A PAPER-BASED DUAL APTAMER ASSAY ON AN INTEGRATED MICROFLUIDIC SYSTEM FOR DETECTION OF HNP 1 AS A BIOMARKER FOR PERIPROSTHETIC JOINT INFECTIONS

Rishabh Gandotra[1], Feng-Chih Kuo[2], Mel S. Lee[3] and Gwo-Bin Lee[1,4]

[1]Institute of Nano Engineering and Microsystems, National Tsing Hua University, Hsinchu, Taiwan

[2]Department of Orthopaedic Surgery, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

[3]Department of Orthopaedic Surgery, Paochien Hospital, Pingtung, Taiwan

[4]Department of Power Mechanical Engineering, National Tsing Hua University, Hsinchu, Taiwan

Abstract

This work demonstrated a novel dual-aptamer assay performed on a paper-based device composed of nitro-cellulose (NC) membrane on an integrated microfluidic platform for detection of a biomarker, human neutrophil peptide 1 (HNP 1) for periprosthetic joint infection (PJI). A fully automated device involved a single loading process for the newly developed sandwich assay for HNP 1 quantification. The primary aptamer was immobilized on the NC membrane where HNP 1 was captured and detected using fluorescent-labelled secondary aptamer. The developed assay is faster (~ 30 min) and requires less volume.

Introduction

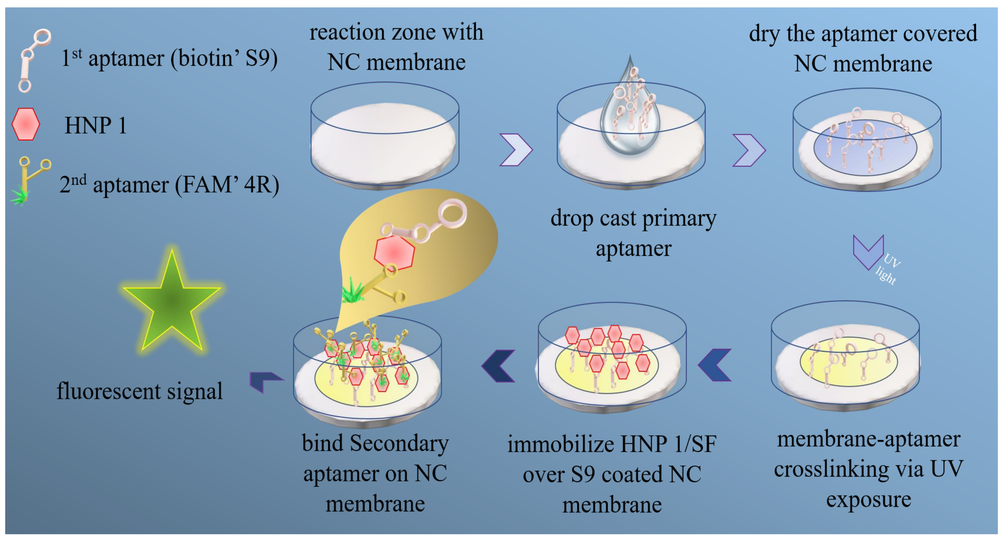


Figure 1: Schematic of the paper-based, dual-aptamer assay. The process involved the crosslinking of a primary aptamer onto a NC membrane via UV crosslinking, followed by HNP 1 capture, secondary aptamer binding and finally fluorescent signals detection.

