PLANAR ALUMINA PURIFICATION OF ^{18}F-LABELED RADIOTRACER SYNTHESIS ON EWOD CHIP FOR POSITRON EMISSION TOMOGRAPHY (PET)

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ABSTRACT

We present planar alumina as a solid phase extraction surface for purification steps of on-chip chemical synthesis. Droplet mixing, by electrowetting and resistive heating, induced sufficient surface interaction with alumina to remove [^{18}F]fluoride ion from a droplet. This mechanism was used for purification of [^{18}F]fallypride (a radiolabeled neurotransmitter for studying Alzheimer's disease with positron emission tomography) after it was produced on chip.

KEYWORDS

Electrowetting, Digital Microfluidics, PET, Radiotracer, Alumina Anodization, Fluoride Removal.

INTRODUCTION

Positron emission tomography (PET) is a highly sensitive type of functional medical imaging that requires administration of a positron-emitting tracer with biological activity. The tracer used establishes the PET imaging application, which can include studies of disease mechanisms, early disease diagnosis, monitoring therapy response, and developing therapeutics [1]. Previously, we demonstrated syntheses of various PET tracers (analogs of sugar, DNA nucleoside, neurotransmitter, and protein-labeling agent) on an electrowetting-on-dielectric (EWOD) chip, but purification steps were performed off chip [2,3]. Motivated towards complete radiosynthesis-on-a-chip, in this report we develop extraction of excess [^{18}F]fluoride on the same chip after tracer synthesis reactions. This is an important purification step to prevent image quality from being diminished by [^{18}F]fluoride uptake in bone.

[^{18}F]fluoride is an ideal radioisotope for PET because of its ability to replace hydrogen or oxygen of a biomolecule. Its intermediate 109.8 minute half-life allows a radiotracer to be produced and transported to nearby imaging sites, but is also short enough to avoid long ionizing radiation exposure to patients or laboratory animals. However, any leftover [^{18}F]fluoride ion from a typical tracer synthesis formulated and injected into a living subject is likely to be taken up into bone, causing a high background signal and complicating interpretations of images [4].

Removal of the excess [^{18}F]fluoride ion after a typical radiotracer synthesis is conventionally performed by flowing the crude solution through a purification column packed with alumina [2,5]. In order to ensure enough surface interaction to trap the free [^{18}F]fluoride, the column must have sufficient amount of alumina, which requires additional solvent volume to separate the radiolabeled probe from the unreacted [18F]fluoride ion with high purification efficiency. Furthermore, the conventional method necessitates additional hardware to flow the crude mixture and solvent through the cartridge, which increases the overall footprint and complexity of the synthesizer. Here we introduce alumina as the dielectric layer of the EWOD cover plate as well as for extraction of the fluoride ion leftover after radiosynthesis on the EWOD chip. An exposed window in the Teflon® coating of the alumina provides sufficient surface area to extract fluoride ion from the sample squeezed within a 150 μm plate gap by a combination of EWOD mixing and resistive heating.

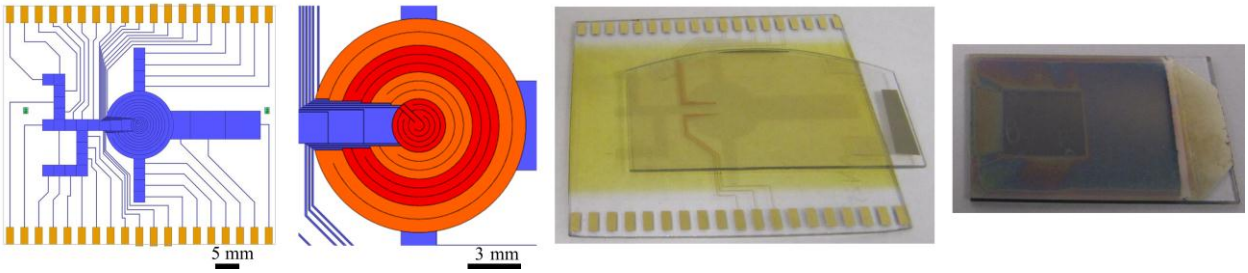

Figure 1: EWOD chip for tracer synthesis and purification. (a) device layout with multifunctional ITO electrodes (blue) and gold contacts (yellow), (b) the reaction site zoomed in to show individually controlled 4 concentric resistive heating rings, (c) assembled EWOD device with ITO/nitride/Teflon®-coated cover plate, (d) cover plate with a window to expose the alumina under Teflon®.

