Herceptin functionalized microfluidic polydimethylsiloxane devices for the capture of human epidermal growth factor receptor 2 positive circulating breast cancer cells

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Building on recent breakthroughs in the field of microfluidic-based capture of rare cancer cells circulating in the blood, the present article reports on the use of Herceptin functionalized PDMS devices designed to efficiently capture from blood cancer cells, overexpressing the tyrosine kinase human epidermal growth factor receptor (HER2). The identification of patients overexpressing HER2 is critical as it typically associates with an aggressive disease course in breast cancer and poor prognosis. Importantly, HER2 positive patients have been found to significantly benefit from Herceptin (Trastuzumab), a humanized monoclonal antibody (MAb) against HER2. Disposable PDMS devices prepared using standard soft lithography were functionalized by the plasma polymerization of an epoxy-containing monomer. The epoxy-rich thin film (AGEpp) thus created could be conjugated with Herceptin either directly or through a polyethylene glycol interlayer. The properties and reactivity toward the monoclonal antibody conjugation of these coatings were determined using x-ray photoelectron spectroscopy; direct conjugation provided a good compromise in reactivity and resistance to biologically nonspecific fouling and was selected. Using the breast cancer cell line SK-BR-3 as a model for cells overexpressing HER2, the immunocapture efficacy of the Herceptin functionalized PDMS was demonstrated in model studies. Validation studies confirmed the ability of the device to efficiently capture ($\sim 80\%$ capture yield) HER2 positive cells from full blood. © 2010 American Institute of Physics. [doi:10.1063/1.3480573]

I. INTRODUCTION

Breast cancer remains the leading cause of cancer-related mortality in women and the second most common cancer in the world. In recent years, molecular profiling of cancer cells obtained from biopsy has provided important diagnostic and prognostic cancer markers. Of particular interest is the tyrosine kinase human epidermal growth factor receptor HER2 (also known as erbB-2 or neu). Human epidermal growth factor receptor 2 (HER2) is a proto-oncogene and overexpression due to gene amplification of the HER2 gene in breast cancer, which occurs typically in 20%–30% and plays an important role in the pathogenesis of this disease. It is, in particular, associated with an aggressive disease course in breast cancer patients.^{1,2} Overexpression of HER2 is especially an adverse prognostic factor in patients with positive auxiliary node breast cancer. The discovery of the role of HER2 led to the development of Trastuzumab, a humanized monoclonal antibody against HER2, which is clinically used as an adjuvant therapy in HER2 positive early breast cancer and in the treatment of metastatic disease. Trastuzumab administration lowered the risk of recurrence of early HER2 positive breast cancer by approximately

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50%.³ The HER2 status is routinely assessed using immunohistochemistry analyses of tumor tissues obtained from biopsies aimed at detecting overexpression of the HER2 protein. More recently, fluorescent *in situ* hybridization, which measures the amplification of the HER2 gene, has become an alternative technology to determine HER2 status. Considering the dramatic efficacy of HER2 targeted therapeutic approaches against HER2 positive breast cancer, accurate determination of the HER2 status is critical. The current methodologies are not, however, without faults, and there is a clear clinical need to improve HER2 testing.⁴ In addition, the HER2 status of metastatic tumor has been observed to differ from the one of the primary tumors in about 20% of breast cancer. This discrepancy, which may be related to genetic instability or clonal selection during the metastatic process,² can potentially lead to suboptimal treatment selection with potentially fatal consequences.⁵

Molecular analysis of tumor cells shed from solid tumors and disseminated in the body either through the vascular or lymphatic systems has been recently advanced as an alternative to tissue biopsy.^{6–8} The relevance of circulating tumor cells (CTCs) to achieve noninvasive solid tumor molecular profiling has now been well demonstrated clinically. The HER2 status of patients with recurrent breast cancer could, for instance, be determined from CTCs using real-time polymerase chain reaction after magnetic enrichment of peripheral blood.¹ In agreement with previous finding on HER2 status, discrepancy between the primary and secondary tumors, the HER2 status of CTCs has been found to be different to the one of the primary tumors for patients with advanced disease.²

