

Executive Summary:

The project "Radiochemistry on chip" (ROC) is focused on the fabrication and test of a modular microfluidic platform able to achieve rapid and efficient syntheses of radiopharmaceuticals for Positron Emission Tomography (PET).

The novelty of this projected platform is a "quasi-continuous approach". The production of radiopharmaceuticals starts from a batch of radioactive fluoride (aqueous ^{18}F , 109 minutes half-life) produced at a cyclotron; this production can be performed continuously for 2 hours and can be restarted after this time interval by the introduction of a fresh batch of starting ^{18}F , thus obtaining an unprecedented nearly continuous output of radiopharmaceutical product. Microfluidics has been exploited to reach this outstanding result, since it generally leads to processes with higher yields in shorter times, if compared to traditional vessel-based technologies. In addition, this modality allows the employment of systems having reduced dimensions, thus implying more efficient shielding of radioactivity than in traditional systems and a lower environmental impact due to decreased amount of wasted solvents in cleaning and operation of the apparatus. Compared with other microfluidic devices targeted at producing only 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG), the most common radiopharmaceutical for PET clinical exams, the ROC system has remarkable flexibility due to its modular concept that would allow the synthesis of different radiotracers by easily changing the module interconnections and overall setup.

During the three years of the project, the Consortium was able to fruitfully work and reach the final task in time. At the beginning, an analysis of the materials to be used for the fabrication was performed and the chip design optimized with the aim of obtaining leak free connections in the platform. Then, the several processes involved in a typical radiopharmaceutical production, such as ^{18}F -fluoride pre-concentration, solvent exchange, reactions in microreactor systems, final product purification, have been implemented in separate modules of convenient geometries and optimized with non-radioactive reagents. The functionality of the single chips produced was then tested in radioactive reactions; highly sensitive radiodetectors were implemented. Finally, suitable pumps, valves and tubings were used to interconnect the modular chips and to assemble a fully equipped platform. FDG was synthesized in the prototype platform with incorporation yields comparable to standard methods (about 60%) within ten minutes (time needed to reach the steady state) and continuously for 2 hours. This new approach would allow to obtain a continuous stream of FDG (or of other radiotracer, e.g.: FEC, FET) from which single doses for a single patient study can be drawn, therefore envisaging an unprecedented Dose-On-Demand approach.

Project Context and Objectives:

The aim of this project has been the design, fabrication and implementation of a microfluidic platform able to synthesise radiopharmaceuticals for Positron Emission Tomography (PET) analysis, a technique usually employed in medicine for its unsurpassed sensitivity and specificity. In this diagnostic methodology, a drug with a known biological activity is labelled with a positron-emitting nuclide and injected into a patient; the in vivo imaging of the radioactivity allows monitoring several biological pathways, hence assessing the health of the patient undergoing the exam. The peculiarity of this technique, compared to other nuclear medicine approaches, is that the radiopharmaceutical has to be produced right before the tomographic exam due to the generally short half-life of the positron-emitting species (from minutes to few hours). This feature implies the requirement for quick, easy and reproducible radiochemical procedures that allow obtaining the tracer in purity high enough for final formulation and injection, starting from a suitable precursor and the radionuclide freshly produced by a cyclotron. The general approach requires the use of bulky and complex devices that need to be screened for radioactivity and handled by qualified personnel. The wide spreading of PET all over the world is stressing the need for simplification of the radiosynthetic steps and innovation to achieve a better service and availability to final users of these high profile diagnostic techniques.

We have realized a modular microfluidic architecture that can be used for standard synthesis protocols as well as for R&D of new radiopharmaceuticals. Emerging technologies in the field of micro reactors and micro total analysis systems (μ -TAS) were applied to the synthetic pathways typical for radiopharmaceuticals, in order to improve the traditional approach in terms of efficiency and safety.

To ensure the success of the overall project, we created a strict collaboration between chemists, physics and engineers in an interdisciplinary approach. We built an international network of collaborations between academic partners dealing with the technological side, an industrial partner for checking the system robustness and a clinical institute able to evaluate the end-user outcome. The synergy between all these different expertises allowed us to develop the core technology necessary for implementing the chemical processes involved in radiosynthesis at microscale and to identify the typologies of reactions for evaluating the efficiency and the versatility of the platform produced.

More in detail, PET radiopharmaceuticals are compounds strictly related to molecules of life such as sugars, nucleosides, aminoacids or receptor ligands. These structures must comprise also a positron-emitting nuclide that is inserted in the last steps of the chemical synthesis (during the labelling step), shortly before the patient injection. The atoms amenable for insertion and with suitable radioactive features are commonly ^{11}C , ^{13}N , ^{15}O and ^{18}F . Among all of these atoms, ^{18}F is the most employed because of its relatively "long" half-life (109 minutes) that allows more complex reaction schemes and the possibility of "satellite centers" for the production and selling of the final radiolabelled product to nearby PET centers. Therefore, we concentrated our efforts towards the fabrication of devices able to handle this nuclide, bearing in mind that the technology developed is a base for similar equipment to be used with other nuclides such as ^{11}C (shorter half-life), ^{94}Tc , ^{64}Cu , ^{68}Ga , ^{76}Br (longer half-life) just to name few.

Of the two chemical forms in which ^{18}F can be produced, we focused on the nucleophilic one (fluoride) because of its simpler utilisation and higher specific activity obtainable. $[\text{F}^{18}]\text{F}^-$ is produced by a biomedical cyclotron from enriched $[\text{O}^{18}]\text{H}_2\text{O}$ proton bombardment and is routed to the traditional apparatus for radiosynthesis through plastic tubing systems. This anion is produced as aqueous species and inserted into the pharmaceuticals generally by nucleophilic substitution reactions under proper activation conditions. This activation is generally performed by replacement of water

solution with an organic aprotic solvent and adding selecting group I or II counterions with or without the use of organic kryptands or phase transfer catalysts. The most used system for [^{18}F]F⁻ incorporation utilizes the CH₃CN/H₂O azeotropic distillation for drying the fluoride in presence of K₂CO₃ and Kryptofix 2.2.2 as kryptand agent. The nucleophilicity of such complex is high enough to react with aliphatic good leaving groups and aromatic substrates activated to nucleophilic aromatic substitution. The use of specifically designed precursor, amenable of easy nucleophilic displacement (i.e. bearing good leaving groups such as halogens, nitro, tosylates, etc.) is essential for a good outcome of the labelling reaction; this implies that it is sometimes necessary to use starting precursor that, once labelled with high radiochemical yield, has to be further (and quickly) chemically modified or processed to obtain the final desired product.

