Proof‐of‐concept modular fluid handling prototype integrated with microfluidic biochemical assay modules for point‐of‐care testing

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RESEARCH ARTICLE

Proof-of-concept modular fluid handling prototype integrated with microfluidic biochemical assay modules for point-of-care testing

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Abstract

Large populations around the world suffer from numerous but treatable health issues, caused by either lifestyle choices or environmental factors. Over the past decades, point-of-care testing kits have been developed to circumvent the reliance on laboratories, by allowing users to perform preliminary health or environmental testing from the privacy of their homes. However, these kits heavily rely on the precision of the user to perform the procedures, leading to increased variability in final assessments. To eliminate user-induced errors, we present an integrated, completely sealed, and disposable point-of-care testing prototype that exploits the benefits of microfluidics and 3D-printing fabrication techniques. The palm-sized modular prototype consists of a manually operated fluid handling device that allows precise mixing, filtration, and delivery of fluids to an on-board microfluidic assay unit for subsequent detection of specific biochemical analytes, with a minimized risk of contamination.

KEYWORDS

fluid handling device, microfluidic biochemical assays, point-of-care tests

1 | INTRODUCTION

Currently, the most common at-home infectious disease tests[1] and on-site water quality testing kits[2] are designed to force users to independently collect fluid samples, manually measure and mix specified reagents, and perform assays using the allocated components of the kits. The likelihood of obtaining false positives and erroneous analyses is high even if kits offer the advantage of obtaining the assessments within a few minutes to a few hours. Additionally, the exposed nature of the fluid handling components increases the risk of biocontamination. On the other hand, more precise assessments can be accomplished by shipping the pre-processed fluid samples to a designated laboratory, with the drawbacks of increased costs and prolonged waiting times to obtain the assessments.

To address these issues, we are developing a modular system consisting of two components: (i) the fluid handling device (FHD) module and (ii) an interchangeable assay module. The FHD is designed to allow users to collect and mix a fluid sample with preloaded reagents, filter the mixture, sequentially deliver the processed sample to an on-board microfluidic assay device, and collect the fluid wastes, within a sealed system, thereby reducing user-induced variability and contamination, while obtaining the assay results within a few

