Supplemental Material

Design and Optimization of Coin-shaped Microreactor Chips for PET Radiopharmaceutical Synthesis

Arkadij M. Elizarov*,[1,2] R. Michael van Dam,[2] Young Shik Shin,[2] Hartmuth C. Kolb,[1] Henry C. Padgett,[1] David Stout,[3] Jenny Shu,[3] Jiang Huang,[4] Antoine Daridon,[4] James R. Heath[2]

[1] Siemens Molecular Imaging, Biomarker Research, 6100 Bristol Parkway, Culver City, CA 90230

[2] California Institute of Technology, Division of Chemistry and Chemical Engineering and the NanoSystems Biology Cancer Center, MC 127-72, 1200 E. California Blvd, Pasadena, CA 91125

[3]Department of Molecular and Medical Pharmacology, University of California, Los Angeles, CA 90095

[4] Fluidigm Corporation, 7000 Shoreline Court, Suite 100, South San Francisco, CA 94080

*to whom correspondence should be addressed: **arkadij.elizarov@siemens.com**

Materials and Methods

Mold and Chip Fabrication

The general procedure for the fabrication of integrated, multilayer elastomeric microfluidic chips has been described in the literature *(1)*. Two sets of molds designed for flow and control/vent layers were prepared. The flow layer mold required two photoresists (Figure S1A). AZ-50 XT (AZ Electronic Materials) was first used to form the rounded features of channels (ca. 60 μm tall, rounded to allow complete valve sealing), followed by SU-8 2100 to make the 250 μm tall reactor feature. (It was necessary to process the thinner resist first in order to get good pattern fidelity at the overlap regions.) In order to preserve the AZ-50 XT features during SU-8 development, the former was baked at 210 °C for 3 h. Precisely-controlled temperature ramping was needed to minimize the buildup of thermal stresses. The control layer mold was made from a single SU-8 2015 photoresist (Microchem) producing 25 μm tall features.

Chip fabrication from molds (Figure S1B-D): Flow and control layer molds were

treated with chlorotrimethylsilane (TMS-Cl, >99%, Aldrich) in a vapor chamber for 15 min to provide an easy release of PDMS. GE RTV 615 PDMS (GE Silicones) A and B components *(2)* were mixed in a 5:1 ratio for the top (flow) layer, poured over the flow layer mold and degassed for 20 min in a vacuum chamber, then cured in 80 °C oven for 10 min simultaneously with the control layer. For the control layer A and B components were mixed in a 20:1 ratio followed by spin-coating over the mold at 2000 rpm for 60 s and curing in 80 °C oven for 10 min. The blank layer was prepared by mixing A and B in a 30:1 ratio and coated on an untreated wafer at 4000 rpm for 30 sec, followed by curing in an 80 °C oven for 10 min (Figure S1B). Upon removal from the oven, thick flow layers were cut into individual chips and had holes punched through them for fluid delivery. Afterwards they were aligned on top of control layers and baked for 80 min to achieve bonding (Figure S1C). Upon removal from the molds these 2-layer devices had holes punched in them for the control channels and were placed on top of blank layers. Final 3-layer chips (Figure S1D) were baked for 16 h in an 80 °C oven.

Fully-cured chips were interfaced with reagents and control pneumatics by inserting hollow metal pins into the punched chip ports (as can be seen in Figure 3B) and connecting the other end of the pin to flexible PTFE tubing leading to reagent vials or controlled pressure sources.

Extended Results and Discussion

Ion Exchange column resin selection

A number of resins suitable for ion exchange column packing have been evaluated. Four lead candidates were identified from commercial products: QMA (Waters) used in large scale synthesizers, Source 15Q (Amersham) used in the first reported CRC, MP-1 (Bio-Rad) used frequently in R&D, and AG1-X8 (Bio-Rad). Columns were made inside polyethylene tubing from each resin and used to trap various amounts of radioactivity. The minimal amount of the ion exchange resin necessary to process the entire target load of $[^{18}O]H_2O$ at high (>95%) $[^{18}F]$fluoride trapping efficiency resulted in a 2 μL volume of the resin bed. The percentage of $^{18}F^-$ trapped by the column out of the total amount of $^{18}F^-$ passed through at 1 mL/min was: QMA – 36

%, Source 15Q – 71 %, MP-1 – 98.2 %, AG1-X8 – 99.7%. The latter was further tested for its capacity and release performance. It trapped 99.5% of 32 GBq (876 mCi) of $^{18}F^-$, of which 92.7% was subsequently released with approximately 5 μL of 0.5 M K_2CO_3.

