Antimicrobial Resistance (AMR) has become a global healthcare crisis. Urinary tract infections (UTIs) have contributed significantly to the problem with the frequent empiric prescribing of broad-spectrum antibiotics that are often ineffective or incorrectly selected on the basis of clinical bias. Over 150 million UTIs are thought to occur globally each year and about 8 million UTIs occur in the US yearly, of which an estimated 10% or 800,000 are antibiotic resistant.[1] Many of these arise in clinical settings deemed "complicated" such as in elderly and immunosuppressed patients or those having co-morbid conditions such as indwelling catheters, anatomic abnormalities, diabetes or neurogenic bladders. Such complicated UTIs are often polymicrobial and a potential source of sepsis with increased morbidity and mortality. New Molecular Point of Care test devices will provide guidance to Antimicrobial Stewardship programs to improve therapy selection, patient outcomes and cost efficiency, especially UTIs involving antibiotic resistant bacteria recognized by the CDC as urgent and serious antibiotic-resistant threats.[2] OvaGene's objective is to expand our in vitro diagnostic platform menu to include the detection of nucleic acids from antimicrobial resistant bacteria that cause health-care associated and community-acquired urinary tract infections. That entails clinical validation of identified markers, FDA authorization, and commercialization of our rapid diagnostic and CLIA waivable tests. The intended use of our in vitro diagnostic Multiple Drug Resistant (MDR) device is to diagnose urinary tract antimicrobial resistance at the point of need in less that 30 minutes.

Our easy to use solution performs microfluidic nucleic acid amplified hybridization assays that rapidly detect the presence & quantities of molecular analytes at point-of-need. This MDR device is rapid, low-cost, single-use, and analyzer free, with the ability to perform quantitative, multiplexed molecular testing. Our multiplexed in vitro diagnostic tests leverage Platinum nanoparticle technology for highly sensitive, specific & stable assays. The platform's foundational platinum nanoparticle technology was innovated and patented by Dr. Lidong Qin Ph.D. of Houston Methodist Research Institute.[3],[4],[5] Data for nucleic acids (RNA/DNA) from a small BioSample are transmitted from a mobile device to our cloud service for analysis, reporting, and mapping. We use innovative technologies to deliver low-cost, disposable Rapid Diagnostic Tests (RDTs) that address unmet public health needs. We intend to measure the quantitative

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[1]Harding, G., & Ronald, A. (1994). The management of urinary infections; what have we learned in the past decade? *International Journal of Antimicrobial Agents, 4*(2), 83-88. doi: 10.1016/0924-8579(94)90038-8

[2] National Action Plan for Combating Antibiotic-Resistant Bacteria, Retrieved March 2015, from https://www.whitehouse.gov/sites/default/files/docs/national_action_plan_for_combating_antibotic-resistant_bacteria.pdf pp 60 - 62.

[3]Song, Y., Xia, X., Wu, X., Wang, P., & Qin, L. (2014, November 10). Integration of Platinum Nanoparticles with a Volumetric Bar Chart Chip for Biomarker Assays. Retrieved January 09, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4351795/

[4] Song, Y., Wang, Y., & Qin, L. (2013, November 13). A multistage volumetric bar chart chip for visualized quantification of DNA. Retrieved January 09, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3875332/

[5]Song, Yujun, Yuanqing Zhang, Paul E. Bernard, James M. Reuben, Naoto T. Ueno, Ralph B. Arlinghaus, Youli Zu, and Lidong Qin. "Multiplexed volumetric bar-chart chip for point-of-care diagnostics." *Nature Communications* 3 (2012): 1283. Web.

presence of genes previously demonstrated to correlate with specific AMR, particularly in the Urgent threat level pathogens Carbapenem-Resistant Enterobacteriaceae and the Serious threat level pathogens Extended Spectrum β-Lactamase (ESBL) Producing Enterobacteriaceae and Vancomycin-Resistant Enterococcus[6]. It is also our intention to perform the addition studies to obtain a CLIA certificate of waiver.

