A method for patterned *in situ* biofunctionalization in injection-molded microfluidic devices

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Received 5th May 2010, Accepted 30th June 2010
DOI: 10.1039/c005307d

We developed a method to modify the surface in injection molded polymer microdevices prior to bonding and to pattern biomolecules in the completed microsystem *in situ* by a sequence of simple perfusion steps directly before utilization of the device. This method is compatible with production technology such as injection molding and bonding processes currently employed in the fabrication of polymer microsystems. It solves the problem of the inherent incompatibility of biomolecules with microfabrication technology as it allows for the biofunctionalization step to be performed after completion of the microsystem. Injection molded cyclic olefin copolymer (COC) microfluidic chips were modified by irradiating the surface with UV-light at $\lambda = 185$ nm. This results in the formation of stable acidic groups which were further modified by binding of the extracellular matrix protein collagen type I. Non-irradiated surfaces were modified by binding of Pluronic® F-127 to become non-adhesive. Density of acid groups decreases to 50% within 45 days and to 25% within 19 weeks after irradiation. However, even then the remaining density of functional groups was shown to be sufficient to bind proteins and promote cell adhesion. Selective adhesion of primary hepatocytes on surfaces patterned by UV-irradiation and a biofunctional coating with collagen type I were demonstrated in injection molded microsystems.

Introduction

Drug screening assays currently in use mostly rely on 2D cell cultures which lack predictability with respect to the *in vivo* response as cells tend to change their phenotype under such conditions.[1] As a result, adverse effects of drug candidates are often discovered only late during clinical trials, when compounds are administered to patients and high R&D investments have already been made. Therefore, the ultimate goal in the development of *in vitro* test systems would be to perfectly mimic the *in vivo* situation by employing human primary cells assembled in an organ like fashion to establish physiological cell–cell and cell–matrix interactions. To this end microfluidic devices are particularly well suited for this purpose as they may be designed to enable and support assembly and perfusion of 3D cell assemblies.

Among the different approaches that have been studied so far are (1) mechanical traps,[1–4] (2) electrical, magnetic or light induced forces,[5–7] and (3) chemical clues to promote site specific adhesion of cells.[8–11]

We are developing a microfluidic chip which uses positive dielectrophoresis[12,13] to assemble liver cells into a 3D structure. After assembly the cells in contact with the chip material shall adhere to a collagen coating in the pre-defined assembly areas. Therefore we need to develop a technique for selective protein coating of defined areas inside the chip to allow for patterned cell adhesion under conditions compatible with dielectrophoresis, namely low conductivity media, lacking soluble salts typically included in cell culture media.

The coupling of extracellular matrix proteins to the microchip surface provides cells with a chemical environment that allows them to retain their particular phenotype. It can also be used for patterned cell culture, as cells will preferably grow on surface areas covered with collagen and avoid non-biofunctionalized surfaces.

Several methods for patterned surface modification in microfluidics have been developed for glass surfaces. Folch and colleagues used a polydimethylsiloxane (PDMS) stamp to create patterns of plasma etched glass which allowed the adsorption of biomolecules.[14] After protein adsorption the PDMS stamp was removed and a microfluidic device sealed onto the glass in which patterned cell seeding was performed. Also, a reverse method has been shown by the groups of Jeon[11] and Langer.[15] Here, the surface is completely coated by the biomaterial. Subsequently, a PDMS stamp is applied and the biomaterial is removed by plasma etching from surface areas that are not protected by the stamp. Again, after patterning the stamp is peeled off and the microfluidic channels are bonded to the surface. PDMS stamps are also used for guided cell seeding in microwells[16] or seeding within PDMS channels.[17] All of these approaches allow creating defined cell patterns. As their main disadvantage several alignment and bonding steps need to be performed. Leclerc *et al.*[18] used a PDMS–glass microfluidic system which allowed the cell patterning and seeding without dismantling the system in between. The PDMS cover was used to crush cells underneath in a defined pattern and seed a second cell type onto the free surface.

However, all of these techniques rely on glass–PDMS microfluidics which are not applicable for high volume industrial

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fabrication. In this respect, polymers such as poly(methyl methacrylate) (PMMA), cyclic olefin copolymer (COC) or polystyrene are gaining importance as they are compatible with low-cost mass production techniques like injection molding. It is therefore important to provide methods capable of generating patterned surface biofunctionalization of polymer surfaces. These polymer microfluidic devices typically are fabricated by bonding a planar cover to a 3D-micropatterned substrate. Therefore, a patterned biofunctionalization has to be applied prior to bonding onto non-planar surfaces and has to withstand the bonding process. However, typical biomolecules such as antibodies, enzymes or extracellular matrix proteins are sensitive to temperature, contact with solvents or laser irradiation as are common in current bonding methods. Thus, biofunctionalization after bonding, prior to the usage of the microfluidic chip, would be highly desirable.

It has previously been shown that the surface of cyclic olefin copolymer, polystyrene as well as PMMA may be modified by UV-irradiation at low wavelengths (180–190 nm) to create acidic groups that can be used for patterned protein binding and cell adhesion.[19–23] To date this method has only been used on planar, openly accessible substrates such as cell culture dishes.

We will show how this method can be applied to the biofunctionalization of the inner surfaces of microfluidic systems. We found that the reactive groups are stable over long periods of time and in particular also endure the industrial bonding process. Proteins selectively bind to UV-activated surfaces in the chip while non-irradiated hydrophobic surfaces are passivated by adsorption of the triblock copolymer Pluronic® to suppress cell adhesion. With respect to the overall goal of assembling liver cells in microfluidic devices, we demonstrate a process which allows selective deposition of collagen and subsequent patterned cell adhesion in completed microsystems on surfaces modified by UV-irradiation prior to chip bonding.

