Original Article

Optical Fiber-Based In Vivo Quantification of Growth Factor Receptors

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BACKGROUND: Growth factor receptors such as epidermal growth factor receptor 1 and human epidermal growth receptor 2 (HER2) are overexpressed in certain cancer cells. Antibodies against these receptors (eg. cetuximab and transtuzumab [Herceptin]) have shown therapeutic value in cancer treatment. The existing methods for the quantification of these receptors in tumors involve immunohistochemistry or DNA quantification, both in extracted tissue samples. The goal of the study was to evaluate whether an optical fiber-based technique can be used to quantify the expression of multiple growth factor receptors simultaneously. **METHODS:** The authors examined HER2 expression using the monoclonal antibody trastuzumab as a targeting ligand to test their system. They conjugated trastuzumab to 2 different Alexa Fluor dyes with different excitation and emission wavelengths. Two of the dye conjugates were subsequently injected intravenously into mice bearing HER2-expressing subcutaneous tumors. An optical fiber was then inserted into the tumor through a 30-gauge needle, and using a single laser beam as the excitation source, the fluorescence emitted by the 2 conjugates was identified and quantified by 2-photon optical fiber fluorescence. **RESULTS:** The 2 conjugates bound to the HER2-expressing tumor competitively in a receptor-specific fashion, but they failed to bind to a similar cell tumor that did not express HER2. The concentration of the conjugate present in the tumor as determined by 2-photon optical fiber fluorescence was shown to serve as an index of the HER2 expression levels. **CONCLUSIONS:** These studies offer a minimally invasive technique for the quantification of tumor receptors simultaneously. ***Cancer* 2012;118:2148-56.** *© 2011 American Cancer Society.*

KEYWORDS: double-clad optical fiber, 2-photon excitation, cancer targeting, growth factor receptor quantification, HER2, Herceptin.

INTRODUCTION

The overexpression of growth factor receptors has been implicated in the growth and metastasis of multiple human neoplasms.[1] Cell surface receptors, such as the ErbB family proteins epidermal growth factor receptor (EGFR), human epidermal growth receptor 2 (HER2), HER3, and HER4,[2-5] vascular endothelial growth factor receptor (VEGFR),[2,6] platelet-derived growth factor receptor,[7-10] fibroblast growth factor receptor,[11-14] and insulinlike growth factor 1 receptor (IGFR),[15,16] have been shown to be overexpressed in human cancers. Monoclonal antibodies produced against several of these receptors, such as trastuzumab (Herceptin; anti-HER2), cetuximab (anti-EGFR), and bevacizumab (anti-VEGFR), have either been shown to have therapeutic value in tumor suppression or are undergoing evaluation in clinical trials.[2,17] Assessing the expression of these receptors on tumors is generally performed by conventional immunohistochemical methods or by nucleic acid quantification and determination of the relevant gene copies, in both cases using isolated tumor tissues. The development of a minimally invasive method for the in vivo quantification of these receptors would have utility in determining the value of a targeted therapy directed against these receptors, as well as for the repeated imaging and monitoring of antibody therapy against receptor-expressing tumor.

One of the most extensively investigated tumor-related growth factor receptors is HER2, a disease marker for breast cancer. HER2 is overexpressed in approximately 25% of breast cancers.[18-20] Trastuzumab, a US Food and Drug

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Administration (FDA)-approved humanized antibody against HER2, is currently used for targeted therapy against breast cancer.[21-24] However, treatment with trastuzumab is costly, can be associated with cardiotoxicity,[25,26] and is only effective against tumors with HER2 amplification.[27-29] Therefore, accurate quantification of HER2 expression in tumors before treatment with trastuzumab is crucial to the success of the therapy.

