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High yield and high specific activity synthesis of [^{18}F]Fallypride in a batch microfluidic reactor for micro-PET imaging

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Materials and methods

Tetrabutylammonium bicarbonate ($TBAHCO_3$), 2,3-dimethyl-2-butanol, anhydrous acetonitrile (MeCN, 99.8%), anhydrous dimethylsulfoxide (DMSO, 99.9%), hexanes, ethyl acetate, ammonium formate ($HCOOHNH_4$), triethylamine, ethanol, and methanol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). 2,3-dimethoxy-5-[3-[[(4-methylphenyl)sulfoniyl]oxy]propyl]-N-[[1-(2-propenyl-2-pyrrolidinyl)methyl] (tosyl-Fallypride precursor) and the fallypride standard compound were purchased from ABX Advanced Biochemical Compounds (Radeberg, Germany). tC18 reversed phase cartridge (particle size 37-55 μm) was purchased from Waters, USA and preconditioned with ethanol (1 mL) and water (2 mL) prior to use.

No-carrier-added [^{18}F]fluoride ion was obtained from the UCLA Crump Institute for Molecular Imaging Cyclotron Facility by irradiation of 85% ^{18}O-enriched water with an 11 MeV proton beam using an RDS-112 cyclotron (Siemens Medical Solution, Knoxville, TN). Radioactivity was determined using a calibrated ion chamber (Capintec CRC-15R). A radioactive thin layer chromatography scanner (MiniGITA star) (Raytest USA, Inc, Wilmington, NC) was used to analyze fluorination efficiency, and an analytical-scale high performance liquid chromatography (HPLC) system (Knauer, Germany) was used to purify the [^{18}F]fallypride that was synthesized on the EWOD chip. The analytical-scale HPLC used for purification was equipped with a SecurityGuard C18 column (Phenomenex, Torrance, CA), a Phenomenex Luna reversed-phase C-18 column (250 x 4.6 mm), a variable wavelength UV detector and a radiometric detector (Eckert&Ziegler, Washington DC, USA). For the macroscale radiosynthesis, the semi-preparative HPLC purification was carried using the Phenomenex Luna reversed-phase C-18 column (250 × 10 mm) (Torrance, CA). A separate analytical HPLC was used to measure chemical and radiochemical purity and specific activity.

EWOD chip fabrication

Schematically illustrated in Fig. S-1, an EWOD chip has two plates: the base plate and the cover plate. The base plate has electrodes designed for droplet routing, heating, and temperature sensing. The cover plate has a conductive layer that serves as the ground electrode. Both plates were fabricated from 700 μm thick glass wafers coated with 140 nm of indium tin oxide (ITO) (Semiconductor Solutions LLC), onto which 20 nm of chrome and 200 nm of gold were deposited by evaporation. Photolithography and wet etching of the metal layers were used to pattern EWOD electrodes, heaters, connection lines, and contact pads. A second photolithography step and wet etching removed gold and chromium from EWOD electrodes and heaters. A 2 μm layer of silicon nitride was deposited onto the base plate and a 100 nm layer was deposited onto the cover plate by plasma-enhanced chemical-vapor deposition (PECVD) to serve as a dielectric. A 250 nm Teflon® layer was then spin-coated onto both plates and annealed at 330 ˚C to make both plate surfaces hydrophobic. The cover plate was bonded to the base plate with a 140 μm gap using two layers of double-sided tape (3M Inc.).

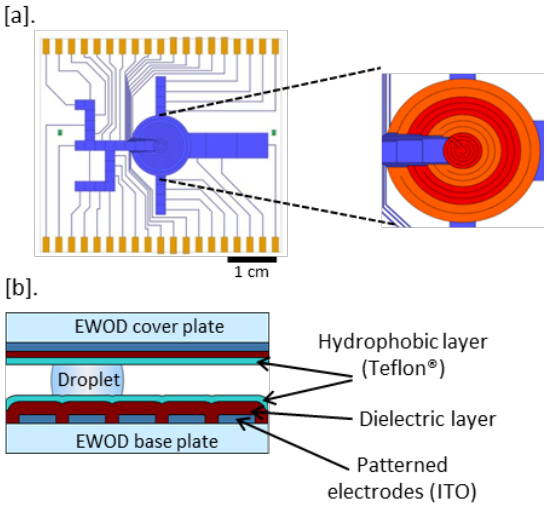

Fig. S-1: [a] Top view schematic of the EWOD chip. Electrodes to control droplet movement and heating are shown in blue. The inset shows the reaction site with four individually controlled concentric ring heaters (red and orange) and [b] Cross-section schematic showing the layers of the EWOD chip (not to scale).

