

Supporting information

Ultrasonic interfacial crosslinking of TiO₂-based nanocomposite hydrogels through thiol-norbornene reactions for sonodynamic antibacterial treatment

Su-Rung Yang^{a, †}, Reuben Wang^{b, c, d, †}, Chen-Jie Yan^a, Yi-Yun Lin^a, Yu-Jia Yeh^b, Ying-Yu Yeh^a, and
Yi-Cheun Yeh^{a*}

^a Institute of Polymer Science and Engineering, National Taiwan University, Taipei, Taiwan

^b Institute of Food Safety and Health, National Taiwan University, Taipei, Taiwan

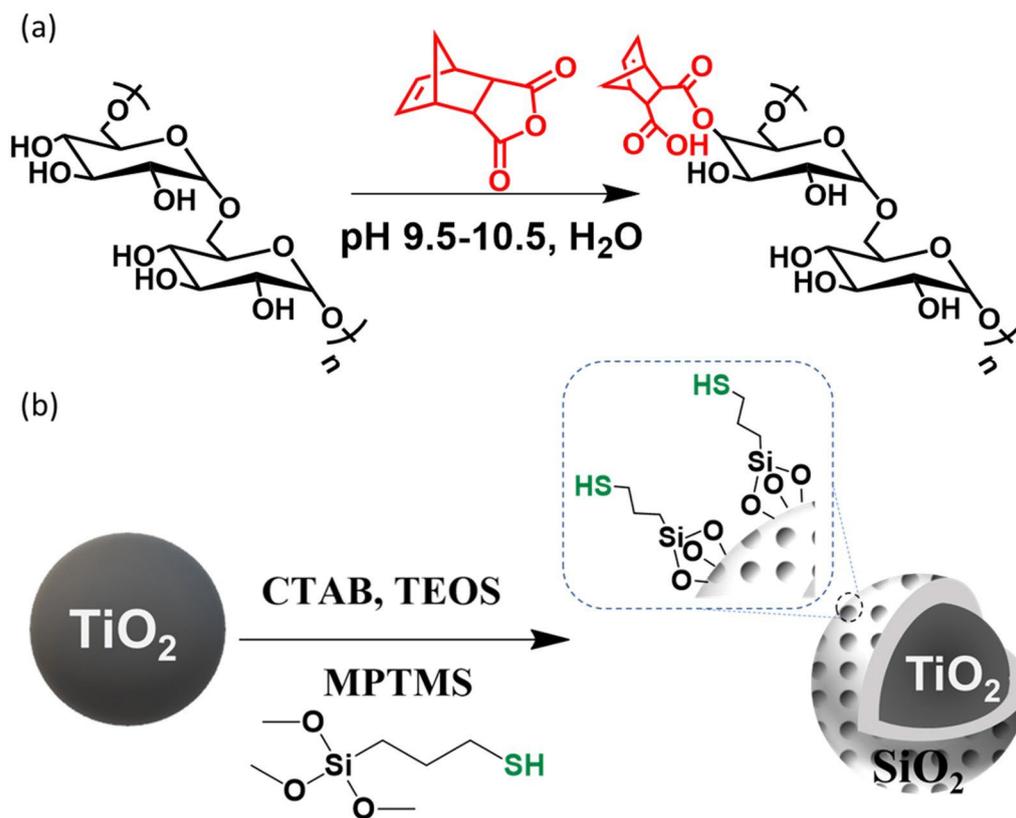
^c Mater of Public Health Program, National Taiwan University, Taipei, Taiwan

^d GIP-TRIAD Master's Degree in Agro-Biomedical Science, National Taiwan University, Taipei, Taiwan