The most commonly used method to assess HER2 expression is in vitro immunohistochemical staining of isolated tumor tissue samples using HER2 antibodies.[30,31] The assessment is based on scoring the histochemical slides as 0 (<10% of any degree of staining), 1+ (>10% incomplete staining), 2+ (>10% weak staining), or 3+ (>10% intense staining),[24] and only cases with 3+ staining are considered for trastuzumab therapy.[22,27-29] However, there are several limitations in the immunohistochemical assessment of HER2, and the results may not always provide accurate decision points for instituting trastuzumab therapy.[24,32-39] The specific scoring of the slides for membrane staining is, at best, semiquantitative and generates only relative numbers even when using computerized algorithm-based grading. The uncertainty in these assessments is evident from the recent amendment by the American Society of Clinical Oncology/College of American Pathologists, where the criterion for the 3+ HER2 is tumors showing ">30% intense staining," although the FDA still recommends a standard of ">10% intense staining."[39-41] Equivocal samples (2+) must be further analyzed by the fluorescence in situ hybridization (FISH) test.[40] Although FISH may give more objective scoring criteria (>6 and <4 copies of the gene as positive and negative, respectively, and 5 copies of the gene as equivocal), it is an expensive and time-consuming technique that requires a highly skilled clinician and has technical challenges including fixation problems, nonintact nuclei, and the inability to preserve slides for further review.[24,39,42-46] Importantly, both the immunohistochemical and FISH methods have the drawback of requiring tissue samples to be removed by invasive surgical procedures. Needle aspiration using 20- to 25-gauge needles can make it challenging to obtain sufficient tissue to do the analyses.[43,47,48] Moreover, the results of the in vitro methods can be influenced by the biochemical changes occurring during the recovery; the overall result of this being that recent studies estimate ~20% of the currently used HER2 quantification results produce inaccurate results.[39,41] Furthermore, these techniques do not assess pathways identified for trastuzumab resistance because of the coexpression and involvement of other receptors, such as IGFR,[49,50] suggesting the need for identifying multiple tumor receptors to predict the utility of trastuzumab therapy.

Our previous studies have shown that a 2-photon optical fiber fluorescence-based detection system can quantify tissue fluorescence both in vitro and in live animals.[51-53] The broad range of wavelengths that can excite the 2-photon excitation also allows fluorescently tagged probes with different excitation/emission wavelengths to be simultaneously quantified using a single laser beam. Therefore, this method has the potential for the simultaneous quantification of multiple different receptors expressed on a tumor.

The hypothesis of this study is that a 2-photon optical fiber fluorescence probe inserted into a tumor can simultaneously quantify 2 different, fluorescently tagged probes targeted to a tumor in vivo, which in turn can serve as an index for the amount of receptors present in the tumor. To test this hypothesis, we synthesized and tested different fluorescently tagged trastuzumab conjugates. Two of these conjugates were intravenously injected into a mouse bearing HER2-expressing tumors, and their fluorescence was quantified using the 2-photon optical fiber fluorescence probe. We show the measurement of the 2 conjugates under receptor-saturating conditions, demonstrating the feasibility of this approach for quantifying the actual numbers of multiple receptors simultaneously.

MATERIALS AND METHODS

Materials

The carboxylic acid, succinimidyl esters of Alexa Fluor 594 (Excitation/Emission: 590/617; $\varepsilon = 92{,}000\ cm^{-1}\ M^{-1}$), Alexa Fluor 633 (Excitation/Emission: 632/647; $\varepsilon = 100{,}000\ cm^{-1}\ M^{-1}$), and Alexa Fluor 660 (Excitation/Emission: 663/690; $\varepsilon = 132{,}000\ cm^{-1}\ M^{-1}$), was obtained from Invitrogen (Carlsbad, Calif). Trastuzumab was purchased from the University of Michigan Hospitals. Spectra/Por dialysis membrane (MWCO 3500) and Millipore Centricon ultrafiltration membrane (YM-10) were obtained from Fisher Scientific (Pittsburgh, Pa). MCA207-control and MCA207-HER2 cells were obtained from Dr. Kevin Mc Donaugh at the University of Kentucky.[52] Female, severe combined immunodeficiency (SCID) mice were purchased from Charles River Laboratories (Wilmington, Mass). Dulbecco modified Eagle medium (DMEM) cell culture medium, fetal calf serum (FCS), trypsin-ethylenediaminetetraacetic acid, penicillin/streptomycin, and Dulbecco phosphate-

buffered saline (PBS; pH 7.4) were from Gibco/BRL (Gaithersburg, Md). All other reagents were from Aldrich (St Louis, Mo). The double-clad fiber used in this study had an inner core diameter of 6 μm, with a numerical aperture of 0.15. The diameter of the inner cladding was 123 μm, with a numerical aperture of 0.46 (P6/123DC; Liekki Corporation, Lohja, Finland).

