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COMMUNICATION

Boronate ester cross-linked PVA hydrogels for the capture and H_2O_2-mediated release of active fluorophores

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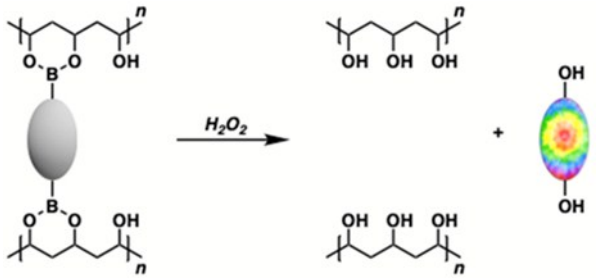
A new set of PVA hydrogels were formed using the boronate ester fluorescent probe, PF1 and the novel boronate fluorescent probe PT1 as the covalent crosslinkers. Treatment with aqueous H_2O_2 allowed triggered release of the fluorescent dye accompanied by complete dissolution of the hydrogel.

Functional hydrogels have generated widespread interest as so-called intelligent devices wherein a specific stimulus can yield a macroscopic change to the self-supporting material.[1, 2] Such constructs offer promise in the area of drug delivery and design of "smart" wound dressings.[3-6] In addition, these functional hydrogels have demonstrated great potential as fluorescent probes for live cell imaging, disease diagnosis and sensing applications with the controlled release of a fluorophore.[7] These constructs have utilised non-covalent interactions such as aromatic–aromatic, hydrogen bonding, and hydrophobic interactions. Unfortunately, these interactions can result in the unwanted leaching of the active molecule from the hydrogel matrix. Next generation systems comprised of a pro-molecule backbone covalently linked to the hydrogel may address these issues by providing a higher local dose and sustained/controlled release of the bioactive molecule.[8] Here, we demonstrate a new set of controlled release materials wherein hydrogen peroxide (H_2O_2) is used as a stimulus to release fluorophores from polyvinyl alcohol (PVA) boronate hydrogels.



Scheme 1 - Cartoon representation illustrating the boronate pro-fluorophore encapsulated within a PVA hydrogel being activated by H_2O_2 to release the active fluorophore.

Boronic acid and boronate esters have found widespread application in material-based applications, in part because of their propensity to bind reversibly with 1,2-and 1,3-diols.[9-19] Such chemistry has been demonstrated *inter alia* using commercially available polyvinyl alcohol (PVA) and diboronic acid crosslinkers to afford functional PVA-boronate hydrogels.[20-25] Boronic acids and boronate esters are well-known to undergo hydrogen peroxide (H_2O_2)-mediated oxidative transformations to afford their corresponding phenol functionalites.[26-28] We envisaged that the use of *bis*-boronate-based pro-molecules as cross-linkers would afford a H_2O_2-responsive hydrogel platform that would allow the controlled and localised release of an active molecule, such as a fluorophore (Scheme 1). It is important to note the boronate functionality is commonly used to mask active therapeutics.[29, 30] Currently, there is considerable interest in functionalized hydrogels wherein a specific stimulus can yield a macroscopic change to the self-supporting material, including for the stimulus-based release of specific payloads.[31, 32,33, 34] However, new approaches to achieving such overarching objectives are still needed.

To address the above need, we have now prepared a new class of H_2O_2-responsive PVA-boronate hydrogels. These systems rely on covalent cross-linking provide solely by a set of constituent H_2O_2-responsive boronate ester fluorescent probes, namely the known fluorophore **PF1**[26] and the novel fluorescent

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probe, **PT1** (Figure 1). The resultant hydrogel constructs **Greenment (Gment)** and **Purplement (Pment)** displayed stability over 7 days in both aqueous solution and in the air; however, upon exposure to aqueous H_2O_2 the polymers were oxidised thus releasing their constituent fluorophores, fluorescein[26] and thionol[35] (Schemes S1 and S2). Complete dissolution of the hydrogel could be effected depending on the specific choice of conditions as detailed below.

