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# Detection of Stress Hormones by a Microfluidic-integrated Polycarbazole/Fullerene Photodetector*

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***Abstract*— A novel photodetector integrated microfluidic system for chemiluminescence (CL) detection is reported. The system incorporates a polycarbazole/fullerene photodiode whose optical characteristics (i.e. dark current, external quantum efficiency and photosensitivity) are described here. Using a CL immunoassay for detecting the stress hormone cortisol, the integrated photodetector achieved a detection sensitivity of 1.775 pA × nM$^{-1}$ and a detection limit of less than 0.28 nM. The device would be a powerful low-cost alternative to silicon photodiode and photomultiplier tube for bioanalytical assays, with potentially wide-ranging applications within point-of-care diagnostics.**

## I. Introduction

Microfluidics has demonstrated its value to develop compact and miniaturized devices for bioanalytical applications. Owing to its merits of reduced analysis times, low-cost fabrication, reduced consumption of reagents and enhanced ability to perform multiplexed analyte detection, microfluidic devices are ideal for on-the-spot or point-of-care (POC) measurements. However, successful implementation of microfluidic POC diagnostics has yet to overcome challenges in integrating optical detection systems into a single microchip [1]. Low-cost but high-sensitivity detection methods are needed as only small quantities of bio-target are usually analysed in a microfluidic chip.

Chemiluminescence (CL) is one of the preferred techniques to detect low bio-target concentrations in microfluidic devices. CL is particularly interesting for POC applications as the CL assay acts as an internal light-source, lowering instrumental requirements and thereby reducing the complexity of the detection systems. Furthermore, the utilization of CL strategies avoids the problematic background interference that is commonly encountered in fluorescence-based assays. The photomultiplier tube (PMT) and silicon photodiode are frequently used for monitoring chemiluminescent reactions [1, 2]. While PMT detectors show high photosensitivity for typical CL emission wavelengths, their large size and high voltage requirements prevents their use as integrated sensors for microfluidic systems. Integrated silicon photodiode may be more suitable in microfluidic environments; however its fabrication is relatively complex and it is not compatible with low-cost microfluidic substrates. Alternatively, organic/polymer photodiodes can be easily realized onto glass or flexible plastic substrates, thus offering new prospects in the area of microfluidic POC diagnostics [2].

The work reported herein demonstrates a thin-film organic photodetector (OPD) based on blend heterojunction 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 ($PC_{70}BM$) as an integrated optical sensor for on-chip microfluidic CL detection. PCDTBT is a poly(2,7-carbazole) derivative with enhanced optoelectronic characteristics, especially higher short-circuit photocurrent and lower dark current when compared to poly(3-hexylthiophene) (P3HT) based OPDs [3]. These characteristics would make the PCDTBT photovoltaic device promising for applications demanding high sensitivity light detection. This work also demonstrates the integration of the PCDTBT:$PC_{70}BM$ optical detector to a poly(dimethylsiloxane) (PDMS) substrate in a microfluidic chip. The remarkable biocompatibility and optical transparency over the entire visible range for the PDMS material is widely acknowledged [4, 5]. The combination of the polycarbazole photodetector with PDMS microfluidic chip structures may provide a promising route towards low-cost POC disposable devices.

Here, the OPD-integrated microfluidic device was tested by application to an on-chip chemiluminescent immunoassay for detecting the cortisol hormone. Cortisol, a glucocorticoid, is the primary stress hormone and is involved in weight and infection control, skin and bone health, and heart function. Low concentrations of cortisol may lead to Addison's disease, often associated with symptoms of fatigue, abdominal pain, muscle loss or even hypotension and coma in severe cases. The developed method was compared with conventional PMT based detection.

## II. System design & Experiments

### A. OPD and Microfluidic System

The schematic layout of the integrated microfluidic device is illustrated in Fig. 1. Fabrication of the 800 μm-thick PDMS fluid flow layer, with the microchannels and CL reaction chamber (~30 μl volume), was realized by PDMS casting with an SU-8 mold. After structuring, the PDMS channel layer was attached to a gold coated Pyrex 7740 glass wafer [6] via carboxyl-amine coupling chemistry [7].

