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Microfluidic single vessel production of hypoxia tracer 1H-1-(3-[¹⁸F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole ([¹⁸F]-FMISO)

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Abstract

We report an automated synthesis of [¹⁸F]-FMISO utilizing a prototype microfluidic radiochemistry module. The instrument allows for production of the tracer with 58% ± 2% (11 runs) decay corrected yield. Total time of production, including synthesis and purification averages 60 minutes. Use of the microfluidic platform results in a specific activity of 138.6 GBq/μmol, which is higher than previously reported for conventional reactors.

Keywords

FMISO; microfluidic; PET

Introduction

[¹⁸F]-fluoromisonidazole, 1H-1-(3-[¹⁸F]-fluoro-2-hydroxy-propyl)-2-nitro-imidazole ([¹⁸F]FMISO) is a radiolabeled imaging agent that has been used to investigate tumor hypoxia with positron emission tomography (PET). FMISO binds covalently to cellular molecules at rates that are inversely related to intracellular oxygen concentration. In hypoxic cells, FMISO is trapped, forming the basis for the use of this tracer to measure hypoxia (Imam, 2010). Because tissue oxygenation may serve as a marker of perfusion, response to radiotherapy and chemotherapy, tumor grade, and prognosis, development of a PET imaging agent for tumor hypoxia is a potentially valuable avenue of investigation.

Presently the most common method for radiolabeling of FMISO in routine synthesis for clinical research use is carried out in macroscale reactors (>5mL). (Lim and Berridge, 1993) The desire for higher yields and specific activity initiated the use of a reactor that would operate under a significantly smaller reaction volume. This has allowed reduced consumption of reagents and consumables, including expensive precursors. Microfluidic technology is well suited for this application and has recently been exploited for radiochemical synthesis (Miller et al., 2010). In the present report, we employ a fourth generation microfluidic prototype instrument (P-IV instrument) developed at Siemens Molecular Imaging to study the synthesis of FMISO in the microfluidic setting. The PIV microfluidics unit uses an anion-exchange column which concentrates the fluoride from a standard high-yield [¹⁸F] niobium or tantalum target and reduces the target volume from several milliliters to less than 50 microliters, to allow true micro scale reactions in the 50μL micro reaction chip without significant losses of [¹⁸F] fluoride. This low volume and high concentration is an important advantage of the PIV microfluidics system, which in part allows the microfluidics system to synthesize [¹⁸F] radiotracers in yields sufficient for

multi-patient batches. The yields demonstrated here would allow distribution of the [18F] radiopharmaceutical through a commercial nuclear pharmacy. The investigation examines the effects of using a single vessel microscale reactor on specific activity, product yield, chemical purity and radiochemical purity of [18F] FMISO.

Experimental

P-IV Prototype of Microfluidic Instrument

The overall reaction pathway is depicted in Figure 1. [18F] Fluoride is trapped on the micro anion exchange column, and then eluted into the microchip reactor for the labeling of FMISO. Once [18F]FMISO labeling has been completed, it is eluted from the microchip and purified via HPLC. A more detailed drawing of the prototype instrument, Figure 2, shows the instrument consisting of two units housed inside a shielded space (hot cell), reagent rack, and a controlling computer mounted outside of the shields. Inside the hot cell, the first unit performs reagent delivery and synthesis in a novel batch microfluidics chip; the second unit is dedicated to semi-preparative HPLC purification and reformulation of the final product. This architecture allows the user to deliver reagents and perform cleaning of the instrument without direct access to the high radiation area inside of the hot cell. The synthetic unit contains several subsystems which are further detailed in the supporting information section. All further details refer to Figure 2 of the PIV microfluidics system.

The reactor, HPLC injector, and reactor tubing were rinsed with water and acetone and then dried using a stream of nitrogen. The semi-preparative HPLC column and formulation system were sanitized with 70% ethanol, USP.

