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A decoy microrobot that removes SARS-CoV-2 and its variants in wastewater

Lai et al. develop a decoy microrobot by camouflaging algae with cell membranes. Without an external motor, the decoy microrobots show fast self-propulsion, allowing for successful “on-the-fly” elimination of SARS-CoV-2 and its variants in wastewater.
Article

A decoy microrobot that removes SARS-CoV-2 and its variants in wastewater

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SUMMARY
The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which can persist in wastewater for several days, has a risk of water-borne-human transmission. The emergence of SARS-CoV-2 variants with increased infection capacity further highlights the need to remove the virus and restrict its spread in wastewater. Here, we report a decoy microrobot created by camouflaging algae with cell membranes displaying angiotensin-converting enzyme 2 (ACE2) for effective elimination of SARS-CoV-2 and its variants. The decoy microrobots show fast self-propulsion (>85 μm/s), allowing for successful “on-the-fly” elimination of SARS-CoV-2 spike proteins and pseudovirus in wastewater. Moreover, relying on the robust binding between ACE2 and SARS-CoV-2 variants, the decoy microrobots exhibit a broad-spectrum elimination of virus with a high efficiency of 95% for the wild-type strain, 92% for the Delta variant, and 93% for the Omicron variant, respectively. Our work presents a simple and safe decoy microrobot aimed toward eliminating viruses and other environmental hazards from wastewater.

INTRODUCTION
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging coronavirus with high infectiousness and fatality1,2 and can spread in a variety of ways, including direct airborne transmission and indirect fomite transmission.3,4 SARS-CoV-2 has been linked to the respiratory system, but it also has the potential to infect the gastrointestinal tract if it remains in feces for lengthy periods of time.5 SARS-CoV-2 has been found in residential wastewater and downstream waterways, in addition to stool samples.6–8 These studies have sparked fears that SARS-CoV-2 infection might spread through wastewater via fecal-oral transmission.9–11 The emergence of variants of concern with higher infection capacity and more concealed symptoms further highlights the urgency of preventing the spread of SARS-CoV-2 and its variants in wastewater.12–14 To combat SARS-CoV-2 contamination in liquid waste, physical, chemical, and biological techniques, including filtration, sedimentation, enzymatic degradation, and disinfection with oxidants or UV, have been proposed and have shown promising outcomes.8,11 Despite that, new techniques for removing SARS-CoV-2 from wastewater that are simple, quick, and effective still need to be explored.8

We report here a biomimetic algae microrobot that is used to effectively remove SARS-CoV-2 and its variants from wastewater. Compared with a static counterpart, the self-propulsion ability of algae microrobots improves target contact and adsorption, as well as localized self-mixing, allowing it to remove contaminants rapidly and
efficiently.15–17 Moreover, benefiting from their facile surface functionalization, the algae microrobots equipped with different functional components have been employed on the removal of extensive aquatic environmental contaminants, including dyes, heavy metals, and pathogenic organisms.18 Despite seeming promising, the existing synthetic microrobotic platforms via chemical and physical methods are constrained by short lifespan, hazardous fuel requirements, complicated external actuation apparatuses, and limited media for operation.19 Biohybrid microrobots, which combine self-moving microorganisms with useful biomaterials, have lately exhibited tremendous promise in large-scale environmental restoration, overcoming limitations experienced by synthetic microrobots.19–21

In this work, angiotensin-converting enzyme II (ACE2)-modified algae (dubbed “ACE2 algae decoy microrobots”) are used in a novel microrobot technique to eliminate SARS-CoV-2. The ACE2 protein is critical in the identification of SARS-CoV-2, with a strong affinity for spike protein S1 subunit (S protein).22–24 and our recent work has demonstrated that engineered cell membrane vesicles displaying ACE2 receptors (ACE2 vesicles) can serve as decoys for SARS-CoV-2 binding and inhibition by competing with host cells.25–27 We herein chose Chlamydomonas reinhardtii as a model algae for our decoy microrobots because of its appealing qualities, such as ease of manufacturing, quick motion, and extended lifespan.28 The decoy microrobots are fabricated by camouflaging algae with ACE2 vesicles (Figure 1A). Without external drive, the resulting ACE2 algae decoy microrobots show fast self-propulsion, enabling effective binding and “on-the-fly” elimination of SARS-CoV-2 and its variants in wastewater (Figure 1B). The scanning electron microscopy (SEM) showed single decoy microrobot and captured SARS-CoV-2 pseudoviruses (Figure 1C).

