NANOZYME-AMPLIFIED LATERAL FLOW IMMUNOASSAY FOR MOLECULAR SIGNATURE DETECTION OF CARDIOVASCULAR DISEASES

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ABSTRACT

Point-of-care (PoC) devices offer the opportunity to decentralize the analysis of biomarkers in biological fluids thus providing patients with more personalized medicine. The golden standard of PoC platforms are lateral flow assays since they are low cost, quick to perform and user-friendly [1]. Here we show the use of a nanozyme-mediated signal readout on a multiplexed PoC lateral flow immunoassay for the diagnosis of cardiovascular diseases. Our aim has been to expand the application of this ultrasensitive detection method towards the development of a multiplexed PoC assay for cardiovascular-related biomarkers to support triage of myocardial injury.

KEYWORDS

Multiplexed, Paper-based, Platinum particles, Cardiovascular diseases

INTRODUCTION

The nanozymes employed here were first reported in ACS Nano in 2019 [2], where they showed a 100-fold signal amplification compared to conventional particle-based lateral flow immunoassays for the detection of a single HIV-related biomarker. Such lateral flow assay differs from conventional ones through the addition of a substrate to the test line that, upon catalysis by the nanozymes, produces a black precipitate (Figure 1A).

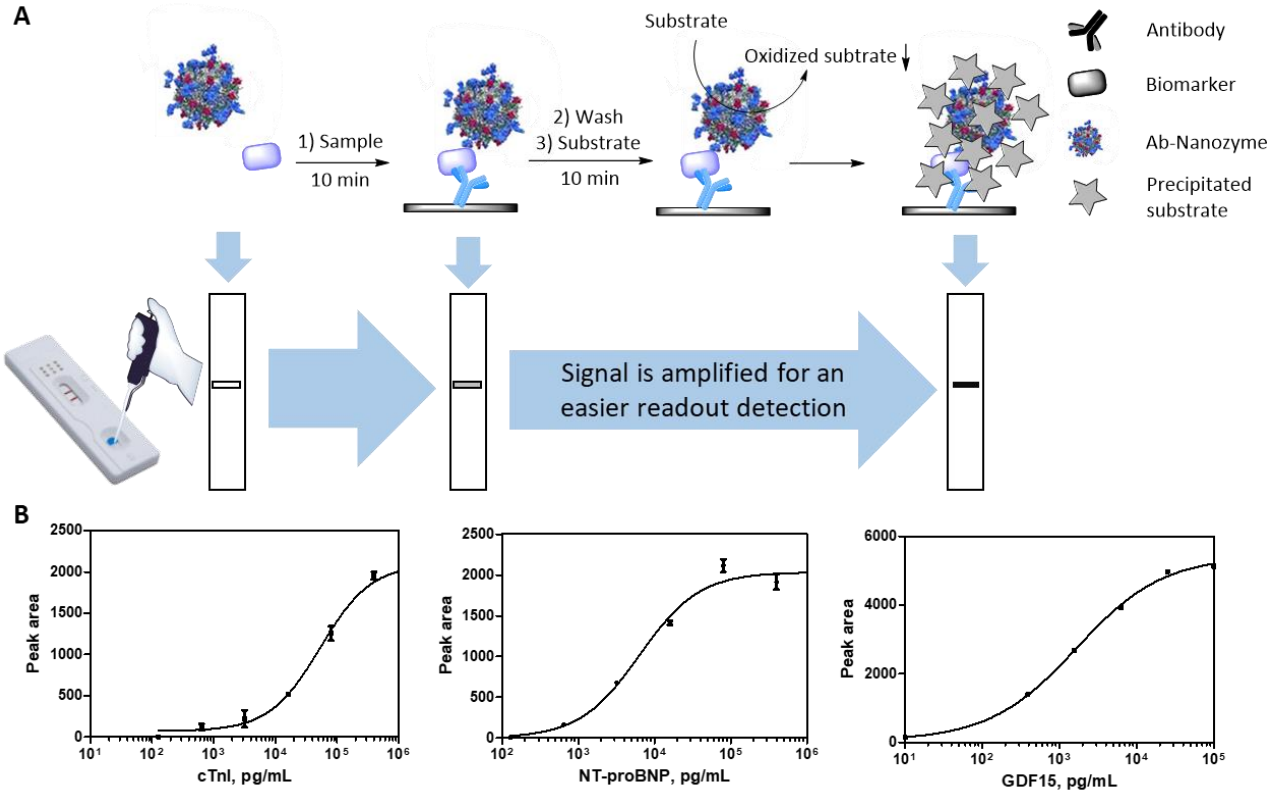

Figure 1: A: *Schematic showing the amplified LFIA. Nanozymes are mixed with a biological sample and, in presence of the biomarker, the complex is formed and the nanozymes bind to the test line.* ***B:*** *Calibration curve of cTnI, NT-proBNP and GDF15. Obtained LOD are 1570, 361 and 110 pg/mL, respectively.*

Nanozymes are platinum particles prepared by seeding Pt onto Au particle cores where the Pt shell confers them peroxidase-like activity that is able to catalyze the colorimetric conversion of a substrate. This strategy addresses a principal limitation of point-of-care systems, poor sensitivity, reaching LODs at a similar level to conventional ELISA.

EXPERIMENTAL