While the presence of CTCs in blood has been known since the mid-1800s, their capture for diagnostic, prognostic, and mechanistic purposes has remained an elusive goal for a long time, mostly due to the absence of efficient technologies able to isolate these cells, which are present at ratios as low as $(1-10)/10^9$ blood cells. Magnetic cell sorting using magnetic beads covalently conjugated with antiepithelial-cell adhesion-molecule (EpCAM) antibodies has been the most successful technology until recently, and a commercially available system has been used clinically with some notable success (CellSearch™ system). Microfluidic CTC capture devices have, however, recently come to the fore, providing enrichment levels up to two orders of magnitude higher than previous methodologies.^{9–11} Using a microfabricated device functionalized with anti-EpCAM, CTCs for instance were isolated in high numbers in 99% of 116 patients with a range of disease including non-small-cell lung, prostate, pancreatic, breast, and colorectal cancers. These novel approaches have generated a tremendous interest in the scientific community and recent progresses in the field have been reviewed recently.^{12–14}

We have recently developed a polydimethylsiloxane (PDMS) based disposable microfluidic CTCs capture device designed to take advantage of the unique features of PDMS, such as compatibility with soft molding techniques, transparency, and permeability to gas. Plasma-based functionalization was used to introduce reactive epoxy functionalities on the internal surface of the PDMS device, which could be further reacted with anti-EpCAM antibodies toward the immunocapture of EpCAM positive cancer cells.¹⁵ We describe here the preparation and validation of a disposable microfluidic device for the capture and detection of cells overexpressing HER2. PDMS device fabricated by standard molding techniques could be functionalized using pulsed plasma polymerization of allyl glycidyl ether. Herceptin was covalently immobilized either directly onto the epoxy-rich interlayer or through polyethylene glycol (PEG) spacers with various molecular weights. Direct immobilization provided the most efficient and straightforward route and was selected for further development of the device.

II. EXPERIMENTAL

A. Preparation of the epoxy-functionalized PDMS devices

The PDMS cell capture devices were prepared based on the procedure reported previously. Briefly, standard photolithographic techniques were used to prepare SU-8-50 masters (MicroChem, USA) for molding of the PDMS device. Cylindrical features with diameters of 100 μm and interspacing of 100 μm were used. A geometric arrangement of the features with a 100 mm

shift after every three rows was selected based on the design reported by Nagrath *et al.*¹⁰ PDMS (SYLGARD 184, Dow Corning, USA) was prepared according to the manufacturer's instructions and casted onto the chlorotrimethylsilane hydrophobized master and baked at 65 °C for 2.5 h. Next, reactive epoxy functionalities were introduced on the surface of the PDMS using the pulsed plasma polymerization of allyl glycidyl ether (>99% obtained from Sigma-Aldrich, USA) as described elsewhere.¹⁶ Standard microscopic glass slides or cover slips were also plasma polymerized. In order to seal the devices, the top surface of the PDMS devices was reacted with polyethyleneimine through an inking procedure, leaving the internal structure unmodified. Upon closing of the PDMS device with either an AGEpp coated glass slide or cover slip, the amine groups introduced on the PDMS surface cross-linked with the epoxy functionalities, thereby efficiently sealing the device.

B. Surface modification and covalent conjugation of Herceptin to PDMS

Silicon samples were cut from 100 mm diameter wafers (Micro Materials and Research Cons. Pty Ltd., Australia) and functionalized with AGEpp as described above. The AGEpp coated samples were washed for 30 s in ethanol to remove unreacted monomers and low-molecular weight species from the films. An ethanolic solution (50 mg/ml) of PEG-diacid with a molecular weight of 600 (Sigma, USA) or 3400 (Rapp Polymere, Germany) was placed on the samples and evaporated in an autoclave at 85 °C forming a thin film of melted PEG on the surface. The samples were left to react overnight before being washed thoroughly with hot ethanol to remove the PEG melt.

Herceptin (Trastuzumab[®]) solutions at a concentration of 100 µg/ml were prepared in 0.14M phosphate buffered saline (PBS). For direct conjugation on the AGEpp samples, Herceptin solutions were placed on the samples and left to react for various amounts of time. Herceptin solution in 1M ammonium sulfate was also tested. To immobilize the Herceptin antibodies on the PEGylated surfaces, terminal carboxyl groups were first activated using *N*-hydroxysuccinimide (NHS) (50 mM) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (100 mM) for 10 min. The activated surfaces were then quickly washed with PBS and exposed to the Herceptin solution for conjugation.

