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A Household LOC Device for Online Monitoring Bacterial Pathogens in Drinking Water with Green Design Concept

Xinyan Zhao, Tao Dong*, *Member, IEEE*

***Abstract*—Bacterial waterborne pathogens often threaten the water safety of the drinking water system. In order to protect the health of home users, a household lab-on-a-chip (LOC) device was developed for online monitoring bacterial pathogens in drinking water, which are in accord with green design concept. The chip integrated counter-flow micromixers, a T-junction droplet generator and time-delay channels (TD-Cs), which can mix water sample and reactants into droplets in air flow and incubate the droplets in the LOC for about 18 hours before observation. The detection module was simplified into a transparent observation chamber, from which the home users can evaluate the qualitative result by naked eyes. The liquid waste generated by the LOC system was sterilized and absorbed by quicklime powders. No secondary pollution was found. The preliminary test of the prototype system met its design requirements.**

I. Introduction

Drinking water is the most important domestic resource that related to human health. Owing to water industry has been highly developed, rare pollutions can threaten water safety of the water system in a city. However, the dangerous factors still exist around the house. In many cities, the sewage and water supply network have been working almost 100 years old. The exchange of water supply network is often not frequent enough to ensure adequate water quality in all houses. Besides, the drinking water and sewage pipes often have imperceptible leaks.[1] The collapse of pipes causes interchange between drinking water and sewage, which can involve local pollutants in the drinking water supply. (Fig. 1) The risk of such an accident increases as time goes on. Since there are a large number of bacteria in human feces, the sewage-polluted water may cause bacterial waterborne diseases. Periodic inspection is essential for reducing the impact of hidden accidents, but high-frequency examinations will increase the costs of domestic consumers. Some online detectors on waterborne bacteria are increased needs for consumers to guarantee water safety in houses. The analytical methods and biosensors for bacterial pathogens have been developed maturely, some of which even appear in textbooks, such as enzymatic methods, immunological methods, plate counting methods etc.[2] Hundreds of detection kits and instruments are available at present and constantly emerging. The commercial kit in this study comes from series products of Royal Biotech™ GmbH Company. In fact, there is no desperate need to develop a new biochip just for a similar analysis of pathogens. However, LOC devices have their merits.[3] LOC devices are miniaturized and automated tools, which are very suitable for unprofessional users.

Various LOC devices for monitoring pathogens have been studied for decades. [4, 5] Recently, Wong C. et al. has developed a DNA chip that can identify 70,000 different viruses and bacteria in one test.[6,7] Besides the immunoassay-based or PCR-based LOC chips, cell-based LOC is another important branch. [8, 9]This kind of system has the potential to work for a long time since cell sensors can conserve a long-term self-renewal capacity. A family-oriented online detector for monitoring bacteria in water is still rare in the market, maybe because such a device has to embody too many restrictive conditions, such as low cost, easy to use, foolproof operation, no danger, no secondary contamination etc. LOC devices are promising to meet almost all conditions, except the cost. Furthermore, biological LOCs are often disposable chips, which naturally reject expensive and time-consuming fabrication methods. For LOC devices, a faster and easier fabrication is the key factor to commercialization. It is a common challenge in the application of microfluidic techniques. Fortunately, a long-term working LOC device has small challenges in the aspect of cost, since it will be used for a long period. Green design is another way to reduce the total cost of products, which is not only to reduce the emissions of harmful substances and the consumption of materials, energy etc., but also to make the separated recyclable components. Especially for home users, the cost and safety of household products is more attractive than the precision of devices.

In this study, a multipurpose cell-based LOC system for monitoring bacteria in water was designed and fabricated with green design concept (Fig. 2). The chip previously served as a cell-based LOC for monitoring toxicants in water, which *Vibrio Fischeri* was used as the living sensor.[10] Here, bacterial pathogens, the targets themselves replaced *Vibrio Fischeri* as sensors in the microfluidic system, which components were also redesigned. Once there is a living bacterium in the water sample, it can be encapsulated in the droplet of culture media and then reproduce itself inside the chip. Its proliferation will change the chemical composition of the surrounded solution, for example, some acid production could change the pH value of culture media. Owing to some special indicative chemicals in the commercial culture media,

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T. Dong (IEEE member: 90889664) is with the department of Micro and Nano Systems Technology (IMST, TekMar), Vestfold University College, Norway. (PhD T. Dong is the corresponding author, e-mail: Tao.Dong@hive.no; phone: +47 3303 7731 fax: +47 3303 11 03).

X.Y. Zhao is with the department of Micro and Nano Systems Technology (IMST, TekMar), Vestfold University College, Norway. (E-mail: Xinyan.Zhao@hive.no)

the activities of living microorganisms can change the color of solution, which exposes the trace of bacterium.

