Microfluidic Systems

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Positron Emission Tomography (PET) and Microfluidic Devices: A Breakthrough on the Microscale?**

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Positron emission tomography (PET) is a powerful noninvasive technique for investigating physiological parameters in the living human and animal body (blood-flow studies, glucose metabolism, receptor properties, drug distribution, and mechanism) after injection of a radiopharmaceutical.[1] These imaging probes are labeled with short-lived radioisotopes (e.g., ^{18}F, $t_{1/2} = 109.7$ min; ^{11}C, $t_{1/2} = 20.4$ min; ^{13}N, $t_{1/2} = 9.96$ min; ^{15}O, $t_{1/2} = 2.07$ min), which necessitates that the reaction process (reaction + purification + formulation + quality control) be as fast as possible. As the chemical reactions are performed on the micro- to nanoscale, special equipment and methods such as miniature reactors and "in-loop" techniques are required.[2] Furthermore, working with radioactivity necessitates careful safety precautions to avoid unnecessary radiation for the operator and the use of computerized systems installed in lead-shielded cabinets (or hot-cells). Because PET radiochemistry represents a relatively new field in chemistry, it is constantly being evolved to improve the techniques for preparing these radiolabeled compounds. Those involved in this task are confronted with enormous challenges as they seek to combine automation with computer science, and fulfill the requirements of the chemical process, radiation shielding, user friendliness, and compactness of the final system to deliver an effective PET chemical production system.

One domain that is constantly expanding and could potentially be a significant help to the PET field is microtechnology and lab-on-a-chip (LOC) technology; the miniaturization of components and equipment with this technology could provide special equipment dedicated to PET chemistry. The appearance some years ago of microfluidic systems[3] for chemical and biological reactions, which contain networks of channels no larger than a few micrometers (10 to 500 μm), offer exciting advantages such as low sample and reagent consumption, acceleration of the reactions, faster analysis, high reproducibility, and automation. These characteristics represent the goals that radiochemists strive to fulfill when synthesizing radiotracers. However, the conventional equipment available today is not always suited to the size or the quantity of material required, which renders the task more complicated than it really should be. Scaling down the chemistry by using microchips or microreactors in this particular PET field could therefore be beneficial, especially considering the timescale, which represents the limiting factor in these syntheses.

In this Highlight, the results of two groups who combined these two modern technologies, microchips and PET, will be presented. Two different approaches are described on the use of LOC technology for radiolabeling of 2-deoxy-2-[^{18}F]fluoro-D-glucose (2-[^{18}F]FDG) by controlling and transferring minute volumes of liquids. The development of the microfluidic technique has also had a significant impact on basic organic synthesis, with potential future applications in the area of PET. One example of such a development is given at the end of this Highlight with work on a gas–liquid carbonylation reaction in the presence of a palladium(0) catalyst.

For some time now, microtechnology has shown its usefulness in chemistry applications ranging from crystallization[4] and drug discovery[5] to pure organic synthesis.[6] The advantages of this method over conventional laboratory techniques are 1) the possibility of controlling and transferring very small quantities of liquids, 2) an increase in specific surface area, giving rise to an enhancement of mass and heat transfer in the system and generating faster reaction kinetics (an advantage that is essential when dealing with short-lived radioisotopes), 3) better product selectivity, and 4) reduced volume of reagents. Minimal resources and space requirements as well as easier shielding represent other practical advantages associated with this technique.

To begin the quest of developing alternative methods for expanding the repertoire of molecular imaging probes, two groups reported the radiosynthesis of commonly used radiopharmaceuticals on microfluidic chips as a proof of principle.

