Supporting Information

Magnetic Glycol Chitin-Based Hydrogel Nanocomposite for Combined Thermal and D-Amino-Acid-Assisted Biofilm Disruption

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SI Table of Contents:

complete biofilm coverage. The biofilm dispersal activities of the individual D-amino acids were evaluated and compared relative to the biomass of the positive controls (saline only treatment) by measuring the absorbance of solubilized crystal violet stain at 595 nm following 24 h incubation. An asterisk (*) indicates that the difference of means are statistically significant at $p < 0.05$ while NS indicates that difference of means are not statistically significant at $p > 0.05$. **(B)** The corresponding crystal violet staining of the remaining biofilms after treatment with the individual D-amino acids; D-methionine (D-met, 50 mM), D-tryptophan (D-trp, 54 mM), D-tyrosine (D-tyr, 2.5 mM), and D-phenylalanine (D-phe, 143.5 mM).

Figure S5. (A) Time-dependent biofilm disruption effects of the D-amino acid mixture of D-tyr, D-trp, and D-phe against biofilms of *S. aureus* showing that a 2 h incubation period results in almost 85% biofilm eradication. Dispersive activity of the 200 mM D-amino acid mixture was evaluated using a 1:22:57 molar ratio of D-tyr, D-trp, and D-phe, respectively. **(B)** The corresponding crystal violet staining of the remaining biofilms after treatment.

Figure S6. Cryo-SEM images of treated (2 h MagDAA gel or 2 h MagDAA gel + 10 min AMF) and un-treated (+ control) bacterial biofilms. The scale bars of the insets represent 100 μm. The *S. aureus* biofilms were pre-grown on 400-mesh Formvar-coated Cu grids and imaged using a desktop Phenom ProX SEM operated at 5 kV using a temperature controlled sample holder set at -25 ^{o}C.

Figure S7. (A) The cumulative release of the D-amino acids in the hydrogel without magnetic field, and **(B)** with magnetic field actuation, monitored at the OD of 280 nm where the D-amino acids strongly absorb.

Figure S8. (A) Biofilm disruption activities of lower concentrations of D-AAs with and without AMF excitation, and **(B)** the corresponding representative crystal violet staining of the remaining biofilms following treatments. The asterisk (*) indicates that the difference of means are statistically significant at $p < 0.05$.

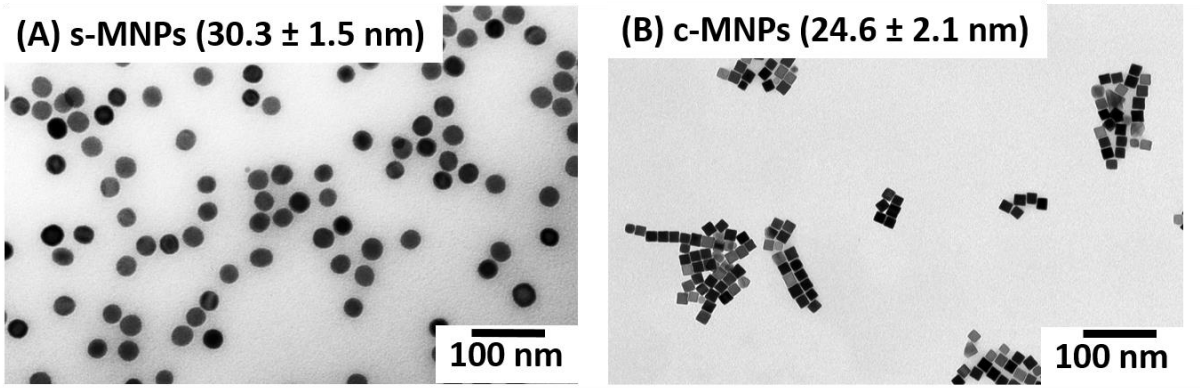


Figure S1. TEM images of the synthesized **(A)** spherical magnetic nanoparticles (s-MNPs) and **(B)** cubic magnetic nanoparticles (c-MNPs) showing wider viewing area.

