Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting Information

Bottom-Up Biofilm Eradication Using Bacteriophage-Loaded Magnetic Nanocomposites: A Computational and Experimental Study

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The supplementary information includes:

Numerical model construction and calibration.

The biofilm cross-section was represented by a two-dimensional checkerboard (100 grids width × 12 grids height) (Fig. 5A). In each layer the ratio of *E. coli* (red grid) to *P. aeruginosa* (green grid) was set as 2:1 to reflect the measured ratio. The numerical model considers the major phage activities in biofilm including phage infection (Eqn. 1), phage diffusion (Eqn. 2), and phage degradation/trap (Eqn.3). Bacterial proliferation was not considered since the growth of tested bacteria was negligible at biofilm in PBS medium within the treatment time (6 h). After successful infection phages replicate by burst size (β) in latent period (τ) and then diffuse in vertical or horizontal directions.

α determination: The infection rate coefficient (α) was calculated based on empirical formula as a function of phage adsorption rate coefficient (Egn. S1). [1] The adsorption rate coefficient (η) of phage towards *E. coli* and *P. aeruginosa* were measured by phage adsorption tests. [2]

$$\frac{\alpha}{\alpha_0} = \frac{exp(-\eta T/L_{box}^3)}{exp(-\eta_0 T/L_{box}^3)} \qquad \text{(Eqn. S1)}$$

where α_0 is infection rate coefficient of phage P1 to host *E. coli*, η_0 is adsorption rate coefficient of phage P1, T is the time period of phage adsorption, and L_{box} is the limit of phage distribution in the model (L_{box} = 30 μm).[1]

λ determination: The horizontal diffusion rate coefficient (λ_H) used diffusion coefficient of carboxylated nanoparticles with similar hydrodynamic radius in biofilm.[3]

$$\frac{D_{bio}}{D_0} = B\exp\left(-A \cdot R_h^2\right) \qquad \text{(Eqn. S2)}$$

Where D_{bio} is the diffusion coefficient in the biofilm, D_0 is the diffusion coefficient in water, R_h is the hydrodynamic radius of the particle, B and A are the structural constant related to the

heterogeneity of the biofilm network (A = 50 μm^{-2}, B = 0.73).[3]

δ determination: The horizontal phage degradation/trap rate coefficient (δ_H) was the degradation rate ensuring the coexistence of biofilm and phage.[1, 4, 5] Since the dual species biofilm and free phage PEB1 coexisted in 6 h, the horizontal phage degradation/trap rate coefficient (δ_H) was set as the maximum degradation rate coefficient (Eqn. S3) ensuring the coexistence of biofilm and phage.[4, 6]

$$\delta_{max} = log(\beta(1 - exp(-\lambda\Delta t)))\frac{1}{\Delta t} \quad \text{(Eqn. S3)}$$

Where β is phage burst size, λ is phage diffusion rate coefficient, and Δt is time step.[6]

PNCs physically disrupted the biofilm when penetrating the biofilm (Fig. S4), which facilitated phage propagation in vertical direction (Fig. S1B). We herein defined a facilitation coefficient (F) to increase phage diffusion rate coefficient (Eqn. S4) and decrease phage trap rate coefficient (Eqn. S5) in vertical directions according to Fick's law.[7]

$$F_\lambda = N_\lambda \times \frac{D_N}{L} \quad \text{(Eqn. S4)}$$

$$F_\delta = N_\delta \times \frac{D_N}{L} \quad \text{(Eqn. S5)}$$

Where N_λ is diffusion normalizing coefficient, N_δ is degradation normalizing coefficient, D_N is the hydrodynamic radius of accumulative space excavated by PNCs, and L is the length of unit grid.

The biofilm width (L_1) was set as 50 μm and the initial free phage number (N_0) was set as 304 by fitting the biofilm removal efficiency of free phages after 2, 4 and 6 h. The large PNCs had the same number of phages loaded evenly onto each particle. The numbers of phages onto each sized PNCs were based experimental results. The other sized PNCs were set to have the same surface area for phage loading. The PNC parameters were summarized in Table 2.

