

Colorimetric point-of-care detection of *Enterococcus faecalis* in urinary tract infection

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Summary:

Urinary tract infections are among the most common bacterial infections, often diagnosed through culture-based methods, which are time-consuming and costly. In this work, we developed a rapid, specific, and accurate point-of-care colorimetric sensor for indirect detection of *Enterococcus faecalis* in urine samples via its metabolite, L-lactate. The probe consists of silica nanoparticles loaded with MnO₂, functionalized with the enzyme lactate oxidase. The probe allows both visual qualitative analysis and spectroscopy quantitative analysis.

Keywords: Point-of-Care device, Colorimetric sensor, Silica nanoparticle, Urinary tract Infection, ASSURED

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting up to 150 million people every year [1]. Currently, diagnosis is often made using culture-based methods, which are time-consuming, leading to the administration of antibiotics without a clear diagnosis [2]. Point-of-care (POC) devices may enable a fast and accurate UTI diagnosis, thereby improving treatment efficacy [3]. Colorimetric sensors, used as POC devices, present several advantages, including a colour change visible to the naked eye [4], being a simple, specific, and sensitive solution that supports the development of rapid, cost-effective, user-friendly, and affordable diagnostic tools that do not require complex or expensive equipment.

In this work, we developed a fast and cost-effective colour-based indirect method for the detection of *E. faecalis* in urine samples, through targeting its metabolic, L-lactate. The sensing probe (Lac@MnSNP) consists of silica nanoparticles loaded with manganese dioxide (MnSNP) and functionalized with the enzyme lactate oxidase. In the presence of L-lactate, an enzymatic

cascade is triggered, with the production of hydrogen peroxide (H₂O₂). The presence of H₂O₂ reduces MnO₂ to MnO/Mn²⁺, resulting in a visible colour change from brownish to colourless. The probe also allows a quantitative detection, through the monitoring of MnO₂ reduction by measuring the decrease in absorbance at 400 nm, apart from the qualitative detection.

Results

MnSNP particles were synthesized through the reverse microemulsion method and characterized through DLS and TEM, producing a homogeneous suspension of nanoparticles with an average size of 158 ± 3 nm and a zeta potential of -34 ± 1 mV. The functionalization of MnSNP with lactate-oxidase led to an increase in the dynamic size to 1438 ± 52 nm, with the core particle size remaining similar to MnSNP (153 ± 1 nm for MnSNP and 105 ± 1 nm for Lac@MnSNP). The difference in dynamic size can be explained by the increase in surface charge in Lac@MnSNP to -13 ± 1 nm, which can promote nanoparticle agglomeration.

Lac@MnSNP at 2 mg/mL was treated against H₂O₂ with concentration ranging from 0 μ M to

100 μM , to evaluate the detection efficacy of the probe. Results are reported as relative absorbance, calculated as the absorbance intensity at each H_2O_2 concentration divided by the untreated sample $[I_n/I_0]$. The absorbance signal of Lac@MnSNP decreased with increasing L-lactate concentration, decreasing in signal to 0.70 at 50 μM of L-lactate, and a calculated LOD of 31 μM ($R^2 = 0.94$) (Fig. 1). These results confirm that Lac@MnSNP effectively catalyzes L-lactate, allowing a quantification through the detection of generated H_2O_2 . The decrease in absorbance indicates the presence of *E. faecalis*, via the detection mechanism of the probe.

Urine is a complex matrix containing various components that could interfere with the probe's performance. To assess the potential matrix effect of the sample in the probe, Lac@MnSNP was tested in an artificial urine medium (AUM). Lac@MnSNP presented similar behaviour in both media, having a decrease in signal to 0.68 at 50 μM L-lactate in AUM, with an LOD of 47 μM ($R^2 = 0.86$), compared to 31 μM in DI water (Fig. 1). These findings indicate that the complex composition of AUM influences the sensitivity of the probe. Nevertheless, the probe remains suitable for the intended application, since bacteria associate with UTIs produce 59–241 $\mu\text{g/mL}$ of L-lactate during UTIs [5], whereas normal urine does not typically contain L-lactate [6].

As a proof-of-concept, the responsiveness of the probe was evaluated, testing 2 mg/mL of Lac@MnSNP against the lactate-producing bacteria *E. faecalis* (Fig. 2). The increase of concentration of *E. faecalis* led to a linear decrease of the absorbance signal, reaching a saturation at 10^3 CFU/mL. Additional increase of *E. faecalis* to 10^4 and 10^5 CFU/mL resulted in higher absorbance signals, likely due to the detection range of the probe being exceeded. For bacterial concentrations up to 10^3 CFU/mL, it was calculated concentrations of L-lactate of 56 μM . The calculated concentrations of L-lactate are above the LOD of the probe (31 μM), showing the potential of the probe in detecting bacterial load up to 10^3 CFU/mL. Further details are available in the full manuscript [7].

Conclusion

We present a colorimetric probe for the indirect detection of *E. faecalis* in urine samples, based on its metabolite, L-lactate. This strategy enhances the reliability of detection in comparison to conventional pH-based methods. Additionally, the proposed method fulfills several criteria of the ASSURED criteria [2], being cost-efficient, with a simple preparation of the probe and the use of simple instrumentation for the measurement.

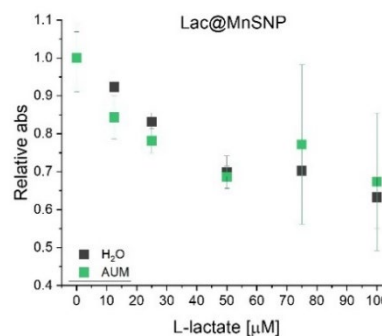


Fig. 1. Responsiveness of 2 mg/mL of Lac@MnSNP against L-lactate, in artificial urine medium (AUM) or water, after 4 h.

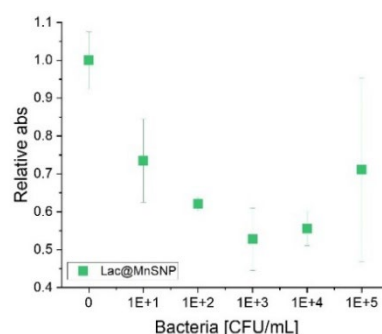


Fig. 2. Responsiveness of 2 mg/mL Lac@MnSNP against *E. faecalis*, after 5 h incubation

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