

Cite this: *Chem. Commun.*, 2019, 55, 15129Received 3rd October 2019,
Accepted 5th November 2019

DOI: 10.1039/c9cc07759f

rsc.li/chemcomm

Reaction-based indicator displacement assay (RIA) for the development of a triggered release system capable of biofilm inhibition†

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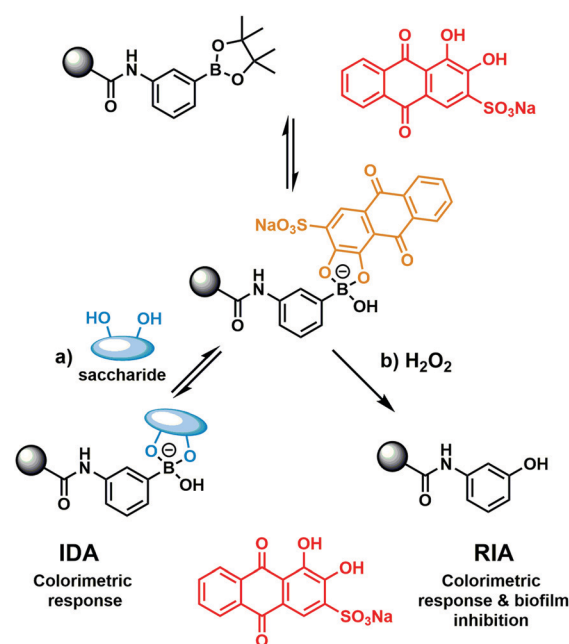
Here, a reaction-based indicator displacement hydrogel assay (RIA) was developed for the detection of hydrogen peroxide (H₂O₂) via the oxidative release of the optical reporter Alizarin Red S (ARS). In the presence of H₂O₂, the RIA system displayed potent biofilm inhibition for Methicillin-resistant *Staphylococcus aureus* (MRSA), as shown through an *in vitro* assay quantifying antimicrobial efficacy. This work demonstrated the potential of H₂O₂-responsive hydrogels containing a covalently bound diol-based drug for controlled drug release.

Dye displacement assays exploit the chemoselective reactivity of certain chemical moieties and the reversible binding of dye molecules to a specific receptor.¹ Such chemistry has begun to find widespread use with marked enhancement over traditional sensing assays.^{1–8} More complex systems containing multiple dyes also offer new paradigms for microarray development.⁹ Not surprisingly, dye displacement assays have been elegantly employed by a number of research groups. These constructs often rely on boronic acid systems as the receptor (host) subunit with a 1,2- and 1,3-diol guest.¹⁰

Previously our group has developed boronate-based hydrogel systems as dye displacement assays (borogel) for monosaccharide detection.^{11,12} As shown in Scheme 1, the commercially available 1,2-diol dye Alizarin Red S (ARS) was shown to successfully bind to the boronate hydrogel and result in a colour change from red to orange. Upon the addition of a monosaccharide, the

competitive displacement of ARS was observed with concomitant observation of an increase in absorption at 513 nm in solution (ARS wavelength).

Aryl boronic acids/boronate esters are well known to undergo hydrogen peroxide (H₂O₂)-mediated oxidation to form the corresponding phenol.¹³ This unique synthetic transformation has been exploited in organic synthesis and fluorescence sensing.¹³ We thus envisaged that modification of the previously developed ARS hydrogel bound indicator displacement assay (IDA) would yield a multimodal detection platform for the



Scheme 1 (a) Previously developed hydrogel bound dye displacement assay (IDA) for the detection of monosaccharides.^{11,12} (b) Present work – the development of a hydrogel bound reaction-based indicator displacement (RIA) assay for the detection of H₂O₂ and for the inhibition of MRSA biofilm formation.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9cc07759f

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Fig. 3 Biofilm inhibition of *S. aureus* MRSA252 when treated with 2 mM H₂O₂ and solution containing released ARS from ARS-**PBA**-based gel using 2 mM H₂O₂ (3 h incubation). Experiments were repeated using three biological replicates, and error bars indicate standard deviation. Statistical significance of biofilm inhibition was assessed by performing a one-way ANOVA using GraphPad 7.0. **** $p \leq 0.001$ relative to untreated control.