EWOD Chip Operation

EWOD actuation voltage for droplet movement was generated from a 10 kHz signal (33220A waveform generator, Agilent Technologies) amplified to 100 V_{rms} (Model 601C, Trek). The voltage was applied selectively to desired electrodes from droplet movement by individually addressable relays (AQW610EH PhotoMOS relay, Panasonic) that were controlled by a LabVIEW program using a digital I/O device (NI USB-6509, National Instruments). A second digital I/O device (NI USB-6259, national Instruments) was used to control a multichannel heater driver that was designed and built in house to measure and maintain feedback-controlled temperatures over the chip's four individual heaters. The chip's central electrodes each had two connections, such that they could be used for either EWOD actuation or for temperature measurement and Joule heating.

Macroscale automated radiosynthesizer

For the macroscale automatic radiosynthesis of [^{18}F]fallypride, our group utilized the ELIXYS radiosynthesizer (Sofie Bioscience, USA). The one-pot synthesis of [^{18}F]fallypride in this study was performed using one of the three available reaction vessels. The crude product was loaded into the HPLC injection valve after synthesis and subsequently purified via semi-preparative HPLC.

Radiosynthesis of [^{18}F]fallypride on EWOD chip

Tetrabutylammonium bicarbonate ($TBAHCO_3$) solution was prepared by diluting the $TBAHCO_3$ stock solution (10 µL, 75mM) in MeCN/H_2O (25:15 v/v) to reach a final concentration of 15 mM. 10 µL of $TBAHCO_3$ (15 mM) was added to the [^{18}O]H_2O/[^{18}F]fluoride solution (40 µL; ~590 MBq) (non-decay

corrected). A stock solution of the tosyl-fallypride precursor (154.8 mM) in MeCN was prepared. Using a micropipette, 5µL of the precursor stock solution was diluted with thexyl alcohol (5 µL) to obtain a final precursor concentration of 77 mM.

Two microdroplets (2.5 µL each) of the TBA[^{18}F]F complex were transported to the reaction site by EWOD actuation, followed by the addition of a 1 µL MeCN wash droplet. The fluoride loading process was repeated with an additional one microdroplet (2 µL) of the TBA[^{18}F]F complex followed by the addition of 3 µL MeCN wash droplet to reach a total on-chip radioactivity of ~290 MBq (non-decay corrected). The on-chip radioactivity was calculated by subtracting the radioactivity in the source vial before and after the fluoride loading process. The TBA[^{18}F]F complex solution was heated to 105°C and held for 1 min to evaporate the solvent. Subsequently, one cycle of azeotropic distillation was performed by adding a 12 uL MeCN droplet to the dried residue and heating at 105°C for 1 min. Upon activation of the TBA[^{18}F]F complex, two microdroplets of the tosyl-Fallypride precursor solution (2 µL each, 77mM) were transferred to the dried TBA[^{18}F] complex on reaction site at room temperature. The reaction mixture was gradually heated to 100°C and held for 7 min to perform the fluorination reaction. After the fluorination reaction, the cover plate was removed and the crude product was extracted using 16 µL of MeOH. A small amount of the crude product was used for radio-thin layer chromatography (radio-TLC) and radio-HPLC analyses. The crude radiochemical yield was calculated by multiplying the fluorination efficiency obtained from the radio-TLC by the extraction efficiency measured using the dose calibrator. The percent ratio of the [^{18}F]fallypride peak, which travelled 30 mm from the baseline on the TLC plate was defined as the fluorination efficiency. The extraction efficiency was calculated by the ratio of radioactivity that was collected into the Eppendorf tube from the chip to the total radioactivity of the entire chip (before extraction) as measured using the dose calibrator. The remainder of the crude product was injected directly onto an analytical HPLC with UV detector (305 nm) and γ-ray detector. The [^{18}F]fallypride was separated using an isocratic elution of MeCN/$HCOOHNH_4$ (aq, 25 mM) 65:45 v/v and 1% of triethylamine at a flow rate of 1 mL/min. The retention time of [^{18}F]fallypride was found around 11 minutes. The [^{18}F]fallypride fraction (~ 2 mL) was collected and diluted with water (15 mL), and then passed through a preconditioned t-C18 cartridge to remove the HPLC mobile phase. The [^{18}F]fallypride was then eluted from the cartridge using ethanol (1.5 mL) with quantitative efficiency. The ethanol was evaporated at 75°C under nitrogen flow for about 10 minutes. The dried residue was reformulated in 0.5 mL of saline for micro-PET imaging. The chemical and radiochemical purity of the formulated [^{18}F]fallypride was analyzed using a different analytical HPLC equipped with the C18 Luna column at 1.5 mL/min and at a UV wavelength of 254 nm (Fig. S-2).

