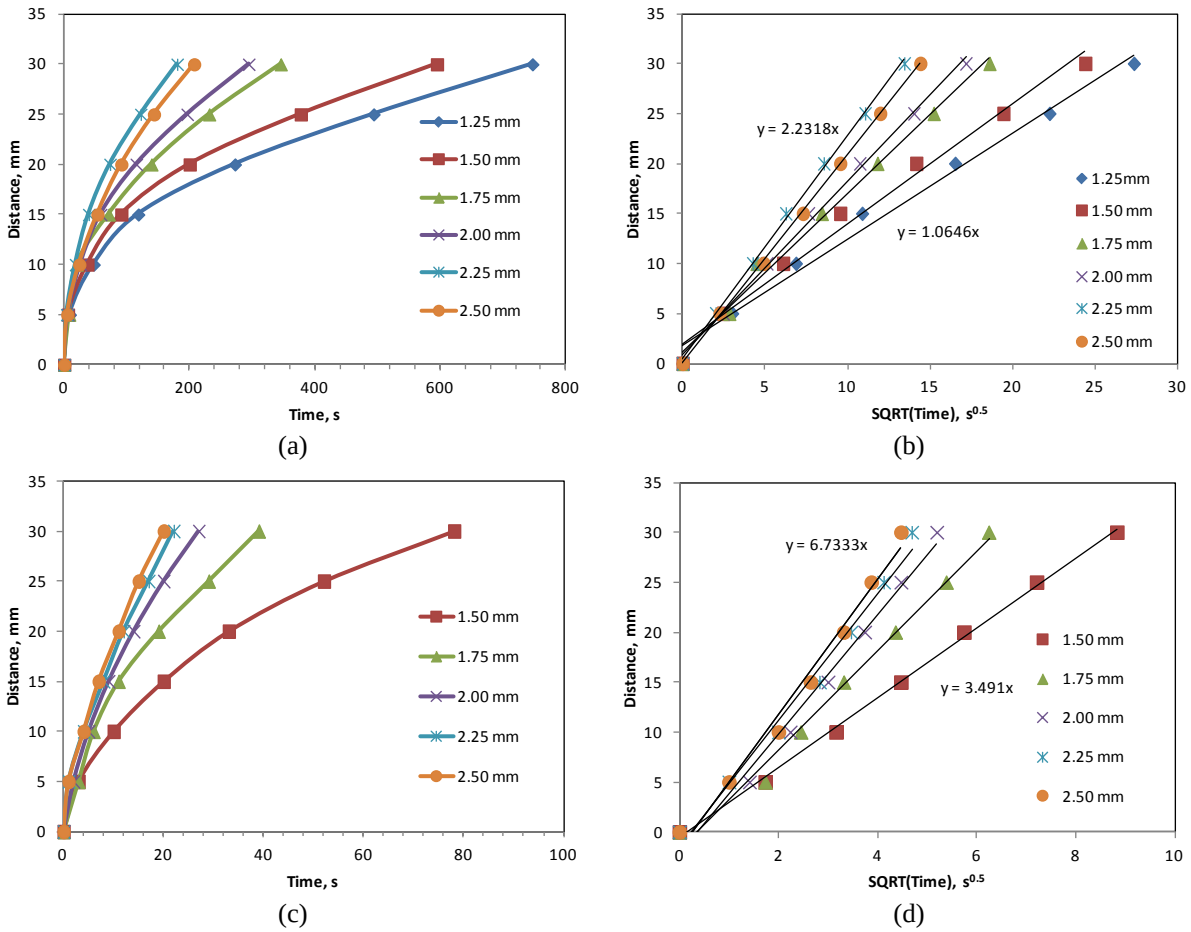


Figure 7: Comparison of the position (x) of the fluid front as a function of time, for the capillary flow in paper-defined channels with different printed widths ("theoretical width" – width that was designed with drawing software)

Table 2
Structural and flow characteristics of the fibrous substrates developed in this experiment

Sample	0.x	1.x	2.x	3.x	4.x	5.x	W
Fibrous composition	100% Porosanier unbeaten	100% Porosanier beaten	75% Porosanier +25% NSBK	50% Porosanier +50% NSBK	25% Porosanier +75% NSBK	100% NSBK	Whatman no. 1
Tickness, μm	188.3	183.9	161.7	156.7	137.8	128.3	180
Density, g/cmc	0.406	0.415	0.473	0.488	0.554	0.595	0.489
Porosity, ε %	72.96	72.30	68.50	67.49	63.03	60.31	67.41
Travelling time, s (30 mm, width 2 mm)	27	29	33	51	66	98	294

Considering absolute time scales, travelling times of the fluid along the straight part of a channel of 30 mm long and 2 mm width were of ~20 s (in the case of the paper sheet from unbeaten Porosanier pulp); ~51 s (for the paper substrate from 50% Porosanier pulp and 50% NSBK pulp); ~98 s (for the paper substrate from 100% NSBK pulp) and ~294 s (for the Whatman No.1 filter paper substrate used as reference) (Table 2).