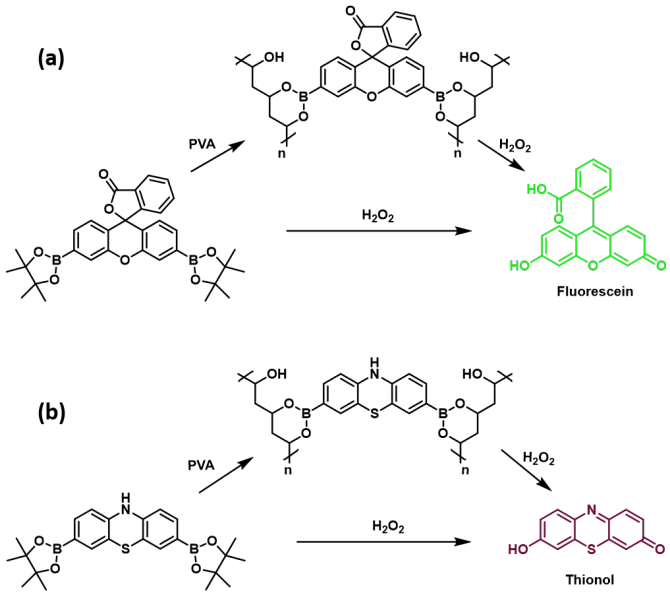

Figure 1 - (a) H_2O_2-responsive fluorescent probe **PF1** and its corresponding H_2O_2-responsive PVA hydrogel **Gment**. **(b)** H_2O_2-responsive fluorescent probe **PT1** and its corresponding H_2O_2-responsive PVA hydrogel **Pment**.

PF1 was prepared following literature procedures.[26] The novel fluorescent probe **PT1** was synthesized through the dibromination of commercially available phenothiazine (**1**) using Br_2 (5 equiv.) in acetic acid at room temperature, giving the desired product in 74% yield. Subsequent Suzuki-Miyaura borylation using potassium acetate, bis(pinacolato)diboron, and $Pd(dppf)Cl_2$ afforded **PT1** in 43 % yield.

With **PF1** and **PT1** in hand, UV and fluorescence analyses were performed. Upon exposure to aqueous H_2O_2 at concentrations as low as 125 µM, **PF1** exhibited a colour change from clear to green with an increase in absorption at 490 nm and an increase in fluorescence emission at 520 nm, which corresponded to the release of fluorescein (Fig. S1 and S2). Whereas, exposure of **PT1** to H_2O_2 in an analogous manner led to a colour change from clear to purple and a concomitant increase in the absorption intensity at 595 nm and an increase in fluorescence emission at 610 nm. These optical changes reflected the release of free thionol as confirmed by high resolution mass spectrometry, Fig. S3-S5. It is important to note that in this work, we have focused on the use of these boronate-based hydrogels as materials whose controlled release may be triggered by H_2O_2. However, previous reports have demonstrated the greater reactivity of boronate-based fluorescent probes towards peroxynitrite ($ONOO^-$).[16, 36-39] Therefore, it is likely that if used in cellular applications, both **PF1** and **PT1** could have a role to play in the fluorescence imaging of both $ONOO^-$ and H_2O_2, albeit not necessarily in a species specific manner.

Next, the **Gment** and **Pment** PVA-hydrogels were prepared by mixing a solution of either **PF1** or **PT1** (100 mM) in dimethylsulfoxide (DMSO) with a DMSO solution of 10% PVA (low molecular weight; purchased commercially) in a 1:1 ratio. This solution was then heated to induce gelation, followed by heating at 60 °C overnight in an oven. The resultant gels were washed with hexanes to remove the displaced pinacol and water to remove excess DMSO. These self-supporting gels proved physically robust and stable in air and could be stored in aqueous media (PBS, pH 7.4) without degradation for 7 days until used Fig. S6-S8.

The ability of **Gment** or **Pment**-based PVA-hydrogel to release the corresponding dye in the presence of H_2O_2 was then evaluated. This was done by submerging the chosen hydrogel (200 ± 10 mg) in aqueous solutions containing different concentrations of H_2O_2. As shown in Figure 2, exposure of **Gment** gels to H_2O_2 (0 – 1 mM) led to a dose-dependent increase in the fluorescence emission intensity. The colorimetric nature of **Gment** was then tested by placing the hydrogel (200 ± 10 mg samples) in an aqueous solution of H_2O_2 (1 mL, 1 mM). A change in colour from colourless to green ensued. Analysis of the UV-Vis absorption revealed an increase in two absorption peaks at 450 nm and 490 nm (Fig. S9). The absorption peak at ~ 450 nm is tentatively assigned to the release of monoboronate **PF3**[40] and the absorption peak at 490 nm corresponds to the release of fluorescein. Based on this result, we believe that oxidation of only one boronate linkage is required to release the fluorescent cargo from the PVA-hydrogel system (Scheme S3).

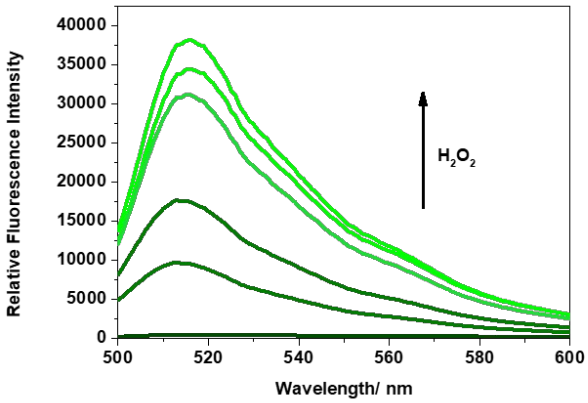

Figure 2 – Fluorescence spectra of the supernatant of **Gment**-based PVA- hydrogels exposed to various concentrations of H_2O_2 (0 – 1 mM) in PBS, pH 7.4. Measurements were taken after 5 min at 25 °C. λ_{ex} = 472 (bandwith: 16 nm) on a BMG Labtech CLARIOstar® plate reader.