Challenges to the successful clinical implementation of our Multidrug Resistance (MDR) platform are those of molecular diagnostics in general: a cultural inertia by physician providers to utilize molecular diagnostics despite the demonstration of improved dynamics of time and sensitivity/specificity, the ongoing need for compelling prospective validation studies compared to the existing "gold standards," and challenges secondary to cost and inadequate reimbursement which in turn inhibit practical implementation and replacement of old testing strategies.[7] Specific to the OvaGene MDR platform, we must show that nucleic acid testing on our platform is translatable for the initial Proof of Concept prototype, that the initial nucleic acid testing performed on blood is transferrable to urine (or other body fluids) and that we design and perform clinical trials in parallel fashion demonstrating superior resistance identification to urine C&S.

WHO has published areas of concern regarding scientific and technical barriers to success in developing effective RDT's.[8] Standard techniques of mid-stream urine collection and proper storage are critical and basic to accurate validation studies. DNA amplification testing using Polymerase Chain Reaction (PCR) is now standard central laboratory testing for multiple different bacteria. Real-time PCR has been used to diagnose UTI and showed success in identifying pathogenic organisms and distinguishing bacteriuria from infection with a 4-hour turnaround.[9],[10] Miniaturized PCA-based technology is thus far limited by the cost and the number of analyte primers used in the assay. Our planned MDR device measures DNA by a chemically amplified hybridization reaction shown to give highly sensitive and specific results and will have up to date inclusive MDR gene primers to limit false negative results.

The current standard of care for diagnosis and confirmation of therapy selection in UTI includes the microscopic examination of urine for pyuria and urine culture with antibiotic sensitivity (C&S) testing to

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[6]National Action Plan for Combating Antibiotic-Resistant Bacteria, Retrieved March 2015, from https://www.whitehouse.gov/sites/default/files/docs/national_action_plan_for_combating_antibotic-resistant_bacteria.pdf pp 60 - 62.

[7]"Thomas, Sarah T. , Carl Heneghan, Christopher P. Price, Ann Van Den Bruel, and Annette Plüddemann. "Point-of-care testing for urinary tract infections ." The National Institute for Health Research Diagnostic Evidence Co-operative Oxford June (2016): n. pag. Web.

[8]"Morel, Chantal, Lindsay McClure, Suzanne Edwards, Victoria Goodfellow, DAle Sandberg, Joseph Thomas, and Elias Mossialos. "Ensuring innovation in diagnostics for bacterial infection." e European Observatory on Health Systems and Policies a partnership hosted by WHO (2016): 40-41. Web.

[9]"Hansen, Wendy L. J., Christina F. M. Van Der Donk, Cathrein A. Bruggeman, Ellen E. Stobberingh, and Petra F. G. Wolffs. "A Real-Time PCR-Based Semi-Quantitative Breakpoint to Aid in Molecular Identification of Urinary Tract Infections." PLOS One April (2013): n. pag. Web.

[10] Van der Zee, Anneke , Lieuwe Roorda, Gerda Bosman, and Jacobus M. Ossewaarde. "Molecular Diagnosis of Urinary Tract Infections by Semi-Quantitative Detection of Uropathogens in a Routine Clinical Hospital Setting." March (2016): n. pag. Web.

identify specific bacteria, provide semi-quantitative assessment and antibiotic susceptibility to available therapies. Urine C&S takes between 24-48 hours for the availability of results. Urine culture is often omitted for uncomplicated UTI in otherwise healthy individuals and a course of empiric antibiotics initiated. Complicated UTI (i.e. age >65, immunosuppressed patients and those having co-morbid conditions such as chronic indwelling catheters and neurogenic bladder) requires a urine culture and sensitivity. Complicated UTI has an increased incidence of AMR and thus a greater risk of urosepsis, septic shock, and death.