X-ray photoelectron spectroscopy (XPS) analyses of the PEGylated and Herceptin conjugated silicon wafer samples were conducted to investigate the surface chemistry. XPS analyses were conducted using a Kratos AXIS Ultra DLD x-ray photoelectron spectrometer with a monochromatic Al K α x-ray source ($h\nu=1486.69$ eV) and a hemispherical analyzer. The pass energy was 20 eV with a resolution of 0.3 eV for high-resolution spectra. Spectra were collected at a photoelectron take-off angle of 90°. Binding energies were referenced to the C 1s hydrocarbon carbon peak at 285.0 eV to compensate for surface charging effects. Component fitting of high-resolution spectra was performed using CASAXPS version 2.3.12 software. Shirley-type backgrounds were used and components constrained to a full width at half-maximum between 0.9 and 1.5 eV. The peak fits used 70% Gaussian/30% Lorentzian peak shapes.

C. Model studies of the capture of HER2 positive CTCs on Herceptin conjugated PDMS

For model studies, flat PDMS samples were epoxy-functionalized as described. The AGEpp coated samples were washed for 30 s in ethanol to remove low-molecular weight species from the films. Prior to immobilization of the antibodies, the surfaces were quickly equilibrated with the coupling buffers (PBS and 1M ammonium sulfate/PBS). Herceptin solutions were left to react with the epoxy-rich surfaces for 4 h. The samples were placed in well-plates and small drop (35 µl) breast cancer cells (SK-Br-3 cell lines) in culture medium at 10⁵ cell/ml concentration were deposited on the samples. After 30 min incubation, gentle washing was applied to remove unbound cells. The average number per unit area of cells captured on the Herceptin conjugated surfaces was determined using optical microscopy. AGEpp functionalized PDMS incubated in PBS for 24 h and 1% bovine serum albumin (BSA) in PBS (2 h) were used as controls.

FIG. 1. Schematic of the Herceptin—CTC capture PDMS device.

D. Capture of HER2 positive cells in full blood

A syringe pump (model KDS-212-CE, KDS-210, KD Scientific, USA) was coupled to the outlet of the CTC capture device. Conjugation of the antibodies in the internal volume of the devices was performed by injecting 200 μl of Herceptin solution which was left to react for 4 h. The devices were then washed with PBS and then incubated for 1 h with a 1% BSA solution. Prior to the start of the experiments, calcein was used to fluorescently label the SK-BR-3 cells as per the manufacturer instructions. 5 ml of blood from healthy male donors was collected into a vacutainer containing ethylenediaminetetraacetic acid. The blood was kept at 4 °C and used within 2 h after collection. The blood was spiked with the calcein labeled breast cancer cells at 2×10^3 and 2×10^4 cells/ml and circulated within the device at a flow rate of 16 $\mu\text{l}/\text{min}$ for 30 min. 5% BSA solution in PBS was then circulated in the device at 16 $\mu\text{l}/\text{min}$ for 20 min and then 100 $\mu\text{l}/\text{min}$ for 40 min to wash off blood cells with a prior to fixation step with 4% formalin. A Leica SP5 spectral scanning confocal microscope was used to image the device. The number of fluorescent cells identified as SK-BR-3 cells captured inside the device was counted from 50 randomly chosen low magnification fields of view at different sections of the device and used to determine the capture efficiency.

III. RESULTS AND DISCUSSION

Microfluidic-based approaches for the capture of cancer cells circulating in peripheral blood have generated the hope of a paradigm shift in diagnostic and prognostic studies of cancer. These devices typically present a 3D structure presenting monoclonal antibodies which, under the right hydrodynamic conditions, enable the immunospecific binding of target cells (Fig. 1). In our previous study, we have shown that PDMS microfluidic CTC capture devices can be prepared using plasma-based functionalization of the elastomeric material.¹⁵ The reactive epoxy functionalities introduced on the internal structure of the device could be conjugated with anti-EpCAM antibodies, which provided efficient capture of various cancer cell lines in buffer and full blood. Extending this previous work, the immobilization of Herceptin toward the immunospecific capture of target cells overexpressing HER2 is reported here. Two different immobilization routes have been tested. In a first approach, the reactive epoxy groups of the AGEpp coated device have been reacted to nucleophilic residues of the MAb. With the aim of improving the interfacial properties of the devices, an alternative methodology was also investigated based on the introduction of a dense PEG interlayer. The introduction of PEG interlayers on biomaterials is the most widely used approach to eliminate/reduce biologically nonspecific protein adsorption events.

