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Microfluidic continuous-flow radiosynthesis of [¹⁸F]FPEB suitable for human PET imaging

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Abstract

The synthesis of fluorine-18 labeled 3-fluoro-5-[(pyridin-3-yl)ethynyl] benzonitrile ([¹⁸F]FPEB) for imaging metabotropic glutamate receptor subtype type 5 (mGluR5) was achieved with a commercial continuous-flow microfluidics device. This work represents the first positron emission tomography (PET) radiopharmaceutical that is suitable for human use with this technology. We also describe a validated synthesis of [¹⁸F]FPEB with a commercial reactor-based system.

Continuous-flow microfluidics (a synergy between microfluidics and continuous flow chemistry) has exciting advantages over traditional radiochemical synthesis.^{1–4} Such microfluidic technologies can offer low sample and reagent consumption, faster kinetics, high throughput screening of reaction conditions, high reproducibility, enhanced product yields and facile automation. Implementation of such a method has significantly accelerated the process of synthesizing labeled compounds and positron emission tomography (PET) radiopharmaceuticals.¹ To date, over fifty radiolabeled compounds have been synthesized in continuous-flow microfluidic devices¹ for PET applications including [¹⁸F]SFB, [¹⁸F]FMISO, [¹²⁴I]doxorubicin, [⁶⁴Cu]DOTA-cyclo(RGDfK) and [⁶⁸Ga]NOTA/DOTA-cyclo(RGDfK). Our group has recently demonstrated “microfluidic flow hydrogenation” i.e., ¹⁸F-labeling and subsequent hydrogenation to efficiently synthesize ¹⁸F-labeled compounds by integrating two commercially available continuous-flow microfluidic systems.⁵

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[‡]Electronic Supplementary Information (ESI) available: Automated synthesis of [¹⁸F]FPEB using GE Tracerlab FXFN; QC procedure; Nanotek plumbing diagram, production log, macro sequence and ICP-MS.

The first reported syntheses PET radiotracers in a microfluidic device were published nearly a decade ago and demonstrated that this technology is capable of providing multi-doses of fluorine-18 (^{18}F ; $t_{1/2} = 109.7$ min) labeled compounds.^{6,7} A recent report described a prototypic batch-reactor microfluidic device to prepare [^{18}F]fallypride for application in a human PET study.⁸ Despite extensive preclinical imaging with radiotracers prepared by continuous-flow microfluidics, there is still no published PET radiopharmaceutical prepared for human use by flow chemistry.

The goal of the present work was to synthesize ^{18}F -labeled 3-fluoro-5-[(pyridin-3-yl)ethynyl] benzonitrile ([^{18}F]FPEB; **2**, Scheme 1) suitable for clinical studies by the most commonly used microfluidic flow platform in radiochemistry (NanoTek[®]; Advion, Inc.). Fluorine-18 labeled FPEB is a metabotropic glutamate receptor subtype 5 (mGluR5) antagonist with high potency, selectivity and brain penetration in rodents, nonhuman primates and human subjects.^{9–13} Our preliminary PET imaging studies with this radiopharmaceutical, as prepared using a commercial automated radiosynthesis unit (GE Tracerlab FX_{FN}; see ESI), have focused on well characterized patients suffering from Alzheimer's disease (AD). Several lines of evidence point to a critical role for mGluR5 in the pathologic process of AD. Oligomeric A β reliably induces abnormal accumulation and over-stabilization of mGluR5 receptors, which is up-regulated in AD, and co-localizes with A β in AD.¹⁴ Figure 1C shows [^{18}F]FPEB binding observed in default network cortices and in medial temporal cortex and hippocampus, in a subject with early mild cognitive impairment.

While several precursors have been developed for the radiosynthesis of [^{18}F]FPEB,^{9a,10a,12,16–18} our laboratory has validated the production of this radiotracer using a commercially available precursor, 3-nitro-5-[(pyridin-3-yl)ethynyl] benzonitrile (**1**; ABX GmbH), and optimized its production (see ESI). Radiochemical yields for this reaction are low (1–5% uncorrected, relative to starting ^{18}F -fluoride) as this nucleophilic substitution reaction at the *meta*-position occurs on a non-activated aromatic ring. It is noteworthy that improved conversions have been noted with diaryliodonium precursors in a continuous flow microfluidic reactor.¹⁹ In the present work, we utilized a NanoTek[®] system to perform ^{18}F -labeling of [^{18}F]FPEB. For the proof of concept this system was integrated to a GE Tracerlab FX_{FN} unit to carry out semi-preparative HPLC purification and formulation.