Methods

Synthesis and purification of trastuzumab-Alexa Fluor conjugates

Our previous studies have shown the targeting of a fluorescein isothiocyanate (FITC)-trastuzumab conjugate into mouse tumors.[52] As FITC is known to be highly photounstable, reliable quantitative information may not be possible with FITC. To overcome this problem, in the current study we synthesized Alexa Fluor conjugates with known photostable properties. To a 5 mg/mL solution of trastuzumab-PBS we slowly added the Alexa Fluor dyes in dimethylsulfoxide (5× excess), mixing them well using a magnetic stirrer. The reaction mixture was stirred continuously overnight at room temperature, and the products were subjected to column chromatography using a Sephadex G25 column (1 × 30 cm) equilibrated with 10 mM phosphate buffer pH 7.0, performed at 4°C. One-milliliter fractions were collected, and the absorbance of the collected fractions was determined at 280 nm as well as at the absorbance maximum for each dye. In Figure 1A is given a typical elution pattern for the separation of the free dye from the conjugates, shown for the trastuzumab-Alexa Fluor 633 conjugate, with similar conjugate and free dye elution volumes obtained for the other conjugates synthesized. Appropriate conjugate fractions were collected and concentrated using a Centricon (Millipore, Bedford, Mass; 10 K molecular weight cutoff) filter device. The protein concentration of the sample was determined using a bicinchoninic acid reagent (Pierce, Rockford, Ill), following the vendor's protocol.

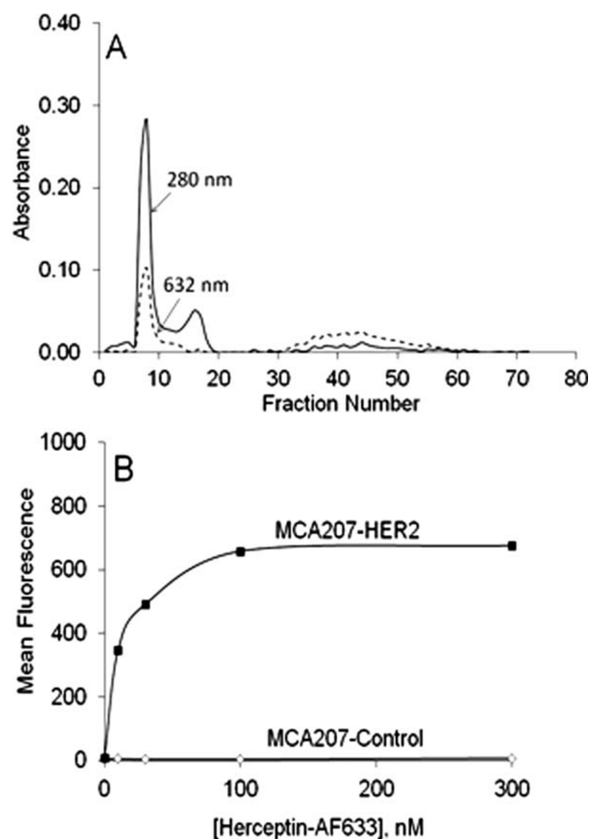

Figure 1. (A) Gel filtration purification of the trastuzumab-Alexa Fluor 633 conjugate is shown. The conjugate was synthesized as described in Methods, and 1 mL of the reaction mixture was applied onto a 25-cm Sephadex G25 column equilibrated with 10 mM phosphate buffer, pH 7.0. One-milliliter fractions were collected, and the absorbance of the fractions was determined at 280 nm (solid line) and at 632 nm (dashed line). In this typical experiment, fractions 6 to 10 (trastuzumab-Alexa Fluor 633) were pooled, concentrated, and used for biological studies. The peak eluted at fractions 30 to 60 represent the free dye, and that which eluted between fractions 12 to 20 may represent free trastuzumab. (B) Flow cytometric analysis of the cellular binding of trastuzumab-Alexa Fluor 633 is shown. The indicated cells were plated in 24-wells and incubated with different concentrations of the conjugate for 1 hour at 37°C. The cells were rinsed, and the Alexa Fluor fluorescence of 10,000 cells was measured in a flow cytometer.