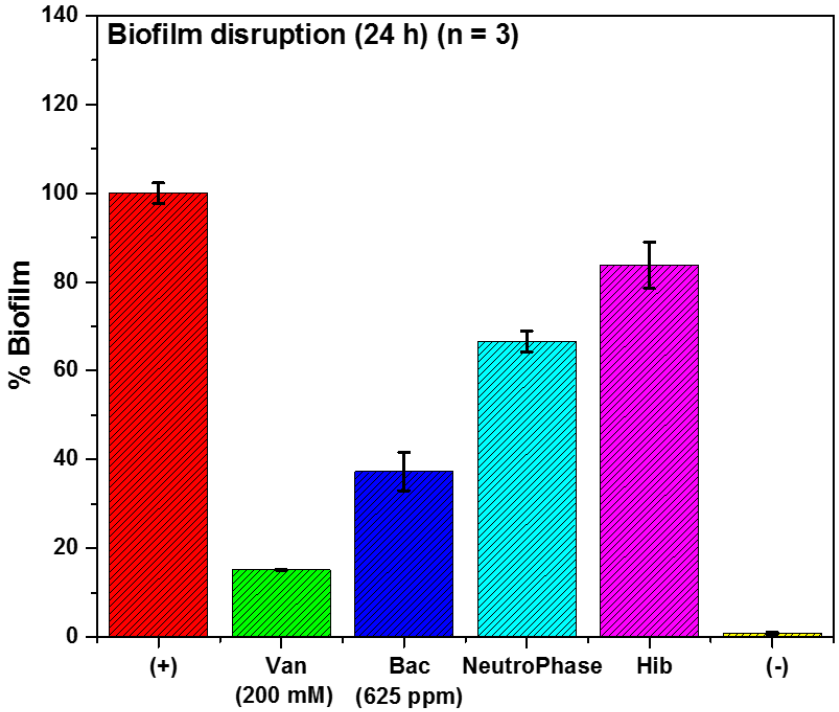


Figure S2. *S. aureus* biofilm disruption activities of vancomycin (Van), bacitracin (Bac), NeutroPhase, and Hibiclens (Hib) after a 24 h incubation period.

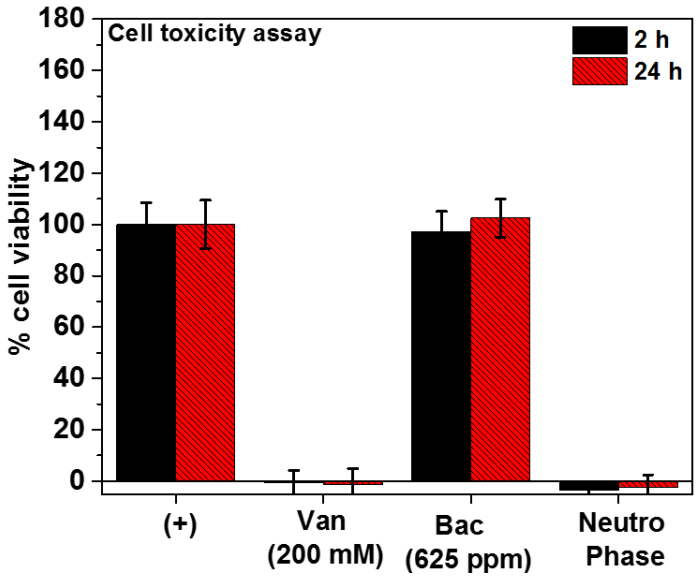


Figure S3. Time-dependent *in vitro* cell cytotoxicity assay of vancomycin (Van), bacitracin (Bac), and NeutroPhase at 2 h and 24 h cell incubation time points. The viability of HeLa cells was determined by exposure to media supplemented by the antibiotics for the specified time (2 h and 24 h) at 37 ^{o}C in 5% CO_2. Cell viability was determined using the PrestoBlue assay by measuring absorbance at 570 and 600 nm. The percent viability is represented relative to non-treated controls.

