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Abstract

Multianalyte-Compatible Lysis for the Detection of *P. aeruginosa* and IL-6 via Lateral Flow Immunoassay †

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Abstract: The development of new multianalyte biosensors that can detect multiple classes of biomolecules is highly desirable and will greatly improve medical diagnostics. In the field of infectious diseases, for example, it is beneficial to detect pathogens via nucleic acid analysis together with host immune response markers. In this work, we present a multianalyte-compatible lysis using antimicrobial peptides (AMPs). This strategy enables the simultaneous detection of bacterial DNA and inflammatory biomarkers via multianalyte lateral flow immunoassay (LFIA).

Keywords: wound diagnostics; paper-based multianalyte biosensor; isothermal amplification

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1. Introduction

Wound infections represent a huge problem for patients and the healthcare system. Their early diagnosis is fundamental for a sufficient wound care. In this regard, it is important to differentiate between colonized and infected wounds [1]. Currently, there are no biosensors available that enable (i) the detection of wound pathogens and (ii) the differentiation between active and inactive infections. To tackle this need, we recently developed a novel multianalyte biosensor that enables the simultaneous detection of bacterial DNA and interleukin-6 (IL-6) from a single sample [2]. To realize our vision of a fully integrated biosensor, a multianalyte-compatible lysis strategy is of uttermost importance. Here, we report the usage of antimicrobial peptides (AMPs) for the lysis of *Pseudomonas aeruginosa* (*P. aeruginosa*) and subsequent detection of both biomarkers via LFIA.

2. Materials and Methods

AMPs were used to lyse *P. aeruginosa* (Figure 1A). For a proof-of-principle investigation, 105 CFU/mL *P. aeruginosa* and 500 ng/mL IL-6 were incubated for 5 min at room temperature in a lysis buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1% Brij 35) containing 50 µM of the AMP cecropin P1 (CP1). Next, the crude lysate (containing IL-6 and lysed bacteria) was added to the recombinase polymerase amplification (RPA) reaction (Figure 1B). *P. aeruginosa* DNA was amplified and labeled at 37 °C for 20 min, which was compatible with protein stability. Subsequently, IL-6 and the labeled amplicons were detected via LFIA (Figure 1C). The validity of the test results was assessed by flow controls (FC) and an internal amplification control (IAC). Details regarding the multianalyte LFIA have been described elsewhere [2]. AMP-based lysis was compared with bead beating (2 × 20 s 6800 rpm).

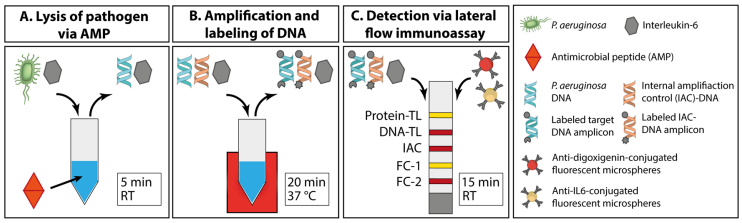

Figure 1. Workflow for the simultaneous detection of *P. aeruginosa* and IL-6. (**A**) Multianalyte-compatible lysis via AMPs. (**B**) Protein-compatible isothermal amplification and labeling of bacterial DNA and internal amplification control (IAC). (**C**) Simultaneous detection of IL-6 and labeled amplification products via LFIA.

3. Results and Discussion

Our recently reported paper-based multianalyte biosensor enables the simultaneous detection of bacterial DNA and IL-6 [2]. For the simultaneous detection of pathogens and inflammatory biomarkers, a multianalyte-compatible lysis strategy is of uttermost importance. Here, we report the usage of CP1, an AMP, for the lysis of *P. aeruginosa* and subsequent detection of bacterial DNA and IL-6 via LFIA (Figure 1). We show that in presence of IL-6 and/or *P. aeruginosa*, a signal is generated at the corresponding test lines (TL) (Figure 2), which constitutes a positive test result, whereas for non-lysed samples containing *P. aeruginosa*, only a low signal is generated, which is comparable to samples without *P. aeruginosa*. Compared with bead beating, a signal decrease of about 31 ± 11% was observed for samples containing *P. aeruginosa*. Thus, further optimization is required. In summary, these promising results indicate that AMPs can offer a fast and simple multianalyte-compatible lysis strategy that paves the way towards a point-of-care multianalyte biosensor for wound diagnostics.

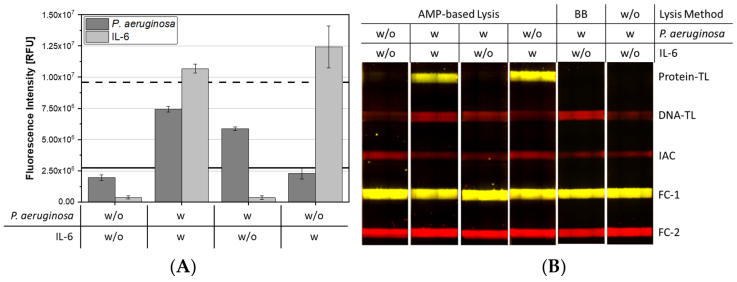

Figure 2. AMP-based lysis for the simultaneous detection of *P. aeruginosa* and IL-6. (**A**) Intensity of protein-TL and DNA-TL for samples with (w) or without (w/o) IL-6 and/or *P. aeruginosa*. The solid and dashed lines represents the fluorescence intensity at the DNA-TL of non-lysed samples and samples lysed via bead beating (BB). Depicted are the mean and standard deviations; n = 3. (**B**) Corresponding fluorescence images of the multianalyte LFIA.

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