Dipsticks are variably used to test for leukocyte esterase (a measure of urine leukocyte presence and inflammation) and nitrites (a bacterial metabolic product and hence an indication of bacteriuria). A negative dipstick is helpful in excluding UTI, but a positive test lacks sensitivity due to possible contamination or asymptomatic bacteriuria without true infection. There are several other technologies in various stages of development that may provide significant diagnostic advantages to standard culture but are not currently cleared for clinical use or are uncommonly deployed: Flow Cytometry, mass spectrometry (MALDI-TOF MS), qPCR assays in various forms, isothermal microcalorimetry, and various Biosensor arrays. Various protein biomarkers such as Procalcitonin, C-Reactive Protein, and Heparin-binding protein have not yet proven their clinical worth and are seldom utilized by US physicians to guide therapy.[11]

Our platform will provide rapid quantitative analysis of established genes found in urine specimens correlating with bacterial resistance. Proof of concept studies have shown high sensitivity and specificity with a wide dynamic range of detection down to the SNP level for nucleic acids.[12] We have successfully demonstrated the performance of accurate amplified ELISA protein assays on this platform as well.

Urine culture provides semi-quantitative bacterial counts and combined with sensitivity testing gives a phenotypic picture of the dominant bacteria currently manifesting in the urinary track. This is made more challenging if multiple organisms are present. Molecular testing of urine has the potential to give a more rapid and accurate identification of the resistant bacteria present but also has the potential to identify "inducible resistance"; i.e. bacteria that have the genetic mutations that may manifest resistance before they are phenotypically manifested (but may do so upon the implementation of broad spectrum antibiotic coverage).

The empiric utilization of broad spectrum antibiotics in both humans and animals has contributed to alterations in the microbiome and given rise to antibiotic resistant microbes. The emergence of these multidrug-resistant organisms has rendered the use of broad spectrum antibiotics problematic in the primary care setting of uncomplicated UTI as well as with complicated UTI's. WHO's Global Analysis of AMR in E. coli and K. Pneumoniae showed significantly increased mortality for those bacteria showing

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[11]Fritzenwanke, Moritz, Can Imirzalioglu, Trinad Chakraborty, and Florian M. Wagenlehner. "Modern diagnostic methods for urinary tract infections." Sept (2016): n. pag. Web.

[12]Song, Y., Wang, Y., & Qin, L. (2013, November 13). A multistage volumetric bar chart chip for visualized quantification of DNA. Retrieved January 09, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3875332/

ESBL and CRE resistance criteria.[13] New reports of resistance have included the presence of the MCR-1 gene indicating Colistin resistance in Escherichia coli for the first time in the US, confirming the truly global presence of yet another severely threatening AMR problem.[14],[15]

Time is of the essence in identifying these AMR bacteria. Urine C&S takes 24+ hours which includes newer routes of identifying bacteria once grown as colonies in culture. The OvaGene MDR assay will identify gene markers of resistant bacteria in under 30 minutes, offering a significant advantage for initial choice of effective and appropriate antibiotic therapy. It can be used in correlation with other diagnostic techniques including standard C&S. The goal is to rapidly choose a targeted antibiotic or combination of antibiotics that addresses the presence of resistant pathogenic bacteria and institute appropriate therapy immediately.

The OvaGene MDR platform was established by Dr. Qin, the inventor, using microfluidic, nanoparticle technology capable of highly sensitive and DNA specificity detection down to the SNP level with multiplex DNA detection on the same platform. The use of pre-deposited platinum nanoparticles is used to perform platinum catalyzed cascade signal amplification to detect as low as 20 pM DNA targets. Oxygen gas generated by the platinum nanoparticle interaction with hydrogen peroxide drives the volumetric readout of this sensitive sandwich DNA hybridization reaction. The assay was shown to a wide dynamic range of reaction. The multiplexing capability was confirmed using 30-nucleotide sequences of Bacillus anthracis, Ebola virus, and Tubercle bacillus. (see Appendix)

In summary, the use of platinum nanoparticle driven DNA amplified hybridization has produced a platform for DNA analysis that is highly sensitive and specific with a wide dynamic range capable of multiplexing analysis. This is a unique approach to DNA analysis that does not require usual PCR technology with PCR related deficiencies.

We have successfully miniaturized and are commercializing molecular-based lab assays onto our platforms card/chip in the ELISA format for the detection of antigens, antibodies, and nucleic acids with excellent sensitivity, specificity, and selectivity. In our fully integrated microfluidic card, the assay sequencing, reagent mixing, incubation timing and washing are all reproducibly controlled. We store all of the reagents on the card. (Both dried-down in reaction wells, and wet in blister packs.)