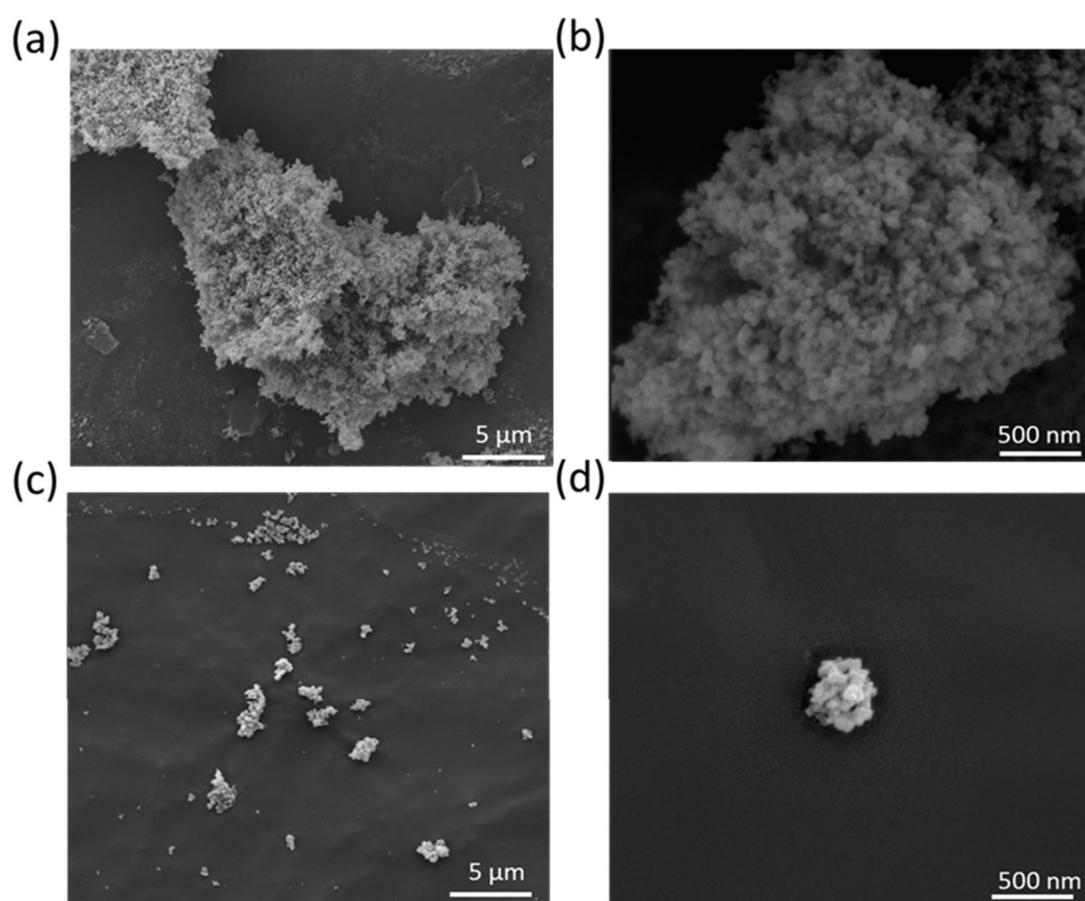
* Corresponding author E-mail: yicheun@ntu.edu.tw

† These authors contributed equally to this work.

Note – ESI updated 08/08/2023 to correct error in Figure 10(a) as requested by the authors.



Scheme S1. Synthetic schemes of (a) Nor-Dex and (b) TiO₂@MS-SH NPs.



(e) **Left: pure TiO₂ Right : TiO₂@MS-SH**



Figure S1. Representative SEM images of (a, b) pure TiO₂ and (c, d) TiO₂@MS-SH NPs. (e) Photographs of NPs dispersed in aqueous solution (left: TiO₂ NPs; right: TiO₂@MS-SH NPs).

