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Lab-on-a-Chip Technology for Integrative Bioengineering

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***Abstract*-Recent progress of lab-on-a-chip technology is challenging for the development of nanobiotechnology and integrative bioengineering. In this work, several novel microfluidic devices for biotechnology and bioengineering, based on the synergetic integration of miniaturization technology to biology, chemistry, and medicine, are described. Currently, we focus on the development of a nanobiosensor, microfluidic device and lab-on-a-chip as a new platform for biological sample processing, separation, and detection, including optoelectrofluidic manipulation, hydrophoretic separation, magnetophoretic assay, and cell-based assay. The final application of these research activities covers several aspects of biomolecular diagnostics, micro total analysis system (μTAS), cell-based screening platform, nanobio device, etc.**

BACKGROUND

Micro/nano fluidics has been a key technology for the realization of micro total analysis systems (μTAS) or lab-on-a-chip as well as the next generation bio-tools for drug discovery, diagnostics, and tissue engineering. This research area covers the design and development of miniaturized devices that manipulate liquid samples at nanoliter volumes, allowing biological assays to be integrated and accomplished on a small scale with minimum time and cost. In particular, recent lab-on-a-chip technologies have widely been introduced in the fields of cellular assay and drug discovery because microfluidics can provide an *in vivo*-like microenvironment, continuous perfusion, and high-throughput screening.

This paper reviews several microfluidic platform technologies for biotechnology and bioengineering developed in the Nanobiotech Laboratory at KAIST. Special topics focus on a novel nanobiosensor, microfluidic device, and lab-on-a-chip as a new platform for biological sample preparation and detection, including optoelectrofluidic manipulation, hydrophoretic separation, and magnetophoretic assay. In addition, several microfluidic cell-based assay platforms, including cytotoxicity test, drug permeability test, and biomechanical analysis of cells or embryos, were also presented.

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CURRENT RESULTS

Optoelectrofluidic manipulation platform: Optoelectrofluidics refers to the motion of particles or molecules and their interactions with an optically induced electric field and surrounding fluid (Fig. 1). This concept has already been demonstrated through the electrophoresis of colloidal particles using an ultraviolet light pattern projected onto an indium tin oxide (ITO) electrode [1]. A photoconductive material deposited on a metal plate electrode can also be used to induce a nonuniform electric field, which results in an electrokinetic motion of particles and fluids, only with a weak white light source [2−5] or a conventional laser source [6, 7]. Although optically induced dielectrophoresis (DEP) has frequently been employed to manipulate individual microparticles, light induced ac electroosmosis (ACEO) has recently attracted a great deal of attention because of its capability to rapidly manipulate nanoparticles or molecules, which are too small to manipulate using DEP force proportional to the particle volume. Recently, we demonstrated the rapid and selective concentration of microparticles by combining several electrokinetic mechanisms and electrostatic interactions [8]. When a dynamic image pattern is projected into a specific area of a photoconductive layer, virtual electrodes are generated, resulting in electrokinetic motion of micro/nanoparticles under a nonuniform electric field. By using a compact, lens-integrated liquid crystal display (LCD)-based optoelectrofluidic platform, we have characterized the frequency-dependent phenomena of the optoelectrofluidic concentration of microparticles due to the image-driven ac electrokinetics, including DEP and ACEO. The particle movements resulted from the frequency-dependent behavior according to the particle diameter. This new platform may be a widely usable integrated system for optoelectrofluidic

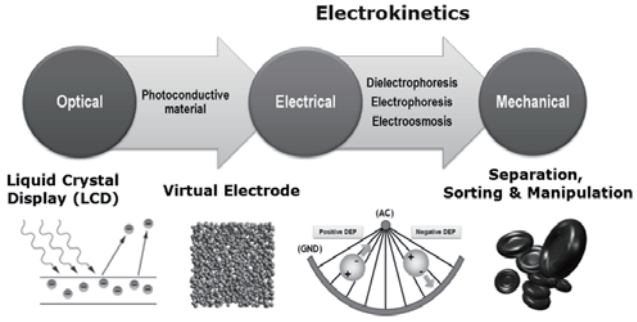

Fig. 1. Principle and concept of optoelectrofluidics.

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manipulation of micro/nano particles including colloidal particles [9].

