Microfluidic Preparation of a ^{89}Zr-Labeled Trastuzumab Single-Patient Dose

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^{89}Zr-labeled antibodies are being investigated in several clinical trials; however, the time requirement for synthesis of clinical doses can hinder patient throughput because of scheduling difficulties. Additionally, low specific activity due to poor labeling efficiency can require larger amounts of the radiopharmaceutical to be administered, possibly leading to adverse side effects. Here, we describe the design and evaluation of a microfluidic reactor capable of synthesizing a single clinical dose of ^{89}Zr-labeled antibody. ^{89}Zr-labeled trastuzumab was chosen for this validation because it is currently being evaluated in clinical trials for imaging human epidermal growth factor receptor 2–positive cancer patients. **Methods:** A microreactor fabricated from polydimethylsiloxane/glass was silanated with trimethoxy(octadecyl) silane to reduce antibody adsorption. Desferrioxamine-p-benzyl-isothiocyanate (DFO-Bz-NCS) was conjugated to trastuzumab in an 8:1 molar ratio following the literature procedures using aseptic techniques. Radiolabeling was performed by pumping ^{89}Zr-oxalate and DFO-Bz-trastuzumab into the microfluidic reactor at a total rate of 20 μL/min in ratios varying from 1:37 to 1:592 mg:MBq at 37°C to achieve optimal labeling. **Results:** Silanated reactors showed low antibody adsorption in comparison to unmodified reactors (95% monoclonal antibody recovered vs. 0% recovered). Labeling of the modified trastuzumab was shown to be achievable at a specific activity above the reported literature value of 220 MBq/mg. A high radiochemical purity was achieved without an incubation period at specific activities of less than 148 MBq/mg; however, specific activities up to 592 MBq/mg could be achieved with an incubation period. Clinical doses were able to be prepared and passed all quality control guidelines set by the Food and Drug Administration. Samples were sterile, colorless, and radiochemically pure (100%); maintained the ability to bind to the intended receptor; formed a minimal amount of aggregates (1%–4%); and were completed within 45–60 min. **Conclusion:** ^{89}Zr-labeled trastuzumab for use in a clinical setting was synthesized in a microfluidic reactor in under an hour while reducing the amount of handling required by a technician. Use of this compact platform not only could enable the use of radiolabeled antibodies to become a common practice, but also could spread the use of radiolabeled antibodies beyond locations with cyclotron facilities.

Key Words: microfluidics; ^{89}Zr; antibodies; trastuzumab; GMP

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The use of monoclonal antibodies (mAbs) in the clinic is quickly growing. In 2012, there were 12 mAbs accepted in the United States for the treatment of tumors; since then, 15 more mAbs have been approved for use (*1,2*). To monitor target expression, patients often have to go through several invasive biopsies to obtain samples for immunohistochemistry. To avoid invasive procedures, the noninvasive technique of immuno-PET for detecting and evaluating tumors has been developed. Immuno-PET uses mAbs modified with positron-emitting isotopes to track the localization of antibodies, allowing clinicians to evaluate the treatment efficacy swiftly and adjust treatment methods as needed.

Antibodies are known to have a long biologic half-life depending on the isotype of the mAb (2–21 d) (*3*). Optimal tumor-to-nontumor ratios are typically achieved 2–4 d after injection, severely limiting the radionuclides available for use (*4,5*). Matching this biologic half-life to an isotope with an appropriate half-life is key to having a functioning radiotracer. Too short of a half-life will result in poor image quality, whereas a half-life that is too long will needlessly increase radiation exposure to the patient. Currently, the 2 most common methods of producing mAb radiotracers are radioiodination and the use of a bifunctional chelator to secure a radiometal to the mAb. ^{124}I's half-life of 4.18 d is well suited for monitoring mAb circulation; however, because of dehalogenation it can be used only with noninternalizing mAbs (*6*). Additionally, the decay of ^{124}I involves the release of high-energy positrons (687 and 974 keV), decreasing the image resolution. Common radiometals such as ^{99m}Tc, ^{86}Y, and ^{68}Ga have short half-lives, making them unfavorable for labeling mAbs. ^{89}Zr has a half-life of 3.27 d, an attractive decay pathway, and quickly clears from the body when chelated, making it well suited for labeling mAbs (*7*).