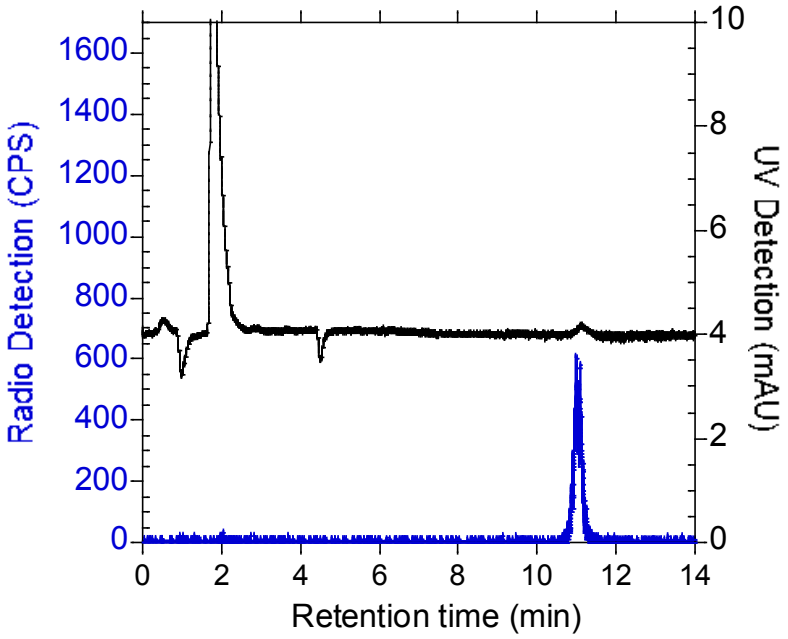

Fig. S-2: HPLC chromatogram of the reformulated [^{18}F]fallypride synthesized on the EWOD microchip.

Radiosynthesis of [^{18}F]fallypride on a macroscale reactor

The macroscale radiosynthesis of [^{18}F]fallypride was performed on the ELIXYS^{TM} automated radiosynthesizer (Sofie Biosciences, Inc., USA). For comparison of the fluorination yield and the specific activity between the micro- and macroreactor, both syntheses were performed under exactly the same conditions (with the exception of the volume of solvents and reagents). Both syntheses were performed on the same day using the same batch of [^{18}F]fluoride ion to control for day-to-day variability of the specific activity of the [^{18}F]fluoride ion. The automated synthesis of [^{18}F]fallypride began with the addition of the aqueous [^{18}F]fluoride ion in [^{18}O]H_2O (740 MBq; 43 µL) into the tetrabutylammonium bicarbonate (0.22 mL; 75 mM) solution that was diluted in MeCN (0.55 mL) and water (0.23 mL). The mixture was then delivered to the reaction vessel using pressurized nitrogen. Solvent was evaporated by heating to 110°C for 8 min under a stream of nitrogen and vacuum. Additional MeCN (1 mL) was added to the reactor for subsequent azeotropic distillation at 110 °C for 2.5 min. To the dried [^{18}F]TBAF residue, a solution of tosyl-fallypride precursor (30 mg, 0.058 mmole), dissolved in a 50:50 mixture of thexyl alcohol and MeCN (500 µL total), was added. The reaction mixture was heated at 105°C for 7 minutes. A small aliquot of the crude [^{18}F]fallypride mixture was taken for radio-TLC analyses using 50:50 methanol/ethyl acetate (1% of triethylamine) as the mobile phase. The remainder of the crude product was heated to remove solvent and then diluted in the HPLC mobile phase before being injected onto a semi-preparative HPLC column (Phenomenex Luna C18, 5µm, 250 x 10 mm). The HPLC conditions for the purification were MeCN/$HCOOHNH_4$ (aq, 25 mM) 55:45 v/v and 1% of triethylamine at a flow rate of 5 mL/min using a UV detector at 254 nm and a γ-ray detector. The average decay-corrected radiochemical yield of [^{18}F]fallypride was 83±10% (n=2).

