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HIGH-THROUGHPUT ROLL-TO-ROLL PRODUCTION OF BIO-FUNCTIONALIZED POLYMER COMPONENTS

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Abstract

Within the last decades, a growing number of miniaturized solutions for biological and chemical analysis processes have been developed by scaling these analyses down to a lab-on-a-chip (LoC) level, thanks to sensitive and rapid testing abilities of LoCs. In parallel to that, a significant need for high-throughput and cost-efficient production of LoC components arises. Our roll-to-roll (R2R) pilot-line for imprinting micro- and nano- structures onto polymers in various designs provides high-throughput manufacturing solutions for polymer based flexible LoC components. The structure imprinting (<1 µm resolution) is performed on polymer foils with ~125-200 µm thickness using a long-lasting stamp. The system also enables accelerated 19 and localized bio-functionalization with molecular probes (e.g., DNA) using low quantities of biomaterials with a photoactive, commercial linker. Without a surface pre-functionalization, efficient generation of DNA micro-arrays are done and probe DNAs can be tethered successfully onto structured polymer components in a continuous and rapid micro-spotting process.

Keywords*: roll-to-roll UV nanoimprint lithography, polymers, DNA, protein, high-throughput micro-array spotting, bio-functionalization.*

Introduction

Roll-to-roll (R2R) UV nanoimprint lithography (NIL) for micro- or nano- patterning of large polymer surfaces provides performing rapid pattern transfers, from one roll to another. In literature, R2R UV-NIL processes have been demonstrated in many diverse fields. [1-5] Our R2R imprinting technology enables high-throughput production of diverse polymer components. The photograph of our complete pilot line was given in Fig. 1. Not only we provided a complete process chain for first-time realization of production lines for lab-on-chip components but also, we bio-functionalize these imprinted polymer components in a high-throughput manner. Prior to any bio-functionalization process, R2R imprinting is performed in a R2R UV-NIL unit (Fig. 2A). A polymer foil (Fig. 2A, in purple color) coated with a custom-developed our custom-developed and uncured, NILcure JR21 photoresin (Fig.2A, in yellow color) enters inside the imprinting unit, at where it is imprinted by a roller stamp (i.e., a shim) carrying the inverse of the desired structures.

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Fig. 1. Our high-throughput R2R pilot line for producing micro / nano- structured polymers.

During the R2R UV-NIL imprinting, an adjustable, rubber-coated counter roller pushes the foil up to support the imprinting process. Simultaneously, the structures are cured from the foil backside using a UV lamp. Inset in Fig.2A also shows a closer-look at the imprinting process, illustrating the photoresin on a polymer foil (in gray color) using a shim (in dark gray color); wrapped over the roller web. Using this technique, we can produce robust, micro- or nano- structured polymer components in high-throughput. [6-9] With our imprinting technology, structuring the resin with down to 1000 nm in depth and 200 nm in width is possible. [6] Furthermore, high-throughput bio-functionalization of structured or unstructured polymer foils can be performed with our large-scale micro-spotting unit (e.g., hundreds of LoC components / minute). Our DNA / protein micro-spotter (Fig.2B) allows a continuous and multiplexed spotting process following the R2R imprinting. Thanks to multiple piezo dispense capillary (PDC) nozzles, multiplexed spotting is possible via this system. The R2R micro-spotting line that is planned to be combined with our R2R imprinting line in the future. Withal, the semi-automated R2R micro-spotting line enables DNA or protein micro-spotting onto large area un/structured polymer foils at pre-defined locations (Fig.2C). The line is composed of three main parts: an unwinder roller, a large-scale micro-array spotter and a rewinder, which wraps the polymer foil back. The micro-spotting is performed automatically via a glass, PDC nozzle dispensing the droplets (shown in purple and green colors) with desired spotting configurations and parameters (Fig. 2D-E). Fig. 2E shows the drop camera view of the micro-spotter showing a PDC nozzle dispensing a droplet of DNA solution. The green rectangle defines the region-of-interest in which the droplet should be located for a successful micro-spotting. The droplet center is 450 μm away from the glass nozzle tip. A spotting solution, including a water soluble and photoactive terpolymer [10], which gets cured in the presence of UV light at 254 nm (for DNA molecules), is utilized to perform easy attachments of the probe bio-molecules providing no necessity for a pre-functionalization of the polymer surface. The spotting is performed at room temperature in a humidity controlled atmosphere. Hence, via our technology, cost-efficient bio-functionalization using small volumes of biomaterials (e.g., single-stranded DNAs (ss-DNAs), antibodies) is possible.