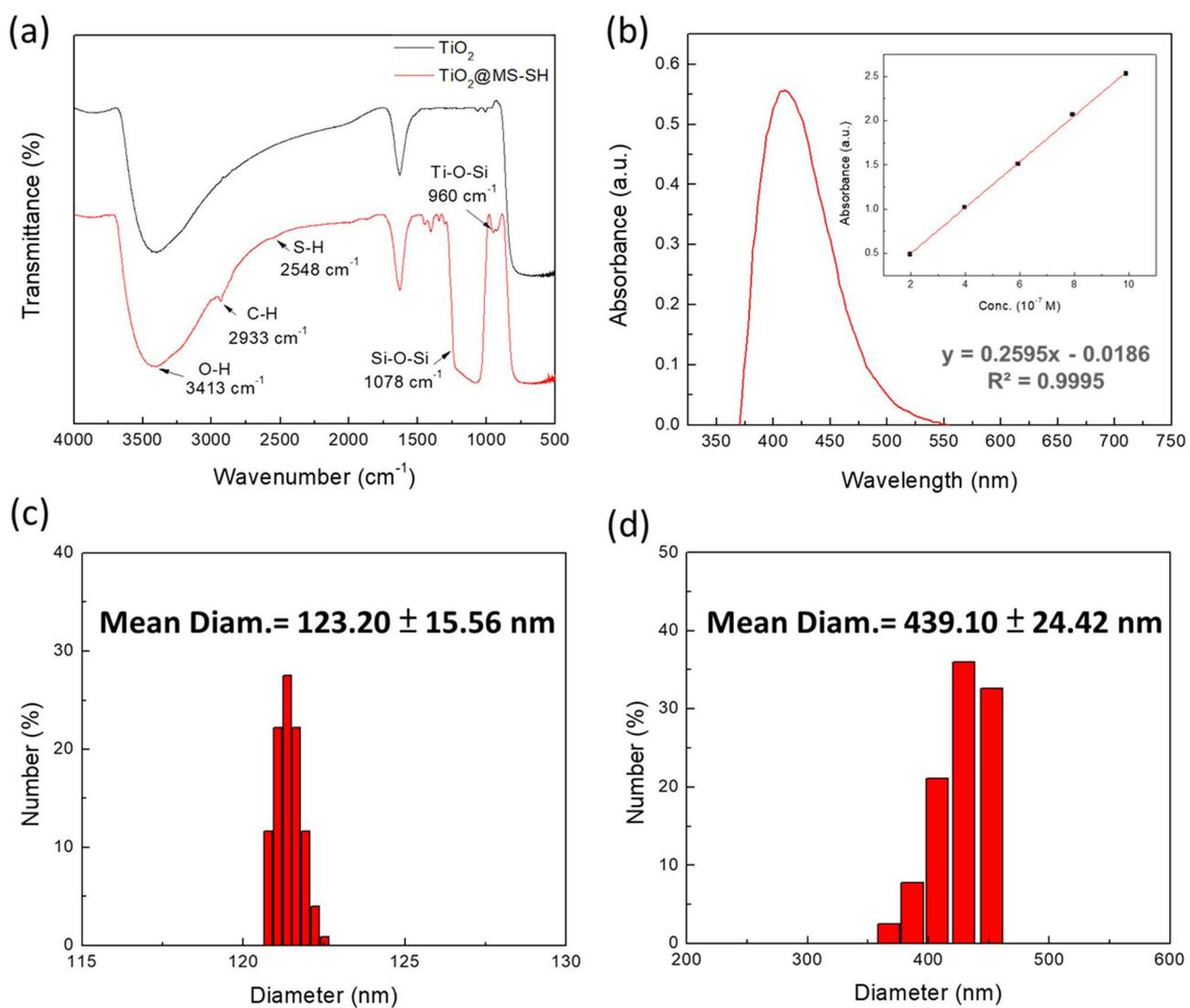


Figure S2. (a) FT-IR spectra of TiO₂ and TiO₂@MS-SH NPs. (b) Quantification of the thiol group of the TiO₂@MS-SH NPs using Ellman's assay. Hydrodynamic diameter distribution of (c) TiO₂ and (d) TiO₂@MS-SH NPs.

