An Integrated Passive-Flow Microfluidic Biosensor With Organic Photodiodes For Ultra-Sensitive Pathogen Detection In Water

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***Abstract*— This work reports on integrated passive-flow optical microfluidic devices to detect waterborne pathogens in the field. Ring-shaped organic photodiodes were integrated to a capillary-induced flow microfluidic channel for monitoring chemiluminescent sandwich immunoassays enhanced by gold nanoparticles. The integrated device yielded a resolution of 4×10^4 cells/mL for the detection of *Legionella pneumophila*, which represented a 25-fold improvement over chemiluminescence detection devices employing no gold-nanoparticle enhancement. This work demonstrates the feasibility of a low-cost but highly sensitive lab-on-a-chip device amenable for point-of-use applications.**

I. Introduction

Outbreaks of waterborne infectious diseases still occur. Better prevention of outbreaks would require the realization of pathogen detection in the field [1, 2]. However, the development of detection technology for in-the-field use encounters a specific type of challenge not commonly faced in laboratory analysis: the technology should be simple, inexpensive and it should be highly sensitive to the target.

Progress in microfluidics has enabled the development of lab-on-a-chip devices that offer high detection sensitivity to a range of waterborne pathogens [3]. However, the portability of these devices is significantly compromised by the use of external pumps and tubing. Furthermore, the high-cost of micro-pumps and high-voltage operation of electroosmotic pumping prevent their successful application to disposable devices. Passive microfluidic systems employing capillary flow may be an ideal choice for point-of-use applications, because of their inherent simplicity and user-friendly operation [4]. In addition, capillary-flow based microfluidic sensors can easily be realized using low-cost thermoplastic or polydimethylsiloxane (PDMS) substrates [5].

Detection of pathogens in the laboratory is often conducted using optical technology based on absorbance, fluorescence or chemiluminescence. Although these optical methods have been employed in lab-on-a-chips, detection is conventionally performed by off-chip photomultiplier tubes, charge-coupled devices and microscope optics or relatively expensive on-chip optoelectronics [6]. The successful adoption of optical microfluidic devices demands for the realization of reasonably priced, sensitive, and compact systems. In response to this demand, we have demonstrated the feasibility of using organic photodiodes (OPDs), originally intended for solar panel manufacturing, as optical sensors in integrated microfluidic systems [7]. Further, the OPD technology was also shown for optical microfluidic detection of chemiluminescence assays [8]. The simplicity and low-cost fabrication of OPDs would pave the way for the realization of truly in-the-field analysis systems.

Immunoassay technology is generally exploited in microfluidic pathogen detection [3], and its combination to chemiluminescent optical methods may be ideal to portable lab-on-a-chip devices. However, well-established microfluidic immunoassays are sometimes not sufficiently sensitive to applications requiring detection of few pathogen organisms in the water samples [1]. This limitation may be overcome by the employment of gold nanoparticles (AuNPs) to improve the sensitivity of chemiluminescent immunoassays [9]. Besides the use of AuNPs in sensitive fluorescence spectroscopy or surface plasmon resonance biosensors, these nanoparticles can enhance the luminescent reactions of horseradish peroxidase (HRP) and luminol in the presence of stable peroxide, typically used in chemiluminescent biosensing.

This work presents an innovative point-of-care device concept incorporating passive microfluidics and OPD detection. For concept demonstration, a trial capillary-driven flow microfluidic device was developed with integrated low-density array of highly sensitive photodetectors. In this study, *Legionella pneumophila* was targeted by chemiluminescent sandwich-type immunoassays enhanced by AuNPs. *Legionella* is an important waterborne pathogen whose detection demands for analysis of very low organism concentrations in water. The performance of the integrated microfluidic biosensor was characterized by a finite element method simulation and a series of assay experiments. Herein, we demonstrate that a simple but highly sensitive in-the-field pathogen test device can be implemented with standard microfluidic architectures and with low-cost optical sensing.

