Automated Microfluidic-Chip-Based Stand-Alone Instrument for the Synthesis of Radiopharmaceuticals on Human-Dose Scales

R. Michael van Dam[1,2], Arkadij M. Elizarov [1], C. Edward Ball[1], Clifton Kwang-Fu Shen[1], Henry Padgett[1], Hartmuth Kolb[1], Jason Rolland[4], Larry Diener[3], Dirk Williams[3], Brian Edgecombe[3], Jennifer Jeffries[3], Diana Stoianova[3], Tony Stephen[3], Farshad Motamedi[3], James R. Heath[2]

[1]Siemens MI Biomarker Research, Culver City, CA, USA (Email: mikevandam@gmail.com)
[2]California Institute of Technology, Pasadena, CA, USA;
[3]Materia, Inc., Pasadena, CA, USA
[4]Liquidia Technologies, Inc., Research Triangle Park, NC, USA

ABSTRACT

We describe an automated, stand-alone, microfluidic instrument for the multi-step chemical synthesis of radiopharmaceuticals such as probes for positron emission tomography (PET).

In earlier work, we developed a proof-of-principle PDMS microfluidic chip with a coin-shaped reactor, and have demonstrated the synthesis of 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) on a scale and of quality sufficient for *in vivo* imaging in mice and rats. In order to produce FDG in amounts suitable for imaging at the human scale, we completely redesigned the chip architecture and operation.

A new generation chip and a novel fluidic adapter were integrated into a working prototype instrument, operated by a PC-104 based controller and software written in FIX32. We have demonstrated the synthesis of FDG up to 16mCi. Other biomarkers can be made by replacing reagents and tuning process parameters in the software interface.

Keywords: microfluidics, radiopharmaceuticals, PET, FDG, synthesis

1 INTRODUCTION

Although only a single PET probe, FDG, is presently in widespread use, numerous other useful probes have been discovered and show enormous potential [1,2]. Most of these probes are very difficult to synthesize and purify and thus their use is limited to a few research facilities.

Striving to increase the accessibility and diversity of PET probes, we have designed and built a prototype of an automated instrument suitable for universal nucleophilic biomarker radiolabeling. This instrument could be used in both research and clinical settings to make desired compounds on demand, or to optimize synthesis conditions of existing and novel probes by tuning process parameters.

The prototype is based on microfluidic chip technology, with chemical reactions taking place inside a low-volume (5µL) reactor. Compared to conventional systems, the chip enables the use of much higher concentrations of radiolabels such as $^{18}F^-$ ion (F-18), and thus theoretically much higher reaction yields. In addition, overall synthesis time of short-lived PET probes is decreased, due to higher reaction rates, and because the small reactor dimensions reduce the times required to add/mix reagents, change temperature, evaporate solvents, and elute the product.

2 MICROFLUIDIC CHIP

The microfluidic synthesis chip contains a closed "coin-shaped" reactor surrounded by six channels and valves for flow of reagents and product. The reactor can be heated to facilitate reactions, and solvents can be exchanged by evaporating across the gas-permeable membrane in the ceiling of the reactor then refilling with the next reagent in the new solvent. We describe elsewhere in detail the motivation for many aspects of the overall chip design [3].

Our latest generation chip is fabricated from three layers—a flexible "gasket layer" between a rigid lower "fluid layer" and a rigid upper "vent layer" (Figure 1). These layers are held together by bolts rather than chemical adhesion, an assembly strategy that simplifies fabrication, permits re-use of chip parts, and greatly increases the variety of materials that we could evaluate and optimize *in situ*. Replacement of PDMS with new materials has solved problems encountered in our earlier chip technology [3-6] that stemmed from the fundamental incompatibility of PDMS with organic solvents and reagents.

