An Automated Microfluidic Paper-Based Analytical Device for Chemiluminescence Immunoassay

Jihong Sun[1,2], Sixuan Duan[1,2], Ruiqi Yong[1], Hang Yuan[1],
Sanli Liu[1,2], Kai Hoettges[2], Junhui Zhu[3], Mark Leach[1,2], Pengfei Song[1,2,*]
[1] School of Advanced Technology, Xi'an Jiaotong-Liverpool University, Suzhou, China
[2] Department of Electrical and Electronic Engineering, University of Liverpool, Liverpool, UK
[3] School of Electronic and Information Engineering, Suzhou University of Science and Technology, Suzhou, China
* Contact: pengfei.song@xjtlu.edu.cn

Xi'an Jiaotong-Liverpool University 西交利物浦大學

46TH ANNUAL International Conference of the IEEE Engineering in Medicine and Biology Society — Orlando 2024 — July 15-19, 2024

Poster No. B3P-336

ABSTRACT

This paper introduces a highly integrated microfluidic paper-based analytical device (μPAD) with a reliable and programmable rotary valve and automated injection system. By controlling the rotation of the valve, different regions on the μPAD can be connected or disconnected, allowing effective reagent transport to the test zone. To address the limitations of traditional chemiluminescent immunoassays (CLIA), which require expensive equipment and extensive manual operations, we use a smartphone to read the results and control the device. As a proof-of-concept, we detected rabbit IgG under optimized experimental conditions, achieving a limit of detection of 3.58 pM.

INTRODUCTION

Chemiluminescence (CL) on microfluidic paper-based analytical devices (μPADs) offers low limits of detection (LOD) and high reproducibility [1].

- Traditional CL measurements require expensive photodetectors (*e.g.*, photomultiplier tubes and charge-coupled devices).
- Smartphones, equipped with high-resolution cameras and powerful computing capabilities, simplify CL detection [2].
- Our method offers comparable sensitivity to chemiluminescence immunoassay (CLIA) using magnetic nanoparticles (4 pM).

METHODS

- Our device introduces pre-mixed reagents at precise intervals, including luminol, H_2O_2 and enhancers, during the detection process through a highly integrated injection system (Fig. 1).

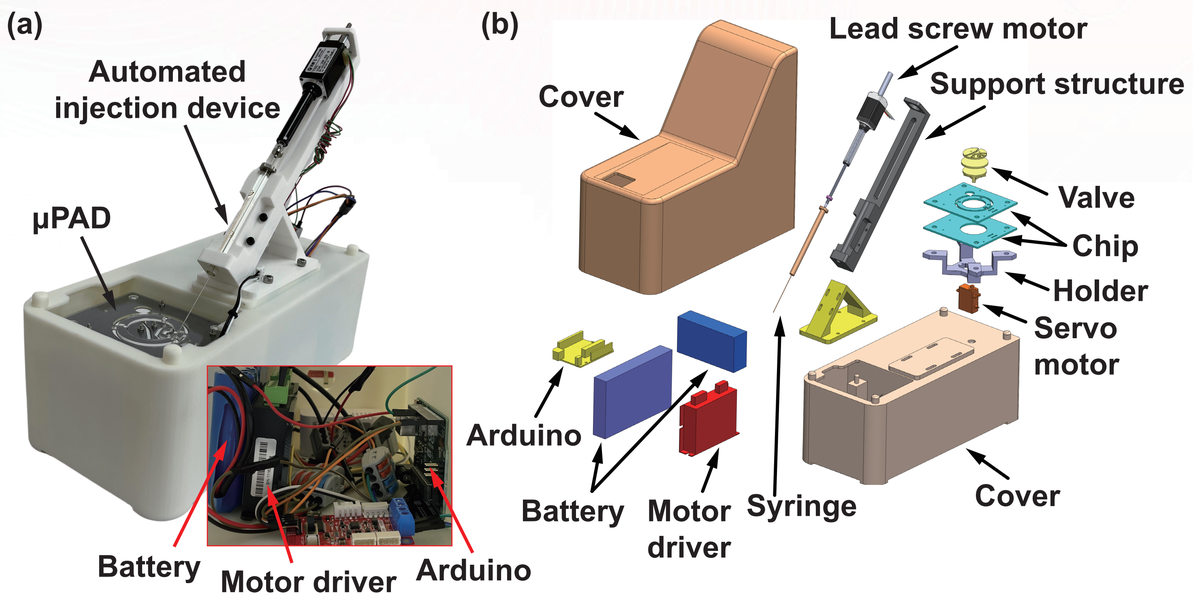

Fig. 1. (a) The photo of the developed device and its internal components. (b) Exploded diagram of the entire system.