RESULTS
Fabrication of decoy microrobots
The manufacture of decoy microrobots is divided into two steps: first, prepare genetically modified ACE2 vesicles, and then, coat the ACE2 vesicles onto the algae. Transducing ACE2 onto 293T cells was the initial step in creating genetically modified 293T/ACE2 cells.25 The significant expression of ACE2 on modified cells was validated by immunofluorescence imaging and flow cytometry (Figures 2A

Figure 1. ACE2 algae decoy microrobots remove SARS-CoV-2 and its variants in liquid waste
(A) Schematic illustration showing the preparation of ACE2 algae decoy microrobots by coating algae with genetically engineered ACE2 cell membrane vesicles. 
(B) Schematic illustration showing that the resulting ACE2 algae decoy microrobots capture SARS-CoV-2 and its variants in liquid waste by interacting with S protein. 
(C) SEM image showing that single decoy microrobot captures pseudovirus SARS-CoV-2. Scale bar, 3 μm.
and 2B). To retrieve cell membrane vesicles, the intracellular substance was extracted using a mixture of osmotic lysis, physical rupture, and density gradient centrifugation.29 The ACE2 vesicles were then created by repeated ultrasonic treatment and extrusion of 293T/ACE2 cell membranes via mini-extruder.30 The algae were then camouflaged with a layer of ACE2 vesicles by gentle magnetic stirring of the algae and ACE2 vesicles.31,32 After the coating, dynamic light scattering (DLS) exhibited that the zeta potential of algae increased from −22 mV to −17 mV (Figure 2C), and transmission electron microscopy (TEM) revealed a layer of 10 nm outer lipid shell on the decoy microrobots (Figures 2D and 2E), demonstrating successful coating of ACE2 vesicles on the algae. Moreover, western blot analysis showed that the ACE2 algae decoy microrobots contained specific protein markers of ACE2 (Figure 2F), further demonstrating the successful fabrication of decoy microrobots.

Motion behavior of decoy microrobots
After that, the motion behavior of the ACE2 algae decoy microrobots was then explored. The speeds of bare algae and ACE2 algae were 90 μm/s and 88 μm/s, respectively (Figures 3A and 3B), implying that the functionalization procedure has little impact on the movement of algae. Notably, individual ACE2 algae tracking trajectories mirrored the very steady motion of decoy microrobots in water (Figures 3A and Video S1). The ACE2 algal decoy microrobots were generated in pure water and then transferred to other aquatic media to evaluate their mobility. They moved quickly (>85 μm/s) in tris-acetatephosphate (TAP) medium, pure water, and river water (Figure 3C) without requiring any additional fuel. In addition, surface functionalization of algae with ACE2 vesicles had a negligible effect on algae survival (Figure S1), and decoy microrobots displayed long-term mobility in both pure and river water (Figure S2). Such robust, fast, and continuous movement of these decoy
microrobots could expedite their interaction with the viral S protein, improving precise binding and removal of target threats in wastewater.