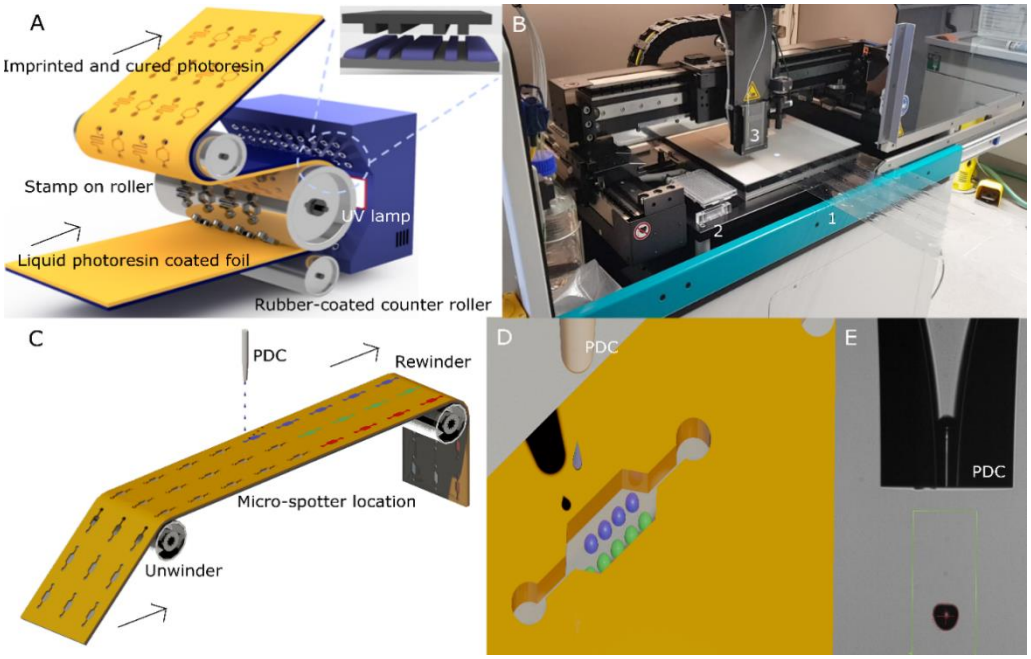

Fig. 2. High-throughput R2R production of bio-functionalized polymers. **(A)** A drawing of our R2R UV-NIL. **(B)** Our high-throughput DNA / protein micro-spotter. 1, 2 and 3 refer to a R2R imprinted foil entering to the micro-spotter in the spotting unit, a DNA/protein source plate and a holder for PDC nozzles, respectively. **(C)** A drawing of the micro-spotting unit with a PDC nozzle. **(D)** A closer-look at the PDC nozzle in action. **(E)** The drop camera view of the micro-spotter showing the PDC nozzle dispensing a droplet of DNA solution.

In this study, we examined the micro-spotting efficiency of a model single-stranded DNA onto polymer foils, which were R2R coated with our NILcure JR21.

Experimental

Materials

For the micro-spotting study, unstructured polymer foils coated with our custom-developed, acrylate-based photoresin, NILcure JR21 were used. Polyethylene terephthalate (PET, Melinex ST506 foil with 125 µm thickness) and polystyrene (PS) foils (190 µm thickness), used in this study, were purchased from DuPont Teijin Films (UK) and 4titude (Wotton, UK), respectively. SciPoly3D DNA conjugation kit (Scienion AG, Germany) was utilized for the immobilization of single-stranded DNA (ss-DNA) molecules on the polymer foils. During micro-spotting, a custom-designed, cyanine 5 (Cy5) labelled ss-DNA (i.e., 25 nt Cy5-R2R oligo) was utilized. The Cy5-R2R oligo (5'-Cy5-GCA TGG TTC AAA AGT GAA AGA CAA A-3') was synthesised with high purity by Microsynth (Austria). In washing tests, phosphate buffered saline (PBS, as tablets, Sigma-Aldrich, Germany) was utilized. During the study, molecular biology (MB) grade water (Carl Roth, Germany) was used.