II. Material and Methods

A. Chip design and fabrication

The chip is composed of three domains, including two counter-flow micromixers, a T-junction droplet generator and six TD-Cs (Fig. 3, Inset A). Only one micromixer and one TD-C were required here (Fig. 3, Inset B). In consideration of the cost of microfabrication, the previous chip is not updated at present. The counter-flow micromixer inherits the design of micro-concentrator to force two fluids to encounter in the opposite directions for efficient mixing, where the counter-flow fluid goes through the channels at the bottom layer and then penetrate into the other fluid through the trilobite-shaped units. [11, 12] Two counter-flow micromixers can be used to mingle three different reagents. But in this study, only micromixer 2 was employed to mix water sample and the reagent solution. The final mixture is encapsulated in droplets through the T-junction structure and driven inside a TD-C when the relative outlet is open. Six TD-Cs with the same depth (100μm) but different width (100~200μm) are incorporated in the proposed prototype chip to investigate the stability of the droplet flow, which length of TD-Cs ranges from 0.83m to 1.18m. [13, 14] Except the glass cover, the chip was fabricated on 4-inch silicon wafers with silicon bulk micromachining.[14, 15] The silicon layers of chip were bonded by Si–Au–Si thermal bonding process.[16] Subsequently, silicon-glass anodic bonding was conducted to connect the silicon chip to a Pyrex 7740 glass on the top.[17]

Due to the need of home users, the detection module was simplified into an observation chamber, from which the users can evaluate the detection result by naked eyes. Although such a method is not able to quantitatively measure the exact number of pathogens in water, it can give a clear message to warn home users of dangers in drinking water. Further activities on these issues could be taken by some specialized companies. The principle of observation chamber is demonstrated in Fig 4. The observation chamber was fabricated of polymethylmethacrylate (PMMA) by KT-40 laser engraving machine (Ketai Laser Tech.®, Liaocheng city, China), which core structure is a thin channel of 35mm long and 2 mm in diameter. The chamber was coated by the shade layer except two ends of the thin channel. The users can directly check the color of droplets from one end, while the light source is arranged at the other end.

B. Sampling module and other accessories

The non-clogging micro-concentrator invented by our group was employed as the continuous sampling module for this online monitor, which characteristics were reported before.[11, 12] The sampling module can be connected with the drinking water pipeline and continually work to prevent possible large particles in water from blocking the channels inside LOC devices. To provide sufficient oxygen supply for bacteria in droplets, the air flow was adjusted through a piezo valve (EasyTech® Auto. Tech. Co. Ltd., Nanjing city, China) and digital syringe pumps (Fusion 200, Chemyx®, Stafford, TX; Genie™ Plus, Kent Scientific®, Torrington, CT). The simulation of periodic air flow in the control of droplet flow was performed in COMSOL® and verified in the test.

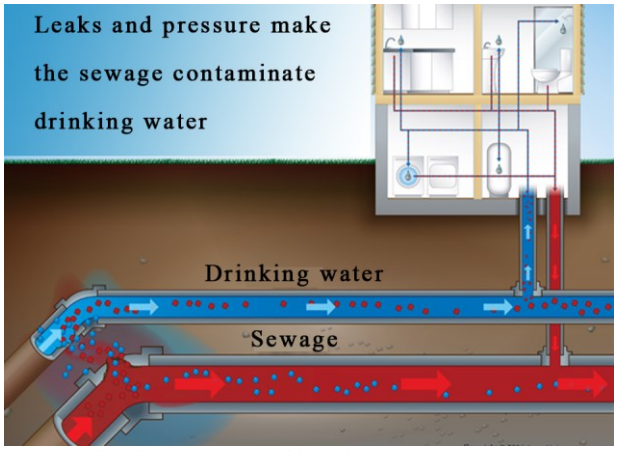

Figure 1 The diagram of possible pollution in the drinking water supply. [1]

C. Microbiological tests on the LOC

Water sample, detection reagents and aseptic air flow were pumped into the microfluidic chip. The experimental system was based on a microbiological analytical Kit (Total viable count®, Royal Biotech™ GmbH, Germany). The tests depend on the color change formed in the culture medium if living microorganisms exist. Sterilized water was used as the negative control, while the testing samples, 100 cell/mL *E. coli* cell solutions were injected by digital syringe pumps. The volumetric flow rates of solutions were initially set at 5μL/hour, and the rate of air flow was about 25μL/hour, which pulse frequency was adjusted with requirement of experiments. Based on the color of droplets observed from the observation chamber, qualitative results from 15 volunteers were collected and analyzed. Waste liquid dripped on quicklime powders in the waste reservoir.

D. Microbiological tests of living bacteria in waste

After waste liquid absorbed by quicklime powders, the mixture was neutralized by acetate buffer (pH 3.5) first. After that the liquid mixture was sampled to test living bacteria by the Total viable count® kit.

