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Measurement and Analysis of Vibrio Fischeri Cell-based Microfluidic Device for Personal Health Monitoring

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Abstract— **The cell-based microfluidic chip was designed and fabricated as a low-cost detector to continuously monitor toxicants in drinking water or human urine samples, which is expected to be an important component of a household health monitoring system in the future. The bioluminescent bacterium, *Vibrio Fischeri*, was selected to validate the function of device. Water samples and *Vibrio fischeri* cells were mixed and encapsulated into droplets in air flow, which can guarantee sufficient oxygen supply for cells in droplets. Preliminary tests were performed using copper ion (Cu^{2+}) as the model toxicant. The droplet system was measured and analyzed at various flow rates in different observation chambers. Both deionized water and human urine samples were tested in the cell-based device. Interestingly, a strong relation between the R.L.U. (Relative Luminescence Units) in the observation chamber and the minute concentration of toxicant (Cu^{2+}) was found using deionized water as solvent, whereas the relation was insignificant using human urine as solvent. This study showed the *Vibrio fischeri* cell-based device might be reliably employed as an early-warning system for the safety of drinking water. However, *Vibrio fischeri* is not competent to detect dangerous materials in a complex biofluid. With the replacement of cell sensors, the microfluidic device might be functional to analyze urine samples in theory.**

I. Introduction

The safety of drinking water is essential for personal health. Although trace amounts of toxicants in water are usually not fatal, they could gradually erode human health unwittingly. Therefore, continuous detection of toxicants in drinking water is one of the important tasks for the household health monitoring system in the future. On the other hand, the detection of dangerous substances in human urine could reflect the personal health conditions to some extent.[1] When the collection and automatic analysis of urine sample could be integrated into the future intelligent toilet, the composition of urine may become an important source of information for personal health monitoring. After all, urine sample can be easily collected and the daily amount of urine is large enough to support macroanalysis or microanalysis. The dramatic changes of trace ingredients in the urine can reflect some changes in the human body.

Two analysis tasks mentioned above are dependent on the detection of trace amounts of dangerous substances in the aqueous solution, especially continuous analysis methods. Options for the rapid analysis of chemical contaminants in water are often deficient. Thorough analytical methods for a broad range of organic and inorganic chemicals are available, but they usually require complex instrumentation that is not readily available in the household situation. Traditional approaches, which are based on chemical or physical analyses, allow highly accurate determination of the exact composition in samples. But there are still several disadvantages to be employed in a household monitoring system, such as high reagent consuming, time-consuming, slow responses, etc. [2] The use of cell sensors can rapidly evaluate the toxicity of water instead of measuring concentrations of individual chemical constituents, which is a possible alternative to traditional analytical approaches.

Biology-based toxicity sensors can provide rapid assessments of water quality and contribute to water security investigations. Some cell sensors of bioengineering can specifically response to appointed substances.[3] Thus, cell-based devices are able to monitor various water samples with suitable cell sensors, which usually evaluate cellular cytotoxic responses for a broad range of sensing capabilities, including the detection of unknown materials. [4, 5] For instance, Frense *et al.* reported immobilized algae cells was employed as optical biosensors to detect environmental pollutants.[6] Rodriguez et al. employed C. vulgaris cells as the biosensors for rapid monitoring of primary-source drinking water.[7] Wei et al. even reconstructed two bioengineering cell sensors for detection of genotoxic chemicals.[8] Compared with other biosensors, bioluminescent bacteria, *Vibrio fischeri*, have a larger population size, higher growth rate, lower cost, easier cultivation, and more rapid response to a broad range of toxicants. Bioluminescent bacteria are found widely in nature and their light emission can be applied as a sensitive, rapid, and safe assay in several biological systems.[9] However, bioluminescent sensors suffer from the poor sensitivity of detectors because the precise determination of luminescence often requires large and expensive instrumentation.