The main steps during radiosynthesis and their implementation into integrated modules can be summarized as follows:

- concentration of the aqueous [^{18}F]F⁻ species via anion exchange columns
- one or more steps of heating (for mixing, dehydration,...)
- one or more steps of mixing for addition of reagents, solvents,...
- final product purification, via microfluidic separation

Under these considerations, the required modules are:

- Pre-concentration of H¹⁸F
- Chemical Reactions
- Product purification
- Solvent exchange
- Detection

Whilst targeting the demonstration of a modular chip device, all these points have been addressed separately and the interconnections between the various modules projected ahead.

Modules for pre-concentration (a), mixing of reagents (b) product purification (c) and solvent exchange (d) were achieved by fabricating microfluidic devices. In their simplest form, these devices consist of a network of micrometer sized channels (typical dimensions in the range 10 - 300 μm) etched into a hard substrate. The microchannels are connected to a series of reservoirs containing chemical reagents, to form the complete device or "chip" with overall dimensions of a few cm. Reactions are performed efficiently inside such microchannels due to the fast and well controllable mixing of reagents. Gas/liquid separation processes for achieving solvent exchange are also possible in glass microchannels of suitable geometry. In our platform, pre-concentration and final product purification were obtained by miniaturised anion exchange chromatography.

Micro detectors for radioactivity were developed (e) and located in the platform for monitoring continuously the radioactive flow.

For chemical reactions as well as for the separation and evaporation steps, fluids need to be moved through the microchannels in a controlled manner. This was achieved by conventional pressure

pumping using syringes. Reaction times and sequence of sample introduction were defined by microchannel layout and pumping velocities. Additionally, temperature control is crucial, particularly during the evaporation step. Real-time monitoring of pressure and temperature were realised through automation: in the platform the flow velocity is regulated by an electronic interface directly acting on valves and pumps, according to the synthetic pathway chosen by the radiochemist.

After identifying the tools needed to implement radiochemistry on chips, we optimised the synthetic steps with a non-radioactive isotope of the labelling agent in separated modules, thus reducing the risks for the operators and then we replicated the whole process with radioactive reagents into well-shielded micro reactors by qualified personnel. In the meantime, the operating modules were assembled in the microfluidic platform. The final step was the synthesis of the target radiopharmaceutical, 2-[^{18}F] fluoro deoxy glucose (FDG), in the assembled device.

In conclusion, as final target, we produced a prototype of modular microfluidic micro reactor for radiochemistry able to manipulate reagent concentrations and reaction interfaces in both space and time within the channel network thus providing a level of reaction control, which is not attainable in traditional bulk reactors. In addition, the reduced volume of the micro reactors, minimising waste whilst ensuring the continuous production of radiopharmaceuticals, enables the efficient shielding from radiation to occur allowing radiochemical reactions to be carried out more safely.

Project Results:

The implementation of a microfluidic platform for the synthesis of radiopharmaceuticals needed a number of steps and many efforts from builder and user side. As described previously, the operations of pre-concentration, solvent exchange, mixing for labeling and hydrolysis reactions, purification were performed at microscale. In order to give a complete overview of the main results, we first summarize the studies made by users to implement the operating steps on chip and then those related to the platform implementation. Finally, results on FDG synthesis in the assembled platform are reported.

Operation to be implemented on chip: the Pre-concentration module

We demonstrated results on the design and implementation of three different microfluidic modules for preconcentration and activation of [^{18}F]-fluoride:

1. A batch process where anion-exchange (AE) polystyrene particles are packed into a glass microdevice (Fig. 1a). Their efficiency for the trapping and elution of fluoride was investigated and successfully tested with radioactive fluoride, with the process requiring less than 4 min; furthermore the regeneration of the resin was tested up to 40 times with no decrease in efficiency for fluoride trapping and elution over the experiments.
2. A batch method based on magnetic forces, where trapping and elution of fluoride was achieved by magnetic particle plug formation. A glass chip with 128 parallel channels for multiple plug formation was used together with three neodymium-iron-boron (NdFeB) magnets (Fig. 1b). 1 mL of fluoride [^{19}F -] solution ($1\text{ }\mu\text{g mL}^{-1}$) was pumped through the magnetic particle plug and $0.5\text{ }\mu\text{g}$ of fluoride was successfully trapped. Subsequently 80 % of the trapped fluoride could be eluted with a solution of K_2CO_3 . The same device was successfully tested with radioactive fluoride where it was possible to trap 38% of fluoride and subsequently more than 95 % of fluoride was eluted in a solution of Acetonitrile / K_2CO_3 .
3. A continuous flow method that operates by utilizing the laminar flow regime, whereby a series of alternating reagents are generated in the x-direction and magnetic particles deflected in the y-direction via an external magnetic field, passing through the different streams where trapping and elution of fluoride takes place on the surface of the particles. We are currently optimizing the trapping and elution of the fluoride via this technique, after optimisation of a suitable fluorinated surface treatment to prevent sticking of the positively charged magnetic particles with the partially negatively charge of the glass substrate.

At the end, only the process based on anion exchange resin was chosen to be implemented in the platform due to its higher yields and rates.

Operation to be implemented on chip: the Solvent-exchange module

Continuous solvent exchange requires twice in the synthesis of FDG. The first solvent exchange step from an aqueous to an organic medium is called SEHxL while the step vice versa is named SELxH. Various applicable exchange techniques were chosen and evaluated with respect to their feasibility of miniaturization.

For a continuously operating solvent exchange module different approaches were tested regarding their feasibility of integration into a microfluidic platform. Furthermore the performance in terms of evaporation efficiency and process time was evaluated.