EXPERIMENTAL

Both the substrate (device) and top (cover) plate of our parallel-plate EWOD chip were fabricated from 700 μm-thick glass wafers coated with 140 nm indium tin oxide (ITO) (Semiconductor Solutions). The ITO on the device plate was patterned to form multifunctional electrodes for heating, temperature sensing, and EWOD driving (Figure 1) [2]. The device plate was further evaporated and patterned with chrome (20 nm) and gold (200 nm) to form EWOD electrodes, connection lines, and contact pads. Silicon nitride was deposited by PECVD, thick (2 μm)

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on the device plate and thin (100 nm) on the cover plate, to form the main dielectric for each plate. Teflon® (250 nm) was spin-coated and annealed at 340˚C under vacuum to make the surfaces hydrophobic.

The purification cover plate was fabricated by evaporating aluminum (65 nm) onto glass slides. Nonporous alumina was grown by anodization with a constant current step (1 mA, 20 min) and a constant voltage step (60 V, 1 hr). A conductive but transparent 15 nm aluminum layer was left beneath the grown alumina (80 nm) (Figure 2). Bake steps (500˚C, 3 hours) were used before and after anodization to induce γ'-Al_2O_3 crystalline formation [6]. Teflon® (Teflon AF 1600) was spin-coated and patterned by lift-off of low-tack tape to open a window for the underlying alumina. Repeatable movement of droplets on alumina-exposed regions was accomplished with water, acetonitrile, dimethyl sulfoxide, and methanol solutions using 90 $V_{rms,10\ kHz}$.

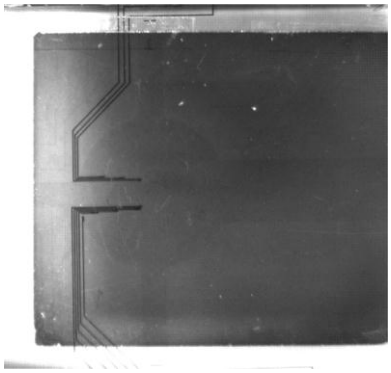

Figure 2: Video image of exposed alumina cover plate assembled over EWOD plate. The 15 nm aluminum electrical ground layer is transparent.

Table 1: Adsorption of fluoride ion on various cover-plate surfaces.

Sample	% Radioactivity on cover plate		
	No mixing or heating	*EWOD mixing, no heating*	*EWOD mixing + heating*
Alumina (baked 6 hrs at 500˚C), after extraction	61%	95%	97%
Alumina (baked 6 hrs at 500˚C), after water wash	61%	81%	94%
Alumina (baked 3 hrs at 500˚C)	43%	Not tested	Not tested
Alumina (no baking)	17%	Not tested	Not tested
Bare glass	17%	Not tested	Not tested
Teflon®-coated glass	0%	Not tested	Not tested

Fluoride adsorption on various cover-plate surfaces was examined by squeezing cyclotron-produced [^{18}F]fluoride ion in water droplets between parallel plates with a 150 μm gap for 10 minutes (Table 1). The loaded radioactivity was measured in a dose calibrator (CRC-25R, Capintec). The plates were then separated and washed with 20 μL of water before the radioactivity of each plate was again measured. Fluoride adsorption was quantified as the ratio of radioactivity left on the plate compared with the radioactivity initially loaded. Fluoride trapping on an alumina cover plate baked at 500˚C was also examined with EWOD mixing (30 sec) and resistive heating (85˚C, 3 min).

We synthesized [^{18}F]fallypride as the tracer to demonstrate on-chip removal of [^{18}F]fluoride (Figure 3). [^{18}F]fallypride is a dopamine receptor antagonist, useful for studying Alzheimer's and Parkinson's disease. It was synthesized in our EWOD chip with a Teflon®-coated ITO cover plate. First, a mixture of TBAH/[^{18}F]fluoride (7 μL) was pipetted onto the chip, moved to the heater by EWOD actuation, and heated at 105˚C for 1 minute. For fluorination, 4 μL of fallypride precursor dissolved in thexyl alcohol was loaded by pipette, moved to the heater, and heated at 100 ˚C for 7 minutes.

