Full-wafer in-situ fabrication and packaging of microfluidic flow cytometer with photo-patternable adhesive polymers

Koen de Wijs^{1,2} · Chengxun Liu¹ · Bivragh Majeed¹ · Karolien Jans¹ · John M. O'Callaghan¹ · Josine Loo¹ · Erik Sohn¹ · Sara Peeters³ · Ruben Van Roosbroeck³ · Tomokazu Miyazaki⁴ · Kenji Hoshiko⁴ · Isao Nishimura⁴ · Katsuhiko Hieda⁴ · Liesbet Lagae^{1,2}

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Abstract Integration of microelectronics with microfluidics enables sophisticated lab-on-a-chip devices for sensing and actuation. In this paper, we investigate a novel method for in-situ microfluidics fabrication and packaging on wafer level. Two novel photo-patternable adhesive polymers were tested and compared, PA-S500H and DXL-009. The microfluidics fabrication method employs photo lithographical patterning of spin coated polymer films of PA or DXL and direct bonding of formed microfluidics to a top glass cover using die-to-wafer level bonding. These new adhesive materials remove the need for additional gluing layers. With this approach, we fabricated disposable microfluidic flow cytometers and evaluated the performance of those materials in the context of this application. DXL-009 exhibits lower autofluorescence compared to PA-S500H which improves detection sensitivity of fluorescently stained cells. Results obtained from the cytotoxicity test reveals that both materials are biocompatible. The functionality of these materials was demonstrated by detection of immunostained monocytes in microfluidic flow cytometers. The flexible, fully CMOS compatible fabrication process of these photo-

patternable adhesive materials will simplify prototyping and mass manufacturing of sophisticated microfluidic devices with integrated microelectronics.

Keywords Microfluidics · Microfabrication · Autofluorescence · Flow cytometer

1 Introduction

Lab-on-a-chip devices are capable of replacing traditional benchtop assays with the integration of microelectronics and mechanical components. In these chips with applications ranging from basic miniaturized sensing to actuation, microfluidics is an important enabler for this leap forward in technology. For example, electrodes can be used for direct impedance spectroscopy (Ayliffe et al. 1999; Holmes et al. 2009; Tang et al. 2017) or more sophisticated sensors can be constructed for sensing of magnetic, optical, electrochemical and thermal stimuli (Adiguzel and Kulah 2012; Luka et al. 2015). The duplication of sensing elements on chip will enable high throughput sample screening. On top of that, integration of actuators for cell manipulation can create fully enclosed on chip cell sorter devices. Recently, cell sorter devices based on acoustic (Li et al. 2015), vapor bubble jet flow (De Wijs et al. 2017) or micromechanical switch (Linton and Oram 2013) are demonstrated. In addition to the small footprint, these microfluidic cell sorters remove the biosafety concern of aerosols generated during sorting in traditional fluorescent activated cell sorters (Robinson and Roederer 2015). Combining integrated sensors and actuators with microfluidics enables sophisticated lab-on-a-chips.

The fabrication of disposable lab-on-a-chip devices demands precise alignment of microfluidic structures to sensors and actuators in a process that is compatible with mass

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✉ Koen de Wijs
koen.dewijs@imec.be

¹ imec, Kapeldreef 75, B-3001 Leuven, Belgium

² Physics Department, KU Leuven, Celestijnenlaan 200D, B-3001 Leuven, Belgium

³ JSR micro NV, Technologielaan 8, B-3001 Leuven, Belgium

⁴ JSR corporation, 1-9-2 Higashi-Shinbashi, Minato-ku, Tokyo 105-8640, Japan

fabrication. Even more, the material should be bio-compatible and fully compatible with standard CMOS fabrication processes to integrate microelectronics for more complicated lab-on-a-chip devices. Currently, many high precision microfluidic structures are fabricated using lithographic processes in silicon or glass, and the form factor of those microdevices has to remain small for achieving low cost of goods. Extremely low-cost microfluidics fabrication is achieved with polymers, but integration of microelectronics with these materials is often quite complex and precise alignment is an issue (Steigert et al. 2008). Though a variety of solutions are available for the creation of microfluidics, a single material and protocol which allows both in-situ lithographic microchannel fabrication and reliable fluidic packaging of these microdevices is highly desirable. When lithographic processes are used to precisely define the fluidic channels on the microdevice, the integration of the microdevice with ultra-cheap plastic cartridges becomes a non-critical and non-precise gluing step that can be performed in a standard assembly line.