Externally mounted reagent vessels allowed for the re-use of the reagent vessel during the synthesis of [18F] FMISO without entering the high radiation field. At start of synthesis, the reagent vessels were loaded with the following:

K222 vial: 30 μ l of 0.16M K222 dissolved in extra-dry acetonitrile

KHCO₃ vial: 14 μ l of 0.5M KHCO₃ dissolved in sterile water for injection, USP

Precursor vial: NITTP (4mg $\pm$ 1mg) in 45 μ l of extra-dry acetonitrile

Water vial: 10mL of sterile water for injection, USP

Following the elution of the [18F]fluoride into the chip, 45 μ l of extra-dry acetonitrile was added to the K222 vial to bring the material into the chip for an azeotropic dry. After the NITTP precursor was added, the precursor reagent line was re-used to bring in 40 μ L of 1N HCl.

2.2 Synthesis of FMISO

The synthesis of FMISO was fully automated using a novel microfluidics radiochemistry system with a dedicated 50 μ L reactor. [18F]Fluoride was trapped on a reusable micro anion exchange column and was eluted into the chip containing the microreactor using a 30 μ l of 0.16M K222 and 14 μ l of 0.5M KHCO₃ mixture. The [18F] fluoride solution was dried at 105°C under a stream of nitrogen for 210 seconds, then cooled to 25°C. An azeotropic dry was performed using 45 μ l of extra-dry acetonitrile at 105°C under stream of nitrogen for 210 seconds, then cooled to 25°C. The precursor, NITTP (3–5mg) was added in a 45 μ l volume of extra-dry acetonitrile. Fluorination occurred at 160°C for 180 seconds. To allow for the deprotection solution of 1N HCl to be added, the acetonitrile was evaporated at 70°C for 140 seconds. The 40 μ l of 1N HCl was then added to the chip and heated to 150°C for 250 seconds.

2.3 Purification and final formulation of [18F] FMISO

Following deprotection, the reaction solution was eluted onto the semi-prep HPLC column using 1mL of sterile water for injection, USP. Semi-prep HPLC purification using the Phenomenex Luna C18(2) 5 μ 10 $\times$ 250mm column, mobile phase 8% EtOH:10mM Sodium Phosphates, pH 6, isocratic flow rate 5mL/min. [18F] FMISO elutes at ~6.55 minutes. [18F] FMISO was collected in a sterile 30mL vial which contained an additional 5mL of mobile phase, for a total product volume of ~10mL. The final [18F]FMISO formulation was filtered through a Millex-GP 0.22 μ m filter

Results

A total of 11 runs were performed once the [18F] FMISO chemistry was developed for the microfluidics scale. Radiochemical yields for [18F] FMISO based on starting [18F] activity was 38% $\pm$ 2%, decay corrected yield for the 60 minute synthesis and purification time was 58% $\pm$ 2%. Radiochemical yield was found to be independent of starting [18F] fluoride activity over the range of 7.4 – 55.5 GBq. Higher yields of [18F] FMISO were obtained using 5mg of NITTP precursor, at a radiochemical yield of ~56%. [18F] FMISO radiochemical purity was ~99% obtained on all analytical HPLC analyzed runs, which were the final four [18F] FMISO runs completed. Chemical purity was ~95% with no observed NITTP. We report a specific activity average of 138.6 GBq/ μ mol of the four runs. Endotoxin and sterility tests were not conducted; all other tests were within USP <823> limits applied to human use tracers.

Discussion

Conversion of the conventional [18F] FMISO chemistry (5 ml reactor) to microfluidics scale (50 μ l reactor) increases the concentration of the reagents and their likelihood of interaction. Changes in surface-to-volume ratio and lack of active mixing also affect relative rates of the processes occurring in the reactor. As a result it is hard to make viable predictions of the reaction conditions required for the micro-scale reactor. Optimization of the reaction parameters must be performed using the microreactor itself.