Removal of SARS-CoV-2 S protein by decoy microrobots
Subsequently, we systemically explored the performance of ACE2 algae decoy microrobots in removing SARS-CoV-2 S protein. As predicted, increasing the density of decoy microrobots boosted the speed and effectiveness of the procedure for removal, with $5 \times 10^7$ mL$^{-1}$ of decoy microrobots removing 91% of 4 ng/mL of S protein from pure water in less than 24 h (Figure 3D). Furthermore, we compared the capacity of different algae groups to eliminate S proteins, including active ACE2 algae, dead ACE2 algae, bare algae, and cell-wall-deficient algae, and we discovered that active ACE2 algae showed remarkably effective binding and 86% elimination effectiveness after 8 h of continual movement (Figure 3E). After 16 h of operation, the dead ACE2 algae and active bare algae lacking ACE2 had a removal efficiency of 68% and 34%, respectively, suggesting the importance of algal motion and ACE2 alteration in the speed and effectiveness of removing S protein. The intraparticle diffusion model proposed by Weber and Morris was used to calculate removal effectiveness as follows:

$$q_t = kt^{1/2}$$

where $k$ is the rate constant for intraparticle diffusion, which contains both S protein diffusion ($k_S$) and microrobot diffusion ($k_{microrobot}$) as

$$\frac{1}{k} = \frac{1}{k_{microrobot}} + \frac{1}{k_S}$$
According to $k_{\text{passive}} < k_{\text{active}}$ ($k_{\text{passive}} = 9.129 \times 10^{-7} \text{ mg}^{-1} \text{ min}^{-1/2}$, $k_{\text{active}} = 1.57 \times 10^{-6} \text{ mg}^{-1} \text{ min}^{-1/2}$), active ACE algae eliminate the S protein by active migration. Nonspecific binding of algae surface coupled with the existence of different ligands (e.g., carboxyl or amino groups) is believed to be the cause of S protein removal by bare algae.\(^{31}\) Additionally, the ACE2 algae decoy microrobots also exhibited robust S protein removal in different media, including TAP medium, distilled water, and river water (Figure 3F), indicating the great potential of this attractive microrobot platform in environmental remediation in complicated surroundings.

### Removal of SARS-CoV-2 pseudovirus by decoy microrobots

To examine the efficacy of the ACE2 algal decoy microrobots on virus elimination, we used SARS-CoV-2 pseudoviruses, which are a viable option to real SARS-CoV-2 for study.\(^{25,34}\) The influence of robotic concentration on the efficacy of SARS-CoV-2 pseudovirus removal from pure water was initially examined, and as expected, the removal efficacy of the ACE2 algae decoy microrobots gradually increased, from 19% to 90% of $8 \times 10^8$ viral copies mL\(^{-1}\) of SARS-CoV-2 pseudoviruses within 24 h (Figure 4A), upon increasing the robot density from $10^4$ mL\(^{-1}\) to $10^7$ mL\(^{-1}\). The viral elimination kinetics of active ACE2 algae, dead ACE2 algae, bare algae, as well as cell wall-deficient algae in pure water were also investigated. The active ACE2 algae effectively removed 90% of pseudoviruses after 4 h treatment (Figure 4B); in contrast, dead ACE2 algae, bare algae, and deficient algae removed 76%, 46%, and 25% of pseudoviruses after 12 h operation, respectively. In addition, the ACE2 algae decoy microrobots also exhibited robust pseudovirus removal in both pure and river water (Figure 4C), ensuring further practical application of these decoy microrobots in a complicated environment. Overall, our findings show that fast algal mobility for virus contacting and the ACE2 receptor...
for virus binding both play important roles in extremely effective virus removal in wastewater samples.