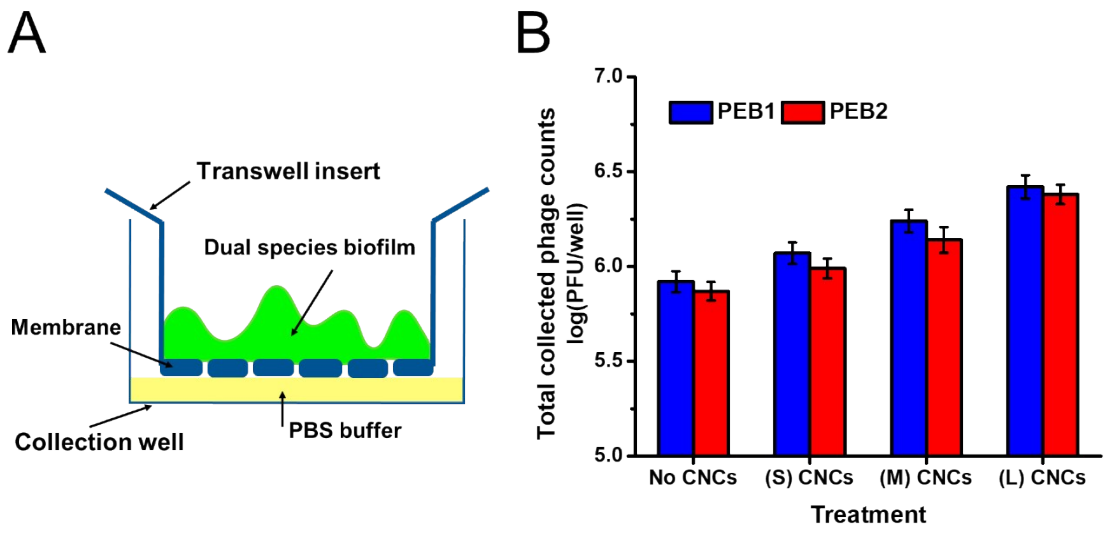

Figure S1. Phage biofilm diffusion assays. (A) Schematic illustration of phage biofilm diffusion assay. One dual species biofilm of *E. coli* and *P. aeruginosa* was established on the 0.45 μm membrane for 48 hours. (B) PEB1 and PEB2 propagated through the biofilm with and without CNCs disruption. The CNCs added on the biofilm were 0.1 μg. CNCs with larger sizes more significantly facilitate phage propagation through the biofilm.

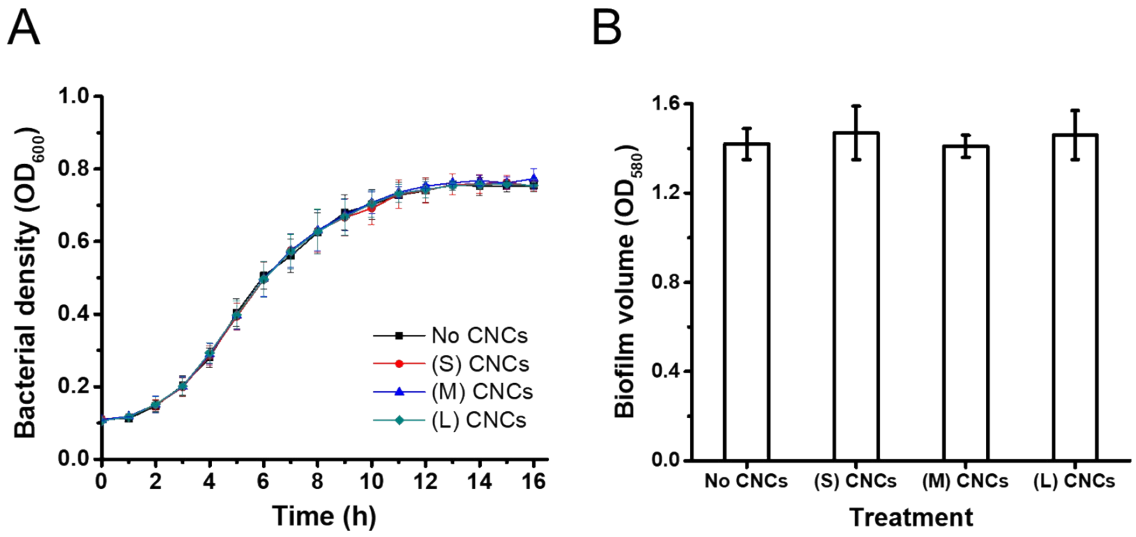

Figure S2. Influence of CNCs on bacterial growth and biofilm formation. The CNCs alone at the tested condition (1.0 μg/mL) had no noticeable bactericidal or antifouling effects based on (A) bacterial growth curves and (B) crystal violet biofilm assay.