II. Integrated Device Concept Design

The design of trial passive opto-microfluidic device is depicted in Fig. 1. The microfluidic chip is composed of a hybrid of 1-mm thick glass and 750 μm thick PDMS. The PDMS layer incorporates a microchannel network with four capture zones. In each capture zone the microchannel is

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covered by AuNPs immobilized onto the 1-mm glass slide. The channel network was originally designed to contain only one inlet (~1-mm wide) and one outlet (~1-mm wide), thus simplifying reagent and sample handling. An indium tin oxide (ITO) coated glass (1-mm thick) containing four ring-shaped OPDs is finally attached to the transparent PDMS beneath the hybrid microfluidic chip. Each 4.5-mm diameter OPD is aligned with the corresponding capture zone. Blends of poly [N-9`-heptadecanyl-2, 7-carbazole-alt-5,5 -(4`,7`-di-2-thienyl-2`,1`, 3`-benzothiadiazole)] (PCDTBT) and [6,6]-phenyl C_{71}-butyric acid methyl ester ($PC_{70}BM$) were used for the active layer of the OPDs. Diodes of PCDTBT:$PC_{70}BM$ may be optimal candidates for in-the-field optical detection due to their high responsivity and stability under ambient conditions of temperature, oxygen and humidity [2].

III. Capillary-flow Microfluidic Simulation

PDMS normally forms hydrophobic surfaces with water, where no capillary flow can be developed. However, the use of oxygen plasma treatment processes [5] confers hydrophilicity to the PDMS channels by the significant reduction of the contact angle from 115˚ to 60˚ [10]. In this study a finite element method simulation (performed in COMSOL Multiphysics) was used to analyze the marching of the capillary meniscus within the plasma-treated PDMS microchannel. The method simulation employed a laminar two-phase flow, and the variable Volume Fraction (V_f) was used to determine the position of the meniscus within the channel. V_f was defined as

$$V_f = (1+\varphi)/2, \quad (1)$$

where φ is a dimensionless phase field variable that describes the water-air interface as a transition region stepping between -1 (gas phase) to 1 (liquid phase).

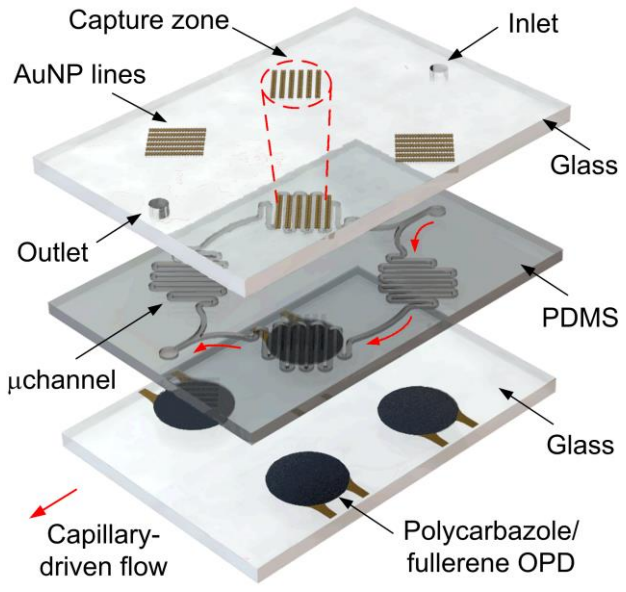

Figure 1. Layout of the integrated passive-flow optical microfluidic device comprising the glass cover with immobilized AuNPs, PDMS capillary-flow microchannel network and array of ring-shaped OPDs prepared on ITO coated glass.