Vent

Solvent evaporation steps are the most taxing on the CRC materials and designs, and so were extensively explored. On-chip solvent exchange involves balancing the valve capabilities and actuation pressures against other pressures involved in solvent evaporation step. Control channel pressures required for valve actuation are in the range of 15 psi. MeCN vapor pressure at the temperatures used in radiosynthesis can reach 23 psi. This leads to a 38-psi minimum valve actuation pressure which has to counteract both of these. The pressures required for safe operation would have to exceed 50 psi, and if applied over time, can easily damage the CRC by either layer delamination or valve membrane rupturing. We explored two serpentine vent designs (below and above the reactor) and two different processes for shortening the solvent evaporation/exchange process. The first approach involved flowing N_2 through the vent to carry away solvent vapors, and the second relied on application of vacuum in the vent. Not surprisingly, the second approach allowed faster evaporations taking place at reduced pressures. Reduction of vapor pressure also allowed to perform evaporations at lower temperature. Placing the vent below the reactor was easier from a fabrication standpoint, but resulted in a lot of vapor being directed into the bulk of PDMS above the reactor making complete vapor removal from the chip slow and inefficient. The vent placed above the reactor dramatically reduced the time needed for complete evaporations of MeCN and water, and resulted in significantly fewer valve failures.

Mixing Methods

(a) Manifold. As liquids are delivered to the reactor, they tend to enter rapidly as the reaction chamber expands, but then they propagate slowly as the gas is displaced out of the reactor across the membrane. If one solution is already in the reactor and the other one is entering by this method, they will not mix efficiently because diffusion-based mixing will have to overcome large distances and the slow-moving front does not carry enough inertia to laminate with the solution already in reactor. Thus, to facilitate the

mixing a manifold with 6 ports evenly spaced around the circumference was designed, which allowed the brief rapid entrance to take place at 6 positions simultaneously. Unfortunately, this approach had minimal effect in fluorination reactions because in laminar flow regime at low Reynold's number, mixing of viscous high-concentration solutions was diffusion limited. Fluorination yields did not differ between cases when precursor was introduced through a single channel and through the manifold (without vacuum).

(b) Vacuum-assisted mixing. This method relies on inertia of rapidly entering liquid. After the first reagent is delivered to the reactor by dead-end filling, all valves are closed and vacuum application in the vent pumps the remaining gas out of the reactor. Elastomeric structure of PDMS allows the reactor to cave in and shrink in volume. (Since the whole chip is soft, it can deform to allow this.) At the same time the other reagent is driven up to a closed valve at one inlet of the reactor. Once that valve is opened, the reagent rapidly shoots into the reactor (accelerated by the vacuum created by the elastic restoration of the reactor shape), thus introducing a turbulent-like effect the entering liquid laminates densely with the solution already in the reactor yielding instantaneous mixing. Vacuum assistance has shown a measurable improvement in the uptake of [^{18}F]fluoride into organic phase. When evaporation of acetonitrile from K222 solution was followed by precursor entrance without vacuum assistance, a large part of fluoride stayed behind and could not participate in the fluorination reaction. This was demonstrated by removing the intermediate with acetonitrile after fluorination followed by removal of residual fluoride with water. The latter portion was much smaller in runs where vacuum-assisted mixing was employed.

(c) Chemically-assisted mixing. This is a method that is applicable in special cases allowing the necessary reagents. Aqueous HCl is delivered to the reactor to perform hydrolysis. Upon entrance, it engages in a CO_2-yielding acid/base reaction with K_2CO_3 in MeCN solution of [^{18}F]TAG. The out-gassing agitates the reaction mixture, causing vigorous back-and-forth swirling at the interface between the two solutions, which leads to rapid disappearance of this interface and a homogeneous reaction mixture. The value of this approach was demonstrated by comparing acidic and basic hydrolysis. The latter produced no out-gassing and the interface between the two solutions remained

visible throughout the entire step. As a result, a lot of unhydrolyzed intermediate was recovered unlike the acid hydrolysis where the reaction is stirred by CO_2 generation. (Both hydrolysis methods have worked similarly in conventional setups *(3)*.)