Materials and methods

Polymer

Microscopy slides and injection-molded microchips made of cyclic olefin copolymer (COC) Topas 5013 were obtained from microfluidic ChipShop (Jena, Germany).

Chemicals and proteins

Nile blue sulfate (Nile blue A, C.I. 51180) was obtained from Roth (Karlsruhe, Germany). Rhodamine B isothiocyanate (Cat. No. R1755), Collagen type I from rat tail (Cat. No. C-7661) and Pluronic® F-127 (Cat. No. P-2443) were obtained from Sigma-Aldrich (Munich, Germany). Calcein AM (Cat. No. C1430) was obtained from Invitrogen (Karlsruhe, Germany).

Nile blue dye solution was prepared by adding 0.06 mg Nile blue sulfate to 200 ml of a 0.1 M sodium dihydrogenphosphate buffer at pH 6.

Collagen type I was labeled with rhodamine B by adding 200 μl of rhodamine B isothiocyanate (1 mg ml^{-1} in DMSO) to a solution of collagen type I (1 mg ml^{-1} in 1 M acetic acid) and stirring overnight. Excessive dye was removed by dialysis against 1 M acetic acid, supplemented with sodium bromide (10 mM).

Collagen type IV, human, was obtained from BD Bioscience (Cat. No. 354245, Lot. No. 34774).

Low conductivity sucrose medium for adhesion experiments contained $CaCl_2$ (27 mg l^{-1}), $MgSO_4$ (13 mg l^{-1}), sodium pyruvate (55 mg l^{-1}), glucose (1 g l^{-1}) and sucrose (106 g l^{-1}). This particular low conductivity medium composition was developed to enable cell manipulation by positive dielectrophoresis, which requires media conductivity to be lower than cell cytosol conductivity. As this low salt condition results in stress for the cells it was necessary to show that cell adhesion to the modified surface is nonetheless possible.

Surface preparation and UV-modification

The COC chip surface was cleaned with isopropanol and dried under nitrogen flow. Subsequently, the COC surface was irradiated through a mask using a low pressure mercury lamp (Heraeus Noblelight, Hanau, Germany, NIQ lamp, $\lambda = 185$ nm, quartz tube, 5 W) to achieve site specific formation of reactive carbonic acid groups (Fig.1). Illumination was carried out in lab atmosphere for 20 min at a distance of 4 cm between lamp and COC surface. At these irradiation time and distance a maximum density of acidic groups was observed by using a Nile blue dye assay (details about procedure and data will be published elsewhere).

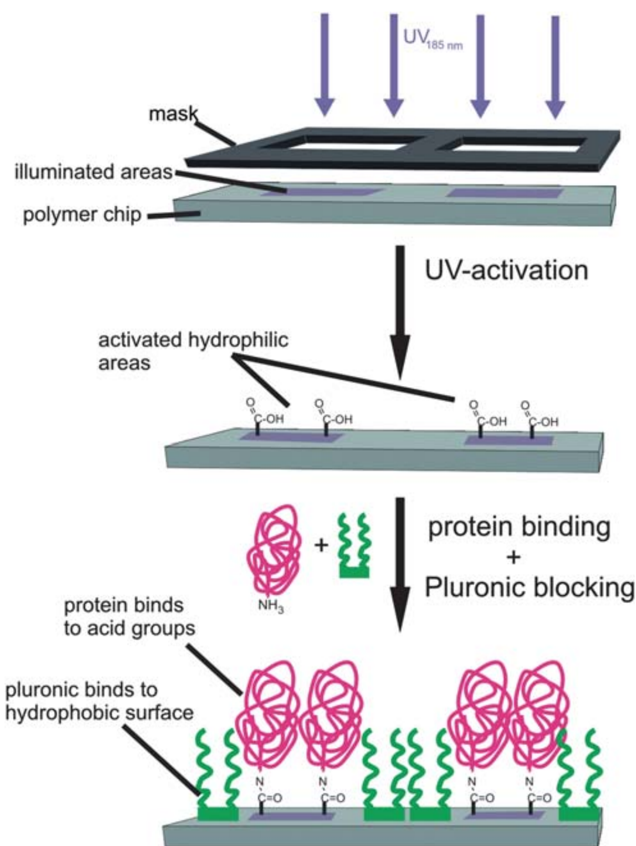

Fig. 1 Process scheme applied for patterned surface biofunctionalization in microfluidic devices by UV-irradiation and subsequent binding of ECM proteins and Pluronic® F-127.

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Measurement of acid group density

The cationic dye Nile blue A was used to measure the number of acidic groups produced by UV-irradiation.[24] It binds *via* ionic interaction to the deprotonated carbonic acid groups and will dissociate at low pH.

UV-irradiated COC slides were incubated in the Nile blue dye solution for 20 min. Unbound dye was washed away with deionized water and the colored slides were dried under nitrogen flow. The bound dye was removed by discoloring the slides for 5 min in a volume of 5 ml of 30% acetic acid. The extinction at the maximum absorption wavelength $\lambda_{max} = 638$ nm ($\varepsilon = 12.2 \times 10^4$ l mol^{-1} cm^{-1}) of Nile blue A in 30% acetic acid was measured using a UV/Vis-spectrometer. The concentration of Nile blue in the remover and the quantity of dye bound to the chip surface were calculated. Under the assumption of an ideal dye binding (no double layers, no steric hindrance) the quantity of dye at the surface can be equaled to the quantity of acidic groups.