Huge advancements in the miniaturization and handling of molecular biochemistry have moved microfluidics forward. The process of manipulating, creating, amplifying or modifying nucleic acids (DNA/

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[13]Fukuda, Keiji. "Antimicrobial Resistance Global Report on Surveillance." World Health Organization (2014): 18-19 37-39. Web.

[14]McGanna, Patrick, Erik Snesruda, Rosslyn Maybanka, Brendan Coreya, Ana C. Onga, Robert Clifforda, Mary Hinklea, Timothy Whitmanb, and Emil Leshoa And. "Patrick McGann." Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF Plasmid: First Report of mcr-1 in the United States. N.p., 01 July 2016. Web. 09 Jan. 2017.

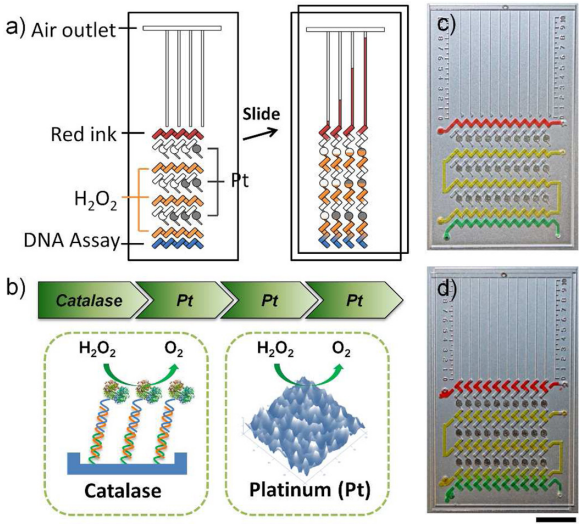
[15]Mediavillaa, José R., Amee Patrawallab, Liang Chena, Kalyan D. Chavdaa, Barun Mathemac, Christopher Vinnarda, and Lisa L. Deverd. "José R. Mediavilla." Colistin- and Carbapenem-Resistant Escherichia coli Harboring mcr-1 and blaNDM-5, Causing a Complicated Urinary Tract Infection in a Patient from the United States. N.p., 30 Aug. 2016. Web. 09 Jan. 2017.

RNA) in microfluidic design applications have become increasingly sophisticated. Detecting target pathogens uses amplification techniques and in-situ hybridization to identify the nucleic acids of the identified targets. Innovative methods for nucleic acid amplification, passive timing, and sequencing methods will all be miniaturized. We have partnered with a world leader in microfluidic contract design and manufacturing. They have fourteen years of experience and have completed more than 1,000 projects for more than 250 clients around the world. Together we are translating these techniques and augmenting the capabilities of our simple-to-use diagnostic device to perform in-situ hybridization assays at point-of-need. The user will interact with our advanced molecular platform only to add the sample and read the result; all results will be digitally sensed and synced with a cloud service. Our device will be easy to use and CLIA waivable.

Please note:

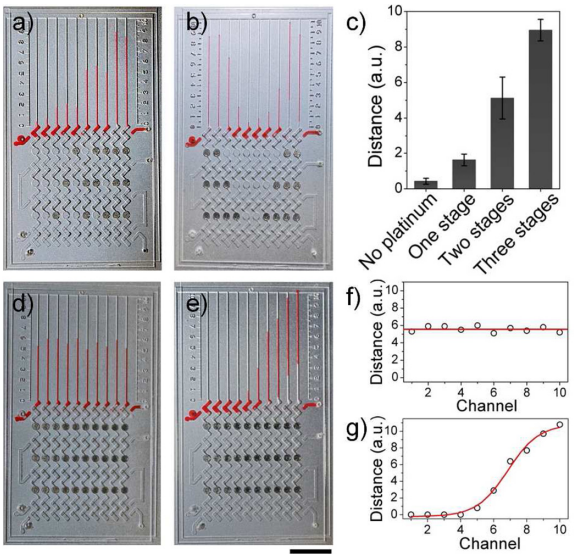
- All studies involving clinical research will be monitored for inclusion tracking of women, minorities, and children.
- We will be in compliance with policies related to the use of Vertebrate Animals, and biosafety issues.
- We have licensed the work of Lidong Qin from Houston Methodist Research Institute. The patent is titled "Multiplexed volumetric bar-chart chip for point of care biomarker and analyte quantitation"; US patent number 20140106346 A1.

