OPTICAL MICROSYSTEM FOR FLOW AND STOPPED-FLOW ANALYSES OF ACTIVITY OF ENZYMES DEFICIENT IN RARE GENETIC DISORDERS

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ABSTRACT

Early and accurate diagnosis of rare disorders is a major challenge, even for trained specialists. Finding innovative, accurate diagnostic methods and high-throughput, cost-effective tools are crucial to improve quality of life and observe further progress of medicine.

This paper describes a simple polymeric microsystem with optical detection for flow and stopped-flow analyses of activity of α-galactosidase A deficient in Fabry disease. The principle for the determination of α-galactosidase A activity was fluorometric measurement of a protonated form of 4-methylumbelliferone released in the enzymatic reaction. The experimental set-up and the design of the detection module allowed determination of the product in the first minutes of the reaction. The presented results make the microsystem a promising tool for point-of-care applications.

KEYWORDS

Early diagnostics, point of care, lysosomal storage disorders, optical detection, flow analysis, stopped-flow analysis.

INTRODUCTION

Lysosomal storage disorders (LSD) are a group of over 50 rare inherited disorders that result from defects in lysosomal function [1]. All of these have in common the deficient activity of a specific lysosomal enzyme, e.g. β-glucocerebrosidase (Gaucher disease), α-glucosidase (Pompe disease), α-galactosidase A (Fabry disease) [2]. Most of LSD can be identified only by specialists, usually after long, frustrating medical examinations and missed diagnoses [2]. While some currently available therapies (such as enzyme replacement, substrate deprivation, chemical chaperon, or gene therapy) have great potential, their efficacy will rely heavily upon the early and accurate diagnosis of the disorder, ideally, before neurologic symptoms arise. Early detection of absent or minimal enzyme activity offers the best opportunity for therapeutic intervention to prevent irreversible damage of organs and premature death.

However, currently used methods are inaccurate, time-consuming, and limited to specialized laboratories. There are still too many false-positive and false-negative test results. The reliability and accuracy of the methods used to determine the activities of lysosomal enzymes and classify patients for a proper therapeutic group need urgent improvement.

The aim of this work was to design and develop a microfluidic system for laboratory diagnostics of lysosomal storage disorders. We present a microfluidic system with integrated optical elements for flow and stopped-flow analyses of activity of α-galactosidase A using cultured human fibroblasts.

EXPERIMENTAL & RESULTS

A schematic view and a picture of the system are presented in Figs. 1 and 2, respectively. The three-layered thermally bonded PMMA microsystem fabricated by micromilling consists of a module for hydrodynamic focusing of cell suspension, a 250 μm wide and 500 μm high mixing microchannel, and an optical detection module connected to an external light source and a detector via optical fibers. Two exciting and one collecting optical fiber were inserted into the system after bonding using guiding microchannels and sealed with UV glue.

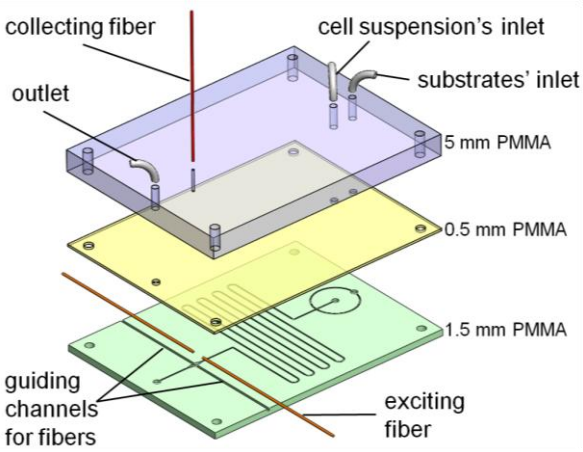

Figure 1. Schematic view of the microsystem.

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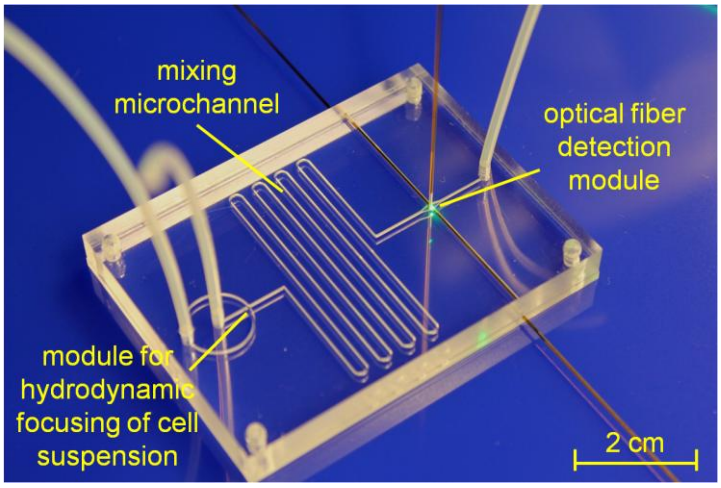

Figure 2. Picture of the microsystem.

The middle PMMA layer was introduced for precise positioning of the collecting quartz fiber and to improve S/N and LOD of the method by filtering most of light below about 400 nm including the exciting light (320 nm) and possible fluorescence by the substrate (here: 375 nm) while allowing fluorescence from the product to pass (445 nm). The spectra for the collected light for a two- and a three-layered device are compared in Fig. 3.