Two-photon optical fiber fluorescence system instrumentation

We used a recently developed solid-core double-clad optical fiber, having improved sensitivity for quantification of low-level tissue fluorescence.[53] The length of the fiber used was approximately 1 m. Femtosecond laser pulses (20 mW) generated from a Ti:sapphire oscillator (Mira 900; Coherent, Santa Clara, Calif) with a pulse duration of 150 fs and center wavelength of 800 nm were used as the excitation source; the pulses were prechirped using a grating pair to precompensate the dispersion of the optical fiber, so the pulse duration at the excitation point was also close to 150 fs. Double-clad fiber was directly inserted into the dye solutions, cell pellets, and the tumor through a 30-gauge needle. The fluorescence signal was collected back through the same fiber and sent through band pass filters before being detected using a photon counting photomultiplier tube (PMT). The signals from the PMT were then analyzed by a computer to generate the 2-photon optical fiber fluorescence photon counts.

Biological studies

MCA207 cells were maintained in DMEM media supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin, under 5% CO_2. Flow cytometric analysis of the cells was performed as we have described previously, using the Flow Cytometry Core facility of the University of Michigan.[52] For the in vitro 2-photon optical fiber fluorescence studies, cells plated in 75-mL flasks were trypsinized, rinsed, and resuspended in PBS containing 0.1% bovine serum albumin. For the in vitro analyses, the cells in suspension were treated with different concentrations of the conjugates for 1 hour at 37°C, rinsed, and centrifuged to collect the cell pellets. The double-clad fiber was then inserted into different regions of the cell pellet, and the 2-photon optical fiber fluorescence counts were collected as previously described.[53]

For the in vivo studies, MCA207-control or MCA207-HER2 cell tumors were developed in 7-week-old SCID mice as described before.[52] The mice were housed in a specific pathogen-free animal facility at the University of Michigan Medical Center in accordance with the regulations of the University's Committee on the Use and Care of Animals as well as with federal guidelines, including the Principles of Laboratory Animal Care. The animals were fed ad libitum with Laboratory Autoclavable Rodent Diet 5010 (PMI Nutrition International, St Louis, Mo). The cells were trypsinized, rinsed, and injected as a 0.1-mL suspension using sterile PBS. The tumors were allowed to reach 0.7 to 0.8 cm in diameter before analysis. Two nanomoles (~1.3 μM initial circulating concentration for 1.5 mL of mouse blood volume) of the conjugates in 0.1 mL of PBS or PBS alone as a control were administered through the tail vein. Fifteen hours later, the mice were euthanized with isoflurane, and the tumors were immediately excised and frozen. The 15-hour time point was selected based on our previous observation that a trastuzumab-FITC conjugate saturates the MCA207-HER2 tumor between 4 and 24 hours.[52] The optical fibers were inserted into the tumor through a 30-gauge needle, with the tip of the fiber placed at the proximal end of the needle hole. The needle with the fiber was sonicated in a bath sonicator between tumor sample measurements. The fluorescence of multiple internal regions of the tumors was recorded for 60 seconds during insertions of the needle through 2 to 3 mm tumor depth, manually controlled by a 2-dimensional translational stage.