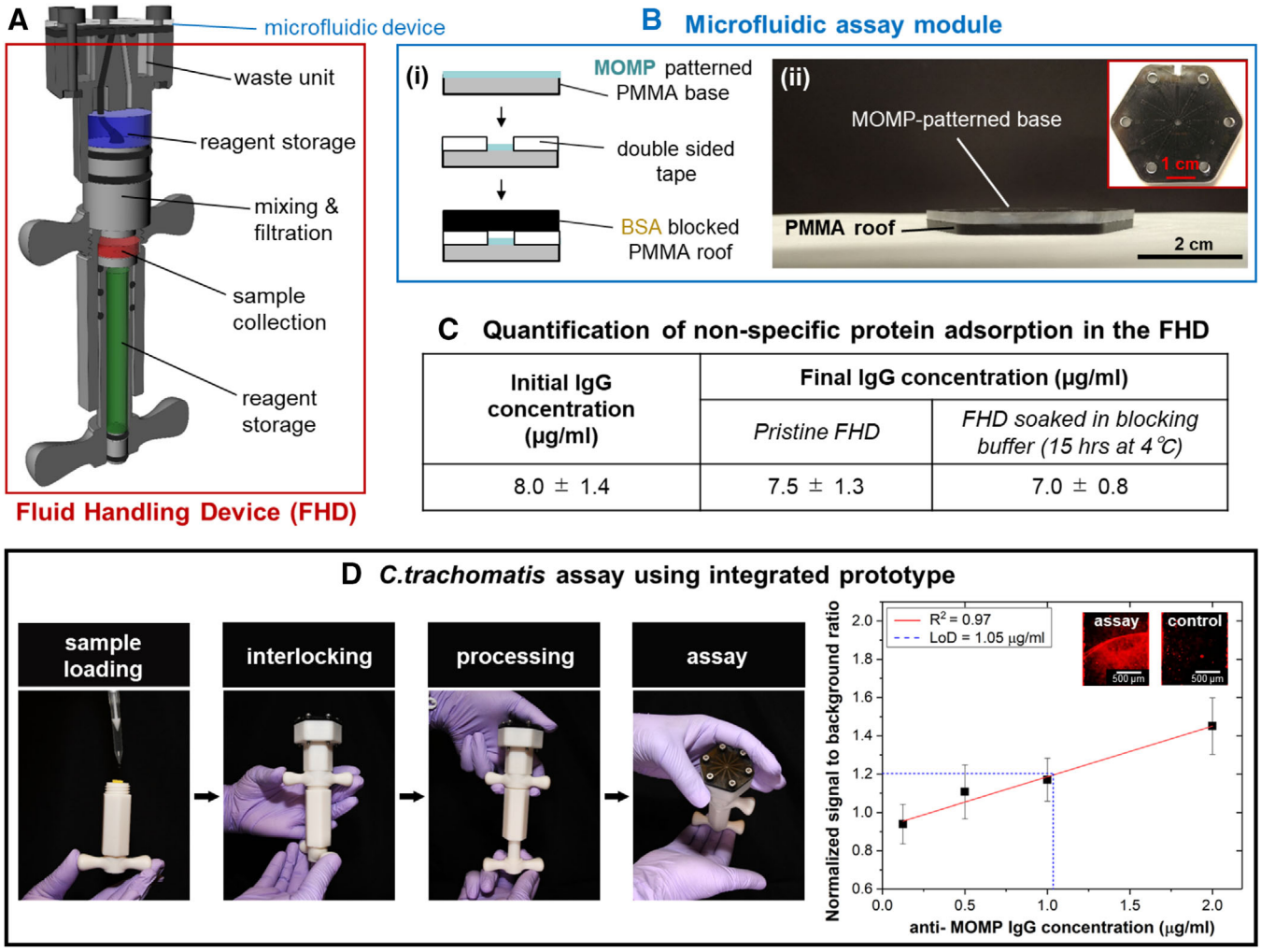

FIGURE 1 Schematic illustrating the (A) components of the fluid handling device (FHD), (B) fabrication strategy of the microfluidic assay module, (C) quantification of non-specific protein adsorption in the FHD. (D) Images depict the processing steps for an anti-MOMP IgG fluorescence assay using the integrated prototype. The graph and fluorescence images depict the linear range of detectable anti-MOMP IgG concentrations with the integrated prototype.

minutes. After the completion of the tests, the user can simply dispose the sealed prototype as designated laboratory waste. As a case study, we carried out biochemical assays to detect antibodies specific to bacterial *Chlamydia trachomatis* infections using our integrated prototype. Specifically, buffer samples doped with *C. trachomatis* specific immunoglobulin G (IgG) antibodies[3] were mixed with on-board fluorescently labeled antibodies in the FHD and assayed in poly(methyl methacrylate) (PMMA) microfluidic assay devices, patterned with antigenic major outer membrane proteins (MOMP) of *C. trachomatis*.[4] Varying concentrations (125 ng/mL - 2 µg/mL) of anti-MOMP IgGs could be detected and reproducibly differentiated from negative samples using our manually powered prototype within 15 min. The efficiency of this proof-of-concept test demonstrates the capability of our integrated prototype to enable precise, reproducible, and rapid diagnosis, with minimized risk of contamination.

2 | EXPERIMENTAL

The FHD body (Figure 1A) was fabricated using the Form2 stereolithography 3D printer (Formlabs, USA) and white V4 photocurable resin (made of methacrylic acid esters). The custom-made components have a piston-cylinder relationship that allows them to slide concentrically within one another, for delivering fluid to chambers without leakage.[5] For the non-specific protein adsorption studies, three sets (n) of FHD were soaked in N101 blocking buffer (1:5, N101:phosphate-buffered saline (PBS)) for 15 h at 4°C. Fluorescently labeled IgG solutions (1.5 mL) were loaded into the reagent storage units of either blocked or pristine FHD bodies (n = 3). The concentrations of antibodies in the eluates were quantified using the NanoDrop™ 2000c spectrophotometer (ThermoFisher Scientific, Japan), post fluid processing using the FHD.

The microfluidic chlamydia assay module was fabricated by first covalently immobilizing MOMP (50 μg/mL) onto PMMA substrates, using our developed air-plasma enhanced bio-functionalization strategy.[6] Next, double-sided adhesive tapes were cut to form microchannels using a carbon dioxide laser cutter (VLS 3.50, Universal Laser Systems, USA), and bonded to the MOMP-functionalized PMMA strips on one side and IPA-cleaned PMMA strips on the other side to complete the microfluidic device (Figure 1B). The bare surfaces of the PMMA microchannels were finally blocked with 1% weight/volume of bovine serum albumin, to prevent non-specific adsorption of proteins, and attached to the upper portion of the FHD using M3 bolts to complete the assembled prototype.