III. Results and Discussion

The simulation results indicated that the flow rates of the air and liquid should have the same order of magnitude, otherwise the stabilization of droplet flows could not be guaranteed, which phenomenon has been observed in the tests. However, the detection of aerobic microorganisms requires abundant oxygen supply. Thus the air flow rate has to be increased many times over the liquid flow. The piezo valve was introduced to meet both requirements. Relative high-speed air flow was turned into a pulse one.(Fig. 5) In the resting period of air flow, a new droplet could be leisurely generated at the T-junction structure, while in its active period, high-speed air flow will push the new droplet forward and generate a large volume of air phase behind the aqueous

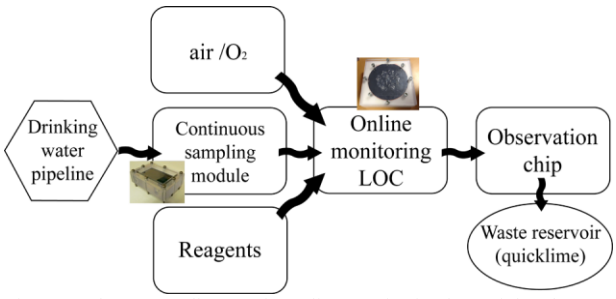

Figure 2 The system diagram for online monitoring bacterial pathogens in drinking water. The system is mainly composed of three microfluidic modules, which will be integrated together in further development. The first one is the continuous sampling module (see the photo [11, 12]). It comes from a developed micro-concentrator that can be normally open to the drinking water pipeline and allow water sample to flow through. Only the soluble molecules and particles smaller than 12μm are able to penetrate into the filtrate. The 2nd module is the chip for online monitoring, which will mix the sample and reagents under aerobic conditions. The 3rd one is an observation chip. Finally, waste liquid was absorbed by quicklime powders.

phase, which solves the problem of oxygen supply. It is interesting that the pulse frequency of the piezo valve seems to have an upper limit; otherwise it will make the resting period be shorter than the intrinsic time of droplet forming. In that case, the droplet flow turns into an irregular mixture, which could disturb the final observation.

The on-chip biological tests have similar results as the description of the Total viable count® kit (Royal Biotech™ GmbH, Germany). The sterilized water sample generated blue droplets in the observation chamber, whereas the *E. coli* cell samples resulted in yellow droplets, in line with expectations. The bacterial assay using waste quicklime powders revealed negative results. It is demonstrated that the liquid waste absorbed by quicklime powders has been sterilized. Moreover, we adjusted the velocity of droplet flows to keep droplets inside the chip within a series of period, which is defined as Retention Period (RP). The total volume of LOC system can be estimated and measured. It contains the volume of microchannels inside TD-C, observation chamber (about 150μL), and the joints for connection. Preliminary results of observation on *E. coli* cell samples were proposed in Fig 6, which indicates that the retention period of 18 hours might be suitable for observation to naked eyes. If a low-cost sensitive photodetector were available, the retention period could be reduced greatly. Obviously, the sensitivity of the detection system can be adjusted by the incubation time, or the flow rates of the droplet flow. The lower flow rates will generate the higher sensitivity of detection and vice versa. However, the contrary is the case for the response time of the LOC. Based on the current situation, it is a contradiction to improve its sensitivity and shorten its response time. With the help of some quantitative instruments, the change of color in the solution can be used to calculate the concentration of pathogens. It's possible to update the detection module into a quantitative one in the future, if the home users demand. Another important factor in the manual of the kit is the temperature. Regularly, a temperature controlling module should be developed within the system, but it is still reasonable to save it since the device is designed to be used inside the house. The users can choose the module according to the circumstances.

Given the operating parameter herein, the annual consumption for the continuous-working LOC device is calculated, which includes 44 mL of detection reagents, 250 mL of drinking water sample, 220 mL aseptic air, and 150g quicklime etc. The quicklime is able to react violently with water, hence kill the pathogens inside droplets, which product is innocuous slaked lime. Therefore, there is no secondary pollution using this device for a long time. Once the online monitoring system was contaminated by bacterial waterborne pathogens, the microfluidic chip and observation chamber should be replaced, whereas the other accessories continue to

