Coin-Shaped Reactor in Microfluidic Devices Used for Radiopharmaceutical Synthesis

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ABSTRACT

A microfluidic device has been designed and optimized for the multi-step synthesis of PET (Positron Emisssion Tomography) probes. Its PDMS elastomer-based architecture centered around a (5 μL) coin-shaped reactor has novel features that facilitate mixing, solvent exchange, product collection and overall synthetic efficiency. New microfluidic properties and processes have been demonstrated in this reactor. Novel mechanisms, such as chemically-assisted mixing and vacuum evaporation across a membrane are utilized. The device presented here, the size of a penny, is the first device in its class to produce radiopharmaceuticals of sufficient quality and quantity (over 2 mCi) to be validated by *in vivo* imaging. It demonstrates the potential to produce multiple human doses of radio-pharmaceutials, such as ^{18}FDG ([^{18}F]2-fluoro-2-deoxy-D-glucose) in the near future.

Keywords: microfluidics, radiopharmaceutical, PDMS, microreactor

1 INTRODUCTION

Microfluidic devices have demonstrated significant advantages over large-scale reactors in a number of applications[1]. Use of microfluidics in radiopharmaceutical synthesis[2] is beneficial for several reasons – speed, automation, efficiency and the need to handle small quantities of material are among them.

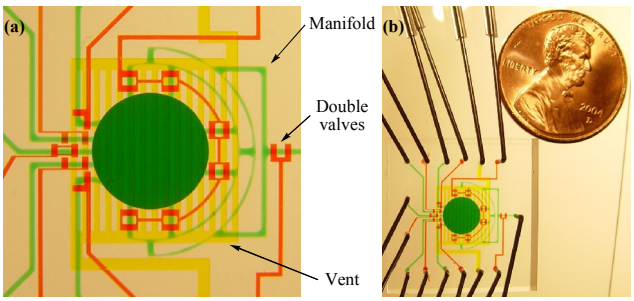
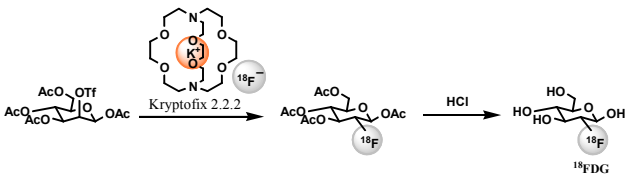


Figure 1: Core of the "coin-shaped reactor" chip (a) and full chip next to a penny (b), filled with food dyes with flow channels in green, valves in red and vacuum vent in yellow.

Positron Emission Tomography[3] (PET) utilizes biomarkers incorporating short-lived isotopes such as ^{18}F ($t_{1/2}$=110 min). Therefore, the number of useful probes is severely limited to the simplest ones that can be synthesized, purified and delivered to the patient in a clinic (from the radio-pharmacy) within a very short period of time. A microfluidic device used in such an application can not only carry out several synthetic steps much more rapidly and efficiently than is possible in a macroscopic lab, but also (being small and portable) can be operated in proximity to any PET-scanner eliminating the delivery time wasted en route from the pharmacy to the clinic. It is also important in radio-synthesis that the device can be operated remotely (or even fully automated) without the need for physical interaction on the part of the technician, allowing it to be completely shielded and minimizing the exposure of personnel to radiation. Since ^{18}F has a very high specific activity (1.71 x 10^9 mCi/mmol), only nanogram quantities of it are needed even for human imaging. Such amount can be easily yielded by a device presented here (Figure 1) with a "coin-shaped reactor". It can carry out multi-step syntheses since the elastomer properties and the chip design can create and support different conditions in the same reaction chamber during distinct steps of the synthetic process.



Sceme 1: Synthesis of ^{18}FDG.

For demonstration purposes ^{18}FDG (2-[^{18}F]fluoro-2-deoxy-D-glucose[4], a cancer biomarker) was chosen because it is the most widely used PET probe, whose synthesis (Scheme 1) involves several steps, and can be easily compared in its efficiency to a well-optimized macroscopic commercial process.[5] The latter requires an entire laboratory and introduces dilution factors which are necessary only for material handling rather than for the chemistry. The microfluidic device presented here has had its ^{18}FDG synthesis validated[6] with a mouse imaging[7] experiment and has been used for synthesis of other biomarkers. Its universal nature and ease of modification

make it valuable not only for convenient on-demand supply of commercial molecular probes, but also for research and development of new ones.