In this study, a prototype chip was designed and fabricated to provide a continuous monitoring cell-based sensor for a broad range of toxicants in aqueous solutions. It was composed of two micromixers, a T-junction droplet generator and six time-delay channels (TD-Cs) *Vibrio fischeri* was employed here as the cell sensor. Besides, the photomultiplier tube (PMT) based detection module was also installed to

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quantify weak bioluminescent signals. The cell-based device will be an important component of a household health monitoring system, demonstrated in Fig. 1. The droplet system was preliminarily analyzed using copper ion (Cu^{2+}) as the model toxicant.

II. Material and Methods

A. Chip design and fabrication

The chip was composed of three circular layers with the diameter of 65 mm (Fig. 2, Inset a). Three kinds of domains were found in the middle layer, including two counter-flow micromixers, a T-junction droplet generator and six TD-Cs. The counter-flow micromixer inherited the design of micro-concentrator to make two fluids encounter in the opposite directions and lead to efficient mixing. [10, 11] Two counter-flow micromixers here were used to mingle three different reagents through the inlets A, B, and C (Fig. 2, Inset b, c). The final solution was encapsulated in droplets through T-junction structure and driven inside a TD-C when the relative outlet was open. Six TD-Cs with the same depth (100μm) but different width (100~200μm) were incorporated in the proposed prototype chip to investigate the influence of the aspect ratio on the stability of the droplet flow. [12, 13] The length of TD-Cs ranges from 0.83m to 1.18m. The middle and bottom layers of the chips were fabricated using 4-inch silicon wafers with standard micro-fabrication process. [14, 15] The two silicon wafers were bonded together by Si–Au–Si thermal bonding process.[16] Subsequently, silicon-glass anodic bonding is conducted to connect the middle layer to a Pyrex 7740 glass on the top.[17]

B. PMT-based detection module

A PMT-based photosensing module H10723-01 (Hamamatsu® Photonics, Hamamatsu City, Japan) was selected for the detection of the emission spectrum of bacterial bioluminescence, which mainly ranges from 400 nm to 600 nm. [18] The photosensing system is demonstrated in Fig. 3. A series of observation chambers were fabricated on PMMA (polymethylmethacrylate) by KT-40 laser engraving machine (Ketai Laser Tech.®, Liaocheng city, China), which shapes are all cylinders of 8mm diameter, but the depths of observation chamber are various. Thus, the top of observation chambers could match the effective area of H10723-01 PMT. After observation chamber was covered by a transparent sealing tape (Biovendis®, Mannheim, Germany), the PMT module will be assembled on the top of chamber and wrapped by shade materials. Luminescence signals of Vibrio fischeri cells in the droplet flow were measured within various observation chambers.

C. Toxicant tests on the chip

Inlets and outlets of the microfluidic chip were connected by polytetrafluoroethylene tubes. The design of experimental system was provided in Fig 3. Air, sample, 2% (w/v) NaCl solution and 1×10^5cells/mL *Vibrio fischeri* cell solution (BioToX™ Kit, Aboatox Oy®, Masku, Finland) were injected by digital syringe pumps (Fusion 200, Chemyx®, Stafford, TX; Genie™ Plus, Kent Scientific®, Torrington, CT).

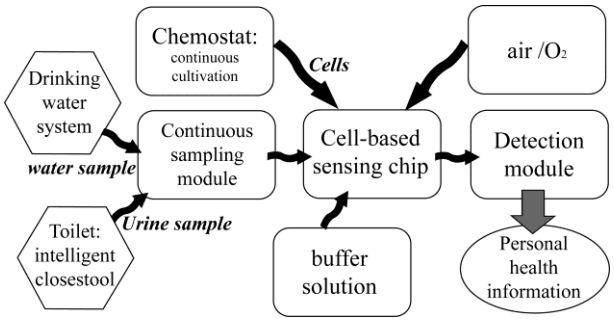

Figure 1 The schematic diagram of a household health monitoring system in the future. The cell-based sensing chip is the core of this system, which can continuously measure aqueous samples using an unfailing supply of cell sensors generated by a chemostat.[18] Both drinking water and personal human urine samples will be analyzed and health information could be evaluated through detection results.