In this project, several approaches to solvent exchange were evaluated: membrane-supported evaporation, electrodialysis, liquid-liquid extraction and continuous-stripping-evaporation. The two latter methods were most successful and are reported in detail in the following.

Experiments were either done with PDMS/glass devices or with glass devices. Furthermore, to validate the methods, we developed a detection method as described below.

Detection methods

At first, suitable detection methods for monitoring the efficiency of the exchange modules without the need of radio-labelled compounds were established.

For the characterization of the modules, the concentration of solvents (here: acetonitrile and water) had to be detected. Due to the different polarity of these solvents, the ratio could be determined by adding a fluoro-solvatochromic dye. Since commercially available dyes were not sensitive enough, a dye with a greater spectral shift (1-methyl-4-[(oxocyclohexadienylidene) ethylidene]-1,4-dihydropyridine, MOED) was synthesised. It is now feasible to measure the ratio between acetonitrile and water with a precision of 1 % in a standard spectrometer.

This method can also be applied as an online detection method to obtain the mixing ratio at specific position in the microchip and hence, further characterize the exchange of solvents. For this, we used an epifluorescent microscope. Here, laser light (Ar-ion laser, excitation wavelength 488 nm) is focused by the objective to excite the sample solution. The fluorescence is collected by the same objective and detected by a fibre-coupled spectrometer. This optical configuration facilitates imaging of the mixtures with high sensitivity and a spatial resolution of about 1 μm .

Besides, for the first exchange step the detection of fluoride is necessary and is accomplished by ion chromatography (IC), having a detection limit below 1 ppm.

Liquid-Liquid-extraction of acetonitrile

The extraction of acetonitrile from the acetonitrile/water mixture can be achieved by addition of butyl-acetate. Butyl-acetate is not miscible with water and hence, can be separated from the target aqueous phase. In first macroscopic batch experiments the expected potential of the butyl-acetate was proven.

The studies performed in the 1st generation solvent exchange glass devices (Cb-5 device, produced in Lecce CNR-NANO) showed a fast and efficient extraction of acetonitrile. The flow rate ratio of a 50% w/w acetonitrile (MeCN) solution compared to butyl-acetate (BuAc) was varied (3:2; 1:1; 2:3). Furthermore the overall flow rate was increased from the left to the right diagram (50 $\mu\text{L}/\text{min}$, 100 $\mu\text{L}/\text{min}$, 150 $\mu\text{L}/\text{min}$). The increase of the flow rate results in a shorter residence time in the microchip without decreasing the performance. Overall the extraction of the 50% solution ends for all conditions in a reduction of acetonitrile to approximately 15% in about 2 to 3 seconds. Under these conditions, the efficiency and speed of extraction is mainly determined by the amount of butyl-acetate.

Due to the regulation for drug synthesis the acetonitrile extraction with butyl-acetate would require an additional cleanup and detection method. Therefore, this approach was declined for the FDG synthesis and not further optimized.

Acetonitrile evaporation

For the solvent exchange modules, evaporation by means of an additional gas was investigated. Briefly, in this method nitrogen is introduced to the liquid stream of acetonitrile and water. In this way an annular flow of nitrogen gas surrounded by the liquid mixture is created. Since the transfer of volatile compounds strongly depends on the surface area, downscaling of this method should be very efficient.

For the investigation of the optimum evaporation conditions various parameters like nitrogen pressure, feed flow rate, flow rate ratios and temperature were varied systematically. The results from experiments at various nitrogen flow rates (given by the nitrogen pressure) and a constant feed flow rate were performed.

Best conditions were found at a nitrogen pressure of 1.2 bar. At these conditions the separation was most stable and we found a reduction of acetonitrile from 50% w/w to 30% w/w. Next, the influence of the feed flow rate on the evaporation was investigated. A range of flow rates between 100 and 10 $\mu\text{L}/\text{min}$ was investigated at constant nitrogen pressure.

The results show clearly that a decrease of the applied feed flow rate leads to an increase of acetonitrile evaporation. At 100 $\mu\text{L}/\text{min}$ feed flow rate around 45% acetonitrile is still remaining after passing the device while at a feed flow rate of 10 $\mu\text{L}/\text{min}$ there is just 18% acetonitrile left. These findings are a consequence of the increased residence time inside the chip. Besides the evaporation efficiency, short process times are of great importance for the overall ROC-platform and hence, as a compromise, 30 $\mu\text{L}/\text{min}$ were chosen as a feed flow rate for further experiments. At the flow rate of 30 $\mu\text{L}/\text{min}$ 25% acetonitrile remained after evaporation, but at the same time the process is 3 times faster than at flow rates of 10 $\mu\text{L}/\text{min}$, i.e. the process requires 5 seconds.

Tests at different temperatures were performed to find the optimum temperature for the process. The increase of temperature increases the evaporation efficiency of the process up to the temperature at which the liquid mixture evaporates, which causes flow instabilities or complete loss of the liquid. In the current design the optimum working conditions are at 60 °C.

To summarize, the developed stripping evaporation device has its operation optimum at a nitrogen pressure of 1.2 bar and a feed flow rate of 30 $\mu\text{L}/\text{min}$ while the temperature should be in the range of 60 °C. Using these conditions a decrease of the acetonitrile content from 50% down to 25% could be shown in one single step. Nevertheless a complete removal of acetonitrile will not be possible in one single step. However, the reinjection of the solution collected after the first evaporation step yield a further reduction of the acetonitrile content down to 7%. A serial operation of these evaporation devices will finally lead to a complete solvent removal.

Separation of gas/liquid and liquid/liquid phases

After the transfer of acetonitrile to another liquid phase such as butyl-acetate or to the gas phase, a separation step is required to obtain finally the aqueous liquid. Based on a design recently presented by Günther et al.¹, different channel geometries were tested to realize the phase separation. Thin

spikes (20 μm width) or capillaries were integrated at one or both sides of the main channel. Due to the hydrophilicity of the chip material (fresh prepared and plasma-cleaned PDMS) the hydrophilic liquid passed these capillaries (due to the capillary forces) while the hydrophobic liquid or gas remains in the main channel. This effect is used to efficiently separate the two phases with different hydrophilicity from each other.