As shown in Figure 3, **Pment** PVA-hydrogels exposed to various concentrations of H_2O_2 (0 – 1 mM) also led to a dose-dependent increase in the fluorescence intensity at the emission maximum of 610 nm. The colorimetric nature of **Pment** was then tested by placing the hydrogel (200 ± 10 mg samples) in an aqueous solution of H_2O_2 (1 mL, 1 mM). A readily discernible change in colour was observed from colourless to purple with an increase in the absorption intensity at 595 nm (See ESI - Figs. S9 - S11). In comparison to one another, **Gment** was found to be more

sensitive to H_2O_2 than **Pment** (Figs. S12 and S13). This finding is reflected in **Gment** having a lower Limit of Detection ((LoD) - **Gment** = 0.12 mM, LoD **Pment** = 0.33 mM). However, it is important to note, these calculated LoD values are dependent upon incubation times.

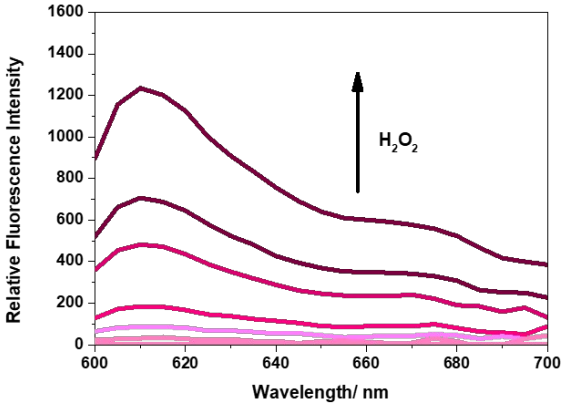

Figure 3 – Fluorescence spectra of the supernatant of **Pment**-based PVA- hydrogels exposed to various concentrations of H_2O_2 (0 – 1 mM) in PBS, pH 7.4. Measurements were taken after 5 min at 25 °C. λ_{ex} = 570 (bandwith: 16 nm) on a BMG Labtech CLARIOstar® plate reader.

Notably, subjecting the hydrogels to an aqueous solution of H_2O_2 (100 mM) resulted in the complete dissolution of the hydrogels into solution, as shown in Figure 4. Of note is that commercially available 3% H_2O_2 sold for consumer use is approximately 980 mM. The present work thus demonstrates the potential utility of boronate-based PVA polymers as a smart material for the masking and facile release of easy-to-visualise fluorophores using a readily accessible trigger. Lastly, an MTT assay with A549 cells was carried out using the **Gment** gel. At concentrations up to 50 µg/mL, A549 cells displayed at least 80% viability, thus demonstrating minimal acute cytotoxicity in this well-studied cell line (Fig. S14). We believe these findings provide further support for the suggestion that the present approach may prove useful in achieving the controlled delivery of fluorescence-based diagnostics and active pharmacophores.[29]

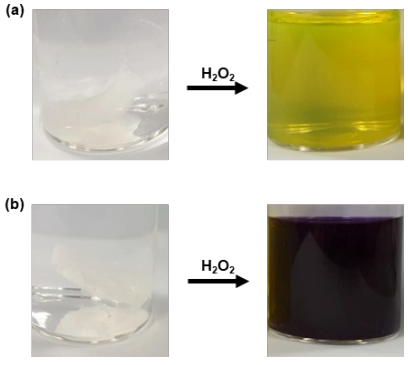

Figure 4 – (a) **Gment** and (b) **Pment** PVA-hydrogels before and after 1.5 h exposure to 100 mM H_2O_2 in PBS, pH 7.4.

In conclusion, we report here the synthesis of a new H_2O_2-responsive *bis*-boronate fluorescent probe, **PT1**, and the synthesis of the previously reported H_2O_2-responsive fluorescent probe **PF1**. Both **PT1** and **PF1** were successfully used as diboronic acid crosslinkers to form air and aqueous stable PVA-based hydrogels. Exposure of these initially colourless and non-fluorescent systems to aqueous solutions of H_2O_2 allowed for the controlled release and activation of the encapsulated fluorophore. We believe these systems represent a masking and delivery strategy that has the potential to achieve the controlled and localised release of boronic acid-based sensors and pro-drugs.[29]

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Conflicts of interest

There are no conflicts to declare.

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