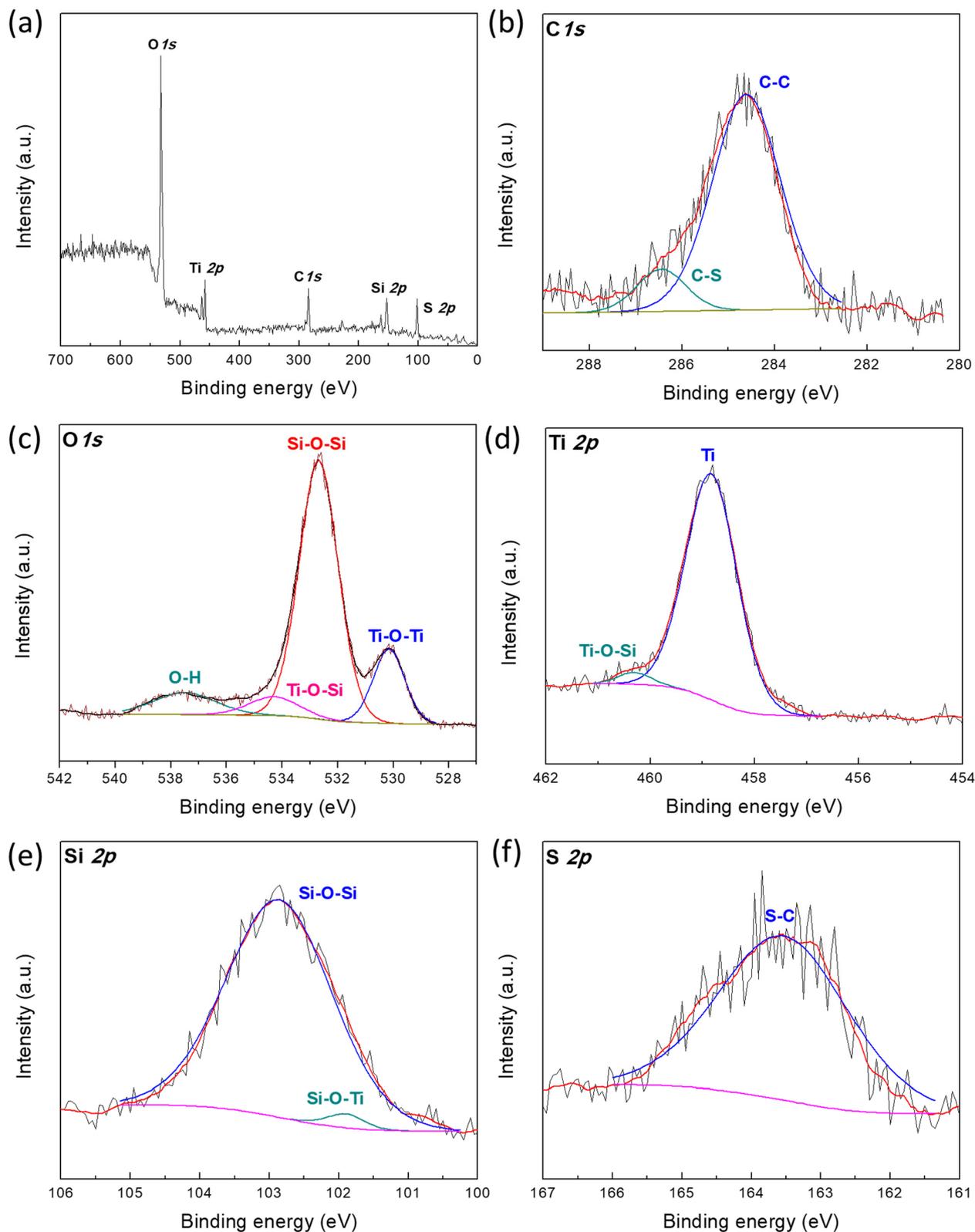


Figure S3. (a) XPS survey data of $\text{TiO}_2@MS-SH$ NPs. High-resolution XPS spectra for (b) C 1s, (c) O 1s, (d) Ti 2p, (e) Si 2p, and (f) S 2p of $\text{TiO}_2@MS-SH$ NPs.

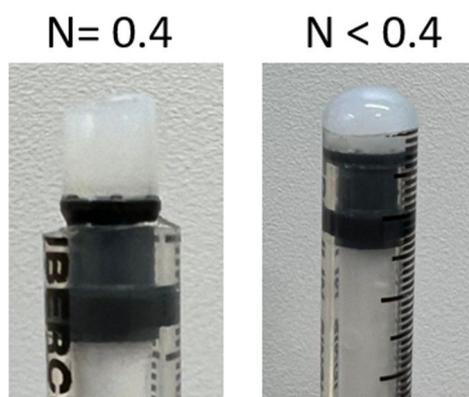


Figure S4. Stable gelation of $\text{TiO}_2\text{@MS-SH/Nor-Dex}$ NC hydrogels was formed in the presence of DTT with thiol/norbornene ratio $N = 0.4$.