Widely reported PDMS is often used with soft lithography for rapid prototyping, but it is not optimized for large area processing and incompatible with CMOS processing (Duffy et al. 1998; Steigert et al. 2008). Additionally, this material and other siloxane based materials such as SINR-3570PF-20 are too flexible for many microfluidic applications because of the inherent flexibility of silicon polymer chains (Voronkov et al. 1978). SU-8 seems to be a perfect candidate, but it requires an additional adhesive for closing of channels (Bilenberg 2004; Blanco 2004). This requires extra process steps and increases costs. The ideal material for microstructure fabrication is an adhesive polymer that also can be used for closing of the microchannel. Adhesive polymers have the advantage of affordable fabrication with only processing of the bottom substrate and bonding at usually low temperatures, which makes them compatible with CMOS processing (Niklaus et al. 2001). Nevertheless, many well-known adhesives are not fully compatible with our microelectronics fabrication line. Examples of these incompatible materials are polyimide which requires high curing temperatures of over 250 °C (J. Kim et al. 2011). Other incompatible materials are BCB (benzocyclobutene) and OSTE which require the use of solvent based developers (Saharil et al. 2013; Scheck and Achen, <http://www.dow.com/scripts/litorder.asp?filepath=/618-00345.pdf>).

Recently, we have demonstrated a full wafer fabrication process for microfluidic cell sorters with the photo-patternable adhesive polymer PA-S321 (Majeed et al. 2014; De Wijs et al. 2017). This photo-patternable adhesive polymer enclosed microfluidic channels in a single process step. The material was spin coated to the desirable thickness and patterned with photolithography to form microfluidic channels precisely aligned to microheaters on the microdevice. The fluidic channels were closed by applying moderate heat and pressure to the substrate possessing the fluidic structure and a

second transparent glass cover plate. The bonding process is compatible with CMOS processing and can be executed on standard bonding equipment. With this process, disposable microfluidic cell sorters were fabricated that tackle biosafety and cross-contamination issues of traditional cell sorters. Inspired by the results of PA-S321, two new photo-patternable polymers, PA-S500H and DXL-009 are developed. PA-S500H allows patterning of thicker microfluidic channels and DXL-009 is a low autofluorescent material. Many polymers are autofluorescent, particularly when excited by UV and even visible light (Hawkins and Yager 2003; Wabuyele et al. 2001; Llopis et al. 2004). The reduced fluorescent background signal of DXL-009 improves detection of dim fluorescence signals during in flow detection of cells.

In this paper, we report two novel photo-patternable adhesive polymers PA-S500H and DXL-009 for the direct patterning and packing of microfluidic devices. First, the fabrication process flow was described including spin coating, UV lithography and die-to-wafer thermal bonding. Afterwards, the adhesive strength was characterized and compared for both materials with a shear bonding and fluidic leakage test. The fluorescent emission spectra as well as the absorption spectra were measured and compared to SU-8. Furthermore, the biocompatibility of both materials was assessed. Finally, we demonstrated the usability of PA and DXL fabricated micro channels for microfluidic flow cytometry applications.

2 Experimental methodology

2.1 Materials

The PA-S500H material and DXL-009 photo-patternable and adhesive materials were obtained from JSR Micro NV (Haasrode, Belgium). Both materials are negative tone (chemically amplified) photoresists consisting of an acrylate type polymer. The autofluorescence intensity of DXL009 has been decreased by reducing the aromatic moieties. For DXL, an additional adhesion promoter JSP-002 was used (JSR Micro NV). SU-8 3010 was obtained from Chimie Tech Services. Glass covers with sand blasted holes for fluidic interconnections were acquired from Micronit.