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Channels and chamber were sealed by placing a 1 mm thick PDMS slab and a 1 mm thick glass slide in conformal contact with the structured PDMS layer. The sealing of this hybrid microchip was achieved by exposing the PDMS layers to oxygen plasma prior to chip assembly. Two inlet ports, an air-bleed port and an outlet port were added to the cover glass slide, which comprised capillary reservoirs that were connected to the channel ends through drilled access holes.

The cover glass slide was previously coated by a 100 nm-thick indium tin oxide (ITO) layer for preparing the polycarbazole photodetector. The OPD whose structure is depicted in Fig. 2 was localized in the vicinity of the CL reaction chamber. For OPD fabrication, the ITO-coated glass substrate that had been pre-treated with UV ozone were deposited with a 40 nm-thick layer of the poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS) by spin coating. Then an 120 nm-thick active layer was spin cast from a chloroform solution of PCDTBT:$PC_{70}BM$ (1:4) on top of the PEDOT:PSS layer. The film was dried at 60°C for 1 h in a nitrogen-glove box. Subsequently, the LiF/Al cathode was thermally evaporated with a thickness of 100 nm using a shadow mask. Encapsulation of the OPD was conducted using a pressure-sensitive barrier foil. The external quantum efficiency (EQE) of the PCDTBT:$PC_{70}BM$ device was determined using a TLS1509-X150 monochromatic light source and a Keithley 236 Source Measure Unit (SMU) for measuring the photocurrent. Light intensity was corrected via a calibrated Si photodiode. The current-voltage characteristics at varying levels of illumination were measured using the 236 SMU and a 405-nm laser diode.

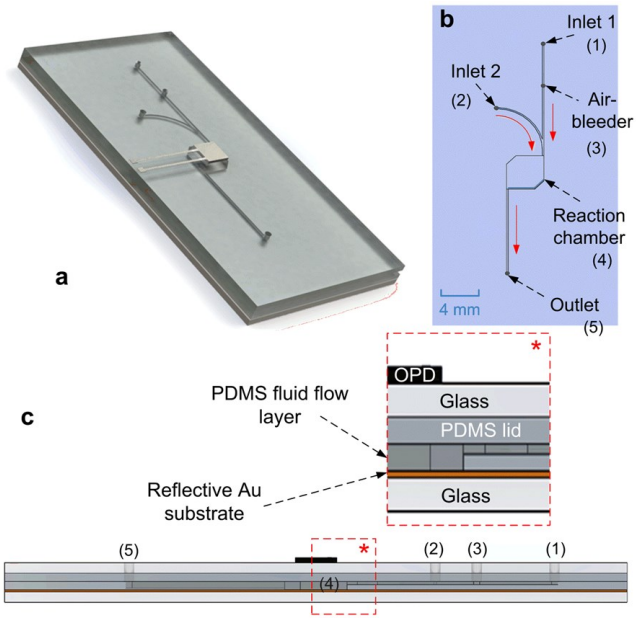


Figure 1. (a) A 3D schematic ilustrating the OPD-integrated microfluidic chip with the top view and side view shown in (b) and (c), respectively. The hybrid microchip mainly comprised two inlets, a 800 µm deep reaction chamber, an an outlet. The channels connecting the inlets and chamber are 250 µm wide, 300 µm deep; whilst the channel connecting the outlet and chamber is 250 µm wide and 650 µm deep. The active area of the polycarbazole photodetector is 0.16 $cm^2$.