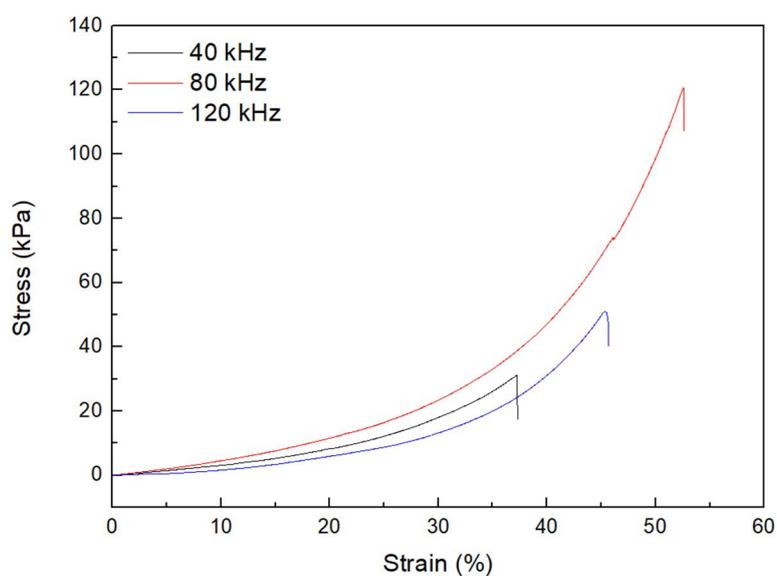


Figure S5. Representative stress-strain curves of $\text{TiO}_2\text{@MS-SH/Nor-Dex}$ NC hydrogels prepared with varied ultrasound frequencies.