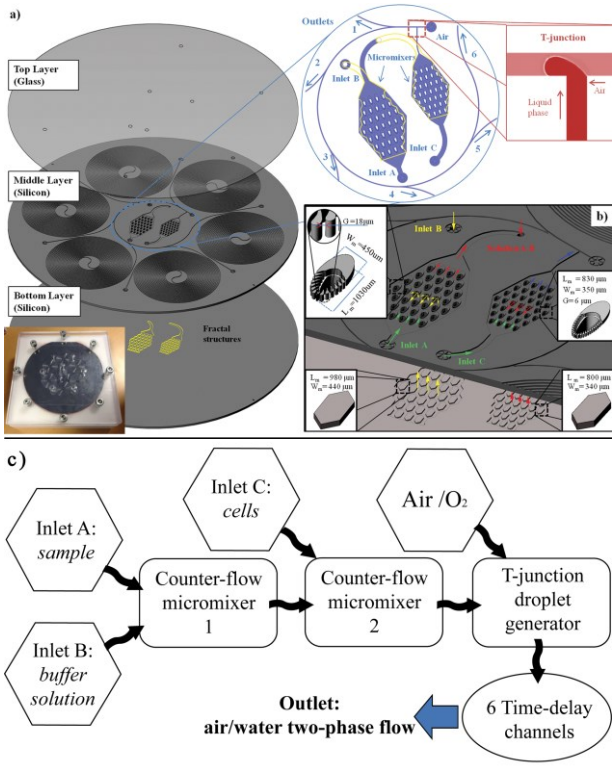

Figure 2 The structure of cell-based microfluidic chip. (a) Three domains on the middle layer, including counter-flow micromixers, T-junction droplet generator, TD-Cs. The photo graph of the chip was shown at the corner. (b) Schema of the counter-flow micromixer, which provides specification about counter-flow unit (on the middle layer) and pillars (on the bottom layer). The working principle of counter-flow micromixer is similar to the counter-flow micro-concentrator in the previous publication, [10] but the inlet and outlet were reverse. c) The schematic structure of the cell-based device. Sample, buffer solution, cell sensors and air flow are mixed into an air/water two-phase flow before detection.

The volumetric flow rate were precisely controlled and recorded. Based on the results of simulation, the volumetric flow rates of water sample, 2% NaCl solution, Vibrio fischeri cell solution and air flow always kept the ratio of 1:1:2:4. Thus, $\mu_{droplet}$, the droplet flow rate, was defined as 4 times that of *Vibrio fischeri* cell solution.

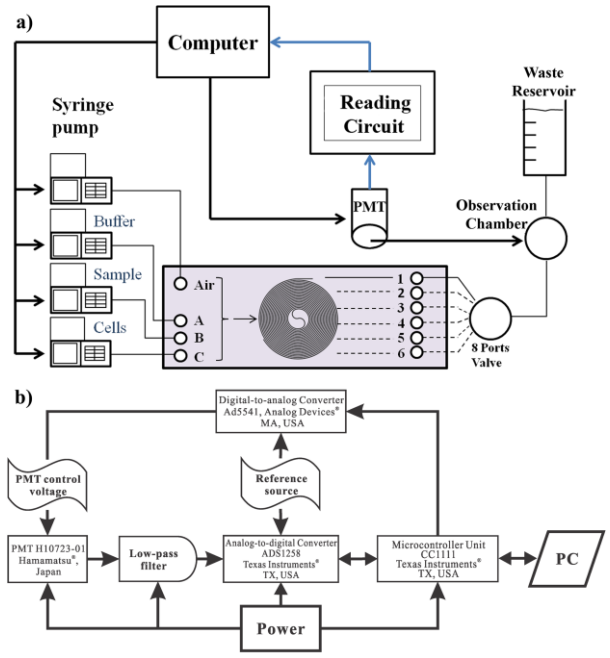

Figure 3 The diagram of experimental system. (a) The sketch map of the testing system. (b) The structure of PMT-based photo-sensing system. The H10723-01 PMT is an ultrasensitive detection module with low-power consumption and a low-noise level, which sensitivity covers from 230 nm to the near infrared region. Finally, the digital signals were transported into a computer (PC).