The fluid layer (Figure 2a) contains a central cylindrical depression (5mm diameter, 250µm deep) that serves as the reaction chamber, and six grooves (250µm wide, 250µm deep) that serve as fluid inlet and outlet channels. These

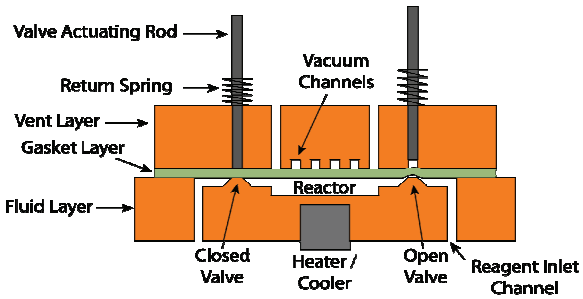

Figure 1: Cross-section of the microfluidic chip illustrating the main features in each layer and the architecture of the on-chip diaphragm valves.

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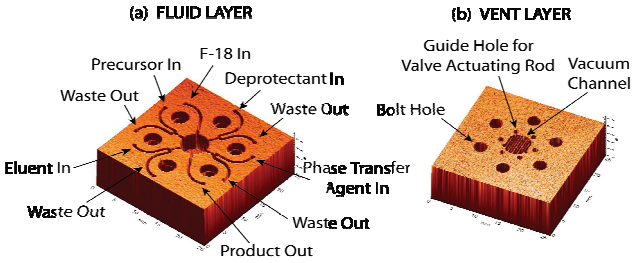

Figure 2: (a) Fluid layer and (b) vent layer imaged by an FRT MicroProf® profilometer (vertical dimensions exaggerated). The central reactor in the fluid layer is surrounded by six valve seats and microchannels, four of which have a T-junction (part of the priming mechanism). The vent layer contains a serpentine vacuum channel and holes to align the valve actuating rods on top of the valve seats. Also visible in both images are the six bolt holes used to hold the layers together.

channels each contain ramped "walls" near the reactor, constituting the valve seats for the on-chip diaphragm valves (two are visible in Figure 1). In addition, the fluidic layer contains a cylindrical counterbore directly beneath the reactor into which a heat-transfer rod is inserted to control the reactor temperature. Small ports are drilled from the outer end of each inlet/outlet channel through to the bottom surface of this layer, where individual O-rings will seal each port to the adapter interface in the instrument.

The gasket layer is a thin flat sheet of flexible material that serves three functions—it acts as a permeable gas-exchange membrane between the reactor in the fluid layer and the vacuum channel in the vent layer; it functions as the diaphragm in the on-chip microvalves, and it serves to seal the microchannels in the bottom and top layers (Figure 2).

The vent layer (Figure 2b) contains a rectangular groove (250µm wide, 1mm deep) following a serpentine path. During solvent evaporations, vacuum is applied to this channel to facilitate the removal of solvent vapors transported across the gas-exchange membrane.

The integrated diaphragm microvalves are closed by metal rods (1mm diameter), guided by holes in the vent layer, and driven by miniature pneumatic cylinders (FESTO EG2.5-10-PK-2). Six valves around the reactor control the flow of reagents and product, or can all be closed during heated reaction and evaporation steps.

2.1 Materials

Both the fluid and vent layers and adapter interface were fabricated from pDCPD, a rigid, transparent polymer developed by Materia Incorporated. This polymer has good machining properties, allowing rapid prototyping of intricate chip designs by CNC, including minute chamfers to remove sharp burrs from channels and valve seats.

The gasket layer must be both strong (for reliable valve operation) and gas-permeable (for rapid evaporation of H_2O and CH_3CN). Numerous materials were evaluated but best results were obtained with a layered composite of 25-30µm of polydimethylsiloxane (PDMS, Dow-Corning Sylgard® 184) on top of 15-20µm of an optimized perfluoropolyether [7] (Liquidia Technologies FluoroCur™), each cured separately after spin-coating. The PDMS is very permeable and provides strength; the FluoroCur™ is less permeable but protects PDMS against attack by F-18.

Extensive experiments confirmed resistance to solvents, stability above 140°C (peak temperature of reactions and evaporations), and compatibility with reactions. Less than 3% adsorption of F-18 (ion, intermediates, or product) onto pDCPD and less than 1% onto FluoroCur™ was measured.

3 INSTRUMENT DESIGN

3.1 Microfluidic Adapter Interface

To connect to the external (macroscopic) fluid handling system, the chip is installed on a plastic fluidic adapter manifold (Figure 3). Tubing connects to the adapter via threaded fittings and the chip seals to a flat surface of the adapter via a set of perfluoroelastomer O-rings, a connection designed to facilitate rapid chip installation.

