EWOD MICRODEVICES FOR SYNTHESIS OF ^{18}F -LABELED TRACERS FOR POSITRON EMISSION TOMOGRAPHY (PET)

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ABSTRACT

We demonstrate the drying (i.e., removing water content) of cyclotron-produced ^{18}F fluoride solutions and synthesis of 2- ^{18}F fluoro-2-deoxy-D-glucose (^{18}F FDG) and 1- ^{18}F fluoro-4-nitrobenzene (^{18}F FNB) as example tracers on an electrowetting-on-dielectric (EWOD) platform using multifunctional electrodes (for heating, temperature sensing, and EWOD driving). This is an important step toward the development of an affordable, universal synthesizer for imaging tracers used in Positron Emission Tomography (PET).

INTRODUCTION

Positron Emission Tomography (PET) is a highly sensitive type of noninvasive functional medical imaging for specific and quantitative measurement of biochemical activity in patients for diagnosis of cancer or Alzheimer's, monitoring of cancer treatment, visualization of immune responses, and many other clinical and research applications [1], [2]. It requires the administration of a chemical tracer labeled with a positron-emitting isotope, such as fluorine-18 (^{18}F). We report the development of a droplet microfluidic device for synthesis of ^{18}F -labeled PET tracers, based on electrowetting-on-dielectric (EWOD) technology enhanced with localized on-chip heating capability.

Microfluidics in general is attractive for PET tracer synthesis, because shorter reaction times reduce radioisotope decay and typical radiolabeling reactions produce only nanograms of tracer [3]. However, EWOD presents additional advantages: (1) With no tubes and discrete pumps, the overall system is simple and compact, easier to clean, and more reliable [4]. (2) Microfluidic sequences are configured on a generic chip electronically (i.e., no hardware adjustment needed), allowing synthesis of a variety of tracers with one system. This is a stark contrast to the current state of the art, in which system setup must be adjusted for different types of probe synthesis. (3) Our chip is made open to operate in air (i.e., not oil-filled), enabling effective evaporations for exchange of solvents, which is critical for many radiosyntheses that are water-sensitive but begin with cyclotron-produced ^{18}F fluoride in ^{18}O H₂O. (4) The chip surface is more chemically inert and thermally stable (vs. PDMS, polycarbonate). The resulting system enjoys enormous reductions in the size and weight of radiation shielding (typically lead), and cost (below \$5K expected vs. the conventional \$50K-\$250K). Currently, although over 1400 PET labeling compounds have been published in literature [5], only several are available to imaging centers by commercial radiopharmacies. As an affordable and flexible synthesizer, this chip would empower final users to produce tracers of their choice locally on-demand, eventually making PET imaging as readily available as CT and MRI.

We have demonstrated the drying (i.e., removing water content) of ^{18}F fluoride solutions – the most critical step in synthesis of most ^{18}F -labeled radiotracers – using an EWOD chip with multifunctional electrodes, which allow heating and temperature sensing as well as the electrowetting actuation. Two ^{18}F -labeled radiotracers were reliably synthesized. The first, 1- ^{18}F fluoro-4-nitrobenzene (^{18}F FNB), is used to label various

antibodies and peptides. The second, 2- ^{18}F fluoro-2-deoxy-D-glucose (^{18}F FDG), is a labeled-sugar that is the most important PET tracer and commonly used in the diagnosis and monitoring of cancer [6].

DEVICE AND EXPERIMENTS

EWOD Device Fabrication

Assisted by multifunctional (heating, temperature sensing, and EWOD driving) electrodes developed for MADLI sample preparation [7] our new device performs drying and reaction steps for radioactive tracer production. Instead of the etched hydrophilic patterns of [7], we used a circular resistor pattern enclosed by the EWOD electrode (Figure 1c) for centering residue from evaporating droplets. Hydrophilic patterns were avoided because, unlike the MALDI case, droplets must be moved after reactions are completed. Many radiolabeling reactions require low vapor pressure solvents, which presented the main challenge. These solvents could not be stored in a sealed device due to the need to remove water during evaporation steps. Instead, solvents were loaded in the EWOD chip at their time of use.

Figure 1: EWOD chip for probe synthesis. (a) with cover plate on, (b) device layout, and (c) heater layout.