Protein binding and blocking with Pluronic®

In order to determine the optimal protein binding conditions a COC substrate was UV-activated with a macrospot (1 mm diameter) array using a shadow mask. Protein binding experiments were performed by covering the slide with a 12 well ProPlate™ Multiarray Slide System (BioCat, Heidelberg, Germany) with each well containing 4 UV-activated spots. The surface in each well was incubated with 100 µl of a solution containing collagen I in various concentrations and Pluronic® (1 mg ml^{-1}) (in HEPES 10 mM, pH 5). In addition, protein binding experiments without Pluronic® were performed. The binding of the fluorescence labeled collagen type I to UV-activated and non-activated surfaces, respectively, was imaged with a fluorescence microscope (Zeiss Axio Imager®) and fluorescence intensity was quantified using ImageJ (version 1.35d). Fluorescence of the labeled protein on UV-activated and non-activated surfaces was corrected to the background fluorescence of UV-activated surface and untreated COC, respectively. In order to quantify the protein binding and successful Pluronic® blocking the ratio of protein fluorescence intensity on UV-activated *vs.* non-activated surfaces was computed. For each condition 8 spots were averaged.

Cell adhesion after long term storage

For determining the long term stability of the surface activation we did not use primary human hepatocytes but the human hepatocyte cell line HepG2, as a large number of cells were needed, and no cell type specific differences on long term stability of the UV-activation are expected. HepG2 were cultured in Eagle's Minimum Essential Medium supplemented with 1% non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, penicillin (100 U ml^{-1}), streptomycin (100 µg ml^{-1}) and 10% fetal calf serum (PAA, Germany). Cell culture was incubated at 37 °C and 5% CO_2. HepG2 cell suspensions were prepared by rinsing the adherent cells with Ca/Mg free phosphate buffered saline (PBS−) and using a solution of trypsin (0.5 mg ml^{-1}) and EDTA (0.22 mg ml^{-1}) in PBS− to detach cells from the culture flask. Counting was performed in a cytometer. Cells were centrifuged at 250*g* for 5 min and resuspended in low conductivity sucrose medium.

In order to assess cell adhesion properties of UV-activated COC surfaces after long-term storage, small COC slides (1.5 × 1.5 cm^2) were irradiated and stored in laboratory atmosphere for several months. The slides were attached to the bottom of a 12 well using double-sided adhesive tape and incubated with collagen type I (10 µg ml^{-1})/Pluronic® (1 mg ml^{-1}) solution (in 10 mM HEPES, pH 5) for 40 min at 4 °C. After removing unbound protein by washing with HEPES (10 mM, pH 7.4), HepG2 cells were seeded at a density of 5 × 10^5 cells per well and allowed to adhere for 30 min. Non-adherent cells were removed by washing with PBS+. Attached cells were stained with Coomassie (10 min, RT, 1× washing with PBS+) and DAPI (0.5 µg ml^{-1}) (10 min, RT, 1× washing with PBS+, 1× washing with deionized H_2O). For quantitative analysis the cell numbers on four different areas on a slide were counted using ImageJ and the results from three slides for each condition were averaged.

Cell adhesion on patterned COC slides

For all other cell experiments we used cryopreserved human hepatocytes obtained from the research group of Prof. Gebhardt at the University of Leipzig or from Tebu-Bio.

Cryopreserved cells were thawed and diluted on ice by adding cold thaw medium (William's E, supplemented with 0.1 µM dexamethasone, 2 mM glutamine, 100 nM insulin, 100 U ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 10% fetal calf serum (v/v), 100 µM trolox, 15 U ml^{-1} DNase 1) in portions of 0.5, 1, 2 and 3 times of the starting volume in 3 min intervals. Cells were centrifuged (5 min, hepatocytes at 50*g*, endothelial cells at 250*g*) and suspended in plating medium (thaw medium without DNase 1). Cell vitality was quantified by a trypan blue counting. The plating medium was removed by centrifugation and the cells were resuspended in low conductivity dielectrophoresis medium.

Static cell patterning experiments were performed in the ProPlate™ Multiarray on COC slides UV-activated with a spot shadow mask. Each well was incubated with 100 µl of a collagen type I (10 µg ml^{-1}) and Pluronic® (1 mg ml^{-1}) solution (in HEPES 10 mM, pH 5) for 40 min at 4 °C and washed with HEPES buffer (10 mM, pH 7.4). Human hepatocytes were seeded at a density of 5 × 10^5 cells per well in low conductivity medium and were allowed to adhere for 30 min at 37 °C. After detaching the ProPlate™ non-adherent cells were removed by washing with PBS+. Attached cells were stained with Coomassie (10 min, RT, 1× washing with PBS+) and DAPI (10 min, RT, 1× washing with PBS+, 1× washing with deionized H_2O). Each spot was imaged using a fluorescence microscope (Zeiss Axio Imager®). ImageJ was used for quantitative cell counting. Counting results from at least seven cell adhesion areas were averaged for each condition.

Statistical significance was determined using one-way ANOVA (analysis of variance) with Tukey/Kramer's *post-hoc* test ($^*p < 0.01$). Error bars represent SEM (standard error of mean).