## B. *Chemiluminescence Detection Protocol*

The chemiluminescent immunoassay was developed on top of the Au coated glass substrate in the reaction chamber, as illustrated in Fig. 2. After the microchannels and reaction chamber were washed with phosphate buffered saline (PBS), 20 µl of 0.1 µg/ml anti-human cortisol antibody was added into the microchip through the Inlet 1. The antibody solution was incubated for 2 h to allow the immobilization of the probe antibody on the chamber surface. This surface was then blocked using Thermo Scientific StartingBlock™ T20 Blocking Buffer, followed by rinsing with PBS. A 100 µl aliquot of several concentrations of a human cortisol solution was injected into the microchip through the Inlet 1, and was incubated within the reaction chamber for 15 min. After PBS washing, 20 µl of 0.02 µg/ml horseradish peroxidase (HRP) labelled secondary antibody was introduced into the chip through the Inlet 2, to avoid contamination [8]. Thereafter, SuperSignal® chemiluminescent working solution was transferred to the chip by the Inlet 1, and the light generated from the reaction of HRP with SuperSignal® substrate was detected by the OPD. The chemiluminescent signal was thus recorded as a photocurrent signal.

To realize the immunoassay protocol, a flow system was designed as shown in Fig. 2. In this setup, the air-bleed and outlet ports are connected to a vacuum flask and then to a vacuum pump. The pressure inside the flask determines the fluid flow within the microchip. Here, the air-bleeder is used to remove small gas bubbles that may scatter the recorded chemiluminescent signal. A valve system comprising three OMNIFIT© 8-way valves was additionally set to control the delivery of reagents and sample into the microfluidic chip.

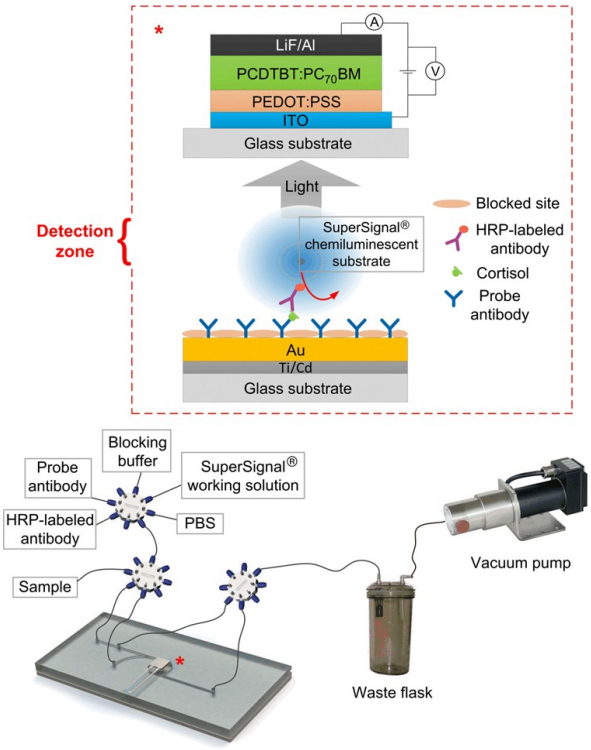


Figure 2. Structure of the PCDTBT:$PC_{70}BM$ device and experimental setup for the chemiluminescent immunoassay.

## III. Results & Discussion

### A. Optoelectronic Characteristics

Prior to application of the integrated photodetectors to on-chip chemiluminescence detection the optical properties of the PCDTBT:$PC_{70}BM$ blend heterojunction photodiodes were characterized.

The measured EQE over the wavelengths between 300 and 800 nm is shown in Fig. 3. EQE values of approximately 60% were achieved by the present OPD for wavelengths ranging from 400 to 600 nm, and peak maximum of ~70% at around 410 nm was observed. Importantly, this closely corresponds to emission spectrum of used chemiluminescent substrate (peak maximum around 425 nm). The bias dependence of the photocurrent at three different illumination levels is depicted in Fig. 4. Typical dark current densities with no applied bias were ~$2.8 \times 10^{-9}$ mA $cm^{-2}$. This value is comparable to the dark current density for a commercially available Si photodiode (model S6430-01, Hamamatsu) measured by the same equipment (data not shown).