Thus, designing paper substrates with desired porosities allows the control of the flow profiles of an aqueous solution through paper-defined channels on an absolute time scale by a factor of *10. The experimental results were compared with those obtained by other researchers,[5] but our findings suggest that accurate control of fluid transport processes with designed structure papers is possible.

CONCLUSION

The current study has demonstrated the possibility of using the popular wax-printing technology to fabricate miniaturized paper-based analytical devices with microfluidic structures. The patterning technique, in combination with the consideration of device geometry and the influence of paper structure, led to a model paper-based microfluidic device.

A novel concept to controlling fluid transport within microfluidic papers, by using paper sheets with designed structure, has been developed. Laboratory-made handsheets, consisting of different pulps, were prepared and further modified with small channels using hydrophobic barriers that have been designed inside paper substrates by wax printing. The influence of different parameters, such as fibrous composition and channel width, on the flow rates through the channel was investigated by monitoring the capillary-driven fluid flow of aqueous solutions.

REFERENCES

[1] G. M. Whitesides, *Nature*, **442**, 368 (2006).
[2] P. Tabeling, "Introduction to Microfluidics", Oxford University Press, New York, 2005, pp.70-129.
[3] A. Manz, J. C. Fettinger, E. Verpoorte, H. Lüdi, H. M. Widmer *et al*., *J. Chromatog. A*, **593**, 253 (1992).
[4] P. N. Nge, C. I. Rogers and A. T. Woolley, *Chem Rev*., **113**, 2550 (2013).
[5] A. Böhm F. Carstens, C. Trieb, S. Schabel and M. Biesalski, *Microfluid. Nanofluid*., **16**, 789 (2014).
[6] A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, *Angew. Chem., Int. Ed*., **46**, 1318 (2007).
[7] A. W. Martinez, S. T. Phillips and G. M. Whitesides, *Proc. Natl. Acad. Sci. U.S.A*., **105**, 19606 (2008).
[8] S. Klasner, A. K. Price, K. W. Hoeman, R. S. Wilson, K. J. Bell *et al*., *Anal. Bioanal. Chem*., **397**, 1821 (2010).
[9] D. A. Bruzewicz, M. Reches and G. M. Whitesides, *Anal. Chem*., **80**, 3387 (2008).
[10] K. Abe, K. Suzuki and D. Citterio, *Anal. Chem*., **80**, 6928 (2008).
[11] K. Abe, K. Kotera, K. Suzuki and D. Citterio, *Anal. Bioanal. Chem*., **398**, 885 (2010).
[12] X. Li, J. Tian, T. Nguyen and W. Shen, *Anal. Chem*., **80**, 9131 (2008).
[13] X. Li, J. Tian and W. Shen, *Cellulose*, **17**, 649 (2010).
[14] E. M. Fenton, M. R. Mascarenas, G. P. López and S. S. Sibbett, *ACS Appl. Mater. Interfaces*, **1**, 124 (2008).
[15] W. Wang, W.-Y. Wu and J.-J. Zhu, *J. Chromatogr. A*, **1217**, 3896 (2010).
[16] Y. Lu, W. Shi, L. Jiang, J. Qin and B. Lin, *Electrophoresis*, **30**, 1497 (2009).

[17] E. Carrilho, A. W. Martinez and G. M. Whitesides, *Anal*. *Chem*., **81**, 7091 (2009).
[18] V. Leung, A.-A. M. Shehata, C. D. M. Filipe and R. Pelton, *Colloids Surf. A*, **364**, 16 (2010).
[19] X. Li, J. Tian, G. Garnier and W. Shen, *Colloids Surf. B*, **76**, 564 (2010).
[20] J. L. Delaney, C. F. Hogan, J. Tian and W. Shen, *Anal*. *Chem*., **83**, 1300 (2011).
[21] J. Olkkonen, K. Lehtinen and T. Erho, *Anal*. *Chem*., **82**, 10246 (2010).
[22] W. Dungchai, O. Chailapakul and C. S. Henry, *Analyst* (*Amsterdam*), **136**, 77 (2011).
[23] G. Chitnis, Z. Ding, C.-L. Chang, C. A. Savran and B. Ziaie, *Lab*. *Chip*., **11**, 1161 (2011).
[24] H. Nanko, A. Button and D. Hillman, "The World of Market Pulp", Appleton, WI, USA, WOMP, LLC., 2005, pp. 16-17.
[25] B. Blaznik, D. Gregor-Svetec and S. Bračko, *Cellulose Chem*. *Technol*., **51**, 755 (2017).
[26] J. Hao, C. Deng, X. Wang and J. Hu, *BioResources*, **9**, 4336 (2014).
[27] Y. Chen, J. Wan, Q. Wu, Y. Ma and M. Huang, *Cellulose Chem*. *Technol*., **50**, 1061 (2016).
[28] E. Carrilho, A. W. Martinez and G. M. Whitesides, *Anal*. *Chem*., **81**, 7091 (2009).
[29] E. W. Washburn, *Phys*. *Rev*., **17**, 273 (1921).
[30] M. Àngels Pèlach, M. Delgado-Aguilar, M. Alcala, J. Puig, A. Blanco *et al*., *Cellulose Chem*. *Technol*., **50**, 449 (2016).