MINIMIZING PARASITIC REACTIONS FOR ENZYME-CONTROLLED METABOLIC PATHWAYS INVESTIGATED IN BIOMEMS

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ABSTRACT

This paper reports design improvements to minimize parasitic reactions in bioMEMS for studying sequential, site-specific enzymatic reactions. Interconnect reservoirs were eliminated by employing aligners on prototype mold to guide packaging. Flow directions for *in situ* enzyme assembly and subsequent enzymatic reaction were separated by employing a cross-channel design. These improvements efficiently suppressed parasitic reactions by trapped enzyme in the interconnect reservoirs and non-specifically bound enzyme on microchannel walls.

KEYWORDS: parasitic reaction, enzyme immobilization, non-specific binding, dead-volume, cross-channel design, packaging aligner

INTRODUCTION

We have recently reported programmable assembly and efficacy of a metabolic pathway enzyme in prepackaged bioMEMS device [1], where precursor S-adenosylhomocysteine (SAH) was converted into S-ribosylhomocysteine (SRH) and adenine by site-specific assembly of Pfs enzyme in a microchannel. This strategy exploits programmable electrodeposition of chitosan as a platform for subsequent spatially distributed assembly of multi-step enzymatic pathways within a closed bioMEMS device (Fig.1(a)-(b)). However, parasitic reactions by enzyme retained in dead volume of the microfluidic network (e.g., reservoirs) or non-specifically bound on microchannel walls impedes our intent to spatially localize the enzymatic reactions on the electrode (Fig.1). Here we report design improvements to eliminate dead volume and minimize non-specific binding on microchannel surfaces (Fig.2).

THEORY AND DEVICE DESIGN

Homogeneous parasitic reactions occur when fluid flow patterns retain unbound enzyme in dead volume regions such as fluidic interconnect reservoirs, where they catalyze substrate and generate products after the substrate introduction has stopped, persisting even through subsequent device flushing. Interconnect reservoirs between on-chip microchannel and external tubing are commonly used to compensate for packaging misalignment. We implemented a packaging technique that fabricates aligners on the prototype mold to guide microfluidic packaging (Fig.3), which eliminates the interconnect reservoir dead volume while offering convenient ways of connecting external tubing to microfluidic channels [2]. We have demonstrated that

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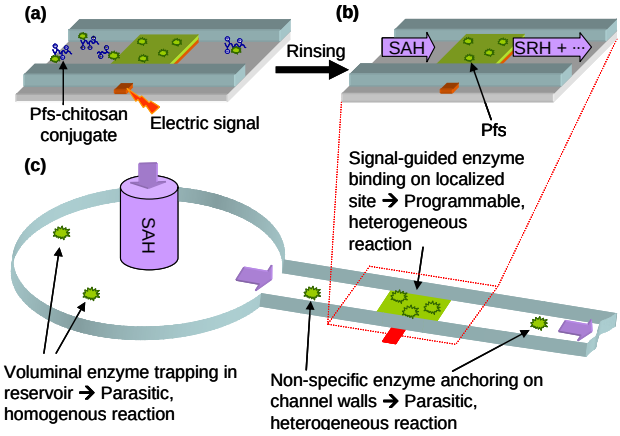

Figure 1. Parasitic reactions in microfluidics due to enzyme trapping in interconnect reservoirs and non-specifically bound on microchannel walls.

these improvements to the packaging are easily engineered into existing designs, adding only one additional mask layer for the mold manufacture.

Heterogeneous parasitic reactions also occur involving enzyme non-specifically bound to microchannel sidewalls. In our designs enzyme assembly sites comprise only 0.2% of the total microchannel surface, yet the site-specific assembled enzyme converts ≥3X more substrate into products than non-specific bound enzyme [1]. To further reduce heterogeneous parasitic reactions, we implemented the cross-channel device as in Fig.2(c)) to separate flow directions for (a) *in situ* enzyme assembly and (b) subsequent enzymatic reaction.

