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Microfluidics without channels: highly-flexible synthesis on a digital-microfluidic chip for production of diverse PET tracers

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EXECUTIVE SUMMARY

Positron emission tomography (PET) imaging is used for fundamental studies of living biological organisms and microbial ecosystems in applications ranging from biofuel production to environmental remediation to the study, diagnosis, and treatment monitoring of human disease. Routine access to PET imaging, to monitor biochemical reactions in living organisms in real time, could accelerate a broad range of research programs of interest to DOE. Using PET requires access to short-lived radioactive-labeled compounds that specifically probe the desired living processes.

The overall aims of this project were to develop a miniature liquid-handling technology platform (called "microfluidics") that increases the availability of diverse PET probes by reducing the cost and complexity of their production. Based on preliminary experiments showing that microfluidic chips can synthesis such compounds, we aimed to advance this technology to improve its robustness, increase its flexibility for a broad range of probes, and increase its user-friendliness.

Through the research activities of this project, numerous advances were made.

- Tools were developed to enable the visualization of radioactive materials within microfluidic chips.
- Fundamental advances were made in the microfluidic chip architecture and fabrication process to increase its robustness and reliability
- The microfluidic chip technology was shown to produce useful quantities of an example PET probes, and methods to further increase the output were successfully pursued.
- A "universal" chip was developed that could produce multiple types of PET probes, enabling the possibility of "on demand" synthesis of different probes
- Operation of the chip was automated to ensure minimal radiation exposure to the operator

Based on the demonstrations of promising technical feasibility and performance, the microfluidic chip technology is currently being commercialized. It is anticipated that costs of microfluidic chips can be dramatically reduced in the future by leveraging economies of scale in the microelectronics industry, leading to low cost production of PET probes.

SUMMARY OF PROJECT ACTIVITIES

Original Aims

Our overall goal is to develop novel, user-friendly, and low-cost radiosynthesizers based on microfluidic chips to reliably supply positron-emission tomography (PET) radiotracers for a broad range of applications that are of interest to DOE programs, allowing users to produce, at the point-of-use, whichever tracers best suit their applications. PET imaging is used for fundamental studies of living biological organisms and microbial ecosystems in applications ranging from biofuel production to environmental remediation to the study, diagnosis, and treatment monitoring of human disease. Based on preliminary experiments showing that electrowetting-on-dielectric (EWOD) microfluidic chip technology can synthesize radiolabeled compounds, we aimed to advance this technology to improve its robustness, increase its flexibility for a broad range of tracers, and increase its user-friendliness through the following specific aims.

Aim 1*: Build a system combining droplet operation and radioactivity monitoring on chip to facilitate rapid advancement of EWOD technology for tracer production.*

Aim 2: *Develop a reagent loading interface for EWOD radiosynthesis chips*.

Aim 3: *Optimize the process for drying [^{18}F]fluoride, the first step in the production of most F-18-labeled radiotracers.*

Aim 4: *Develop and characterize unit operations on EWOD chips (evaporation zones, reaction zones, mixing zones, etc.).*

Aim 5: *Develop and provide EWOD radiosynthesizer chips*.

Aim 6: *Develop and provide preliminary prototype system*.

Results

Objectives for the overall grant period have been met. Specifically, the following results have been achieved.

Aim 1:

An EWOD chip development/test platform was designed and built. For visualization purposes, the chip is installed in a holder within a light-tight enclosure. A rotatable mirror above the chip directs light to either a conventional CCD camera for observation of the chip under normal illumination, or to a sensitive CCD camera for collection of faint Cerenkov light produced during positron-decay of radioisotope-containing liquids being manipulated within the chip. This optical design is an improvement upon our previously published work: (1) The use of the mirror in conjunction with longer focal-length lenses enables placement of radiation shielding between the chip and the CCD, reducing CCD noise and damage by direct gamma irradiation; and (2) The mirror allows the use of the second camera, which provides superior images and faster video rates (compared to the sensitive CCD camera) for monitoring operations on the chip. We calibrated the Cerenkov setup by loading droplets of liquid containing [F-18]fluoride into an EWOD chip and integrating the total light output in a region of interest (ROI) as a function of time. The calibration curves were found to be very linear and to depend slightly on the solvent used (i.e. water, acetonitrile or dimethyl sulfoxide). After calibration, it is possible to quantify the amount of radioactivity within an ROI at any time during the synthesis if the solvent is known. The entire setup was placed behind a compact lead L-block in a fume hood to provide radiation shielding for operator safety while minimally hindering pipetting of reagents to the chip. In addition to the imaging system, electronics were developed to apply voltage to individually-addressed electrodes to cause droplet transport or mixing, or to apply current to perform local heating. To reduce the size, cost and complexity of our previous control setup a custom circuit board was designed as a compact replacement for the signal generator, voltage amplifier, digitally-controlled switch box, and data acquisition module. Leveraging separate funding, remaining functions (temperature control, impedance detectors, etc.) were integrated into a single control circuit board. The product of this aim is a control and visualization system that can be used for developing EWOD microfluidics for radiochemistry and PET applications.

As we started using the Cerenkov imaging system on a routine basis, small discrepancies between measurements of radioactivity from Cerenkov images and a dose calibrator were noted. We considered various factors that could affect the accuracy of the Cerenkov measurement, including variation in the gap height between the two EWOD substrates as well as droplet properties such as type of solvent, solute concentration, and liquid coloration. Using Monte Carlo simulations, we found that the majority (>2/3) of the positron energy available to produce Cerenkov photons is deposited in the glass plates of the EWOD chip, and only a small fraction deposited in the droplet. Thus, variations in index of refraction due to solute concentration or type of solvent were not expected to significantly impact Cerenkov light output. Indeed, experiments with varying ratios of acetonitrile, dimethylsulfoxide, and water showed Cerenkov output to have only a small dependence on composition. Variations in gap height from the nominal height of 150 µm were also explored. For gap heights as low as 100 µm or as high as 200 µm, only a maximum of 5% change in signal or less was observed, depending on the type of solvent. Droplet coloration, on the other hand, can have a significant impact on results. Coloration blocks light produced in the droplet as well as light produced in the glass plate furthest from the light detector and leads to a significant underestimate of the radioactivity in the actual synthesis. Indeed the biggest discrepancy between radioactivites computed from Cerenkov images and measurements from dose calibrator occurred after radiosynthesis steps associated with a significant color change, e.g. the yellow/brown color that appears after the fluorination reaction. These studies helped us to understand limitations of the Cerenkov method, and could assist future research efforts to enable accurate Cerenkov measurements despite coloration in solutions. Despite the limitations, the use of in situ Cerenkov imaging has led to numerous fundamental insights about the microscale chemistry process and has facilitated optimization of chip designs and process parameters (see below).

Aim 2:

To eliminate the need for manual pipetting of reagents to the chip and thereby increase safety and ease of use, we investigated approaches for automated reagent dispensing. Since many reagents involved in the radiosynthesis of tracers for PET are volatile or sensitive to moisture/air, they must be stored in sealed containers until needed. We developed and compared 2 approaches for dispensing small volumes of reagents from septum-sealed vials.

In the first approach, the reagent is drawn into a glass syringe and dispensed via tubing to the chip. A tiny blunt needle was positioned by a custom fixture so that its opening was located adjacent the gap between the two substrates of the chip. To compensate for evaporation of volatile liquid at the needle tip, the syringe pump advances the liquid until it is detected by a sensitive, on-chip liquid sensor, then pumps the desired volume of the reagent in through the side of the chip. Sensing is based on the change of AC current needed to drive the EWOD electrode due to impedance difference of air and liquid. With correction for evaporation, accuracy of dispensing was dramatically improved. Using this system to deliver acetonitrile on-demand at multiple points through the synthesis, production of 2-[F-18]fluoro-2-deoxy-D-glucose ([F-18]FDG) with reduced manual intervention was achieved.