The gap between macrofluidic and microfluidic volumes is bridged by a novel mechanism utilizing the on-chip microvalves and portions of the microfluidic inlet channels to allow rapid automatic priming of reagent lines up to the reactor (

Figure 4). Priming saves valuable time by eliminating the need to push large volumes of air trapped in the tubing through the gas-exchange membrane as each reagent is added by dead-end filling.

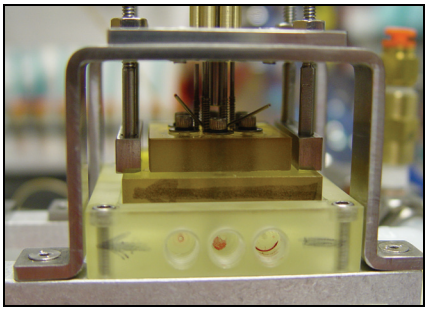

Figure 3: Photograph of microfluidic chip clamped to the adapter manifold to form a tight seal at the O-ring interface.

3.2 Temperature Control

An insulated metal heat transfer rod is inserted into the bottom of the microfluidic chip, the tip of the rod sitting only about 0.5mm below the reactor, to provide localized heating (Figure 5). The top portion of the rod contains a 50W Watlow FireRod® cartridge heater and a thermocouple, and the base of the rod contains a network of channels through which cold air can be supplied by the attached Exair vortex tube. Active heating and cooling elements are controlled by an Ogden ETR3400-4111115 temperature controller. This system can switch the rod

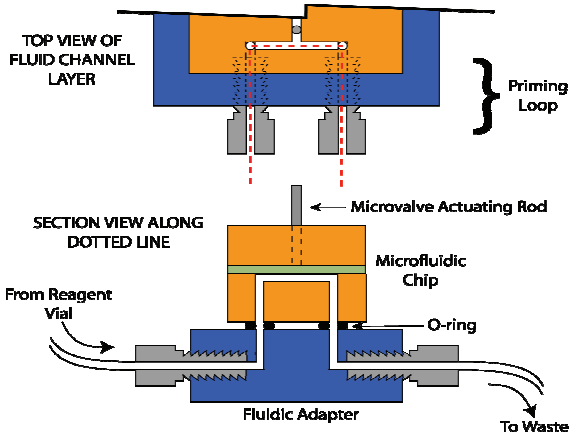

Figure 4: Operation of priming mechanism in the fluidic adapter interface. When the on-chip microvalve is closed, the reagent flows into the chip, through the "loop" and out to waste, allowing rapid elimination of any trapped air in the line. Once the line is primed, the reagent can be instantly delivered into the reactor by opening the on-chip microvalve and simultaneously closing a valve in the external waste line to provide a back-pressure. The connections to the chip and external fluidic system can be seen clearly in the lower figure.

temperature between room temperature and 140°C in about 45sec in either the heating or cooling direction. Experiments in which a thermocouple was placed inside the microreactor allowed us to calibrate the temperature lag between the tip of the heat transfer rod and the reactor.

3.3 Vacuum System

The two ends of the channel in the upper layer of the microfluidic chip are connected to a single-stage vacuum pump (KNF MPU953) and a pressure supply, each controlled by a 2-way valve. A charcoal trap in the vacuum line collects liquid and is mounted in a dose calibrator to monitor F-18 losses due to formation of volatile compounds during synthesis. An approximate reading of the pressure inside the vent channel is provided by an electronic gauge, allowing occlusions to be detected and evaporation progress to be monitored.

3.4 Fluidic System

The complete fluidic system is shown in Figure 6. Reagents are pre-loaded into vials containing a fluid delivery line extending to the bottom of the vial and a pressure delivery line in the headspace of the vial. Reagents are pushed through the system by pressurized nitrogen (typically 15-30psi) under the control of reagent pressure valves. Between each reagent vial and the chip adapter interface, the reagent line joins a selection valve that determines whether the reagent or a wash solvent is delivered to the chip. Reagents that use a priming loop have an associated waste line running out from the adapter interface to a common waste manifold and 2-way valve.