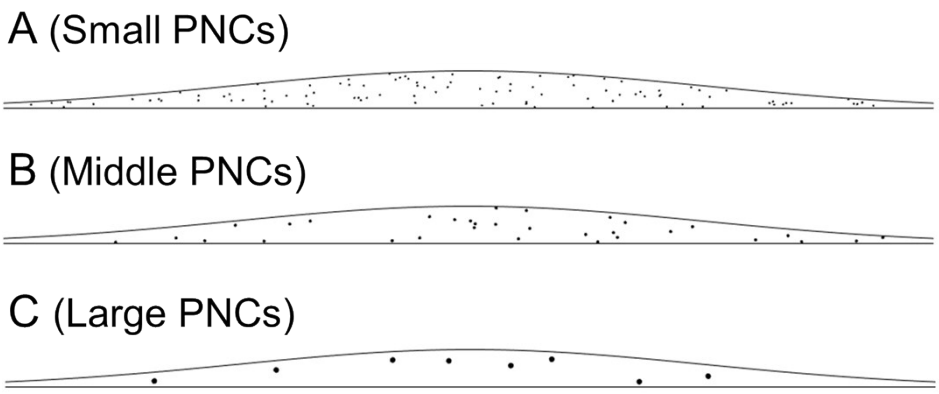

Figure S3. Distribution of PNCs at the bottom of simulated biofilm. PNCs are randomly dispersed at the biofilm bottom layers following one-dimensional normal distribution.

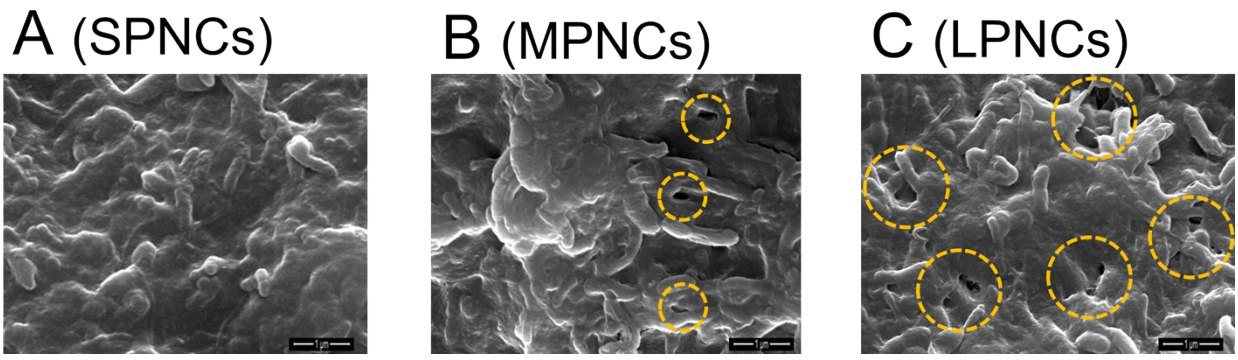

Figure S4. Physical disruption of biofilm by different sized PNCs. Large PNCs produced more holes in the biofilm when penetrating the biofilm under magnetic field compared with small PNCs. Scale bar is 1 μm.