The results obtained from the evaporation tests indicated that a high nitrogen pressure (up to 1.6 bar) is necessary to support the evaporation process. To accomplish the separation with these high pressures, we used thin side capillaries with lower heights than the main channel.

To fabricate this micro device with channels in different heights a double layer technique was introduced. Using two different layers for the main channel and the capillaries enables an independent variation of the geometries and heights. A complete separation of the gas and liquid phases was achieved using the new double layer approach for all relevant flow conditions.

Integration of heating module

Finally, for all evaporation experiments the increase of temperature is necessary. Currently, the second generation of μ -heater devices fabricated by CNR-NANO are used. In these μ -heater devices a thin layer of gold is evaporated onto a glass slide, which is then used for resistive heating of the whole fluidic chip.

In conclusion, different solvent exchange modules were tested and evaluated regarding their suitability. As a result, stripping of solvents in an annular flow regime and the liquid-liquid-extraction of acetonitrile with butyl-acetate were further investigated. All modules are operating continuously. A module for stripping evaporation of solvents was developed and after optimisation of the flow conditions a significant reduction of acetonitrile from 50% down to 25% in one single step and in only 5 seconds was shown. Due to the short timescale of this process a series of evaporation steps will lead to a complete removal in an adequate time.

Solvent exchange with radioactivity

The chip built by ETH was tested as stand-alone (not integrated in the platform), mainly for having a further proof of the absence of a liquid breakthrough in the gaseous outlet. Therefore a radioactive solution (actually a fluoride complex one) was routed to the liquid inlet, while both outlets were collected into two ice-cooled vials. It was evident that virtually no radioactivity escaped from the gas outlet, as further proof of the delicate but useful mechanism of gas/liquid separation, that is the main operating issue with the current Solvent Exchange device.

Operation to be implemented on chip: labelling and hydrolysis modules

A Labtrix start 'micro reactor' system from Chemtrix BV has been used throughout the project to perform studies over the temperature range of 25 to 180°C, with a back pressure regulator set to 15bar to eliminate solvent evaporation.

The two steps were initially optimised separately. For step 1, the fluorination of 1 to 2, the inlets the two solutions (both in acetonitrile) were introduced through. All studies were conducted on a micro reactor with a 10 μ l volume over the area of the heated element.

The flow rates at which the solutions were introduced to the micro reactor were varied to give different residence times of the reaction over the heated element.). It can be seen that at each residence time the conversion increased as the temperature increased up to 150°C after which decomposition of both 1 and 2 were observed. The conversion was higher at longer residence times, the optimum conditions were found to be a 60s residence time and a temperature in the range of 125 - 175°C where the conversion seemed to plateau. The ratio of fluoride to 1 was also investigated, as in hot chemistry the mannose triflate is available in vast excess. As the amount of mannose triflate is increased the conversion to 2 increases (reported in terms of percentage with regard to starting concentration of fluoride, to be comparable to radiochemistry conditions).

The Labtrix start system was then taken to CNR (Pisa) and 'hot' chemistry studies were performed. The fluorination was optimised first, this was done at both a 30s and 60s residence time over a temperature range of 50 to 150°C. The reaction was monitored by both radio TLC and radio HPLC. As expected the conversion for radiochemistry was much higher, probably due to large excess of starting materials and radio detection methods. The trends observed are the same as those seen with non radioactive chemistry, conversion increases as temperature increases. The maximum conversion was observed at 125°C, above which decomposition occurs.

The lower conversion at longer residence times is thought to be due to prolonged exposure of 2 to high temperatures causing decomposition, which was not observed in non-radioactive chemistry due to the low conversions. An investigation into residence time was conducted. They show that at a residence time as short as 3s the conversion does not drop below that observed at 30s; this fast conversion is due to the large excess of mannose triflate present reacting with the fluoride as soon as it is delivered into the micro reactor.

The hydrolysis of 2 to 3 was then investigated using radiochemistry. The hydrolysis was carried out in the same micro reactor as the fluorination, and was introduced via a third inlet after the 10 μ l reactor volume over a heated element in which the fluorination occurs.

The fluorination conditions were set to a 12s residence and a temperature of 125°C, the ratio of sodium hydroxide was altered by varying the flow rate at which it was introduced (the lower the ratio of sodium hydroxide the longer the residence time). As the concentration/ratio of sodium hydroxide increases, the conversion to 3 increases.

It has been successfully demonstrated that production of ¹⁸F-FDG can be achieved on a micro reactor and in a platform similar to the ROC device.

The Labtrix start system was then used to perform other reactions that are of interest in PET chemistry. It was decided to start with the fluorination of 4 to 5 as two PET chemicals can be made from this point, by either reacting 5 with tyrosine to give 6 or with dimethyl ethanol amine to give 7.

The fluorination results show that a shorter residence time gives a higher conversion, with the optimum conversion of approximately 28% being achieved with a residence time of 30s at 150°C.

The next reaction to be investigated was the conversion of 5 to 6 with the addition of tyrosine and sodium hydroxide in DMSO.

The optimisation results for the production of 6 from 5 show that production of 6 is higher at longer residence times. The production also increases as the temperature increases. However at longer residence it was observed that at temperatures above 150°C, decomposition of the both 5 and 6 were occurring. The optimum conditions for the production of 6 from 5 were found to be a residence time of 120s at a temperature of 150°C.

The conversion of 4 to 5, and then 5 to 6 have been successfully performed and optimised on a micro reactor. This highlights the versatility of the ROC platform to perform not only the FDG production but also other reactions of interest in PET chemistry.

Operation to be implemented on chip: the purification module

The aim of this part of the project was to develop microfluidic modules for the purification of [18F]FDG produced via the ROC platform. Conventionally, this is achieved via the use of solid-phase extraction (SPE) cartridges, e.g. a Chromabond Set V cartridge (ABX, Germany), through which the solution is pumped. Such cartridges contain a series of resins, each of which are used to remove contaminants based on certain types of interactions. Here, the dam-type chip design utilised for the pre-concentration module was also applied to the purification modules by filling the chambers with the SPE resins: PS-H+ (cation exchange resin), PS-HCO₃ (anion exchange resin), ALOX N (alumina, normal phase resin), and HR-P (reverse phase resin).