Outlet tubes were fixed to an 8-way valve (Hamilton®, Bonaduz, Switzerland) and one of specific observation chambers. After the observation chamber was filled with gas-liquid two-phase flow, the detection module started to work. The 'Response Time' of the sensing device is defined as the period from the moment that the first droplet is generated to the moment that the observation chamber is filled with such kind of droplets. The toxicity of water samples and urine samples was evaluated through the intensity of emitted light measured by the PMT-based detection module. The bioluminescence data were analyzed using Microsoft Excel® and OriginPro® software as before.[19] For continuous monitoring aqueous samples, the detection module reads the data every a specific period. This period is equal to that all droplets in the observation chamber are expelled by new ones, which is defined as 'Sampling Interval'.

$CuSO_4$ solutions were serially diluted in deionized water and in urine samples from three healthy male volunteers, which were used in the testing. Deionized water and 2% NaCl were employed as two blank solutions respectively. 60mg/L CPC (Weifa®, Oslo, Norway) in water was selected as positive control solution, because CPC, cetylpyridinium chloride, is an easy-to-obtain spectrum bactericidal drug that can kill *Vibrio fischeri* and eliminate the bioluminescence efficiently. Fresh solutions of *Vibrio fischeri* were made just before use.

III. Results and Discussion

After many attempts, the properties of on-chip cell sensors were made clear, and so were the appropriate operating parameters of the system. When the observation-chamber (volume: 20μL) and TD-C No. 6 (width: 200μm) were employed, six kinds of water sample solutions were tested in the cell-based sensing device at a series of droplet flow rates (Fig. 4, Inset a).

With the same operating parameters, the R.L.U. in the group of 2% NaCl is the highest, because 2% NaCl solution is approximately equal to *Vibrio fischeri* isosmotic solution; the R.L.U. of Deionized water group descended about 25%; the group using 0.05mg/L Cu^{2+} solution showed very similar results to those in deionized water group. However, the solutions of 0.5mg/L Cu^{2+} and 2.5mg/L Cu^{2+} showed significant toxicological effects on *Vibrio fischeri* cells. Especially when $\mu_{droplet}$ was below $40\times10^{-12}m^3/s$, the R.L.U. measured would be less than 50% of that in the isosmotic group. The results of positive control group (60mg/L CPC) were in line with expectations, which bioluminescent signals were close to the noise level. When $\mu_{droplet}$ was $40\times10^{-12}m^3/s$, toxicological effects in water can be distinguished easily. In addition, we also tested different observation chambers and TD-Cs using serially-diluted $CuSO_4$ in deionized water. It is found that using a smaller observation chamber would result in larger relative errors (Fig. 4, Inset b), whereas the switch of different TD-Cs have no significant impact on the results (Fig. 4, Inset c). In the future work, the cell-based system will be examined and evaluated by other toxic chemicals and heavy metal ions, such as Hg^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+} etc.

The urine samples with/without Cu^{2+} spiked were tested using the same operating parameters. The results (Fig. 4, Inset d) showed that urine samples are more similar to 2% NaCl solution to *Vibrio fischeri* cell sensors. Furthermore, Cu^{2+} ions in urine samples almost lost the toxicity, compared with the experiments in water samples. This phenomenon can be explained that Cu^{2+} ions were neutralized by proteins in the urine. When proteins are removed from urine sample in advance, we found the biofluid samples showed similar results as that of water samples. Besides, the biuret reaction in biochemistry also indicates that proteins are capable of forming complexes with copper ions. Therefore, *Vibrio fischeri* is not suitable for analyze natural urine samples, and should be replaced by others cell sensors, for example the yeast biosensors for genotoxic chemicals.[8] The device can still work as a urine analyzer if the mentioned yeast sensors are employed here.