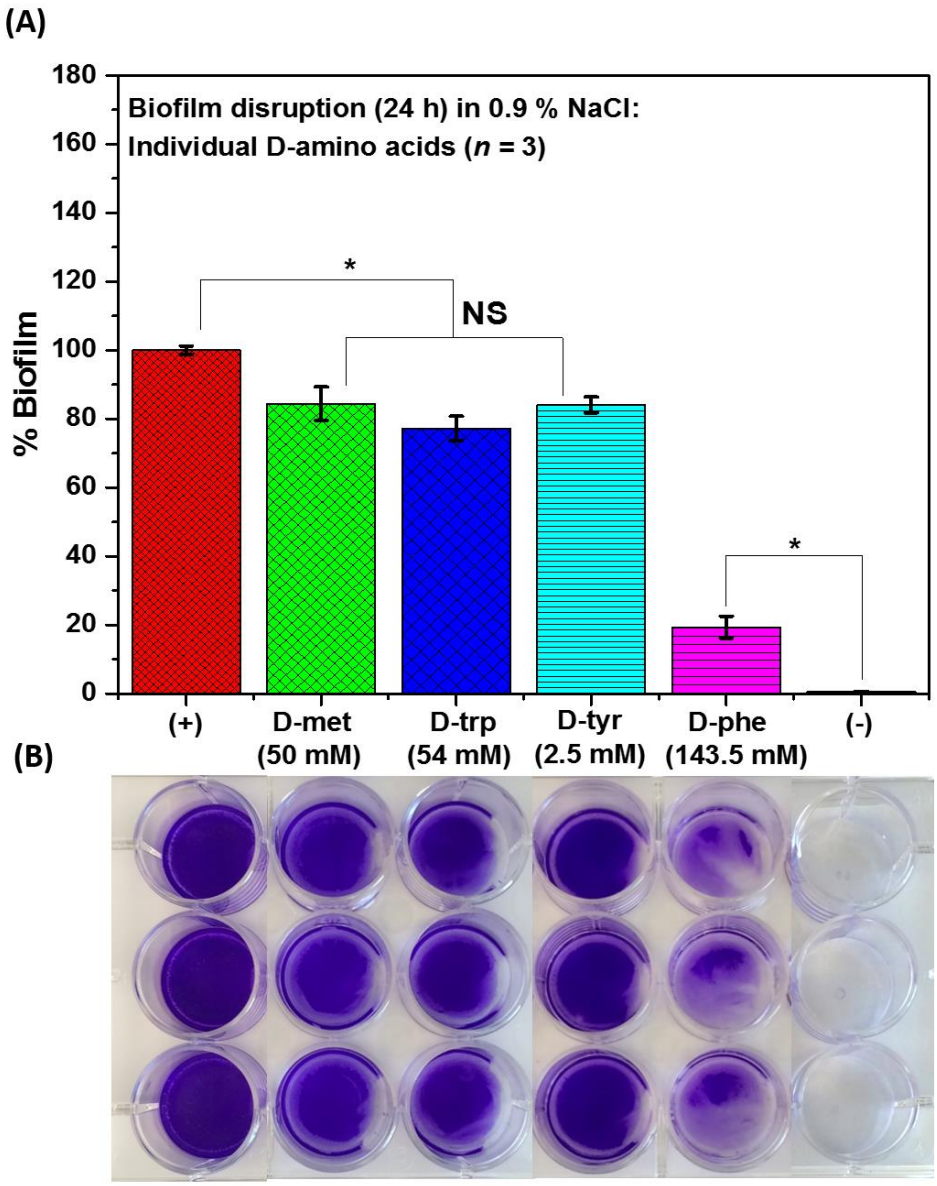