Firstly, the ability of such microfluidic modules to remove fluoride from solutions was determined by passing consecutive 1 mL aliquots of [19F]fluoride through the chips. The anion exchange resin, PS-HCO₃, was tested initially, and it was found that 15 µg of fluoride in pH neutral water could be removed from the system before a loss of efficiency was observed, corresponding to 610 ng of fluoride per mg of resin. However, since the [18F]FDG prepared by the ROC platform would be base-hydrolysed, the PS-HCO₃ resin was tested again but with the fluoride now in 15 mM sodium hydroxide. It was found that the sodium hydroxide eluted the fluoride from the resin, resulting in very little trapping, and similar tests on the PS-H+ and HR-P resins yielded comparable results. The ALOX N resin, on the other hand, was able to trap typically >80 % of the first 18 µg of fluoride in 15 mM NaOH before significantly losing efficiency, equivalent to a trapping capacity of 320 ng of fluoride per mg of resin prior to the efficiency drop. Thus, these results showed that in the presence of sodium hydroxide, only the ALOX N resin was sufficient for the removal of fluoride from a solution. As an added note, a brief study of the PS-H+ resin showed that it was able to neutralise 15 mM NaOH, though only <200 µL per mg of resin. However, since this cation exchange resin is the first in the series of resins in [18F]FDG purification systems, this means that until the PS-H+ is saturated the resultant solution will be pH neutral, therefore allowing removal of fluoride by the PS-HCO₃ resin. Hence, the amount of PS-H+ in a purification device may be important if optimal removal of [18F]fluoride is desired by both the PS-HCO₃ and ALOX N resins.

Subsequent investigations concerned the purification of real [18F]FDG product mixtures using either a commercial Advion Nanotek system, or the ROC platform. Two types of purification module were tested for the [18F]FDG synthesised by the Advion device: a single purification chip containing all four resins, or a train of four purification chips that each featured one resin (essentially doubling the amount of each resin compared to the single purification chip).

These results showed that while less than 50 % of the total radioactivity in the unpurified mixture was due to [18F]FDG, after being passed through either of the purification modules the amount of [18F]FDG was typically around 90 %, thereby demonstrating successful purification of the product. However, the single chip device was only able to purify 2 to 3 mL of solution before [18F]fluoride started to break through due to saturation of the resins. On the other hand, 4 to 5 mL of solution were purified by the train of chips due to the greater volume of resins present, and potentially more solution could have been purified still. However, it was found that the level of acetylated-[18F]FDG ([18F]ACY-FDG) was slightly too high in each case, most likely due to the relatively high volume of acetonitrile in the reaction mixture due to the lack of a solvent exchange step after the fluorination reaction (in order to simulate reaction conditions in the ROC platform and described in the section "Running FDG in ROC platform"). The presence of acetonitrile would thus elute [18F]ACY-FDG from the reverse-phase resin, HR-P, reducing the trapping efficiency of the resin and allowing greater amounts of [18F]ACY-FDG to pass through the purification modules.

A real [18F]FDG sample was also produced using the ROC platform, and 1 mL aliquots of this solution were passed through a train of purification chips that was integrated to the ROC system. Here, three TLC readings were taken and compared to three readings of the unpurified product. While the percentage contribution of [18F]FDG was only 53 ± 15 % in the unpurified solution, after being passed through the purification device the [18F]FDG now accounted for 86 ± 3 % of the total radioactivity, demonstrating successful purification of the radiotracer synthesised by the ROC platform. Of particular note was the lack of [18F]fluoride present in the purified product solution, though the level of [18F]ACY-FDG remained high due to the aforementioned issues concerning the relatively high volume of acetonitrile present, caused by the absence of a second solvent exchange step.

In conclusion, a [18F]FDG purification module was developed that consisted of a train of four purification chips, each filled with one SPE for removing different components from the product mixture. It was found that the PS-HCO₃ resin was suitable for fluoride removal only in pH neutral solutions, while in basic conditions only the ALOX N resin would suffice. It was also determined that whether the solution passing through the purification device is neutral or basic, depends on the amount of PS-H⁺ resin present, which was able to neutralise the basic solution being pumped through the device until it was saturated. Finally, real [18F]FDG samples were prepared via a commercial Advion Nanotek system and by the ROC platform, and in both cases the product was purified to a large extent by the purification modules. However, the degree of purification was not sufficient to produce a [18F]FDG solution that accounted for greater than 95 % of the total radioactivity, as is required by the European Pharmacopeia (2008) for safe injection into a patient. This could be achieved, however, by decreasing the amount of acetonitrile present in the final product solution, either by the addition of a solvent exchange step after the fluorination reaction, the removal of acetonitrile via another means prior to introduction into the purification module, or possibly by greatly increasing the amount of HR-P reverse-phase resin present in the purification modules.

Platform assembly

Chip fabrication

The first step towards the fabrication of micro reactors for synthesis of radiopharmaceuticals has been the choice of materials compatible with radioactivity. ^{18}F -fluoride is a nuclide that decays mainly by positron emission; this particle may interact with the channel walls and strongly damage them. This phenomenon is also observed in the synthetic apparatus traditionally employed in radiochemistry and limited by the replacement of the damaged components.

Glass, pyrex and silicon are standard materials for microreactors because of their highly solvent and temperature resistant properties. Being also used in traditional apparatuses, they are supposed to be relatively stable to radioactivity. Polydimethylsiloxane (PDMS) and SU-8 are recently emerging as alternative to standard glass/silicon microreactors being cheaper and easily to be produced but their resistance to radioactivity is unknown. Fluoropolymers are a form Teflon-like materials to be deposited as coating on glass/PDMS. They are supposed to increase the radiochemical resistance of such substrates, being Teflon used in traditional radiosynthetic apparatuses for tubings and valves.