The dynamic control of local molecular concentration was also achieved by using several frequency-dependent optoelectrofluidic phenomena such as optically induced ACEO, DEP and electrostatic dipole interactions [10]. Optoelectrofluidic fluorescence microscopy, wherein an optoelectrofluidic device is integrated into a conventional fluorescence microscopy, made it possible both to modulate and to detect the molecular concentration in a localized area at the same time. In another application, we have demonstrated a sudden decay of molecular concentration in a localized area by optoelectrofluidics in a few hundred Hz frequency range. On the basis of this approach, the measurement of diffusion using different-sized biomolecules has been performed [11]. This technique would be a useful tool for analyzing electrokinetic behavior of molecules as well as studying molecular diffusion kinetics. In addition, the sudden change of local molecular concentration can be applied for several biological and chemical applications such as cellular chemotaxis and optoelectrofluidic immunoassay.

Hydrophoretic separation platform: The development of microfluidic separation technologies is one of the major issues in the area of lab-on-a-chip and micro total analysis system. However, previously-reported passive methods for particle separation cannot separate microparticles without the aids of sheath flows and complex channel networks. As shown in Fig. 2, we proposed a new microfluidic separation scheme, "hydrophoresis", which uses slanted or anisotropic obstacles to induce hydrodynamic interaction between the obstacles and the particles subjected to rotational flows induced by the obstacles [12−14]. By exploiting the slanted obstacles in a microchannel, we can eliminate the needs of sheath flows and complex channel networks. In addition, we can generate a lateral pressure gradient so that microparticles can be deflected and arranged along the lateral flows induced by the gradient. The equilibrium positions of the particles by the hydrodynamic interactions depend on their size. The slanted obstacle as a microfluidic control element in a microchannel is analogous to the electric, magnetic, optical, or acoustic counterparts in that their function is to generate a field gradient. The approaches using hydrophoresis were successfully applied to the particle sizing [12], isolation of white blood cells from red blood cells [13], and sheathless particle focusing [15, 16]. Recently, we also reported a hydrophoretic device that uses rotational flows induced by regularly patterned obstacles only on the top wall for separating biological samples, including DNA molecules [17] and mammalian cells [18]. Continuous separation of micrometer and submicrometer particles was achieved using the obstacles, demonstrating the potential of hydrophoresis for biological sample preparation on the micro- and nanoscales, with the advantages of continuous flow and sheathless passive operation [17, 19]. From a practical point of view, the hydrophoretic device is simple, noninvasive, effective, and does not require any external power for particle and cell separation. Since our method is based on intrinsic pressure fields, we can eliminate the need for external potential fields to induce the movement of particles. Therefore, this hydrophoretic method will offer a new opportunity for power-free and biocompatible particle control within integrated microfluidic devices

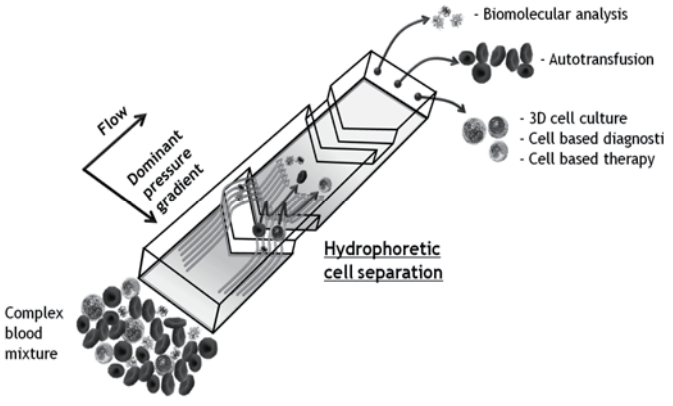

Fig. 2. Schematic of hydrophoretic separation based on the slanted or anisotropic obstacles to induce hydrodynamic interaction between the obstacles and the particles.