(d) Pressure-assisted mixing. In some cases with highly viscous solutions (which are not uncommon with high concentrations and low volumes), even after lamination created by the above method one can see unmixed regions that may take a very long time to diffuse. At this point, since diffusion is the only mechanism left, it can be accelerated by pressure and temperature. Pressurizing the reactor through the vent while heating it completes the mixing (while the corresponding reaction between the two solutions can be already starting in the mixed regions). In some cases, where too much solvent has been evaporated from the intermediate, the residue left is too thick for chemical assistance to induce enough effect. In these situations (which were identified visually) pressurizing the vent has driven hydrolysis to completion.

(e) Agitation. As one solution enters the reactor containing another solution, the driving pressure is pulsed rapidly. This leads to alternating expansions and contractions of the reactor, which allows the two solutions to be mixed by the time the reactor has been filled. Also, vacuum and pressure can be alternated in the vent over the closed reactor leading to the changes in reactor shape, which at a particular frequency mix the contents. Agitation did improve the mixing when used by itself without any of the above methods. The improvement was validated by fluorination yields. However, its side effect was partial loss of the reaction mixture into the mixing channel (used top deliver cold reagent). Therefore, this method was abandoned in view of others that offered similar benefits without losses or the complexity of operation.

Product elution

There is an element of chip design that allows removal of reactor contents with a minimal amount of solvent. This is important because purification that has to follow radiosynthesis is sensitive to the total volume of crude reaction mixture. The approach taken here, like some of the mixing methods, relies on inertia. The entrance and exit ports (9 and 10 in Figure 2) for flushing the product are placed tangential to the reactor circumference. At a particular flow rate this allows a sweeping wave of solvent to push

all contents out in a concentrated manner *(4)*. One more element of design that assures efficient product elution is the difference in cross sections of entrance and exit channels. The incoming channel (250 x 45 μm) being larger than the exit (200 x 45 μm) assures a positive pressure in the reactor during elution which further improves the flow patterns. The difference in the volume of eluent necessary for complete product removal has varied almost 10-fold between the tangential channel elution (25 μL) and that with channels perpendicular to the circumference (220 μL)

Material compatibility and reduced radiosynthesis validation

During prolonged heating or after a series of evaporations, PDMS valves would often get stuck in their closed state and would not re-open when their actuation pressure is relieved. This problem was solved by coating *(5)* all flow channels with thin layer of Chemraz®, which allowed the valves to re-open easily after extended heating at temperatures above 120 °C. At the same time gas permeability of the vent membrane was virtually unaffected by this coating. Valve failure was further reduced by introducing double valves, separated from one another by 300 μm, on all channels (Figure 3B).

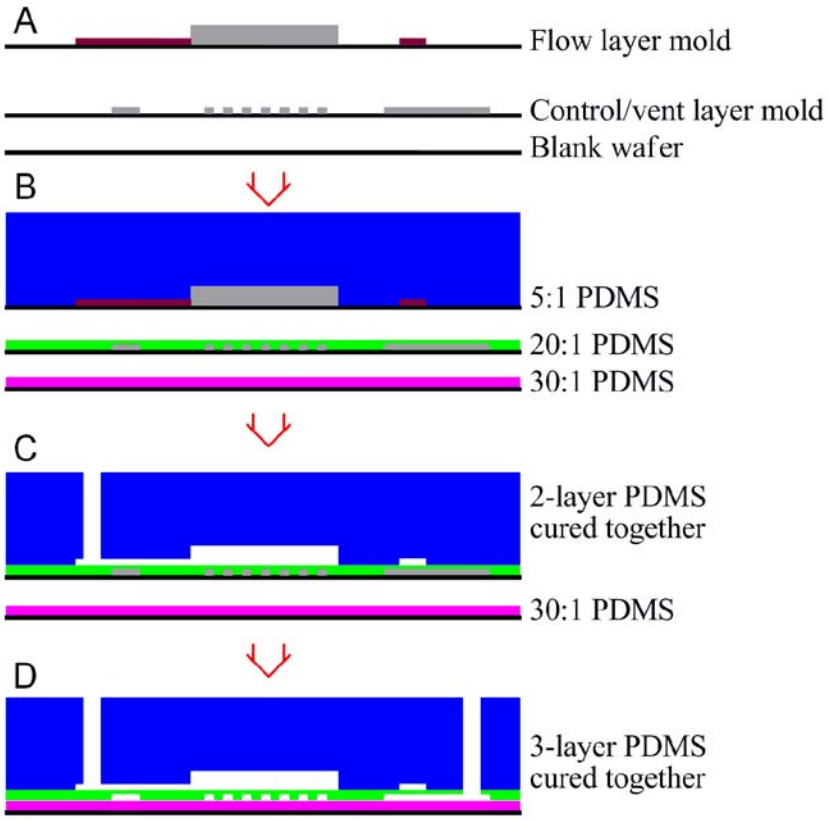
However, further issues with PDMS material were uncovered. Although we demonstrated that 10s of GBq (100s of mCi) of [^{18}F]fluoride could be delivered into the 5-μL reactor, it quickly became apparent that PDMS was incompatible with aq. K_2CO_3 solution at elevated temperatures. During water evaporation (Step 3) it was etched, leading to burst valves and layer delamination. In addition to that, PDMS was also found to be reactive towards [^{18}F]fluoride, yielding volatile ^{18}F-labeled species and reducing the yield of [^{18}F]FDG synthesis. The yield calculated from [^{18}F]fluoride available for reaction is based on all activity eluted out of the chip at the end of synthesis. It does not account for ^{18}F that is lost in reaction with PDMS. While the former has shown consistent results, the losses varied dramatically (from 5 to 95%) presumably driven by the variation in the Chemraz® coating quality/thickness. Partial mitigation of these effects was achieved with Chemraz® coating. It allowed chips to stay intact with about 50% success rate, while ^{18}F losses persisted. While such performance was sufficient for design validation and demonstration of a fully integrated process (as described in the