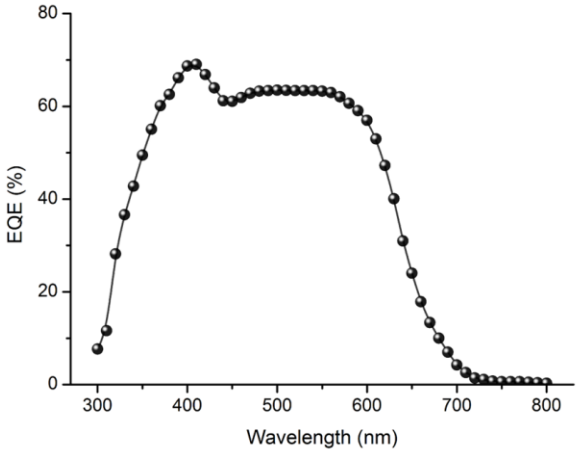


Figure 3. Spectrum of external quantum efficiency (EQE) for the PCDTBT:$PC_{70}BM$ photodetector.

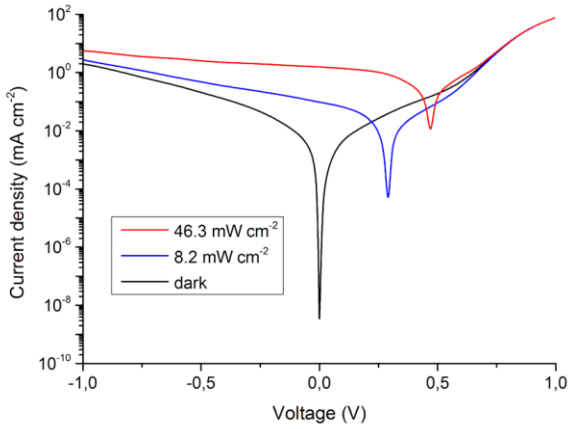


Figure 4. Current-voltage characteristics of the PCDTBT:$PC_{70}BM$ photodetector under dark conditions and two different levels of 405 nm monochromatic illumination.

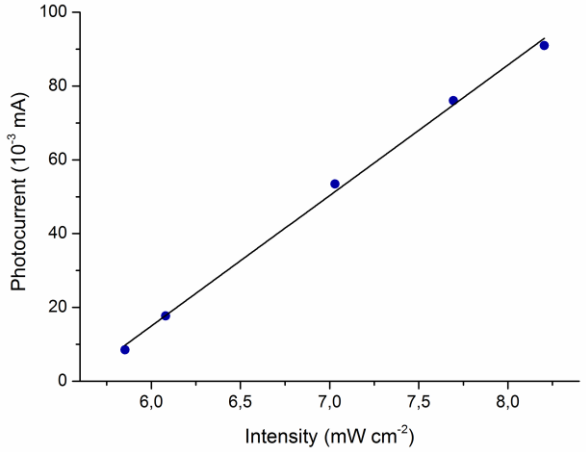


Figure 5. Relationship between short-circuit photocurrent for the PCDTBT:$PC_{70}BM$ device and the light intensity from a 405-nm diode.

To assess the photosensitivity of the PCDTBT:$PC_{70}BM$ photodetector, a 405 nm monochromatic light was used as model light for the chemiluminescence of SuperSignal® substrate. Fig. 5 demonstrates the light intensity dependence of the short-circuit photocurrent, which varies linearly over the full range investigated ($R^2$ = 0.997). From the slope of the line plotted in Fig. 5, the photosensitivity of the present OPD was calculated as 0.22 A/W for the 405 nm wavelength. This contrasts with the photoresponsitivity (0.10 A/W at 425 nm) obtained by a OPD prepared from P3HT:PCBM [9].

### B. Fluid Flow Testing

The optimization of the fluid flow protocol was conducted prior to analyte detection by the integrated lab-on-chip device. Enhanced photocurrent was achieved when the reagents and chemiluminescent substrate were added into the microchip system under a pressure inside the vacuum flask between 40 and 50 kPa, which was controlled by the valves and pump. Furthermore, no fluid leakage was observed during processing, indicating the robustness of the device.