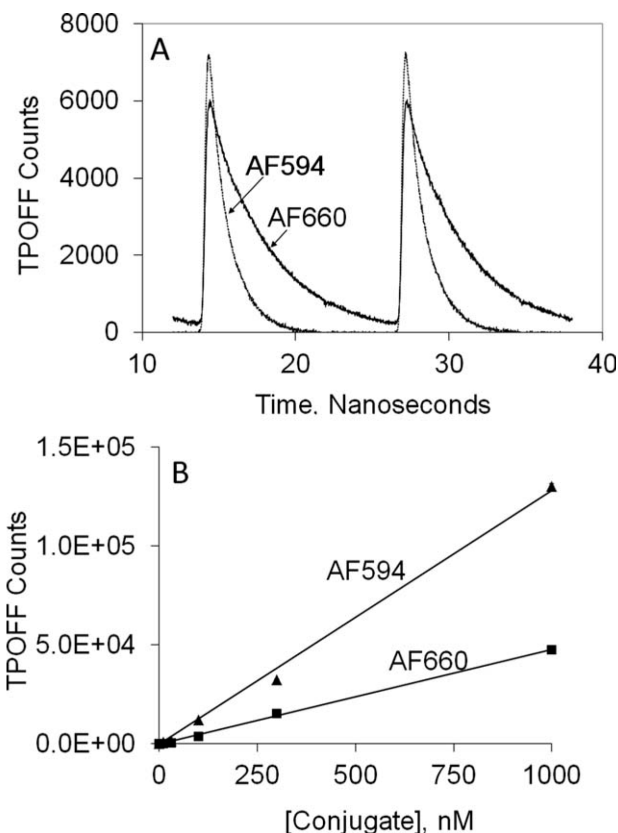

Figure 2. Linearity of the 2-photon optical fiber fluorescence (TPOFF) measurements of standard solutions of the dyes Alexa Fluor 594 and Alexa Fluor 660 is shown. The double-clad fiber was dipped into solutions of the 2 dyes in phosphate-buffered saline, pH 7.4, and the TPOFF counts were taken for a period of 60 seconds. (A) The fluorescence decay curves of 300-nM solutions of the 2 dyes are shown. (B) Mean TPOFF counts per second obtained for different concentrations of the 2 dyes are shown, corrected for buffer background.

The statistical significance of differences among the groups was analyzed by the Student-Newman-Keuls test, with significance calculated at $P < .05$.

RESULTS

We initially verified if the newly synthesized products were biologically active by flow cytometry analyses using purified conjugates. As demonstrated in Figure 1B, the trastuzumab-Alexa Fluor 633 conjugate bound to the MCA207-HER2 cells in a dose-dependent and saturable fashion, whereas the MCA207-control cells failed to bind to the conjugate, confirming the retention of its binding activity. The binding onto the MCA207-HER2 cells was completely blocked by a 10-fold excess of free trastuzumab (data not shown), indicating the receptor specificity of the conjugate.

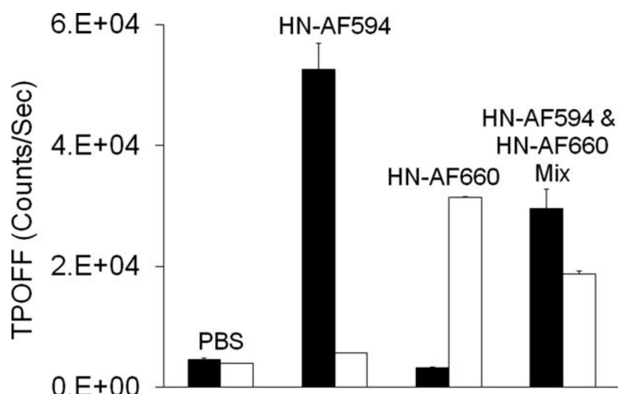

Figure 3. Simultaneous 2-photon optical fiber fluorescence (TPOFF) measurement of the mixture of the 2 trastuzumab conjugates (Herceptin [HN])-Alexa Fluor 594 and HN-Alexa Fluor 660 is shown. The TPOFF of the 2 conjugates prepared in phosphate-buffered saline (PBS) buffer was measured either individually (30 nM each), or as a mixture of the 2 (15 nM each), using a single excitation laser beam, and acquiring the TPOFF counts using 2 different filters, 525 ± 125 nm (solid bars) and 680 ± 15 nm (open bars), selected for capturing the specific fluorescence of HN-Alexa Fluor 594 and HN-Alexa Fluor 660, respectively.