3 | RESULTS AND DISCUSSION

The FHD is a two-piece locking system (Figure 1A) that incorporates sample collection, sample dilution, filtration, and fluid delivery into one easy-to-use manually operated device. The FHD is the base module for our point-of-care testing system, where it serves as a platform to secure different analysis/detection modules for subsequent biochemical tests. The reagent storage cartridge is capable of housing 1- 3 mL of primary reagents, used for sample dilution and priming for the biochemical assay. Once the sample is deposited, the lower portions of the FHD are locked with the upper portions that consist of secondary reagents (0.5-3 mL) employed during the assay. Sample dilution and mixing is then performed by manually pulling down on the ergonomic buffer cartridge tabs. This action induces a vacuum pressure within the mixing cylinder and forces the primary reagents to flow into the mixing chamber, thereby mixing and diluting the deposited sample. The diluted sample is then subsequently passed through the upper portion of the FHD for filtration and assay within the on-board microfluidic assay module, while allowing the waste fluid to be collected in the waste unit (Figure 1A). Finally, the secondary reagents are delivered to the microfluidic assay module by a final compression step, completing the assay and allowing the user to visualize the results. The photographs illustrating the processing steps are depicted in Figure 1D.

To test if our integrated prototype can carry out effective biochemical assays efficaciously, a proof-of-concept antibody assay was performed to detect bacterial *C. trachomatis* infections.[7] PBS samples (4 mL) doped with 2 μg/mL of anti-MOMP immunoglobulins (IgGs) served as the fluid sample and blank PBS served as the negative control, which were processed with six individual FHD modules, respectively (three for each condition). The samples were loaded onto the lower portion of the FHD and mixed with the primary reagent buffer (2 mL) comprising fluorescently-labeled detection antibodies, as described above. The mixed samples were filtered through the upper portion of the FHD and delivered to MOMP patterned microfluidic assay modules to detect the presence of the anti-MOMP IgGs in the fluid sample. The microfluidic assay module was washed by delivering the secondary wash buffer (0.05% Tween-20 in PBS) to remove unbound biomolecules, and imaged via fluorescence microscopy for qualitative analysis. The inner layers of the FHD body showed minimal non-specific protein adsorption, with an insignificant difference between the blocked and pristine units (Figure 1C). As seen in Figure 1D, the integrated prototype enabled the detection of anti-MOMP IgGs with a linear concentration range of 125 ng/mL - 2 μg/mL, and a limit of detection[8] of 1.05 μg/mL. The assay could be reliably differentiated from the negative controls reproducibly in each device, thereby demonstrating the potential of our prototype for point-of-care testing applications.

Current research is focused on minimizing sample volume requirements by optimizing FHD design that minimizes dead volume, and improving the sensitivity of the microfluidic assay modules. Additionally, the microfluidic assay modules are being engineered to test multiple biochemical analytes from a single biological sample with our integrated device.

4 | CONCLUSION

By exploiting the benefits of microfluidics and state-of-the-art fabrication techniques using 3D printing, we developed a completely integrated, easy-to-use, hand-held, manually powered and disposable fluid processing, and biochemical assay device. The potential of our prototype to serve as point-of-care testing devices was demonstrated by detecting antibodies specific to *C. trachomatis* infections. In the future, we envision the inclusion of multiplexed microfluidic assay chambers within one single device, to allow detection of multiple bioanalytes from one single sample, thereby reducing both the testing time (<30 min) and the cost (< US$25 for multiple tests), for discrete at-home testing.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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REFERENCES

1. N. P. Pai, C. Vadnais, C. Denkinger, N. Engel, M. Pai, *PLoS Med.* **2012**, *9*, e1001306.
2. J. Y. Park, L. J. Kricka, *Anal. Bioanal. Chem.* **2014**, *406*, 5631.
3. S. A. Morré, C. Munk, K. Persson, S. Kruger-Kjaer, R. van Dijk, C. J. L. M. Meijer, A. J. C. van den Brule, *J. Clin. Microbiol.* **2002**, *40*, 584.
4. A. Badamchi-Zadeh, P. F. McKay, B. T. Korber, G. Barinaga, A. A. Walters, A. Nunes, J. P. Gomes, F. Follmann, J. S. Tregoning, R. J. Shattock, *Front. Immunol.* **2016**, *7*, 162.
5. K. Toda-Peters, A. Q. Shen, S. Sathish, D. Lee, C. J. Galvin, K. Funakoshi (Okinawa Institute of Science and Technology School Corporation), *US Patent Application number: 62/509,244*, **2017**.
6. S. Sathish, N. Ishizu, A. Q. Shen, *ACS Appl. Mater. Interfaces.* **2019**, *11*, 46350.
7. S. Sathish, K. Toda-Peters, A. Q. Shen, in *Proc. 23rd Int. Conf. Miniatur. Syst. Chem. Life Sci*., Basel, Switzerland, October, **2019**.
8. D. A. Armbruster, T. Pry. *Clin. Biochem. Rev.* **2008**, *29*, S49.

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