^{89}Zr-labeled mAbs have been examined both preclinically and in clinical trials extensively (*8–12*). One antibody in particular, trastuzumab, more commonly known under the trade name Herceptin (Genentech), is currently used in the treatment of metastatic breast cancer. It binds to domain IV of the overexpressed human epidermal growth factor receptor 2 (HER2), inhibiting angiogenesis via blocking signaling pathways resulting in reduced tumor growth (*13*). In a trial administered by Dijkers et al., ^{89}Zr-labeled trastuzumab has shown great promise. The radiotracer was able to detect several metastatic tumors in a patient that were previously unnoticed (*14*). Furthermore, it was noted that some known tumors did not retain the labeled trastuzumab. These tumors when biopsied showed a lack of the HER2 that binds trastuzumab, confirming the specificity of the imaging agent to HER2 expression.

The use of ^{89}Zr-trastuzumab in clinical trials requires the radiopharmaceutical to be produced via good manufacturing practices.

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Production using good-manufacturing-practice procedures is costly, needs a large amount of laboratory space, and requires a significant amount of time to perform. The lengthy protocol for the preparation of a clinical dose causes technicians to be unavailable to produce other radiopharmaceuticals and adds time constraints, further hindering patient scheduling, thus putting a limit on the number of patients able to be scanned in a day. To reduce the cost, laboratory space requirements, and dose production time, we have turned to microfluidics to create a dose on demand system for labeling mAbs with ^{89}Zr.

Microfluidic devices have been developed for use in a wide variety of biomedical applications including the synthesis of radiotracers (*15–18*). Most commonly applied to ^{18}F and ^{11}C labeling, these devices are often composed of small channels ranging from 10 to 500 μm wide, containing various geometries to increase stochastic mixing (*19,20*). The small reaction volumes enable fine control over reaction conditions, such as temperature and mixing, while computerized control of flow and reagent concentration increases the reaction reliability and reproducibility. Reactions can be performed in either continuous flow or batch-mode, providing high yields under milder conditions than conventional methods (*18,21*). In comparison, conventional labeling with radiometals often requires dilution of reagents for proper handling, leading to picomolar concentrations requiring an excess of bifunctional chelator–biomolecule conjugates and long reaction times to achieve acceptable reaction yields. Here, we report the fabrication and validation of a polydimethylsiloxane (PDMS)/glass microreactor for radiolabeling mAbs with ^{89}Zr. To the best of our knowledge, this represents the first use of a microreactor to radiolabel an antibody with ^{89}Zr, as well as the first example of the preparation of a clinical dose using such a system.

MATERIALS AND METHODS

Trastuzumab (Herceptin) was purchased from Roche. Desferrioxamine-p-benzyl-isothiocyanate (DFO-Bz-NCS) was purchased from Macrocyclics. Zeba desalting columns and the bicinchoninic acid assay kit were purchased from Thermo Scientific. Reagent preparation and the trastuzumab conjugation procedures were performed under good laboratory practice conditions. Materials and solvents for the conjugation and labeling procedures were sterile and endotoxin free. Deionized water was produced in-house using a Millipore Milli-Q water system. All other chemicals were purchased from Sigma-Aldrich unless stated otherwise. The SKBR3 cell line was purchased from the American Type Culture Collection. Reagents for cell culture were purchased from Life Technologies unless stated otherwise. The number of cells used in the in vitro assays was determined using a Cellometer Auto T-4 Cell Counter from Nexcelom. Radioactivity associated with cells was counted in a Beckman 8000 γ-counter from Beckman Coulter Inc. Silicon wafers were purchased from University Wafer. SU-8 2050 photoresist was purchased from MicroChem. Microbore PTFE tubing (0.012″ inner diameter, 0.030″ outer diameter) was purchased from Cole-Parmer. Microliter flow modular pump components (syringe pump, a pump driver circuit, and a power supply) were obtained from Harvard Apparatus. A Kapton-insulated thin film heater, CN740 temperature controller, and an SA1-RTD probe were purchased from Omega Engineering. The CRC-712 M radioisotope dose calibrator was purchased from Capintec Inc. and used for the measurement of radioactivity. Silica instant thin-layer chromatography (iTLC) paper was purchased from Agilent Technologies. Analytic size-exclusion high-performance liquid chromatography (HPLC) was performed on an Agilent 1200 Series using a TSKgel Super SW 3000 size-exclusion column. The flow rate was 0.25 mL/min, with a mobile phase consisting of 50 mM phosphate buffer (pH 7) with 150 mM NaCl and 0.1% polysorbate-20. Endosafe PTS and the *Limulus* Amebocyte Lysate test cartridges were purchased from Charles River Laboratories.