Magnetophoretic assay platform: Magnetophoresis is a phenomenon explaining particle migration driven by magnetic force exerted on a particle. Magnetic force induced by an external magnetic field exerts an object to move toward the denser or sparser magnetic field. All particles exhibit their own magnetic properties according to their chemical compositions. The intensity and direction of magnetic force depend on the magnetic properties of the materials and surroundings of them, including diamagnetism and paramagnetism [20]. In a magnetic biosensor, magnetic microbeads and magnetic nanoparticles have been used as a solid support and a labeling, respectively [21, 22]. We developed a new immunoassay system based on the magnetophoretic mobility of a microbead, depending on the amount of associated superparamagnetic nanoparticles under magnetic field gradient in a microfluidic channel [23, 24]. Fig. 3 shows basic principle of magnetophoretic immunoassays. By measuring the magnetophoretic deflection velocity of microbeads as the signal for the presence of analytes, the multiple analytes (such as allergen-specific IgEs in patient samples) in a microchannel are simultaneously quantified by conjugated nanoparticles as a label. Because magnetophoresis is also influenced by magnetic field gradient, the detection sensitivity of this assay system can be improved to the sub-femtomolar concentration range using an enhanced magnetic force from the ferromagnetic microstructures in a microfluidic device [24, 25]. This technology has been successfully applied to develop a magnetophoretic, continuous purification platform that rids single-walled carbon nanotubes (SWCNTs) of superparamagnetic iron-catalyst nanoparticles [25]. We also

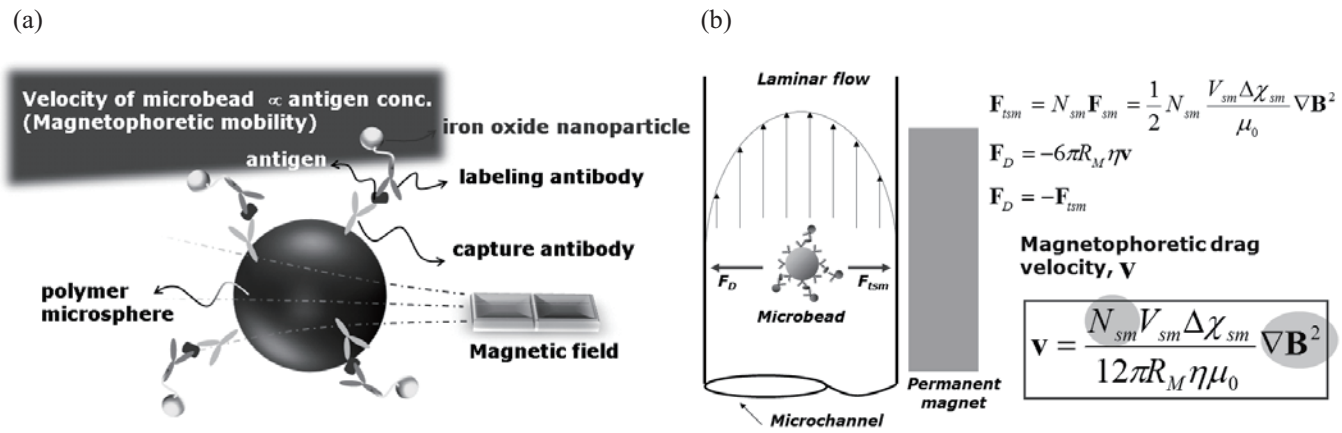

Fig. 3. Principle of magnetophoresis. (a) Schematic of magnetophoretic immunoassays and (b) magnetophoretic drag velocity in a microchannel.

demonstrated an ultrasensitive magnetophoretic assay for prostate-specific antigen (PSA) using magnetic nanoclusters (MNCs) as a signal amplifier [26]. The developed system enabled detection of PSA as low as 50 fg mL^{-1} with a detection limit of 45 fg mL^{-1}. It is expected to be effectively applied to the detection of a target analyte with low abundance.

Recently, we have developed an isomagnetophoretic immunosensor platform which adjusts the dynamic range and resolution according to the concentration of target biomolecules. Isomagnetophoresis is an improved magnetophoretic method which discriminates materials with subtle magnetic susceptibility difference employing the magnetic susceptibility gradient across a microchannel applied by magnetic field [27]. Because the magnetophoretic mobility of a particle is in proportion to the magnetic susceptibility difference between a particle and surrounding solution, a particle migrates and stays at the net position where the magnetic susceptibility of a particle and the surrounding solution are equal under the magnetic susceptibility gradient of the surrounding solution. With this technology, we can enlarge the linear response range in the dynamic range which is characterized by conventional magnetophoresis. Quantitative analysis using each pixel of charge-coupled device (CCD) was carried out by measuring the positions of microbeads in the outlet of a microfluidic device [28]. The developed immunosensor platform could be useful to make a patient-specific diagnosis in a variety of clinical fields.