main article) from dilute [^{18}F]fluoride to [^{18}F]FDG, it has triggered an intense search and optimization of alternative materials *(6,7)* for the chip (which will be described in another publication). In the meantime, most of validation of the CRC functionality past fluoride drying has been accomplished with [^{18}F]fluoride dried off-chip. (Anhydrous [^{18}F]KF/K_2CO_3/Kryptofix 2.2.2 in MeCN was produced by *explora* RN *(8)* nucleophilic radiochemistry module.)

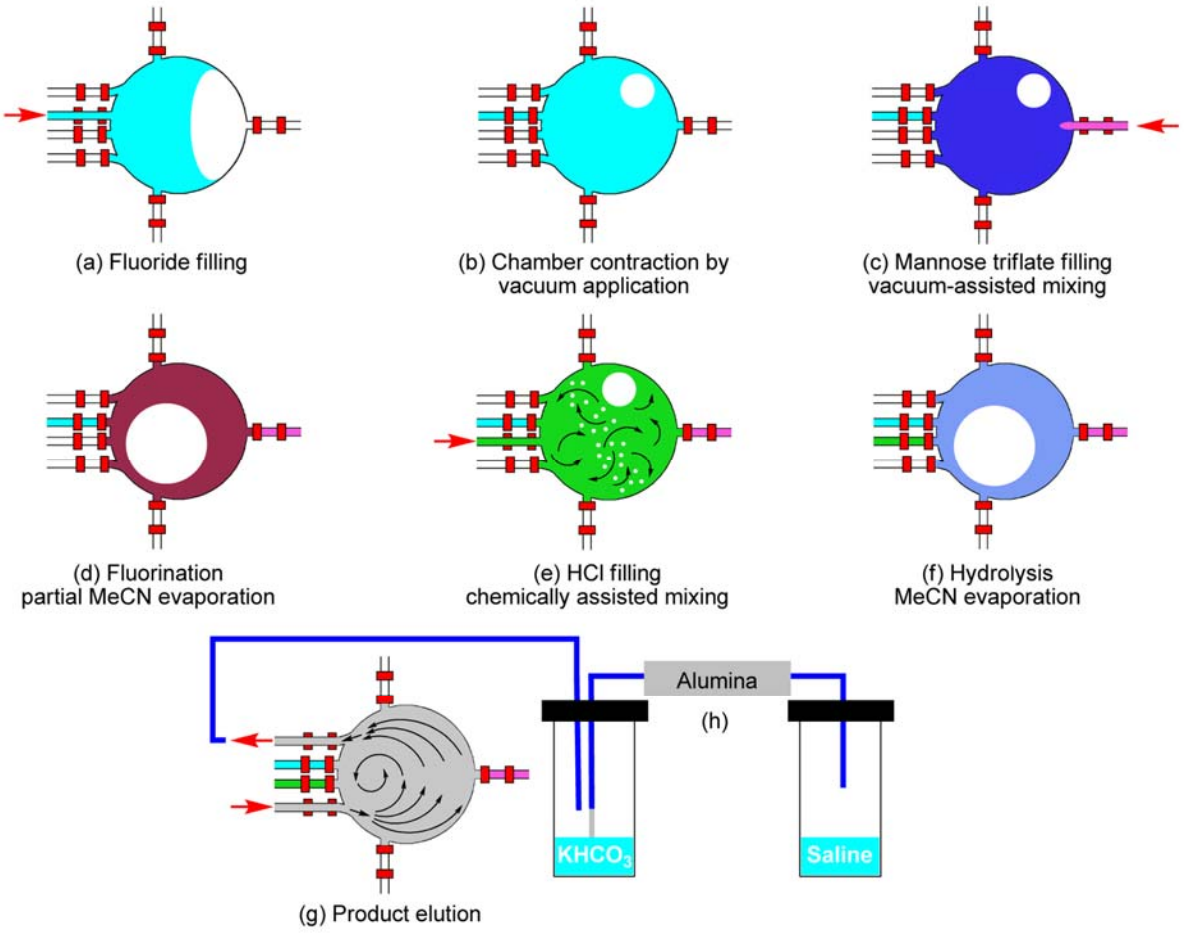
Optimization has yielded the following protocol, outlined in Figure S2. First, the solution of [^{18}F]KF/K_2CO_3/Kryptofix 2.2.2 in MeCN is delivered to the reactor (Figure S2a), by opening appropriate valves. The gas displaced by the liquid is removed across the gas-permeable membrane by the application of vacuum to the vent over the top of the reactor. As the fluoride solution fills ⅔ of the reactor, the valves are closed, and the vacuum is allowed to pump the remaining gas out of the reactor (Figure S2b). Next, mannose triflate solution in dry MeCN is delivered through a channel on the opposite side of the reactor (Figure S2c) which relies on vacuum-assisted mixing. After the reactor is full, all valves are closed (Figure S2d) and the chip is heated at 65 °C for 3 min to accelerate the reaction of mannose triflate with [^{18}F]fluoride forming the intermediate tetraacetylated [^{18}F]fluoro-glucose compound ([^{18}F]TAG). Vacuum is applied in the vent to evaporate some solvent on order to create space in the reactor for the acid in the next step. When the volume of about 2/3 of the reactor is empty with all [^{18}F]TAG concentrated in the remaining solution, the chip is cooled and 3M HCl is added inducing chemically-assisted mixing (Figure S2e). The reactor is closed and heated (Figure S2f) for another 2 min at 75 °C to remove the remaining acetonitrile and allow hydrolysis of [^{18}F]TAG to [^{18}F]FDG to proceed to completion. Upon cooling to ambient temperature, the contents are eluted into a tangential channel with minimal volume of