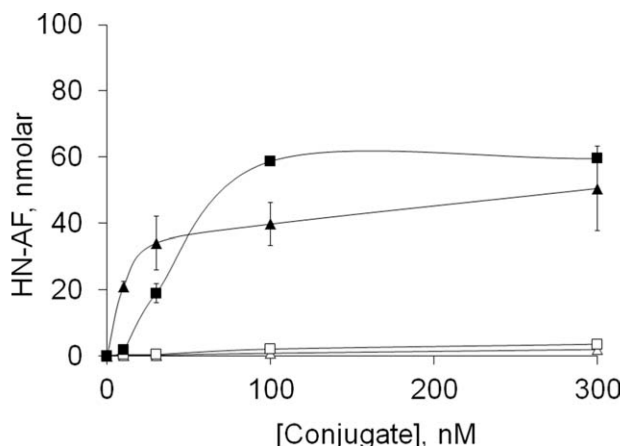

Figure 4. In vitro targeting of trastuzumab (Herceptin [HN]) conjugates HN-Alexa Fluor (AF) 594 (triangle symbols) and HN-AF 660 (square symbols) in human epidermal growth receptor 2 (HER2)-negative (open symbols) and HER2-positive (filled symbols) MCA 207cells was determined by 2-photon optical fiber fluorescence measurements. A suspension of 5 × 10^6 cells in phosphate-buffered saline containing 0.1% bovine serum albumin was incubated with different concentrations of the conjugate for 1 hour at 37°C, the cells were rinsed and centrifuged, and the 2-photon optical fiber fluorescence counts were taken in different regions of the cell pellet. The data are given as the nanomolar concentration in the cell pellet, calculated from the 2-photon optical fiber fluorescence counts obtained for the standard conjugate solutions.

We selected the 2 dyes, Alexa Fluor 594 and Alexa Fluor 660, which are widely separated on their emission spectra, for further in vitro and in vivo 2-photon optical fiber fluorescence studies. Initially we verified the linearity of the 2-photon optical fiber fluorescence counts, and as given in Figure 2, the mean 2-photon optical fiber fluorescence counts of the 2 dyes were linear up to 1000 nM. Conjugates of trastuzumab with these dyes were then used to examine the utility of the 2-photon optical fiber fluorescence to simultaneously measure the fluorescence in solution by comparing their 2-photon optical fiber fluorescence measured individually and as a mixture. For this, the fluorescence emitted by the 2 conjugates was measured using appropriate filters that selectively collect the individual fluorescence of the 2 dye molecules in the mixture. A mixture of 15 nM each of the 2 conjugates gave half of the 2-photon optical fiber fluorescence counts given individual conjugates measured separately at 30 nM each (Fig. 3), demonstrating the quantitative nature of the fluorescence measured in the mixture by 2-photon optical fiber fluorescence. The double-clad fiber probe was then used to quantify the targeted fluorescence of the 2 conjugates in cell pellets obtained from the cell lines. The conjugates bound to the MCA207-HER2 cells in a dose-dependent and saturable fashion, whereas they completely failed to bind to the MCA 207-control cells, which lack the HER 2 receptor (Fig. 4), confirming the preliminary flow cytometric data (Fig. 1B).

To gather proof of concept for the simultaneous targeting of 2 different conjugates as a strategy for receptor quantification, we administered the 2 Alexa Fluor-labeled conjugates into mice bearing HER2-postive and HER2-negative tumors. The conjugates were intravenously injected into mice either individually or as their mixture (2 nmol each, and in the mixture a total of 4 nmol combined), and the fluorescence accumulated in the tumor after a 15-hour time point was quantified by 2-photon optical fiber fluorescence measurement. In support of the in vitro targeting (Fig. 4) and our previous tumor targeting of trastuzumab-FITC,[52] both trastuzumab-Alexa Fluor conjugates were specifically targeted into the MCA207-HER2 tumors, whereas they failed to bind to MCA207-control tumors (Fig. 5).

The tumor fluorescence of each conjugate was quantified using appropriate filters that collected the specific fluorescence emission of the conjugate administered (Fig. 5A and B). When the 2 conjugates were injected into the mice as a mixture, both could be quantified simultaneously (Fig. 5C), similar to the demonstrated quantification of the 2 mixtures initially verified in solutions (Fig. 3). Importantly, we observed that the 2 conjugates competed for the receptor, thereby giving only half the

accumulated fluorescence in the tumor when given as mixture versus when given to them individually, confirming the HER2-specific binding of the conjugate in the tumor.

Knowing the 2-photon optical fiber fluorescence of the standards of the trastuzumab-Alexa Fluor, the average tumor fluorescence obtained can be converted to the average concentration of the conjugates in the tumor. As shown in Figure 5C, the 2 conjugates when administered individually showed an average concentration of about 60 nM, which is similar to the saturated levels obtained in the in vitro uptake measured in the cell pellets (Fig. 4). This suggests that at the 2-nmol doses of the conjugates injected into the mice, the entire tumor HER2 was fully occupied by each of the 2 conjugates. This is further supported by the observation that at a total of 4 nmol combined administration of the 2 conjugates, there is nearly half of the total fluorescence of that injected individually. As trastuzumab is known to bind to HER2 at a 1:1 ratio,[54] the concentration of trastuzumab bound at the saturating concentration is equivalent to the concentration of the average number of HER2 receptors present in the tumor. As the data given in Figure 5 are derived from the measurement of 4 to 5 separate regions of the tumor interior while the needle was moved vertically over a span of 2 to 3 mm during each measurement, the average fluorescence shown is representative of several thousand cells. Therefore, the concentration of the HER2 derived from Figure 5C is a fair representation of the average HER2 in the whole tumor mass.