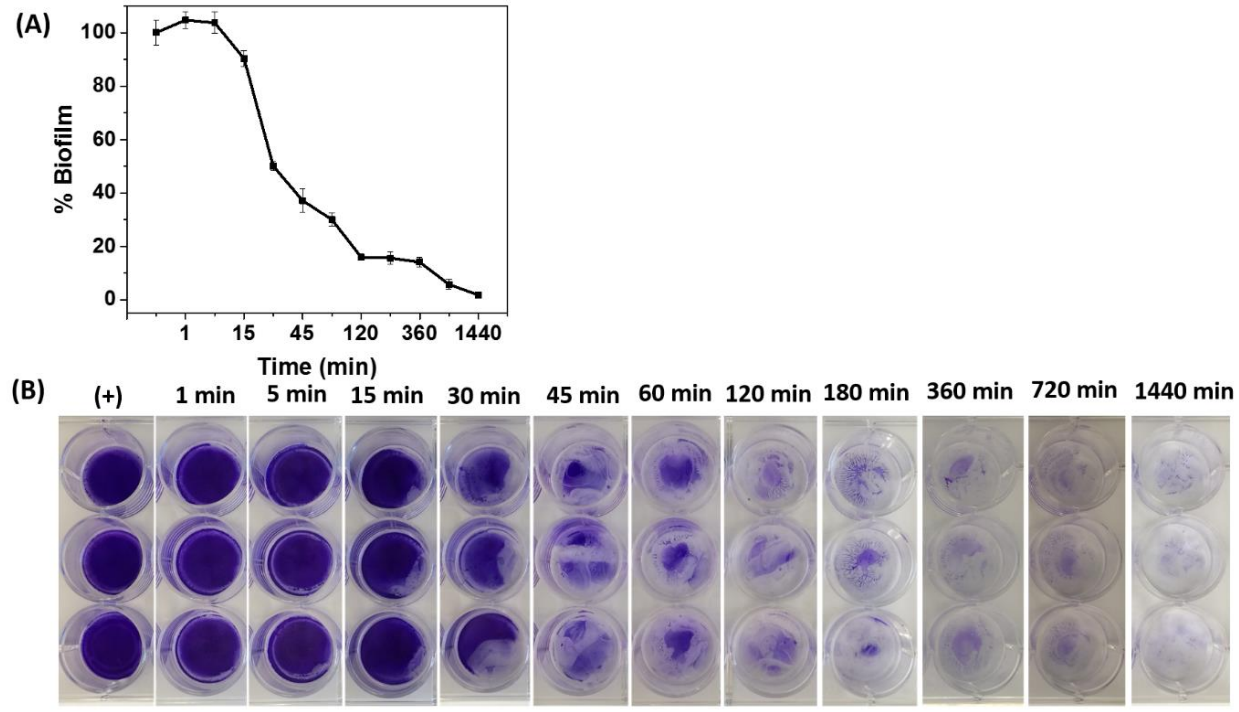