2.2 Device fabrication

Microfluidic flow cytometer devices were fabricated using a procedure previously reported by our group (Fig. 1a) (Majeed et al. 2014). In short, 400 nm SiO₂ was thermally grown on the 200 mm silicon wafer. Secondly, 400 nm Al was deposited and patterned to form the microheater elements for cell sorting and electrical connections of 20 devices per wafer. To insulate the metal heater structure from liquid in experiments, 1.5 μm SiO₂ was deposited with chemical vapor deposition (CVD). A

Fig. 1 Fabrication process of the microfluidic flow cytometer with integrated microheater for micro bubble-jet sorting. **a** Schematic of the wafer scale fabrication process for cell sorter chips. **b** Spin speed curve of PA and DXL for single and double layer spin coating. The more viscous PA requires fewer spin coating layers for thick channels. **c** 200 mm

microfluidic cytometer wafer before dicing with 20 glass cover dies bonded on wafer level (left), chip packaged and wire-bonded to PCB (right) and inset of microfluidic channels precisely aligned to microheaters inside the side chambers

second lithography step was applied to open the bond pads for electrical connections to the microheaters.

To fabricate the microfluidic structures, the photo-patternable adhesive material PA was spin coated on the wafer for 30 s at 1000 rpm to form a 30 μm thick film (Fig. 1b). Next, a soft-bake at 110 °C for 180 s was applied, followed by an UV exposure through a microfluidic mask at a dose of 3500 mJ/cm². The development consisted of a post exposure bake at 110 °C for 300 s, 2 times 60 s puddle with TMAH (2.38 wt%) and a rinse with DI water for 60 s. The last step in the formation of closed microfluidic channels is the bonding of glass dies to the PA material on the silicon wafer in a two-step process. In the first step, 20 glass dies with holes are individually bonded to the wafer with a pick and place tool. During this bonding step, the bottom chuck was heated to a temperature of 100 °C and during die bonding 4900 N was applied for 2 s. After the wafer was fully populated with 20 glass dies, a final full wafer bonding step was performed at 200 °C for 30 min while applying 300 N per die. The temperature was applied to the top and bottom chuck. The wafer was diced and the individual chips were packaged onto printed circuit boards (Fig. 1c).

Except from some minor changes, the process flow for the photo-patternable DXL is very similar to the above described process flow of PA. In contrast to the PA, first an adhesion promotor JSP-002 was used. DXL was spin coated at 1000 rpm for 35 s, followed by a soft-bake at 100 °C for 180 s. To obtain a similar thickness as for PA, a double coating step (i.e. 2 × 35 s at 1000 rpm) was necessary (Fig. 1b) followed by a soft-bake at 110 °C for 180 s and exposure at a lower UV dose of 500 mJ/cm². No post exposure bake was applied.

2.3 Bonding strength

To evaluate the bonding strength of PA and DXL, two methods were used, i.e. shear testing and fluidic leakage tests. For shear testing, die to die level assembly was carried out by flip chip bonding (SET FC150) of 8 × 8 mm² glass dies to patterned PA and DXL dies. Thermocompression bonding conditions of 200 °C (for alignment and bonding) for 5 min with a pressure of 1 MPa were used. The bond shear strength was measured with a Condor 250 XYZTEC.

Fluidic leakage tests were performed with devices fabricated out of DXL and PA. Fluidic port connectors (LabSmith)

were glued onto the 3 inlet holes on the glass covers and 360 μm PEEK tubing (LabSmith) was used to connect to a dual track syringe pump. The sample consisted of blue ink to check the hydrodynamic focusing during the tests. A pressure gauge (DPG1000B-100G, OMEGA Engineering) was connected close to the chip in order to measure the fluidic inlet pressure.