Antibody Adsorption Protection

To prevent antibody adsorption, the microfluidic reactor was treated with trimethoxy(octadecyl)silane. The PDMS-glass reactor was activated with a hydrogen peroxide/hydrochloric acid solution (H_2O_2/HCl/H_2O 1:1:5, 1 mL) with a flow rate of 20 μL/min at 37°C for 30 min. The chip was washed with 1 mL of H_2O and then dried with air for 5 min. Neat trimethoxy(octadecyl)silane was pushed through the chip at a rate of 20 μL/min at 37°C for 15 min. The reactor was then washed with 1 mL of dimethyl sulfoxide (DMSO), 1 mL of H_2O, and then 1 mL of acetone at a rate of 25 μL/min. After being washed, the reactor was dried with air for 5 min and annealed at 70°C for 2 h under vacuum to cure the surface.

Synthesis of DFO-Trastuzumab

The conjugation of DFO-Bz-NCS to trastuzumab in an 8:1 molar ratio was performed using aseptic techniques and previously reported literature procedures (*22,23*). Trastuzumab (21 mg/mL) was conjugated to DFO-Bz-NCS dissolved in DMSO in 0.1 M sodium carbonate buffer (pH 9) at 37°C for 1 h. The resulting trastuzumab-DFO conjugate was purified via a gel filtration spin column (molecular weight cut off, 40 kDa) during which the buffer was exchanged to 1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. The product was then sterile-filtered into 0.5-mL doses in a laminar flow hood and stored in a freezer at −80°C. The bicinchoninic acid assay was performed to determine the concentration of the resulting antibody.

Radiolabeling of DFO-Trastuzumab with ^{89}Zr

Production of ^{89}Zr-oxalate was performed in-house, diluted with an equal volume of 1 M HEPES buffer and neutralized to a pH range previously reported for optimal radiolabeling (pH 6.8–7.2) with 2 M sodium hydroxide and 2 M hydrochloric acid (*24,25*). The reaction solution consisting of trastuzumab-DFO (10 mg/mL), ^{89}Zr-oxalate (370 MBq/mL), and 1 M HEPES buffer was pumped into a single channel reactor (total volume, ~2.81 μL) from separate inlets in varying ratios of ligand to metal at a total flow rate of 20 μL/min. Ratios of mg trastuzumab-DFO to MBq of ^{89}Zr greater than 1:148 were incubated for 1 h at 37°C within the reactor (by halting the flow), whereas lower ratios of mAb:^{89}Zr were pumped through at a continuous rate. After the reaction, the reactor was flushed with 1 M HEPES buffer to wash out the remaining ^{89}Zr-trastuzumab; the product was collected in a microcentrifuge tube and the radiochemical yield was confirmed by instant thin-layer chromatography.

Preparation of Clinical ^{89}Zr-Trastuzumab Dose

A single dose of trastuzumab-DFO was removed from a −80°C freezer and thawed for 30 min. ^{89}Zr-oxalate was obtained in-house as previously described. The trastuzumab-DFO, ^{89}Zr-oxalate, and 1 M HEPES buffer were combined at 37°C in a 1:37:0.1 ratio at a rate of 20 μL/min for 15–25 min and collected in a microcentrifuge tube. While the labeling was occurring, a gel filtration spin column was equilibrated with sterile saline. After the labeling was finished, 50 μL of 50 mM diethylenetetraaminepentaacetic acid was added to the labeled antibody and the mixture was incubated for 5 min at 37°C to complex unreacted ^{89}Zr. The mixture was then purified with the prepared spin column and placed in a sterile laminar flow hood. The purified product was filtered through a sterile 0.22-μm pore-size syringe filter into a sterile vial and diluted to 2.5 mL with sterile saline to produce the patient dose. Purity was confirmed by iTLC using a 50 mM diethylenetetraaminepentaacetic acid solution as the running buffer and by size-exclusion HPLC.

