A Digital Revolution in Radiosynthesis

The rapid growth in the use of PET has led to a concomitant demand in the availability of imaging tracers and research directed toward more efficient and flexible radiosynthetic routes. The production of multiple PET tracers presents a challenge to radiopharmaceutical production facilities; production of each tracer uses a specialized synthesis module optimized for that particular tracer, with each module located in a shielded hot cell. In an ideal world, there would be sufficient space for as many synthesis modules as needed to meet the clinical and research demand for PET tracers; the real world, however, imposes its own constraints. In many cases, a low demand for a particular novel tracer would preclude the purchase or development of a synthesis module for its preparation, so the development of a synthetically flexible device capable of producing a single dose on demand would be of great utility. The development of microfluidic reactors for the production of radiopharmaceuticals, easily capable of providing a single dose, provides a means to greatly expand tracer synthesis for both clinical and research needs.

See page 321

Microfluidic reactors generally fall into 3 broad classes—continuous flow, droplet, and batch—and can vary in design from something as simple as a single channel with multiple inlets to exceedingly complicated devices with numerous valves and reaction chambers capable of performing multiple chemical operations in sequence. Continuous-flow devices consist of one or more streams of reagents coming together and reacting, whereas droplet devices produce a droplet of one or more reagents surrounded by an inert carrier fluid with the desired reaction occurring within the droplet. A batch device is perhaps the most commonly used design in radiopharmaceutical production, with the reagents delivered into a reaction chamber and then allowed to react, followed by elution from the device. Microfluidic reactors can be fabricated from a variety of materials but perhaps most commonly the elastomer poly(dimethoxysilane). They comprise enclosed microchannels (normally 10–500 μm wide or tall), mixing units, heaters, and pumping systems and are able to control and process chemical or biologic reactions in a continuous flow manner or batch mode. Microfluidic radiolabeling:

- Offers the ability to manipulate small volumes, which mitigates issues associated with dilution effects.
- Offers efficient mixing, which greatly improves reaction kinetics.
- Provides an exquisitely fine level of control over reaction conditions, such as concentrations and temperature, enabling reliable and reproducible labeling.
- Presents a much smaller overall footprint of the system, drastically reducing the volume that requires shielding.

The report by Javed et al. in this issue of *The Journal of Nuclear Medicine* (*1*) represents an important contribution in the development of a mechanically robust microfluidic radiosynthesis device based on the electrowetting-on-dielectric (EWOD) principle. This all-electronic device requires no fluidic systems (pumps, channels, or valves) and is capable of producing a batch of ^{18}F-labeled radiopharmaceutical on the single-dose scale. EWOD devices are constructed from inorganic materials and consist of 2 parallel plates separated by a gap (~150 μm in this case): a bottom plate patterned with electrodes and coated with dielectric and hydrophobic perfluoropolymer layers and a conductive cover plate serving as the ground electrode also coated with dielectric and hydrophobic perfluoropolymer layers. Droplets of liquid are sandwiched into a disk shape between the 2 plates, and electrical potential is applied to individual or multiple electrodes, which modifies the surface tension of the liquid causing the droplet to flow in the direction of reduced surface tension. By programming the activation of electrodes, operations such as droplet generation, transport, splitting, and merging of individual droplets can be performed anywhere on the chip. In previous work, an EWOD device was used to synthesize FDG (*2*), whereas in this work the authors present an optimized synthesis of 3′-deoxy-3′-^{18}F-fluorothymidine (^{18}F-FLT) in a decay-corrected radiochemical yield of 63% in 63 min and an average specific activity of 2,100 GBq/μmol. Rather than simply reporting a successful microfluidic synthesis of ^{18}F-FLT, the authors also subjected the produced tracer to all of the quality control tests required by the U.S. Pharmacopeia for human use: pH, chemical purity, residual solvent analysis, and pyrogenicity.