2.4 Characterization of device autofluorescence

The autofluorescence of SU-8, DXL and PA microfluidic chips was examined using an epi-fluorescence microscope (Zeiss Axio Examiner) equipped with three fluorescent filter sets: red (Zeiss 43 HE, Ex: 550/25 nm, dichroic: FT 570, Em: 605/70 nm), green (Zeiss 46 HE, Ex: 500/25 nm, dichroic: 515LP nm, Em: 535/50 nm) and blue (Zeiss 49, Ex: 365, dichroic: 395LP nm, Em: 445/50 nm). The chips were excited with a 365 nm LED or 505 nm LED of a Zeiss Collibri LED light source and the fluorescent light was collected with 10×0.3 N.A. microscope objective. The collected light was detected with a cooled CCD camera (Zeiss, AxioCam ICm) and the intensity was compared to borofloat glass of 1 mm thick.

2.5 Fluorescence emission and absorbance spectra

To collect the fluorescence emission and absorbance spectra, both DXL and PA were spin coated on a 4-in. quartz wafer to a final thickness of 15 μm . As a reference, 15 μm thick SU-8 3010 was fabricated according to manufacturer's procedure (Microchem 2006). The fluorescence spectra of PA, DXL and SU-8 were measured by a spectrofluorometer (HR800 Horiba Jobin-Yvon) with a 100×0.9 N.A. Olympus objective. The optical absorption spectra were measured using a SpectraMax (Molecular Devices) UV-Vis spectrophotometer.

2.6 Cytotoxicity determination

An extraction cytotoxicity test, based on the ISO 10993–5 standard, was performed on DXL and PA samples. The PA and DXL samples were spin coated on a 4 in. quartz wafer at 1000 rpm and developed as described previously. As a negative control a bare quartz wafer was used and as a positive control the DXL material film was cured at a reduced temperature of 70 $^{\circ}\text{C}$ for 2 h. Samples and glass rings were sterilized with 70% EtOH and the glass rings were glued to the samples for the confinement of medium. 1.3 ml of Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% FBS and 1% penicillin streptomycin was added to PA, DXL and control samples. The extraction test was performed at 37 $^{\circ}\text{C}$ and lasted for 48 h. Each test extract was then placed into three separate wells of a 96-well plate in which

mouse embryonal fibroblasts (MEF) had been propagating at 37 $^{\circ}\text{C}$, 5% CO_2 . These samples were incubated at 37 $^{\circ}\text{C}$ for 48 h. Afterwards the wells were examined microscopically to determine morphological changes of the cells. To further assess cell viability, the cells were stained with Calcein AM and viability was measured with an Infinite M1000 plate reader (Tecan).

2.7 Microfluidic flow cytometry

Blood samples were obtained from a healthy donor with informed consent and the study was approved by the Medical Ethical Committee of UZ/KU Leuven (Ref. S57599). All experiments were performed in compliance with the relevant local laws and institutional guidelines. Blood was drawn into an 8 ml Vacutainer CPT tube (Sodium Citrate, BD Biosciences) and subsequently inverted and centrifuged at $1600 \times g$ for 30 min. The peripheral blood mononuclear cell (PBMC) fraction was transferred to a conical tube and phosphate-buffered saline (PBS) was added to bring the volume to 15 ml. Cells were washed twice at $300 \times g$ for 15 min at room temperature and re-suspended in 10 ml PBS. A second wash took place at $300 \times g$ for 10 min. The supernatant was aspirated and the cell pellet was re-suspended in 200 μl of PBS supplemented with 0.5% BSA and 2 mM EDTA. The monocytes in the PBMC fraction were fluorescently stained with 10 μl of PE Mouse Anti-Human CD14 (BD Biosciences) for 1 h, and subsequently washed and re-suspended in PBS with 0.5% BSA and 2 mM EDTA.