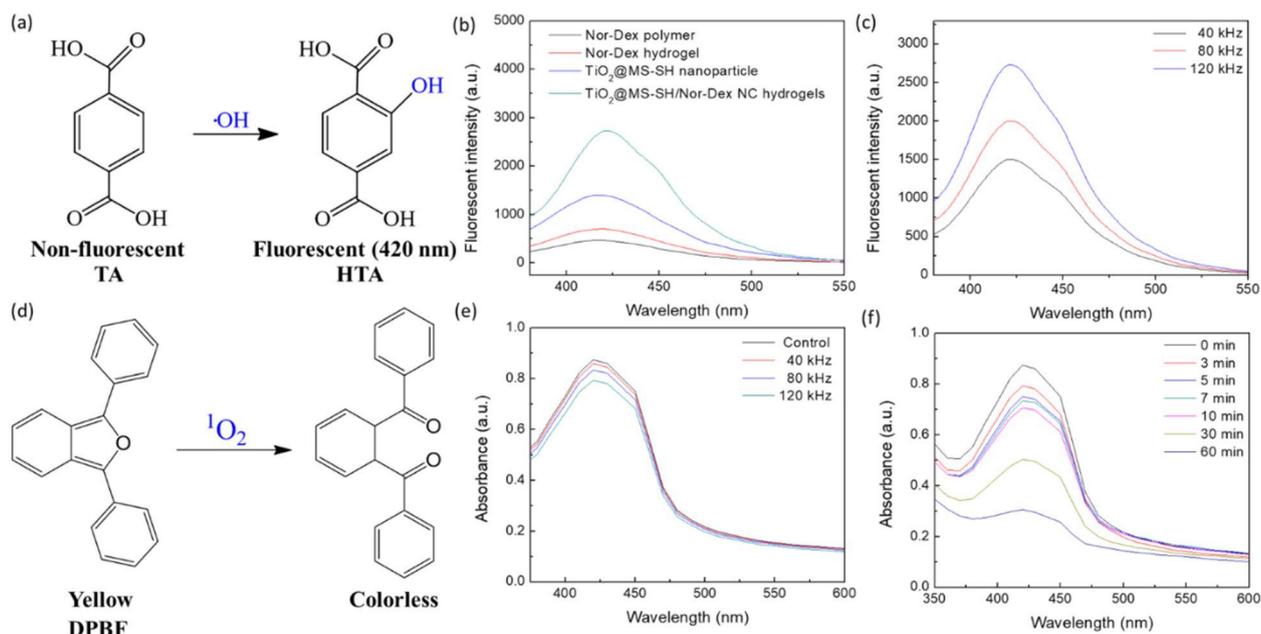


Figure S6. Measurements of ROS generation in the solution. (a) Hydroxyl radical ($\cdot\text{OH}$) reaction with terephthalic acid (TA) and radical production under ultrasound irradiation. (b) Fluorescent spectra of TA acid with different samples (i.e., Nor-Dex polymer, Nor-Dex hydrogel (crosslinked with DTT), TiO_2 @MS-SH NPs, and TiO_2 @MS-SH/Nor-Dex NC hydrogels) under 120 kHz ultrasound irradiation. (c) TiO_2 @MS-SH/Nor-Dex NC hydrogels under varied frequencies of ultrasound irradiation. (d) The reaction of singlet oxygen ($^1\text{O}_2$) with DPBF. The decay UV-vis absorption curves of DPBF in aqueous solution as a function of the (e) varied frequencies and (f) ultrasound irradiation time.

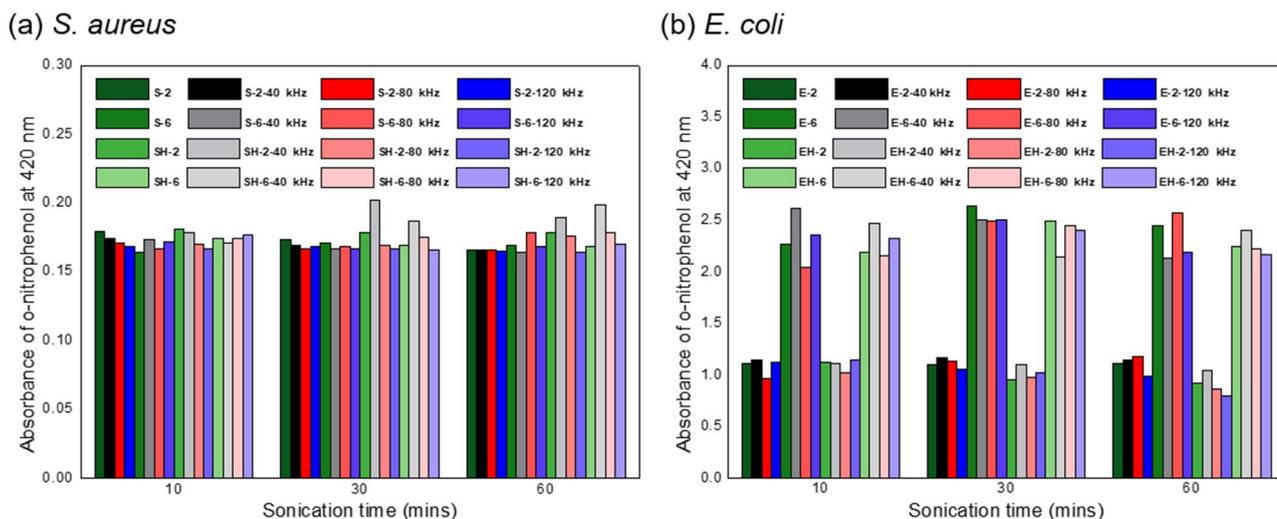


Figure S7. Cell membrane permeability of (a) *S. aureus* and (b) *E. coli* in the absence and presence of $\text{TiO}_2\text{@MS-SH/Nor-Dex}$ NC hydrogels under various ultrasound frequencies and sonication times. S: *S. aureus*; E: *E. coli*; SH: *S. aureus* incubated with hydrogel; EH: *E. coli* incubated with hydrogel. S-2 (or SH-2, E-2, and EH-2) and S-6 (or SH-6, E-6, and EH-6) indicate the absorbance of *S. aureus* (or *E. coli*) suspensions with ONPG obtained after the reaction time for 2 and 6 hr, respectively.