IV. Conclusion

The analytical device and methods developed here are based on the measurement of bioluminescent intensity of *Vibrio fischeri* cells in a large observation chamber. It is conservative and robust. This *Vibrio-fischeri*-based device could be used as a continuous early-warning system for monitoring heavy metal irons or toxic chemicals in drinking water. However, it is not competent enough to analyze human urine samples. Replacing some suitable cell sensors on the platform, the microfluidic device might be still promising to monitor personal health conditions. Besides, the proposed cell-based system is no potential for detection of waterborne bacteria or viruses.

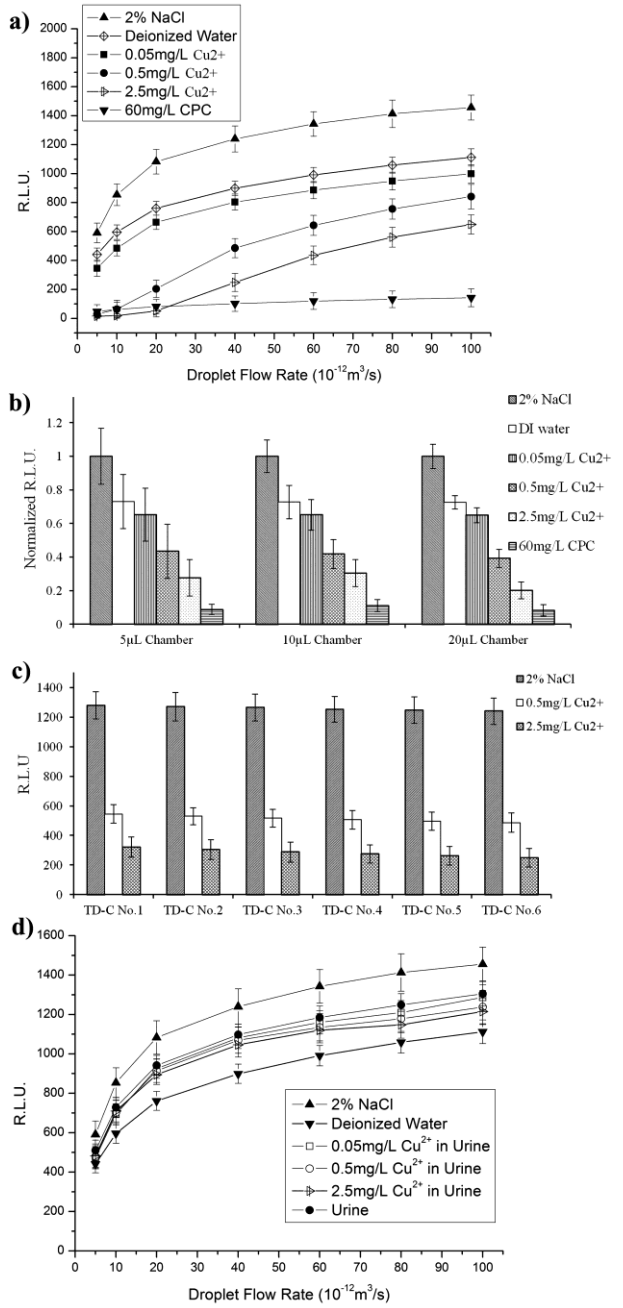

Figure 4 Toxicity testing results of the *Vibrio-fischeri*-based sensing device. (a) Six Cu^{2+} solutions of water were tested at a series of $\mu_{droplet}$. (b) Observation chambers (Volume: 5μL, 10μL, 20μL) were tested respectively. (c) Parallel tests of six TD-Cs. (d) Urine samples with/without Cu^{2+} spiked were analyzed.

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REFERENCES

[1] M. Truong, B. Yang, and D. F. Jarrard, "Toward the detection of prostate cancer in urine: a critical analysis," J Urol, vol. 189, pp. 422-9, Feb 2013.

[2] P. J. Marriott, P. Haglund, and R. C. Y. Ong, "A review of environmental toxicant analysis by using multidimensional gas chromatography and comprehensive GC," Clinica Chimica Acta, vol. 328, pp. 1-19, Feb 2003.

