AN AUTOMATED CENTRIFUGAL MICROFLUIDIC SYSTEM INTEGRATED WITH ETALON SENSOR FILMS FOR RAPID IMAGE ANALYSIS BASED DETECTION OF HORMONES IN MILK

Yuting Hou[1], Rohit Mishra[2], Menglian Wei[1], Nicholas Balasuriya[1], Jens Ducrée[2], Michael J. Serpe[1] and Jed Harrison[1*]

[1]Department of Chemistry, University of Alberta, Canada and

[2] FPC@DCU - Fraunhofer Project Centre for Embedded Bioanalytical Systems at Dublin City University, Ireland

Rohit Mishra and Menglian Wei contributed equally to this work.

ABSTRACT

Microfluidic technology can provide cost-efficient, user-friendly devices for applications in the life sciences, environmental monitoring and the agrifood, through miniaturization and automation. In Alberta, undetected estrus is the leading reason for poor reproductive efficiency in dairy herds. Therefore, accurately identifying estrus by monitoring related hormones will be ultimately beneficial. Herein, we developed an automated centrifugal microfluidic disc aimed at realizing low-cost, easy-to-use, rapid on-site hormone detection in aqueous solutions. The workflow is designed with direct delivery of milk to the device, integrated sample cleanup, followed by on-disc analysis with embedded color sensor films and a simple camera.

KEYWORDS: Centrifugal Microfluidics, Color sensor, Etalons, Automation, Integration, Point-of-use, Dairy industry, Progesterone

INTRODUCTION

Microfluidic devices have demonstrated their potential for a broad range of applications in environmental monitoring, biopharma, food safety and clinic diagnosis [1]. Attempts have been reported in developing micro-devices for detection of hormones by immunoassay which avoid the employment of complex instrumental methods such as high-performance liquid chromatography and liquid chromatography/gas chromatography-mass spectrometry. However, immunoassay still requires multiple incubation/washing steps, and often uses radioactive materials. The alternative fluorescence readout relies on specialized equipment such as fiber optic probes and spectrometers unsuitable for point-of-use scenarios. We plan to adapt etalon-based technology with a centrifugal device format to provide simple user-operation, including a sample clean-up, single-step incubation, followed by delivery of wash and stimulus reagents, to give an on-disc display that can be read by a simple camera via image analysis. The etalon-based technology is a novel type of color sensor that has never been integrated into a microfluidic platform and offers considerable opportunity for on-disc sensing with an optical readout.

EXPERIMENTAL

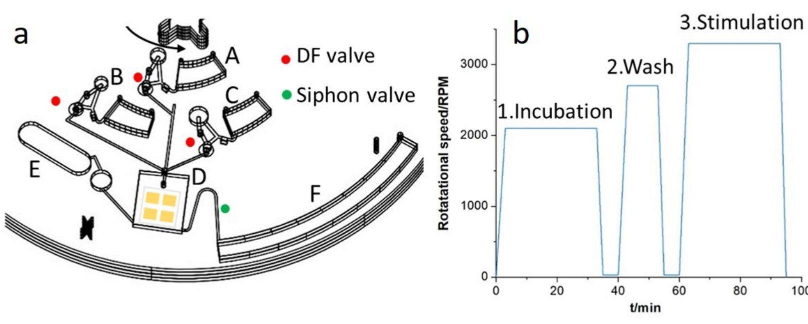

Figure 1: 3D view of design for each unit (a) and the flow control spinning operation protocol procedure (b).

This fluidic device consists of four layers of PMMA-poly (methyl methacrylate) and four layers of PSA-pressure sensitive adhesive. Features are defined by laser cutter and layers are then laminated [2]. Dissolvable film (DF)-based pneumatic valves and a siphon valve [3] are utilized to automate sample preparation and delivery (Fig.1a). For each on-disc procedure, 190-µL volumes of milk, washing buffer and 1.5 M NaCl solution are preloaded into chambers A, B and C, respectively. Solutions in chambers A, B and C are sequentially released through DF valves to chamber D, where the etalon film is integrated. Spin rates of 2100, 2700 and 3300 rpm, respectively, are used to release the solutions in a stepwise fashion. The times for incubation and washing are indicated in Figure 1b.