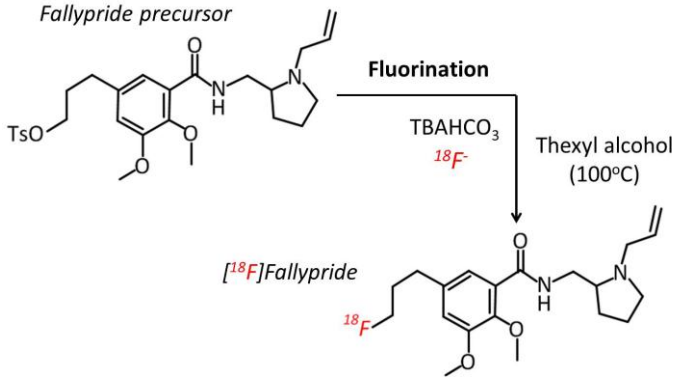

Figure 3: Synthesis of [^{18}F]fallypride.

At the end of synthesis, the ITO/nitride cover plate was replaced with an exposed alumina version. For this demonstration, crude product was collected using a micropipette with 10 μL of MeOH and MeCN and analyzed by radio-TLC (MiniGITA star, Raytest) to quantify the ratio of [^{18}F]fluoride compared with the [^{18}F]fallypride product. Crude product (10 μL) was then transferred back onto the chip and squeezed between the parallel plates. An EWOD mixing sequence (30 seconds) and heating (65˚C, 4.5 minutes) were used to facilitate adsorption of ions onto the exposed alumina surface. The remaining droplet was collected by syringe after removal of the alumina cover plate. Radiochemical purity (proportion of total radioactivity labeled onto desired radiotracer) was analyzed by radio-TLC.

RESULTS AND DISCUSSION

The radio-TLC results of the crude product and the final product are shown in Figure 4. The crude product

radio-TLC shows a radiofluorination efficiency of 88%, which is typical for our on-chip [^{18}F]fallypride synthesis. After the fluoride removal step, the final product radiochemical purity was 100%. The radio-TLC showed that all of the unreacted [^{18}F]fluoride was removed from the crude reaction mixture.

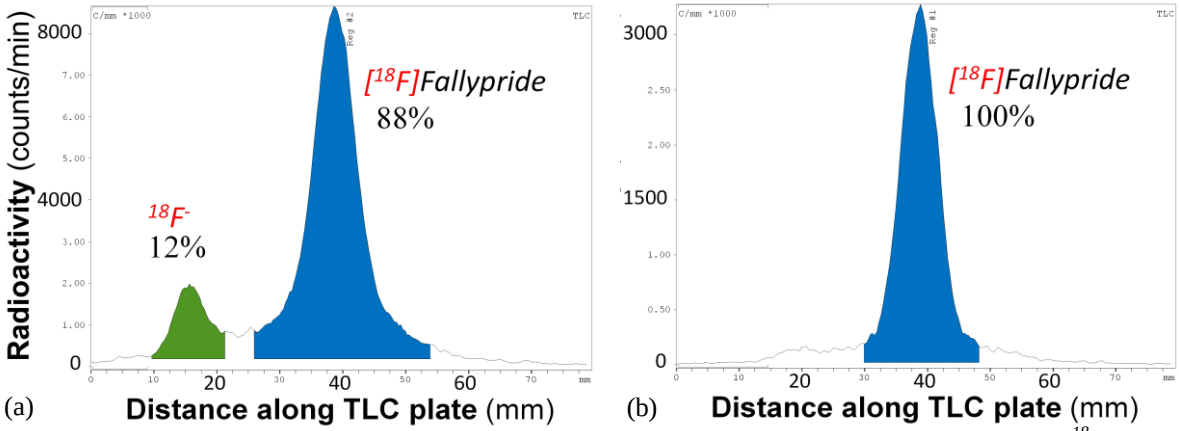

Figure 4: Radio-TLC analysis of: (a) crude product to determine the radiofluorination efficiency of [^{18}F]fallypride, (b) the purified product after extraction using the alumina exposed cover plate.

The reported demonstration of planar alumina as a solid-phase extraction surface shows that with adequate mixing, purification can be performed on chip in a parallel-plate EWOD device using only surface modifications, eliminating the use of a packed column. We designed the alumina cover plate to remove [^{18}F]fluoride for our application, but with further processing the alumina can be prepared for adsorption of other ions, amines, and aromatic compounds.

CONCLUSION

We have reported a simple on-chip purification method to remove fluoride. Future work will focus on characterizing the alumina surface and optimizing the extraction so only one cover plate is needed. With proper design, potentially other chemical species can also be extracted on chip during multi-step synthesis.

ACKNOWLEDGEMENTS

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