For the synthesis of 2-[^{18}F]FDG (Scheme 1), which is the most widely used clinical PET tracer for imaging tumors in oncology,[7] Gillies et al.[8] applied the simplest form of microreactors: the assembly of three layers of thermally bonded soda-lime glass plates containing three holes as reactants inlets, an etched mixing disk in which the

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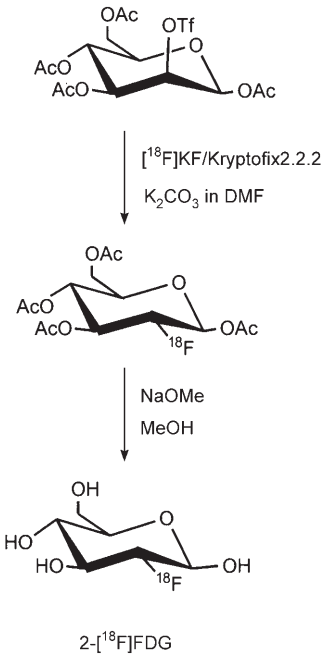

Scheme 1.

reaction occurs, and an outlet (Figure 1). To synthesize 2-[^{18}F]FDG, two microfluidic reactors were linked together in sequence and connected to a series of reservoirs containing the diluted starting materials. Each reactor was designed for one particular reaction, and the process was driven by the continuous flow of reactants through these microreactors (Figure 2). The first reactor was connected to two reservoirs: reservoir 1 containing [^{18}F]fluoride obtained from the cyclotron (500 MBq), and a mixture of KF/Kryptofix 2.2.2/K_2CO_3 (Kryptofix 2.2.2 = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) in di-

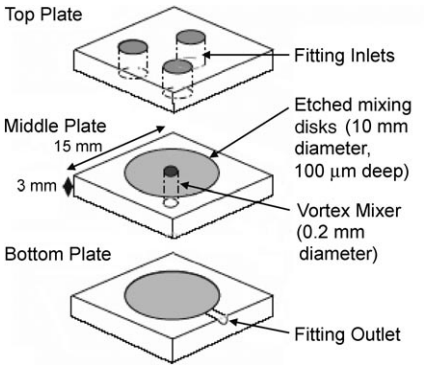

Figure 1. The construction of a microreactor incorporating a three-tier system of inlets, reactor, and outlet. Reprinted with permission from Elsevier.

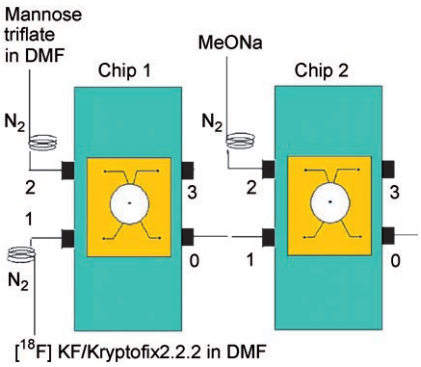

Figure 2. Experimental setup for the production of 2-[^{18}F]FDG in a microfabricated reactor. Chip 1: Radiolabeling of the mannose triflate percursor; chip 2: hydrolysis step to give 2-[^{18}F]FDG. Reprinted with permission from Elsevier.

methylformamide (DMF), and reservoir 2 containing the protected mannose triflate in DMF. The acetonitrile (ACN) usually used for the synthesis of FDG was substituted with DMF because of incompatibilities between ACN and the polymer microfabricated device. The contents of the reservoirs were hydrodynamically pumped under a flow of nitrogen at a flow rate of 250 $\mu L\,s^{-1}$ into device 1, where the fluorination took place. The protected [^{18}F]fluorodeoxyglucose was then pumped into the second reactor, where it was hydrolyzed with a solution of sodium methanolate contained in reservoir 3 to give crude 2-[^{18}F]FDG. The authors produced this radiopharmaceutical in only a few seconds in a 50% radiochemical yield together with 20–30% of unhydrolyzed product and 10–20% of unreacted [^{18}F]fluoride.