In situ protein binding and cell patterning in a COC–PDMS microfluidic system

Experiments were performed to demonstrate the feasibility of biofunctionalization for guided cell adhesion in a closed

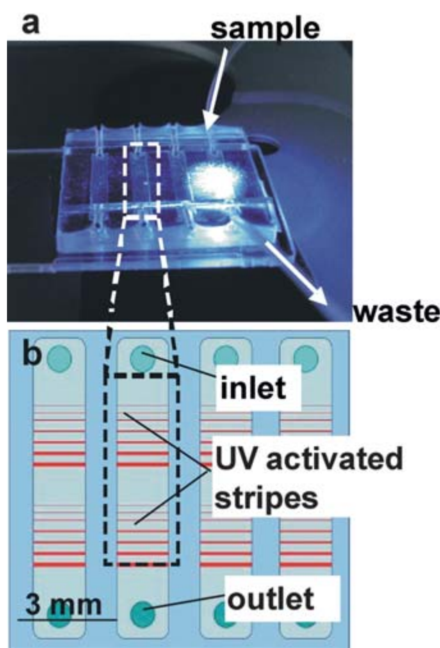

Fig. 2 (a) Microfluidic system consisting of a COC slide preactivated by UV-illumination and a channel structure made of PDMS elastomer shown in microscopic setup. (b) Schematic depiction of functionalization pattern on the COC slide.

microfluidic device. For this purpose a COC substrate was UV-activated using a shadow mask with stripe shaped openings. Following irradiation a PDMS channel structure was bonded onto the slide using a thin film of a silicone-based adhesive (RS, Moerfelden-Walldorf, Germany, Cat. No. 555-588). The chip design is shown in Fig. 2. Protein binding was carried out in two rinsing steps using a syringe pump. First a solution of rhodamine B labeled collagen type I (10 μg ml^{-1}) and Pluronic® (1 mg ml^{-1}) (in HEPES 10 mM, pH 5) was introduced at a flow rate of 10 μl min^{-1} for 10 min. Subsequently, HEPES buffer (10 mM, pH 7.4) was flushed through the channels at a rate of 20 μl min^{-1} to remove unbound protein. Binding of fluorescence labeled proteins was imaged using a fluorescence microscope (Nikon Eclipse Ti, Melville, USA).

After protein binding cell attachment was performed. Primary hepatocytes in low conductivity sucrose medium at a density of 3.5×10^5 cells per ml were pumped through the system at a flow rate of 10 μl min^{-1}. At this flow rate hepatocytes were able to settle in the channel and to adhere on the channel bottom coated by protein. Non-adherent cells were removed by rinsing with sucrose media at a flow rate of 20 μl min^{-1} for 5 min. Higher flow rates were avoided, to limit shear stress experienced by the cells.

Protein binding and cell adhesion in an injection molded COP-microchip

Injection molded COP-microchips were irradiated by UV-light (185 nm) through a shadow mask in our lab and send to microfluidic ChipShop for bonding with a COP-foil as cover. The completed, bonded chips were perfused with rhodamine labeled collagen type I and Pluronic® at a rate of 7 μl min^{-1} for 45 min and rinsed with buffer at a rate of 15 μl min^{-1} for 20 min using a syringe pump. Binding of fluorescence labeled protein was observed using a fluorescence microscope (Nikon Eclipse Ti).

For cell culture the biofunctionalization was performed as described above, however, collagen type IV was used as extracellular matrix protein. Human hepatocytes in low conductivity dielectrophoresis medium were flushed into the chip at a rate of 50 μl min^{-1} and assembled by positive dielectrophoresis by applying an electric field of 30 kV m^{-1} and 350 kHz. After completion of dielectrophoretic assembly of cells the field was turned off and the medium was changed to plating medium and the device was continuously perfused at a rate of 3 μl min^{-1}.

Results and discussion

Long term stability of acidic groups and its effect on cell adhesions

The long term stability of the surface modification is critical since it will determine the shelf life of completed microdevices. We observed that surface density of acidic groups, measured with the Nile blue dye assay, decays over time (Fig. 3). This may be due to diffusion of the functional groups into the bulk of the polymer.[25] Also esterification with volatile compounds comprising alcohol residues may contribute to the loss of active acidic groups as the slides were stored under lab atmosphere. However, even after 45 days, 50% and after 130 days about 25% of the initial density of acid groups are retained (Fig. 3). This density of acid groups has to be related to the maximum achievable density of proteins. The molecular weight of a single collagen type I molecule, which was used as protein matrix in our research, is about 200 kDa. In solution aggregates of an average weight of about 800 kDa are found.[26] Using the estimated size of a single collagen type I molecule of about 300 nm length and 1.5 nm width the maximal achievable density of collagen molecules on the surface amounts to 3.6×10^{-15} mol mm^{-2}. Accordingly, immediately after UV-irradiation the ratio of acid groups per mm^2 to the number of collagen molecules per mm^2 would be 6.8×10^4 and it would still be 1.9×10^4 after 130 days of storage. Assuming that a collagen type I molecule only needs a few acid groups to bind to the surface, the protein binding—and thus cell adhesion—capacity should still be sufficient after storing the UV-activated surface for several months. This was confirmed by protein binding and cell seeding on long term stored UV-activated COC-slides which

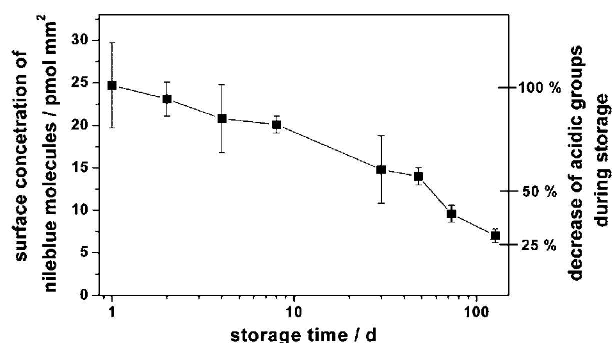

Fig. 3 Density of acidic groups on COC surface as a function of storage time after UV-irradiation.