Figure 1



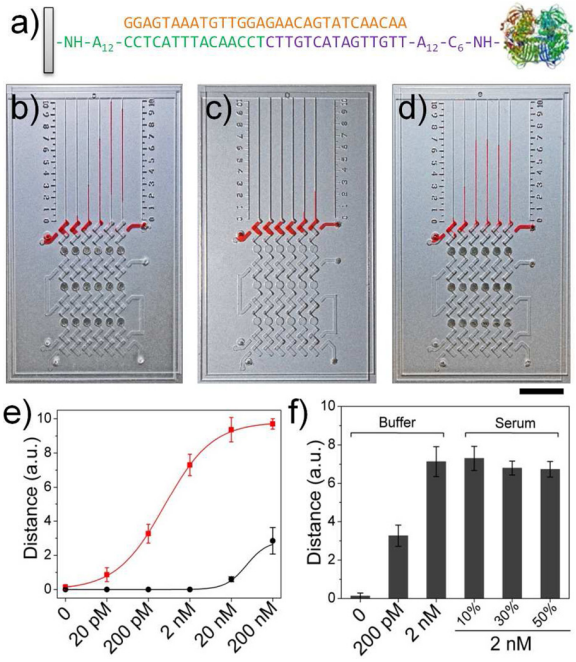
Working principle of the MV-Chip. (a) Schematic view of a typical MV-Chip for DNA assay. The detection units with platinum amplification (black circles) show larger bar chart advancement than those without amplification. (b) 'Rocket-like' propelling mechanism of the MV-Chip. Catalase introduced by DNA hybridization is the initiator and three stages of rough platinum films (Pt) amplify the signals. (c & d) The representative flow path of each reagent before and after an oblique slide: red lane (ink), yellow lane (H2O2), and green lane (DNA assay). Scale bar is 1cm for c) and d).

Figure 2



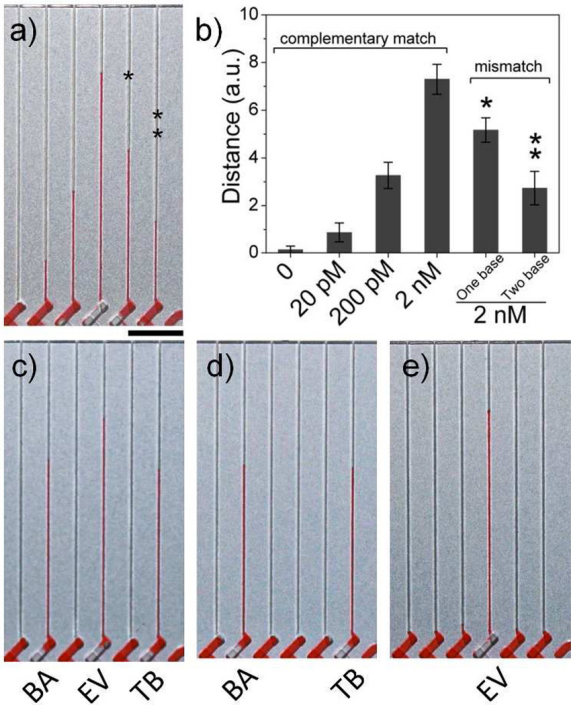
Validation of signal amplification for MV-Chip. (a & b) Selectively deposited circle wells with platinum films (dark dots) to produce amplified signals. (c) Bar chart results without or with one, two, or three stages of platinum amplification. Data are obtained from (a) and (b). (d) A uniform concentration of catalase generates uniform readout distance in the presence of 10 μg mL–1 catalase and 0.25 M H2O2. (e) Bar chart images of diffusing 50 μg mL–1 catalase with three stage amplification in the presence 0.25 M H2O2. The dark dots are platinum films in (d) and (e). Scale bar is 1cm for a), b), d) and e). (f & g) the uniformed advancements and sigmoid curve obtained from (d) and (e) respectively.

