STORAGE AND REACTIVATION OF ENZYMES IN A DISPOSABLE, SELF-CONTAINED LAB-ON-A-CHIP SYSTEM

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ABSTRACT

For a point-of-care chip, it is important to have reactants stably stored on-chip. In this study, deposition and drying of a solution of three enzymes have been examined. The time for diffusion/rehydration of fluorescently labelled mouse IgG in the dried enzyme solution was ~10 minutes. Microchips with native cyclic olefin copolymer (COC) surfaces showed large adsorption of mouse IgG, while polyethylene glycol (PEG) coated surfaces showed adequate protein resistance. Successful amplification on chip in 500 nl reaction chambers was obtained for spotted and dried enzymes when mixed with 0.5% PEG 8000.

Keywords: NASBA, mRNA amplification, fluorescence detection, diagnostics

1. INTRODUCTION

In this work, which is part of a project aimed at the development of a self-contained disposable microchip, we have explored an approach for stabilization and storage of three labile enzymes (AMV-RT, RNase H and T7 RNA polymerase) in a single formulation on-chip for the isothermal amplification of mRNA and ssDNA by nucleic acid sequence-based amplification (NASBA) [1]. Similar work has been performed by Weigl and co-workers [2] for air drying and reactivation of PCR mixes in conventional macro scale volumes using 96-well plates. While PCR is only dependent on the activity of one enzyme for amplification, NASBA requires 3 functional enzymes. NASBA has previously been demonstrated on-chip with premixed reagents [3]. The work reported herein evaluates the reactivation of enzymes after drying on-chip. To our knowledge, this is the first time the NASBA enzymes have been successfully dried and reactivated in a microchip. For self-contained systems, both liquid and dried reagents have been proposed as plausible solutions for long-term on-chip storage of reagents [4, 5].

2. EXPERIMENTAL

For the experiments, COC chips were employed Figure 1 (a). The microchips were O_2 plasma activated prior to coating with 0.5% PEG in methanol (Sigma Aldrich Norway AS, Norway). The NASBA reagents were provided in the PreTect HPV-Proofer kit (NorChip AS, Norway). A stabilizing agent, PEG 8000 (Sigma Aldrich Norway AS), was added to the enzyme solution prior to spotting and drying. For the enzyme wall adsorption and the diffusion experiments, fluorescently labelled mouse IgG (Southern Biotech, Birmingham, AL) was used as a model molecule. The microchips were spotted with enzymes, dried in room temperature for up to 2 days before they were sealed with adhesive tape. The amplification reaction was performed by manually mixing the NASBA reagents, including the positive control sample of human papillomavirus (HPV) type 16. The mixture was first incubated at 65˚C for 3 minutes followed by 3 minutes at 41˚C. The microchip containing the dried enzymes was adjusted to 41˚C before 1 µl reaction mixture was applied to each

reaction chamber on the microchip. A custom-made instrument recorded the fluorescent signal of the amplification reaction at 41˚C [3].

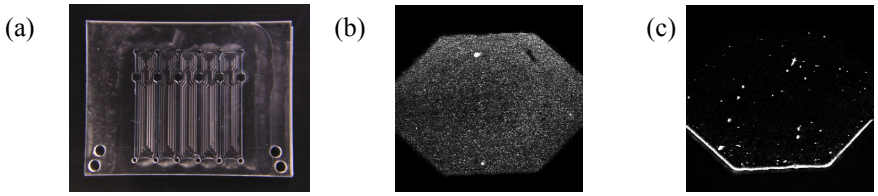

Figure 1. (a) COC microchip fabricated using hot embossing. The outer dimensions of the chip were 43 mm × 32 mm, while the dimensions of the reaction chambers were 1.6 mm × 1.6 mm × 0.2 mm, corresponding to a volume of 500 nl. (b), (c) Adsorption of fluorescently labelled mouse IgG on native COC and PEG coated COC surfaces. (b) The native COC surface was only plasma activated. No amplification could be observed for microchips with native COC surfaces. (c) The PEG coated COC surface showed adequate resistance to the fluorescently labelled IgG. PEG coated surfaces gave successful amplification reactions.