R2R Printing of NILcure JR21

In our R2R pilot line, our custom-developed NILcure JR21 photoresin is reverse rotogravure printed onto a polymer foil (i.e., PET or PS). NILcure JR21 coating is done with a customized base-coater (BC44, Coatema Coating Machinery, Germany) at a web speed of 5 meter/min. For this study, a flat unstructured nickel shim without any topography was used in the UV NIL imprinting unit. Thus, without performing the UV-NIL structuring, NILcure JR21

on the polymer foils was cured using a mercury lamp with a light intensity of 2.2 W/cm^2. With these settings, a flat, uniform coating of the polymerised imprint NILcure JR21 was produced on the polymer foils.

High-Throughput DNA Biofunctionalization

The spotting solution was prepared using 75 µl sciPOLY3D SOL2D1, 30 µl sciPOLY3D SOL1, 42 µl MB grade water and 3 µl R2R-Cy5 oligo stock solution (100 µM). The ss-DNA stock solution was prepared in MB grade water. The spotting was performed using a customized, medium-scale micro-array spotter (sciFLEXARRAYER S12, Scienion AG, Germany), which enables spotting with 8 different nozzles simultaneously. Prior to the micro-spotting, the spotting solution was provided in a 384 well-plate (V-shaped, sciPLEXPLATE, Scienion AG, Germany), placed and continuously cooled at 25 ˚C inside the micro-array spotter. The presented results were generated with chips spotted with the following parameter: an inert glass piezo dispense capillary (PDC70 Type 1 for DNA spotting, Scienion AG, Germany) was used to dispense the spotting solution with a drop per spot, onto the NILcure JR21 coated polymer foil. The spot pitch and the drop volume were 180 µm and 410 ± 10 µl, respectively. The spotting was performed at room temperature with a 38 ± 2 % relative humidity. The spots were cured at 254 nm using a NU-72KM model UV-lamp (Benda, Germany) with different curing times of 2 min, 12 min or 26 min.

In order to investigate micro-spotting efficiency, two different washing tests were applied: a thorough washing test and an incubation test. In the washing test, the spotted polymer foils were rinsed with 150 µl of 1x PBS solution. In the incubation test, the foils were incubated for 1 hour at room temperature, in 1x PBS solution, inserted inside a 1.5 ml Eppendorf™ tube, placed on a laboratory shaker. Following all washing tests, the foils were washed with MB grade water and were dried at ambient air.

Visualization of the DNA Micro-Spots

A fluorescence microscope (BX51 model, Olympus-Lifescience, Germany) with a mercury lamp (U-RFL-T 102 model, Olympus-Lifescience) was used to visualize the ss-DNA microspots. All images were taken using a Cy5 filter (650 nm excitation maximum and 667 nm emission maximum) and a 10X objective. All images were created in Cell^D software with 50 seconds of integration time. The fluorescence intensities (a.u.) were calculated for the red channel using GIMP 2.10.10 software. Using four different Cy5-R2R microspots of each image, the average pixel intensity was calculated. For each sample, the background signal was calculated by averaging the pixel intensities from four different non-spotted (i.e., dark) regions. The background signals were subtracted from each micro-spot signal for each measurement. Then, mean values of each measurement were calculated. Final data was presented by a single mean value ± its standard deviation.