Immunoreactive Fraction Determination

The in vitro binding characteristics (immunoreactive fraction) of ^{89}Zr-trastuzumab was determined by the Lindmo assay (*26*). SKBR3

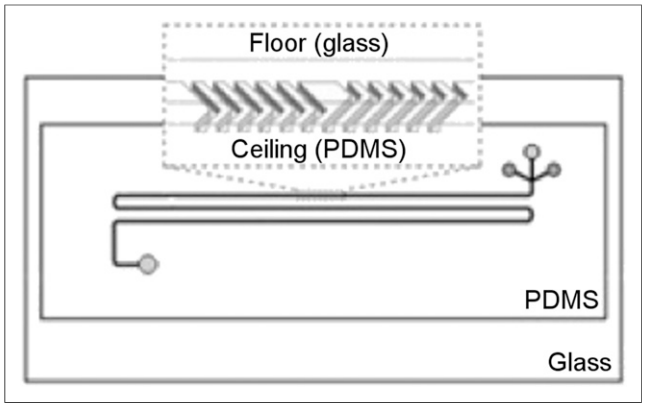

FIGURE 1. Microfluidic device used for continuous flow labeling.

(HER2-positive) cells were cultured in Dulbecco modified medium with 10% bovine serum and gentamycin (50 mg/mL) in a humidified incubator with 5% CO_2 at 37°C. Cells were harvested with trypsin and used at a concentration of 5×10^6 cells/mL. A sample of the radiolabeled trastuzumab was diluted with a 1% bovine serum albumin solution until the final count in 50 μL of the mixture was 800–5,000 cpm. Fifty microliters of the final dilution were combined with cell concentrations ranging from 0.5 to 5×10^6 SKBR3 cells in phosphate-buffered saline and allowed to incubate for 1 h with gentle agitation. The cells were then pelleted via centrifuge, washed with cold phosphate-buffered saline 3 times, and counted for ^{89}Zr activity. The binding was calculated as the ratio of bound activity to the total amount of activity and was background corrected.

Pyrogenicity Test

An aliquot (10 μL) of the final ^{89}Zr-Herceptin in saline was tested for the presence of bacterial endotoxin using a *Limulus* Amebocyte Lysate Test. The sample was further diluted with saline by 100 times before the analysis. The test was performed using an Endosafe-PTS from Charles River Laboratories.

RESULTS

The reactor was composed of a single snaking channel with a staggered herringbone design developed by Stroock et al. to aid in passive mixing and increase radiolabeling yields (*19*). The device consisted of a single 200 × 100 μm serpentine channel 4 cm in length containing 900 total herringbones and a total volume of approximately 2.81 μL (Fig. 1). To check the compatibility of the microreactor for use with ^{89}Zr, ^{89}Zr-oxalate was injected into the microreactor and then flushed with 1 M HEPES buffer (pH 7); the entire microreactor was then placed in a dose calibrator and counted to determine the amount of ^{89}Zr retained. The silanated microreactors did not absorb any of the radiometal and were fully compatible with ^{89}Zr.

Antibody adsorption was overcome by the passivation of the surface via silanization (*27*). This process involved activating the reactor surfaces with an acidic hydrogen peroxide solution followed by neat trimethoxy(octadecyl)silane at 50°C. The reactor was then flushed with DMSO, Milli-Q water, air dried, and then annealed at 70°C. After being annealed, the reactor was flushed again with DMSO, deionized water, and then 1 M HEPES buffer before use. After silanization it was found that 95% of the antibody was recoverable after passage through the reactor. Keeping the pH of the reactor near 7 reduced the antibody adhesion further as well.

With the starting reagent adsorption issue addressed, we then moved our focus to the radiolabeling of trastuzumab-DFO under various conditions (Table 1). DFO-Bz-NCS conjugation was performed under aseptic conditions, and the concentration was determined to be 10 mg/mL by bicinchoninic acid assay. A labeling yield of greater than 90% was achieved using a continuous flow for specific activities up to 148 MBq/mg. Performing this labeling using conventional methods (using vials in a thermomixer at 37°C) and the same reaction time resulted in a much lower yield of 36.5%. Incubation within the reactor allowed for a significant increase of the maximum specific activity in comparison to conventional methods (incubation within a vial), increasing it from 220 to 592 MBq/mg (*28*). The immunoreactive fraction was checked to ensure that the antibody integrity was maintained using the Lindmo assay and was found to be 99.3% (Supplemental Fig. 1; supplemental materials are available at http://jnm.snmjournals.org).

Because the goal of the project is to eventually use this technique for labeling in the clinic, we then needed to test whether the device could be used to create clinical doses. Three validation runs were performed, with each product being held to the same standards set by the Food and Drug Administration as a clinical dose. Preparation of the patient dose was completed within 45–60 min and passed all specifications (Table 2).