Cell-Based assay platform: The *in vivo*-like micro environments may play an important role for drug screening and development [29]. As orally administered drugs must be absorbed from the intestine into the blood circulation, permeability and cytotoxicity assays of drug candidates have been widely used in the early screening stages of drug discovery. To realize the cell-based assays in a microchannel, we used two different approaches such as 3D cell culture [30−32] and microhole-trapped cells [33] in a microfluidic device. In particular, a microvalve-assisted patterning platform was demonstrated to provide a new method for investigating cellular dynamics by generating a linear concentration gradient of a drug as well as to realize 3D cell culture in a microenvironment [32]. Micofluidics-based cytotoxicity tests using human hepatocellular liver carcinoma cells (HepG2) or human hepatocytes were also performed in real-time monitoring with exposure to different drug concentrations. In a drug permeability assay system using a microfluidic device, a microhole array structure for cell trapping was exploited by mimicking the intestinal epithelial cell membrane, considering the *in vivo* delivery path of drugs in humans [33]. With the use of trapped cells, the integrated system including toxicity assay could be used as a valuable tool in drug discovery, and its applicability will be extended to include ADME/Tox drug properties.

As another cell-based approach, a new kind of microfluidic biomechanical device for compressive stimulation and lysis of cells was developed. The physical forces to which living cells are most commonly exposed are fluid shear, pressure, and stretch. These mechanical stimulations influence the physiological and pathological condition of the organism, which induces many aspects of human health and disease. Cancer cells are also known to have a less extensive internal cytoskeleton than healthy cells, so behave differently when squeezed. When a mechanical stress was applied to the cells with the deflection of the poly(dimethylsiloxane) (PDMS) membrane between two microchannels, we found that compressed cancerous cells were left with a series of bulges across their surface [34]. But the healthy cells looked very different, being covered with worm-like projections. This technology can also be used to spot the difference between cancerous cells and healthy ones by squeezing them until they deform - a discovery that could lead to a cheap tool for cancer detection [35]. We also demonstrated a novel microfluidic *in vitro* cultivation system for bovine embryos that improves their development using a partially constricted channel that mimics peristaltic muscle contraction (Fig. 4a) [36]. Very recently, a novel microfluidic multiplexed immunohistochemistry

(MMIHC) platform [37] was demonstrated to give a new insight of clinical application of the cell-based microfluidic platform (Fig. 4b). The MMIHC platform for quantitative IHC was designed to have four microchannels, and four biomarkers known as a prognostic indicator in breast cancers were examined by using four breast cancer cell lines (MCF-7, SK-BR-3, AU-565, and HCC70) to confirm the massive multiplexed IHC.

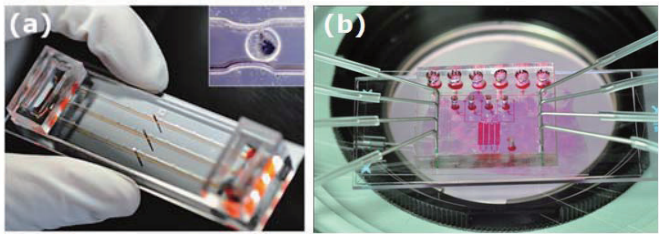

Fig. 4. (a) A microfluidic *in vitro* cultivation system for bovine embryos. Inset shows an embryo after penetrating the constriction area of a microchannel. (b) A tissue slide compatible microfluidic device for immunohistochemistry.

REFERENCES

[1] R. C. Hayward, D. A. Saville, and I. A. Aksay, "Electrophoretic assembly of colloidal crystals with optically tunable micropatterns," *Nature*, vol. 404, pp. 56-59, 2000.

[2] W. Choi, S. -H. Kim, J. Jang, and J. -K. Park, "Lab-on-a-display: a new microparticle manipulation platform using a liquid crystal display (LCD) ," *Microfluid. Nanofluid*., vol. 3, pp. 217-225, 2007.