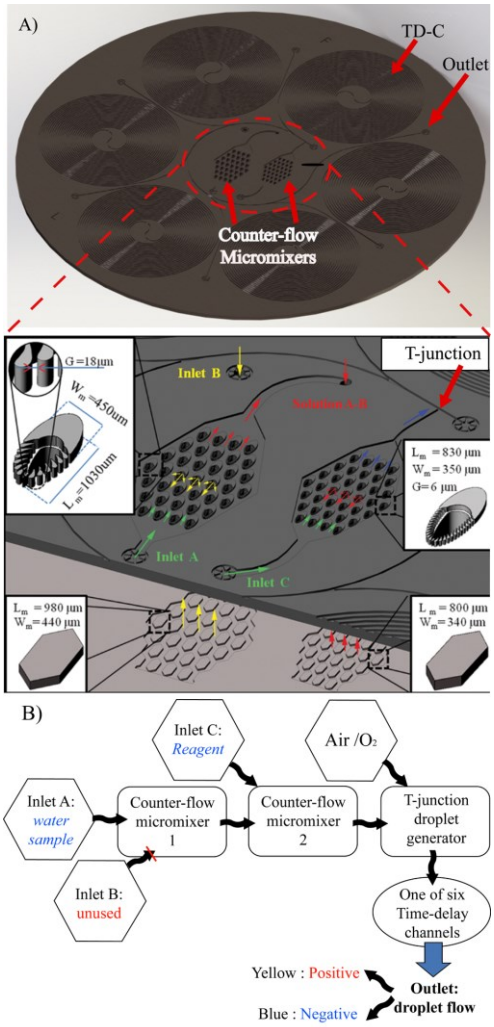

Figure 3 The structure of online-monitoring chip. (a) The main layer of the chip, including two counter-flow micromixers, T-junction droplet generator, six time-delay channels (TD-Cs); the schematic structure of the counter-flow micromixer is also presented. The counter-flow unit is characterized by an elliptical cylinder profile with an inlet port in the center which is comprised of a solid rounded front-end section and a barrier section. The second solution will go beneath the main layer first, and then pass through the inlet port inside the counter-flow unit, at last, rush out from the gap between tiny pillars. [10] (b) Only the second counter-flow micromixer was used here. The air flow and mixed solution form a gas-liquid two-phase flow at the T-junction structure. The color of droplets is the signal of detection.

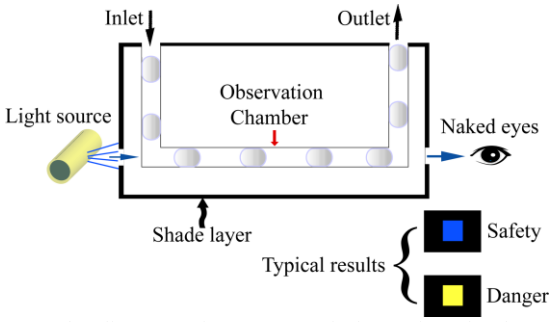

Figure 4 The diagrammatic structure of observation chamber. The experimental system was installed in a dark cassette, which mainly composed of a small LED white-light bulb and an observation chip coated by tinfoil. The chip is connected to the online-monitoring LOC and a waste reservoir outside the cassette. From the sole observation hole of that cassette, observers could check the color of light that passed through droplets in the observation chamber. Compared with standard colorful pictures in the manual of Total viable count® kit, judgments could be made.

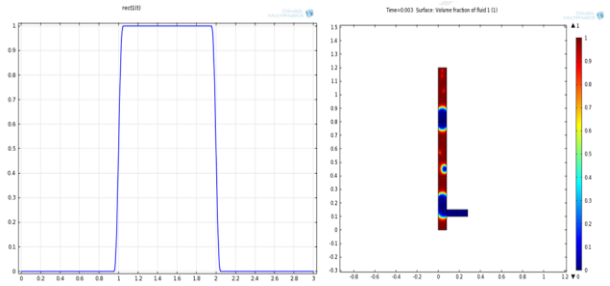

Figure 5 Typical simulation results of water-droplet formation in the 2D T-junction model under the condition of a periodic air flow.

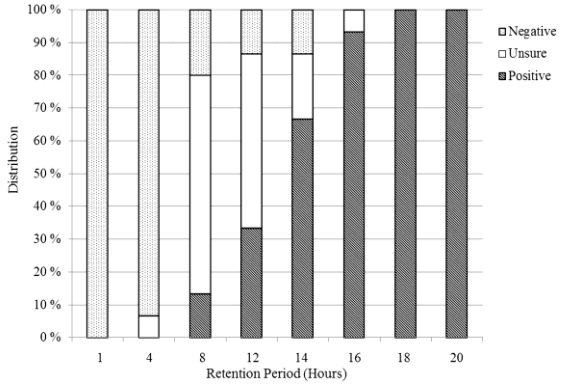

Figure 6 Judgment distributions of 15 volunteers on the observation of system validation. 100 cell/mL E. coli cell solutions were employed as polluted water sample. Varied results with eight different RPs were obtained.

work. All these features are in line with the concept of green design. The main challenge of the system is the processing costs of the involved microfluidic chips. In further study, the chip will be fabricated by economic methods and materials, such as injection molding on PMMA. The cell-based LOC is a compatible platform for multifarious living-cell-based diagnostic kits. The strategies of compatibility make this LOC device much easier to be commercialized, and maybe much easier to be accepted by both the related suppliers and the consumers.

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