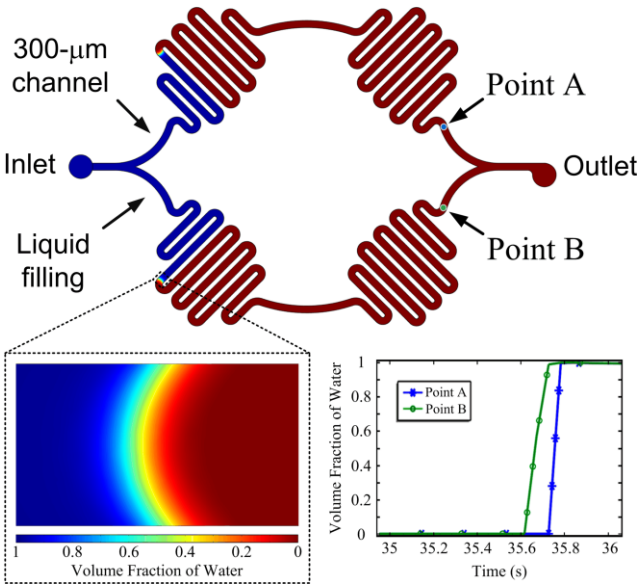

Figure 2. Simulated capillary-driven flow within the PDMS microchannel network using a finite element method. The inset shows the capillary meniscus in detail. The 2D graph represents the temporal variation of the Volume Fraction of filling water, calculated using eq. (1).

The results for V_f are shown in Fig. 2. The two branches of the 300-μm wide microchannel were filled almost simultaneously. There was a filling time difference of only 0.1 s as suggested by the temporal variation of V_f depicted in the 2D graph. Furthermore, there was no fluid receding during the filling process, thus demonstrating the continuous marching of the meniscus along the channel.

IV. Micro/Nano-fabrication

The PDMS microfluidic chip was fabricated by standard PDMS replica molding with a SU-8 master. The surface of PDMS was pre-treated with oxygen plasma before the chip was bonded to the plasma-treated cover glass. Immobilization of AuNPs onto the cover glass followed modification of the glass surface with amine groups (using 3-aminopropyltriethoxysilane) and modification of the AuNP surface with carboxyl groups (using dithiodipropionic acid). Further, terminal carboxyl groups on the AuNPs were covalently coupled with primary amines on the capture antibody. This procedure is summarized in Fig. 3(a).

The ring-shaped OPDs were prepared on a ITO coated glass by a two-step spin coating of films of poly(3,4-ethylenedioxythiophene):polystyrene sulfonate) (PEDOT:PSS) and PCDTBT:$PC_{70}BM$, followed by thermal evaporation of LiF/Al. OPD fabrication has followed a protocol previously described in [7].

V. Assay Procedures & Measurements

Sandwich-type chemiluminescent immunoassays were developed on the AuNP surface modified with antibody, as shown in Fig. 3(b). First, the AuNP-antibody complex captured the pathogen which was previously spiked in deionized water (DI) samples. The pathogen sample was added into the integrated device via simple pipetting and

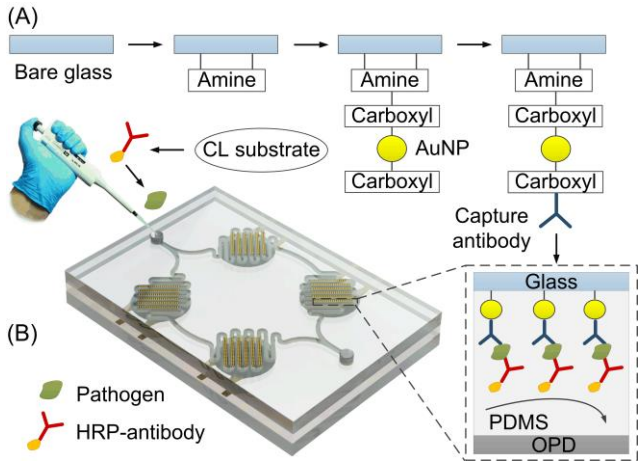

Figure 3. (a) Treatment procedure of the glass surface for the immobilization of AuNPs and capture antibody. (b) Summarized testing setup scheme for the development of the AuNP-enhanced chemiluminescent immunoassay.