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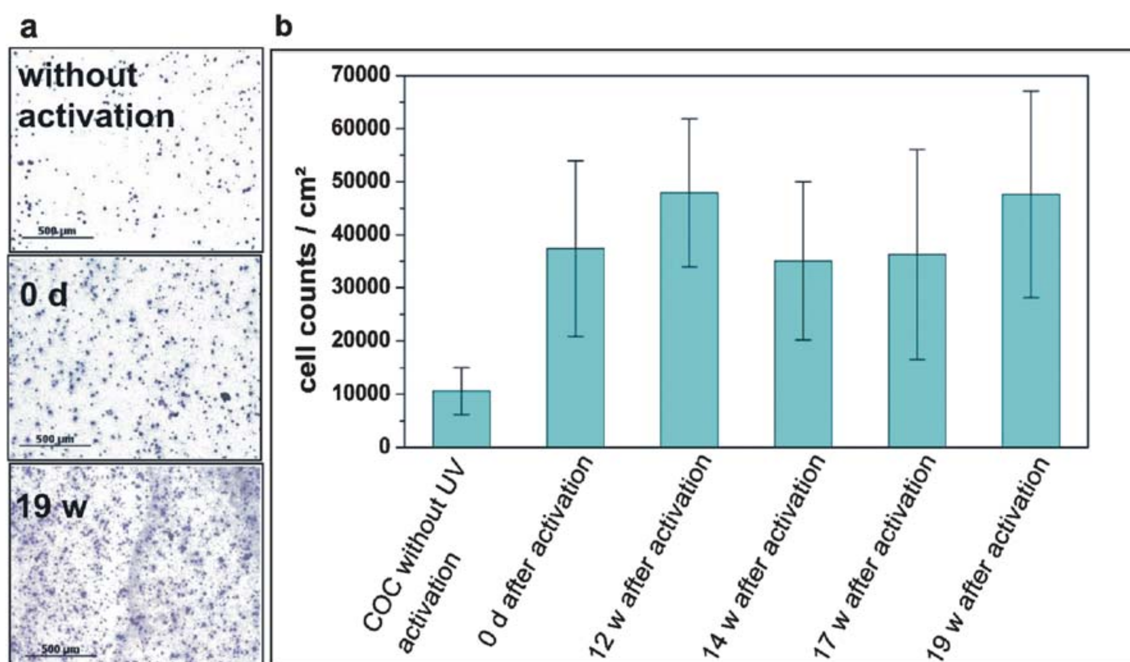

Fig. 4 Cell adhesion on UV-activated COC surfaces after different storage periods. (a) Cell adhesion on COC-surfaces without UV-activation and after different storage periods (representative micrographs). (b) Quantitative analysis of cell adhesion. Cells were stained with Coomassie and counted with ImageJ. For each condition three slides were seeded with cells, and 4 micrographs per slide were averaged.

showed that cell adhesion did not decrease even after 130 days of storage (Fig. 4).

Protein binding and blocking with Pluronic®

The optimal protein binding result would be represented by high protein coverage on activated surface areas—indicated by high fluorescence intensity of the rhodamine labeled collagen, and no binding to non-activated areas—resulting in low fluorescence. The contrast of rhodamine fluorescence after protein binding of activated to non-activated areas is shown in Fig. 5 for protein binding with and without Pluronic® and for three protein concentrations each. Without blocking by Pluronic® the contrast decreases in the case of high protein concentration due to increased protein binding to the non-activated surface. If, however, the protein solution contains Pluronic® the contrast increases with increasing collagen concentration. Pluronic® adheres to the non-activated hydrophobic surface and prevents unspecific protein binding while protein binding to the activated spot is still high.

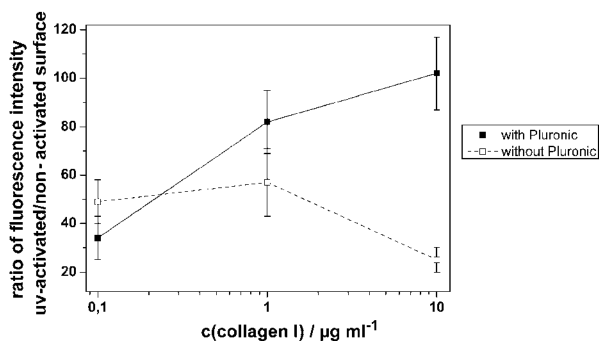

Fig. 5 Ratio of fluorescence intensity of UV-irradiated and non-irradiated COC surface after treatment with rhodamine labeled collagen type I with and without Pluronic® blocking.

Cell patterning on COC

The same spot array was used to examine cell patterning. If no protein/Pluronic® treatment was performed in the UV-activated spot array, cells randomly adhered to the surface (Fig. 6a). In contrast, if an incubation with collagen type I and Pluronic® was carried out, human hepatocytes adhered to the UV-activated, protein coated spots and did not attach to the surrounding areas blocked with Pluronic® (Fig. 6b). Fig. 6c shows the quantification of cell adhesion. After incubating a non-irradiated COC surface with collagen type I cell adhesion increased significantly due to unspecific binding of the protein to the hydrophobic surface. Adding a Pluronic® treatment significantly decreased cell adhesion, as Pluronic® blocked the hydrophobic areas from unspecific protein binding. On the other hand Pluronic® did not prevent protein binding to the hydrophilic, UV-irradiated COC surface. Treatment of UV-irradiated COC with a mixture of collagen type I and Pluronic® resulted in a significant improvement of cell adhesion. Thus, this method enabled site specific cell adhesion and prevention of unspecific binding at the same time.