Figure S4. (A) The effects of individual D-amino acids against *S. aureus* biofilm disruption. The biofilms were grown from *S. aureus* bacterial cell cultures that were incubated overnight and diluted to an optical density (OD) at 595 nm of 0.1 and were further diluted 100x (~10^5 CFU/mL) in modified tryptic soy broth (3% NaCl, 0.5% glucose). Each well was filled with 2 mL of the diluted bacterial solution and incubated at 37 ^oC for an additional 24 h to form complete biofilm coverage. The biofilm dispersal activities of the individual D-amino acids were evaluated and compared relative to the biomass of the positive controls (saline only treatment) by measuring the absorbance of solubilized crystal violet stain at 595 nm following 24 h incubation. An asterisk (*) indicates that the difference of means are statistically significant at $p < 0.05$ while NS indicates that difference of means are not statistically significant at $p > 0.05$. **(B)** The corresponding crystal violet staining of the remaining biofilms after treatment with the individual D-amino acids; D-methionine (D-met, 50 mM), D-tryptophan (D-trp, 54 mM), D-tyrosine (D-tyr, 2.5 mM), and D-phenylalanine (D-phe, 143.5 mM).



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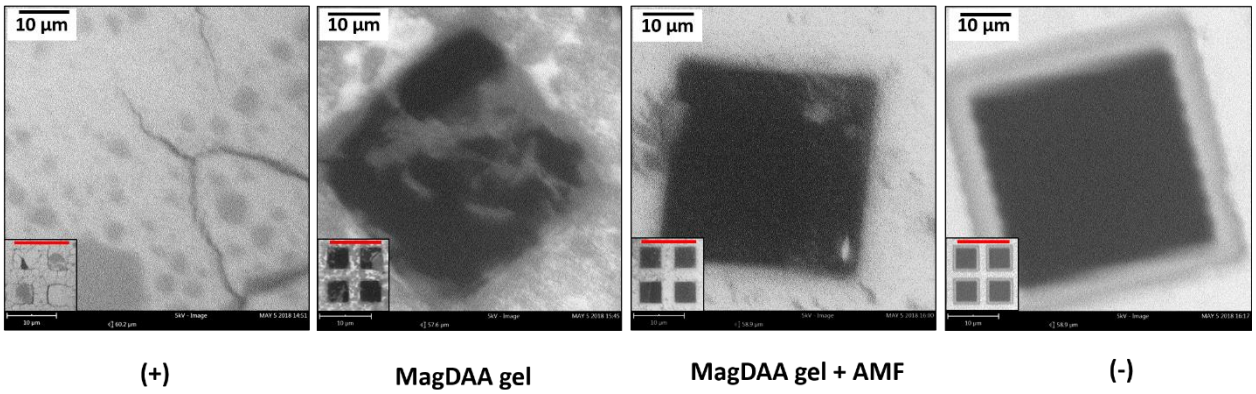