TABLE 1
Conditions for Labeling Trastuzumab-DFO with ^{89}Zr (n = 3)

mAb:Metal (mg:MBq)	Trastuzumab-DFO (mg/mL)	^{89}Zr (MBq/mL)	Total flow rate (μL/min)	Residence time (min)	iTLC labeling yield
1:37	10	370	20	30	100%
1:37	10	370	20	0.15 (9 s)	100%
1:74	10	370	20	0.15 (9 s)	100%
1:148	10	370	20	0.15 (9 s)	92%
1:148 (Benchtop)	10	370	—	0.15 (9 s)	36.5%
1:296	10	370	20	60	100%
1:370	10	370	20	60	100%
1:592	10	370	20	60	100%

Continuous flow through reactor takes 9 s for material to flow out of outlet.

TABLE 2
^{89}Zr-Trastuzumab Clinical Dose Specifications/Results

Test	Specification	Results
Filter membrane integrity test	≥50 psi	55–60 psi
pH	5.0–6.5	5.5
Appearance and color	Clear; colorless	Clear; colorless; no particulate matter
Strength (radioactivity concentration)	3.7–37 MBq/mL	18.5–37 MBq/mL in 2.5 mL
Specific activity	≥7.4 MBq/mg	20.7–37 MBq/mg
Radioimmunoassay	≥65%	72.4%–96.5%
Radionuclidic purity	≥99.5%	100%
Radiochemical purity	≥95%	100%
Protein aggregation	≥80% monomer	96%–99% monomer
Bacterial endotoxin	≤175 EU/V (where V is the maximum total dose)	<50 EU/mL
Sterility	Sterile (no visible growth)	Sterile

DISCUSSION

Trastuzumab is currently used in the clinic as a treatment of primary and metastatic breast cancer tumors; however, there is a need for a way to evaluate whether a patient will respond to therapy. Trastuzumab labeled with ^{89}Zr is currently being investigated in several locations for this purpose, and reports have shown promising results (*10,14,29*). With the imminent increase in the use of ^{89}Zr-labeled antibodies in the clinic, we set out to develop a method for creating a dose on demand. This will allow for an easily controlled method to generate single-patient doses quickly while simultaneously reducing the technician's exposure to the activity.

The microreactor was fabricated out of PDMS and glass for 3 reasons. The Food and Drug Administration has approved PDMS as a material for the use in medical devices, which means ease of translation into clinical use. Previously, we have shown that these microreactors are compatible with common radiometals such as ^{68}Ga and ^{64}Cu; thus, we expected to see a minimal loss of ^{89}Zr to the reactor (*17,30*). This is different in comparison to ^{18}F, which is well known to be incompatible with PDMS because of the absorption of ^{18}F into the polymer (*20,31*). We were happy to find no loss of ^{89}Zr to adsorption within the reactor. Last, methods of surface functionalization are well described in the literature, making fabrication and surface modifications relatively easy to perform (*18,27*). This proved necessary because without surface modification there was significant loss of mAb due to adsorption. The ease of fabrication and compatibility with both biomolecules and radiometals make PDMS an attractive material for fabricating microreactors for radiopharmaceutical production.

In our previous work, we demonstrated the labeling of bovine serum albumin-DOTA with both ^{68}Ga and ^{64}Cu using a microfluidic device. This labeling required a short incubation time but showed an improvement in radiolabeling yields when compared with conventional methods. When labeling trastuzumab-DFO with ^{89}Zr, however, we were able to achieve high radiolabeling yields at clinically relevant specific activities without an incubation time. This result is likely due to the DFO chelator being an open chain. The lack of steric hindrance in the open chain chelators allows for a quick reaction in comparison to the cyclic structures of DOTA and NOTA. The labeling of trastuzumab-DFO was performed using the conditions in Table 1. For the continuous flow labeling, radiochemical yields began to fall off at radiolabeling ratios greater than 1:148 mg:MBq. As a comparison, labeling the same antibody conjugate using the conventional method at a 1:148 mg:MBq without an incubation period gave a 36.5% radiochemical yield (Fig. 2). The ability to achieve high radiochemical yields in this manner displays the superior mixing achieved through the use of the herringbone pattern and the increased reaction speed from the rapid heat transfer and smaller diffusion distances commonly observed in microfluidics.

