

# ON-CHIP PURIFICATION OF [<sup>18</sup>F]FDG IN POSITRON EMISSION TOMOGRAPHY RADIOTRACER SYNTHESIS

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## ABSTRACT

We demonstrate the use of microfluidic modules for purification of the Positron Emission Tomography (PET) radiotracer, 2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose ([<sup>18</sup>F]FDG), as part of an integrated microfluidic Radiochemistry-On-Chip (ROC) platform for complete radiotracer synthesis. Two different modules were applied to the purification of [<sup>18</sup>F]FDG as synthesized by a commercial system and by initial tests of the ROC platform itself.

## KEYWORDS

[<sup>18</sup>F]FDG, Positron Emission Tomography (PET), purification, radiochemistry, solid-phase extraction (SPE).

## INTRODUCTION

Positron Emission Tomography (PET) is a molecular imaging technique involving the injection into a patient and subsequent monitoring of a radioisotope (e.g. [<sup>18</sup>F]fluoride) labeled drug molecule (radiotracer), and is employed as a diagnostic tool in oncology, cardiology, and the neurosciences. Although many different radiotracers are available, the most common is glucose labeled with [<sup>18</sup>F]fluoride: 2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose, better known as [<sup>18</sup>F]FDG. [<sup>18</sup>F]FDG, as well as other radiotracers, is manufactured via the use of automated synthesizers housed within heavily shielded and bulky “hot cells”. Due to the half-life of the radioisotope and the limited quantities produced by a cyclotron prior to radiotracer manufacture, each step of the synthesis must be carried out rapidly and with high efficiency. In recent years, microfluidic devices have shown great promise in the synthesis of PET radiotracers thanks to rapid synthesis times and minimal shielding requirements [1-3]. However, most reported devices focus only on the synthesis step of the procedure or, to a lesser extent, the radioisotope pre-concentration step prior to the synthesis, neglecting other key processes in the production that could equally benefit from miniaturization. In particular, on-chip purification of the final radiotracers has not been explored, instead being achieved off-line via conventional solid-phase extraction (SPE) cartridges with large dead volumes. As part of a European Union FP7 project and in collaboration with Siemens, we have been developing a “Radiochemistry-On-Chip” (ROC) platform that incorporates all aspects of the synthetic procedure into a modular microfluidic platform. Previously, we presented a module for the pre-concentration of [<sup>18</sup>F]fluoride from irradiated water for subsequent radiotracer synthesis [4,5]. Here, we extend this technique further and demonstrate microfluidic modules for the purification of [<sup>18</sup>F]FDG, synthesized by (i) a commercial Advion NanoTek system utilizing a capillary-based microreactor, and (ii) initial tests of the ROC platform itself.

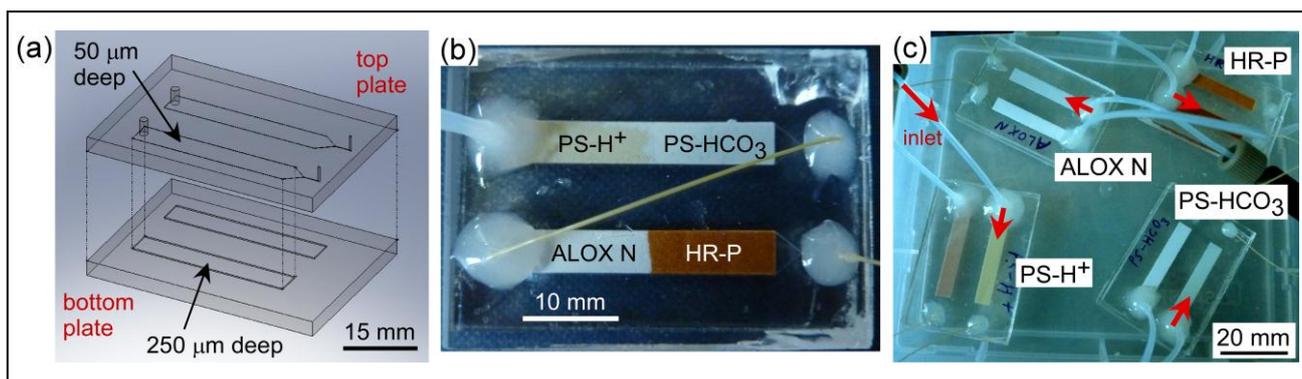


Figure 1: (a) Schematic of the chip design, consisting of a bottom plate etched to a depth of 250 μm, and a top plate etched to 50 μm. When bonded, they formed a 300 μm deep chamber with a shallow triangular section acting as a dam to trap particles. (b) Single module containing all four SPE particle types required for [<sup>18</sup>F]FDG purification. Each chamber was filled with two of the resins, and the outlet of the upper chamber was routed into the inlet of the lower chamber. (c) Train of purification modules, with each chamber containing only one type of particle, thereby doubling the trapping capacity compared to the single module. The outlet of each module was connected to the inlet of the next, allowing solutions to pass through each chamber consecutively.