allowed to enter the capture zones via capillarity. After sample incubation and one-step washing with DI water, the immune complex interacted with HRP-conjugated detection antibody for one hour approximately. Chemiluminescence was generated by adding the CL substrate formed by 1:1 mixture of luminol/enhancer and stable peroxide solution. The intensity of chemiluminescence was measured immediately with a dual-channel source measure unit (SMU, Keithley series 2600) which monitored the response of the integrated OPDs. Simultaneous monitoring of four OPDs was conducted by linking two dual-channel SMUs in parallel using a master/slave configuration. The master unit was connected to a PC via GPIB interface for data analysis.

VI. Results & Discussion

A. Assay characterization

First tests of AuNP-enhanced assays were conducted to optimize the exposure time of the sandwich immune complex to the CL substrate. The assays targeted 3×10^7 cells/mL of *L. pneumophila* and the measurements of photocurrent from one OPD were performed applying different reverse bias voltages to the OPD. The results in Fig. 4 showed that a 3 minute incubation time of the CL substrate within the passive opto-microfluidic device may offer the optimal signal-to-noise ratio for the AuNP-chemiluminescence detection. Furthermore, the results demonstrated that the signal-to-noise ratio was degraded by applying the reverse bias. This may be explained by the enhancement of the inherent shot noise of the OPD.

The incubation time of *L. pneumophila* with the AuNP-antibody complex was also varied in further assay tests. It was found that incubation times above 30 minutes did not lead to significant improvement of the signal-to-noise ratio. Besides, the point-of-use detection of pathogens often requires minimization of the assay duration. Thus, pathogen incubations of 30 minutes may be optimal for the AuNP-chemiluminescent sandwich immunoassays.

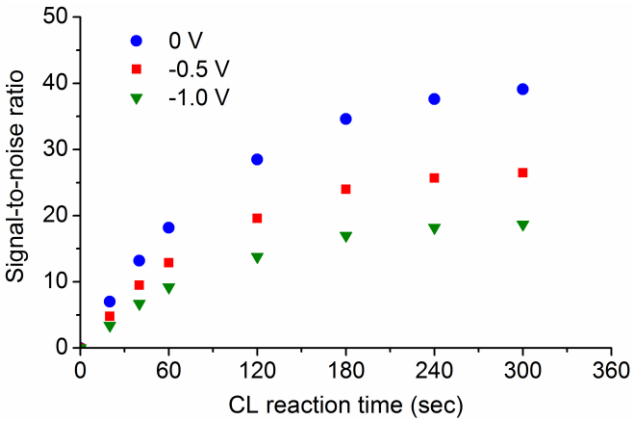

Figure 4. Characterization of the AuNP-enhanced CL immunoassay applying different bias voltage to the OPD using a Keithley 2600 SMU. The signal-to-noise ratio was determined for the detection of 3×10^7 cells/mL *Legionella* at various exposure times to the CL substrate.

B. Legionella detection

Various concentrations of *L. pneumophila* in DI water ranging from 1×10^5 to 5×10^7 cells/mL were tested with the passive opto-microfluidic device. For comparison additional tests were conducted employing no AuNPs immobilized on the cover glass. For all cases *Legionella* was targeted in one capture zone and photocurrent response was monitored from one OPD. The dose-response curve for AuNP-enhanced chemiluminescent immunoassays is plotted in Fig. 5. From the curve the detection limit was estimated to be 4×10^4 cells/mL. This value was 25-fold lower than that obtained with chemiluminescent immunoassays using no AuNPs. The estimated limit-of-detection was the concentration corresponding to a signal-to-noise ratio of 3. Besides the enhancing effect on the chemiluminescence, the AuNPs do not compromise the simplicity of the immunoassay procedure.