The benefit of using a continuous flow is the reduced time it takes to label a large amount of antibody. With this method, it is possible to obtain 1.5 mg of trastuzumab labeled with 55.5 MBq of ^{89}Zr in roughly 15 min, as opposed to the 1-h incubation time required by the current methods. Increasing the ratio between the MBq of ^{89}Zr and mg of trastuzumab further speeds up the process, ending in a product with a specific activity of 148 MBq/mg with a 92% labeling yield in roughly 4 min. This time saving enables more flexibility when scheduling a patient, which can aid in the transition of these radiopharmaceuticals to the clinic. The ability to control the labeling via computer not only shields the technician from the dose, but also reduces error and ensures that the procedure is easily reproduced.

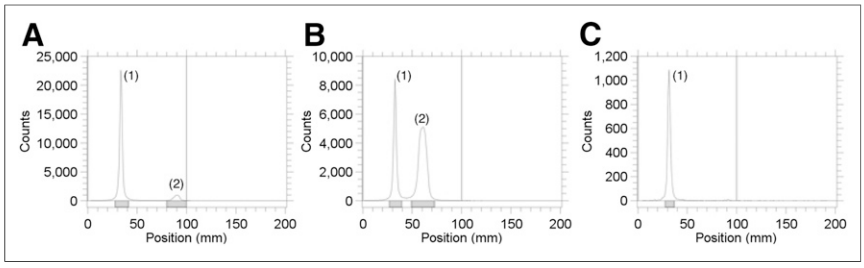

FIGURE 2. iTLC results. (A) Results from labeling at 1:148 ratio using a continuous flow through microreactor. (B) Results from labeling at 1:148 ratio using conventional labeling methods without incubation period. (C) Results from labeling at 1:592 ratio after 1-h incubation in microreactor. (1) = ^{89}Zr-labeled trastuzumab; (2) = free ^{89}Zr.

TABLE 3
Immunoreactivity Results

Date performed	Validation number	Immunoreactivity (%)	Trastuzumab-DFO batch
06/14/14	N/A	99.3	12102010
04/07/15	1	96.5	01302014
05/07/15	2	72.4	09292014
05/13/15	3	94.4	09292014

N/A = not applicable.

Allowing an incubation time of 1 h enhanced the specific activity of the final product while maintaining a high labeling yield, reaching a maximum specific activity of 592 MBq/mg. This is a significant improvement in comparison to the current literature value of 220 MBq/mg and could be useful if applied to mAbs with significant side effects (*28*). A higher specific activity will enable physicians to administer a smaller amount of mAb, potentially eliminating toxic effects while still being able to visualize the localization of the labeled mAb. Cleaning the device is also a concern if it is to be used in clinical production. The reactors can be flushed with 1 M nitric acid without harm to clean the system quickly. We have found that the reactors need to be flushed with buffer to return them to pH 7 after cleaning to neutralize the reactor. Failure to neutralize the reactor before labeling results in low radiochemical yields.

To ensure that the radiolabeled antibody is able to bind to the intended receptor, we performed the Lindmo assay on HER2-positive SKBR3 cells. The total/bound activity was plotted versus 1/normalized cell concentration, and the *y*-intercept was used to determine the immunoreactivity. As expected, the results showed the labeled trastuzumab had a high affinity for the HER2-positive cells with high confidence. This retention of the antigen-binding properties shows that there are no adverse effects from the labeling process and suggests that the process could be used for the production of radiopharmaceuticals.

The purpose of this system was to automate the production of ^{89}Zr-trastuzumab for human use. To ensure that the system would be able to produce a patient dose, we performed 3 validation runs, which produced doses between 46.25 and 92.5 MBq in 2.5 mL of saline. All samples were prepared using aseptic techniques with the exception of the labeling process, which was performed within the microreactor. These validation runs were held to the strict quality control methods set by the Food and Drug Administration that would be required for a human dose. The diluted solution developed no particles and did not change color during the preparation process, and the pH was unchanged from that of the saline. As we observed with the smaller samples, the immunoreactivity was unaffected by the procedure and passed the required more than 65% (Table 3). The assays in validation runs 2 and 3 were performed using the same batch of trastuzumab-DFO. The different results display the variability of target expression in cells and the difficulty in reproducing results using the Lindmo assay. Radiochemical purity of the samples was checked using iTLC and confirmed to be 100%. Size-exclusion HPLC confirmed that a minimal amount of aggregates had formed during the labeling process (Fig. 3). The solution was sterile and free of endotoxins, and the filter membrane was still intact after filtration was finished.