[3] H. Hwang, Y. -J. Choi, W. Choi, S. -H. Kim, J. Jang, and J. -K. Park, "Interactive manipulation of blood cells using a lens-integrated liquid crystal display based optoelectronic tweezers system," *Electrophoresis*, vol. 29, pp. 1203-1212, 2008.

[4] H. Hwang, J. -J. Kim, and J. -K. Park, Experimental investigation of electrostatic particle particle interactions in optoelectronic tweezers," *J. Phys. Chem. B*, vol. 112, pp. 9903-9908, 2008.

[5] W. Choi, S. -W. Nam, H. Hwang, S. Park, and J.-K. Park, "Programmable manipulation of motile cells in optoelectronic tweezers using a grayscale image," *Appl. Phys. Lett*. vol. 93, pp. 143901, 2008.

[6] P. Y. Chiou, A. T. Ohta, and M. C. Wu, "Massively parallel manipulation of single cells and microparticles using optical images," *Nature*, vol. 436, pp. 370-372, 2005.

[7] A. Jamshidi, P. J. Pauzauskie, P. J. Schuck, A. T. Ohta, P. Y. Chiou, J. Chou, P. Yang, and M. C. Wu, "Dynamic manipulation and separation of individual semiconducting and metallic nanowires," *Nat. Photonics*, vol. 2, pp. 86-89, 2008.

[8] H. Hwang and J. -K. Park, "Rapid and selective concentration of microparticles in an optoelectrofluidic platform," *Lab Chip*, vol. 9, pp. 199-206, 2009.

[9] H. Hwang, Y. -H. Park, and J. -K. Park, "Optoelectrofluidic control of colloidal assembly in an optically induced electric field," *Langmuir*, vol. 25, 6010-6014 (2009).

[10] H. Hwang and J. -K. Park, "Dynamic light-activated control of local chemical concentration in a fluid," *Anal. Chem*., vol. 81, 5865-5870, 2009.

[11] H. Hwang and J. -K. Park, "Measurement of molecular diffusion based on optoelectrofluidic fluorescence microscopy," *Anal. Chem*., vol. 81, 9163-9167, 2009

[12] S. Choi and J. -K. Park, "Continuous hydrophoretic separation and sizing of microparticles using slanted obstacles in a microchannel," *Lab Chip*, vol. 7, pp. 890-897, 2007.

[13] S. Choi, S. Song, C. Choi, and J. -K. Park, "Continuous blood cell separation by hydrophoretic filtration," *Lab Chip*, vol. 7, pp. 1532-1538, 2007.

[14] S. Choi and J. -K. Park, "Optically coated mirror-embedded microchannel to measure hydrophoretic particle ordering in three dimensions," *Small*, vol. 5, pp. 2205-2211, 2009.

[15] S. Choi and J. -K. Park, "Sheathless hydrophoretic particle focusing in a microchannel with exponentially increasing obstacle arrays," *Anal. Chem*, vol. 80, pp. 3035-3039, 2008.

[16] S. Choi, S. Song, C. Choi, and J. -K. Park, "Sheathless focusing of microbeads and blood cells based on hydrophoresis," *Small*, vol. 4, pp. 634-641, 2008.

[17] S. Choi, S. Song, C. Choi, and J. -K. Park, "Hydrophoretic sorting of micrometer and submicrometer particles using anisotropic microfluidic obstacles," *Anal. Chem*. vol. 81, pp. 50-55, 2009.

[18] S. Choi, S. Song, C. Choi, and J. -K. Park, "Microfluidic self-sorting of mammalian cells to achieve cell cycle synchrony by hydrophoresis," *Anal. Chem*. vol. 81, pp.1964-1968, 2009.

[19] S. Choi and J. -K. Park, "Tuneable hydrophoretic separation using elastic deformation of poly(dimethylsiloxane)," *Lab Chip*, vol. 9, pp. 1962-1965, 2009.

[20] N. Pamme, "Magnetism and microfluidics," *Lab Chip*, vol. 6, pp. 24-38, 2006.

[21] S. P. Mulvaney, C. L. Cole, M. D. Kniller, M. Malito, C. R. Tamanaha, J. C. Rife, M. W. Stanton, and L. J. Whitman, "Rapid, femtomolar bioassays in complex matrices combining microfluidics and magnetoelectronics," *Biosens. Bioelectron*., vol. 23, pp. 191-200, 2007.