We selected a molecular signature of three biomarkers (hs-cTnI, NT-proBNP and GDF15) for the detection of myocardial injury. This signature should provide a better patient stratification in primary healthcare settings and thus a better patient treatment and outcome. Our approach aimed to meet the standards of lab-based techniques. To achieve the desired LOD, in the pg/mL range, the appropriate immunoreagents were selected and the nanozymes redesigned to achieve further signal amplification. Commercial antibodies were purchased and used to functionalize both the nitrocellulose paper and the nanozymes to form a sandwich complex that produces the signal in presence of the biomarker.

RESULTS AND DISCUSSION

The concentration of immunoreagents was optimized and reported LODs of 1570, 361 and 110 pg/mL for hs-cTnI, NT-proBNP and GDF15 respectively (Figure 1B). Furthermore, PoC analysis of troponins (Tn) has always focused on TnI which is not specific to a cardiovascular event. hs-cTnI has been employed in order to achieve higher clinical specificity and sensitivity. The assay specificity was tested by mixing all the particles and adding only one of the biomarkers demonstrating low crossreactivity of the immunoreagents (Figure 2A). Finally, we optimized the nanozyme design to produce a higher signal amplification since hs-cTnI rule-out guidelines recommend a lower LOD than the one we obtained. It was observed that the catalytic activity as a function of surface area of the smaller particles was higher than the bigger ones (Figure 2B). Further studies of the particle morphology revealed that smaller Pt seeding concentration produced a higher porosity and thus a higher catalytic activity.

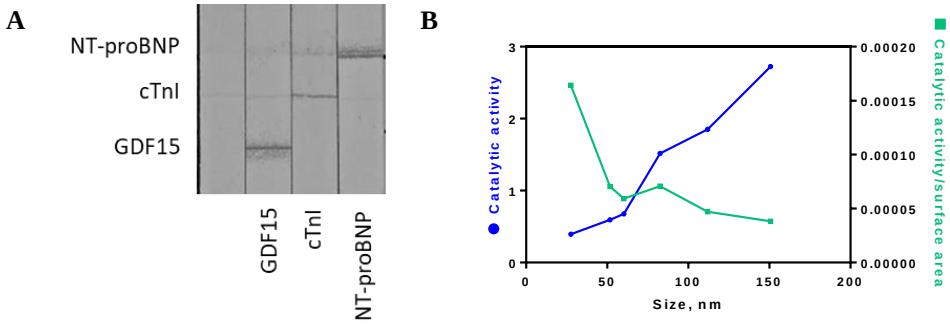

Figure 2: A: *Picture of the lateral flow strips showing high specificity for each antigen.* ***B:*** *Comparison of the catalytic activity of the particles depending on their size. Left axis compares the catalytic activity with the same concentration of particles, right axis has the correction for particle surface area.*

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