The main distinction of the second approach is that the liquid does not contact any active components (i.e. pumps or valves); instead, the fluid path from the reservoirs to the chip is made from inexpensive components that can be disposable (to eliminate the need for cleaning). Inert gas drives liquid up through a tube toward the chip, where it is detected by impedance change. When this occurs, pressure is removed, and gravitational force acting on the column of liquid within the tube pulls excess liquid from the chip, leaving only a volume of liquid covering the activated sensing electrode. Pneumatic loading approaches found in the literature are not suitable for chemistry applications because Teflon-wetting liquids such as organic solvents would spontaneously flood the EWOD chip. Tube and electrode geometry, and operating pressure were optimized for a diverse set of liquids with different properties. Water, as well as several organic solvents and ionic solutions were successfully loaded as demonstrations and exhibited good droplet volume reproducibility.

Another aspect of this Aim was to evaluate methods for increasing the amount of [F-18]fluoride loaded into the chip. One strategy is to sequentially load droplets (10-20 µL in volume), each time evaporating the solvent to make room for the next droplet. Using Cerenkov imaging and a dose calibrator, we confirmed that the total amount of activity on-chip matched the sum of activities in individual droplets. While suitable for modest increases in loaded radioactivity, this method is not scalable to large volumes (e.g. ~1mL of cyclotron target), due to limitations of the droplet evaporation time and the large number of droplets that would be needed. As an alternative approach, instead of squeezing the initial droplet between the plates (i.e., into a disk shape), we also evaluated a second method in which we loaded and heated a much larger droplet adjacent to the chip. The droplet was loaded on the extended surface of the bottom plate near the edge of the top electrical ground plate, and allowed to maintain its hemispherical shape. (This shape allows a much larger volume to be loaded onto the same chip footprint.) A 200 µL droplet of fluoride with base and phase transfer catalyst was heated outside of the gap and then pulled between into the gap within the chip by EWOD actuation after its volume reduced to 5 µL. Within the gap, the droplet could shrink more or be completely dried with further heating. The total heating time for the 200 µL droplet was 12 minutes, but the 10 minutes outside of cover plate could potentially be reduced with the addition of a dedicated heater outside of the cover plate. Calibrated ion chamber measurements before and after concentration showed that negligible radioactivity was lost during the whole process. We also evaluated a third strategy based on trapping of [F-18]fluoride on a custom-made, miniature, quaternary methyl ammonium (QMA) cartridge and releasing in a small volume of eluent. Using 10 mg of QMA resin packed inside tubing, we have demonstrated reliable trapping from 1 mL (1000 µL) of [F-18]fluoride in [O-18]water and release into 25-50 uL of eluent (acetonitrile, water, K_2CO_3, Kryptofix K222) with >90% overall efficiency. The eluted activity could then be loaded into the chip via the pneumatic loading mechanism. The latter approach is the most scalable, potentially enabling the radioactivity within the entire cyclotron target volume to be concentrated into a droplet compatible with the chip. Furthermore, it is directly compatible with the pneumatic reagent loading mechanism described above.

A final aspect of this Aim was to develop a method for collecting the final PET tracer from the chip. Unfortunately, during the radiosynthesis process, the wetting properties of the Teflon layer become altered and it is difficult to subsequently transport liquid away from the reaction site. To overcome this challenge, we developed a simple approach for efficient collection of the crude radiolabeled compound from the chip. Droplets of water (or other collection solvent, such as MeOH/H_2O) are loaded into the chip via an unused reagent loading inlet, and transported to the heater site to dissolve/dilute the product. The entire reaction site is filled up to ensure collection of as much of the product as possible. A vacuum is then applied to one of the other unused reagent vials (empty), and electrodes leading from the reaction site to the corresponding hole are sequentially activated in a cyclic fashion. The combination of this actuation and the airflow generated by applying vacuum was sufficient to move the diluted product toward the reagent loading site, and down through the needle into the empty vial. We observed the diluted droplet to remain intact during this collection process, thus leaving negligible residual liquid behind in the chip. If desired, these steps could be repeated to rinse the reaction site a second time and ensure high recovery of the radioactive product. An important advantage of this approach was that it did not require modification of the original chip design, and furthermore, it was compatible with the reagent loading setup with minor changes (i.e. supplying vacuum to one vial instead of pressure).

