Electronic supplementary information (ESI)

Solvent resistant lab-on-chip platform for radiochemistry applications

Christian Rensch,*[a] Simon Lindner,[b] Ruben Salvamoser,[a] Stephanie Leidner,[b] Christoph Böld,[a] Victor Samper,[a] David Taylor,[a] Marko Baller,[c] Stefan Riese,[d] Peter Bartenstein,[b] Carmen Wängler,[b,e] and Björn Wängler,*[f]†

COC material

COC 6017-S04 (TOPAS® Advanced Polymers GmbH, Germany) was utilized for injection moulding of rectangular blanks (Rodinger Kunststoff-Technik GmbH, Germany) with outer dimensions of 100mm x 100mm x 2mm. COC 6015 (TOPAS® Advanced Polymers GmbH, Germany) was used for manufacturing of insert bodies for resin on-chip integration. COC 6015 foil (TOPAS® Advanced Polymers GmbH, Germany) at 100µm thickness was obtained for on-chip valve realization.

Milling

All microfluidic structures on each layer are created utilizing a four axis computerized numerical controlled (CNC) milling machine (MDX-540 SA, Roland DGA Corp., USA). A custom clamp manifold enables easy insertion of the COC blank and subsequent fully automated two-sided milling of the microfluidic chip layer (outer dimensions 95mm x 60mm x 2mm). At cutting tool diameters ranging from AD 100µm to AD 2mm, the feeding rate is varied between 1500mm/min to 700mm/min at rotational speeds between 5.000 rpm and 12.000 rpm. Custom milling tools are utilized for manufacturing of conic fluid I/O ports at < 3 seconds processing time per port. The milling machine is controlled by a SolidCAM interface (SolidCAM GmbH, Germany), providing a seamless transfer of 3D computer aided design (CAD) drawings created in SolidWorks (Dassault Systèmes SolidWorks Corp., USA).

Cleaning

All chip layers were exposed to a 10 minute ultrasonic bath in de-ionized water (Bransonic® 2510EMTH, Branson Ultrasonics, USA) for removal of milling residuals. Subsequently, each layer is rinsed for 3 min. in isopropyl alcohol and blow dried utilizing nitrogen gas. COC foils were cut to size, the protective polyethylene terephtalate (PETg) layers removed, the foil rinsed with isopropyl alcohol and subsequently placed 5 minutes in a fume hood for drying.

[a] *GE Global Research, Freisinger Landstrasse 50, 85748 Garching bei Munich, Germany. Fax: +49-89-5528-3181; Tel: +49-89-5528-3613; E-mail: rensch@ge.com*
[b] *University Hospital Munich, Department of Nuclear Medicine, Ludwig Maximilians-University, 81377 Munich, Germany.*
[c] *Department of Biomedical Sciences in Micro- and Nanotechnology, University of Applied Sciences Kaiserslautern - Zweibrücken, Amerikastr. 1, 66482 Zweibrücken, Germany.*
[d] *GE Healthcare, Schaimburgstraße 3, 48145 Münster, Germany.*
[e] *Biomedical Chemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, 68167 Mannheim, Germany; Tel: +49-621-383-2067; E-mail: bjoern.waengler@med.uni-heidelberg.de*
[f] *Molecular Imaging and Radiochemistry, Department of Clinical Radiology and Nuclear Medicine, Medical Faculty Mannheim of Heidelberg University, 68167 Mannheim, Germany.*

Resin on chip

The insert bodies are of simple geometry and milled from COC 6015 (TOPAS® Advanced Polymers GmbH, Germany), enabling compatibility to all process chemicals and low cost injection moulding. Insert bodies were designed to carry 46mg resin weight, comparable to conventional cartridges (L: 12mm, B: 5mm, H: 5mm, Bore: Ø4mm). The silica-based resin material was extracted from the respective standard cartridges and manually transferred to the COC6015 insert bodies creating phase transfer inserts (46 mg resin material from Sep-Pak® Light QMA Carbonate, Part No. 186004540, Waters Corp.) and purification inserts (42 mg resin material from Sep-Pak® Light C18, Part No. WAT023501, Waters Corp.). Resin weights were measured by a high precision scale (TP-214, Denver Instrument GmbH, Germany). Each insert body is closed by two Polytetrafluoroethylene (PTFE) frits (Part No. 302831, Reichelt Chemietechnik GmbH + Co., Germany).

On-chip valves

The valve seat has a central bore diameter of 250µm and a 50µm wide circular sealing edge. The valve can be prototyped utilizing high precision milling techniques and is suitable for injection moulding. FEP disks (OD 4 mm) were punched out of 127µm thick FEP foil (DuPont FEP, part No. #536-3996, RS Components GmbH, Germany) and subsequently positioned on the valve structures after being enclosed by the COC 6015 foil during chip assembly.