The influence of the background autofluorescence signal was investigated in PA and DXL microfluidic flow cytometer devices. The PBMCs were infused in the devices and hydrodynamically focused in lateral direction by sheath fluid from the two side channels (Fig. 2a). Cells passed the detection point at a flow speed of 0.3 m/s. As a reference, the sample was compared with a flow cytometer (BD FACSCanto). The recorded fluorescent and scatter information from both the cytometer and the microfluidic devices were processed on FlowJo. Doublets and platelets were removed on scatter plots for monocyte fraction determination.

All flow cytometry experiments were performed using a custom designed optical microscope setup. A 532 nm CW laser (OBIS FP 532LS, Coherent) was focused into the microfluidic channel through a 20×0.42 NA lens objective (Mitutoyo Plan Apo objective) (Fig. 2b). The emitted fluorescence and scatter light from passing cells was captured with the same objective. The fluorescent light passed through the dichroic 550LP mirror and 550LP bandpass filter onto a 50:50 beam splitter. There it was split into a CCD camera (AxioCam ICm, Zeiss) for observation and a PMT (Hamamatsu H10722) with 585/40 nm bandpass filter for fluorescent detection. The back scattered light was reflected on the dichroic mirror and partially reflected with a beam splitter. At the back focal plane

Fig. 2 Principle of the microfluidic cytometer. **a** Schematic of microfluidic flow cytometer chip. Buffer fluid from the side inlet channels hydrodynamically focusses the cells in the sample to the center of the channel. The scatter and fluorescence signal from cells that pass the laser detection spot is captured off-chip with a microscope setup. The microheater for cell sorting in the side chambers is not used for the

a bar was placed to block reflected laser light. In this way only scattered light from passing cells was captured onto a PMT (Hamamatsu H10722). Both scatter and fluorescent signal were sampled and processed on a FPGA (National Instruments USB-7855R).

3 Results and discussion

3.1 Microfluidic channel quality

Microfluidic cytometers were fabricated on wafer scale using two novel photo-patternable adhesive materials, DXL and PA (Fig. 1). Both materials were used for channel formation and bonding material. For cell based cytometry applications, a channel thickness in the range between 20 and 100 μm is desired. The fabricated microfluidic cytometers had a 30 μm thick channel, which is sufficient for our cytometry application that targets blood cells. This thickness was obtained by the two spin-on photo-patternable polymers with a single layer spin of PA and a double layer spin of DXL (Fig. 1b). The spin-on procedure allows the precise alignment of micro channels to underlying sensors and actuators. With this method fluidic structures are created in which microheaters for integrated cell sorting are well aligned to fluidic chambers (Fig. 1c). An additional benefit of both materials over commonly used PDMS and SU-8 is that they are free of solvents which prevents adhesion to optical masks. We optimized the critical fabrication parameters for DXL and PA, i.e. exposure, bonding pressure, temperature and time, to obtain straight channels without channel deformation after cover glass bonding (Fig. 3). The rigid structure of both materials ensured the formation of perfect vertical walls. After optimization of the lithography parameters, aspect ratios of 1:1 could be obtained.

flow cytometry measurements. **b** Schematic of optical setup for in-flow detection of fluorescent cells. BB: beam blocker; BFP: back focal plane; BP: band pass filter; BS: beam splitter; DM: dichroic mirror; LP: long pass filter; PMT BS: PMT for back scatter detection; PMT FL: PMT for fluorescent detection at 585/40 nm