water driven by 12 psi N_2 pressure and entering the other tangential channel (Figure S2g). Subsequent purification required elution into a vial containing 45 uL of 1M $KHCO_3$ solution to neutralize the acid. After the two solutions have mixed, a second line containing a column (Figure S2h) with 190 mg alumina was lowered into this vial. The vial was pressurized once again (through the chip) to send the contents into the final product vial through the alumina column.

[^{18}F]FDDNP

To stress the potential of the described devices as a universal platform for radiopharmaceutical synthesis, the chip was used to make another biomarker. Alzheimer's disease PET probe, 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-napthyl}ethylidene)malonitrile ([^{18}F]FDDNP) *(9)* was successfully prepared using the chip of the same design and positively identified by analytical HPLC *(10)*. It should be possible to produce other PET-probes requiring nucleophilic chemistry in the fluorination step on this CRC with minimal modification (requiring only addition of extra channels at most). The design is capable of sustaining a range of temperatures since it can balance the vapor pressures with a vacuum vent.



Supplemental Figure 1. PDMS chip fabrication by the off-ratio method. (a) Preparation of flow and control layer molds by photolithography. (b) Top PDMS layer is cast, bottom two are spin-coated. All three are partially cured. (c) Flow layer is peeled off the mold and placed on top of the control layer followed by further curing. (d) Flow and control layers are peeled off the control layer mold and placed on top of the blank layer followed by complete curing.



Supplemental Figure 2. Schematic of injectable [^{18}F]FDG radiosynthesis process using a coin-shaped reactor chip.

References

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(4) Thorough analysis of flow inside the microreactor by empirical and theoretical methods has been performed it is covered in a separate manuscript (in preparation).

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