- Captured photos of CL results using a smartphone exposed for 30 seconds, and analyzed CL intensity using ImageJ (Fig. 2).
- The rotary valve on the μPAD controls the on/off state of microchannels, achieving the connection of various regions (Fig. 3).

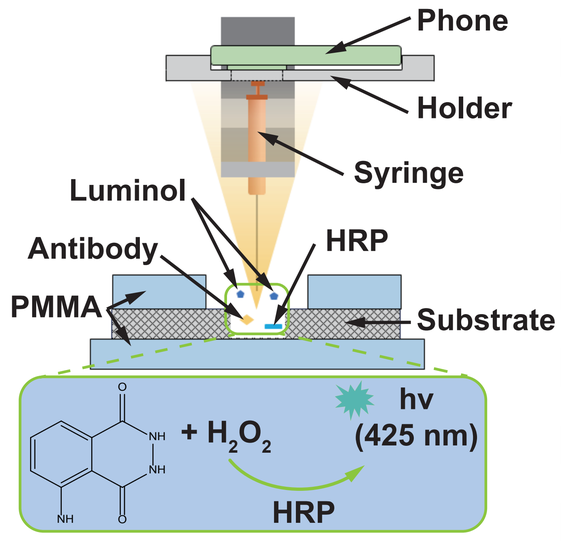

Fig. 2. Cross sectional view of μPAD.

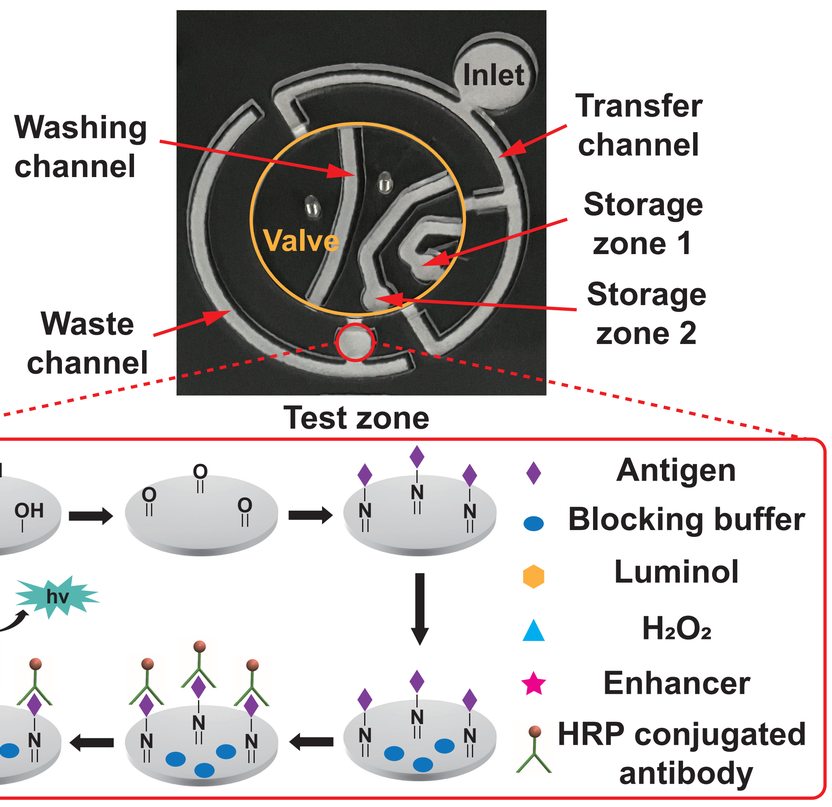

Fig. 3. The photo of developed μPAD consisted of a rotary valve and a surrounding chip with the protocol of CL ELISA in the test zone.

RESULTS

Detected rabbit IgG by direct ELISA on our μPAD (Fig. 4): LOD = 3.58 pM; R^2 = 0.997

- Optimized experimental conditions (H_2O_2 concentration at 0.1 M, HRP-conjugated antibody concentration at 150 μg/mL, and plasma treatment time of 4 minutes).