The valve design was characterized for maximum pressure capability under realistic test conditions. Therefore, simplified valve test structures were designed and pressurized until membrane failure. One test structure consists of an OD 6mm chamber which is equal to the outer diameter of one valve, surrounded by a concentric venting channel at a radial distance of 2mm between the chamber and the channel (Fig. 1).

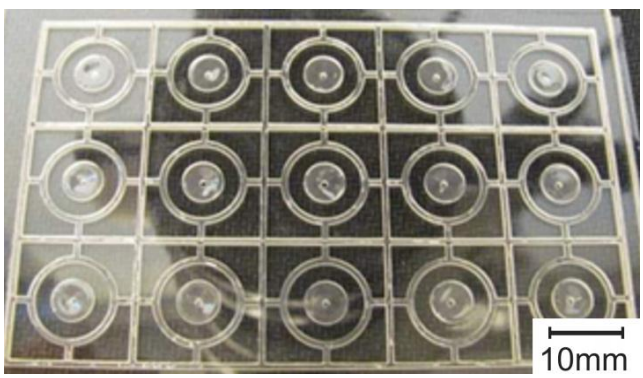

Fig. 1 Valve test structures for maximum pressure assessments.

The radial distance defines the maximum delamination length to fatigue upon pressurization of the chamber and was chosen

according to a valve-to-valve pitch of 4mm in the current chip design. The chamber and the venting channel are covered by the COC 6015 membrane according to the bonding parameters used for chip manufacturing. A LabView controlled test setup consisting of a syringe pump (Cavro® XLP 6000, Tecan Group Ltd., Switzerland equipped with 10ml glass syringe filled with air) is used for chamber pressurization until rupture of the membrane or delamination towards the venting channel, whereas the system pressure is continuously monitored by an in-line pressure sensor. In order to determine valve stability across multiple switching cycles, another test setup has been employed utilizing test chips with an array of functional valves (Fig. 2).

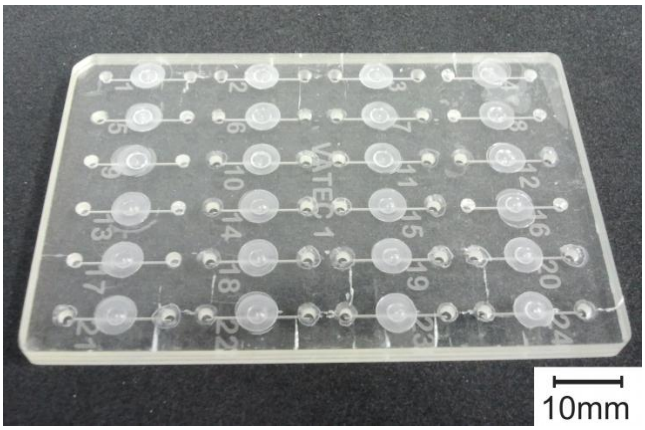

Fig. 2 Test chip for valve cycle performance and valve leakage rate with n=24 test valves per chip.

Each valve is connected to a separate input and output. A high resolution linear actuator (M-230.25, Physik Instrumente PI GmbH & Co. KG, Germany) equipped with a force sensor (SML-25, Interface Inc., USA) is utilized for valve opening and closure at a defined plunger force (accuracy ±0.1N) and vertical position (accuracy ≤ ±1.0µm). For each valve under test, the linear actuator is calibrated and subsequently operated under absolute position control, hence, material deformation of either the membrane or the valve seat results in a measureable change of the closing force. Mass flow controllers (MTC Massflow Controller, Analyt-MTC Messtechnik GmbH, Germany) are connected to each valve input and output respectively. A nitrogen gas flow with a pressure of 2 bar is applied to the flow path and can be switched on and off by a motor valve. All hardware elements are automated by a custom LabView-based test software running 50 consecutive open-close cycles per valve. For each cycle, plunger force, plunger position, system pressure and gas flow are recorded.

The valve leakage rate was determined by connecting the valve outlet with a short ID 250µm PEEK capillary to a low dead volume ID 200µm needle submerged in 2-propanol. The valve under test is closed and pressurized by nitrogen at 2 bar from the input. Subsequently, the needle tip is visually observed for bubble formation. Under the assumption of spherical gas bubbles with a diameter close to the outer diameter of the needle being visually detectable, the theoretical dead volume of one gas bubble is 0.1 µL. The bubble formation was observed for 60s, hence, one bubble occurring during that time roughly correlates to a gas leakage rate of 0.1µL/min, presenting the lower detection limit of this simplified approach.