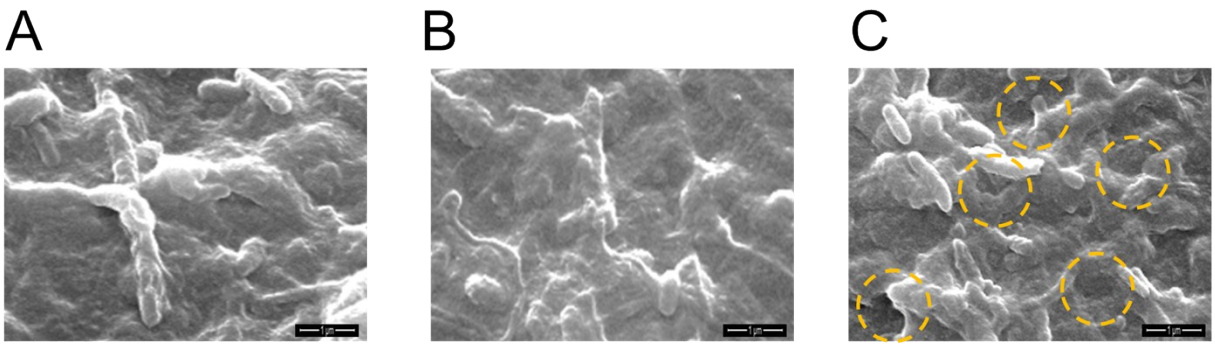

Figure S5. Biofilm disruption due to PNCs movement in vertical or horizontal directions. (A) Intact biofilm surface without PNCs disruption (control), (B) Undamaged biofilm surface after PNCs horizontal movement in biofilm, and (C) Disrupted biofilm surface after PNCs vertical movement from biofilm surface to bottom. Scale bar is 1 μm.

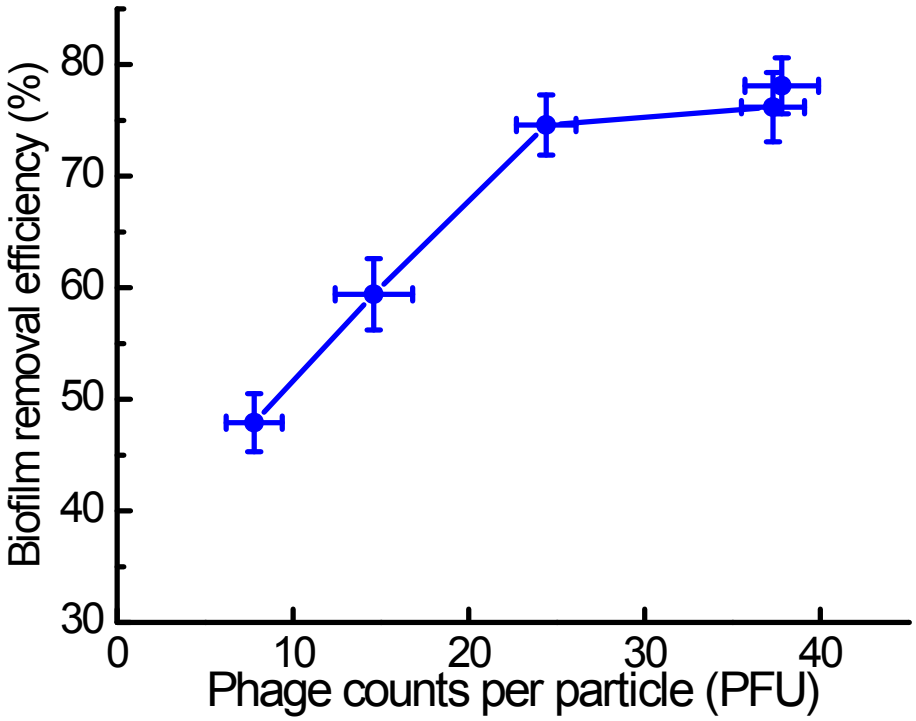

Figure S6. Influence of phage loading number on PNCs biofilm removal. Increased phage loading onto large PNCs did not resulted in more biofilm removal efficiency when phage counts exceeded more than 20 phages per particle.