XPS analysis was used to investigate the surface modification of AGEpp PDMS with homobifunctional diacid-PEG with two different molecular weights (600 and 3400). Elemental analysis

FIG. 2. (a) High-resolution XPS C 1s spectra of AGEpp films deposited on PDMS before (AGEpp) and after reaction with PEG-COOH₂ 600 (AGEpp PEG 600) and PEG-COOH₂ 3400 (AGEpp PEG 3400). The dashed lines show binding energies of carbon atoms in a C—C/C—H environment (285 eV), C—O/C—N (286.5 eV), and O—C=O (289.3 eV) environments. [(b) and (c)] Static water contact angle after 24 h.

showed an increase of the oxygen percentage on the surface after grafting of the PEG molecules (26.5% versus 29% for PEG600 and 32.5% for PEG3400) suggesting that at least for PEG3400, a dense and thick polymeric ethylene glycol rich layer was obtained. This was confirmed by analyses of the XPS C 1s core level spectra shown in Fig. 2. Significant increases in ethylene glycol moieties (characteristic peak at 286.5 eV) were observed after grafting of both PEG600 and PEG3400. A significant shoulder at about 289.3 eV (6% of total C) that can be attributed to carboxylic groups could be observed for the PEG600, in good agreement with theoretical predictions (7% of the carbon in O—C=O environment). Water contact angle measurements performed using the sessile drop technique [Figs. 2(b) and 2(c)] showed that PEGylation increased significantly the hydrophilicity of the surface when compared to native PDMS and AGEpp coated PDMS (49° for PEG600 and 42° for PEG3400 versus 105° for PDMA and 68° for AGEpp PDMS). The small extent of contamination of the PEG interlayers with low-molecular weight siloxane moieties, which can be inferred by the presence of a small amount of Si on the surface (~1% as detected by XPS elemental analysis), can be expected to impact on the wettability of the samples.

Next the reactivity of the AGEpp and PEGylated AGEpp surfaces toward covalent immobilization of Herceptin was investigated. XPS was used to sensitively quantify surface-bound MAb, which was measured as the percentage increase in surface nitrogen. In good agreement with our previous study using EpCAM, Herceptin could be readily immobilized on the AGEpp functionalized surfaces. A significant increase in the amount of surface nitrogen (from 0% to 5.4%) was indeed observed after incubation of the AGEpp samples in a 100 $\mu\text{g}/\text{ml}$ Herceptin solution for 4 h, which represent about 50% protein coverage of the surface as estimated using a standard XPS overlayer model.¹⁷ Carbodiimide chemistry was used to activate the terminal carboxylic groups on the surface grafted PEG molecules and the NHS activated PEG was then reacted with Herceptin. Nitrogen percentages increased to 3.5% and 1% for PEG600 and PEG3400, respectively, confirming the immobilization of the MAb onto the PEGylated surfaces, although at lower densities than through direct immobilization on AGEpp interlayers. In a previous study, a dense layer of diacid-PEG3400 molecules grafted using a similar solvent-free method to superparamagnetic nanopar-

FIG. 3. Change in nitrogen percentage as determined by XPS analyses. (1) After conjugation with Herceptin for AGEpp, PEG-COOH₂ 600 functionalized AGEpp (NHS/EDC activation) and PEG-COOH₂ 3400 functionalized AGEpp (NHS/EDC activation). (2) After 1 h incubation in a 5% serum solution diluted in PBS for AGEpp, PEG-COOH₂ 600 functionalized AGEpp, PEG-COOH₂ 3400 functionalized AGEpp, PDMS control (resistance to biologically nonspecific fouling).

tics and further conjugated with a MAb afforded highly specific detection of cell death.¹⁸ A significantly higher MAb immobilization yield was however obtained (up to 6% N) which may be explained by the higher reactivity of the PEG interlayer in solution and higher accessibility of the terminal carboxylic groups when grafted on nanoparticles.