2 EXPERIMENTAL

2.1 Design

Elasticity and gas permeability of PDMS (polydimethylsiloxane)[8] allow several important features to be realized in devices fabricated from it. The "coin-shaped reactor" utilized in this microfluidic chip has been designed to handle multi-step syntheses with high efficiency.

The "coin-shaped reactor" chip outlined in Figure 2 has the following dimensions. The reactor is a cylinder 5 mm in diameter and 250 μm tall. The radiator vent is composed of 250x100 μm channels separated from the reactor by a 100 μm membrane. Fluid channels are 250 μm wide by 45 μm tall with curved top surfaces (except for the eluent entrance channel, which is 300 μm wide). Push-up valves are used with cross section areas of 250x250 μm with 25 μm membranes. The device was fabricated using three different molds for the control, flow, and vent layers.[9]

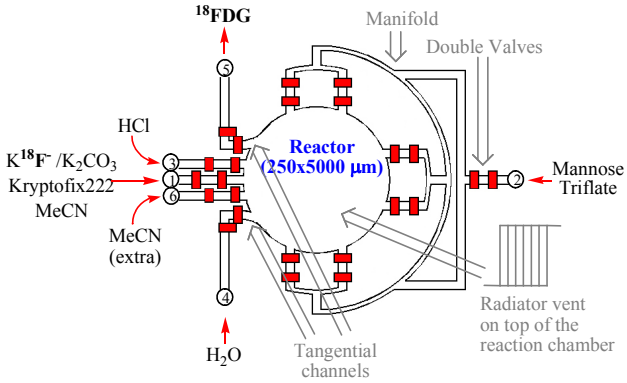


Figure 2: The core architecture of the "coin-shaped reactor" chip. The channels are labeled by the numbers in order of their use in ^{18}FDG synthesis.

The shape of the reactor has been determined by several requirements. Since solvent exchange is required for the synthesis, rapid evaporation calls for a large surface area achieved by the flat reactor, separated by a gas-permeable membrane from the vent, to which vacuum can be applied. Vacuum evaporation done in such a manner takes place rapidly and the vapors are removed from the chip instead of staying in the elastomer matrix. In case of water vapors, residual gas could easily quench moisture sensitive reactions following aqueous ones. The optimal volume of the reactor has been calculated to be 5 μL based on the fluoride concentration available and the goal to yield over 100 mCi of product (10 human doses). The flat cylindrical shape of the reactor enables efficient elution of products and facilitates reagent mixing in several steps. The reactor can deform when full vacuum is applied to the vent above it and pull solutions inward when restoring its shape after one of the valves is opened. Rapid fluid entrance allows inertia to dominate over diffusion in the mixing with the solution already inside the reactor making it very efficient. Mixing is also facilitated by the use of a 6-channel manifold for mannose triflate, which allows simultaneous introduction of reagent from all ports by having all of them equidistant from the first splitting point and using two sets of valves, one for filling the manifold and the second for filling the reactor.

Elution efficiency is achieved by tuning two parameters: angle and speed of fluid entrance and exit. Both of these factors have been studied by eluting fluorophore solutions out of the reactor with pure water and recording the time and volume of eluent necessary to reduce fluorescence by 95%. We have demonstrated that when entrance and exit channels tangential to the reactor circumference are used, significantly less eluent is needed than when the channels are perpendicular to the circumference. (At 30 psi fluid pressure tangential elution took 88 μL of eluent and 3.7 s, while perpendicular elution required 256 μL and 27 s.) As for the flow, it has been demonstrated that while high rates are required for fastest elutions, lower rates result in lower amounts of eluent needed. The pattern of elution at low pressure is different from that of a fast elution. In the former case the fluid takes a shortcut from entrance to exit which gradually grows in volume washing out the rest of the chamber, while in the latter case inertia allows the entering fluid to follow a trajectory of a wave along the far wall of the reactor, with the middle clearing last. The improvement on time with fast elutions was more significant than that on volume with slow ones. Tangential elutions at 5 psi (73 μL, 53 s) and 30 psi (88 μL, 4 s) fluid pressure suggest that during synthesis operation a little volume efficiency may easily be sacrificed for a several-fold gain on speed. Since elution is the last step of the reactor cycle, a dilution can be tolerated, while use of short-lived radio-isotopes makes time one of the most critical aspects.

Pneumatic valves have been well characterized[10] in a number of applications. Since the chip presented here involves evaporation of relatively large volumes of solvent, the valves have to withstand extra vapor pressure. Introduction of backup valves allows the pressure to equilibrate quickly on both sides of the first valve, enabling it to respond to sudden pressure changes without de-pressurizing the reactor.