the lag phase of growth (0 h), similar to other reports in the literature for Alizarin.²¹ However, ARS was unsuccessful in the inhibition of *P. aeruginosa* PAO1 and *E. coli* NCTC 10418 biofilms at concentrations below 100 μM (see Fig. S14–S16, ESI[†]). Additionally, H₂O₂ inhibited biofilm formation, albeit at much higher concentrations, prevents growth at 5 mM for *S. aureus* MRSA252 and *S. aureus* H560, 10 mM for *E. coli* NCTC 10418, and 100 mM for *P. aeruginosa* PAO1 (see Fig. S17–S19, ESI[†]). H₂O₂ (2 mM) with ARS (50 μM) acted synergistically, effecting biofilm inhibition of *S. aureus* MRSA252 when added during the lag phase (Fig. S20, ESI[†]). Unfortunately, this combination was unable to inhibit biofilm formation at all other growth phases for each bacterial strain (Fig. S21 and S22, ESI[†]).

We next turned our attention towards the ability of the **PBA-ARS** hydrogel system to inhibit MRSA biofilm formation. Initial control experiments were carried out. **PBA-ARS** gel incubated in PBS solution for 3 h was shown to result in no biofilm inhibition, which indicates the requirement of H₂O₂ for ARS release and no off-target gel toxicity (Fig. S23, ESI[†]). Control “blank” acrylamide gels were subsequently tested to evaluate the requirement of the boronic acid units for the H₂O₂-mediated release of ARS. Following the usual ARS-loading protocol (see ESI[†]), acrylamide gels loaded with ARS (ARS uptake through passive diffusion – see Fig. S2, ESI[†]) were treated with H₂O₂. No biofilm inhibition was observed, which illustrated the requirements of the boronic acid units for H₂O₂-mediated release of ARS from the hydrogel (Fig. S24, ESI[†]). To capitalize upon the H₂O₂-mediated release of ARS from the boronic acid containing polyacrylamide hydrogel, **PBA**-based gels (0.1 g comprising of 2.5×10^{-4} M ARS) were incubated with H₂O₂ (2 mM) for 3 h to achieve maximum ARS release (cf. Fig. 1). The resultant ARS release was then applied to *S. aureus* MRSA252 at the lag phase. Remarkably, this resulted in complete biofilm inhibition (Fig. 3) thus demonstrating the potential of the **PBA**-based gels as a “smart” wound dressing.

In summary, we have developed a multimodal reaction-based indicator displacement (RIA) hydrogel assay for the

detection of H₂O₂ with concomitant release of ARS for antimicrobial application. The greatest reactivity towards H₂O₂ was observed for the **PBA**-based gel compared to the **BOB**-based gel, attributed to attenuated reactivity of the cyclic **BOB**-boronate ester. In addition, the antimicrobial efficacy of each assay component was evaluated with the aim of developing a “triggered release” antimicrobial hydrogel. ARS was discovered to be a potent biofilm inhibitor in combination with hydrogen peroxide against *S. aureus* MRSA252, with the ARS loaded **PBA**-based gel successfully inhibiting biofilm formation. These results lead us to suggest that **PBA**-based gels, in combination with an early bacterial detection system for a MRSA biomarker, might find use as a “smart” wound dressing capable of preventing MRSA biofilm formation.²⁶

The authors would like to thank the EPSRC for grant EP/R003556/1. BLP would also like thank James Tudor and Mr and Mrs Watson for additional funding. GTW would like to thank the EPSRC and Public Health England. ACS, ATAJ and NTT wish to thank the EPSRC for funding on Smartwound plasma – EP/R003939/1. TDJ wishes to thank the Royal Society for a Wolfson Research Merit Award. We would like to thank James T. Brewster II at Harvard college for helpful discussions and suggestions to improve the manuscript.

Conflicts of interest

No conflicts of interest.

Notes and references

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