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Title: Optimization of Radiochemical Reactions Using Droplet Arrays

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**, all done

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 25
Number of Shots: 54

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Michael van Dam:** Current automated radiosynthesizers are designed to produce large batches of widely used radiopharmaceuticals such as FDG. Due to the limited number of syntheses possible per day and relatively high reagent consumption, these systems, however, are not well suited for performing synthesis optimization studies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Alejandra Rios:** With this technique, throughput is significantly increased by performing up to 16 small-scale reactions in parallel, and reagent consumption is reduced 100-fold. Furthermore, by performing reactions in parallel, fewer batches of radioisotope are needed to complete a study.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Travis Holloway:** Increased throughput enables wider exploration of reaction conditions with greater number of replicates each, compared to the use of conventional instruments. While this protocol shows the optimization of precursor concentration in the synthesis of [^{18}F]Fallypride, the technique can be used to optimize other conditions and other radiopharmaceuticals.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Planning and optimizing the study

2.1. Begin by fabricating batches **[1]** of multi-reaction microdroplet chips **[2]** from 4-inch silicon wafers using standard photolithography techniques **[3]**.

2.1.1. Glass petri dish with cut chips making the whole wafer, with one of the chips slightly off the aligned chips.

2.1.2. ECU: Zoom in to that chip to show the reactions sites.

2.1.3. LAB MEDIA: Figure 1 B.

2.2. In this protocol, the high-throughput optimization of precursor concentration is demonstrated with the synthesis of the radiopharmaceutical [^{18}F]fallypride *(pronounce "F-18-Fallypride")* **[1]**. Sixteen simultaneous reactions can be performed on a single chip. The conditions to be compared are mapped to the reaction sites **[2]**. *Videographer: This step is important!*

2.2.1. LAB MEDIA: Figure 2.

2.2.2. LAB MEDIA: Figure 3 A.

3. Preparation of reagents and materials for optimizing the radiosynthesis of [^{18}F]fallypride

3.1. Prepare a stock solution of the reaction solvent, consisting of thexyl alcohol and acetonitrile in a 1 to 1 by volume mixture. Ensure that the volume is enough to create the planned dilution series **[1]**. Prepare a 30-microliter stock solution of precursor in the reaction solvent with the maximum concentration to be explored **[2]**.

3.1.1. Talent transferring from labeled bottle of thexyl alcohol and another transfer from a MeCN bottle to a labeled container with reaction solvent.

3.1.2. Talent adding reaction solvent to the ABX bottle with 77 mM written on it.

3.2. From the precursor stock solution and reaction solvent, perform 2x serial dilutions in a set of microcentrifuge tubes to prepare the different concentrations of the precursor solution **[1]**.

3.2.1. Talent making the first dilution in a labeled tube.

3.2.2. Rack of labeled tubes with corresponding concentrations.

3.3. Prepare another set of microcentrifuge tube to collect each crude reaction product, using a permanent marker to label each tube with a unique number **[1]**. Ensure that the total number of microcentrifuge tubes matches the number of conditions multiplied by the number of replicates **[2]**.

3.3.1. Talent labeling a few tubes.

3.3.2. Labeled microtubes in a rack.

3.4. Prepare a 10-milliliter stock of collection solution comprising of 9 to 1 methanol to deionized water **[1]**. Aliquot 50 microliters each into an additional set of 16 microcentrifuge tubes, labeled as collection solution **[2]**.

3.4.1. Talent adding methanol and water to a 9:1 MeOH:H_2O labeled container.

3.4.2. Talent aliquoting the collection solution into tubes pre-labeled with C.S.

3.5. Prepare a [^{18}F]fluoride *(pronounce 'F-18-Fluoride')* stock solution by mixing around 7 millicuries of the [^{18}F]fluoride source with 56 microliters of 75 millimolar tetrabutylammonium bicarbonate and diluting with DI water up to 140 microliters.

3.5.1. Talent adding base and water to a tube labeled activity or [18F] stock, then adding [^{18}F]fluoride source.

4. Parallel synthesis of [^{18}F]fallypride with different precursor concentrations

4.1. Using a micropipette, load an 8-microliter droplet of [^{18}F]fluoride *(pronounce 'F-18-Fluoride')* stock solution on the first reaction spot of a multi-reaction chip **[1]**. Measure the activity of the chip by placing it in a dose calibrator **[2]** and record the time at which measurement is conducted **[3]**. *Videographer: This step is important!*

4.1.1. Talent loading droplet on the first reaction spot.

4.1.2. Talent placing the chip in a dose calibrator and measurement displayed.

4.1.3. Talent recording measurement in spreadsheet.

4.2. Remove the chip from the dose calibrator **[1]** and load an 8-microliter droplet of [^{18}F]fluoride stock solution on the second reaction spot **[2]**. Measure the activity on the chip and record the time at which measurement is conducted. Repeat this process for all other reaction sites **[3]**.

