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Supporting Information

A H₂O₂-Free Depot for Treating Bacterial Infection: Localized Cascade Reactions to Eradicate Biofilm in Vivo

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Materials
Graphite was provided by Sinopharm Chemical Reagent (Shanghai, China). Hemin, CaO₂, alginate, and CaCl₂ were purchased from sigma. Chitosan was purchased from BBI Life Co., Ltd. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) strains were purchased from Chuanxiang biotechnology, Ltd. (Shanghai, China). Other reagents and solvents were achieved from Beijing Chemicals (Beijing, China). Ultrapure water (18.2 MΩ, Millipore Co., USA) was used throughout all experiments.

Instruments
SEM images were obtained with a Hitachi S-4800 FE-SEM. TEM images were acquired with a TECNAI G2 transmission electron microscope (Philips Electronic Instruments Co., the Netherlands) at 200 kV. AFM measurements were performed on Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). UV–Vis spectroscopy was carried out on JASCO V-550 UV-Vis spectrophotometer. Fluorescence measurements were carried out on a JASCO FP-6500 spectrofluorometer. Fluorescence images were performed on an Olympus BX-51 optical equipped with a CCD camera. Thermogravimetry (TGA) was performed with a Pyres 1 TGA apparatus (Perkin Elmer, MA) at a heating rate of 10 ºC/min from 25 to 1000 ºC under a nitrogen atmosphere. Photographs were taken with a Canon digital camera.

Cleavage of polysaccharides
Chitosan hydrogels were prepared with glutaraldehyde crosslinking. Chitosan (1%) was dissolved in 500 μL of NaAc (0.1 M, pH 5.5) and incubated with 2% glutaraldehyde at 25 °C for 1 h. The hydrogel was washed with water to remove uncrosslinked chitosan. After contacting with filter paper for 1 h, the mass of hydrogel was recorded. Hydrogel were treated with as-prepared depots for 6 h. The corresponding mass of remaining gel was also recorded.

Cleavage of nucleic acids and proteins
To investigate the degradation of DNA, *S. aureus* genome DNA was extracted with a bacteria genome DNA kit. Unless indicated otherwise, nucleic acid cleavage assays were performed at 37°C for 48 hours in 50 μl NaAc buffer (0.1 M, pH 5.5) containing various depots. In these and later experiments, nucleic acid cleavage products were identified with agarose gel electrophoresis and ethidium bromide staining. To obtain whole-cell proteins, *S. aureus* cells were disrupted by sonication, and soluble supernatant was obtained by centrifugation (13,000 rpm for 15 min). Protein cleavage assays were performed at 37 °C for 48 hours in 50 μl NaAc buffer (0.1M, pH 5.5) containing CaO₂/H-G@alginate prior to separation with SDS-PAGE and Coomassie staining.
Figure S1. X-ray diffraction patterns that reveal decomposition of CaO$_2$ and the formation of Ca(OH)$_2$.

Figure S2. (a) The absorbance of I$_3^-$, a product of the reaction with KI and H$_2$O$_2$, is taken to quantify H$_2$O$_2$ concentration. (b) The calibration curve was obtained from the absorbance of I$_3^-$ based on the fixed KI concentration with different concentration of H$_2$O$_2$.

Figure S3. Representative TEM image of GS.
Figure S4. AFM images of (a) GS and (b) H-G.

Figure S5. TGA of GS (red), hemin (black) and H-G (blue).

Figure S6. The effect of pH on the peroxidase-like activity of (a) hemin and (b) H-G with 2.5mM ABTS as the substrate. Error bars were taken from three parallel experiments.
Figure S7. Plots of the ABTS oxidation reaction rates with H$_2$O$_2$ catalyzed by H-G (red, 6.25 μg/mL) and equivalent hemin (black, 2.56 μg mL$^{-1}$) versus the concentration of H$_2$O$_2$.

Table S1. The comparison of K$_m$ and V$_{\text{max}}$ with H-G and hemin.