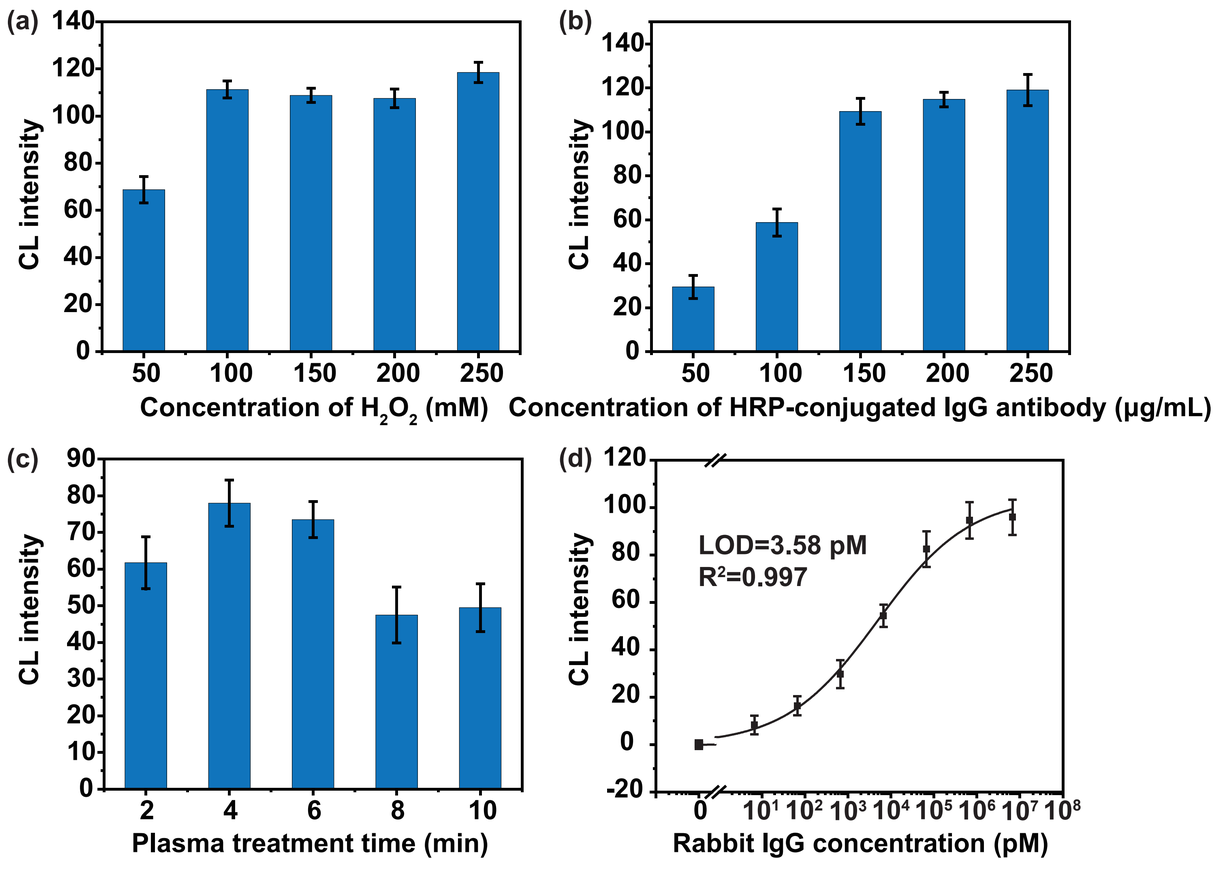

Fig. 4. (a-c) Optimized experimental conditions include the concentrations of H_2O_2 and HRP-conjugated IgG antibody, and the oxygen plasma treatment time. (d) Calibration curve of CL intensity vs rabbit IgG concentration (N=5).

CONCLUSION

This paper introduces a smartphone-based μPAD for automated CLIA. As a proof-of-concept, the LOD of rabbit IgG on this μPAD reached 3.58 pM.

Acknowledgements:
This work is supported by Natural Science Foundation of the Jiangsu Higher Education (22KJB460033), Jiangsu Science and Technology Programme - Young Scholar (BK20200251), XJTLU RDF project (RDF-18-02-20), XJTLU AI University Research Centre, Jiangsu Province Engineering Research Centre of Data Science, Cognitive Computation at XJTLU, and SIP AI innovation platform (YZCXPT2022103), the State Key Laboratory for Manufacturing Systems Engineering via the open project (SKLMS2023019), and the Key Laboratory of Bionic Engineering, Ministry of Education at Jilin University.

Ruiqi Yong
Undergraduate Student (Year 3)
Xi'an Jiaotong-Liverpool University
Email: ruiqi.yong1118@gmail.com
Web: https://ruiqiyong.github.io/

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