Results and Discussion

In order to study the ss-DNA immobilization on the NILcure JR21 resin, the micro-arrays were created on the NILcure JR21 coated PS and PET foils. Prior to the micro-spotting, the foils were roto-gravure printed with acrylate-based NILcure JR21 resin in our R2R pilot line. The foils were spotted in the medium-scale micro-array spotter, using a PDC nozzle suitable for DNA spotting applications. The Cy5-R2R spot size was ~ 150 µm after the micro-spotting process. Two different washing methods were applied and the spots were checked under the fluorescence microscope, before and after the washings. For each polymer foil type (i.e., PET and PS), 9 individual samples were prepared applying 3 different UV curing times (i.e., 2, 12, 26 min). To determine the optimal curing time for each polymer foil, Cy5-R2R micro-arrays were prepared with 2, 12 and 26 min UV curing times (Fig.2A-F). Signal

intensities (a.u.) obtained from all unwashed (UW) samples, were not altered dramatically showing that the curing time has a low impact on Cy5 intensity arising from the DNA micro-spots (i.e., bleaching of Cy5 during the curing process was negligible).

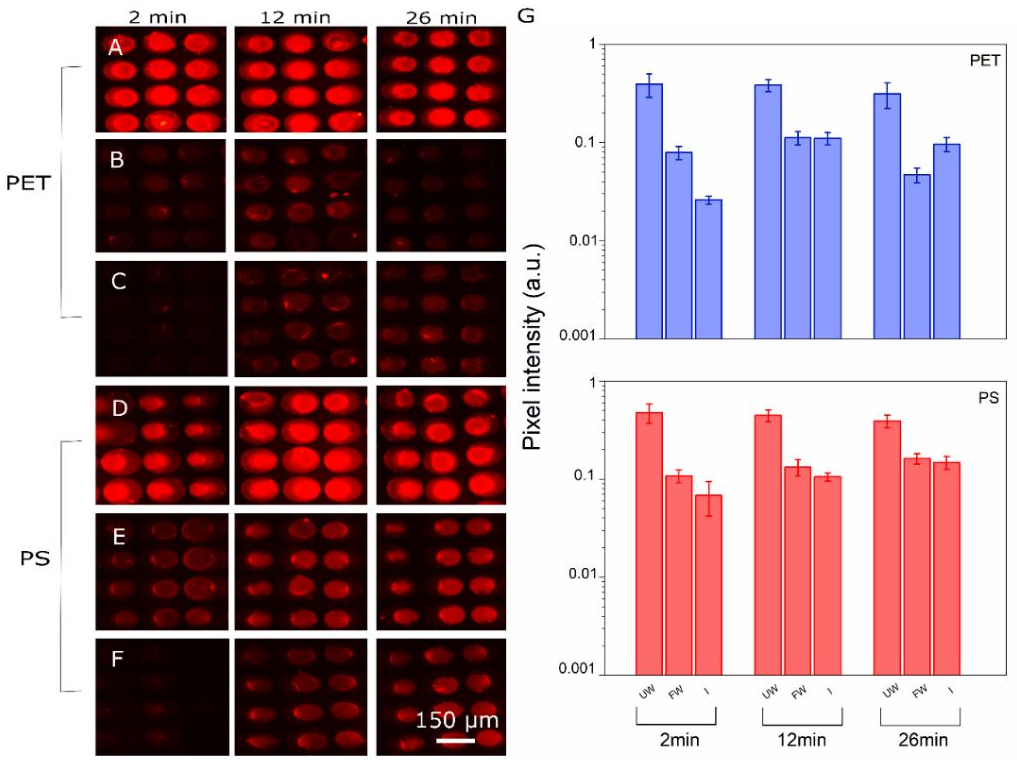

Fig. 2. Verification of the Cy5-R2R bio-functionalization of polymer foils in a continuous micro-spotting process. For the PET foils, **(A)**, **(B)** and **(C)** show the images of unwashed, thoroughly washed and in 1x PBS incubated samples, respectively. From **(D)** to **(F)**, the fluorescent images were presented in the same logical order for the PS foils. **(G)** Comparison of fluorescence intensities (a.u.) obtained on using the different treatment conditions.