**Removal of SARS-CoV-2 and its variants by decoy microrobots**

Finally, we tested the performance of our ACE2 algae decoy microrobots on the elimination of SARS-CoV-2 and its variants from liquid waste. The effects of robot density, robot type, operation time, and medium type on the viral removal performance were first investigated. After 4 h of continuous movement, the ACE2 algae at the density of $10^7$ mL$^{-1}$ efficiently removed 95% of $10^9$ viral copies mL$^{-1}$ of SARS-CoV-2 wild-type (WT) strain from pure water (Figure S3). Under optimized condition, each decoy microrobot can remove at most 900 viral copies of SARS-CoV-2 WT strain. After 12 h of operation, dead ACE2 algae and active bare algae had removal effectiveness of 78% and 55%, respectively. Given that the novel SARS-CoV-2 variants have a comparable affinity for the ACE2 receptor,12,35 our ACE2 algal decoy microrobots should be able to effectively remove various viral types from wastewater. As expected, the decoy microrobots demonstrated a broad-spectrum removal of SARS-CoV-2 variants with an efficiency of 95% for SARS-CoV-2 WT strain, 92% for the Delta variant, and 93% for the Omicron variant from wastewater samples, respectively (Figures 4D–4F). After the microrobot treatment, the supernatant of wastewater showed a reduced infection efficiency of host cells for SARS-CoV-2 and its variants (Figure S4). To clean the ACE2 algal decoy microrobots, flocculants (i.e., 0.5% chitosan) were used as a post-treatment,36 and the flocculants were able to separate the decoy microrobots from wastewater without reducing virus removal effectiveness (Figure S5). Additionally, to further investigate the stability of decoy microrobots for the SARS-CoV-2 removal, the decoy microrobots could be reused to achieve a virus removal effectiveness of 90% after 5 repeated cycles (Figure S6), further suggesting considerable potential of our decoy microrobot platform in practical environmental remediation applications.

**DISCUSSION**

In conclusion, we have devised a simple, safe, and successful decoy microrobot for actively removing SARS-CoV-2 and variants from wastewater. The decoy microrobots were fabricated by camouflaging algae with genetically engineered cell membrane vesicles displaying viral entry protein receptor ACE2, while maintaining the motility behavior and the targeting ability of ACE2 algae. The ACE2 algal decoy microrobots that resulted had exceptional mobility capabilities, allowing for “on-the-fly” elimination of SARS-CoV-2 S proteins and pseudovirus in a variety of water matrices. Moreover, because novel SARS-CoV-2 variants have a comparable attachment to the ACE2 receptor, the decoy microrobots demonstrated broad-spectrum SARS-CoV-2 and variants elimination in wastewater. Given the high viral load in our trial ($10^9$ viral copies mL$^{-1}$), each decoy microrobot removed at most 900 SARS-CoV2 viral copies, indicating a high removal effectiveness when compared with typical wastewater viral treatments.11 The post-treatment and reusability of the decoy microrobots were also investigated, revealing great potential for eliminating waterborne harmful viruses from polluted water. Despite the fact that the current platform can eliminate 95% of viruses, converting virus loads in sewage to host illness frequency is problematic. On the other hand, any decrease in viral load will be closely related to viral infectivity management and patient disease.11 Overall, such functionalized algae-based decoy microrobots provide an appealing solution for environmental remediation applications since they use genetic engineering and cell membrane camouflaging techniques to precisely display protein receptors onto natural algae surfaces.32,37 Due to their surface of unique proteins, the decoy
microrobots may be encapsulated with host cell-derived vesicles to adsorb Zika virus, platelet-derived vesicles to adsorb bacteria, or monocyte-derived vesicles to neutralize inflammatory factors. These modified vesicles, when combined with the microrobot, may be able to fulfill their exceptional adsorption capabilities and contribute to the future of the microrobot platform.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact
Further information and requests for resources and procedures should be directed to the lead contact, Prof. Lang Rao (lrao@szbl.ac.cn).

Materials availability
This study did not generate new unique materials.

Data and code availability
All of the data supporting the findings are presented within the article and supplementary information. All other data are available from the lead contact upon reasonable request.

Construction of 293T/ACE2 cells
Genetically modified ACE2 on 293T cells were graciously contributed by Dr. Lanying Du at Lindsley F. Kimball Research Institute, New York Blood Center. Parental and modified cells were seeded onto dishes to confirm the presence of ACE2, and after overnight culture, cells were treated for 30 min with recombinant SARS-CoV-2 spike RBD-Fc protein (Sino Biological, China), followed by 30 min of labeling with secondary antibody PE conjugated goat anti-human IgG (Jackson ImmunoResearch). After being labeled with 4′, 6-dimidyl-2-phenylindole (DAPI), the engineered cells were investigated using confocal laser scanning microscope (CLSM; ZEISS LSM700). Flow cytometry was also employed to validate the presence of ACE2 in 293T/ACE2 cells. After secondary antibody staining, dead cells were removed via 7-AAD viability staining solution. Data were gathered using a CytoFLEX flow cytometer and processed utilizing the corresponding CytExpert software (Beckman Coulter).