A schematic of the radiosynthesis of [^{18}F]FPEB using the Nanotek[®] is shown in Figure 2, A–F. Fluorine-18 labeled fluoride was produced by the $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$ nuclear reaction using a cyclotron (GE PETtrace, 16.5 MeV) and delivered to the microfluidic module. As previously described,⁵ the [^{18}F]fluoride was trapped on an ion exchange resin, eluted using a solution of tetra-*n*-butylammonium bicarbonate ($n\text{Bu}_4\text{NHCO}_3$, 0.075M, 0.6 mL) and delivered to the [^{18}F]fluoride drying concentrator (A). The reaction mixture was dried azeotropically three times by addition of anhydrous CH_3CN under N_2 flow and vacuum over 2 min. The precursor **1** and dried [^{18}F]nBu₄NF (re-solubilized in DMSO) were loaded into storage loop (C) and dispensed into the microfluidic flow reactor (D) at various temperatures and flow rates. The ensuing reaction mixture was transferred into dilution vial (E, pre-loaded with 25 mL of H_2O) and mixed under a stream of nitrogen. The diluted crude mixture was pre-purified on a solid phase extraction cartridge (Waters Oasis HLB Light; F) to remove

DMSO and subsequently transferred to a GE TracerLab FX_{FN} for HPLC purification and formulation (see Fig 2 and ESI).

The microfluidic flow platform enabled rapid optimization of radiolabeling conditions by dispensing a small aliquot of reagent and isotope and performing reaction in a continuous-flow microfluidic reactor. Reaction parameters including temperatures, flow rates and precursor/¹⁸F isotope ratios were varied via two syringe pumps (Figure 2,B). Flow reactions occurred inside coiled silica glass capillary tube (16 μ L internal volume; 2 meter length) encased in a brass housing (Figure 2,D). Fluorine-18 incorporation in DMSO gradually increased from 120 $^{\circ}$ C and reached the maximum radiochemical conversion (10%) at 215 $^{\circ}$ C (Figure 3A). As shown in Figure 3B, flow rate also played an important role in the production of [¹⁸F]FPEB. The conversion increased from 5% to 26% when the flow rate of the reaction was decreased from 80 μ L/min to 5 μ L/min. Although the slowest flow rate gave the highest conversion, considering that the volume of our starting ¹⁸F-fluoride stock solution is 250 μ L, we determined that 10 μ L/min (per syringe pump) was the most practical flow rate for this reaction (total flow rate is 20 μ L/min) and offers a suitable compromise of radioactive decay relative to reaction time (50 min vs. 25 min). In our initial screening, we also determined that the optimal ratio of flow rates (precursor to ¹⁸F isotope) was 1:1.

Our radiochemistry optimization was achieved in one day using one batch of [¹⁸F]fluoride (ca. 20 mCi, 740 MBq) and a small amount (2 mg in 200 μ L of DMSO) of **1** (Fig. 3).

Following the optimization process of [¹⁸F]FPEB, the reaction was seamlessly extrapolated to a production level. In order to scale up the reaction, we doubled the flow rate as well as the reactor length and internal volume. The system was set up with a 4 meter reactor (32 μ L internal volume) and the radiosynthesis was carried out at 215 ± 5 $^{\circ}$ C with a flow rate of 20 μ L/min per syringe pump (total flow rate is 40 μ L/min). To our knowledge, we tested the maximum amount of radioactivity (> 4.5 Ci or 166 GBq of starting ¹⁸F), representing the highest radioactivity concentration (>10 Ci/mL) on a continuous-flow microfluidic system, and no radiolysis was observed by HPLC.

Three consecutive productions of [¹⁸F]FPEB were carried out to validate this radiopharmaceutical for human use (Table 1). Uncorrected radiochemical yields of [¹⁸F]FPEB ($2.1 \pm 0.4\%$ relative to starting [¹⁸F]fluoride) by this method are consistent with those achieved previously,^{9a,10a} and high specific activities were obtained in the final formulation (4.4 Ci/ μ mol) within 75 minutes. HPLC analysis of the formulated [¹⁸F]FPEB product in 10% EtOH in 0.9% sodium chloride revealed high radiochemical purity (>95%). The formulated [¹⁸F]FPEB PET radiopharmaceutical maintained was found to be stable over a period of 6 h. No long-lived isotopes were observed in the final product, as determined by the long lived impurity analysis on a HPGE detector after ¹⁸F-decay. The formulated product had no detectable endotoxin and was sterile. Volatile organic compound analysis was carried out by GC-FID showing residual acetone, CH₃CN, and DMSO below the lower limit of detection, thereby exceeding the International Conference on Harmonisation of Technical Requirement of Pharmaceuticals for Human Use requirements.