The main impetus in this work toward the introduction of a PEG interlayer on the PDMS device was to minimize biologically nonspecific binding events, mainly plasma protein fouling that could lead to activation of the blood coagulation cascades and enhance binding of white blood cells within the device. To test the resistance of the PEGylated AGEpp PDMS samples to biologically nonspecific adsorption events, they were exposed *in vitro* to a 5% serum solution and the amount of adsorbed serum proteins was measured with XPS. The sensitivity of XPS is typically in the range of nanogram of protein per cm² for flat surfaces. The increase in nitrogen percentage resulting from protein fouling after incubation for 1 h in 5% serum is presented in Fig. 3. In agreement with our previous observations, “deactivated” AGEpp surfaces demonstrated a good resistance to serum fouling; although an increase in N% was observed (ΔN 1.9%), fouling remained significantly lower than what is observed on uncoated PDMS used as control (ΔN 11%). The introduction of a dense layer of short (Fw600) diacid-PEG on AGEpp only resulted in modest reduction in serum fouling (ΔN 1.6%). In comparison, the PEG3400 interlayer completely eliminated (to the limit of the sensitivity of XPS) protein fouling (ΔN 0%). The latter is in good agreement with previous reports on the efficiency of dense PEG layers surface grafted using solvent-free procedures.¹⁹ Solvent-free PEG grafting procedures, which aimed at minimizing the excluded volume interaction effects, have been indeed shown to maximize the density of PEG molecules per surface area and consequently to optimize the properties of the PEG interlayers.^{20,21}

The control of nonspecific absorption events is critical in the biomedical field and more specifically, its importance for microfluidic applications has been outlined recently in a number of publications. PDMS silanized with 3-aminopropyl-triethoxysilanes and further reacted with epoxy modified hydrophilic copolymers such as poly(acrylamide-co-Glycidyl methacrylate) was significantly more resistant ($\sim 90\%$ reduction) to the absorption of BSA and lysozyme used as model proteins.²² In another approach, radio frequency glow discharge plasma polymerization of tetraethylene glycol dimethylether was used to create thin PEG-like films on PDMS able to resist

FIG. 4. XPS elemental spectra of AGEpp film conjugated with Herceptin under different experimental conditions. (a) Control AGEpp film; (b) AGEpp reacted for 1 h with Herceptin in PBS; (c) AGEpp reacted for 24 h with Herceptin in PBS; (d) AGEpp reacted for 1 h with Herceptin in 1M ammonium sulfate/PBS. No significant differences were observed in the amount of immobilized antibody based on the nitrogen percentage.

fouling by fibrinogen.²³ The absence of any detectable protein adsorption on the PEG3400 AGEpp surface after exposure to serum solution, which is a more demanding assay than those based on model protein solution, is therefore noteworthy.

The nonfouling properties of PEG interlayers originate in their large excluded volume in aqueous solutions and configurational entropy, which induce strong osmotic repulsion for proteins approaching the surfaces. The surface density, conformation, and molecular weight of the surface grafted PEG chains are the key experimental parameters that govern the properties of the PEG interlayers. The observed difference between the PEGylated surfaces (Fw 600 and 3400) could be related to the overall structure of the polymeric layers as well as to the presence of a relatively high density of carboxylic groups for the PEG600, as shown by XPS analyses.

Based on its superior protein immobilization yield and good resistance to nonspecific fouling, the direct antibody conjugation approach was selected in the further development of the device for selective capture of target cells overexpressing HER2. In order to optimize the conjugation of HER2, a more in depth study was conducted. As shown in Fig. 4, various incubation times were tested, from 1 to 24 h. No significant differences were observed, demonstrating that the antibody immobilization proceeded relatively rapidly on AGEpp. Next the “salting-out” effect was tested using ammonium sulfate to enhance the hydrophobic interactions between the antibody and the surface and facilitate conjugation. In our previous study, the conjugation on AGEpp of lysozyme, used as a model enzyme, was significantly increased by the addition of 1M ammonium sulfate.¹⁶ No difference was however observed in this work with Herceptin, which may reflect mechanistic differences in the surface interactions of these two proteins. To confirm the surface chemistry, high-resolution C 1s XPS spectra were acquired after immobilization of Herceptin on AGEpp. Four components were necessary to simulate the C 1s peak, as shown in Fig. 5. A first component at 285.0 eV was attributed to hydrocarbon. A second component originating from carbon singly bonded to oxygen or nitrogen was found at 286.5 eV. The third and fourth components at 288.2 and 289.1 eV were attributed to carbons in N—C=O/C=O and O—C=O environments, respectively. The drastic increase of the component at 288.2 eV, which can be attributed to the amide carbons of proteins, confirmed the presence of the antibody on the surface.