Small pieces of the above mentioned materials were prepared, treated with different radioactivity doses and characterized by optical microscopy, Quartz crystal Microbalance (QCM) and Atomic Force Microscopy (AFM). Radioactive solutions were injected into glass, Teflon coated glass, SU-8 microreactors and the amount of the retained radioactivity measured.

We found that PDMS reacts with radioactivity generating protrusions and swelling phenomena. Thin Teflon (of about 20nm) coatings don't increase the stability of PDMS because the superficial treatment of plasma and annealing, necessary to activate the PDMS surface before the deposition of the Teflon coating, probably decreases the PDMS stability to radioactivity. Therefore the sample is damaged also after low doses exposure. However, thick teflon films (200nm thick) on PDMS are able to decrease the entity of the damage for low doses, but at high doses they are not strong enough to avoid the formation of protrusions on the underlying PDMS. The resistance of thick Teflon layers to radioactivity is particularly evident on pyrex where no alterations of the deposited layer are detected. Pyrex, glass and Silicon are substrates not affected by these treatments. It is noteworthy that also SU-8 coated silicon substrates are strongly radioactivity-resistant.

From this study it was concluded that bare glass, pyrex, silicon and polymers such as SU-8 and 200nm thick Teflon layers are radioactivity resistant materials and therefore they were considered eligible to fabricate micro reactors for radiochemistry.

Using such materials, three typologies of microreactors (glass, Teflon coated glass and SU-8 coated silicon/pyrex) were produced using standard photolithography, wet etching and sealing (via hydraulic press for SU-8 devices and thermal fusion in the case of glass) procedure and tested flowing inside irradiated water. The test was planned to understand undesired absorption effects of radioactive fluoride in the studied materials. From these tests, it was possible to assess that Teflon coating on glass is beneficial to prevent the small ^{18}F -fluoride adsorption in glass chips and that similar results can be obtained with SU-8 chips.

Another test that was performed was related to temperature, pressure and mechanical resistance of the devices. SU-8 chips were found to be scarcely resistant at temperature/pressure tests, so the attention was focused on glass chips. Even with glass chips Siemens experienced crack problems in the platform. Parameters such as bonding procedure and glass quality were optimized. A number of soda

lime, B270 and borosilicate devices were sent to Siemens to perform temperature/pressure tests. They reported that borosilicate chips are the best choice for fabricating chips to be integrated in the platform.

Additionally, microreactors and solvent exchange chips were equipped with microheaters/sensors to allow controlled heating of liquids in microchannels.

After the fabrication, the sensor elements were calibrated in order to achieve an accurate thermal control. The relation between resistance and temperature was obtained by recording a series of resistance values at different calibration temperatures.

At the end, micro heaters able to stably reach 280°C at V=16V (200°C inside the microchannels) were integrated with microreactors and micro heaters that reach 215°C at 15V allowed to heat liquid inside solvent exchange at 150°C.

Radioactivity detectors

A radiation detector module was developed for the ROC system. The module is used for monitoring of the radioactivity in the waste lines (waste detector) and at any point in the system (flow detector) in order to assess the quality of the process flow.

The development was performed in three stages, from the first prototype (described in D7.1 report), through the second prototype (described in D7.2 report), to the final, third, prototype (described here and in D7.3 report).

Following improvements of the first prototype, the second prototype was produced, comprising:

1. Interchangeable radiation probe,
2. Read-out electronics box,
3. Dedicated software.

Full details of the above components are given in D7.2 report.

Optimization of the second prototype led to the final prototype.

The work on the final prototype included the following main tasks:

1. Improvements of the two gamma radiation probes to avoid connection problems:
2. 13mm x 13mm x 13mm CSI for waste detector
3. 6mm x 6mm x 30mm CSI for flow detector
4. Work on additional beta radiation probe,
5. Further integration and packaging of electronics in a single box,
6. Software modifications as per comments received by the end user (INFN-Pisa),
7. Final detailed software tests and debugging.

The system is based on the scintillation principle. Owing to the use of novel Silicon Photomultiplier technology and know how in electronics design, the following features have been achieved:

1. High sensitivity and accuracy,
2. Low voltage (power),
3. Small size,
4. Low cost.

These features are of interest in several applications, and commercial exploitation of R&D results is planned. This is described in the foreground section of the report.

Platform integration

The Siemens platform, which consists of three main assemblies, was sent to the IFC-CNR Institute in Pisa for real hot test runs in a "hot cell", (therefore for a test run with real radioactive ^{18}F anions in a isolated chamber).

The assemblies are:

The fluidic platform includes four chip housing modules, where the FDG synthesis takes place. A 2-position-6-port switching valve, three mass flow meters, one pressure regulator and four pressure sensors are used to control the different solvents involved. Not yet integrated into the platform is a purification and detection module.

The pumping platform includes five syringe stations, six manual rotary valves for guiding the FDG solvents, several glass bottles for FDG solvent storage and one waste station. The Cetoni pump is vertical positioned to minimize air bubble capture inside the syringes.

The controlling platform includes a Siemens PC computer running a LabView software program to drive the Cetoni pump station and the voltage supply unit to enable a precise temperature and pressure control of the four chip modules involved.

Only the fluidic platform is installed in a "hot cell" near the cyclotron of the IFC-CNR Institute in Pisa.

The highly radioactive solution of ^{18}F in H_2O coming from the cyclotron, is injected directly into the pre-concentration chip module 1 by using an external Advion pump, which is placed inside the "hot cell".

This first injection step transfers the radioactive ^{18}F anions from the water solution onto an exchange resin, which is placed inside the pre-concentration chip. The chamber of the pre-concentration glass chip is filled with commercially available anion exchange resin with a particle diameter of $<100\text{ }\mu\text{m}$. The fluoride of an injection batch of irradiated water is then trapped within the chip.

The water coming out of the pre-concentration chip is pushed into a waste line. After this step, a 2-position-6-port switching valve distributes an acetonitrile/ $\text{K}_2^{222}\text{K}_2\text{CO}_3$ solution into the pre-concentration chip to elute the radioactive ^{18}F anions from the resin. When this is done, the radioactive solution is further pushed into one of the two input ports of the micro-reactor chip 1 in

module 2. The other input port of the micro-reactor chip 1 in module 2 delivers a solution of the precursor for the coupling reaction in MeCN. In this micro-reaction step 1, hence, a hot solution mixture is delivered into the micro-reactor chip and rest water is evaporated.