DISCUSSION

Our study predicts the utility of the 2-photon optical fiber fluorescence fiber probe as a tool to quantify the total amount of overexpressed receptors such as HER2 or EGFR in solid tumors. In addition, this study shows that the intravenous administration of a mixture of

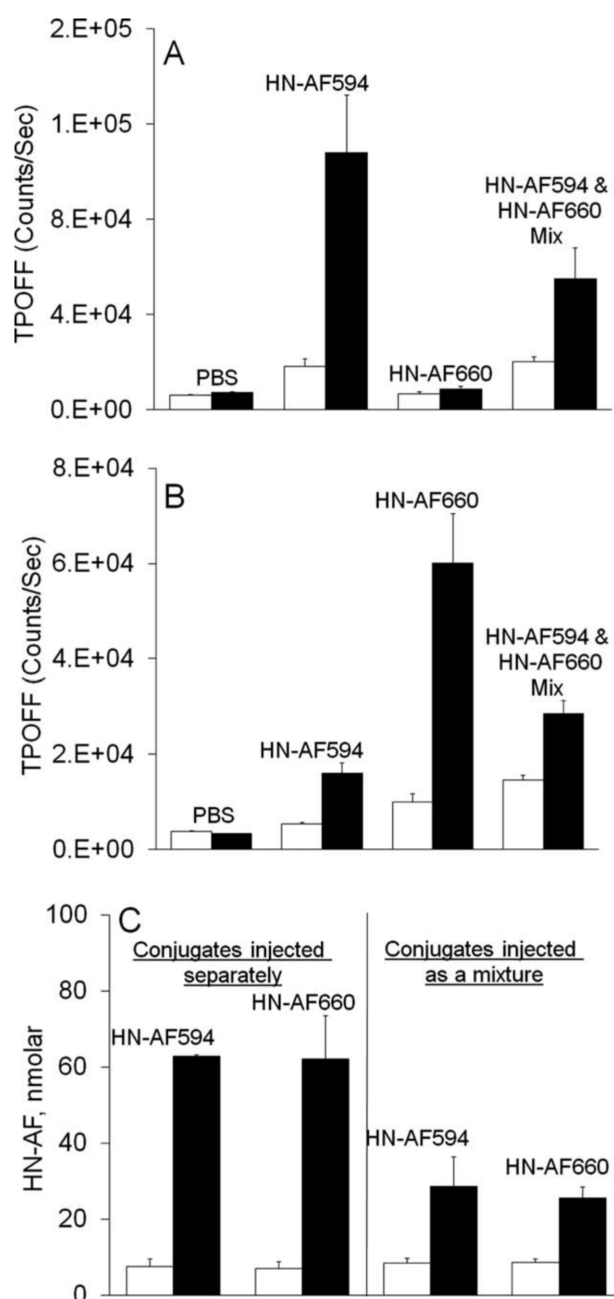

Figure 5. Simultaneous 2-photon optical fiber fluorescence (TPOFF) measurement of the in vivo targeting of 2 trastuzumab (Herceptin [HN]) conjugates HN-Alexa Fluor (AF) 594 and HN-AF 660 in mice xenograft tumors expressing human epidermal growth receptor 2 (HER2) is shown. MCA207-control tumor and MCA207-HER2 tumors were developed in SCID mice, respectively on the left and right flank areas of each mouse. When the tumors reached an average of 0.8 cm in diameter, 2 nmol each of the 2 conjugates was injected through the tail vein, either individually, or as their mixture (total 4 nmol of the 2 conjugates in the mixture). The control mice were injected with the vehicle phosphate-buffered saline (PBS). The tumors were isolated after 15 hours, and the TPOFF counts were determined by inserting the fiber into different regions of the tumor through a 30-gauge needle, using 2 different filters as described in the legend for Figure 3. The open bars represent counts obtained for the MCA207-control tumors, and the solid bars represent the counts obtained for MCA-HER2 tumors. The data given are the mean $\pm$ standard error obtained for 4 to 5 different internal regions of the tumor, each reading having been taken over a period of 60 seconds while the needle was being slowly moved inwardly. (A) Mean TPOFF counts were obtained using the 525 $\pm$ 125 nm filter. (B) Mean TPOFF counts were obtained using the 660 $\pm$ 15 nm filter. (C) The nanomolar concentrations of the 2 conjugates in the tumor were calculated from the TPOFF counts obtained for the standard conjugate solutions.