The EWOD device developed by the authors measures 4.5 × 4.7 cm and performs a total of 3 synthesis steps: fluoride activation, nucleophilic substitution, and hydrolysis. A droplet of ^{18}F-tetrabutylammonium fluoride (TBAF) complex (preformed from tetrabutylammonium bicarbonate and no-carrier-added ^{18}F-fluoride ion) is added to the chip and transported to the reaction site located on a ring of 4 concentric resistive heaters, followed by a drop of acetonitrile, which is then mixed with the ^{18}F-TBAF. The solvents are then azeotroped off at 105°C and the drying process repeated to produce activated fluoride ion. A droplet of the ^{18}F-FLT precursor (3-*N*-Boc-5-*O*-dimethoxytrityl-3-*O*-nosyl-thymidine) dissolved in dimethyl sulfoxide and 2,3-dimethyl-2-butanol is then transferred to the reaction site, followed by a droplet of acetonitrile. The reaction is then heated and allowed to react at 120°C for 3 min, undergoing the fluorination reaction. The third and final step is the hydrolysis of the product, removing the *tert*-butyloxycarbonyl (Boc) and the 4,4′-dimethoxytriphenylmethyl protecting groups with 2 sequential droplets of 1N HCl in acetonitrile at 95°C for 1.5 min. The top plate is then removed, and the crude product extracted with a sequence of water,

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acetonitrile, and dimethyl sulfoxide totaling 268 μL. The crude product is then purified on a custom purification cartridge consisting of a mix of resins and alumina packed into a 1-mL syringe. The ^{18}F-FLT is trapped on the column while the impurities are removed, followed by the final elution of the tracer in 0.5 mL of ethanol into a sterile vial. The ethanol is removed, and the tracer is then formulated for use in sterile saline; the entire process including the cartridge purification occurs in 63 min. Each of the 3 steps was carefully optimized to maximize the radiochemical yield, but no hardware changes to the EWOD device were required, merely optimization of reaction times and droplet volumes.

The reported specific activity of the ^{18}F-FLT produced (average of 2,100 GBq/μmol) is significantly higher (>10 times) than that found in the literature and is attributed to the reduction of radiolysis offered by the small dimensions of the EWOD device. In 2012, Rensch et al. reported the results of both experiment and simulations on the reduction of radiolysis due to the confinement of the radioactivity in small dimensions, as is typical in microfluidic devices (*3*). These studies found that most of the positron energy from ^{18}F is deposited into the walls of microfluidic chips rather than in the reaction mixture when the dimension of the channel (microfluidic chip) or gap (EWOD) is smaller than the positron range (~400 μm for ^{18}F). The high specific activities found by both Rensch and Javed suggest an additional advantage of microfluidics for the production of radiopharmaceuticals.

This work represents an important milestone in the development of a robust, simple-to-operate, microfluidic radiosynthesis device capable of producing dose-on-demand PET tracers. EWOD-based devices have been shown capable of producing ^{18}F-FDG and now ^{18}F-FLT, perhaps a more relevant example of microfluidic radiopharmaceutical production. At most sites, the demand for ^{18}F-FDG is best met by synthesizing a large batch of ^{18}F-FDG; the demand for ^{18}F-FLT would typically be much lower and thus the more economical choice would be to use a dose-on-demand synthesis. As more novel PET tracers translate into the clinic, the radiopharmaceutical production facility is faced with a choice, devote funds and hot cell space devoted to the automated synthesis of a tracer of possible low demand or rely on a largely manual synthesis requiring trained synthetic chemists. Digitally controlled EWOD devices present an important new flexible option. The necessary reagents need to be introduced onto the inlets of the chip at the start; the synthesis program then controls the chemistry, delivering droplets of the correct reagent to the right location on the chip at the correct time. In addition to synthetic flexibility, microscale devices such as this EWOD chip generally are capable of delivering higher specific activities and require smaller amounts of expensive precursors. As devices such as this move from the developmental phase into commercial availability, expect widespread adoption, freeing up hot cell space and expanding the pool of available PET tracers.

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