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A 3D printed RT-LAMP microdevice for the detection of feline calicivirus

Wojciech Kubicki, Justyna Kornicka, Rafał Walczak
Wrocław University of Science and Technology, Faculty of Electronics, Photonics and Microsystems, Department of Microsystems, Janiszewskiego Street 11/17, 50-372 Wrocław, Poland
wojciech.kubicki@pwr.edu.pl

Summary:
This study presents preliminary results of the development and characterization of a 3D-printed microfluidic lab-on-a-chip (LOC) device for rapid molecular diagnostics of feline calicivirus (FCV). The device was fabricated using digital light processing (DLP) technology and printed with biocompatible GR-10 photopolymer resin. The LOC was successfully applied to perform a reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection of FCV, a common pathogen in cats. The results of the RNA amplification reactions confirmed the high potential of 3D printing technology and the applied UV-curable resin with isothermal enzymatic reactions for prospective use in veterinary medicine.

Keywords: LAMP, lab-chip, RNA, 3D printing, DLP, FCV.

Introduction

Lab-on-a-chip technology enables the integration of sample preparation, chemical reactions, separation, detection and many other processes within a single miniaturized device. In recent years, additive manufacturing methods have emerged as a powerful alternative that offers fast, low-cost, and customizable manufacturing of microfluidic chips, especially for research and small-batch production. 3D printing technology allows direct translation of virtual models into functional prototypes without the need for master molds or cleanroom facilities [1]. In this work, a microdevice (LOC, chip) for rapid molecular diagnostics using LAMP method was fabricated using 3D printing DLP technology with biocompatible resin. The chip was applied for RT-LAMP detection of FCV, which is a common and highly contagious pathogen that affects cats, particularly in shelters and multi-cat environments.

Materials and methods

The RT-LAMP chip was designed with CAD software and fabricated using a DLP 3D printer (ASIGA MAX, Asiga) with biocompatible photopolymer resin (GR-10, Pro3dure). The microdevice has dimensions of 50 mm x 25 mm x 2.2 mm and contains four microreactors (25 µL capacity each), which allow simultaneous amplifications, for example, one negative and one positive control, and two DNA/RNA samples (Figure 1). Each microreactor contains an inlet and an outlet for sample introduction and pressure distribution. The fabrication time of a single 3D printed chip is approximately 1 hour, including post-processing.

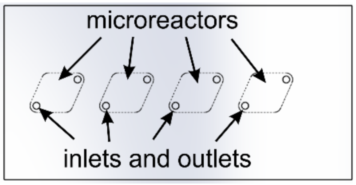

Fig. 1. Layout of the 3D printed LAMP microdevice

A similar device with identical dimensions of the microreactors, but arranged in different locations (Figure 2), was fabricated with borosilicate glass [2], using wet etching and fusion bonding processes (described elsewhere [3]). Here, a glass chip was used as a reference device, which was manufactured using verified glass technology.

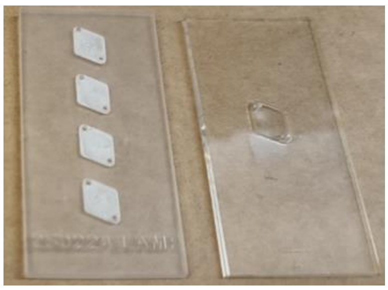

Fig. 2. Ready-to-use chips for RT-LAMP: 3D printed (on the left) and all-glass (on the right)

The microreactors of both devices were loaded with the same volume of LAMP solution, comprising SuperScript™ IV RT-LAMP Master Mix, FIP/BIP, F3/B3 and LoopF/LoopB primers and target RNA. The chips were sealed with PCR adhesive foil and placed on a laboratory hotplate (SD162, Stuart). The amplification reaction was carried out for 30 min at 67°C. Subsequently, the sealing was removed and 1 µL of SYBR Green fluorescence dye was added to each reaction microchamber. The fluorometric signal was measured using a laser-induced fluorescence setup (Figure 3), containing a laser diode (490 nm, 350 mW, Thorlabs) with planoconvex lenses and optical bandpass filter (497 nm, Thorlabs), a CCD board camera with optical long-pass filter (525 nm, Thorlabs), video grabber, a computer with custom detection software (LabVIEW), and a set of 3D printed or commercially available mechanical stages for proper arrangement of the chip.