In situ protein binding and cell patterning in a COC–PDMS microfluidic system

Fig. 7a shows an increase of fluorescence intensity as rhodamine labeled collagen type I binds to the UV-activated stripes on a COC surface in a COC–PDMS microchannel. A measurement of fluorescence intensity in the region of interest (ROI) during

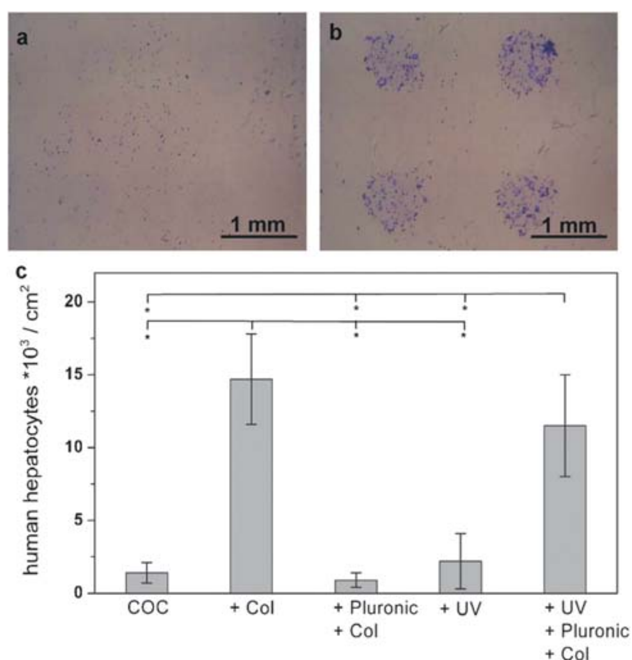

Fig. 6 Adhesion of primary human hepatocytes to a UV-irradiated macrospot array. (a) Without further treatment. (b) After incubation with collagen type I/Pluronic® F-127. (c) Quantitative analysis of cell adhesion to different treated COC surfaces (Col = collagen type I, UV = irradiated with UV-light).

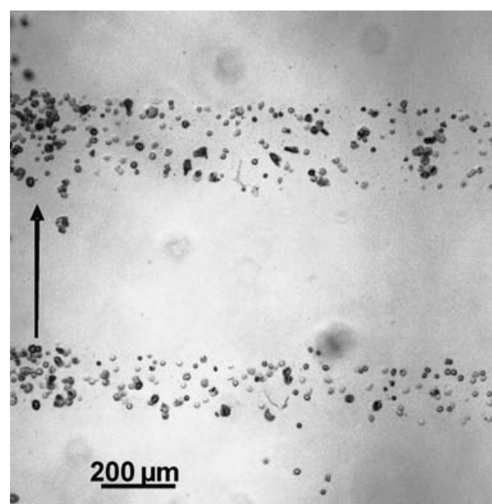

Fig. 8 Pattern of primary human hepatocytes imaged immediately after introduction of the cell suspension into the COC–PDMS channel followed by rinsing with medium. Cells exclusively adhere to UV-activated and collagen coated stripes while cell adhesion is effectively prohibited on areas coated by Pluronic®. Arrow indicates direction of flow.

protein binding and washing is shown in Fig. 7b. In the course of 5 min fluorescence intensity and protein binding reached a saturation limit. After 10 min incubation the medium was changed to a washing buffer to remove unbound protein and the fluorescence intensity reached a stable plateau.

Directly after the protein binding cells can be introduced into the channel. As shown in Fig. 8 hepatocytes introduced into the channel adhered to the stripes on the COC surface previously activated by UV-light and coated by protein. No stopping of flow and sedimentation was needed for cell adhesion. Cell adhesion was confined to the areas coated by protein and no cells adhered in the areas in between which were passivated by Pluronic®.

Protein binding and cell adhesion in an injection molded COP-microchip

The goal of this research was to develop a surface modification and patterning technique applicable to industrial fabricated and bonded microfluidic chips. The stability of the UV-activation with respect to the bonding process was shown in injection molded, UV-activated and bonded COP chips comprising a 3D surface structure as shown in Fig. 9a. The planar surfaces of the

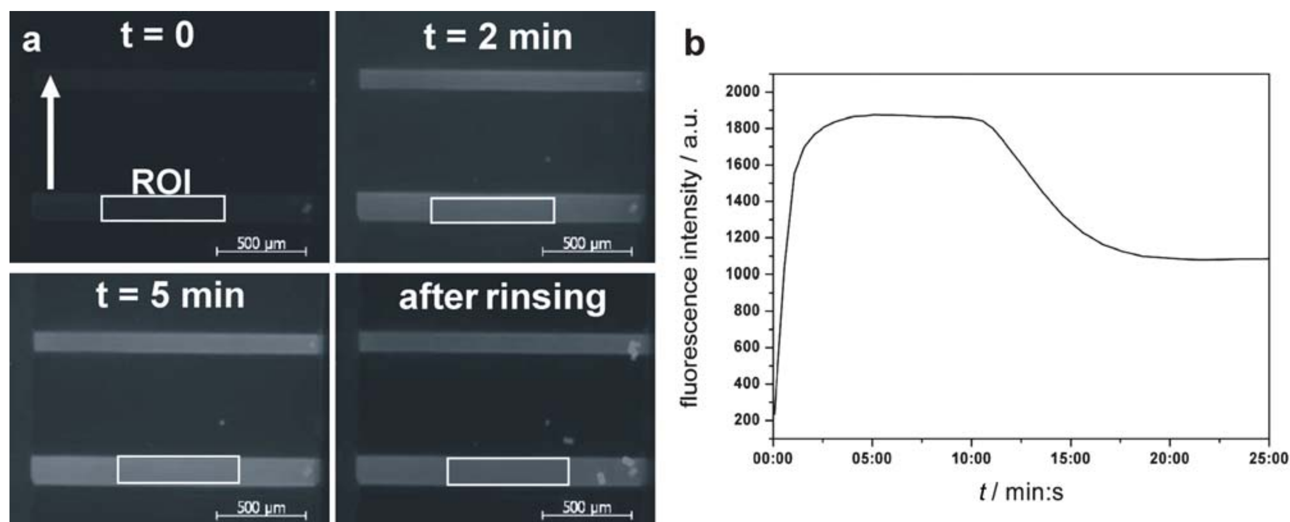

Fig. 7 (a) Rhodamine labeled collagen type I binds to UV-activated stripes in a COC–PDMS channel. (b) Change in fluorescence intensity in the region of interest (ROI) during incubation with collagen/Pluronic® and after washing with HEPES. Arrow indicates direction of flow.