Aim 3:

Several aspects of [F-18]FDG radiosynthesis have been optimized to increase overall yield and reliability. We first optimized fluorination and hydrolysis conditions as a function of reaction parameters (time, temperature, and concentrations of precursor, Kryptofix, and K_2CO_3). In addition, we added to the setup a small needle positioned to direct a gas flow between the two substrates of the chip. In initial experiments, solvent vapor released during evaporations (e.g. during drying of [F-18]fluoride) was observed to condense around the heater due to the lower temperature away from the heater. The gas flow more effectively removes vapor, and thus accelerates evaporation and reduces cross-contamination. Micrographs comparing the distribution of condensed vapor with and without gas flow show a dramatic difference (>50% of region surrounding the reaction site is vapor-free when gas is used.) With these optimizations, reliable synthesis was achieved. We also performed extensive chemical characterization to establish if the tracer produced on chip would pass US FDA quality control requirements for human injection. All tests did pass, and the amounts of Kryptofix, residual solvents, etc. were far below acceptable limits.

We then proceeded to optimize the synthesis. Cerenkov imaging was used to optimize the on-chip mixing process. During solvent exchange processes (e.g., azeotropic drying of [F-18]fluoride), the dried residue must be uniformly redissolved in the new solvent droplet for efficient downstream reactions. Among several strategies - passive diffusion, heating to a modest temperature (60°C) to induce flows within the droplet, and active movement of droplet by EWOD actuation - the latter resulted in homogeneous distribution of radioactivity in the shortest time (~30 sec).

Cerenkov imaging has also been indispensable in further synthesis optimization, specifically in analyzing the losses of radioactivity during the synthesis. For example, contrary to expectations, some radioactivity (determined to be [F-18]fluoride) was observed in the condensation surrounding the reaction site. In terms of radioactive species, the composition of this condensation was found to be mostly unreacted [F-18]fluoride. While the exact mechanism of radioactivity distribution outside the reaction site is unknown, this knowledge enabled the design of a new chip with electrode pathways directed toward the reaction site. These paths collect and transport the condensation back to the reaction mixture on the heater. Significant improvement (~15%) in yield resulted. Cerenkov images have also revealed losses of radioactivity at several points during the synthesis, enabling further optimization. Overall, using [F-18]FDG as an example, we were able to use the Cerenkov imaging technique to enable an overall increase in the crude radiochemical yield from 50±3% (n=3) to 72±13% (n=5).

One further finding was that the specific activity of [F-18]FDG produced on the EWOD chip is significantly higher than that of [F-18]FDG synthesized by conventional macroscale means. Preliminary experiments show a 25-50x improvement. Our hypothesis is that this is due to the smaller reaction volume which reduces contamination by fluorine-19 found in reagents and solvents. We were successful in obtaining separate funding to extensively study this particular issue in more detail. Indeed we confirmed that with microvolume reactions (a few µL), we could routinely obtain specific activities of 20 Ci/µmol or more. Typical values are 1 Ci/µmol. We also established that the presence of a fluoropolymer coating on the chip does not seem to adversely affect the specific activity, even up to hundreds of mCi exposure to the chip surfaces for up to 3 hours (longer than any expected reaction). (In macroscale systems, the use of Teflon tubing and stir bars is known to have a negative impact on specific activity.) Finally, we showed that high specific activity can be achieved, even starting with very low starting radioactivity. In conventional approaches, typically one must use very high starting activities, e.g. >1 Ci, to end up with useful specific activity, even if only a small amount of radioactive probe is needed for experiments. With microvolume synthesis, we showed that high specific activity could be obtained for starting activities as low as 10 mCi. (Lower starting activity has not yet been tested because it would likely result in insufficient quantity of final product.)