Chip design & optimization

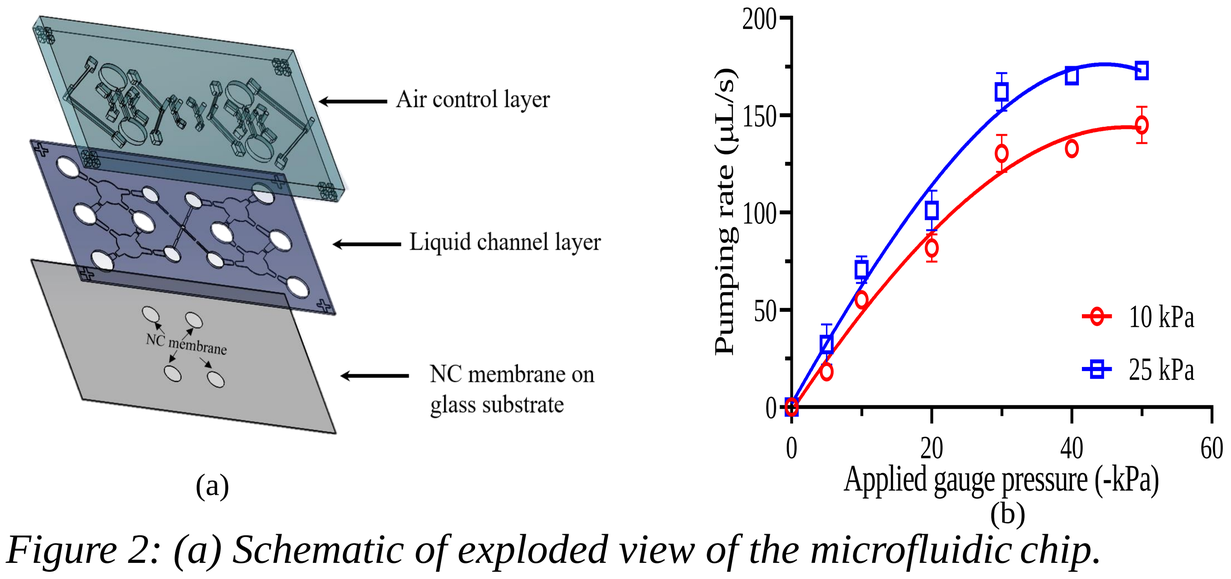


Figure 2: (a) Schematic of exploded view of the microfluidic chip. (b) Pumping rate of micropump at variable gauge pressure (N=3).

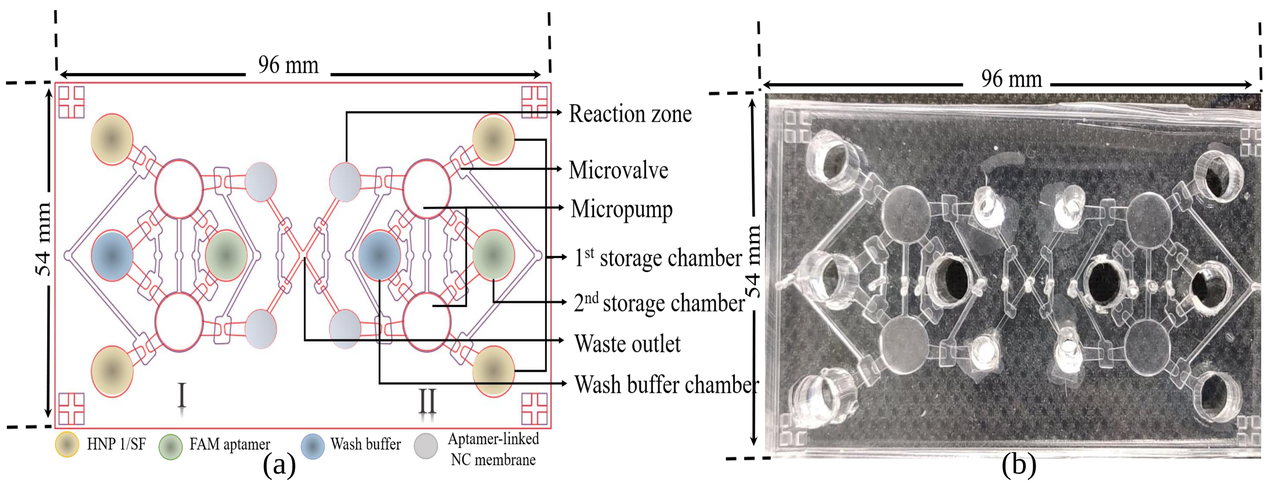
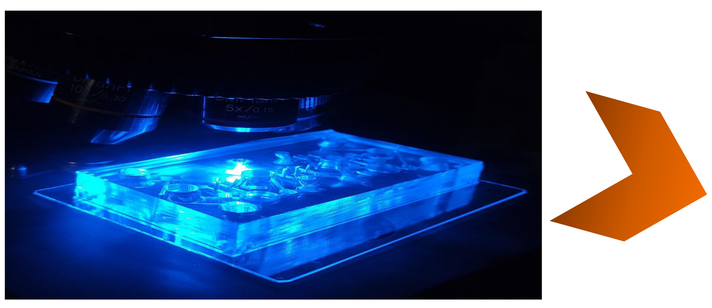


Figure 3: (a) Detailed design and functions of micro-components of the chip and the two identical patterns (I and II). (b) Photograph of the microfabricated chip, which was composed of 12 electromagnetic valves (EMV) ports, 1 micropump, 8 reagent chambers, 4 NC membrane chambers and 1 waste outlet.