After the reaction step 1, the solution is pushed further into a solvent exchange chip module 3. A second input port of the solvent exchange chip is used to flush a precise nitrogen N₂ flow into the solvent exchange channel for continuous stripping evaporation.

The solvent exchange chip as well as both micro-reactor chips are fixed and adjusted upon Minco heater foils and controlled by using Minco heater stat controllers.

The electric contact of the heating foil is solved by using spring contact pins.

The nitrogen flow is pressure controlled to generate an annular flow (regime > 250 mbar) inside the solvent exchange channel for an optimal gas/liquid stream flow. Nearly 300 hundred parallel etched glass capillaries (5µm x 10µm) are connected orthogonal to the main exchange channel at the end of the chips output for an optimal separation of the liquid/gas phase. The gas is pushed into a waste line; the gathered liquid coming out of the capillaries is pushed into the next inlet port of the micro-reactor chip 2 module 4. The second inlet port of the micro-reactor chip 2 delivers the solvent NaOH/H₂O.

Running FDG synthesis in ROC platform

The platform constructed by Siemens was transferred into a hot-cell installed at IFC-CNR in Pisa and tested in the production of 18F-FDG. The prototype hosted 4 different types of modules in the following order: preconcentration, 1st microreactor, solvent exchange, 2nd microreactor.

The initial scheme included a SolEx module but data available from parallel studies conducted with model radiochemical reactions demonstrated that: a) small percentages of water might be tolerated into the radiofluorination step, b) careful choice of the incoming flows into the hydrolysis chip would allow a successful reaction.

Therefore, we first tested the radiofluorination of Mannose Triflate by using a K²²²/K₂CO₃ complex that contained 5% of water in acetonitrile, at 125°C. By analyzing the resulting mixtures by Radio-TLC, we obtained very good yields of incorporation, up to 80%.

This result is important since it allows a substantial simplification of the system, while opposing the traditional feeling that a successful radiofluorination is possible only in rigorously anhydrous conditions. On the other hand, non-radioactive chemistry studies demonstrated that a maximum amount of 20% was tolerated when performing the basic hydrolysis step of the produced intermediate, 18F-Acetylated-FDG (Acy-FDG); this allowed us to eliminate the solvent exchange step also in that case by substitution with a careful flow ratio choice between NaOH_{aq} and the Acy-FDG acetonitrile solution.

Optimization of the hydrolysis solution conducted on the ROC platform found out that the best conditions used room temperature process and a ratio of 2/5 in favour of 0,1M NaOH. By using these parameters, we obtained an unpurified mixture containing 60% of FDG.

This result is very important since the radiochemical yield obtained is comparable to the ones reported in traditional (highly optimized) vessel-based synthesizers (60-80%). This represented that the main core of the project was achieved and the major theoretical and technical difficulties were overcome.

We also tested a purification system based on the dam approach reported before; this method allowed to reduce virtually to 0% the fluoride content of the outlet mixture, but some minor modification are needed in order to improve the purity obtainable from 86% to the minimum required for injection, 95%. Once this step is achieved, the final part (not covered in the planning of the present prototype) should be the implementation of a formulation module, that can be envisaged as a simple mixing with hypertonic saline solution and sterile filtration.

Overall, the system has achieved to yield in 60% radiochemical yield, a 86% pure product; what is totally new is that the platform can operate in continuous mode and is linear with time; this would allow to obtain a Dose-On-Demand of tracer in matter of few minutes of waiting time. The platform is

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Potential Impact:

In terms of perspective application of PET, the use of ^{18}F offers many advantages as demonstrated by the clinical success of FDG. In general, ^{18}F -radiopharmaceuticals can be synthesized in quantities sufficient for the formulation of multiple doses from a single production run and shipment away from the production/cyclotron site (the so called "satellite center" philosophy, usually within a 4 hour delivery time, door-to-door). Moreover, it is worth noticing that the introduction of FDG in 1977 [1] revolutionized the field of oncology and lead to its widespread clinical utilization, was given by the possibility to easily automatize its synthesis as optimized in 1986 [2]. This makes clear the importance of high-yielding and easy to access methodologies to synthesize radiopharmaceuticals for their actual wide clinical testing and utilization.

Nowadays, PET market surveys from BioTech systems, Inc., dated October 2006, predict growth rates for the US-market of the order of 20%. The detail for the US-market is:

- FDG for oncology imaging was USD230M in 2005, and was projected to be USD303.8M in 2006 and USD371.3M in 2007. Growth rate of this market was between %16 and 18% per annum until 2012.
- FDG for myocardial viability imaging was USD15.3M in 2005.
- FDG for neurology imaging was USD13.5M in 2005.

This then gives a total market for FDG in the US in 2005 of USD258.8M.

In the USA, the total production of FDG to date is estimated to be about 1.5 to 2 million doses a year with a market volume of about USD300M (for FDG only). The share of commercial producers has increased to > 90% and the rest is left to the academic PET centers. On average PET satellites having one PET/CT scanner are increasing worldwide and PET centers usually perform >2000 scans per year, 95% of them being oncology patients.

EU is actually paralleling this growth proportionally in all the Schengen area countries. Moreover, there is a growing interest in the use of non-FDG tracers to obtain molecules that can target more specifically (higher specificity than FDG) biochemical pathways involved in many diseases. For each of these radiopharmaceuticals that have been recently identified as biologically relevant, there is high debate on the best synthetic ways and, therefore, on the production automation. FDG yields have already been optimized to a very good level (generally > 60% non decay corrected yield) and FDG precursors are reasonably cheap following two decades of development. There is now a great need of better automation, new radiochemistry strategies and cheap precursors for addressing new radiomolecules: it is therefore in this last wide field that a novel general technology allowing the synthesis of different radiomolecules in an easy, cheap and fast way would have a major impact, and hopefully boost on more specific PET analysis, eventually leading to a better understanding of many diseases.