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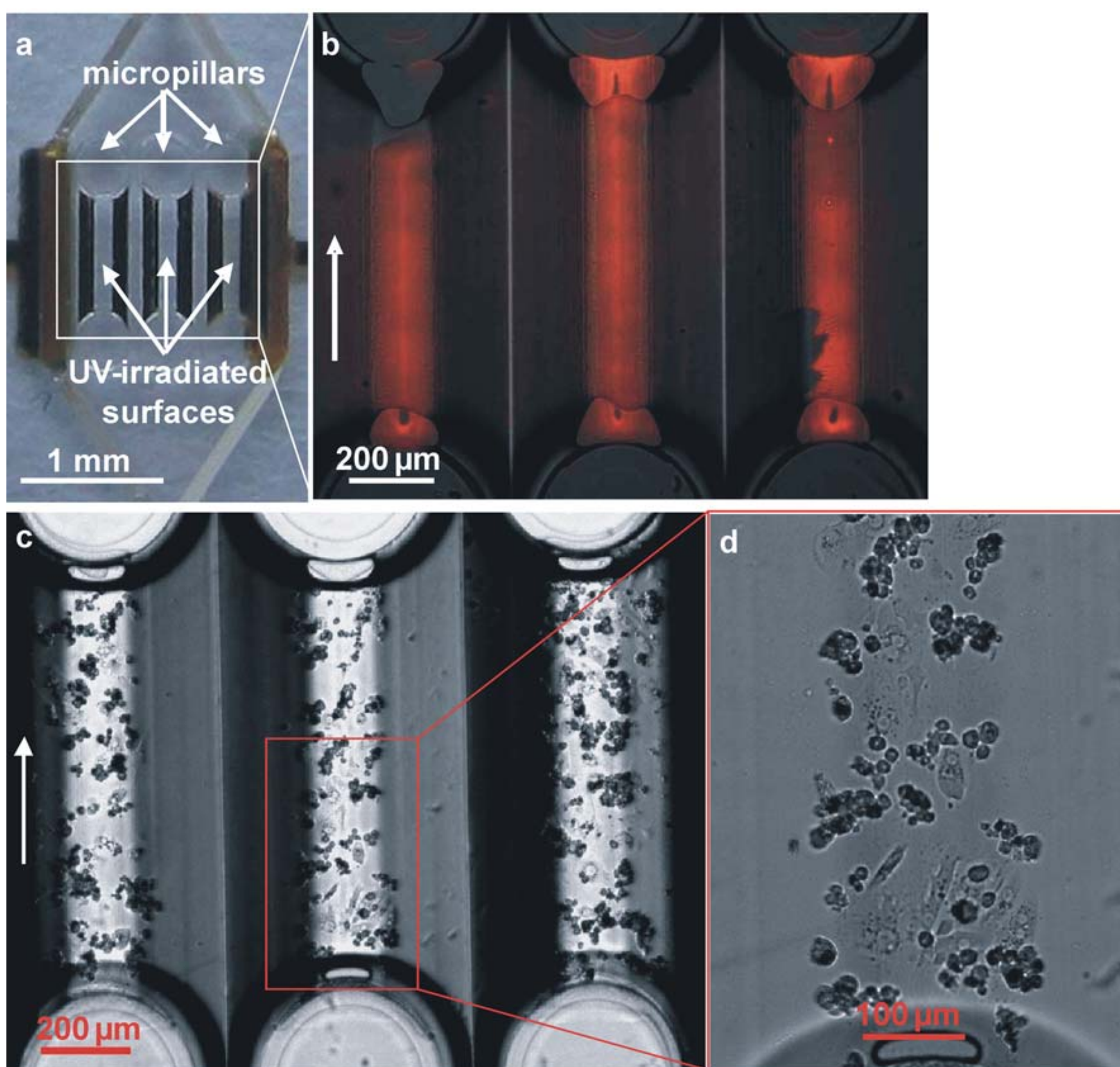

Fig. 9 (a) Cell assembly chamber in an injection molded COP microchip. The areas between the micropillars were UV-irradiated prior to bonding. (b) Rhodamine labeled collagen type I bound to the UV-irradiated surfaces after flushing the chip with collagen/Pluronic®. (c) Primary human hepatocytes assembled and adherent to the protein covered surface after 20 h of cultivation. (d) Morphology of hepatocytes after 20 h of cultivation on collagen IV covered surface. Arrow indicates direction of flow.

bone-shaped structures were irradiated with UV-light through a shadow mask prior to bonding. The completed microdevice was then rinsed with the protein/Pluronic® mixture. Fig. 9b shows the selective binding of rhodamine labeled collagen type I to the previously UV-irradiated surfaces. This indicates that the surface modification by UV-light withstands the industrial bonding process and is also applicable to three-dimensional surface geometries. In the next step hepatocytes were assembled in the microchip by dielectrophoresis. Fig. 9c and d show micrographs of the assembled cells after 20 h of cultivation. Cells are confined to the surface area coated with protein and did not attach to the non-irradiated channel walls. The collagen matrix also proved sufficient to support the formation of a morphology characteristic for hepatocytes (Fig. 9d). The cell assembly consists of a first layer with cells spreading on the collagen surface and a second layer of cells on top exhibiting a more spherical morphology. About 70% of cells still showed calcein positive staining after 20 h of cultivation in the chip.

