Integrated microfluidic platform for utilizing aptamer-based ELISA-like assay for simultaneous detection of multiple cardiovascular clinical samples

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Abstract

Cardiovascular diseases (CVDs) have been documented as one of the leading causes of deaths globally. Early detection may reduce the high risk of sudden deaths associated with CVDs. Current biomedical sensing processes vastly rely on antigen-antibody based detection processes, which have limitations in point-of-care applications. In this work, we developed an integrated microfluidic platform for the simultaneous detection up to six clinical samples using a highly specific aptamer for recognizing the CVD associated protein, **cardiac troponin I** (**cTnI**), followed by an antibody-based chemiluminescence assay in less than 30 minutes. A fully automated on-chip detection of **cTnI** was performed by using only 5 µL of clinical samples from each patient. It may serve a promising tool for CVDs monitoring and diagnosis.

1. Motivation and background

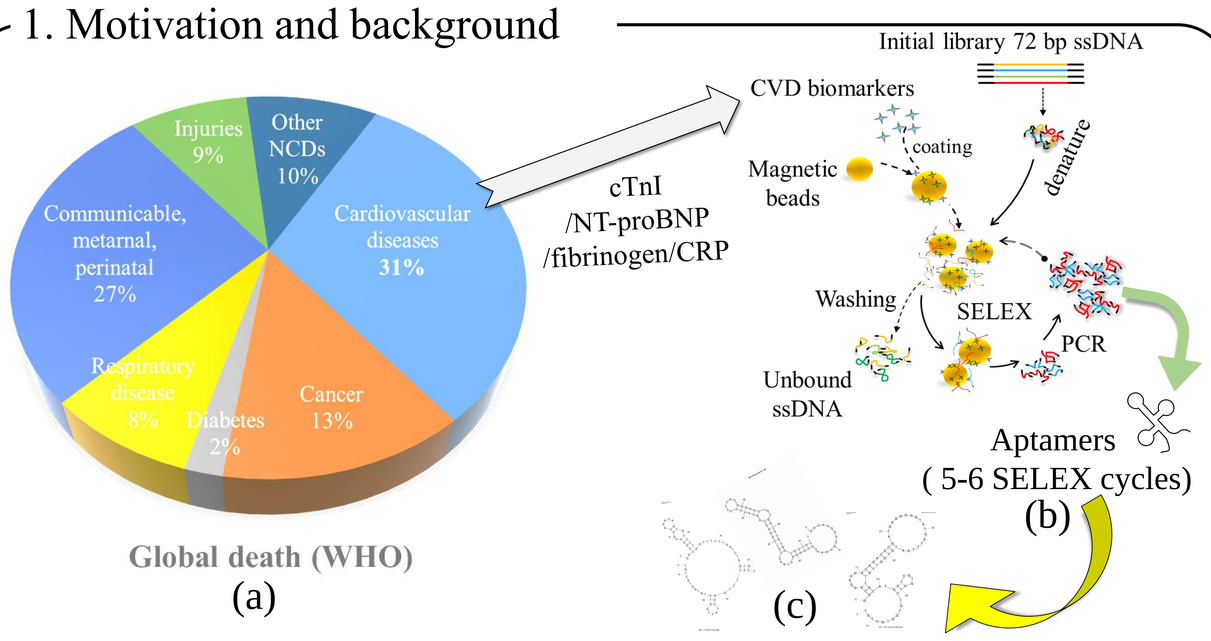


Figure 1: (a) The pie chart explains global cause of death in percentage. (b) Cardiovascular disease biomarkers were chosen and subsequent aptamers were screened by on-chip SELEX. (c) Two-dimensional structures of the aptamers are shown for targeting cTnI.