Figure 3



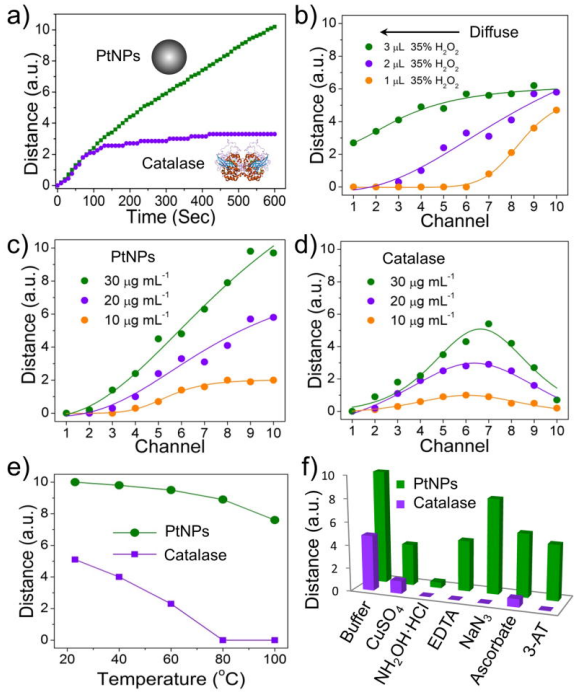
DNA detection using MV-Chip. (a) The sandwich DNA hybridization structures and 30-nucleotide Ebola virus (EV) sequence used in MV-Chip. (b, c) The images of ink advancement for detection of target DNA with b) or without c) platinum amplification. The concentration in each lane corresponds to the values in (e). (d) From left to right: bar chart advancements for detection of 0, 200 pM, and 2 nM target DNA in buffer and 2 nM target DNA in 10 %, 30 %, and 50 % serum. Dark points are platinum films in (b), (c) and (d). Scale bar is 1cm for b), c), and d). (e) The target DNA calibration curves corresponding to ink advancement with (red) or without (black) platinum amplification at 5 min, with the target DNA concentration varying from 20 pM to 200 nM. f) MV-Chip readouts of 0, 200 pM, and 2 nM target DNA in buffer and 2 nM target DNA in 10%, 30%, and 50% serum. The error bars in e and f represent the s. d. of three measurements

Figure 4



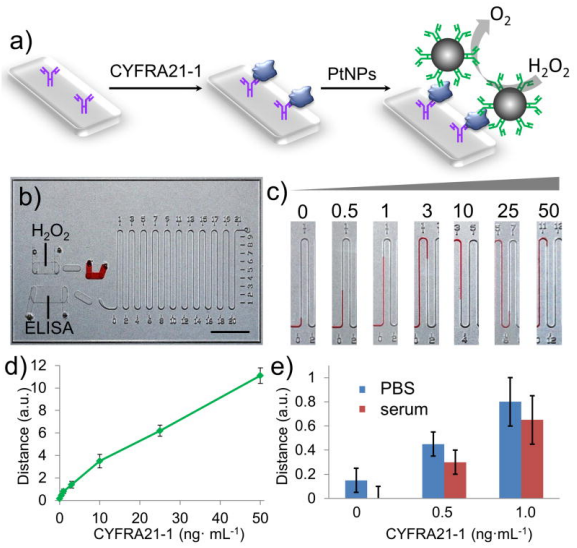
DNA detection using MV-Chip. (a) The sandwich DNA hybridization structures and 30-nucleotide Ebola virus (EV) sequence used in MV-Chip. (b, c) The images of ink advancement for detection of target DNA with b) or without c) platinum amplification. The concentration in each lane corresponds to the values in (e). (d) From left to right: bar chart advancements for detection of 0, 200 pM, and 2 nM target DNA in buffer and 2 nM target DNA in 10 %, 30 %, and 50 % serum. Dark points are platinum films in (b), (c) and (d). Scale bar is 1cm for b), c), and d). (e) The target DNA calibration curves corresponding to ink advancement with (red) or without (black) platinum amplification at 5 min, with the target DNA concentration varying from 20 pM to 200 nM. f) MV-Chip readouts of 0, 200 pM, and 2 nM target DNA in buffer and 2 nM target DNA in 10%, 30%, and 50% serum. The error bars in e and f represent the s. d. of three measurements.