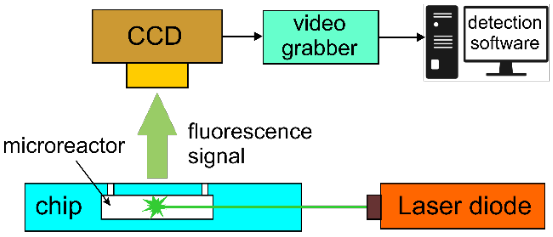

Fig. 3. Scheme of the fluorometric detection setup.

Results

Exemplary images captured by the CCD camera are presented in Figure 4. The software enables image analysis in the selected regions of interest (ROI) to calculate the intensity of the fluorescence signal. For all images, the same location and ROI area were applied to provide comparative results of LAMP output signal detection.

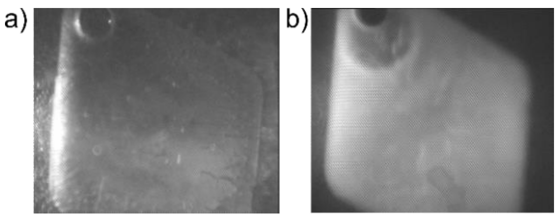

Fig. 4. Images of the 3D printed microreactors after LAMP: a) negative control, b) sample analyzed

The RT-LAMP process was performed for three samples: negative control (NEG) – equine RNA, positive control (POS) – *Feline Calicivirus* (M86379.1) RNA genome fragment 5179-5369 (191 bp), and tested sample (SAM). The RNA of the SAM sample was isolated from FCV-positive feline gingival swabs. The results of the analysis confirmed that a similar fluorescence intensity was obtained for the same sample type, regardless of the chip construction material (Figure 5). In the case of the 3D printed chip, the NEG value is slightly higher, while the POS and SAM values are a little lower in comparison to the glass device. Such differences can be explained by the observed increased intensity of background signal, probably invoked by a higher autofluorescence of the resin. This effect has already been investigated for other polymers [4], but more studies are required for the resin used here.

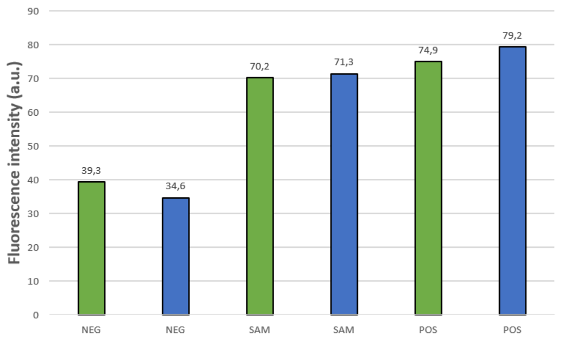

Fig. 5. Results of FCV RT-LAMP performed on the 3D printed device (green bars) and on the all-glass device (blue bars). Abbreviations: NEG/POS – negative/positive control, SAM – sample analyzed

Summary

This study successfully demonstrated the development of a 3D printed microfluidic chip for the application of RT-LAMP in veterinary diagnostics. The device was manufactured using digital light processing technology and printed with biocompatible GR-10 photopolymer resin. The primary objective was to use this device to perform a reverse transcription loop-mediated isothermal amplification assay to detect feline calicivirus, a contagious pathogen that affects cats. To conclude, this study confirms a promising future for the use of 3D printing technology to develop microfluidic devices for molecular diagnostics, offering fast, low-cost, and customizable solutions for veterinary applications. Nevertheless, more studies and optimizations are required to fully harness the potential of this technology.

References

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