In an alternative setup, Quake, Tseng, and co-workers[9] reported the radiosynthesis of 2-[^{18}F]FDG in a much more complex microfluidic chip; the whole synthetic process, including concentration of [^{18}F]fluoride ion on an anion exchange column, solvent exchange from water to dry ACN, fluorination of the mannose triflate precursor, solvent exchange back to water, and acidic hydrolysis, takes place on a single chip no larger than a penny (Figure 3). All these tasks were performed through a tiny network of channels equipped with micromechanical valves, rotary pumps for mixing, and in situ affinity columns. The whole system was monitored by digital control. The chips were fabricated by multilayer soft lithography.[10] The integrated microvalves prevented cross-contamination of reagents and possible leakage between steps of the processes (which can be a problem in flow-through systems such as that developed by Gillies et al.). They were able to produce around 7.4 MBq of [^{18}F]FDG starting from 27 MBq of [^{18}F]fluoride, delivered from the cyclotron, which is a sufficient quantity for imaging of mice. Typically, the synthesis took 14 minutes and gave a 38% radiochemical yield of 2-[^{18}F]FDG with a radiochemical purity of 97.6%. The device used by Quake and co-workers appears to be more reliable for a multiple-step process, although it requires more technical knowledge in the field of microtechnology. In contrast, the device developed by Gillies et al. is much more accessible and has the advantage of being able to trap more [^{18}F]fluoride, which allows larger doses of 2-[^{18}F]FDG.

By way of comparison with these two examples, 25–40 GBq 2-[^{18}F]FDG is produced daily at our PET center in Aarhus in about 25 minutes with a radiochemical yield of 75–80% and a radiochemical purity of approximately 99% by using a GE-Tracerlab MX synthesizer.[11] However, only one production run is performed per instrument per day because of the risk of exposure of personnel to unacceptable doses of radiation from radioactive leftovers in the system.

The work by both Gillies et al. and Quake and co-workers has demonstrated the proof of principle that microfluidic technology in radiochemistry can be successfully applied to produce an important radiotracer that is used daily for oncological investigations. Nevertheless, further optimization to increase the radiochemical yields and purities is required before this interesting technique can be used on a routine basis at a PET center.

In PET chemistry, the use of [^{11}C]carbon monoxide represents an interesting avenue to a whole range of PET probes such as amides, esters, lactams, and lactones. Because of the poor trapping of carbon monoxide in solution, these target molecules are not easy to synthesize, especially when micromolar scale reactions are used. Långström and co-workers elegantly adapted