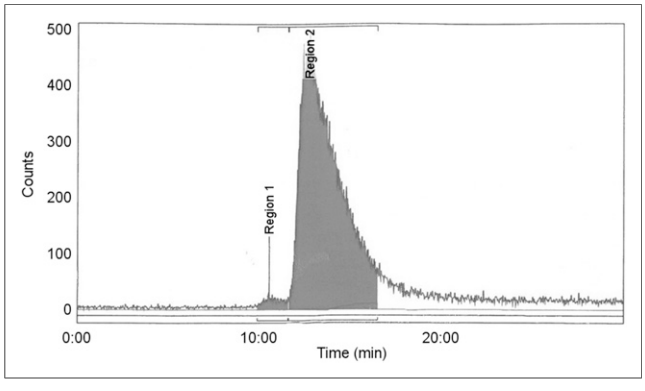

FIGURE 3. HPLC aggregation data. Region 1 shows 2% aggregates, and region 2 shows 98% ^{89}Zr-labeled monomer.

CONCLUSION

Here we report the successful development of a microfluidic platform for labeling antibodies with ^{89}Zr. Trastuzumab-DFO labeled in a continuous flow method resulted in high radiochemical yields at specific activities up to 148 MBq/mg. Using an incubation period enabled a large increase in the maximum specific activity in comparison to conventional methods. Clinical-grade single-patient doses were prepared and passed all quality control specifications including pH, chemical purity, immunoreactivity, and sterility. The small, computer-controlled system in conjunction with a sterile laminar flow hood was able to produce a patient dose in roughly a quarter of the time required in the conventional method, bringing the radiopharmaceutical closer to a dose on demand.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734. This work was supported in part by the DOE BER Integrated Research Training Program of Excellence in Radiochemistry under grant DESC0008432, by 1R01CA161348, and by 1R21CA182945-01. No other potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Scott AM, Wolchock JD, Old LJ. Monoclonal antibodies currently FDA approved in oncology and their mechanisms of action. *Nat Rev Cancer.* 2012;12:278–287.
2. Hematology/Oncology (Cancer) Approvals & Safety Notifications. FDA website. http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm279174.htm. Updated January 28, 2016. Accessed February 4, 2016.
3. Chames P, Regenmortel MV, Weiss E, Baty D. Therapeutic antibodies: successes, limitations and hopes for the future. *Br J Pharmacol.* 2009;157:220–233.
4. Clinical Pharmacology Review of Herceptin, 98-0369. FDA website. http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm091373.pdf. Accessed February 4, 2016.
5. van Dongen GA, Vosjan M. Immuno-positron emission tomography: shedding light on clinical antibody therapy. *Cancer Biother Radiopharm.* 2010;25:375–385.