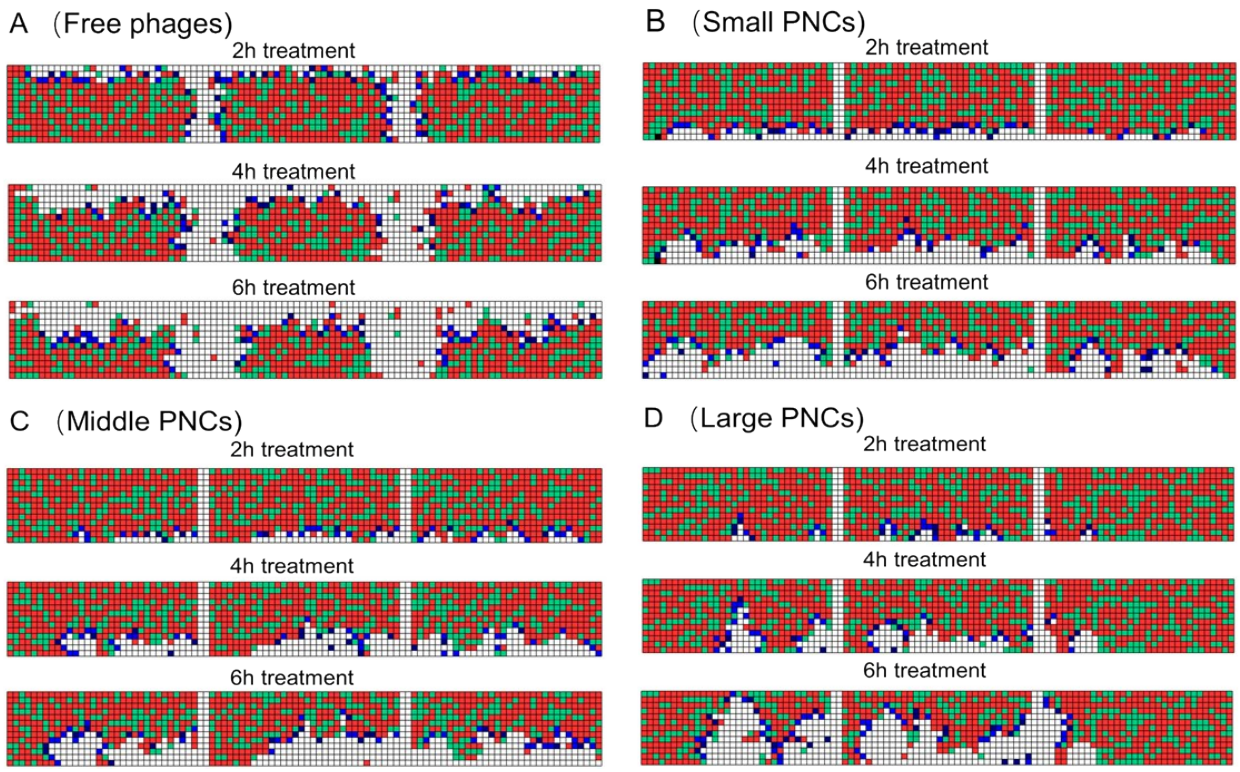

Figure S7. Simulated phage infection patterns by free phages and different sized PNCs. Free phages follow from top to bottom infection pattern while PNCs penetrate the biofilm and initiate infection from the bottom. SPNCs with more infectious centers effectively de-anchor the biofilm within six hours while LPNCs resulted in larger biofilm disruption in the vertical direction.

Table S1. The values and sources of the semi-empirical model parameters.

Symbol	Definition	Values	Sources
L	Unit length of each grid	0.5 μm	Literature [4]
τ	Latent time in *E. coli* Latent time in *P. aeruginosa*	45 min 50 min	Measured
β	Burst size in *E. coli* Burst size in *P. aeruginosa*	80 PFU 100 PFU	Measured
D_N	Width of excavated channel	0-0.5μm	Claculated
Bio%	Biofilm composition of *E. coli* and *P. aeruginosa*	2/3 in *E. coli* 1/3 in *P. aeruginosa*	Measured
N_δ	Phage degradation adjusting parameter	0.1, 1.5, 5.0	Calibrated
N_λ	Phage diffusion adjusting parameter	0.69	Literature [3]

Table S2: Parameters of different sized PNCs considered by the numerical model

NPs Size (nm)	NPs number per biofilm section	Phage loaded per NP	Total phages on PNCs
150	89	4	356
250	32	12	384
500	8	38	304

References

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