HAIRPIN-STRUCTURED PCR ENHANCER FOR DIGITAL MICROFLUIDIC SYSTEMS

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Abstract

This work proposed a novel polymerase chain reaction (PCR) enhancer and testified its function to restore and enhance the fluorescence signal of dsDNA binding dyes for PCR performed on digital microfluidic (DMF) platforms. SYBR Green I (SGI) and other dsDNA binding dyes such as EvaGreen (EG) have a long history of application in quantitative real-time PCR. However, the reduced sensitivity and the false negative signal of PCR on microfluidic chips make them unsuitable as amplification indicators on-chip. The proposed PCR enhancer solved this problem by utilizing its unique hairpin structure with a 3' quencher moiety. The stem of the enhancer acted like a temporary reservoir, holding and protecting the dye molecules from damaging factors that cause their invalidity, and released them only when amplicons with larger size were produced during amplification process. Through this mechanism, the signal of on-chip PCR can be enhanced to a large extent.

Methods

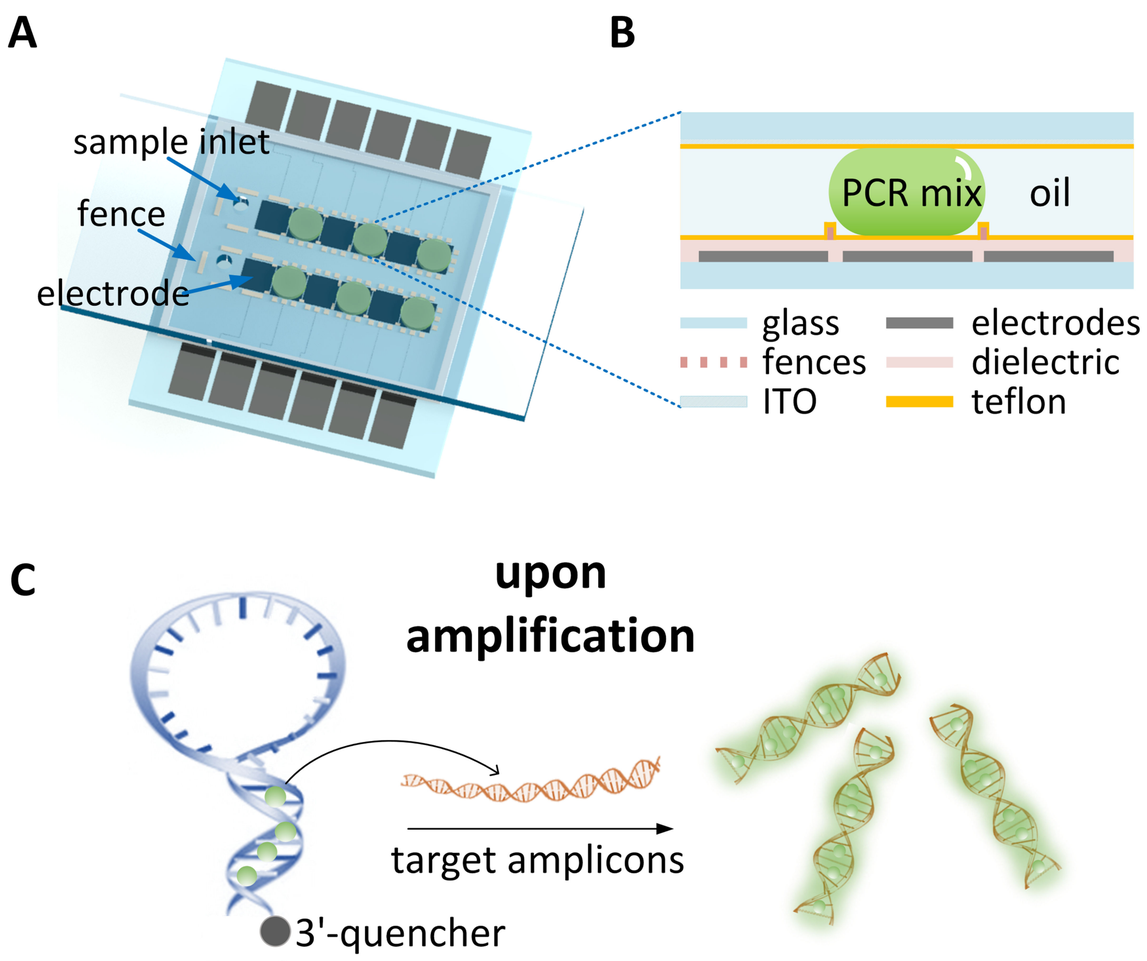

Figure1. Schematic of the applied DMF chip and the enhancer's functioning mechanism. (A) Topview of the DMF chip used in this work. (B) Sideview of the DMF chip. (C) Illustration of the proposed PCR enhancer's mechanism.