2.2 Operation

The synthesis of ^{18}FDG involves two reactions outlined in Scheme 1 – fluorination of mannose triflate followed by hydrolysis of the [^{18}F]TAG intermediate to ^{18}FDG. The number of processes involved is eight (Figure 3): fluoride filling, chamber contraction, manifold filling, triflate introduction into reactor/fluorination reaction, partial

MeCN evaporation, HCl introduction/mixing, MeCN removal, and elution.

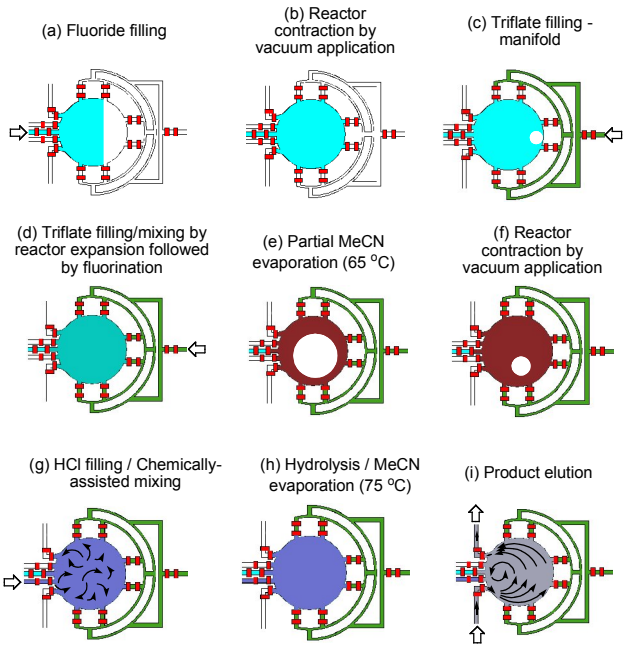


Figure 3: Steps of the "coin-shaped reactor" chip operation involved in ^{18}FDG sysnthesis.

The layout of the microfluidic chip (Figure 2) allows performing all steps in the same “coin-shaped reactor”. All solutions are introduced with 15 psi back pressure, while the valves are actuated by 40 psi. As the solution of $^{18}F^-/K_2CO_3$/Kryptofix 2.2.2 in MeCN[11] enters the reactor through channel 1, all valves are closed except for the one on that channel (Figure 3a). The gas displaced by the liquid is removed across the gas-permeable membrane by the application of vacuum inside the vent over the top of the reactor. As the fluoride solution fills ⅔ of the reactor, the valve on channel 1 is closed, and the vacuum is allowed to pump the remaining gas out of the reactor since the elastomer allows the reactor to cave in and shrink in volume (Figure 3b). At the same time, mannose triflate is brought through channel 2 into the manifold which is first filled up to the second set of valves surrounding the reactor by displacing the gas inside the channels into the PDMS matrix (c). Once it is free of gas the last set of valves (controlled by one input) is opened and the triflate enters the reactor (d). Its entrance is accelerated by the expansion of the reactor (squeezed earlier by application of vacuum to it) as the elastomer restores its original shape. Although efficient microfluidic flow-through mixing mechanisms have been suggested for this reaction recently,[12] rapid introduction of the reagent into the coin-shaped reactor from 6 directions simultaneously assures instantaneous mixing of two solutions inside a closed space. The height of the chamber being 250 μm allows such mixing to proceed and eliminates the need for a stirring mechanism. After the reactor is full, all valves are closed and the chip is heated to 65 °C (e) to accelerate the reaction of mannose triflate with $^{18}F^-$ forming intermediate tetraacetylated [^{18}F]fluoro-glucose compound ([^{18}F]TAG). Since some solvent needs to be removed to create space in the reactor for the acid in the next step, the vacuum is applied in the vent. It allows faster solvent evaporation and reduces the vapor pressure that is exerted on the closed valves and that could lead to their failure. In this step again the chamber collapses inward and reduces its volume upon application of vacuum (f). When about ½ of the reactor is empty, the chip is cooled and 3M HCl is introduced through channel 3 (g). As it enters the reactor, it engages in a vigorous acid/base reaction with the MeCN solution of [^{18}F]TAG and K_2CO_3 on contact. This reaction results in swirling of two solutions at their interface, which leads to their rapid mixing (chemically-assisted). If acetonitrile is evaporated completely in the previous step, it becomes very difficult to dissolve the [^{18}F]TAG residue in aqueous HCl even at elevated temperatures, resulting in low yield of hydrolysis and longer reaction time. Acid pressure is left on, the valve on channel 3 remains open while the heat and vacuum are still on – this creates an environment where acetonitrile continues to evaporate out of the MeCN/H_2O mixture as the acid keeps coming in to occupy the vacated space. After the reagents are mixed (as can be clearly seen from the disappearance of the interface and stopping of the swirling) all valves are closed again and the reactor is heated for another 2 min at 75 °C to remove the remaining acetonitrile and allow hydrolysis of [^{18}F]TAG to ^{18}FDG to proceed to completion (h). Upon cooling to ambient temperature, the contents are eluted into tangential channel 5 with minimal volume of water introduced through the tangential channel 4 (i).