At present, our clinical research studies are routinely carried with the optimized and validated GE Tracerlab FX_{FN} conditions (see ESI) as higher radiochemical yields ($7.0 \pm 2.1\%$; uncorrected and relative to [^{18}F]fluoride) and specific activities ($7.07 \pm 1.38 \text{ Ci}/\mu\text{mol}$) are achieved. The proof of concept microfluidic methodology demonstrates that [^{18}F]FPEB can be validated for human use under an approved U.S. Food and Drug Administration (FDA) Investigational New Drug (IND) application, or equivalent regulatory submission, and offers an alternative technology to prepare PET radiopharmaceuticals.

Conclusions

The synthesis of the mGluR5 radiopharmaceutical [^{18}F]FPEB was validated for routine human use with a commercial reactor-based platform. This synthesis was also achieved using a commercial continuous-flow microfluidic system. This proof of concept work demonstrates that microfluidics flow chemistry systems are capable of synthesizing PET radiopharmaceuticals that are suitable for human use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Preliminary PET data from a 64 year-old subject with evidence of mild memory impairment:
(A) $[^{11}\text{C}]\text{PiB}$, (B) $[^{18}\text{F}]\text{FDG}$, (C) $[^{18}\text{F}]\text{FPEB}$ and (D) MRI showing hyperactivity in the
hippocampus.¹⁵

Fig. 2. Schematics of microfluidic flow platform and parameters for the radiosynthesis of [18F]FPEB: (A) [18F]fluoride drying concentrator, (B) high resolution syringe pump, (C) storage loop (polyether ether ketone), (D) microfluidic flow reactor, (E) dilution vial, (F) solid phase exchange cartridge. Specifications of the microfluidic flow reactor : volume 8–64 μ L, reaction path from 1–8 meter, I.D. 100 μ m, temperature range from 25 $^{\circ}$ C to 220 $^{\circ}$ C, pressure up to 3.45MPa (500 psi).

Fig. 3.

Temperature (A) and flow rate (B) effect of [¹⁸F]FPEB synthesis. (a) Conditions: [¹⁸F]fluoride was trapped on an anion exchange resin (MP1, ORTG, Inc., USA), eluted with *n*Bu₄NHCO₃ (0.075M aqueous solution) and azeotropically dried using standard NanoTek drying macro sequence (see ESI). The temperature effect was screened at a total flow rate of 60 μL/min and the flow rate was evaluated at 210°C. (b) Radiochemical conversion of [¹⁸F]FPEB (*n* = 1 per data point) was determined with radio-HPLC (*t*_R (FPEB) = 7.0 min, *t*_R

(impurity) = 6.2 min, mobile phase: 60/40 CH₃CN/H₂O + 0.1N ammonium formate; Luna 5 μ C18 100 Å, λ = 254 nm, 250 $\times$ 4.6 mm; flow rate: 1 mL/min).

Table 1^aSummary of [¹⁸F]FPEB specifications by continuous-flow microfluidics

Parameters	Results (<i>n</i> = 3)
Synthesis time	75 min (ready for injection)
Isolated product	45 ± 10 mCi at end of synthesis
Visual inspection	Clear, absence of particulates
Radiochemical identity	±10% of FPEB reference standard retention time
Radiochemical purity	95%
Specific activity	4.4 ± 0.3 Ci/μmol
Residual solvent analysis	DMSO < 5 mg/mL
	Acetone < 5 mg/mL
	Acetonitrile < 0.4 mg/mL
	Ethanol < 10% v/v ±10%
pH Assay	5–5.5
Sterile filter integrity test	50 psi
Radionuclidic ID:photopeak	99.5% emission @511 KeV, 1.022MeV, or Compton scatter peaks
Radionuclidic ID:Half-life	105 – 115 minutes
Endotoxin analysis	Not greater than 17.5 EU per injected dose
Sterility testing	No evidence of growth at 14 days post inoculation

^aQuality control was performed on an aliquot drawn from the product vial, and carried out according to an approved protocol at MGH; see ESI;^bThe radiochemical identity and purity were determined with radio-HPLC (*t_R* = 4.97 min, mobile phase: 45:55 Ethanol/H₂O; Waters Nova-Pak C18 4μm, λ = 254 nm, 150 × 4.6 mm; flow rate: 1 mL/min.).