## EXPERIMENTAL

**Purification modules:** The microfluidic purification modules were fabricated from glass using conventional photolithography and wet etching techniques. The chips featured a 300  $\mu\text{m}$  deep chamber (30 mm long and 4.7 mm wide), with a 50  $\mu\text{m}$  deep triangular section at one end forming a dam with which to trap SPE particles (Fig. 1a). Each chip featured two separate chambers. A 1.5 mm diameter access hole allowed the introduction of particles and solutions, whilst a 400  $\mu\text{m}$  diameter hole in the triangular section acted as an outlet. SPE particles were then packed into the chambers, and tubing was glued into the inlet and outlet holes. SPE particles were obtained from ABX (Germany) and consisted of four types: *cation exchange* (PS-H<sup>+</sup>), *anion exchange* (PS-HCO<sub>3</sub>), *normal phase* (ALOX N, alumina), and *reversed phase* (HR-P). Using these, two types of purification module systems were prepared: a single module, and a train of modules. The single module consisted of one chip and utilized both chambers present. The chambers were each filled with two types of SPE particle, in the same order as found in the standard cartridges, with the outlet from the first chamber connected to the inlet of the second chamber such that solutions could pass consecutively through each SPE particle bed (Fig. 1b). The train of modules consisted of four chips, each containing a chamber filled with only one type of resin, and which were connected end-on-end so that solutions flowed from one chip to the next, passing through all four particle beds (Fig. 1c). This train configuration essentially featured twice the amount of SPE particles as the single module. Fig. 1c shows how both chambers on each chip were filled with particles, but only one chamber was used per experiment.

**Synthesis of [<sup>18</sup>F]FDG:** [<sup>18</sup>F]FDG was prepared via the use of two different synthesis systems: (i) a commercial Advion NanoTek instrument, and (ii) initial tests of the ROC platform. In both cases, the same procedure was applied: [<sup>18</sup>F]fluoride was pre-concentrated from [<sup>18</sup>O]-water using anion exchange particles, before being eluted in a solution of Kryptofix 2.2.2/potassium carbonate in acetonitrile. Mannose triflate in acetonitrile was introduced and reacted with the [<sup>18</sup>F]fluoride to form acetylated-[<sup>18</sup>F]FDG (ACY-FDG), before being base hydrolysed with sodium hydroxide to produce crude [<sup>18</sup>F]FDG.

**Purification of [<sup>18</sup>F]FDG:** The crude [<sup>18</sup>F]FDG synthesized via the NanoTek system was pumped through the single purification module in 1 mL aliquots, and the collected solutions were analysed by radio-TLC (thin layer chromatography). The same procedure was repeated for the train of modules, with crude [<sup>18</sup>F]FDG also being analysed for comparison. [<sup>18</sup>F]FDG synthesized by the ROC platform was pumped only through the train of modules in 1 mL aliquots and again analysed by radio-TLC, with three readings of crude product used for comparison.

## RESULTS AND DISCUSSION

**Purification of [<sup>18</sup>F]FDG from NanoTek system:** The radio-TLC plates from before and after the purification modules are shown in Fig. 2. The unpurified product collected from the NanoTek system showed high levels of [<sup>18</sup>F]FDG (47 %) had been produced, but large signals were also observed for unreacted [<sup>18</sup>F]fluoride (<sup>18</sup>F) (25 %) and for partially reacted acetylated-[<sup>18</sup>F]FDG (ACY-FDG) (27 %). When 1 mL aliquots were pumped through the single module, the first 3 mL exhibited very low levels of [<sup>18</sup>F]fluoride and ACY-FDG, while the [<sup>18</sup>F]FDG was present with 91  $\pm$  2 % radioactive purity. However, as more product was pumped through the chip, the [<sup>18</sup>F]fluoride levels increased significantly, reducing the purity of [<sup>18</sup>F]FDG, while the ACY-FDG levels remained relatively steady. The sudden increase in [<sup>18</sup>F]fluoride levels indicated that the ALOX N normal phase particles had become saturated, and so were unable to remove any further [<sup>18</sup>F]fluoride from the crude product solution. Pumping the crude product through the train of modules showed similar results to the first 3 mL that had passed through the single module, with high levels of [<sup>18</sup>F]FDG but very little [<sup>18</sup>F]fluoride and ACY-FDG. However, with twice as much SPE material in the train of modules, more than 5 mL of crude product could be purified to give [<sup>18</sup>F]FDG levels of 90  $\pm$  1 %, with very little ACY-FDG and [<sup>18</sup>F]fluoride, though ACY-FDG levels were starting to increase slightly. These results demonstrate the potential for on-chip SPE-based purification of radiotracers, in this case [<sup>18</sup>F]FDG, as a better means of handling the volumes of product produced by microreactor systems than by employing conventional cartridges. It should also be noted that the purity of [<sup>18</sup>F]FDG was particularly limited by the presence of ACY-FDG (8  $\pm$  1 %), which was due to a high content of acetonitrile present in the final product solution as a result of the production method used. This acetonitrile would have continuously eluted the ACY-FDG from the HR-P reversed phase resin, allowing ACY-FDG to remain in the product solution. In future, the excess acetonitrile could be removed by the addition of a solvent exchange step during the synthesis, which would significantly reduce the ACY-FDG present in the final solution and thereby increase the [<sup>18</sup>F]FDG levels.