Results

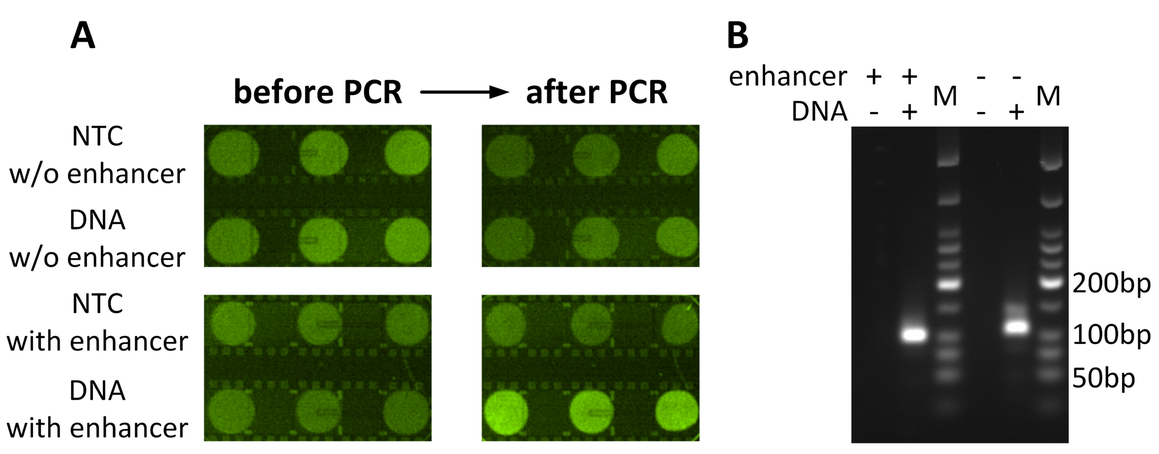

Figure2. Fluorescence images under microscope (A) and gel electrophoresis result (B) of on-chip PCR at 0.4 × SGI.

At a normal SGI concentration such as 0.4 × SGI, the SGI's signal failed to increase, and even decreased a little after on-chip PCR. However, the successful amplification of template DNA was verified by electrophoresis, suggesting that the negative results from the on-chip fluorescence were false negative results. By introducing the enhancer in the mixture, the droplets containing DNA templates brightened up normally after on-chip PCR, with a 150% fluorescence increment.