To validate the selected conjugation strategy, a model cell binding study was conducted. Flat

FIG. 5. High-resolution C 1s XPS spectra after Herceptin immobilization on AGEpp (1 h, PBS). Curve fitted components have been included.

PDMS samples were modified with Herceptin and incubated with the breast cancer cell line SK-BR-3 cells, used as a model for HER2 positive cells. As shown in Fig. 6, samples conjugated with Herceptin were very efficient in capturing the HER2 positive cells. In agreement with the XPS study, no difference was observed between samples prepared in PBS or PBS supplemented

FIG. 6. Capture efficiency of the breast cancer cell line SK-BR-3 on Herceptin conjugated AGEpp. Surfaces incubated with PBS (AGEpp) and 1% BSA (AGEpp BSA) were used as negative control. Binding in presence of excess of free antibody was used to demonstrate binding specificity (AGEpp HER Comp).

FIG. 7. Overlay fluorescence image of fluorescently labeled breast cancer SK-BR-3 captured on the Herceptin conjugated PDMS internal structure (left) and the Herceptin conjugated cover slip (right). The inset shows a differential interference contrast microscopic image of the captured cell.

with 1M ammonium sulfate. To verify the specificity of the cellular binding, three control experiments were performed. First, AGEpp coated PDMS samples were incubated in PBS for 24 h to quench the reactive epoxy groups on the surface. AGEpp samples were also conjugated with bovine serum albumin as described for Herceptin. XPS analyses confirmed the successful conjugation of BSA on the AGEpp surfaces. The third control experiment consisted in a competition binding assay where an excess of free Herceptin in solution was used to inhibit the immunospecific binding of the SK-BR-3 cells onto the Herceptin functionalized AGEpp surfaces. On all control samples, no significant cellular binding could be detected, thereby confirming the immunospecificity of the SK-BR-3 cells binding on the Herceptin modified samples.

The ability of the Herceptin functionalized PDMS devices to capture target cells overexpressing the HER2 proteins from full blood was then tested. PDMS devices were functionalized with AGEpp and sealed with AGEpp coated glass cover slips as described. The internal volume of the device was incubated for 4 h with a 100 $\mu\text{g}/\text{ml}$ Herceptin solution in PBS and then with a 1% BSA solution to passivate the surface. SK-BR-3 cells were first fluorescently labeled with calcein and then used to spike blood collected from a healthy donor at concentrations of 2×10^3 and 2×10^4 cells/ml. Using a syringe pump, the blood was then circulated inside the device at a flow rate of 16 $\mu\text{l}/\text{min}$ for 30 min. The device was then flushed with a 5% BSA solution until complete removal of the blood and then washed with PBS. The devices were then observed using confocal microscopy and differential interference contrast and fluorescence microscopic images of the captured cells were acquired. Fluorescent breast cancer cells could be easily detected either on the PDMS features or on the glass surfaces, as shown in Fig. 7. The cells appeared rounded and due to the transparency of the PDMS, high-resolution differential interference contrast images could be easily collected. Importantly, only a small number of white blood cells were observed inside the devices. The captured breast cancer cells were mostly located toward the proximal side of the device, suggesting that the capture process was rather efficient. The total number of fluorescent cells in the devices was determined through image analyses and the capture efficiency calculated. Independently, of the initial number of cancer cells spiked in the blood samples, about 80% of the injected cells were retained inside the devices. Control experiments with devices coated with BSA only confirmed the specific nature of the capture process.

IV. CONCLUSIONS

These results demonstrate that the covalent immobilization of Herceptin within AGEpp plasma polymerized PDMS devices can be used to capture with a high efficiency cells overexpressing the HER2 gene. This approach could provide a fast and reliable methodology to establish HER2 status for breast cancer patients presenting metastases, thereby enabling the selection of more potent therapy based on Trastuzumab. In addition, while EpCAM functionalized microfluidic

devices have been used with a remarkable success to capture CTCs from a range of tumors,^{10,24} invasive tumor cells have been observed to lose their epithelial antigens as a result of the epithelial to mesenchymal transition,⁶ thereby making EpCAM a questionable CTC marker for invasive tumors. In addition, epithelial positive nontumor cells can be found in peripheral blood, potentially providing false-positive results. The clinical application of microfluidic-based CTC capture devices can therefore be expected to benefit from alternative approaches such as the one presented here, based on the use of immunocapture approaches specific to a particular disease or mutation.

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