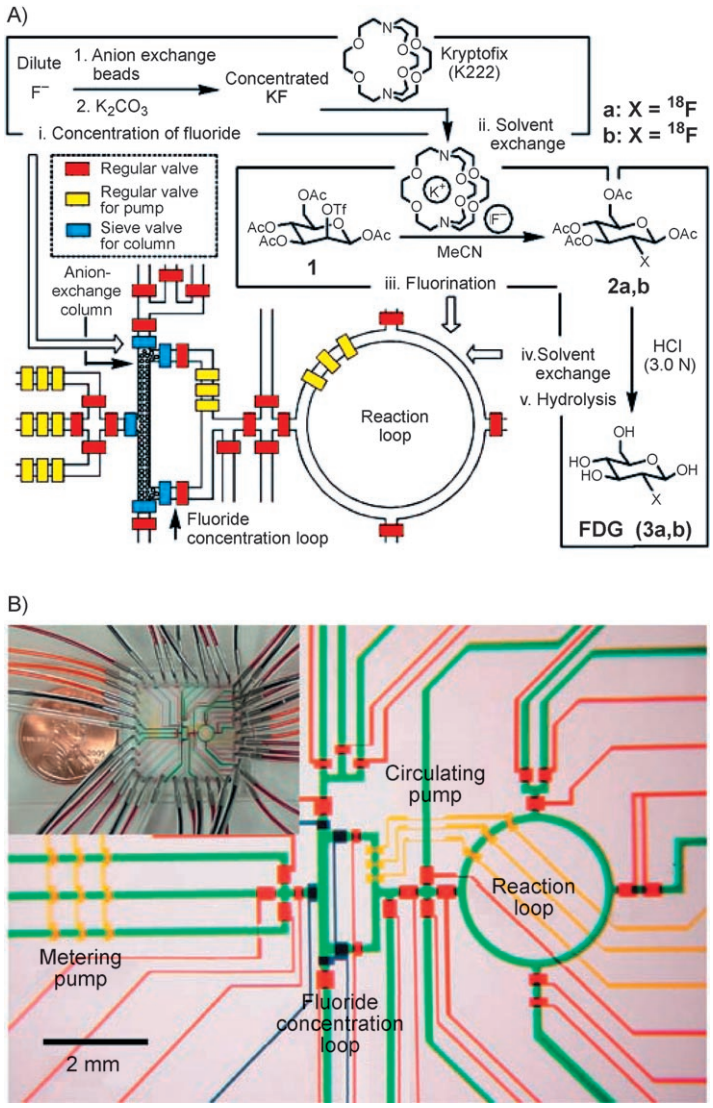

Figure 3. A) Schematic representation of a chemical reaction circuit with five sequential processes used in the production of 2-[^{18}F]FDG. B) Optical micrograph of the central area of the circuit. Inset: View of the device compared with a penny (diameter 18.9 mm). Reprinted with permission from the AAAS.

the high-pressure method to the radiochemistry field using [^{11}C]carbon monoxide gas in the presence of a zerovalent palladium catalyst in microautoclaves.[12] The specialized equipment required (autoclave systems) nevertheless limits its broader use. Hence, a more facile means of synthesizing these interesting [^{11}C]CO-incorporated molecules is still required.[13]

Miller et al. recently demonstrated the possibility of applying microreactors as an alternative method for carrying out gas–liquid phase carbonylation reactions.[14] In their microsystem, two factors help to increase the carbon monoxide solubility and thus facilitate the insertion step of the catalytic cycle: 1) an increased interfacial gas–liquid contact area and 2) an increase in the carbon monoxide pressure produced in the system. These two features allow faster reactions and higher yields relative to their corresponding batch reactions run under pressure. A solution of aryl halide in benzylamine and a palladium catalyst was infused in such a way as to provide a stable annular flow regime into a microfluidic device containing a 5-m-long reaction channel. A steady stream of carbon monoxide gas controlled by a mass-flow controller was then mixed into the chip with heating to generate the *N*-benzylbenzamides after a two-minute chip residence time. The yields obtained were in the order of 46–58%, which are much higher than the yields obtained for the same reaction under high pressure of carbon monoxide over a 10-minute period, which clearly demonstrates the potential of the microreactor method. Undoubtedly, further optimization of the reaction conditions and the catalysts in the microreactors will provide improved yields of carbonylation. The next and very exciting step will then be to investigate the possibility of applying this technique with ^{11}[C]carbon monoxide chemistry.

Irrespective of the degree of complexity of the microfluidic chips discussed herein, the synthesis of the widely used radioactive compound 2-[^{18}F]FDG can be prepared in useful amounts, which demonstrates the adaptability of this technique to PET chemistry. Although the synthesis of this tracer through LOC technology is probably not commercially viable (it is difficult to surpass the well-established robotic synthesis and its rentability), its ability to produce an important tracer will undoubtedly speed up the introduction of the microfluidic systems in radiochemical applications for many alternative reactions.[15] The major problem in radiochemistry will now be to localize the initial applications of this technique. The small size of LOC technology is quite appealing when compared to the large equipment currently required. The next step will be to increase the versatility of LOC technology. For example, will it be possible to apply this technique to 1) [^{11}C]-methylations, one of the key reactions in PET chemistry for the synthesis of neuroreceptor radioligands or 2) carbonylation reactions with [^{11}C]-carbon monoxide? Could one particular chip be used for a particular reaction and be reusable? Will it be facile for nonexperts in microfluidics to prepare such chips? These are but a few of many questions that have to be answered before PET and microfluidic techniques can be combined and revolutionize the microscale. In any event, a "micro"-revolution is slowly taking place and it will be exciting to follow its development in the future.

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