**Purification of [<sup>18</sup>F]FDG from ROC platform:** A train of modules was utilized for the purification of [<sup>18</sup>F]FDG synthesized in initial tests of the ROC platform, and Fig. 3 shows radio-TLC plates of product solution before and after being passed through the modules. The results of the unpurified product show successful [<sup>18</sup>F]FDG synthesis was achieved via the newly developed platform, but also showed high proportions of [<sup>18</sup>F]fluoride and ACY-FDG, yielding [<sup>18</sup>F]FDG levels of 53  $\pm$  15 %. The three aliquots of product solution that had been passed through the train of modules, however, show [<sup>18</sup>F]FDG purity levels of 86  $\pm$  3 % were achieved, with ACY-FDG levels of 14  $\pm$  3 %, while [<sup>18</sup>F]fluoride was completely removed from the solution. This again demonstrates the potential of the train of modules for performing the purification of radiotracers, though as before the [<sup>18</sup>F]FDG purity could be increased further by the addition of a solvent exchange step to the synthesis procedure. Further optimization would also be required to ensure that the quantity of SPE particles is sufficient for the amount of radiotracer to be processed within a microreactor system, in order to avoid saturation as observed for the single purification module.

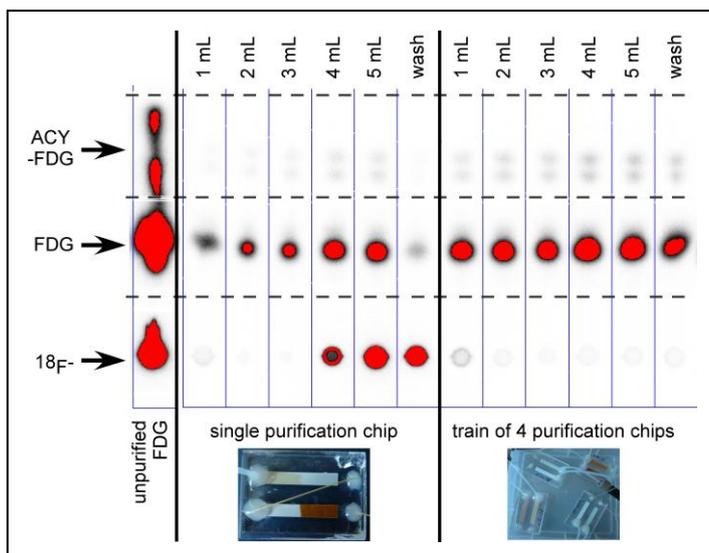


Figure 2: Radio-TLC plates of [ $^{18}\text{F}$ ]FDG produced via a commercial Advion NanoTek system. The unpurified product (on the left) shows high levels of [ $^{18}\text{F}$ ]fluoride ( $^{18}\text{F}$ ) and ACY-FDG impurities. A single purification module was able to purify <4 mL before fluoride started to breakthrough, while ACY-FDG removal remained high. A train of modules was able to purify >5 mL of solution with an [ $^{18}\text{F}$ ]FDG purity of 90 %.

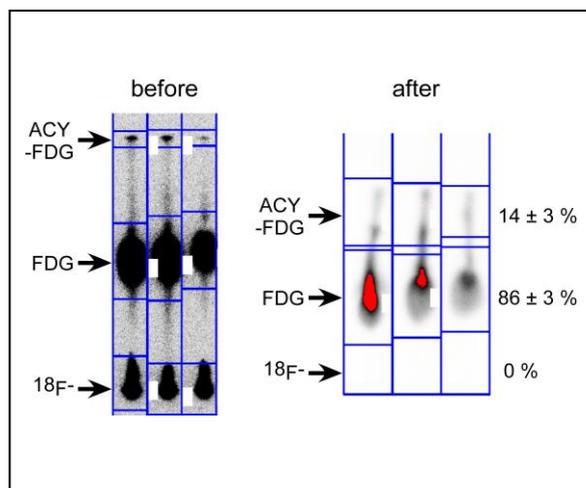


Figure 3: Radio-TLC plates of [ $^{18}\text{F}$ ]FDG synthesized during first tests of the ROC platform. The product mixture before purification (left) showed high levels of [ $^{18}\text{F}$ ]FDG were being produced, but  $^{18}\text{F}$  and ACY-FDG levels were also high. After being passed through a train of purification modules (right), the [ $^{18}\text{F}$ ]FDG purity was 86 %, with fluoride completely removed and only relatively small amounts of ACY-FDG present.

## CONCLUSION

We have demonstrated the on-chip purification of PET radiotracers via the use of microfluidic modules, in particular the purification of [ $^{18}\text{F}$ ]FDG synthesized via a commercial system and the ROC platform. Despite being preliminary trials, the modules already approach the levels of purity needed for [ $^{18}\text{F}$ ]FDG to pass QC tests, and with further optimization will be able to fulfill these requirements. These initial results show great promise for the purification of a range of radiotracers utilizing similar SPE techniques, but in which the same particle types are employed in different amounts and in different orders.

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