2.3 Analysis

As the solution exits, it is collected and analyzed by radioactive TLC. The radiochemical purity of 96% has been observed in eluted ^{18}FDG. While such purity is sufficient for imaging, an extra purification has been performed. The acidity is quenched by a 0.5 M $NaHCO_3$ solution present in the collection vial, since the dose has to be neutral for *in vivo* application. Afterwards the collection vial is pressurized (through the chip) and the contents exit via an alumina column, which traps excess $^{18}F^-$ and other impurities. ^{18}FDG purified in this manner exhibited 99.3% purity (Figure 4a), and was used in mouse imaging experiments, which revealed presence of tumors (Figure 4b). Upon both process and architecture optimization, the chip has been brought to a point where it yielded over 2 mCi of ^{18}FDG repeatably with run times around 20 min.

However, an intrinsic property of PDMS did not allow further increase in output. Mechanical properties of this elastomer such as gas permeability and elasticity are perfect for the application in this case, but we have discovered that a significant amount of $^{18}F^-$ is lost in its reaction with

PDMS yielding volatile fluoro-silane products, which were quantified by placing a charcoal trap in the vacuum line.

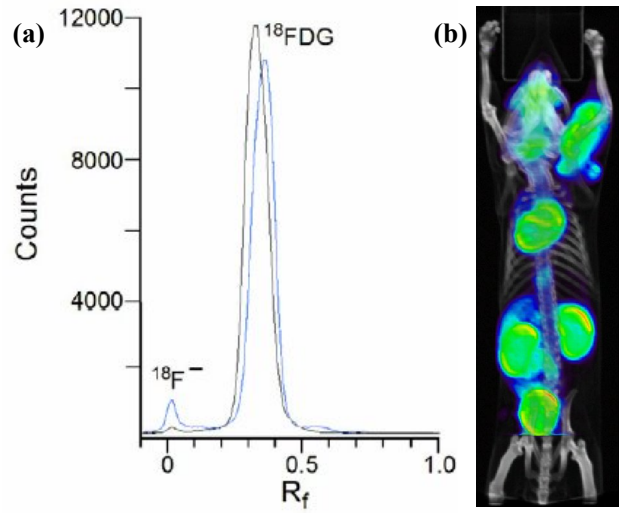


Figure 4. (a) Radio-TLC traces of chip-based ^{18}FDG as eluted (96%) and purified (>99%). (b) Validation of the chip-based ^{18}FDG synthesis by in vivo microPET imaging. (The tumor is located on the right shoulder of the mouse.)

3 CONCLUSIONS

This work has demonstrated a "coin-shaped reactor" chip is capable of carrying out a process, occupying an entire lab in a rapid and efficient manner. Its universal applicability has been illustrated by the synthesis of another biomarker – 2-(1-{6-[(2-[18F]fluoroethyl)(methyl)amino]-2-napthyl}ethylidene)malonitrile (FDDNP)[13] used for Alzheimer's disease detection.

The application of such chips in commercial biomarker synthesis and in research is awaiting the development of a new elastomer which, while preserving the mechanical properties of PDMS would not engage in reaction with ^{18}F$^-$.[14] Otherwise, the design of the chip has been proven appropriate for multi-step synthesis of radio-pharmaceuticals. It has been demonstrated that moisture-sensitive reactions can be carried out inside this chip following aqueous ones, where the water has been removed by the application of a vacuum vent. This demonstrates that much more complex syntheses than the one used for ^{18}FDG production can be performed on the coin-shaped reactor chip, suggesting that potent biomarkers developed, but not used in the clinic because of length of synthesis, can start being evaluated. Compact nature and automation of the device should allow it to be used in the clinic where PET scans are being performed (with proper shielding). In the research applications operating in a manual mode, a variety of syntheses involving more steps can be performed using the "coin-shaped reactor" chip. (Channels can be easily added to the chip layout for longer synthetic processes.)

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