Bonding

The three chip layers containing microfluidic structures, the resin inserts, the FEP valve discs and the cover foil are assembled, aligned and subsequently joined to a microfluidic chip within a single thermal bonding step. A standard hydraulic press (Hydraulic press 25T 54MP250, Maassen GmbH, Germany) was equipped with two electrically heated metal plates, whereas a glass mirror plate was attached to each bonding face. Thermal compression bonding is executed for 45 min. at 162 °C and a bonding pressure of 4.3 MPa. After bonding, the chip is cooled down at a rate of 1.5°C/min. in order to avoid thermal stress at the bonded interfaces.

Reagent storage and transport

Kryptofix eluent, oxalic acid, precursor and aqueous fluoride-18 are provided to the platform from septa capped low dead-volume vials (Certified CD™ Vial, part No. 29307-U, Supelco Analytical, USA). Since conventional luer adapters show dead volumes >10µl, the vials are interfaced to 1/16" PEEK capillary tubing (ID 0.5 mm) by means of a custom low dead volume connector built from a needle (Septoject dental needle, 27G, OD 0.4mm, length 42mm, part No. 01-N1271, Septodont, France) and a MicroTight® adapter (MicroTight® ZDV, part No. UP P-882, Techlab GmbH, Germany). Each vial is connected via the adapter to the chip manifold using a single PEEK capillary line respectively.

Chip designs

Two chip designs were employed for the study: The first design enables multi-use of chips and characterization of each synthesis step during protocol development by utilizing a resin evaluation setup for the QMA and SPE cartridge external to the chip (Fig. 3). The evaluation setup is connected to the chip via capillary tubing. According to figure 4, media on its way to a resin takes a detour exiting the chip (Fig. 4, connectors C13 and C15), passing across the external resin (Fig. 3) and subsequently re-entering the chip (Fig. 4, connectors C14 and C16).

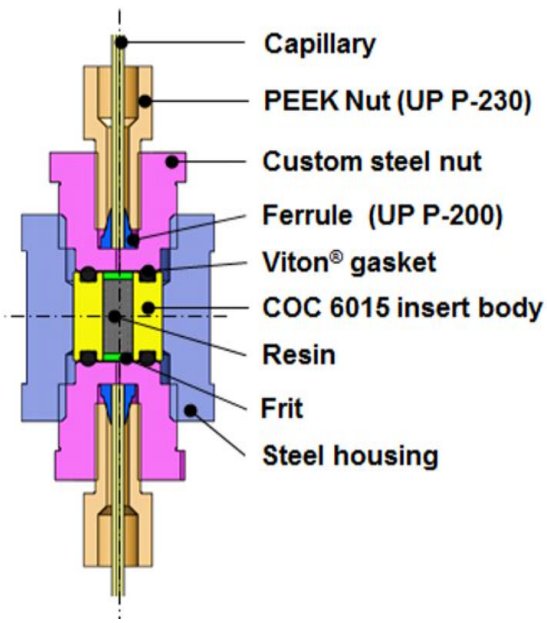

Fig. 3 Test setup for operation with resins external to the chip.

The resin evaluation setup (Fig. 3) contains a COC 6015 resin body with internal dimensions and resin weights identical to the previously described design for on-chip integration. Hence, synthesis protocols could be transferred seamlessly to the second chip design which included both resins on-chip. For the second chip design the fluid path was not connected to C13 – C16 (fig. 4) but "wired" directly to the respective on-chip resin, all other functional system elements remained in the same configuration.

Chemistry processing

Aqueous fluoride-18 was produced via the $^{18}O(p,n)^{18}F$ nuclear reaction by irradiation of enriched [^{18}O] water. A [$K^+\subset$2.2.2]OH- eluent kit was prepared from Kryptofix 2.2.2 (41 mg, 110 µmol), 100 µL aqueous KOH (1 M, 100 µmol) and final lyophilisation for dry deep freeze storage. Upon use, the Kryptofix complex was dissolved in 300 µL anhydrous acetonitrile.

The following steps were performed on the microfluidic platform: (1) Conditioning of QMA cartridge (46 mg resin, Sep-Pak® Light QMA Carbonate, Part No. 186004540, Waters Corp.) utilizing 1 ml H_2O (Tracepur), (2) gas pressure driven loading of aqueous fluoride-18 activity to QMA cartridge, (3) drying of QMA cartridge using 5 sec. nitrogen gas flow, 4 mL of anhydrous acetonitrile and another 5 sec. nitrogen gas flow, (4) elution of fluoride-18 from QMA cartridge utilizing 300 µL of [$K^+\subset$2.2.2]OH^- (100 µmol) in anhydrous acetonitrile, (5) neutralization of fluoride-18 elution mixture by 100µL oxalic acid in anhydrous DMF (0.5 M, 18.75 µmol), (6) addition of SiFA-PESIN precursor dissolved in 100µl anhydrous DMF (5 mM, 25 nmol), (7) radiolabeling reaction at room temperature for 15 min, (8) conditioning of C18 cartridge (46mg resin, Sep-Pak® Light C18, Part No. WAT023501, Waters Corp.) utilizing 4 mL ethanol and subsequent 4 mL of water (Tracepur), (9) dilution of the intermediate reaction mixture by 4 mL HEPES buffer (0.1 M, pH = 4) and transfer to C18 cartridge, (10) rinse of C18 cartridge utilizing 4.9 mL water (Tracepur) to remove solvent residues, (11) elution of purified product in 1.0 mL ethanol to the product vial.