4.2.1. Talent removing the chip from the calibrator.

4.2.2. Talent loading the stock solution on the second reaction spot.

4.2.3. Talent recording measurement in spreadsheet.

4.3. Calculate the activity loaded per reaction spot by taking the activity measurement after loading the radioisotope and subtracting the previous measurement before that site was loaded **[1]**.

4.3.1. Decay-correct chip activity column in the spreadsheet and the adjacent column.

4.4. To align the multi-reaction chip on the heater, add a thin layer of thermal paste on top of the ceramic heater **[1]**. Carefully place the chip on top of the heater using tweezers, aligning the reference corner of the chip with the reference corner of the heater. The

chip should overhang the heater by a small amount **[2]**. *Videographer: This step is difficult and important!*

4.4.1. Talent adding a thin layer of paste to ceramic heater. *Videographer: If possible, film the chip through the window. If not, film with door open and make a note of this.*

4.4.2. Talent positioning the chip.

4.5. Heat the chip for 1 minute by setting the heater to 105 degrees Celsius in the control program to evaporate the droplets **[1]**, leaving a dried residue of [^{18}F]fluoride *(pronounce 'F-18-Fluoride')* and $TBHACO_3$ **[2]**. Then, cool the chip by setting the heater to 30 degrees Celsius and turning on the cooling fan with the control program **[3]**.

4.5.1. Talent setting heating temperature and a timer

4.5.2. Show droplets shrinking.

4.5.3. Talent turning on the fan in control panel. Videographer NOTE: Do not use tk 1

4.6. Using a micropipette, add a 6-microliter solution of fallypride precursor on top of the dried residue on the first reaction site. Repeat this for all other reaction sites on the chip **[1]**. Use the optimization plan to determine which concentration of the dilution series is used for each reaction site **[2]**.

4.6.1. Talent adding a droplet from tube labeled 77 mM to the first reaction site and a droplet from the tube labeled 39 mM to the second reaction site

4.6.2. Droplets on reaction sites.

4.7. Heat each chip to 110 degrees Celsius for 7 minutes using the control program to perform the radiofluorination reaction **[1]**, then cool the chip by setting the heater to 30 degrees Celsius and turning on the cooling fan with the control program **[2]**.

4.7.1. Talent setting temperature and timer to 7 minutes.

4.7.2. Talent turning on the fan in control panel. Videographer NOTE: Use 4.5.3

4.8. Collect the crude product at the first reaction site by adding 10 microliters of collection solution from the designated microcentrifuge tube **[1]**. After waiting for 5 seconds, aspirate the diluted crude product **[2]** and transfer it to its corresponding collection microcentrifuge tube **[3]**. *Videographer: This step is important!*

4.8.1. Rack with labeled vials on a heating platform.

4.8.2. Talent opening a vial labeled C.S and drawing out 10 ul.

4.8.3. Talent adding solution to reaction site, pipetting the solution up and down, then collecting it.

4.8.4. Talent placing the solution in the tube.

4.9. Repeat this process a total of 4 times using the same pipette tip and then close the microcentrifuge tube **[1]**. Repeat these steps to collect the crude product from all other reaction sites on the chip **[2]**.

4.9.1. Talent closing the vial.

4.9.2. Show rack of collection tubes.

5. Synthesis analysis to determine reaction performance and optimal conditions

5.1. To determine the collection efficiency for the first reaction on the chip, place the microcentrifuge tube with the collected crude product of the first reaction spot in the dose calibrator to measure the activity **[1]**. Record the measurement and time of the measurement. Repeat this process for each of the collected crude products**[2]**.

5.1.1. Talent placing the collection tube in the calibrator and measuring activity, with the label on the tube visible.

5.1.2. Talent typing the measurement in spreadsheet.

5.2. Calculate the collection efficiency by dividing the activity of the collected crude product by the starting activity measured for the same reaction site. Repeat this for all other reaction sites on the chip **[1]**.

5.2.1. Calculated column of collection efficiency values in spreadsheet.

5.3. Next, analyze the composition of each collected crude product. With a pencil, draw a line 15-millimeters away from the bottom edge of the TLC plate, and another line 50-millimeters away from the same edge. The first line is the origin line and the second is the solvent front line **[1]**.

5.3.1. Talent drawing the lines on the pre-cut TLC plate, using a ruler.

5.4. Draw 8 small "X"s along the origin line at 5-millimeter spacing to define the sample spotting position for each of the 8 lanes **[1]**.

5.4.1. Talent drawing the X's, using a ruler.

5.5. Using a micropipette, transfer 0.5 microliters of the first crude product **[1]** onto the TLC plate at the "X" for the first lane **[2]**. Repeat this for additional crude products **[3]**, then wait for the spots to dry **[4]**. *Videographer: This step is important!*

5.5.1. Talent opening microcentrifuge tube labeled with the first crude product and drawing out 0.5 ul.

5.5.2. Talent adding crude product from labeled tube to the first X.

5.5.3. Talent opening microcentrifuge tube labeled with the second crude product and drawing out 0.5 ul.

5.5.4. Talent adding second crude product to the second X.

5.6. Develop each TLC plate using a mobile phase of 60% acetonitrile in 25 millimolar ammonium formate with 1% TEA **[1]** until the solvent front reaches the solvent front line **[2]**.