[22] K. Enpuku, D. Kuroda, A. Ohba, T. Q. Yang, K. Yoshinaga, T. Nakahara, H. Kuma, and N. Hamasaki, "Biological immunoassay utilizing magnetic marker and high Tc superconducting quantum interference device magnetometer," *Jap. J. Appl. Phys.*, vol. 42, pp.L1436-L1438, 2003.

[23] K. S. Kim and J. -K. Park, "Magnetic force-based multiplexed immunoassay using superparamagnetic nanoparticles in microfluidic channel," *Lab Chip*, vol. 5, pp. 657-664, 2005.

[24] Y. K. Hahn, Z. Jin, J. H. Kang, E. Oh, M. -K. Han, H.-S. Kim, J. -T. Jang, J. -H. Lee, J. Cheon, S. H. Kim, H. -S. Park, and J. -K. Park, "Magnetophoretic immunoassay of allergen specific IgE in an enhanced magnetic field gradient," *Anal. Chem.*, vol. 79, pp. 2214-2220, 2007.

[25] J. H. Kang and J. -K. Park, "Magnetophoretic continuous purification of single-walled carbon nanotubes from catalytic impurities in a microfluidic device," *Small*, vol. 3, pp.1784-1791, 2007.

[26] Z. Jin, Y. K. Hahn, E. Oh, Y. -P. Kim, J. -K. Park, S. H. Moon, J. -T. Jang, J. Cheon, and H. -S. Kim, "Magnetic nanoclusters for ultrasensitive magnetophoretic assays," *Small*, vol. 5, pp. 2243-2246, 2009.

[27] J. H. Kang, S. Choi, W. Lee, J. -K. Park, "Isomagnetophoresis to discriminate subtle difference in magnetic susceptibility," *J. Am. Chem. Soc*., vol. 130, pp. 396-397, 2008..

[28] Y. K. Hahn, Z. Jin, J. -B. Jang, H. -S. Kim, J. -K. Park, "Magnetophoretic position detection for multiplexed immunoassays using colored microspheres in a microchannel," *Biosens. Bioelectron*., vol. 24, pp. 1870-1876, 2009.

[29] J. H. Yeon and J. -K. Park, "Microfluidic cell culture systems for cellular analysis," *Biochip J*., vol. 1, pp. 17-27, 2007.

[30] M. S. Kim, J. H. Yeon, and J. -K. Park, A microfluidic platform for 3-dimensional cell culture and cell-based assays, *Biomed. Microdevices*, vol. 9, pp. 25-34, 2007.

[31] M. S. Kim, H. Hwang, Y. -S. Choi, and J. -K. Park, "Microfluidic micropillar arrays for 3D cell culture," *Open Biotechnol. J*., vol. 2, pp. 224-228, 2008.

[32] M. S. Kim, W. Lee, Y. C. Kim, and J. -K. Park, "Microvalve-assisted patterning platform for measuring cellular dynamics based on 3D cell culture," *Biotechnol. Bioeng*., vol. 101, pp. 1005-1013, 2008.

[33] J. H. Yeon and J. -K. Park, "Drug permeability assay using microhole-trapped cells in a microfluidic device," *Anal. Chem*., vol. 81, pp. 1944-1951, 2009.

[34] Y. C. Kim, J. H. Kang, S. -J. Park, E.-S. Yoon, and J. -K. Park, "Microfluidic biomechanical device for compressive cell stimulation and lysis," *Sens. Actuators B Chem*., vol. 128, pp. 108-116, 2007.

[35] Y. C. Kim, S. -J. Park, J. -K. Park, "Biomechanical analysis of cancerous and normal cells based on bulge generation in a microfluidic device," *Analyst*, vol. 133, pp. 1432-1439, 2008.

[36] M. S. Kim, C. Y. Bae, G. Wee, Y. -M. Han, and J. -K. Park, "A microfluidic in vitro cultivation system for mechanical stimulation of bovine embryos," *Electrophoresis*, vol. 30, pp. 3276-3282, 2009.

[37] M. S. Kim, T. Kim, S. -Y. Kong, S. Kwon, C. Y. Bae, et al., "Breast cancer diagnosis using a microfluidic multiplexed immunohisto-chemistry platform," *PLoS ONE*, vol. 5, e10441, 2010.