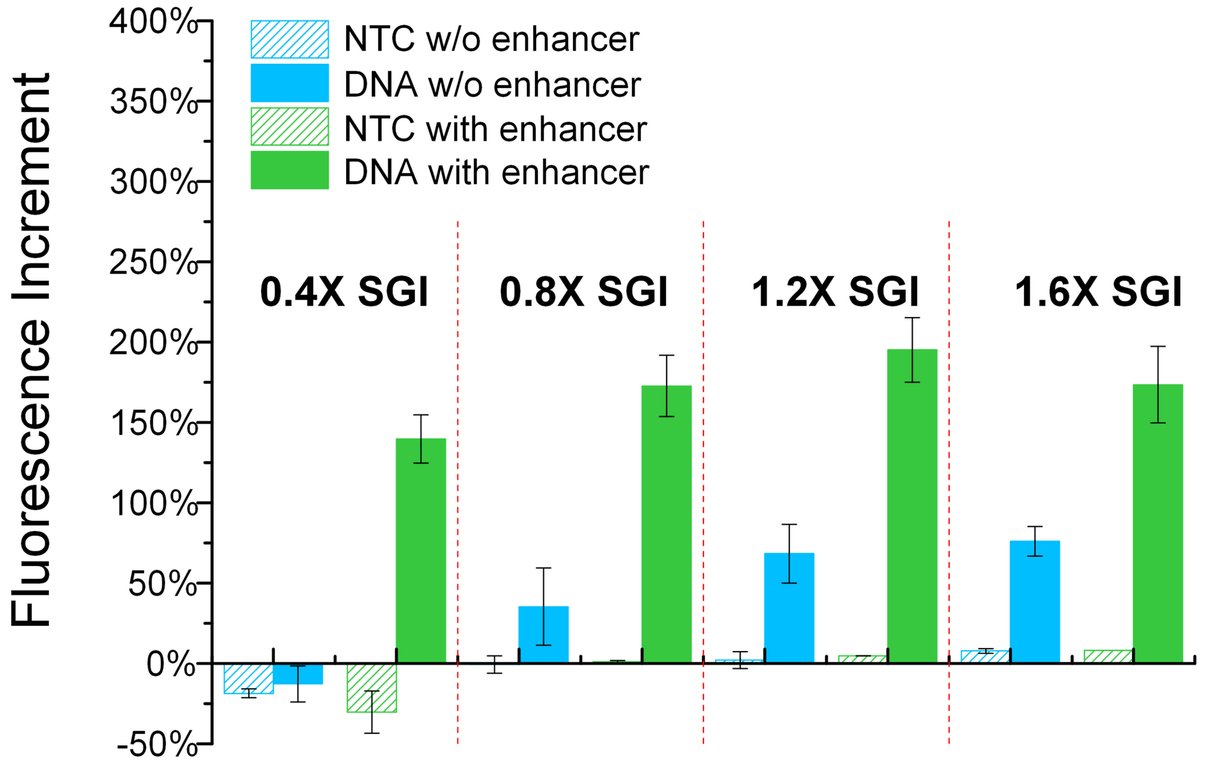

Figure 3. End-point analysis results of on-chip PCR in comparison between samples with and without the proposed enhancer, at 0.4 ×, 0.8 ×, 1.2 ×, and 1.6 × SGI concentrations.

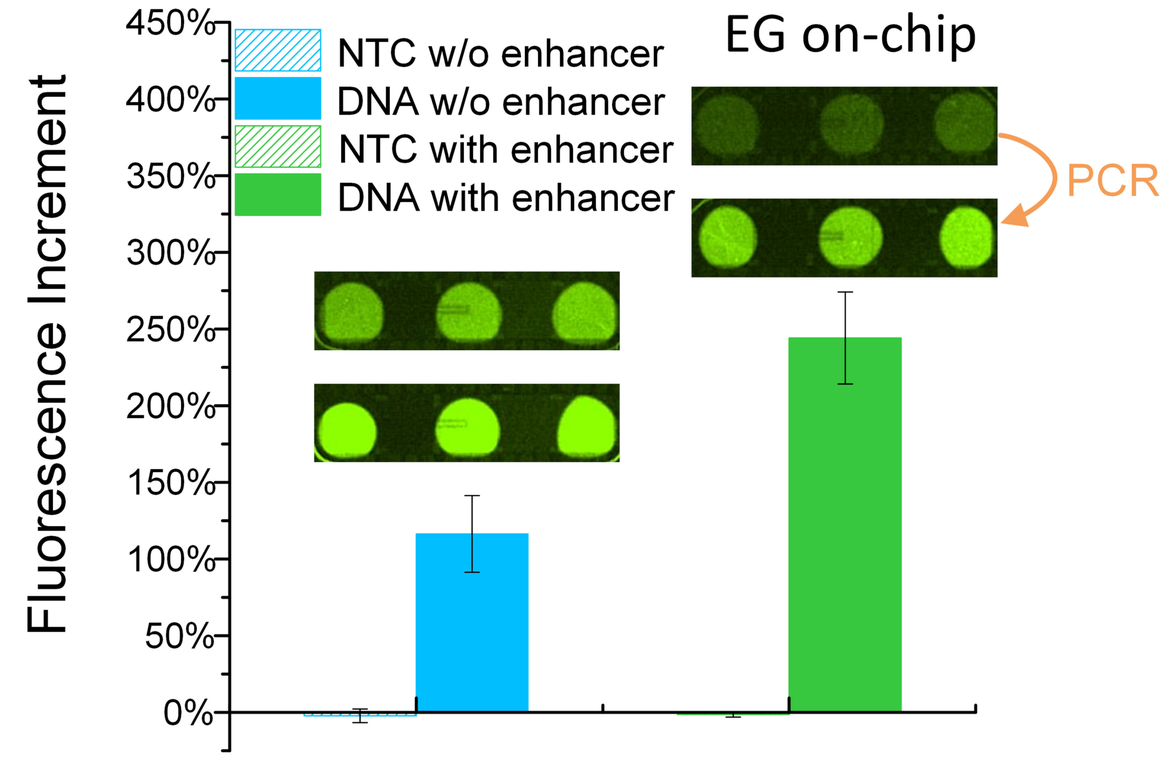

Figure 4. On-chip PCR comparative results for samples with and without the enhancer, at 1× EG. Fluorescence images of positive samples are shown.

Conclusions

This work described and testified a novel PCR enhancer for DMF platforms. With its hairpin structure and the quencher on the 3' end, the enhancer can offer a shelter for dsDNA binding dyes from on-chip damaging factors. It eliminated the false negative results for on-chip PCR at low SGI concentrations, and increased the fluorescence increment at high SGI concentrations. The enhancer also worked for other dsDNA binding dyes such as EvaGreen. In sum, the proposed enhancer can cancel out the incompatibility of dsDNA binding dyes with microfluidic systems, and make them more robust in on-chip applications.

References and Acknowledgements

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