5.6.1. Talent pouring from a vial with the mobile phase and into the glass vial for developing of TLC plates. Videographer NOTE: Do not use tk1 or tk3

5.6.2. Talent placing TLC in chamber with mobile phase and solvent front moving up the plate.

5.7. At that time, remove the TLC plate from the chamber **[1]** and wait for the solvent on the TLC plate to dry, then place the TLC plate in the Cerenkov imaging system **[2]** and cover it with a glass microscope slide **[3]**.

5.7.1. Talent removing TLC plate from chamber once the front reaches the top line.

~~5.7.2.~~ Talent placing TLC plate in Cerenkov imaging system~~.~~

5.7.3. Talent covering the dry TLC plate with a microscope slide, using tweezers.

5.8. Obtain a radioactivity image of each TLC plate by setting the Cerenkov imaging system to a 5-minute exposure **[1]**, then select the produced file of the imaged TLC plate and perform standard image corrections **[2]**.

5.8.1. Talent setting Cerenkov system to a 5-minute exposure and pressing start.

5.8.2. SCREEN: 5.8.2. SCREEN B.mov.

5.9. Use region of interest analysis for the first lane of the first TLC plate. Draw regions around each band visible in the lane **[1]**, then compute the fraction of integrated intensity of each region compared to the total integrated intensity of all regions **[2]**.

5.9.1. SCREEN: 5.9.1. SCREEN B.mov. *Video Editor: Speed this up slightly*

5.9.2. SCREEN: 5.9.2. SCREEN B.mov. 0:00 – 0:12. *Video Editor: Speed this up slightly*

5.10. Determine the fluorination efficiency as the fraction of activity in the [^{18}F]fallypride *(pronounce "F-18-Fallypride")* band. Repeat this analysis for all other lanes on all TLC plates **[1]**.

5.10.1. SCREEN: 5.10.1. SCREEN B.mov. *Video Editor: Emphasize the areas of the screen outlined in the yellow and red boxes.*

5.11. Then, compute the crude radiochemical yield for each reaction **[1]** and choose the optimal precursor concentration by examining the crude radiochemical yield as a function of precursor concentration **[2]**.

5.11.1. SCREEN: 5.11.1. SCREEN B.mov. *Video Editor: Just show the first few seconds, enough to cover the VO.*

5.11.2. SCREEN: 5.11.2. SCREEN B.mov. 0:10 – 0:16.

Results

6. Results: Influence of precursor concentration on the microdroplet synthesis of [^{18}F]fallypride

6.1. Optimization studies of the radiopharmaceutical [^{18}F]fallypride *(pronounce "F-18-Fallypride")* were performed by varying precursor concentration in thexyl alcohol-acetonitrile **[1]**. Reactions were performed at 110 degrees Celsius for 7 minutes. Collection efficiency and sample composition are shown here **[2]**.

6.1.1. LAB MEDIA: Table 1. *Video Editor Emphasize the first column.*

6.1.2. LAB MEDIA: Table 1.

6.2. The fluorination efficiency increased with increasing precursor concentration **[1]**, and the concentration of unreacted [^{18}F]fluoride *(pronounce 'F-18-Fluoride')* decreased **[2]**.

6.2.1. LAB MEDIA: Figure 4 A. *Video Editor: Emphasize the red data points.*

6.2.2. LAB MEDIA: Figure 4 A. *Video Editor: Emphasize the blue data points.*

6.3. There was a small amount of a radioactive side product at low precursor concentrations, which was not formed at the higher precursor concentrations **[1]**. The collection efficiency was nearly quantitative for most conditions, though it dropped slightly at low precursor concentrations **[2]**.

6.3.1. LAB MEDIA: Figure 4 A. *Video Editor: Emphasize the black data points.*

6.3.2. LAB MEDIA: Figure 4 B. *Video Editor: Emphasize the purple data points.*

6.4. The highest crude radiochemical yield was achieved with a 39 millimolar precursor concentration **[1]**. At this condition, the fluorination efficiency was 96%, the crude RCY was 87%, and there was no observed radioactive side product formation **[2]**.

6.4.1. LAB MEDIA: Table 1. *Video Editor: Emphasize the 39mM precursor datapoints (green, red, and purple).*

6.4.2. LAB MEDIA: Table 1. *Video Editor: Emphasize the second row (39mM precursor concentration).*

Conclusion

7. Conclusion Interview Statements

7.1. **Travis Holloway:** It is critical to have a mapped plan of what reaction condition corresponds to which reaction droplet on the chip and to have appropriately labelled reagent tubes and product collection tubes that can be double-checked during the experiment.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.7.1 for mapped plan and 3.3 for labeled reagent tubes*

7.2. **Alejandra Rios:** The procedure can be used for the optimization of other reaction conditions such as amount of base, type of solvent, or reaction volume. It can also be used to optimize the synthesis of other radiopharmaceuticals.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.