Etalons are optical devices constructed by sandwiching a layer of poly(N-isopropylacrylamide)-based microgel between two thin Au films. Their assembly and sensing mechanism has been previously described by Dr. Serpe's group [4]. In brief, Na^+ and Cl^- ions can change the solvation state of the microgel particles in the etalon, causing a change in distance between the Au layers. This causes a change in the visible color of the etalon. P4 in a sample binds to a DNA aptamer immobilized on the top Au layer of the etalon device, hindering the diffusion of Na^+ and Cl^- ions into the microgel layer. Therefore, the amount of color change quantified by RGB values obtained via image analysis is inversely proportional to the concentration of P4 in the sample.

RESULTS AND DISCUSSION

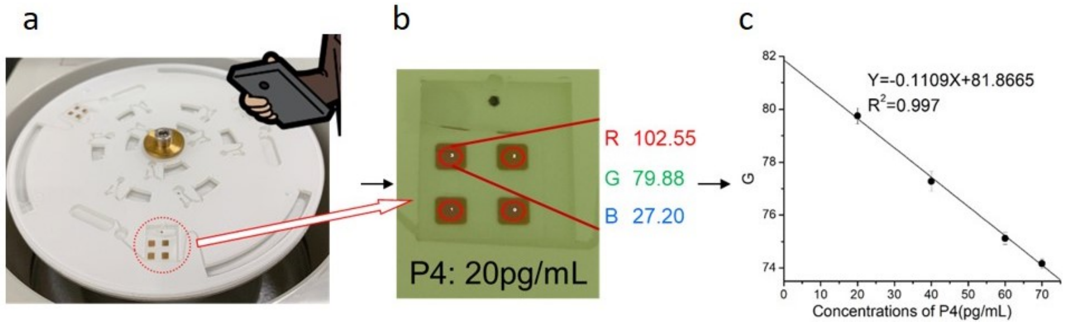

Figure 2: Workflow for on-disc image analysis of hormones in aqueous solution. Actual device and data are shown.

This etalon's selectivity and capability of quantification for P4 have been demonstrated in off-chip work, by directly measuring peak shifts in the reflectance spectrum. To simplify the on-chip readout process, this work employs image color analysis to monitor change in the reflected light color. Compared to monitoring reflectance spectra, image analysis avoids the employment of specialized equipment such as fiber optic probes and spectrometers. Figure 2b demonstrates the RGB of etalon responses to NaCl after incubating with 20 pg/mL P4 in Figure 2a. Also, other concentrations of P4 were studied. The results are shown in Figure 2c as a G channel-based calibration curve for different concentrations of P4 in delivered samples.

CONCLUSION

Our microfluidic device with embedded sample-to-answer sensors automates the detection of hormones in milk samples with ease of operation and flow control and a simple detection strategy. The entire procedure is time-efficient when compared to protocols like ELISA. The device, with integrated P4 DNA aptamer modified etalon sensors provides simple, sensitive and quantitative analysis. The method shows advantages over immunoassay and chromatographic techniques and is well suited to the on-site, cow-side analysis of aqueous samples.

ACKNOWLEDGEMENTS

We thank the Natural Science and Engineering Research Council of Canada, Alberta Agriculture and Forestry Strategic Research and Development Program, Alberta Agriculture Food and Rural Development (AAFRD) Growing Forward 2 Research Opportunities and Development and the Faculty of Science, University of Alberta for funding.

REFERENCES

[1] Y. Zhao, Y. Hou, J. Ji, et al. *Anal. Chem.*, 91, 7570-7577, 2019.
[2] Y. Hou, J. Ji, R. Mishra, et al. Proceedings of Micro Total Analysis Systems 2018, Kaohsiung, Taiwan, 1128-1131, 2018.
[3] R. Mishra, et al. *Sens Actuators B Chem*., 263:668-675, 2018.
[4] Y. Jiang, M.G. Colazo and M.J. Serpe, *Anal. Bioanal. Chem*., 410, 4397-407, 2018.

CONTACT

* D. J. Harrison; phone: +1-780-492-2790; jed.harrison@ualberta.ca