Specific activity analysis

A fallypride standard calibration curve (linear fit, $R^2 = 0.9906$) was generated using known concentrations of fallypride at a UV detection wavelength of 254 nm on an analytical HPLC. The cold masses of fallypride from both syntheses were calculated based on this calibration curve. The range of specific activity reported was based on four experiments on Teflon-glass substrates and one experiment on the macroscale synthesizer starting with ~ 7.4 GBq of stock [^{18}F]fluoride/[^{18}O]H_2O. For the specific activity analysis of [^{18}F]fallypride synthesized on-chip, a modified procedure was performed to obtain sufficient cold mass for UV detection. The synthesis was performed on a Teflon-glass substrate (a low-cost, electrode-less approximation of the EWOD synthesis configuration). Tetrabutylammonium bicarbonate ($TBAHCO_3$) solution (5 µL, 75mM) was diluted in [^{18}F]fluoride/[^{18}O]H_2O (100 µL; ~ 1850 MBq (50 mCi) radioactivity solution to reach a final concentration of $TBAHCO_3$ of 3.75 mM. Tosyl-fallypride precursor (4 mg) was dissolved in 50 µL of 1:1 mixture of MeCN/thexyl alcohol to reach a final concentration of 77 mM. 8 µL droplet of the TBA[^{18}F]F complex and 8 µL of MeCN droplet were added onto the Teflon-glass substrate. The mixture was heated to 105 °C for 1 minute. The fluoride loading and evaporation process was repeated for additional four times to reach a total volume of the $TBAHCO_3$ complex of 40 µL and a total on-chip radioactivity of ~ (590-630 MBq (16-17 mCi) non-decay corrected). Upon activation of the TBA[^{18}F]F complex, the tosyl-Fallypride precursor solution (4 µL, 77 mM) was added to the dried TBA[^{18}F] complex on reaction site at room temperature. The reaction droplet was then sandwiched using a separate Teflon-glass substrate. The gap height was set to ~ 150 µm, mimicking the gap height on the EWOD chip. The reaction mixture was gradually heated to 100°C and held for 7 min to perform the fluorination reaction. After the fluorination reaction, the top Teflon-glass substrate was removed and the crude product was extracted using 80 µL of MeOH. Using a gas-tight 100 µL Hamilton syringe, 50 µL of the crude produce was taken up into the syringe. The radioactivity on the syringe before and after injecting into the analytical HPLC was measured using a dose calibrator. The residual activity remaining on the syringe was subtracted from initial radioactivity to determine the total amount of radioactivity that was injected into the HPLC. The radioactivity of [^{18}F]fallypride injected was estimated by multiplying the total radioactivity by the fluorination efficiency of the reaction based on radio-TLC:

$$[^{18}\mathrm{F}]fallypride\ injected\ into\ the\ HPLC = total\ radioactivity \times \%\ fluorination\ efficiency$$

A representative HPLC chromatogram of the crude [^{18}F]fallypride sample used for specific activity measurement is shown in Fig. S-3.

For the macroscale reaction, the crude mixture was first purified via the semi-preparative HPLC as described above prior to the specific activity analysis. The HPLC condition for the specific activity analyses of both the microscale and macroscale syntheses were 60:40 MeCN / 25 mM ammonium formate plus 1% of triethylamine at a flow rate of 1.5mL/min.

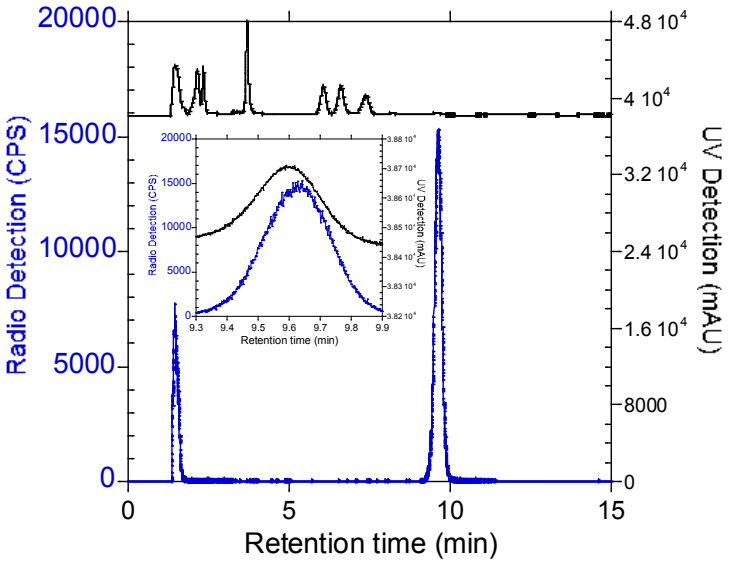

Fig. S-3: Representative HPLC chromatogram of the crude [^{18}F]fallypride synthesized on the EWOD chip that was used for specific activity analysis. The inset shows the magnified region in which the cold mass of fallypride eluted between 9 and 10 minutes.