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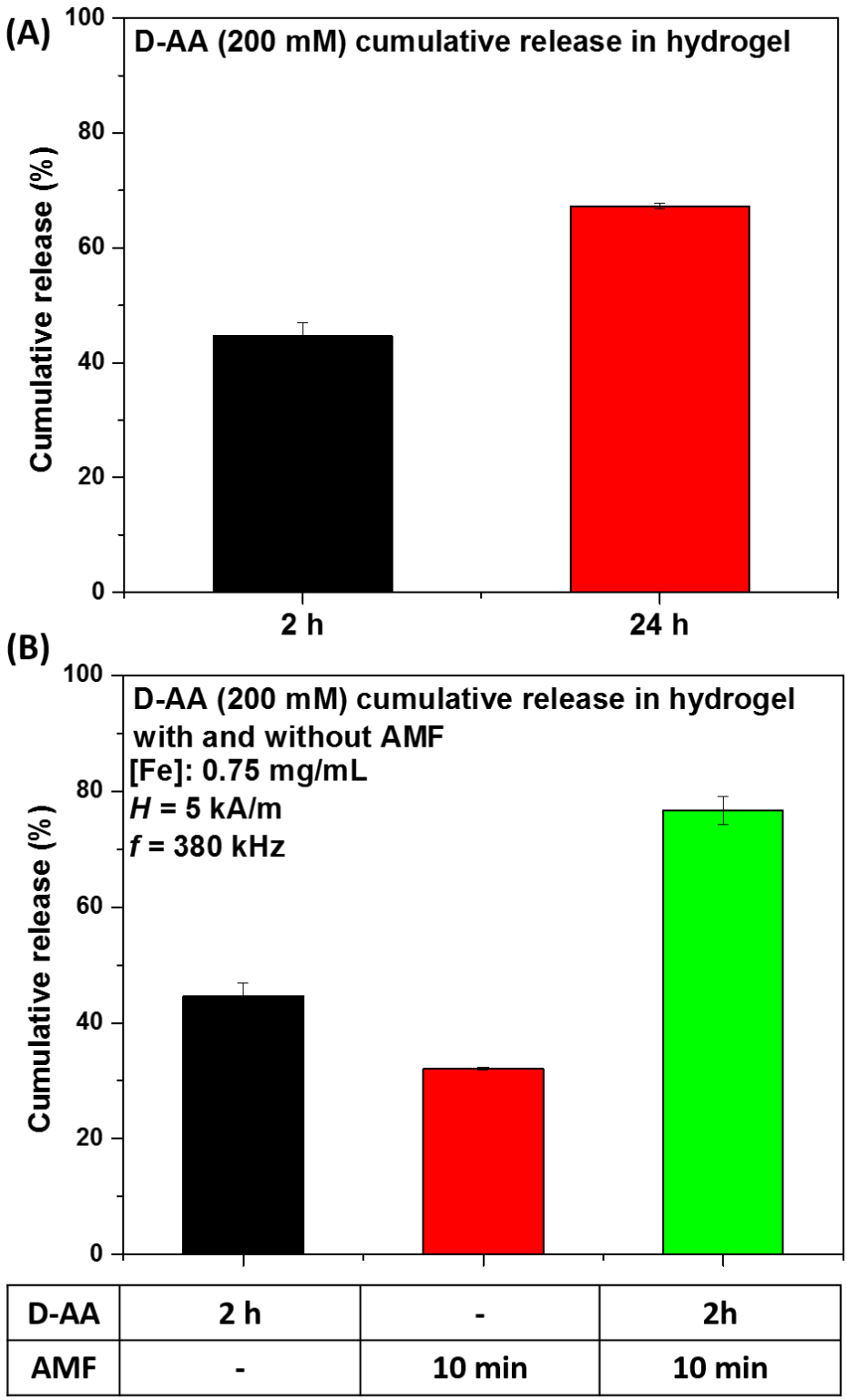

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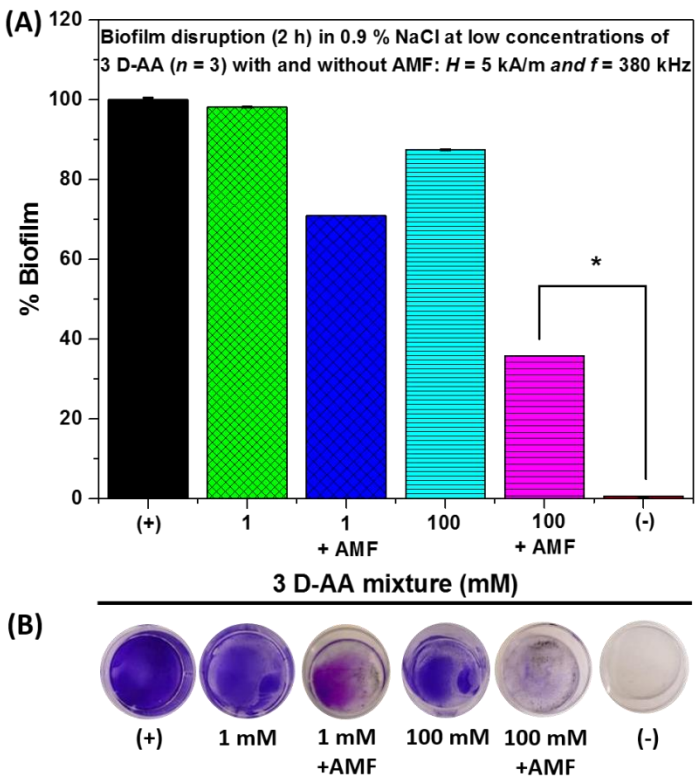

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