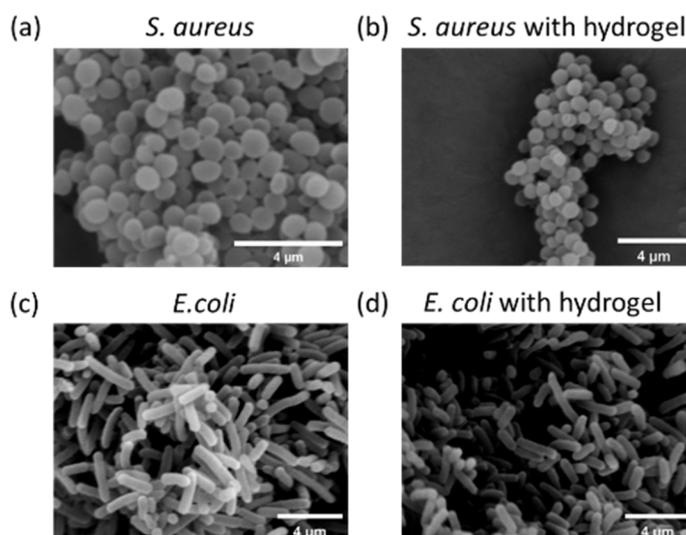


Figure S8. Representative SEM images of *S. aureus* in the (a) absence and (b) presence of $\text{TiO}_2\text{@MS-SH/Nor-Dex}$ NC hydrogels. Representative SEM images of *E. coli* in the (c) absence and (d) presence of $\text{TiO}_2\text{@MS-SH/Nor-Dex}$ NC hydrogels.

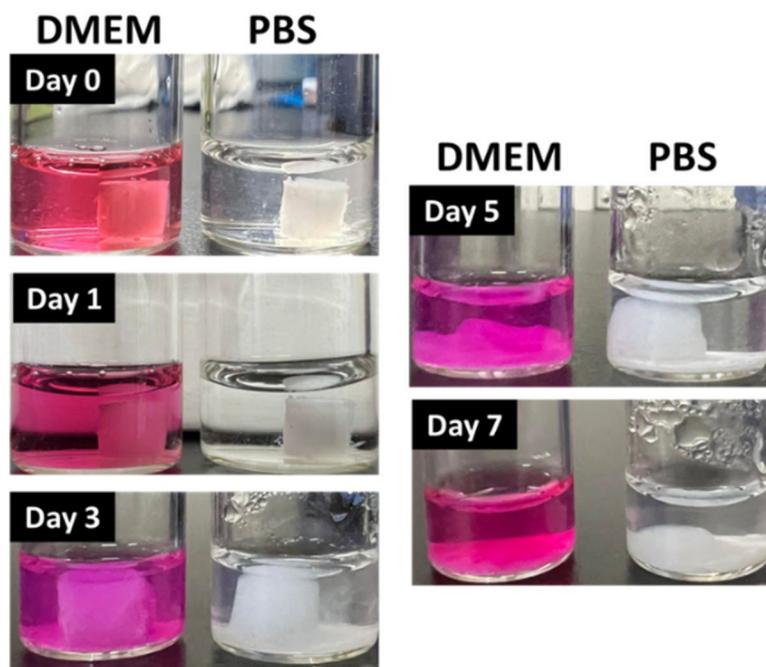


Figure S9. Stability test of TiO₂@MS-SH/Nor-Dex NC hydrogels in serum-containing DMEM and PBS solution.