Aim 4:

As described below in the progress for Aim 5, several iterations of the chip design were made. Many of these were related to improving the performance of evaporation, reaction, and mixing operations. The central feature of the final chip design was a 12mm-diamter heating region, where these three types of operations were performed. With a droplet height (spacing between chip substrates) of 150 µm, this reactor could accommodate ~16µL of solution. The electrodes in the heater region consisted of 4 concentric ring heaters, each with independent feedback control of temperature. Temperature readout for each ring was achieved by measuring its resistance. Calibration was performed for each heater of each chip using a temperature controlled bath. In heating mode, current is passed through each ring electrode via its two electrical connections, but the concentric electrodes could also be used for droplet actuation by applying EWOD-level voltages at both electrical connections. Mixing of the contents of the reaction site could be achieved by actuating the ring electrodes in sequence from inner to outer and back. Using Cerenkov imaging of radioactive solutions, 30 sec of active mixing was found to be adequate.

Though capable of rapid heating, temperature of the heater site was typically ramped up at a rate of about 1°C/s. Higher rates were found to sometimes lead to “bursting” of the droplet at the reaction site. Bursting must be avoided as it distributes some or all of the PET tracer being synthesized off of the EWOD chip electrodes, where it can no longer be manipulated or detected. Since the heating time was a significant portion of the overall synthesis time (and thus impacts the non-decay-corrected yield), we used other funding to study the phenomenon of bursting to see if increases in temperature ramping could be possible. We performed a systematic study of various factors hypothesized to be related to this effect, including temperature, droplet composition, droplet volume, and gap height between the plates of the EWOD chip. It was found that reduced droplet volume and increased gap height can reduce the incidence of bursting, all else being held constant. With small volume droplets, it is even possible to heat droplets to temperatures significantly above the boiling point without incidence of bursting. It is hypothesized that above the boiling point, small vapor bubbles form inside the droplet and expand due to evaporation. For small droplets and larger gap sizes, these vapor bubbles are close to the surface of the droplet and can quickly merge with the surface to relieve their internal pressure before the bubbles reach significant sizes or growth rates and thus are less likely to burst.

In light of these data it was surprising that bursting was found to sometimes occur with temperature setpoints below the boiling point. We observed via infrared thermography that there was significant non-uniformity in the temperature (a range of > 20°C) across the reaction zone. It was hypothesized that minor manufacturing imperfections could lead to local variations that get amplified by positive feedback, likely exceeding the boiling point in “hot spots” on the chip. Off-chip heating will be considered in the future in order to better control temperature, avoid hot spots, and enable rapid and uniform ramping up to “safe” temperatures to speed up the overall radiosynthesis process.

After investigation of unit operation parameters, we demonstrated on a third generation chip that it was possible to synthesize a variety of “one-pot” PET tracers using the same chip design: [F-18]FDG, [F-18]FLT, [F-18]Fallypride, and [F-18]SFB. Leveraging other funding for in-depth study and optimization of the synthesis of specific probes, we achieved overall yields of 37±13% (n=2), 56±8% (n=6), 19±8% (n=5), and 65±11% (n=6), respectively. The demonstration of these four probes establishes that the platform is compatible with diverse probes and the chip design and operation appears to be universal for “one pot” probes. Additional tracers will be studied in the future.

During the development of these synthesis protocols for different tracers, we observed differences in reaction kinetics between macroscale processes and on the chip. For example, we typically required higher (2x to 4x) solute concentrations on the chip compared to the macroscale. One possibly-relevant difference when moving from macroscale to microscale reactions is that the ratio of precursor to [F-18]fluoride decreases. (This is because the concentration of [F-18]fluoride increases, assuming similar level of radioactivity is loaded into the chip.) Perhaps the higher precursor concentrations are needed to counteract this effect. Fortunately, the EWOD platform enables detailed study of such kinetics, and future studies will be aimed at understanding these differences, potentially yielding new insights into microscale batch chemistry (which in turn can enable further optimizations).