3.2 Bonding strength measurements

In order to evaluate the suitability of microfluidic channels out of PA and DXL for lab-on-a-chip applications, the bond strength of devices was tested with shear strength measurements and fluidic leakage tests. The glass transition temperature of PA and DXL is 135 $^{\circ}\text{C}$ and 145 $^{\circ}\text{C}$, respectively. To close the channels with a glass cover, the chip was heated a few minutes above this glass transition temperature while applying an external force as described in the material section. The shear strength measurements were carried out on three samples of each material. The DXL coated dies showed a bond shear strength of 13.1 ± 0.7 MPa and PA coated dies a slightly higher bond strength of 14.4 ± 1.4 MPa. These values demonstrate that the bond strength of these materials is sufficient for microfluidic applications. A common system for microfluidic prototyping is PDMS to glass bonding. The reported bond shear strength of PDMS to glass bonding after oxygen plasma activation is 74 kPa (Xiong et al. 2014). This is around 200 times smaller than DXL and PA. In comparison, SU-8 has been reported to withstand a maximum shear bond strength of 27 MPa (Salvo et al. 2012). Although, this bonding is very strong, SU-8 requires patterning on both substrates whereas the photo-patternable adhesives PA and DXL only require single patterning on the substrate (Blanco 2004).

For our cytometry application, cells pass the detection spot with flow speeds ranging from 0.1 m/s up to 1 m/s. To generate these flow speeds, the inlet sample pressure is operated up to 0.1 MPa. To check if these materials can withstand these pressures, 20 microfluidic flow cytometer devices from each DXL and PA were examined while injecting liquid at increasing pressures until the chip started leaking (Table 1). The measured pressure at which the chip started leaking is divided in three groups. The first group at <0.1 MPa does not allow fluidic operation for microfluidic flow cytometry. Most of the

Fig. 3 SEM cross-section of PA and DXL flow cytometer device at cleaved glass top cover edge. **a** The PA channel is 32 μm high and 200 μm wide and **b** the DXL channel is 500 μm wide and 33 μm high

DXL and PA devices in this group failed at the initial start of the test. The cause of immediate leakage was a bonding failure of certain areas of the cover glass to the substrate. These unbonded areas were visible before injection of liquid. Few devices failed in the pressure range between 0.1–0.3 MPa. The bonding quality of these devices was just strong enough for our cytometry application. Most of the PA and DXL devices were leak free above 0.3 MPa, far above the required 0.1 MPa operation pressure. Fabricated devices that were leak free during operation, were found to be still leak free after several months. These bonding tests indicate that these materials are well suited for microfluidic devices and that they can be used as alternative for PDMS and SU-8 in prototyping applications.

3.3 Optical properties

Depending on the life science application, fluorescence, absorbance or transmission measurements are performed to detect single particles or analyte concentration. For fluorescent detection in flow cytometry, the optical efficiency of detecting immunostained cells in flow depends largely on the background signal level. The flow cell in commercial flow cytometers is often made out of quartz glass to minimize any autofluorescent background signal. For disposable microfluidic devices, it is too costly to make the microfluidic channels out of quartz glass. The largest background signal source of flow cytometer chips is the polymer used to construct the microfluidics. Although the laser spot for fluorescent cell detection does not hit directly the polymer sidewalls, a part of the laser light reflects from the substrate and cover,

which indirectly hits the polymer sidewalls creating the autofluorescent background signal.

Studies were carried out to determine the amount of autofluorescence for SU-8, PA and DXL at blue, green and red light emission in comparison to a bare quartz glass slide (Fig. 4a). The fluorescence signal with 365 nm excitation and blue light emission was considerable larger than the other colors. The measured fluorescent intensity of DXL and SU-8 compared to PA was much smaller in the blue emission and ~4 times less intense in the green emission. For red emission all materials were nearly non-fluorescent. The measured fluorescence of SU-8 was slightly lower than DXL for all three colors. To further characterize the fluorescence of these materials, the fluorescence spectrum of SU-8, DXL and PA films on quartz glass were compared at an excitation of 405, 630 and 785 nm (Fig. 4b–d). At an excitation of 405 nm the fluorescent background signal between 400 and 600 nm is much stronger for PA than for DXL and SU-8 (Fig. 4b). DXL has similar fluorescent intensity as SU-8, but outperforms SU-8 above 500 nm. When excited at 633 nm, SU-8 and PA have similar fluorescence intensity while DXL has lowest signal intensity (Fig. 4c). At even longer wavelength excitation of 785 nm, PA and SU-8 material again show similar signal levels and DXL is again the weakest autofluorescent material (Fig. 4d). It is clear from these data that improved cytometry experiments can be performed using cells labeled with red or infrared fluorescent dyes. The much lower autofluorescence of DXL and SU-8 compared to PA at shorter wavelengths allows the combining of more fluorescent colors which is often required for immunostaining.