Fabrication of ACE2 vesicles
293T/ACE2 cells were homogenized using a Dounce homogenizer after being treated with hypotonic lysis buffer. Following DNase and RNase treatment (Invitrogen), the samples were rotated at 3,200 g for 3 min, and the supernatant was obtained and rotated again at 20,000 g for 30 min before being centrifuged at 80,000 g for 1.5 h. After collecting precipitates, they were rinsed in phosphate buffered saline (PBS) and treated with protease inhibitor tablets, and then processed with ultrasonic treatment for 5 min. Ultimately, samples were extruded via 400- and 200-nm polycarbonate membranes utilizing a mini-extruder technique (Avanti polar lipids) to generate ACE2 vesicles.

Fabrication of decoy microrobots
Green C. reinhardtii algae (strain CC-125 mt+) were generously provided by Dr. Lin Deng at Institute of Molecular Physiology, Shenzhen Bay Laboratory. The algae were transferred from the agar plate to TAP medium (Thermo Fisher) and cultivated at room temperature under cycles of 12 h sunlight and 12 h dark. To fabricate the ACE2 algae decoy microrobots, bare algae were rotated at 500 g for 3 min and rinsed 5 times with pure water to eliminate the TAP medium and then re-suspended.
in pure water. To camouflage the algae with the ACE2 vesicles, the as-prepared ACE2 vesicles were suspended in pure water and mixed with the algae (volume ratio of 1:1) overnight at 4°C with mild magnetic stirring. The resulting ACE2 algae decoy microrobots were rotated at 500 g for 3 min, washed 3 times with TAP medium, and collected for later use.

**Characterization of decoy microrobots**

The zeta potential of decoy microrobots was evaluated using a DLS (Nano-Zen 3600, Malvern Instruments, UK) before and after the ACE2 vesicle coating. TEM (JEM-2100HT, JEOL, Japan) was also used to examine the morphologies of decoy microrobots. The TEM sample of ultrathin section of bare algae and ACE2 algae decoy microrobots was prepared by the engineers (Medical Research Center for Structural Biology, School of Basic Medical Sciences, Wuhan University, China). Western blot was also used for the protein analysis on the decoy microrobots. In a 10% polyacrylamide gel, denatured samples of bare algae and ACE2 algae decoy microrobots were placed. Subsequently, the proteins were conducted transmembrane to polyvinylidene fluoride (PVDF) and blocked using 5% nonfat milk for 1 h. The 4°C overnight incubation of primary antibody was treated for ACE2 (AbClone, China). The PVDF membranes were then incubated for 1 h with a secondary antibody conjugated to horseradish peroxidase (Thermo Fisher), and the blots were observed using a West Pico PLUS Chemiluminescent Substrate Kit (Thermo Fisher).

**Motion analysis of decoy microrobots**

The motion of bare algae and ACE2 algae decoy microrobots was analyzed in different media, including TAP medium, pure water, and river water. After transferring to the river water, the speed of algae was measured at 1, 4, 12, and 24 h. The movies of the algae motion were captured by a Dragonfly Confocal Microscopy System (Andor) coupled with different microscope objectives, a Zyla 4.2 sCMOS camera, and 3D/4D Image Visualization and Analysis Software.