[3] S. Melamed, T. Elad, and S. Belkin, "Microbial sensor cell arrays," Curr Opin Biotechnol, vol. 23, pp. 2-8, Feb 2012.

[4] J. Z. Xing, L. J. Zhu, S. Gabos, and L. Xie, "Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity," Toxicology in Vitro, vol. 20, pp. 995-1004, Sep 2006.

[5] N. M. M. Pires, T. Dong, Z. C. Yang, N. Hoivik, and X. Y. Zhao, "A mediator embedded micro-immunosensing unit for electrochemical detection on viruses within physiological saline media," J. Micromech. Microeng., vol. 21, 115031, Nov 2011.

[6] D. Frense, A. Müller, and D. Beckmann, "Detection of environmental pollutants using optical biosensor with immobilized algae cells," Sens. Actuators, B, vol. 51, pp. 256-260, 1998.

[7] M. Rodriguez, Jr., C. A. Sanders, and E. Greenbaum, "Biosensors for rapid monitoring of primary-source drinking water using naturally occurring photosynthesis," Biosens Bioelectron, vol. 17, pp. 843-9, Oct 2002.

[8] T. Wei, C. Zhang, X. Xu, M. Hanna, X. Zhang, Y. Wang, H. Dai, and W. Xiao, "Construction and evaluation of two biosensors based on yeast transcriptional response to genotoxic chemicals," Biosens Bioelectron, vol. 44, pp. 138-45, Jun 15 2013.

[9] X. Zhao, T. Dong, Z. Yang, N. Pires, and N. Hoivik, "Compatible immuno-NASBA LOC device for quantitative detection of waterborne pathogens: design and validation," Lab Chip, vol. 12, pp. 602-12, Feb 7 2012.

[10] T. Dong, et.al., "Integratable non-clogging microconcentrator based on counter-flow principle for continuous enrichment of CaSki cells sample," Microfluid. Nanofluid., vol. 10, pp. 855-865, Apr 2011.

[11] N. Tran-Minh, et.al., "Design and optimization of non-clogging counter-flow microconcentrator for enriching epidermoid cervical carcinoma cells," Biomedical Microdevices, vol. 13, pp. 179-190, Feb 2011.

[12] Z. C. Yang, E. Halvorsen, and T. Dong, "Power generation from conductive droplet sliding on electret film," Appl. Phys. Lett., vol. 100, 213905, May 21 2012.

[13] T. Dong, Z. C. Yang, and H. L. Wu, "Molecular simulations of R141b boiling flow in micro/nano channel: Interfacial phenomena," Energy Conversion and Management, vol. 47, pp. 2178-2191, Sep 2006.

[14] T. Dong, Z. C. Yang, Q. C. Bi, and Y. L. Zhang, "Freon R141b flow boiling in silicon microchannel heat sinks: experimental investigation," Heat and Mass Transfer, vol. 44, pp. 315-324, Jan 2008.

[15] T. Dong, et.al., "A smart fully integrated micromachined separator with soft magnetic micro-pillar arrays for cell isolation," J. Micromech. Microeng., vol. 20, 115021, Nov 2010.

[16] B. Xiao, et.al., "Integrated micro Pirani gauge based hermetical package monitoring for uncooled VO (x) bolometer FPAs," Microsystem Technologies, vol. 17, pp. 115-125, Jan 2011.

[17] T. Dong and Z. C. Yang, "Measurement and modeling of R141b condensation heat transfer in silicon rectangular microchannels," J. Micromech. Microeng., vol. 18, 085012, Aug 2008.

[18] E. Vetrova, et.al., "A bioluminescent signal system: detection of chemical toxicants in water," Luminescence, vol. 22, pp. 206-214, May-Jun 2007.

[19] X. Zhao and T. Dong, "Multifunctional sample preparation kit and on-chip quantitative nucleic Acid sequence-based amplification tests for microbial detection," Anal Chem, vol. 84, pp. 8541-8, Oct 16 2012.