<table>
<thead>
<tr>
<th>Sample</th>
<th>K$_m$ (mM)</th>
<th>V$_{\text{max}}$ (μM S$^{-1}$)</th>
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<tbody>
<tr>
<td>H-G</td>
<td>2.568</td>
<td>0.185</td>
</tr>
<tr>
<td>hemin</td>
<td>5.371</td>
<td>0.171</td>
</tr>
</tbody>
</table>

The apparent kinetic parameters were calculated using the Lineweaver-Burk plot:

$\frac{1}{v} = \frac{K_m}{V_{\text{max}}} - \frac{[S]}{V_{\text{max}}}$

where $v$ was the initial velocity, $V_{\text{max}}$ was the maximal reaction velocity, $K_m$ was the Michaelis constant, and $[S]$ was the substrate concentration.

Figure S8. a) Schematic illustration of preparing CaO$_2$/H-G@alginate depots and the corresponding photographs. b) Variation of pH of culture medium treated with various doses of CaO$_2$/H-G@alginate. Error bars were taken from three parallel experiments.
Figure S9. Typical SEM images of a-b) alginate, c-d) H-G@alginate, e-f) CaO$_2$@alginate, g-h) CaO$_2$/H-G@alginate respectively.

Figure S10. The UV-Vis spectra of different samples with ABTS (2.5 mM) after 6 h incubation.
**Figure S11.** Optical density at 600 nm of *S.aureus* and *E.coli* after treated with different depots (20 pellets mL\(^{-1}\)) for 3 h and 6 h respectively.

**Figure S12.** *E.coli* after treatment with different depots at 20 pellets mL\(^{-1}\) were cultured on agar plates. The bacterial samples 1-6 represented *E.coli* cells were treated with blank, alginate, H-G@alginate, CaO\(_2\)@alginate, mixed depots, CaO\(_2\)/H-G@alginate respectively. (n=3)

**Figure S13.** *S. aureus* after treatment with different depots at 20 pellets mL\(^{-1}\) were cultured on agar plates. The bacterial samples 1-6 represented *S. aureus* cells were treated with blank, alginate, H-G@alginate, CaO\(_2\)@alginate, mixed depots, CaO\(_2\)/H-G@alginate respectively. (n=3)
Figure S14. Optical density at 600 nm of *S. aureus* and *E. coli* after treated with different treatments for 6 h. The concentration of CaO$_2$/H-G@alginate and vitamin C (Vc) were 20 pellets mL$^{-1}$ and 10 mM, respectively, n=3.

![Figure S14](image)

Figure S15. Inhibiting the formation of biofilm. (a) Biofilms are visualized by crystal violet staining and (b) corresponding quantitative analysis. n=3, *p <0.05; The plate samples showing colonies of *S. aureus* after different treatments. c) Samples 1-6 represented blank, alginate, H-G@alginate, CaO$_2$@alginate, mixed depots, CaO$_2$/H-G@alginate respectively (20 depots/mL). (n=3)
**Figure S16.** Eradicating the formed biofilm. (a) Biofilms were visualized by crystal violet staining and (b) the corresponding quantitative analysis. n=3, *p <0.05; c) The plate samples showing colonies of *S. aureus* incubated with The bacterial samples 1-6 represented blank, alginate, H-G@alginate, CaO\textsubscript{2}@alginate, mixed depots, CaO\textsubscript{2}/H-G@alginate respectively (20 depots/mL). (n=3)

**Figure S17.** LIVE/DEAD stain images of residual biofilms using fluorescence microscopy to analyse the effects of inhibiting the formation of biofilm (a) and eradicating the formed biofilm (b). (Scale bar = 100 μm). Green and red stains indicate viable and dead bacteria respectively. Groups 1-6 represented blank, alginate, H-G@alginate, CaO\textsubscript{2}@alginate, mixed depots, CaO\textsubscript{2}/H-G@alginate respectively.
Figure S18. (a) Photographs of the chitosan gels before and after different treatments. (b) The residual ratio of chitosan gels. (c) Genome DNA and (d) protein were incubated with different depots 48 h respectively. 1-6 represented blank, alginate, H-G@alginate, CaO$_2$@alginate, mixed depots, CaO$_2$/H-G@alginate respectively.

Figure S19. Histological analysis of the health tissue around implanted site. (Scale bar = 100 μm)

Figure S20. Histological sections of heart, liver, spleen, lung and kidney of the mouse. (Scale bar = 50 μm)