*[Sinha et al. **Biosensors and Bioelectronics**, 122, pp. 104-112, 2018]*

2. Aptamer-based ELISA-like assay

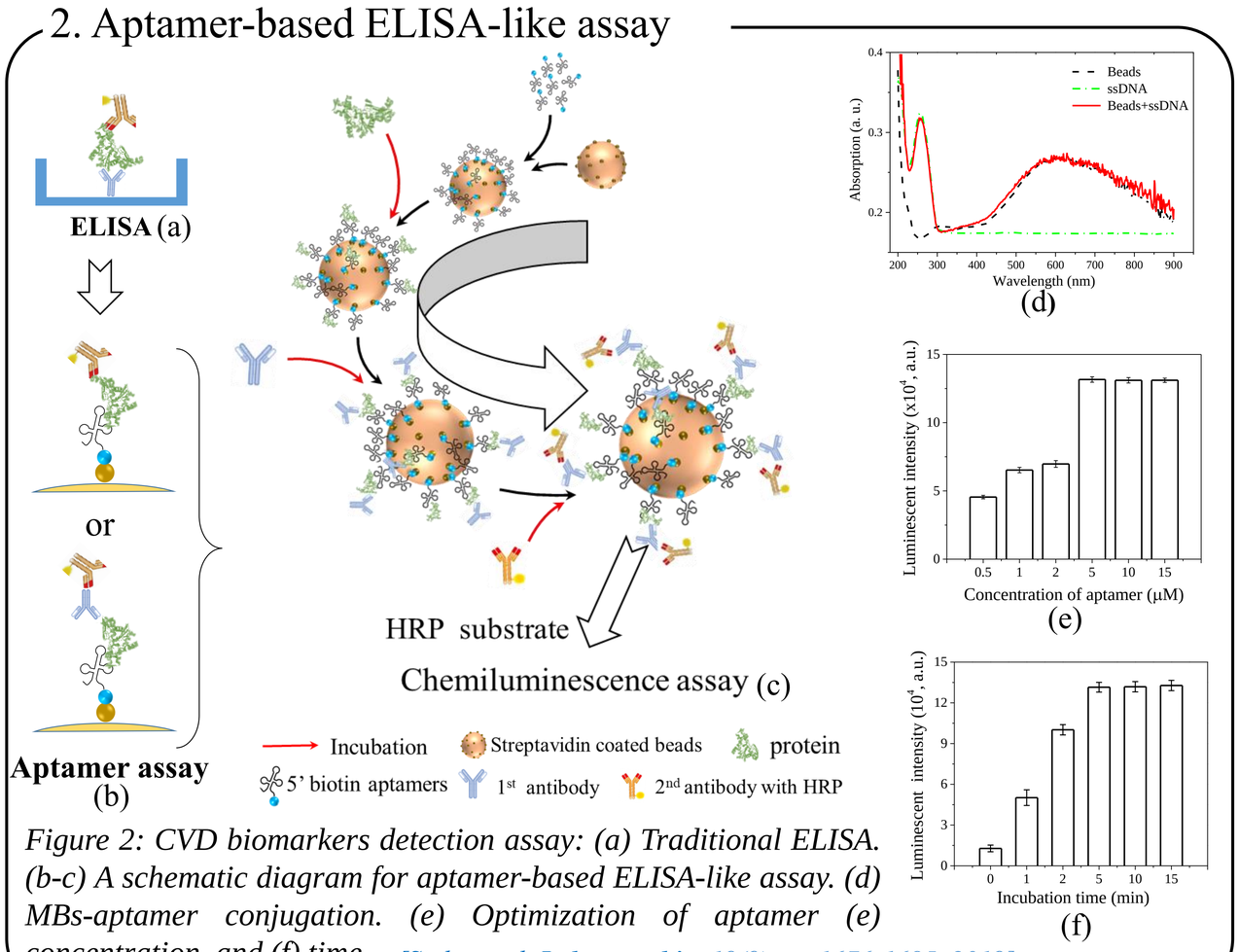
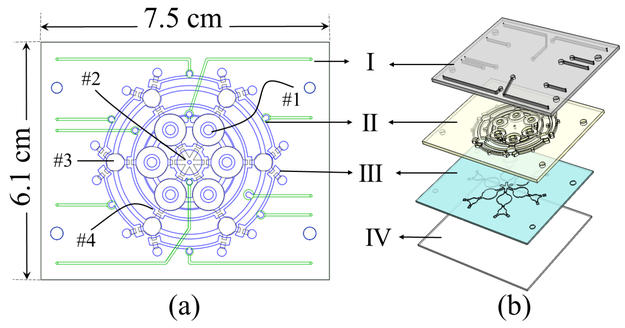


Figure 2: CVD biomarkers detection assay: (a) Traditional ELISA. (b-c) A schematic diagram for aptamer-based ELISA-like assay. (d) MBs-aptamer conjugation. (e) Optimization of aptamer (e) concentration and (f) time. *[Sinha et al. **Lab on a chip**, 19(9), pp.1676-1685, 2019]*

3. Chip design



Microfluidic chip

Figure 3: (a) Schematics of the device and its parts including six open-type micromixers (#1), waste outlets (#2), six micropumps (#3), and normally-closed microvalves. (b) Exploded view of the microfluidic chip which includes air control layers (I, II), liquid control layer and a glass substrate.