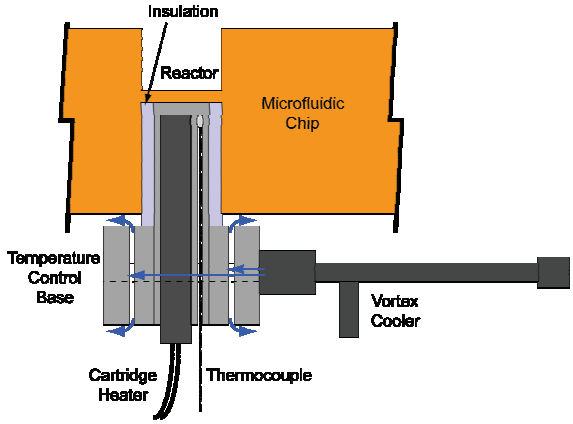

Figure 5: Schematic of chip heating and cooling system.

3.5 Radiation Shielding

Integrated shielding is a critical feature that makes this instrument independent from traditional hot cells and radiopharmacies. In our prototype, shielding consisted of a box built from 18 interlocking 0.565"-thick lead panels (2000lb total). The production instrument will have lead components minimized, shielding only critical components and detectors, thus making it lighter and more portable.

4 INSTRUMENT OPERATION

4.1 Synthesis Process

Synthesis of FDG was carried out according to the following process steps. The many parameters for each step (temperatures, heating times, flow times, etc.) can easily be adjusted to optimize the process.

1) **F-18 concentration.** Dilute F-18 is delivered from the cyclotron in 1.8mL of $[^{18}O]H_2O$ to the F-18 vial, then is flowed through an AG 1-X8 resin exchange column to trap F-18. Concentration by a factor of about 350x is achieved by eluting with 5µL of 0.5M K_2CO_3 directly into the microreactor through the F-18 inlet. By eluting with several plugs of 1-2µL volume separated by nitrogen, 79% of F-18 could be transferred. (We also tried eluting with a stream of K_2CO_3, but although most of the F-18 is released at the "front" of the stream, diffusion is so rapid that only about 5-15% of starting F-18 ended up in the 5µL reactor.) To achieve a low-volume fluid pathway between the K_2CO_3 vial and the chip, small diameter tubing and a miniature rotary injection valve (Rheodyne 9910-000) were used.

2) **Phase transfer.** Water is evaporated by closing all on-chip valves and heating the reactor to 105°C with the vacuum on. Next, the reactor is filled with Kryptofix 222 (K222, 50mg/mL in CH_3CN) to solvate F-18 in the organic phase. Next, the reactor is closed and heated to about 105°C to evaporate solvent. Note that all filling steps involve priming of the reagent line and opening of the on-chip valve while the reagent vial is pressurized. Typically, >10% of the reactor is left empty because the remaining gas bubble is a convenient monitor of evaporation progress.

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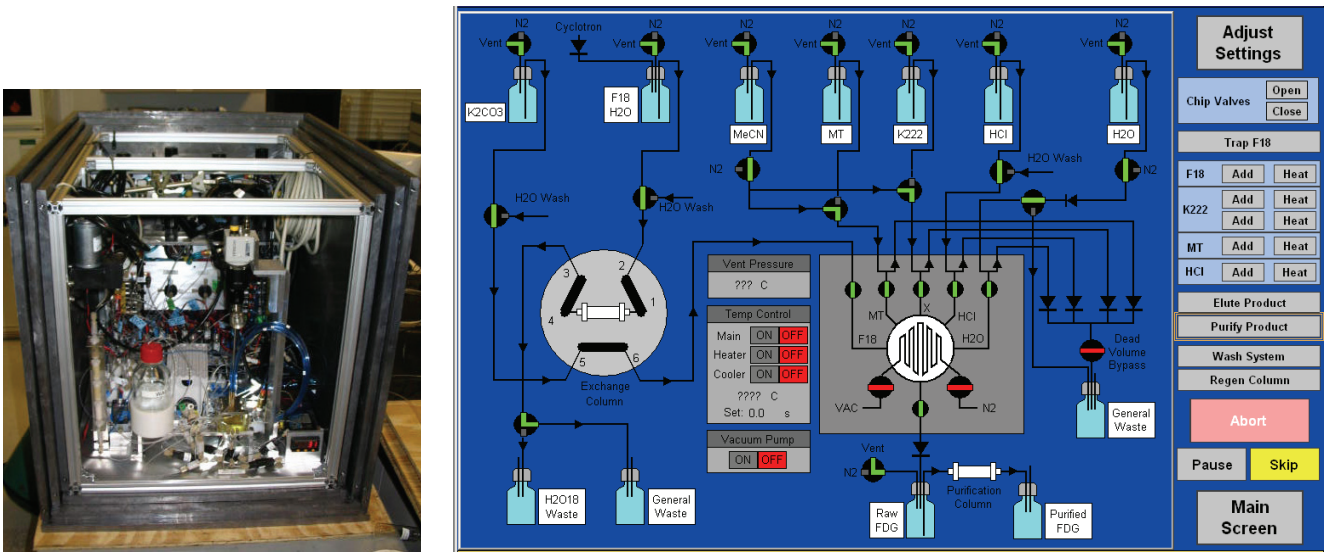