**Algae viability measurement**

The viability of ACE2 algae was explored by using the fluorescein diacetat (FDA) and propidium iodide (PI) fluorescent staining assay, in which FDA was used for active algae staining and PI for dead algae staining. Briefly, to eliminate the TAP medium, freshly prepared ACE2 algae were rotated at 500 g for 3 min and rinsed 5 times with pure water. The cell solution was concentrated and dyed using 1 mL of FDA/PI solution (Thermo Fisher) for 10 min, then rinsed 5 times with pure water. Finally, the cells were suspended in pure water and seeded on confocal dishes (Biofil, China) to test the algae viability. To count the number of living and dead cells and determine the relative ratio, the built-in threshold plugin of ImageJ was utilized. The live/dead tests were performed in triplicate, and each dish was photographed three times.

**Removal of SARS-CoV-2 S protein by decoy microrobots**

To investigate the influence of algal density on S protein removal effectiveness, the ACE2 algae decoy microrobots with different densities of $2 \times 10^6$, $5 \times 10^6$, $1 \times 10^7$, $2 \times 10^7$, and $5 \times 10^7$ mL$^{-1}$ were incubated with 4 ng mL$^{-1}$ of S protein in pure water, and the removal efficiency was tested at 24 h after the incubation. To explore the kinetic profiles of S protein removal effectiveness, the ACE2 algae, dead ACE2 algae, bare algae, as well as cell wall-deficient algae with a density of $5 \times 10^7$ mL$^{-1}$ were incubated with 4 ng mL$^{-1}$ of S protein in pure water, and the removal efficiency was tested at 1, 2, 4, 8, 12, 16, and 24 h after the incubation. To study the environmental effect on the removal efficiency, $5 \times 10^7$ mL$^{-1}$ ACE2 algae decoy microrobots were also incubated with 4 ng mL$^{-1}$ of S protein for 8 h in different media,
including TAP medium, pure water, and river water. To measure the S protein removal efficiency, all the groups were rotated at 500 g for 3 min and quantified by SARS-CoV-2 spike RBD protein ELISA kits (ABclonal).

Removal of SARS-CoV-2 pseudovirus by decoy microrobots
SARS-CoV-2 pseudoviruses were graciously contributed by Dr. Gong Cheng at School of Medicine, Tsinghua University, and developed following the below procedures. The HIV-1 backbone encoding luciferase reporters were co-transfected into 293T cells with S protein expression vectors comprising 293T/SARS-CoV-2/GFP. SARS-CoV-2 pseudoviruses were present in the supernatant, which was collected after 72 h transfection, rotated at 3,000 g for 10 min, and finally kept at −80°C. The ACE2 algal decoy microrobots with varying densities of 10³, 10⁴, 10⁵, 10⁶, and 10⁷ mL⁻¹ were cultured with SARS-CoV-2 pseudovirus in pure water at a concentration of 8 × 10⁸ viral copies mL⁻¹, and the removal effectiveness was assessed 24 h later. At a concentration of 8 × 10⁸ viral copies mL⁻¹ in pure water, the time-dependent removal efficiencies of SARS-CoV-2 pseudovirus by active ACE2 algae, dead ACE2 algae, bare algae, as well as cell wall-defective algae were tested at t = 1, 2, 4, 8, 12 and 16 h post incubation. To study the environmental effect on the removal efficiency, 10⁷ mL⁻¹ ACE2 algae decoy microrobots were also incubated with SARS-CoV-2 pseudovirus at the concentration of 8 × 10⁸ viral copies mL⁻¹ in pure water and river water for 4 h. The specific attaching of SARS-CoV-2 pseudoviruses with the ACE2 algae decoy microrobots was further observed with SEM (6700F, JEOL, Japan). The SEM sample was prepared by the engineers (Medical Research Center for Structural Biology, School of Basic Medical Sciences, Wuhan University, China).