6. Verel I, Visser G, Boerman O, et al. Long-lived positron emitters zirconium-89 and iodine-124 for scouting of therapeutic radioimmunoconjugates with PET. *Cancer Biother Radiopharm.* 2003;18:655–661.
7. Meijs WE, Haisma H, Van Der Schores R, et al. A facile method for the labeling of proteins with zirconium isotopes. *Nucl Med Biol.* 1996;23:439–448.
8. Marquez BV, Ikotun O, Zheleznyak A, et al. Evaluation of ^{89}Zr-pertuzumab in breast cancer xenography. *Mol Pharm.* 2014;11:3988–3995.
9. Doran MG, Watson P, Cheal S, et al. Annotating STEAP1 regulation in prostate cancer with ^{89}Zr immuno PET. *J Nucl Med.* 2014;55:2045–2049.
10. Gaykema SB, Schröder C, Vitfell-Rasmussen J, et al. ^{89}Zr-trastuzumab and ^{89}Zr-bevacizumab PET to evaluate the effect of the HSP90 inhibitor NVP-AUY922 in metastatic breast cancer patients. *Clin Cancer Res.* 2014;20:3945–3954.
11. Dijkers EC, Kosterink J, Rademaker A, et al. Development and characterization of clinical grade ^{89}Zr-trastuzumab for HER2/neu immunoPET imaging. *J Nucl Med.* 2009;50:974–981.
12. Perk LR, Visser O, Stigter-van Walsum M, et al. Preparation and evaluation of Zr-89-zevalin for monitoring of Y-90-zevalin biodistribution with positron emission tomography. *Eur J Nucl Med Mol Imaging.* 2006;33:1337–1345.
13. Albanell J, Codony J, Rovira A. *Mellado B*, Gascon P. Mechanism of action of anti-HER2 monoclonal antibodies: scientific update on trastuzumab and 2C4. *Adv Exp Med Biol.* 2003;532:253–268.
14. Dijkers EC, Munnink T, Kosterink J, et al. Biodistribution of ^{89}Zr-trastuzumab and PET imaging of HER2-positive lesions in patients with metastatic breast cancer. *Clin Pharmacol Ther.* 2010;87:586–592.
15. Oita I, Halewyck H, Thys B, Rombaut B, Vander Heyden Y, Mangelings D. Microfluidics in macro-biomolecules analysis: macro inside a nano world. *Anal Bioanal Chem.* 2010;398:239–264.
16. Javed MR, Chen S, Kim H-K, et al. Efficient radiosynthesis of 3′-deoxy-3′-^{18}F-fluorothymidine using electrowetting-on-dielectric digital microfluidic chip. *J Nucl Med.* 2014;55:321–328.
17. Wheeler TD, Zeng D, Desai A, Onal B, Reichert D, Kenis P. Microfluidic labeling of biomolecules with radiometals for use in nuclear medicine. *Lab Chip.* 2010;10:3387–3396.
18. Li H, Whittenberg J, Zhou H, et al. Development of a microfluidic "click chip" incorporating an immobilized Cu(1) catalyst. *RSC Advances.* 2015;5:6142–6150.
19. Stroock AD, Dertinger S, Ajdari A, Mezic I, Stone H, Whitesides G. Chaotic mixers for microchannels. *Science.* 2002;295:647–651.
20. Elizarov AM. Microreactors for radiopharmaceutical synthesis. *Lab Chip.* 2009;9:1326–1333.
21. Audrain H. Positron emission tomography (PET) and microfluidic devices: a breakthrough on the microscale? *Angew Chem Int Ed Engl.* 2007;46:1772–1775.
22. Vosjan MJ, Perk L, Visser G, et al. Conjugation and radiolabeling of monoclonal antibodies with zirconium-89 for PET imaging using the bifunctional chelate p-isothiocyantobenzyl-desferrioxamine. *Nat Protoc.* 2010;5:739–743.
23. Viola-Villegas NT, Rice S, Carlin S, et al. Applying PET to broaden the diagnostic utility of the clinically validated CA19.9 serum biomarker for oncology. *J Nucl Med.* 2013;54:1876–1882.
24. Wooten A, Madrid E, Schweitzer G, et al. Routine production of ^{89}Zr using an automated module. *Appl Sci.* 2013;3:593–613.
25. Perk RL, Vosjan M, Visser G, et al. p-Isothiocyanatobenzyl-desferrioxamine: a new bifunctional chelate for facile radiolabeling of monoclonal antibodies with zirconium-89 for immuno-PET imaging. *Eur J Nucl Med Mol Imaging.* 2010;37:250–259.
26. Lindmo T, Boven E, Cuttitta F, Fedorko J, Bunn P. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J Immunol Methods.* 1984;72:77–89.
27. Sui G, Wang J, Lee C-C, et al. Solution-phase surface modification in intact poly(dimethylsiloxane) microfluidic channels. *Anal Chem.* 2006;78:5543–5551.
28. Chang AJ, DeSilva R, Jain S, Lears K, Rogers B, Lapi S. ^{89}Zr-radiolabeled trastuzumab imaging in orthotopic and metastatic breast tumors. *Pharmaceuticals (Basel).* 2012;5:79–93.
29. ^{89}Zr-trastuzumab. ClinicalTrials.gov website. http://clinicaltrials.gov/ct2/results?term=^{89}Zr-Trastuzumab&Search=Search. Accessed February 26, 2016.
30. Zeng D, Desai A, Ranganathan D, Wheeler T, Kenis P, Reichert D. Microfluidic radiolabeling of biomolecules with PET radiometals. *Nucl Med Biol.* 2013;40:42–51.
31. Lee C-C, Sui G, Elizarov A, et al. Multistep synthesis of a radiolabeled imaging probe using integrated microfluidics. *Science.* 2005;310:1793–1796.