The product output was analysed (Agilent 1200 HPLC, Agilent Technologies Inc., USA) using a Chromolith Performance column (RP-18e, 100-4.6 mm, Merck KGaA, Germany) at 0 – 100 % acetonitrile plus 0.1 % trifluoro-acetic acid (v/v) over 5 minutes.

The following measurements were taken on the setup with off-chip cartridges: The efficiency for fluoride-18 drying across the QMA cartridge (input vs. usable output activity, decay corrected to **s**tart **o**f **s**ynthesis, SOS) was measured to an average of 78% (standard deviation 14.8%, n = 4). The on-chip radiolabeling efficiency for [^{18}F]PESIN was measured to >60% (n = 4, Agilent 1200 radio-HPLC, Agilent Technologies Inc., USA) which is comparable to conventional processing. The subsequent purification efficiency was measured to an average of 81% (standard deviation 8.1%, n=4, based on activity transferred to product, decay corrected to SOS) at >99% radiochemical purity of the final product [^{18}F]PESIN. The residual activity on-chip after process completion was measured to <14% (n=4, decay corrected to SOS), residuals on QMA cartridges to 11.5% in average (n=4, decay corrected to SOS) and on SPE cartridges to <2.8% (n=4, decay corrected to SOS).

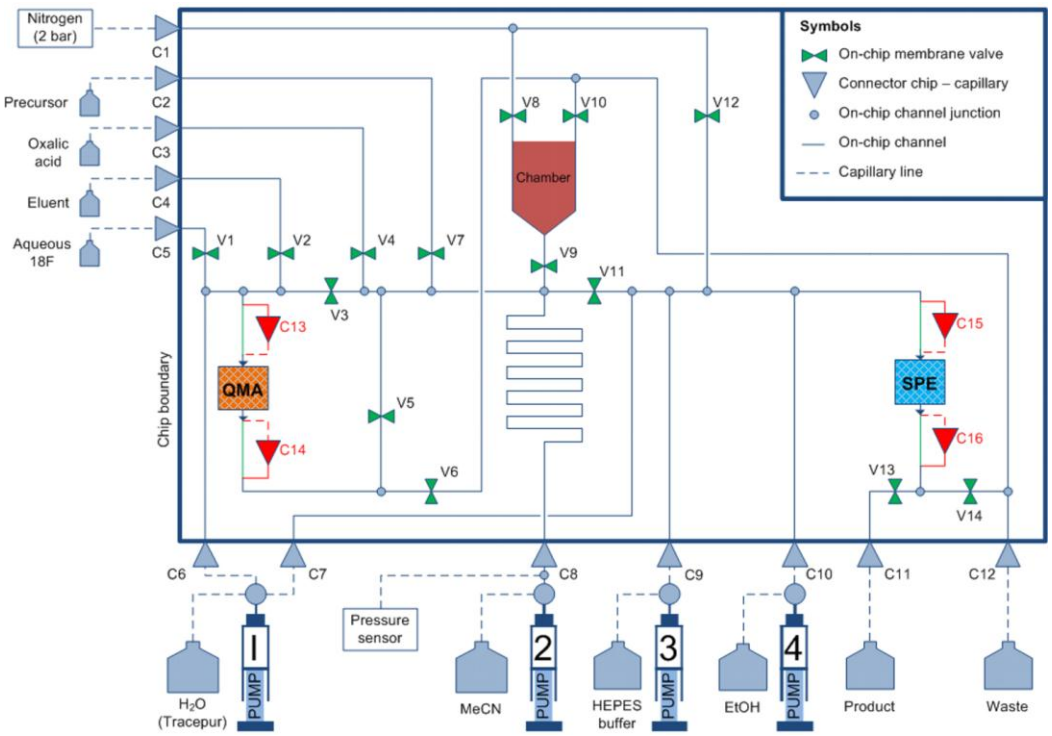

Fig. 4: Schematic layout of microfluidic chip and required hardware periphery. The connectors C13 – C16 are utilized for the setup configuration with external cartridges. For the configuration with QMA and SPE resins integrated on chip, fluids pass directly across the cartridges on chip respectively and connectors C13-C16 are not connected to the fluid path.