Thin films of DXL and PA were coated on quartz glass to measure the transmittance spectra (Fig. S1). DXL and PA have high transmittance at longer wavelengths above 500 nm. Below 500 nm the absorbance of PA increases while DXL remained almost transparent. At even shorter wavelengths below 400 nm the transmittance dropped rapidly for both materials. Neither PA nor DXL microstructures are well suited for absorbance measurements or bright field imaging in the ultraviolet region. In comparison, the transmittance of SU-8 drops more quickly below 400 nm, but further has also good optical clarity above 500 nm (Pai et al. 2007). For applications requiring

Table 1 Fluidic leakage test of PA and DXL microfluidic cytometer devices. Failure pressures are divided into three categories: below (<0.1), suitable (0.1–0.3) and above (>0.3) required inlet pressure for microfluidic flow cytometry

Leakage Pressure (MPa)	PA	DXL
<0.1	20%	25%
0.1–0.3	10%	5%
>0.3	70%	70%

Fig. 4 Fluorescent intensity of SU-8, PA and DXL. **a** Relative intensity for SU-8, PA and DXL cytometer devices for red, green and blue emission. **b-d** Fluorescent spectrum of SU-8, PA and DXL with excitation at 405 nm **b**, 633 nm **c** and 785 nm **d**

fluorescence, absorption or transmission measurements, it is apparent from these measurements that DXL is an excellent candidate, followed by SU-8, while both outperform PA.

3.4 Cytotoxicity

Biocompatibility of lab-on-a-chips is important for studying cell viability, gene activation or cell signalling behavior. Common materials for microfluidic channel formation like glass, silicon and SU-8 are known to be biocompatible (Kotzar et al. 2001; Voskerician et al. 2003). To test the cytotoxic effects of new proposed materials PA and DXL, an extraction test was performed to check possible leaching out of cytotoxic components from these materials as described previously. The confluency of the MEF monolayer in the wells was examined (Fig. S2). No visible morphological differences were visible between negative control sample (quartz glass), PA and DXL. The positive control sample of DXL which was cured at a reduced temperature of 70 °C clearly showed detachment and rounding up of the cells, indicating cell death. The high toxicity of the positive control can be explained by the leaching of toxic

components into the medium, because low temperature curing led to incomplete cross-linking. Full curing of the polymer is therefore crucial to guarantee low toxicity.

A Calcein Am viability test revealed no remarkably difference in viability of MEF cells on quartz glass, PA and DXL. The cytotoxic test of DXL revealed a viability of $103.0 \pm 2.7\%$ and PA $97.8 \pm 3.1\%$ compared to the control sample. This test demonstrated that both materials generated low cytotoxic response from the extractable and therefore can be used for microstructure fabrication for biological applications.

3.5 Microfluidic flow cytometry

We evaluated the performance of DXL and PA in a more complicated model system, a microfluidic flow cytometer device with in flow detection of immunostained monocytes out of a mixture of white blood cells (PBMC). The monocytes were fluorescently labeled with anti-CD14 PE to distinguish them from lymphocytes. The PBMC mixture was infused at a rate of ~ 300 cells/s and focused to the middle of the main fluidic channel of 90 μm by 30 μm with sheath fluid (Fig. 2a). The

Fig. 5 Histograms of PBMC sample with monocytes as CD14+ cells from reference flow cytometer **a**, DXL device **b** and PA device **c**. The fraction of CD14+ monocytes detected with the PA and DXL devices is consistent with the flow cytometer that acts as a reference. The

autofluorescent background signal of PA increases the intensity of the negative and positive fluorescent fraction detected on the PA device compared to the DXL device

cells passed a 532 nm laser detection spot in the middle of the main channel. This generated a back scatter signal to count all cells including unstained cells and a fluorescence signal to count the fraction of monocytes. The counts from PA and DXL devices and reference flow cytometer were compared to check the influence of autofluorescence background signals.