The use of microfluidics in organic chemistry has been demonstrated to give faster reactions and dramatically higher yields with greater selectivity compared with conventional bench top methodology [3, 4]. The enhancement of yields and the speed of processes are due to the integration of all the synthesis, purification and analysis steps into a total system where mixing and transferring procedures are more efficient; this fact is particularly true for radiolabelling reactions in which the

reagents actually used are on a microgram scale. Manipulation of smaller volumes means significantly less use of the exotic chemicals (precursors) currently used.

In the case of FDG, synthesis yields are already commonly 70%, so there is not a strong economic driver there. Similarly, the cost of FDG of precursors (mannose triflate) is actually only a small fraction of the overall cost. So, microfluidic methods do not have a lot of impact on these specific aspects. A different condition concerns dose availability (timing, activity, concentration) at the clinical site where they could even theoretically improve on the current situation, by continuous process flow and simpler dosage form preparation. The situation is indeed very different for new radiopharmaceuticals, such as fluoro-L-thymidine (FLT). Typical synthetic process yields are 25% or less, and the raw material (Boc-Nosyl FLT precursor from ABX, Dresden, Germany) is in the K range per synthesis. Here, microfluidic devices will hold a lot of promises.

Furthermore micro chemical engineering in continuous mode has proven to speed up fabrication times considerably (experience of customers using SiProcess; in chemical development and fabrication reveals factors of 5-10) and going along with higher yield and less efforts time for work up; these aspects are extremely attractive for labeling of costly radiopharmaceuticals with short lived nuclides.

The manipulation of smaller amount of liquids allows to overcome the problem of performing an efficient use of chemicals and cleaning of reactors after each radiosynthesis (thus improving environmental protection); microsystems are also easily shielded by radioactivity, with consequent reduction of the risk level for the operators and considerable decrease of space and resources required by conventional radiosynthetic equipments. Improving control and optimisation may also play an important role in the reduction of fixed costs.

The prototype we have developed may thus be of interest for both academics, and industry as well as have a positive social impact.

Academic interest. In the field of R&D of new radiopharmaceuticals, the high speed and efficiency of processes inside the micro reactors and the flexibility that the modular approach guarantees, allow the exploration of the chemistry with positron emitters which have extremely short half-lives, e.g. nitrogen-13 ($t_{1/2}=9.96$ min) and even oxygen-15 ($t_{1/2}=2.04$ min). An increased number of substrates and reactions can be tested and evaluated in shorter time and with reduced costs.

Industrial interest. Industry may have at least a three-fold interest:

- a) Radiotracers production increase for companies involved in the supply/management of satellite production centers.

On point a), the improvement in production is given by lower precursor costs, quicker personnel training and smaller highly qualified lab space needed: all these features basically allow companies to produce and sell more products with fewer costs. This should eventually foster a denser distribution of production centers all over the EU, an essential requirement for better exploitation of PET diagnosis.

- b) Easier access to radiopharmaceuticals for R&D, such as new diagnostic agents or proof of principle demonstration of completely new drugs for pharmaceuticals companies.

As for point b), it is already well known that the process for a pharmaceutical company to get a new drug into market can be long and expensive; all intermediate processes are charged on the final price of the products and only a few are able to arrive to the end of the long validation procedures. Hence, any methodology capable to provide early answers to the decision making process helps to get new

drugs to the final user in a quicker and cheaper way. PET has shown excellent performances in drug development, in particular by understanding in the mechanism of action of many new drugs by labeling the active molecule with positron-emitting species or by quantitative in vivo pharmacodynamic studies with already acclaimed PET tracers. The possibility to have a great flexibility in the labeling reactions, allowed by the use of modular chip devices projected herein, will therefore give a powerful tool to pharmaceutical companies to complete their validation studies in a quicker and cheaper way.

c) Technology development of microfluidic methods for fine chemistry needs.

Microfluidics poses the way to Process Analytical Technology (PAT); this, along with the use of fewer precursors, less space, higher yields, may be a benefit also in non-radioactive fine chemistry. With its goal of optimizing production processes so that quality becomes an integral element of the process, the FDA initiative "Pharmaceutical Manufacturing in the 21st Century" marks a crucial turning point [5]. PAT not only means a change in thinking in the production process, but also a critical examination of all factors that influence product quality during production. The goals with PAT can be addressed by:

- Quickly gaining knowledge about the key variables of specific processes
- Determining the mechanism to minimize off-spec radiochemicals and maximize product yield
- Creating a high process reproducibility (consistent quality) and early identification of unsuccessful production runs, based on increased process control and understanding
- Controlling even delicate and fast processes 24 hours a day
- Dramatically reduction of Quality Control (QC) for critical low half-life radiochemicals and significant faster approval of doses
- Meeting future regulatory requirements for real-time product release and a risk-based validation approach.

The continuous flow technology in micro reactors developed in this project is a tool to fulfill PAT requirements and maybe used in research laboratories as well as in industrial facilities for the development and production of fine and in particular short living radio chemicals with much higher yield and reliability than today.

Social interest.

Radiopharmaceuticals are a class of drugs that has to be produced and injected in the patients as fast as possible due to their limited lifetime (radioactive half-life). Since they are generally produced in big radioactive batches, any problem affecting the product (failure, out of specification, low yield) may cause product unavailability at clinical site. This situation generates high discomfort to very delicate patients but also high cost and time losses. The apparatus we built allows the synthesis of a single dose of FDG or other radiopharmaceuticals in few minutes, making possible the preparation of a "Dose On Demand" for patients in PET centers. This would allow a more efficient availability and thus significantly improving social benefit and performance of health care systems.

The employment of a small apparatus for radiosynthesis rather than larger traditional systems reduces the amount of chemicals and solvents used during the process and lower waste management.

Additionally, the employment of a small apparatus for radiosynthesis rather than larger traditional systems reduces the amount of washing solvents used during the process and implies lower waste management.

In conclusion, we believe that our prototype guides the way to a possible solution for a routine, high-quality and efficient radiochemistry as an enabler for the breakthrough of novel applications and a facilitated routine use for PET.

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