Device fabrication is shown in Figure 2. 700 μm thick glass wafers coated with 140 nm ITO (TechGophers Inc.) were covered with 20/200 nm of evaporated Cr/Au. Gold and ITO layers were etched to form heaters, EWOD electrodes, connection lines, and contact pads. 1 μm PECVD silicon nitride was deposited as a dielectric, and 1 μm of Cytop® was spin-coated and annealed at 200 $^{\circ}\text{C}$ to make the surface hydrophobic. A cover plate was prepared from 700 μm thick glass coated with 150 nm ITO (Delta Technologies Inc.) to serve as a ground electrode for electrowetting. The cover plate was coated with thinner layers of PECVD silicon

nitride (100 nm) and Cytop® (100 nm). The cover plate was spaced and bonded to the EWOD substrate using 100 μ m thick double-sided tape (3M Inc.).

Figure 2: Process flow to fabricate EWOD substrate.

Solution Preparation

Acetonitrile (MeCN), anhydrous dimethyl sulfoxide (DMSO), Kryptofix 2.2.2, mannose triflate, potassium carbonate (K_2CO_3) and hydrochloric acid (HCl) were purchased from Sigma-Aldrich, and 1,4-dinitrobenzene was purchased from Acros Organics. No-carrier-added [^{18}F]fluoride ion, obtained from the UCLA Cyclotron and Radiochemistry Center, was produced in an RDS-112 cyclotron (Siemens). Aqueous [^{18}F]fluoride ion was treated with K_2CO_3 and Kryptofix 2.2.2, a phase-transfer agent to a final concentration of 56 mM K_2CO_3 , 112 mM Kryptofix 2.2.2 in MeCN and water (5:1 v/v). For preparation of [^{18}F]FNB, a solution of 1,4-dinitrobenzene (20 mg/mL) was used. For preparation of [^{18}F]FDG, solutions of mannose triflate (50 mg/mL) and HCl (1N) were used. For radio-TLC running buffers for [^{18}F]FNB and [^{18}F]FDG, we used dichloromethane/hexane (1:1 v/v) and MeCN/water (95:5 v/v), respectively.

In addition, solvents commonly used for radiotracer synthesis were tested for EWOD compatibility. Repeatable movement, splitting, and merging of droplets was accomplished with MeCN, DMSO, DMF, MeOH, K_2CO_3 (1 M), HCl (1 N), and NaOH (1 N) with voltages below 90 V_{rms} and frequencies ranging 0-20 kHz.

Device Actuation

Multifunctional electrodes were connected to a switch to alternate between EWOD actuation and temperature control. Voltage and current applied to the electrodes were controlled by a LabVIEW program, which sequenced EWOD actuation using a digital I/O device (DAQPad 6507, National Instruments) and performed PID control over heating with a DC source meter (Model 2400, Keithley Instruments).

RESULTS AND DISCUSSION

Synthesis of 1-[^{18}F]fluoro-4-nitrobenzene ([^{18}F]FNB)

Figure 3 illustrates radiosynthesis of [^{18}F]FNB. It is produced by a one-step reaction of dried [^{18}F]fluoride with the precursor

dinitrobenzene in DMSO. On chip, this is accomplished by loading a water/MeCN droplet with ^{18}F , K_2CO_3 , and Kryptofix. The droplet is moved by EWOD to a heater electrode (Figure 4a) and heated at 105°C for 3 minutes (Figure 4b). Next, an azeotropic drying step is repeated 3 times, in which an MeCN droplet is loaded, moved to the heater electrode (Figure 4c), and heated at 105°C for 3 minutes (Figure 4d). A droplet of dinitrobenzene in DMSO is then loaded and moved to the same heater along a different pathway to avoid cross-contamination (Figure 4e). The droplet is subsequently heated to 120°C for 3 minutes for radiolabeling reaction (Figure 4f).

Figure 3: Synthesis of 1-[^{18}F]fluoro-4-nitrobenzene.

Figure 4: Synthesis process to produce [^{18}F]FNB on EWOD: (a) loading and movement of ^{18}F droplet, (b) heating of ^{18}F droplet (105°C, 3 min), (c) loading and movement of acetonitrile, (d) heating of acetonitrile (105°C, 3 min), (e) loading of dinitrobenzene droplet, (f) heating of dinitrobenzene droplet (120°C, 3 min).