Figure 5 shows the histograms from the flow experiments for both DXL and PA devices and the count for the reference flow cytometer. From the histograms it is clear that there is no overlap between CD14+ and CD14- cells. Further, the count of ~21% for both devices was consistent with the count from the conventional flow cytometer, meaning that all monocytes

were detected in both devices. The peak shift to higher values of the CD14+ monocytes and CD14- unstained cells of the PA device compared to DXL device indicates a more intense fluorescent background signal. This result is consistent with the previous fluorescence spectra (Fig. 4).

To further investigate the fluorescent signal quality, the raw fluorescent data were investigated (Fig. 6). When monocytes passed the laser spot, a clear peak was generated even with only a 4 mW laser spot power. The lower autofluorescent level of DXL compared to PA causes a 6 times smaller background signal level. To calculate the signal to noise ratio (SNR), the average background signal is subtracted from the mean fluorescence intensity (MFI) signal of the positive cells:

$$SNR_{dB} = 10 \log_{10} \left(\frac{MFI - Background}{\sigma_{background}} \right) \quad (1)$$

Fig. 6 Fluorescent signal of monocytes from PBMC sample in DXL and PA devices. The recorded signals from the PA and DXL cytometer device show clear peaks for passing CD14 positive monocytes. The PA device has a larger signal offset and noise level due to larger autofluorescence background signal. Photobleaching PA reduces the signal offset, but does not reduce the noise signal level

The SNR of fluorescent stained monocytes on the PA device was 20.2 dB and the SNR on the DXL increases with a factor of two to 23.2 dB. Both signal levels were more than high enough to detect all monocytes from the sample. During optical properties characterization of PA, it was observed that this material quickly bleaches if it was illuminated by a 365 nm LED. To check the consequence of this bleaching effect, a PA device was illuminated at the detection spot for 2 min with the 365 nm LED. This bleaching reduced the background signal level, but the variance of the background noise stayed the same.

4 Conclusion

In this paper, we reported the fabrication process and material characteristics for the two photo-patternable adhesive

polymers, PA and DXL, toward the production of microfluidic electronic devices. The utility of these materials was demonstrated with microfluidic cytometer devices for in flow detection of fluorescently labeled monocytes. The key components of the simple processing scheme were the photo-patternable adhesive polymer and direct bonding to a glass cover by applying pressure and moderate heating. The DXL and PA fabrication process requires no additional adhesive layers, low bonding temperature and yields disposable devices that can contain microelectronics. With this fabrication process, we have addressed an important challenge of combining standard CMOS processing with wafer scale microstructure fabrication for low cost lab-on-a-chips. The compatibility with standard lithography simplifies massive integration of sensors and active elements. The photo-patternable adhesive has bi-functional material property in which the material forms the microstructures and acts as adhesive for channel closing. These material properties together with their compatibility to standard processing make them better suited for easy prototyping and volume production compared to SU-8 and PDMS. Microdevices out of PA or DXL can then easily be assembled with cheap plastic liquid handling devices without the need for precise alignment steps.

The new photo-patternable polymers may find broad application in bio-sensing and on-chip cell sorting where integrated electrodes need to be precisely aligned to fluidic microstructures. For applications in which large biological cells are investigated, thick microfluidic structures can especially be manufactured out of the higher visous material PA. For weak fluorescence applications, DXL improves detection sensitivity compared to PA and SU-8. The bio-compatibility of these materials demonstrates the large potential of DXL and PA for life science applications, for example cell capturing for image cytometry or monitoring of incubated cells with integrated electrodes.

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