Results



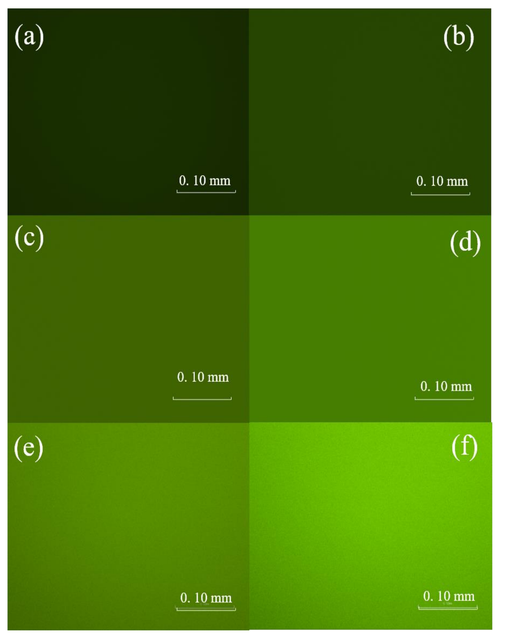


Figure 4: Images observed under a fluorescence microscope for 5 different conditions of HNP 1. (a) Negative control (0 mg/L), (b) HNP 1 (1 mg/L), (c) HNP 1 (2 mg/L) (d) HNP 1 (5 mg/L), (e) HNP 1 (10 mg/L) and (f) HNP 1 (25 mg/L)

Table 1: Clinical sample analysis

Sample No.	HNP 1 concentration (mg/L)	Result	Image Under Microscope
Sample 1	1.5	Negative	
Sample 2	4.7	Positive	
Sample 3	7.5	Positive	
Sample 4	0	Negative	
Sample 5	0.8	Negative	

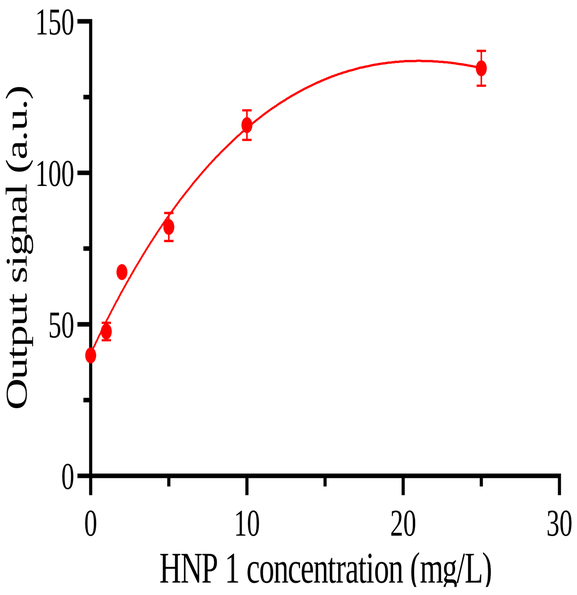


Figure 5: Calibration curve using image quantification (N=3).

Conclusions

A new paper-based, dual-aptamer assay on a microfluidic platform was successfully developed. The aptamer-based sandwich assay increased affinity and specificity for targeting PJI biomarker, HNP 1, without antibody reliability. A fluorescent image-based quantification was used to develop a calibration curve that could be used to analyze the clinical samples. The developed assay is cost-effective and can be performed within 30 min where four samples were analyzed simultaneously on a single chip. The developed microfluidic device can be used for further prognosis and diagnosis of PJI.

Acknowledgements

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Immediate Contact:

Rishabh Gandotra

Ph.D. candidate, iNEMS, NTHU

rishabhgandotra.mbl@gmail.com

*Chair Prof. Gwo-Bin Lee; gwobin@pme.nthu.edu.tw