Conclusion

A procedure for long term stable chemical biofunctionalization of COC surfaces in microfluidic devices by means of patterned UV-irradiation has been developed. The binding of collagen to previously irradiated surface areas and of Pluronic® F-127 to non-irradiated surfaces as well as patterned cell adhesion was shown for different patterns and geometries in (1) conventional Petri dishes, (2) microfluidic systems consisting of a COC slide and a PDMS channel structure, and (3) in an injection molded microfluidic system. This procedure is particularly suitable for the fabrication of polymeric microsystems. Most importantly, sensitive bioactive compounds may be deposited within such microsystems after completion of microfabrication by a simple sequence of rinsing steps prior to cultivation of cells. Applications of this technology are envisioned for devices for substance screening and medical diagnostics (lab on a chip).

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Acknowledgements

COC slides and injection molded COP chips were kindly provided by microfluidic ChipShop, Jena, Germany. We thank Prof. Juergen Ruehe (IMTEK, Freiburg, Germany) for helpful advice regarding polymer surface recovery. Collaboration with microfluidic ChipShop, Keyneurotek AG, BSL Bioservice and University of Leipzig within the *HepaChip* project is acknowledged. Funding for this research was provided in part by the German Ministry for Education and Research, BMBF, through grant 01GG0729.

References

1 A. Sivaraman, J. K. Leach, S. Townsend, T. Iida, B. J. Hogan, D. B. Stolz, R. Fry, L. D. Samson, S. R. Tannenbaum and L. G. Griffith, *Curr. Drug Metab.*, 2005, **6**, 569–591.
2 M. J. Powers, K. Domansky, M. R. Kaazempur-Mofrad, A. Kalezi, A. Capitano, A. Upadhyaya, P. Kurzawski, K. E. Wack, D. B. Stolz, R. Kamm and L. G. Griffith, *Biotechnol. Bioeng.*, 2002, **78**, 257–269.
3 P. J. Lee, P. J. Hung and L. P. Lee, *Biotechnol. Bioeng.*, 2007, **97**, 1340–1346.
4 Y. C. Toh, C. Zhang, J. Zhang, Y. M. Khong, S. Chang, V. D. Samper, D. van Noort, D. W. Hutmacher and H. Yu, *Lab Chip*, 2007, **7**, 302–309.
5 C.-T. Ho, R.-Z. Lin, W.-Y. Chang, H.-Y. Chang and C.-H. Liu, *Lab Chip*, 2006, **6**, 724–734.
6 A. Sebastian, A.-M. Buckle and G. H. Markx, *Biotechnol. Bioeng.*, 2007, **98**, 694–700.
7 A. Sebastian, A.-M. Buckle and G. H. Markx, *J. Micromech. Microeng.*, 2006, **16**, 1769.
8 M. S. Kim, J. H. Yeon and J. K. Park, *Biomed. Microdevices*, 2007, **9**, 25–34.
9 D. R. Albrecht, G. H. Underhill, T. B. Wassermann, R. L. Sah and S. N. Bhatia, *Nat. Methods*, 2006, **3**, 369–375.
10 D. R. Albrecht, V. L. Tsang, R. L. Sah and S. N. Bhatia, *Lab Chip*, 2005, **5**, 111–118.
11 S. W. Rhee, A. M. Taylor, C. H. Tu, D. H. Cribbs, C. W. Cotman and N. L. Jeon, *Lab Chip*, 2005, **5**, 102–107.
12 H. A. Pohl, *Dielectrophoresis*, Cambridge University Press, New York, 1978.
13 H. A. Pohl and J. S. Crane, *Biophys. J.*, 1971, **11**, 711–727.
14 A. Tourovskaia, X. Figueroa-Masot and A. Folch, *Lab Chip*, 2005, **5**, 14–19.
15 A. Khademhosseini, K. Y. Suh, S. Jon, G. Eng, J. Yeh, G. J. Chen and R. Langer, *Anal. Chem.*, 2004, **76**, 3675–3681.
16 A. Khademhosseini, J. Yeh, G. Eng, J. Karp, H. Kaji, J. Borenstein, O. C. Farokhzad and R. Langer, *Lab Chip*, 2005, **5**, 1380–1386.
17 D. T. Chiu, N. L. Jeon, S. Huang, R. S. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 2408–2413.
18 E. Leclerc, K. El Kirat and L. Griscom, *Biomed. Microdevices*, 2008, **10**, 169–177.
19 A. Holländer, S. Kröpke and E. Ehrentreich-Förster, *Plasma Processes Polym.*, 2007, **4**, 773.
20 A. Welle, S. Horn, J. Schimmelpfeng and D. Kalka, *J. Neurosci. Methods*, 2005, **142**, 243–250.
21 A. Welle, *J. Biomater. Sci., Polym. Ed.*, 2004, **15**, 357–370.
22 A. Welle and E. Gottwald, *Biomed. Microdevices*, 2002, **4**, 33–41.
23 J. L. Dewez, J. B. Lhoest, E. Detrait, V. Berger, C. C. Dupont-Gillain, L. M. Vincent, Y. J. Schneider, P. Bertrand and P. G. Rouxhet, *Biomaterials*, 1998, **19**, 1441–1445.
24 A. Hartwig and K. Albinsky, Final Project Report, AiF-Nr. 10900 N/1, Fraunhofer IFAM, Bremen, 1999.
25 D. R. Gagnon and T. J. McCarthy, *J. Appl. Polym. Sci.*, 1984, **29**, 4335–4340.
26 F. H. Silver and R. L. Trelstad, *J. Biol. Chem.*, 1980, **255**, 9427–9433.

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