3. RESULTS AND DISCUSSION

Adsorption of enzymes to the microchip surface, as well as the process of rehydration of the dried enzymes in the liquid sample, were investigated experimentally using confocal laser scanning microscopy (CLSM). Both native COC and PEG coated surfaces were incubated with NASBA reaction mixture including fluorescent IgG at 41˚C for 2.5 hours followed by a washing step with water. The native COC surfaces showed large adsorption of IgG, while PEG coated surfaces showed adequate protein resistance, Figure 1 (b) and (c).

For the diffusion measurements, the enzyme solution was mixed with fluorescent IgG prior to spotting and drying. Rehydration and diffusion of the fluorescent IgG species were essentially completed after approximately 10 minutes, see Figure 2.

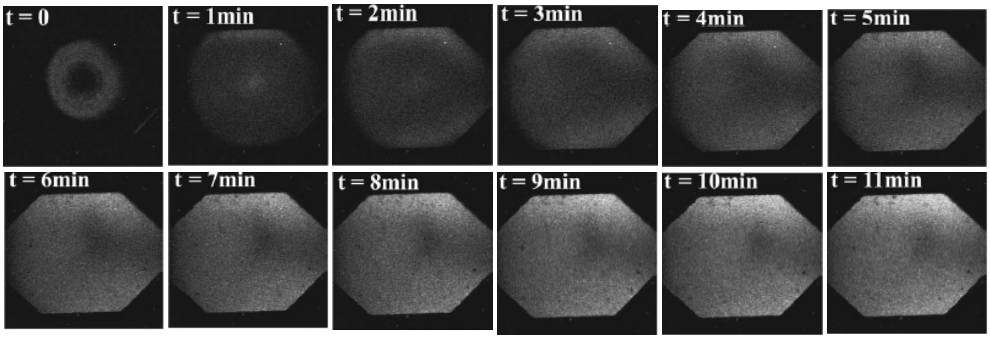

Figure 2. Rehydration of dried enzymes, temporal evolution. The time between frames was 1 minute. The dissolving liquid was a solution of all the NASBA reagents except the enzymes. The dried enzyme spot was inside a 500 nl chamber. All images were taken with the focal plane positioned 150 µm above the bottom wall of the reaction chamber.

The estimated time of diffusion time for a distance of 150 µm was ~3 minutes. The observed longer time required, may indicate that the process was dominated by rehydration and not by diffusion. The fluorescent IgG tracer (150 kDa) modelled the largest enzyme (AMV-RT, 160 kDa).

Figure 3 shows amplification curves of the 500 nl reaction chambers for reactivation of spotted and dried enzymes containing 0.5% PEG 8000. No amplification was observed when PEG 8000 was excluded in the drying procedure. The 10 minutes required for enzyme dissolution was shown to be acceptable.

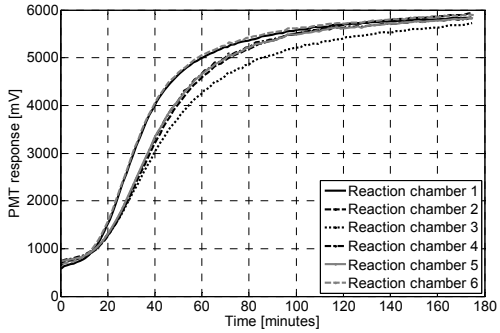

Figure 3. Amplification curves of the six 500 nl reaction chambers run simultaneously on one microchip employing a sample of positive control for HPV type 16 (0.1 μM). The dried enzymes including PEG 8000 were rehydrated after one day at room temperature.

4. CONCLUSIONS

The present work demonstrates on-chip storage of dried enzymes which are reactivated upon rehydration of sample. These results are promising with regard to the development of disposable, self-contained microchips for NASBA.

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