### C. Cortisol Detection

Chemiluminescent immunoassays for the detection of human cortisol were then performed. The chemiluminescent signal due to cortisol concentrations ranging from 0.1 ng/ml to 50 ng/ml was recorded under short-circuit conditions using the 236 SMU. The background level before starting the CL reaction was ~15.4 pA, which was considerably higher than the measured dark current. This was mainly due to the presence of stray light during measurements. Fig. 6 shows the transient response of the CL signal at each of the cortisol concentrations. In each case steady-state was reached approximately 1 min following addition of the SuperSignal® substrate. Plotting the average of the photocurrent at the plateau of the signal (time period between 3 and 6 min) against seven cortisol concentrations, a linear calibration curve was obtained. The detection limit (LOD) was estimated to be <0.1 ng/ml (0.28 nM), which is below the lowest limit of cortisol in blood plasma of normal individuals (typically 80 nM).

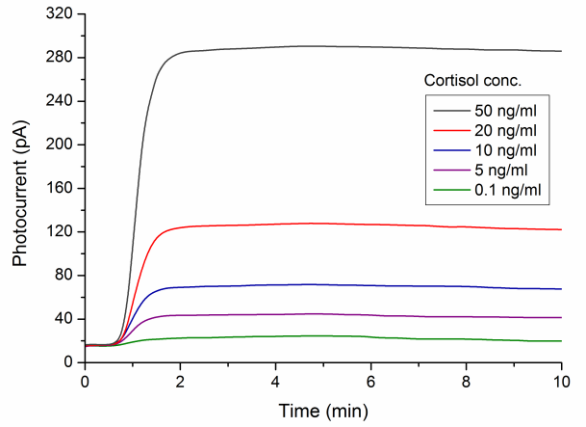


Figure 6. Transient chemiluminescent signals for the on-chip detection of human cortisol at five different concentrations.

TABLE I. SUMMARY OF THE ANALYTICAL FEATURES FOR THE CL IMMUNOASSAY USING OPD AND PMT BASED DETECTION

| **Analytical feature** | **PCDTBT:$PC_{70}BM$ photodetector** | **PMT** |
|---|---|---|
| Linearity, $R^2$ | Y=0.001775X+0.009018 $R^2$=0.997 | Y=0.001415X+0.01578 $R^2$=0.992 |
| Dynamic range (nM) | 0.28 – 249 | 0.28 – 276 |
| [a]Precision (RSD) | 0.54 | 0.39 |
| [b]Detection sensitivity ($pA \times nM^{-1}$) | 1.775 | 1.415 |

a. Determined from the average photocurrent of 1 nM cortisol, n=3

b. Determined from the slope of the calibration curve (average photocurrent vs cortisol conc.)

The performance of the PCDTBT:$PC_{70}BM$ photodetector for the detection of cortisol was compared with a commercial PMT device. The PMT (model H10723-01, Hamamatsu) was fixed above the microfluidic chip, composed of cover glass slide, PDMS (middle) and Au-coated glass substrate (bottom), with the PMT window closed to the CL reaction chamber as near as possible. Results for typical analytical features are summarized in Table 1. No significant difference in terms of LOD and dynamic range was observed between OPD and PMT. Both detection methods provided excellent detection precision (relative standard deviation (RSD) <1%) for 1 nM of cortisol. However, the polycarbazole/fullerene detector showed slightly higher detection sensitivity than PMT.

## IV. SUMMARY

In summary, a novel OPD-integrated microfluidic device was developed for highly sensitive detection of stress hormones. The photodetector, comprised of PCDTBT:$PC_{70}BM$ blend heterojunction as the active layer, showed low dark current, high photosensitivity and enhanced EQE characteristics at wavelengths corresponding to emission spectra of typical CL substrates. The excellent linearity over wide range of analyte concentrations and LOD <1 nM make the developed OPD-microfluidic device promising for monitoring cortisol levels in patients. Nevertheless, the device has to be challenged with physiologically-relevant samples, including blood and saliva, to demonstrate its clinical feasibility. The cost of the OPD-microfluidics integrated system will be much lower than silicon- or PMT-based optical biosensors, which may encourage its use in POC applications.

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