The effects of decoy microrobot density on the SARS-CoV-2 removal
To investigate the impact of algae density on SARS-CoV-2 elimination efficiency, the ACE2 algae decoy microrobots with different densities of 10³, 10⁴, 10⁵, 10⁶, and 10⁷ mL⁻¹ were incubated with SARS-CoV-2 WT strain in pure water at the concentration of 10⁹ viral copies mL⁻¹ and tested the removal efficiency at 24 h after the incubation. To explore the time dependence removal efficiency of SARS-CoV-2, the ACE2 algae, dead ACE2 algae, bare algae, as well as cell wall-defective algae with SARS-CoV-2 in pure water at the concentration of 10⁹ viral copies mL⁻¹ were tested at t = 1, 2, 4, 8, 12, and 16 h after the incubation. To study the environmental effect on the removal efficiency, 10⁷ mL⁻¹ ACE2 algae decoy microrobots were also cultured with SARS-CoV-2 at the concentration of 10⁹ viral copies mL⁻¹ in pure water and river water for 4 h.

Removal of SARS-CoV-2 and its variants by decoy microrobots
SARS-CoV-2 WT strain (IME-BJ01 strain, GenBank No. MT291831) and the Delta (CSTR.16698.06.NPRC6.CCPM-B-V-049-2105-6) and Omicron (SARS-CoV-2 strain Omicron CoV/human/CHN_CVR-I-01/2022) variants were isolated from patients, and experiments involving SARS-CoV-2 WT strain and its variants were carried out in a biosafety level 3 (BSL3) facility. To explore the performance of ACE2 algae decoy microrobots on the elimination of SARS-CoV-2 and its variants, the ACE2 algae decoy microrobots with different densities of 10³, 10⁴, 10⁵, 10⁶, and 10⁷ mL⁻¹ were incubated with SARS-CoV-2 WT strain, the Delta strain, or the Omicron strain at the concentration of 10⁹ viral copies mL⁻¹ for 4 h in pure water. After centrifuging the samples at 500 g for 3 min, the supernatants were collected for RNA quantification using quantitative real-time PCR. Viral RNA was isolated from supernatants using EasyPureViral DNA and RNA Kit (Trans-Gen, China) and quantified using the One Step PrimeScrip RT-PCR Kit (Takara, Japan).
Inhibition of SARS-CoV-2 and its variant infection
To explore the inhibition of SARS-CoV-2 infection after the treatment of decoy microrobots, the ACE2 algae decoy microrobots with different densities of $10^3$, $10^4$, $10^5$, $10^6$, and $10^7$ mL$^{-1}$ were incubated with SARS-CoV-2 WT strain, the Delta strain, or the Omicron strain at the concentration of $10^9$ viral copies mL$^{-1}$ for 4 h in pure water. The supernatant was collected and transferred to 1× PBS; after centrifuging the samples at 500 g for 3 min, 50 μL of the supernatant was added to Vero-E6 cells. After 1 h of incubation at 37°C, the supernatant was substituted for new culture medium containing 2% FBS. After another 48 h, the supernatant was collected, and the inhibition of viral infection was estimated by detecting viral RNA levels.

Post-treatment and reutilization of decoy microrobots
For the post-treatment, the ACE2 algae decoy microrobots were treated by 0.5% chitosan for 10 min. In comparison, the control groups were treated by 500 g centrifugation for 3 min. For the reutilization, the ACE2 algae decoy microrobots with the density of $10^7$ mL$^{-1}$ were incubated with the Delta strain or the Omicron strain at the concentration of $10^9$ viral copies mL$^{-1}$ for 4 h in pure water. The performance of the decoy microrobots on the viral removal was evaluated after 5 repeated cycles.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xcgp.2022.101061.

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AUTHOR CONTRIBUTIONS
J.L., Q.-F.M., and M.T. contributed equally to this work. J.L. and L.R. conceived and designed the experiments. J.L., Q.-F.M., M.T., X.Z., P.P., and L. Du performed the experiments. J.L., Q.-F.M., M.T., L. Deng, J.T., N.J., and L.R. analyzed the data. J.L., Q.-F.M., M.T., J.T., and L.R. wrote the manuscript. All authors have given approval to the final version of the manuscript.

DECLARATION OF INTERESTS
L.R. has applied for patents related to this study (Chinese Patent No. 202210935288.6).

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