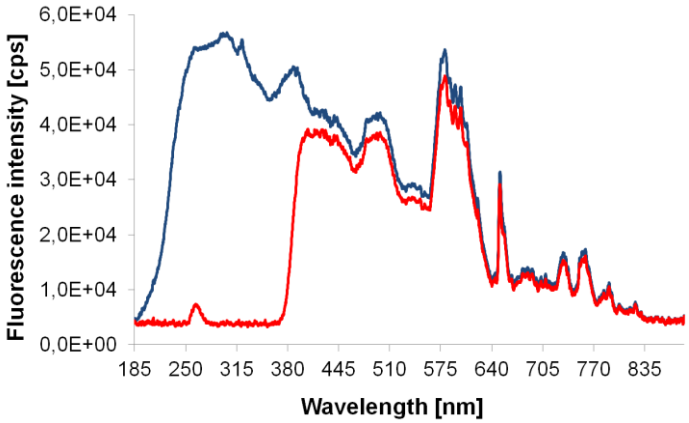

Figure 3. The spectra for the collected light for a two- (blue curve) and a three-layered (red curve) microsystem.

The flow analysis of α-galactosidase A activity is based on the fluorometric measurement of a protonated form of the enzymatic reaction product (4-methylumbelliferone). By changing the flow rates for the cell suspension and the substrate while maintaining a constant ratio, the concentration of the released product after different reaction times could be determined (Table 1, Fig. 4). The α-galactosidase A activity determined with this method was 76.8 ± 9.5 μU/10^6 cells.

Table 1. Flow rates for the reagents introduced to the system while maintaining a constant 1:4 flow ratio, and resulting times for the enzymatic reaction.

Cell suspension [μL/min]	Substrate (side streams) [μL/min]	Enzymatic reaction time [min]
8	32	1
4	16	2
2	8	4
1.6	6.4	5
1	4	8

For stopped-flow analysis the reagent flow was stopped after introduction to the system and the increase of the product's fluorescence in time was measured (Fig. 5). The determined α-galactosidase A activity using this method was 70.6 ± 9.8 μU/10^6 cells, showing good agreement with the flow-based analysis.

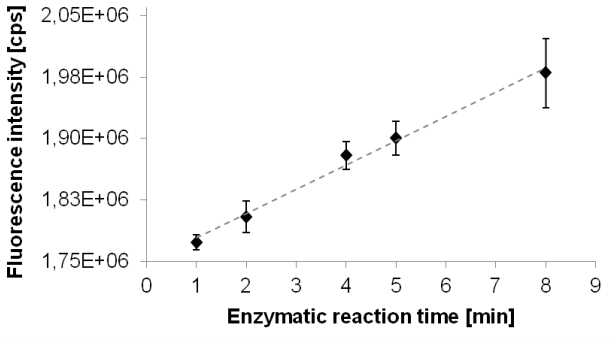

Figure 4. Flow analysis of α-galactosidase A activity. The reaction progress curve was estimated for different times of the enzymatic reaction (1, 2, 4, 5 and 8 min) obtained by changing the flow rates for the cell suspension and the substrate while maintaining a constant ratio.

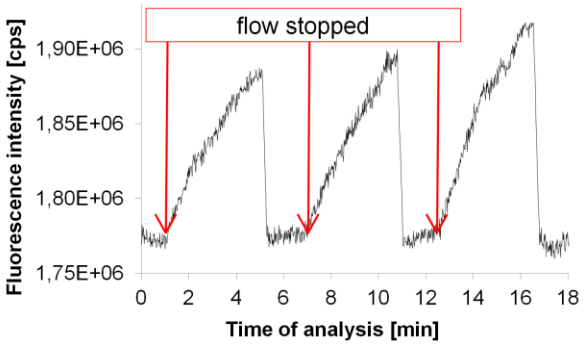

Figure 5. Stopped-flow analysis of α-galactosidase A activity.

CONCLUSIONS

Compared to currently used protocols for α-galactosidase A activity determination [3], the microdevice provides a significant reduction of the analysis time (from several hours to minutes). Detection of fluorescent product is possible shortly after the start of the reaction because of the design of the detection module (LOD=200 nM). Moreover, the experimental set avoided having to terminate the enzymatic reaction with alkaline buffer and sample dilution, with significant effects on the sensitivity of the method. The system is equally suitable to determine the activity of other lysosomal enzymes. Due to its straightforward and low cost fabrication, and the possibility of integration with commercially available detectors, the system holds much promise for point-of-care applications.

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REFERENCES

[1] G. M. Pastores, *Lysosomal Storage Disorders: Principles and Practice*, World Scientific Publishing Company (2009).

[2] H. Zhou, P. Fernhoff and R. F. Vogt, *Newborn Bloodspot Screening for Lysosomal Storage Disorders*, Journal of Pediatrics 159, pp. 7-13 (2011).

[3] A. M. Martins, V. D'Almeida, S. O. Kyosen, E. T. Takata, A. G. Delgado, A. M. Gonçalves, C. C. Benetti Filho, D. Martini Filho, G. Biagini, H. Pimentel, H. Abensur, H. C. Guimarães, J. G. Gomes, J. Sobral Neto, L. O. D'Almeida, L. R. Carval-ho, M. B. Harouche, M. C. Maldonado, O. J. Nascimento, P. S. Montoril, R. V. Bastos, *Guidelines to Diagnosis and Monitoring of Fabry Disease and Review of Treatment Experiences*, Journal of Pediatrics 155, pp. S19-S31 (2009).

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