Figure 5



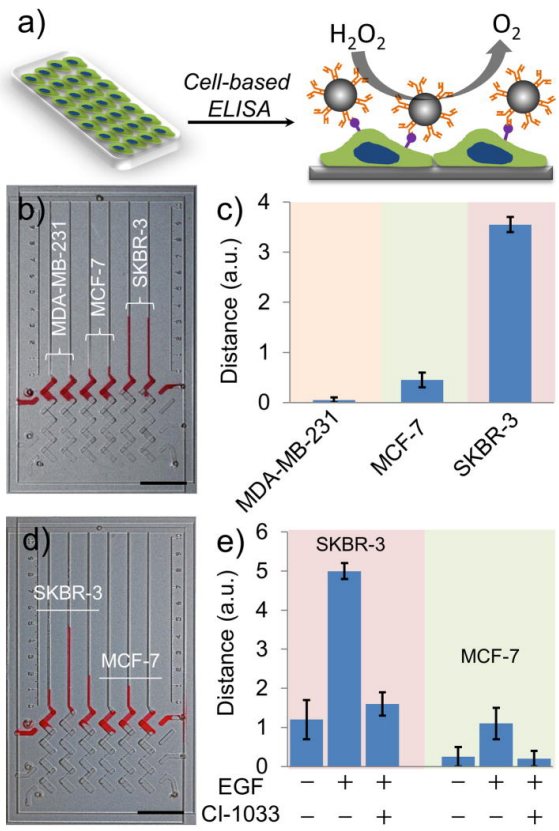
Comparison of PtNPs and catalase for catalyze oxygen gas production in 10-plex V-Chip. a) Time-dependent ink advancements with the application of 20 μg·mL−1 PtNPs and catalase. H2O2 concentration is 35% or 4% respectively. b) Bar chart advancements after diffusing different volumes of 35% H2O2 in the presence of 20 μg mL−1 PtNPs. c, d) Bar chart advancements of diffusing 2 μL of 35 % H2O2 in the presence of different concentration of PtNPs (c) or catalase (d). e) Catalytic activity of PtNPs and catalase after treatment at different temperatures for 30 min. f) Catalytic activity of PtNPs (5×) and catalase (1×) in the absence or presence of 5 mM CuSO4, NH2OH·HCl, EDTA, NaN3, ascorbate and 3-amino-1, 2, 4-triazole (3-AT).

Figure 6



PtV-Chip for detection of CYFRA21-1. a) A standard sandwich ELISA was employed in PtV-Chip for biomarker detection. CYFRA21-1 (blue) was added in the assay wells in the bottom plate and bound to the capture antibodies (purple). The PtNP-antibody complexes were loaded into the assay chamber to form sandwich structures. To detect the target biomarkers, PtNP was conjugated with the probe antibody to react with the hydrogen peroxide and produce oxygen gas. b) Image of PtV-Chip after loading reagents and samples. Scale bar, 1 cm. c) Images for single-plex V-Chip results in the absence or presence of different concentration of CYFRA21-1 (0.5, 1, 3, 10, 25, 50 ng·mL−1). d) The CYFRA21-1 calibration curve corresponding to ink advancement at 10 min, with the CYFRA21-1 concentration varying from 0.5 to 50 ng·mL−1. e) Detection results of spiked CYFRA21-1 in PBS buffer and serum. The error bars in d and e represent the SD of three measurements.

Figure 8



Cell-based ELISA for detection of HER2 and pHER2 using PtV-Chip. a) Schematic mechanism for cell-based ELISA for cell surface biomarker detection using PtV-Chip. Cells were seeded in the assay wells in the bottom plate. Antibody conjugated PtNPs were used to target the cell surface biomarkers and to react with the hydrogen peroxide to produce oxygen gas. b), c) PtV-Chip detection of HER2 expression on 1,000 MDA-MB-231, MCF-7, or SKBR-3 cells. d), e) PtV-Chip detection of pHER2 expression on SKBR-3 and MCF-7 cells treated with or without 100 ng·mL–1 EGF and 5 mM Cl-1033. Scale bar, 1 cm for (b) and (d). The error bars in b and d represent the SD of three measurements.