Micro-PET imaging

The imaging study was conducted under the protocols approved by the Chancellor's Animal Research Committee at the University of California Los Angeles. A total of four healthy balb-c mice were imaged: two for the [^{18}F]fallypride synthesized on the EWOD chip, and two for the [^{18}F]fallypride synthesized on the macroscale synthesizer. The mice were anesthetized with isoflurane (4%) and were injected with [^{18}F]fallypride via tail vein and scanned for 60 minutes under isoflurane anesthesia. Two mice were injected with 2.9 and 4.4 MBq (79 and 119 μCi) of [^{18}F]Fallypride that were synthesized on the EWOD chip. The other two mice were injected with 7.4 and 3.5 MBq (201 and 94 μCi) of [^{18}F]fallypride that were synthesized on the macroscale synthesizer. The microPET images were acquired using a small animal PET scanner (MicroPET Inveon and Focus, Siemens, Knoxville), followed by a microCT scan (microCAT II, Siemens, Knoxville). The microPET and microCT scans were co-registered to yield a single image that was displayed using AMIDE(*1*) and images were reconstructured using a 3D filtered back-projection algorithm for quantitation. Regions of interest of the left and right striatum were drawn to calculate the ratio of specific binding to non-specific binding. The cerebellum was used as the reference region to measure the non-specific binding of [^{18}F]fallypride. In both cases, the left and right striatum (n=2 for each experiment) was clearly visualized (Fig. S-4). The average binding ratios (striatum/cerebellum) of [^{18}F]fallypride that were synthesized on the macroscale radioasynthesizer and the EWOD microfluidic radiosynthesizer were calculated to be similar (Fig. S-5).

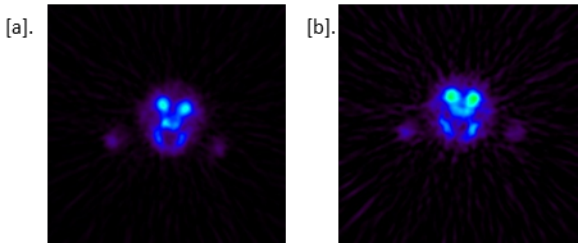

Fig. S-4: Representative micro-PET images of the striatum in the brain of a mouse using [^{18}F]fallypride prepared on the (a) EWOD microfluidic radiosynthesizer and (b) macroscale radiosynthesizer

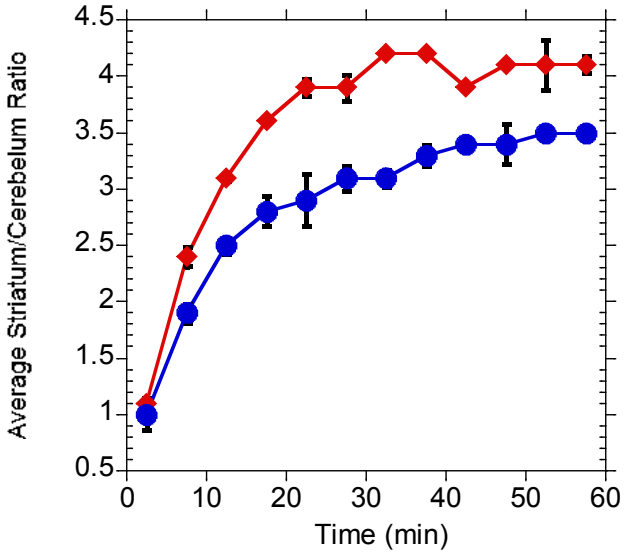

Figure S-5: Average ratios of [^{18}F]fallypride binding in the striatum versus the cerebellum as a function of scan duration for the microPET experiment. The upper line represents the average binding ratio of [^{18}F]fallypride that was synthesized on the macroscale radiosynthesizer, while the lower line represents the average binding ratio of [^{18}F]fallypride that was synthesized on the EWOD chip. The microPET experiments were performed on two different mice. Error bars (black line; greater than 1%) were plotted based two imaging experiments using [^{18}F]fallypride obtained from each synthesis approach.

Reference

1. Loening AM, Gambhir SS. Amide: A free software tool for multimodality medical image analysis. *Mol Imaging.* 2003;2:131-137.