Figure 6: (Left) Photograph of operational instrument with microfluidic chip installed. The 18"x20"x24" system is shown partially enclosed in lead shielding. (Right) Graphical control interface illustrating the fluid pathways (black lines) that connect system components. Electronic valves are represented by black circles with red or green lines, and check valves are represented by diode symbols. The grey circle at the left is the rotary injector and the grey rectangle represents the microfluidic chip (six small circles are the on-chip valves). The interface allows manual operation by clicking on components to switch states, or automatic operation by selecting unit operations along the right side, each triggering a script to execute a sequence of steps.

3) **Fluorination reaction.** The precursor mannose triflate (25mg/mL in CH_3CN) is then introduced into the reactor where it mixes with F-18/K222 and is reacted at 105°C to form the intermediate fluoro-1,3,4,6-tetra-O-acetyl-D-glucose (FTAG). Heating continues until about 65% of the solvent has evaporated.

4) **Deprotection reaction.** 1M HCl is then added to the reactor to deprotect the FTAG and yield FDG. After filling the reactor, the contents are heated to 105°C for 10 min. As in other steps, HCl is introduced while there is still CH_3CN from the previous step left in the reactor in order to facilitate improved dissolution and mixing.

5) **Elute product from reactor.** The FDG inside the reactor is eluted with 10-15 mL water into a collection vial, optionally passing through a purification column.

After synthesis, all lines can be washed and dried to avoid cross-contamination on subsequent runs or to avoid dripping on the O-ring interface when chips are exchanged.

4.2 Automation and Computer Control

The prototype is operated by a PC-104 based controller connected to a PC running our custom control software, written in FIX32. The software can access individual digital outputs (2-way and 3-way valves, on-chip valves, temperature controller enable, heater enable, cooler enable, vacuum enable, rotary injector) and analog outputs (temperature setpoint). Analog inputs (reactor temperature, vent channel pressure, radiation levels) are scaled to engineering units for monitoring on the main screen.

In addition to the interactive graphical interface in Figure 6, dozens of scripts were written to automate the process steps described in the previous section. Each subprogram performs a sequence of simple operations such as changing the state of a valve, waiting for a fixed amount of time, or waiting for particular value of an input (e.g. heating until the reactor reaches a specified temperature).

5 RESULTS AND CONCLUSIONS

We have designed, constructed, and demonstrated an instrument for automated radiopharmaceutical synthesis. The PET probe FDG was synthesized at scales up to 16mCi (sufficient for a human dose). This stand-alone system was fully automated, in principle allowing synthesis of a desired biomarker on demand with the push of a single button. Using similar microfluidic technology we have also demonstrated the synthesis of 2-(1,1-dicyanopropen-2-yl)-6-(2-[^{18}F]fluoroethyl)-methylamino)-naphthalene (FDDNP) and 3'-[^{18}F]fluoro-3'-deoxy-L-thymidine (FLT).

This instrument is being commercialized by Siemens Molecular Imaging and is expected to help researchers to develop new probes and to enable wider deployment of these probes by allowing for on-site synthesis.

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