C. Optical detection reproducibility

Further AuNP-chemiluminescent immunoassays were conducted to analyze the reproducibility of the OPD

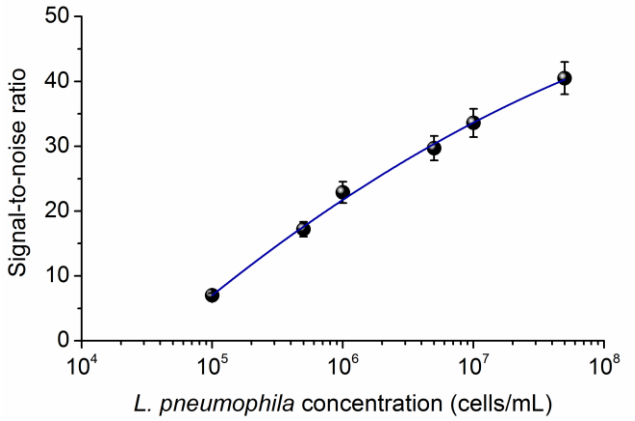

Figure 5. Calibration curve for the detetion of *Legionella* in water using the integrated passive-flow optical microfluidic device and employing the AuNP-enhanced chemiluminescent immunoassay. Error bars represent standard deviation for triplicate assays.

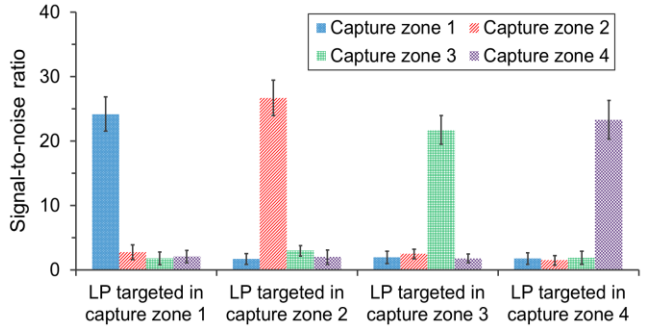

Figure 6. Histogram of results for the detection of 1×10^6 cells/mL *Legionella* (LP) targeted in different capture zones. The tests were conducted by measuring the photocurrent of the four OPDs in parallel. Error bars represent standard deviation for triplicate detection tests.

detection. Four cover glass slides were individually prepared with the AuNP lines arranged in different capture zones. Thus, for each test, one capture zone was used to target the pathogen sample (1×10^6 cells/mL *L. pneumophila*) while the other three zones were left blank. The responses of the four OPDs were measured in parallel for all tests. Figure 6 indicated that no significant variation in the detection of *Legionella* was encountered between the different capture zones. The variation in signal-to-noise ratio did not exceed 10%. Moreover, the signal-to-noise ratio was below 3 (detection limit) for all blanks.

For all experiments the microchannel was entirely filled by capillarity-induced flow in less than 60 seconds. This total filling time guarantees that the chemiluminescence achieves its maximum after complete filling of all capture zones. Immobilizing different antibody on the surface of AuNPs may allow the detection of different pathogens. To demonstrate the multiplexed detection [11], future work will address the parallel detection of four pathogens using the four capture zones of the integrated device.

VII. Conclusion & Outlook

A simple ultrasensitive optical microfluidic device for point-of-use pathogen detection is described in this study. Capillary-driven flow, OPD detection and AuNP-enhanced chemiluminescent immunoassays were incorporated into a single lab-on-a-chip for the first time. Finite element method simulation was used to demonstrate the capillary flow within a plasma-treated PDMS microchannel. The AuNP chemiluminescent immunoassay with OPD detection showed a detection limit of 4×10^4 cells/mL for the targeted *L. pneumophila*. Further parallel photocurrent measurements demonstrated the reproducibility of *Legionella* detection in four different capture zones. The integrated device proposed here holds promise for simultaneous detection of various pathogens in one sample. However, further work may consider the incorporation of sample concentration mechanisms, such as immunomagnetic separation [12], as the detection of pathogens in water commonly involves analysis of complex samples.

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