For PET foils, as compared to intensities (a.u.) of UW samples, after both the flush (i.e., thorough) washing (FW) and incubation (I) tests (for 1 hour at 25˚C), the pixel intensities were decreased by ~ an order of magnitude in 2 min and 26 min cured samples (Fig.2G-PET). However, the signal decrease was below an order of magnitude in 12 min cured PET samples, both after FW and I tests as compared to UW sample signal. Likewise, a decrease around an order of magnitude in the signal intensities (a.u.) were observed for PS samples after all testing (Fig.2G-PS). Each bar column is a mean data, averaged from 4 different intensity measurements, shown in logarithmic scale. The background values were subtracted. UW, FW and I refer to unwashed, flush washed and incubated samples, respectively. For 12 min and 26 min curing times, the signal intensity was also observed in the same range for all testing. The results revealed a successful, rapid and robust immobilization of Cy5-R2R DNAs on PET and PS foils. Furthermore, 12 min was found to be an optimum curing time to create stable DNA micro-arrays on our polymer components (i.e., PET and PS foils).

Conclusion

High-throughput ss-DNA micro-spotting was successfully performed on the R2R photoresin printed polymeric foils, without applying pre surface bio-functionalization. The DNA spots were robust on the polymer surfaces even after the washing tests. In the light of the results we obtained, as a next step we will study immobilisation of probe ss-DNA molecules onto a R2R UV-NIL structured NILcure JR21 on a polymer foil, following imprinting of fluidic structures in order to demonstrate a low-cost and high-throughput manufacturing of polymer foil-based diagnostic chips.

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References

[1] N. Kooy, K. Mohamed, L. T. Pin, O. S. Guan, *A Review of Roll-to-Roll Nanoimprint Lithography*, **Nanoscale Research Letters, 9(1)**, 2014, p. 320.

[2] A. V. Shneidman, K. P. Becker, M. A. Lukas, N. Torgerson, C. Wang, O. Reshef, M. J. Burek, K. Paul, J. McLellan, M. Loncar, *All- Polymer Integrated Optical Resonators by Roll-to-Roll Nanoimprint Lithography*, **ACS Photonics, 5(5)**, 2018, pp. 1839-1845.

[3] C. H. Chuang, D. M. Lu, P. H. Wang, W. Y. Lee, *Fabrication of antireflective polymer films with subwavelength structures using Roll to Roll Nanoimprint Lithography*, **IEEE**, Symposium on Design, Test, Integration and Packaging of MEMS/MOEMS (DTIP), 2016.

[4] P. Yi, C. Zhang, L. Peng, X. Lai, *Flexible Silver-Mesh Electrodes with Moth-Eye Nanostructures for Transmittance Enhancement by Double-Sided Roll-to-Roll Nanoimprint Lithography*, **RSC Advances**, **7**, 2017, pp. 48835-48840.

[5] J. G. Ok, Y. J. Shin, H. J. Park, L. J. Guo, *A step toward next-generation nanoimprint lithography: extending productivity and applicability*, **Applied Physics A, 121**, **(2)**, 2015, pp. 343-356.

[6] M. Leitgeb, D. Nees, S. Ruttloff, U. Palfinger, J. Götz, R. Liska, M. R. Belegratis, B. Stadlober, *Multilength Scale Patterning of Functional Layers by Roll-to-Roll Ultraviolet-Light-Assisted Nanoimprint Lithography*, **ACS Nano, 10(5)**, 2016, pp. 4926 – 4941.

[7] B. Stadlober, C. Leiner, D. Nees, A. Haase, U. Palfinger, L. Kuna, S. Ruttloff, M. Smolka, J. Hesse, M. Sonnleitner, C. Sommer, *R2R-UV-nanoimprinting as a powerful mean for large-area fabrication of freeform micro-optical elements*, **Optical Design and Fabrication 2019** (Freeform, OFT), OSA Technical Digest (Optical Society of America), paper OT1A.7, 2019.

[8] D. Nees, S. Ruttloff, U. Palfinger, B. Stadlober, *Experiments towards Establishing of Design Rules for R2R-UV-NIL with Polymer Working Shims*, **Proc. SPIE** 9777 Alternative Lithographic Technologies VIII, 97770D, 2016.

[9] M. Smolka, S. Ruttloff, D. Nees, C. Prietl et al., *High Throughput Roll-to-Roll Production of Microfluidic Chips*, **Proceedings of Eurosensors, 2(13),** 2018, p. 1054.

[10] Z. Li, T. Feizi, *The Neoglycolipid (NGL) Technology-based Microarrays and Future Prospects*, **FEBS Letters, 592**, 2018, pp. 3976-3991.

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