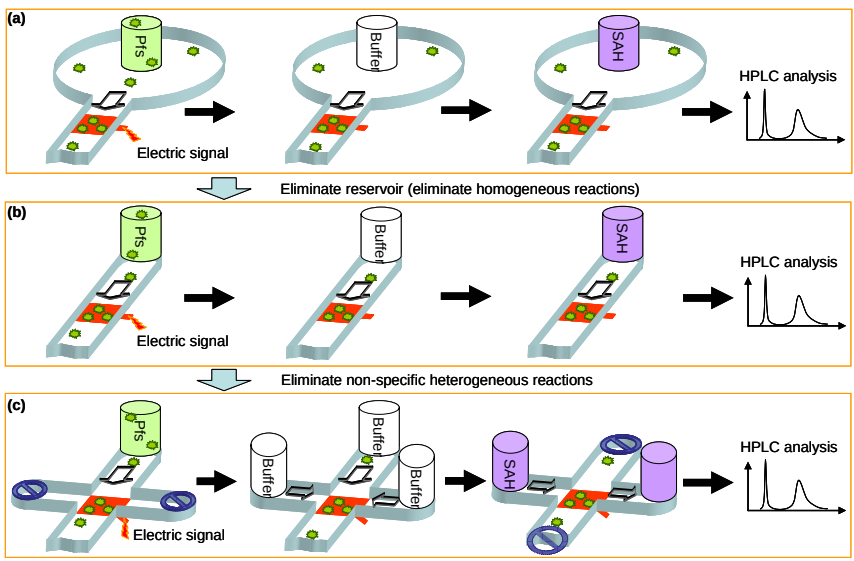

Figure 2. Parasitic reactions in microfluidics due to enzyme trapping in interconnect reservoirs and non-specifically bound on microchannel walls.

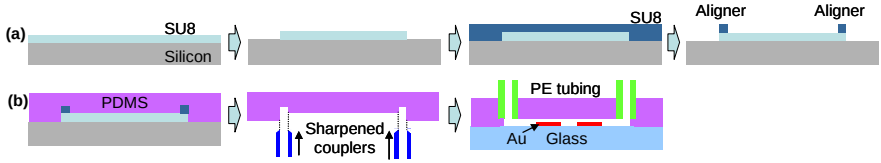

Figure 3. Minimization of parasitic reactions. (a) Mold fabrication, (b) packaging.

RESULTS AND DISCUSSION

By eliminating the interconnect reservoirs, system time response as measured downstream has improved 2-3X, clearly suppressing homogeneous parasitic reactions in the interconnection reservoirs. By separating the microchannels for enzyme assembly from those for the subsequent enzymatic reaction so that only the intended reaction site (assembly electrode) is common to both microchannels, the interaction of substrate with the non-specifically bound enzyme on the enzyme assembly cross-flow channel walls is diminished.

The design improvements depicted in Fig.2 reduce background parasitic signal substantially, as shown in Fig.4. The final results show that the signal-to-background (S/B) ratio has increased from 0.72 to 1.28 by eliminating the packaging reservoirs and further to 2.43 by separating the flow direction of enzymatic reaction from that of enzyme assembly step, netting a 340% increase in S/B ratio. We believe these design improvements will promote progress in using bioMEMS as a powerful platform for metabolic engineering and its various applications.

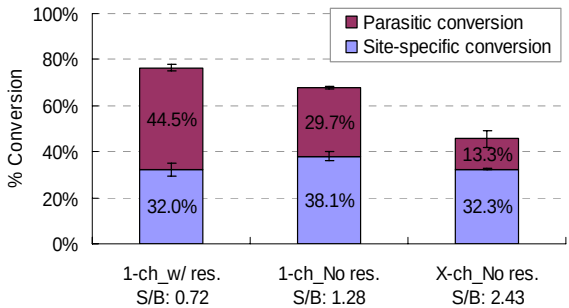

Figure 4. Minimization of parasitic reactions.

ACKNOWLEDGEMENTS

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REFERENCES

[1] X.L. Luo, A.L. Lewandowski, H.M. Yi, G.F. Payne, R. Ghodssi, W.E. Bentley and G.W. Rubloff, *Lab on a Chip*, **8**, pp. 420-430 (2008).

[2] X.L. Luo, A.L. Lewandowski, D. Berlin, G.F. Payne, R. Ghodssi, W.E. Bentley and G.W. Rubloff, *Proc. First Shenyang International Colloquium on Microfluidics*, Shenyang, China, pp. 204-207, (2007).