Probe synthesis on EWOD device is analyzed by removing the cover plate and transferring the product droplet with a capillary onto a thin-layer chromatography (TLC) plate. [^{18}F]FNB is separated from free fluoride by TLC with a running buffer of 90% MeCN and 10% H_2O . Composition of radioactive components is then measured with a radio-TLC scanner (miniGITA Star, Raytest). Conversion to desired product, [^{18}F]FNB, averaged $78 \pm 12\%$ ($n=8$ runs) with results ranging as high as 96% as shown in Figure 5.

Figure 5: Radio-TLC results showing product purity after on-chip drying and reaction to produce [^{18}F]FNB. The left peak represents unbound [^{18}F]fluoride. The right peak represents the desired product, [^{18}F]FNB.

Synthesis of 2- ^{18}F fluoro-2-deoxy-D-glucose ([^{18}F]FDG)

Figure 6 illustrates radiosynthesis of [^{18}F]FDG, a two-step reaction. The first step is a fluorination reaction in which the precursor manose triflate in MeCN is labeled with dried [^{18}F]fluoride to produce a fluorinated intermediate, 2- ^{18}F fluoro-1,3,4,6-tetra-O-acetyl-d-glucose ([^{18}F]FTAG). The second step is a deprotection reaction in which HCl is added to remove the acetyl protecting groups.

Figure 6: Synthesis of 2- ^{18}F fluoro-2-deoxy-D-glucose ([^{18}F]FDG).

The [^{18}F]FDG synthesis process using EWOD is shown in Figure 7. After loading of fluoride, azeotropic drying, shown in Figure 7b, is repeated 6 times using acetonitrile to ensure residual water will not interfere with fluorination reaction. Fluorination reaction is accomplished by loading a precursor droplet of mannose triflate in acetonitrile. The precursor droplet is moved to the fluoride and heated to 85°C for 5 minutes (Figure 7c). During the fluorination reaction, more acetonitrile droplets are added to maintain a liquid phase during reaction. Fluorination is followed by solvent evaporation and hydrolysis (Figure 7d). A $1\ \mu\text{L}$ droplet of 1 N HCl was added 3 times over the 5 minute deprotection reaction to replenish the droplet as it evaporated. If a more constant acid concentration is desired, water droplets can be added instead.

Figure 7: Synthesis process on EWOD: (a) loading of ^{18}F droplet at cover plate edge, droplet movement to heating site, and evaporation of ^{18}F droplet by resistive heating (105°C , 3 min), (b) loading of acetonitrile droplet at cover plate edge, droplet movement to heating site, and evaporation by heating (105°C , 3 min), (c) loading of mannose triflate droplet at cover plate edge, droplet movement to heating site, and heating for radiolabeling reaction (85°C , 5 min), (d) loading of HCl droplet at cover plate edge, droplet movement to heating site, and heating of droplet to remove protecting groups (95°C , 5 min).

Product was recovered with a capillary after removing the cover plate, and ^{18}F -labeled compounds were separated by TLC and measured with a radio-TLC as was done for analysis of [^{18}F]FNB synthesis. As shown by the example result in Figure 8, conversion to desired product was high (84.9%).

Figure 8: Radio-TLC results showing crude product purity after on-chip drying and reaction to produce [^{18}F]FDG. The left peak represents unreacted [^{18}F]fluoride. The second peak from the left shows 84.9% of the radioactivity incorporated into the desired product, [^{18}F]FDG. The rightmost peak represents the intermediate compound. The third peak from the left represents side products from incomplete hydrolysis.

CONCLUSION

We have demonstrated the synthesis of 1- ^{18}F fluoro-4-nitrobenzene (^{18}F FNB) and 2- ^{18}F fluoro-2-deoxy-D-glucose (^{18}F FDG) from cyclotron-produced ^{18}F radioisotope solutions using an EWOD device with multifunctional electrodes for heating. Greater quantities of radioactive tracers can be produced for imaging by handling larger concentrations of ^{18}F droplets or running processes in parallel. Future work will be towards automating droplet loading to the chip and developing a more generic synthesizer EWOD chip for the production of additional PET tracers.

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