Aim 5:

Guided by experience performing radiochemical reactions on EWOD, several generations of chips were developed. The initial chip included 1 mm EWOD electrodes, a square loop path for cyclical transport of droplets for mixing, and 2 multifunctional heating electrodes. The resistive heating electrodes contained a circular resistive heater within a square EWOD electrode. The dielectric layer was a 1 um-thick PECVD silicon nitride. Cytop was chosen as the surface hydrophobic coating because it has been shown to have better lifetime performance for electrowetting than Teflon under certain conditions.

For the second generation, the EWOD electrode length was increased to 2 mm to quadruple the volume carried by each electrode from 200 nL to 800 nL when using a 0.2 mm plate gap. The heater diameter was enlarged from 0.7 mm to 12 mm so that larger volumes of [F-18]fluoride (and hence higher amounts of radioactivity) could be loaded. The heater consisted of 4 concentric circular resistive heating elements, each with its own feedback control of temperature. Compared to a single-element heater, this multi-element heater was able to center the droplet and maintain its temperature more uniformly as the droplet shrinks during evaporations. The droplet could be mixed by sequentially actuating the concentric heating elements with EWOD voltage, eliminating the need for a square loop path for mixing. The silicon nitride thickness was increased to 2 um to limit pinholes in the dielectric (which can lead to electrolysis and chip failure). The hydrophobic coating was switched to Teflon because its higher glass-transition temperature makes it more stable for high temperature reactions than Cytop, thereby expanding the range of radiochemical reactions possible. Although Cytop has better electrowetting lifetime under DC conditions, we performed an in-depth study of Cytop, Teflon, and Fluoropel and found that under AC conditions (as used here to move organic solvents), the lifetime is similar for all materials. Thus, switching from Cytop to Teflon is not expected to compromise the lifetime of the chips.

The third generation chip has a smaller footprint of 3.5x3.2 cm compared to 5.0x4.3 cm so that 4 chips can be made from a 4 inch wafer instead of only 2. The pitch between contact pads was reduced from 2.54 mm to 1.27 mm to increase contact pad density so that only one edge needs to be connected for electrical signals. Although the heater diameter was maintained at 12 mm, each of the 4 concentric heating elements, made of thin indium tin oxide (ITO), inside the heater was slightly widened to account for changes in electrical properties of ITO after annealing the Teflon topcoat and draw similar power as the second design. After the Teflon anneal, ITO resistivity varied less than 0.3% after further heating cycles below 250°C.

Additional chip modifications were made while optimizing the pneumatic reagent loading mechanism. In particular, we inverted the normal configuration so that the patterned electrode layer (EWOD substrate) was on top, while the ground layer (cover substrate) was on the bottom. Holes drilled through the cover allow fluid access via needles from the off-chip reagent storage vials located below. Linear arrays of electrodes connect each inlet hole to the central heater in the chip. The two electrodes closest to the inlet hole serve as "inlet electrodes"- they are actuated by EWOD while liquid is pumped into the chip, and serve to detect liquid and also retain a defined amount of liquid. The distance from the inlet hole to the inlet electrodes was optimized to ensure liquid is detected before flooding occurs and that the loaded droplet can be reliably separated from the excess liquid removed by gravity. Remaining electrodes along the path were optimized to ensure reliable transport from the inlet electrode to the heater.

Finally, in terms of fabrication, we explored alternative dielectric materials to address inconsistency observed in the silicon nitride dielectric layer properties (i.e., some batches contained pinholes that led to dielectric breakdown and chip failure). Metal oxides can be created by anodization on the surface of suitable metal electrodes, and the resulting structures exhibit a current limiting behavior that is compatible with EWOD within certain voltage, polarity, and frequency constraints. In fact, because an electric field is involved in the initial formation of the oxide layer, such devices can exhibit self-healing properties under some conditions. Operation outside the permitted voltage range (as needed for radiosynthesis on chip), however, results in rapid deterioration. To solve this problem, we developed a "mirrored" structure, including anodized dielectric on both the actuation electrodes and on the ground electrode on the other substrate. This approach was demonstrated with aluminum electrodes and alumina dielectric. The mirrored structure ensures that one of the two electrodes is always under the correct polarity for the current limiting effect, and can be operated without the usual voltage and frequency restrictions. Such an approach is promising for high reliability EWOD radiosynthesis devices. Before this approach can be routinely used, however, certain practical issues must be addressed. For example, the use of non-transparent electrodes limits visualization of the droplets within the chip, hindering the development and optimization of synthesis processes.