The high level of automation and ability to perform multiple runs in a day can reduce the optimization time. The prototype instrument employed for the current study is designed around a distributed architecture paradigm: the parts of the instrument that the user needs to access are located away from the high radiation area. As a result, upon completion of one run, the user can set-up the instrument for the next run. Theoretically, the number of runs done without direct access to the instrument is only limited by the waste collector volume. The duration of the clean cycle for the prototype is 45 minutes; thus the next run can be started within 1 hour following the completion of a prior synthesis.

As a result of our optimization study we found that the radiolabeling efficiency is dependent on the precursor concentration. Decay corrected yield increases from ~20%, in case for 2 mg of precursor, and then plateaus at ~55% in case of 5 mg. In this range of the precursor concentrations it is assumed that the precursor is present in vast excess to the fluoride anion; therefore there should be no correlation between the radiochemical yield and initial precursor amount. We speculate that a competing decomposition process occurs faster than the fluorination and an elevated precursor amount allows the precursor to persist longer in the reaction mixture. Our speculation is supported by the fact that use of a milder base (KHCO₃ as opposed to K₂CO₃) resulted in improved radiochemical yields. Elevated concentration of the radioactivity might lead to the inferred severe radiolysis. It was previously reported that nitroimidazole compounds are prone to radiolytic decomposition (Raleigh and Liu, 1984)

Use of a 50 μ l reactor vs. a 5 ml reactor is equivalent to a 100-fold increase in starting radioactivity in terms of concentration and is anticipated to result in a higher radiolysis rate. In our study, we observed linear dependence of the amount of produced radiotracer on the amount of the starting radioactivity. This was observed in the range of starting activity from 7.4 to 55.5 GBq. One potential explanation of the low rate of radiolysis is the increased surface-to-volume ratio of the micro reactor as compared to macroreactors. The increased surface area of the vessel will result in a decreased lifetime of a radical and therefore a reduced rate of radiolysis. It is also likely that the reduction of energy deposited by positrons contributes to the observed trend. A positron can travel more than 2.4 mm away from the site of generation, diffusing on average for 0.6 mm before reacting with an electron (Bailey et al., 2002). The reactor chamber of P-IV prototype is only 3 mm thick, therefore positrons emitted inside the chamber deposit a considerable portion of their kinetic energy outside of the reaction mixture.

We report an average specific activity of 138.6 GBq/ μ mol, which is higher than the previously reported average for conventional reactors of 34 GBq/ μ mol. (Kämäräinen et al., 2004) We believe the higher specific activity of [18 F] FMISO reported using this microfluidics synthesizer compared with macroreactors is in part due to the reduced surface area of the fluorinated polymers the [18 F] fluoride solution is in contact with during the synthesis.

Our reported synthesis time of 60 minutes including HPLC purification is comparable to macroscale cassette based synthesizers which utilize a solid-phase extraction based purification and report an average synthesis time of approximately 60 minutes. (Tang et al., 2005) We selected HPLC purification for our synthesis based on the ability of a HPLC column to be remotely cleaned and conditioned, allowing for several runs per day without entering the high radiation field. Although cartridge based solid-phase extraction purification offered a potentially shorter overall synthesis time, it was ruled out, as it would have required the operator to enter the high radiation field to replace the cartridges prior to the next synthesis. Our total synthesis is shorter than macroscale reactor based systems which utilize HPLC purification. Our yields for the synthesis of an average radiochemical yield of 58% are equal to that previously reported in the literature. (Kämäräinen et al., 2004; Tang et al., 2005) An advantage of our system is that unlike current commercially available macroscale radiochemistry systems, either cassette or reactor based, our system allows for multiple runs per day without requiring entry into the hot cell containing the high radiation field.

Conclusions

[F-18] FMISO can be produced using a batch microfluidic reactor. The production process can be fully automated and the resulting product conforms to the criteria of purity set by USP for clinical tracers. The process features an average 58% decay corrected yield starting from as high as 55 GBq of [18 F] Fluoride. These results highlight advantages of chip microreactor based microfluidic technology for clinical and pre-clinical production of FMISO.

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Figure 1.
Flow chart of FMISO microfluidic radiochemistry.

Figure 2.
Flow chart of the P-IV synthesis unit