fluorescently tagged antibodies would allow the simultaneous quantification of 2 or more receptors. The currently practiced immunohistochemical and FISH methods for quantification of the HER2 rely on in vitro analyses of tissues removed during surgery or by needle biopsy. The latter method often requires repeated insertion of larger-gauge needles to retrieve sufficient tissue sample needed for analyses. As the 2-photon optical fiber fluorescence probe can be inserted into the tumor using an ultrafine 30-gauge needle, this method can serve as an alternative, minimally invasive method to quantify the receptors by using appropriate fluorescent ligands administered intravenously. The 2-photon optical fiber fluorescence method also has the potential advantage that it quantifies the native HER2 in live cells without introducing experimental artifacts because of tissue processing and storage, limitations inherent in the conventional methods. The biologically relevant HER2 is only that which is expressed on the cell surface and not that seen in the cell interior. As the systemically administered HER2 antibody accumulates in the tumor via extracellular binding after delivery from the circulation, the fluorescence measured will be proportional to the cell surface-expressed HER2. Although in the current study the 2-photon optical fiber fluorescence measurements were made in isolated tumors, based on our previous findings on the feasibility of tumor fluorescence quantification in live animals,[52] we believe these studies can be extended to receptor quantification in live animal tumors.

The application of the 2-photon optical fiber fluorescence method for receptor quantification relies on the tumor-specific binding of the ligands (eg, trastuzumab) onto the receptor, which may be dependent on the ligand pharmacokinetics, clearance from the normal tissues, tumor penetration, and tumor size. Although the variability of pharmacokinetics and tumor availability may compromise the usefulness of this method to correctly translate the observed fluorescence to tumor receptor levels, further validation of this technique by determining the kinetics of the ratio of the fluorescence of tumor versus blood and tumor versus adjacent tissue (eg, normal breast tissue, the fluorescence of which can be simultaneously recorded during insertion of the needle into the breast) may establish its clinical utility. In support to this view, several recent studies in animal tumor models have shown high specific uptake of radiolabeled HER2 antibody in the tumor, with tumor to tissue/blood ratio reaching a maximum from 48 to 72 hours,[55] and HER2-positron emission tomography scanning is currently being evaluated as a possible imaging tool for HER2-positive breast tumor.[56] One drawback of the trastuzumab antibody used in this study is its observed cardiotoxicity.[25] Nonetheless, any observed toxicity problems can be solved by choosing ligands that bind to the receptor without activating any cellular signal transduction pathways. One such possibility is the design of a fluorescently labeled dendrimer nanoparticle carrying polyvalent small molecule peptides or scFv-Fc fragments that bind to the HER2 without mediating any cellular signal activation pathways.[53] For clinical application, it is also crucial that the toxicity of the conjugated dye is thoroughly examined. Multiple insertions of the needle through different areas within the tumor are needed for the accurate quantification of average receptor number in the whole tumor volume. However, if the tumor position within the breast tissue is correctly identified by magnetic resonance imaging or computed tomography scan, it is possible that the needle can be inserted into multiple internal tumor regions without having to pull out the needle completely from the breast or even the tumor by choosing different angles to direct the needle from the same insertion point on the tumor surface during a single breast insertion.

In conclusion, our study shows the applicability of a novel optical fiber method to quantify overexpressed receptors in a tumor simultaneously by a few insertions of the 2-photon optical fiber fluorescence probe using an ultra-fine 30-gauge needle.

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CONFLICT OF INTEREST DISCLOSURES

J.R.B. holds an ownership position in PhotonAffinity, Inc., and is the coinventor of technologies that the University has licensed to PhotonAffinity, Inc. Some of these technologies are involved in this research. PhotoAffinity, Inc. had no role in the study design, data collection and analysis, decision to publish, or preparation of the article.

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