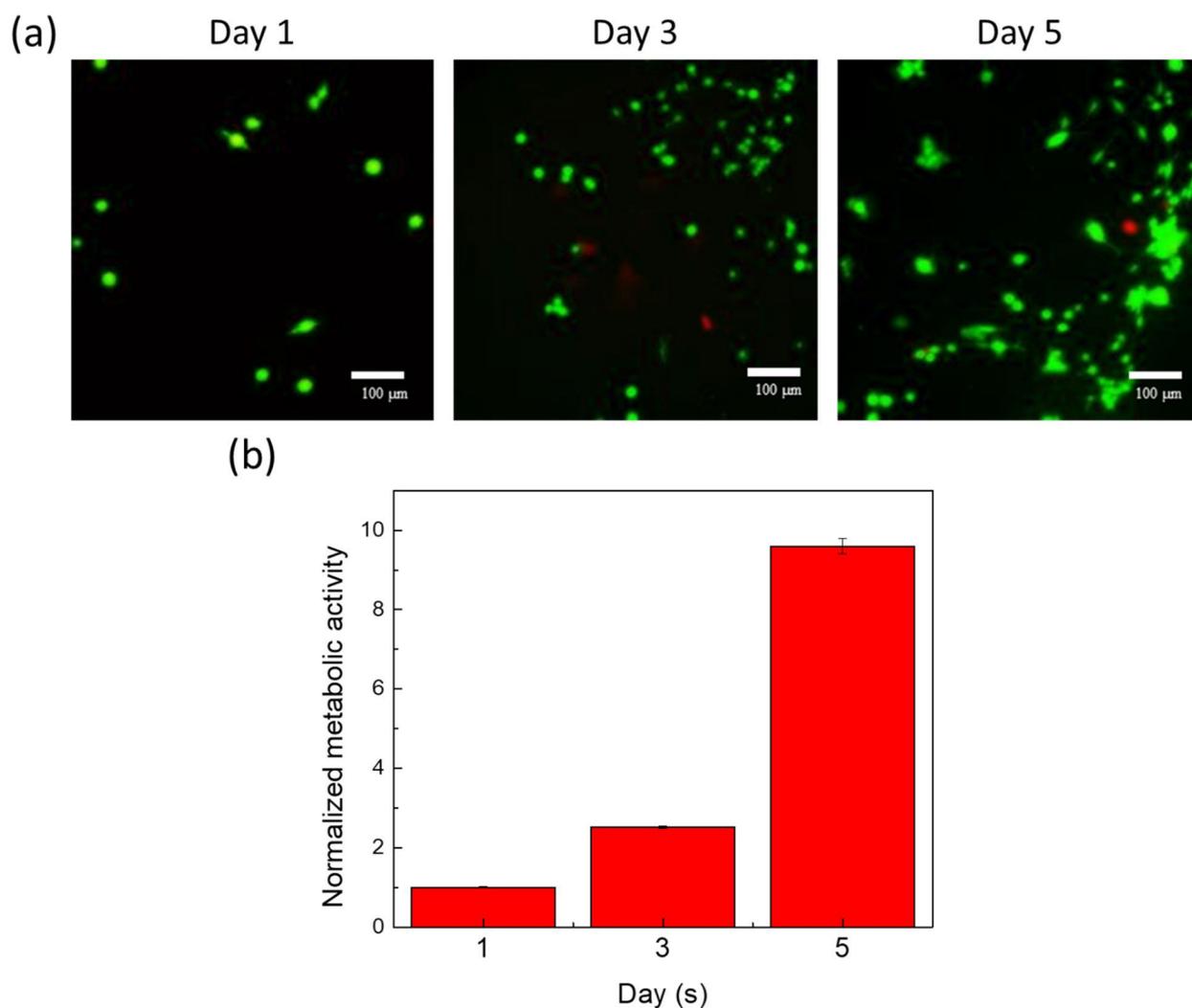


Figure S10. Cytocompatibility tests of the extract solutions obtained from the $\text{TiO}_2@\text{MS-SH}/\text{Nor-Dex}$ NC hydrogels. (a) Fluorescence images of the live/dead staining of MEFs at different time points, where calcein-AM stained live cells green while ethidium homodimer-1 stained dead cells red. Scale bar: 100 μm . (b) Metabolic activity of MEFs cultured in the extract solutions obtained from the NC hydrogels.

Table S1. Summary of the sample conditions of TiO₂@MS-SH/Nor-Dex NC hydrogels used in the compression tests.

Tests ^{a,b}	TiO₂@MS-SH concentration (wt%)	KPS concentration (wt%)	Frequency (kHz)
TiO ₂ @MS-SH concentration	0, 0.5, 1, 1.5	1	120
KPS concentration	1	0, 0.25, 0.5, 1.25, 1.5	120
Frequency	1	1	40, 80, 120

^a The Nor-Dex polymer was fixed at 10 wt% in each test.

^b The thiol/norborene ratio was fixed at 0.4 in each test.