4. Microfluidic device characterization

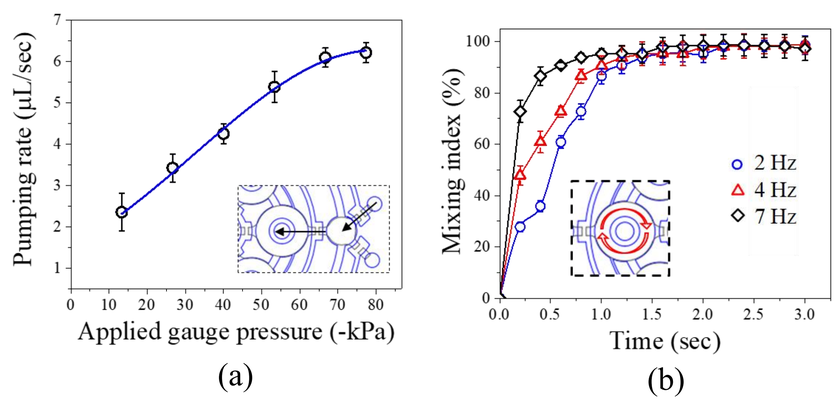


Figure 4: (a) Pumping rate of the micropump, and (b) mixing index of the open-type micromixer

5. On-chip process steps

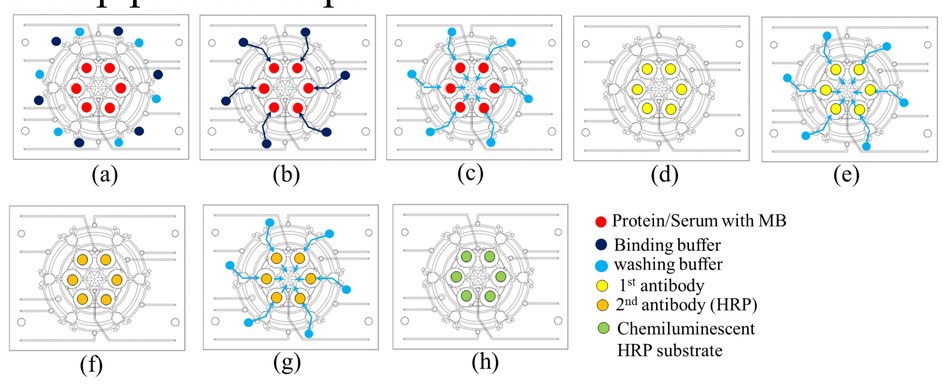


Figure 5: Schematics of the on-chip aptamer-based ELISA-like chemiluminescence assay. (a) Biotinylated aptamer was coated onto streptavidin-coated magnetic beads; (b) Incubation of pure proteins or serums with beads and subsequent washing; (c) Transportation and mixing of primary antibody and washing; (d) Transportation of secondary antibody; (e) Finally, chemiluminescence assay with chemiluminescent HRP substrate was performed.

6. Calibration curves

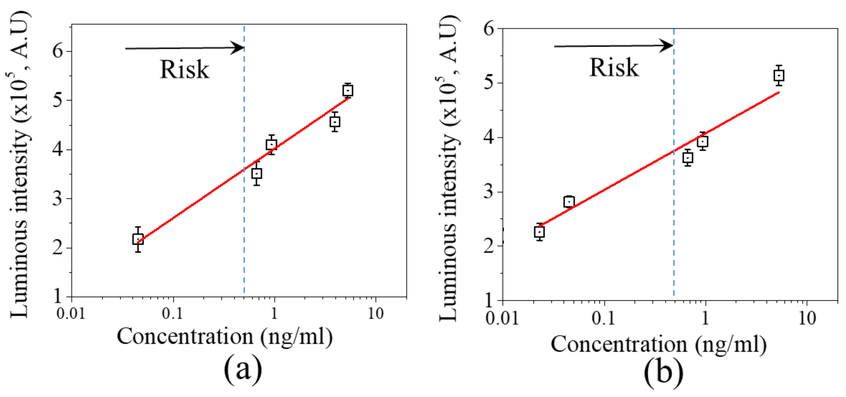


Figure 6: (a) calibration curve using pure cTnI protein and (b) clinical serum samples of known concentrations.

Conclusions

This study has successfully demonstrated a new automatic microfluidic system for the measurement of cardiac troponin I, which is an important CVD biomarker, from clinical samples using an aptamer-based ELISA-like assay. With this approach, the microfluidic system can achieve rapid detection of cTnI from just 5 µL of human serum samples without any pretreatment in 30 min. This study could replace antibodies in the conventional assay and could be useful for future point-of-care systems.

Acknowledgements

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