Aim 6:

We have combined several of the above elements into a laboratory prototype to learn about the challenges of automation and integration. In particular the prototype incorporates pneumatic loading of multiple reagents, a first-generation [F-18]fluoride concentration system, and vacuum-assisted PET tracer collection. Development of this system has guided several aspects of chip design and operation and has enabled increased automation of the overall synthesis process, depending less on visual feedback from a human operator. With this laboratory prototype, we have successfully performed the 1-step synthesis of [F-18]fallypride as a demonstration. After collection of the product from the chip into a vial, it can be purified and then formulated for injection.

Several challenges were highlighted in the current system: (i) the chip is difficult to align and install; (ii) after product collection from the chip, the product had to be manually transferred to the purification step; (iii) due to the reagent loading mechanism, a substantial amount of shielding is still needed. With separate funding, improvements to all of these aspects are being pursued.

Additional Achievements

To enhance the utility of the EWOD platform for chemical synthesis of PET tracers, we developed an additional on-chip unit operation: solid-phase extraction (SPE). SPE is a type of purification process. The on-chip purification of [F-18]Fallypride was used as an example. After synthesis, radio-TLC analysis of the crude samples reveals two radioactive peaks, one corresponding to the desired [F-18]Fallypride (~84%) and the other corresponding to unreacted [F-18]fluoride (~16%). One way to eliminate [F-18]fluoride from any PET tracer is to pass the crude mixture through a SepPak cartridge containing alumina particles. The [F-18]fluoride will bind to the alumina particles and remain in the cartridge as the fluid, containing the [F-18]Fallypride, passes through. To demonstrate on-chip purification, we loaded a slurry of alumina particles (80 µm diameter) onto the chip. This droplet was merged with the crude reaction droplet after on-chip [F-18]Fallypride synthesis and mixed using electrowetting actuation. Finally, the combined droplet was then pulled across an on-chip “fence” consisting of pillars spaced by 60 µm (smaller than the particle size), leaving the particles on one side and the purified liquid on the other. Radio-TLC analysis of the purified droplet showed it to be nearly free of [F-18]fluoride (i.e. <1%).

This technique may also hold potential as a critical tool for on-chip analysis of reaction outcomes. If it can be assumed that the alumina particles completely trap the [F-18]fluoride and none of the desired product, then the chip can be subjected to Cerenkov imaging after separation of the liquid from the beads to determine yield. The radioactivity signal associated with the filtered beads represents the amount of [F-18]fluoride and the radioactivity signal associated with the purified droplet represents the amount of the desired probe. These two values can be used to compute the radiochemical conversion of the reaction.

Summary

Overall, all objectives have been met and several advances were made to enable production of diverse radiolabeled molecules on the EWOD digital microfluidic platform, and to enable the study and development of small-scale reactions. The achievements of this research have laid the groundwork for Small Business Innovative Research awards which seek to commercialize this technology.

Products and Papers

Patent Applications:

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- G.J. Shah, Pei Yuin Keng, R. Michael van Dam. Disposable world-to-chip interface for digital microfluidics. U.S. provisional 61/657570 filed Jun 8, 2012.

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Other products:

- EWOD microfluidic chip platform (including control system, software, Cerenkov imaging system, and radiation shielding)

- Prototype syringe-pump based reagent loading system for EWOD chips (single reagent)

- EWOD microfluidic chip platform prototype incorporating pneumatic-based pumping of multiple reagents, [F-18]fluoride concentration, and product collection

- EWOD chip design (and photomasks for fabrication) capable of synthesis of diverse PET tracers