Membrane Nanoparticles Derived from ACE2-Rich Cells Block SARS-CoV-2 Infection

Cheng Wang, Shaobo Wang, Yin Chen, Jianqi Zhao, Songling Han, Gaomei Zhao, Jing Kang, Yong Liu, Liting Wang, Xiaoyang Wang, Yang Xu, Song Wang, Yi Huang, Junping Wang, and Jinghong Zhao*

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ABSTRACT: The ongoing COVID-19 pandemic worldwide necessitates the development of therapeutics against SARS-CoV-2. ACE2 is the main receptor of SARS-CoV-2 S1 and mediates viral entry into host cells. Herein, membrane nanoparticles (NPs) prepared from ACE2-rich cells were discovered to have potent capacity to block SARS-CoV-2 infection. The membranes of human embryonic kidney-239T cells highly expressing ACE2 were applied to prepare NPs using an extrusion method. The nanomaterials, termed ACE2-NPs, contained 265.1 ng mg⁻¹ ACE2 on the surface and acted as baits to trap S1 in a dose-dependent manner, resulting in reduced recruitment of the viral ligand to HK-2 human renal tubular epithelial cells. Aside from affecting receptor recognition, S1 translocated to the cytoplasm and induced apoptosis by reducing optic atrophy 1 expression and increasing cytochrome c release, which was also inhibited by ACE2-NPs. Further investigations revealed that ACE2-NPs efficiently suppressed SARS-CoV-2 S pseudovirions entry into host cells and blocked viral infection in vitro and in vivo. This study characterizes easy-to-produce membrane nanoantagonists of SARS-CoV-2 that enrich the existing antiviral arsenal and provide possibilities for COVID-19 treatment.

KEYWORDS: SARS-CoV-2, spike, ACE2, cell membrane-based nanoparticles, kidney

The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 85,000,000 individuals worldwide, resulting in more than 1,800,000 deaths.1 Fever, cough, and myalgia or fatigue are common symptoms of COVID-19 in patients.2 Once the illness worsens, acute respiratory distress syndrome, respiratory failure, sepsis, and acute kidney injury are common lethal complications.3 In addition to supportive and symptomatic care, development of therapies that target viral pathogenic processes, including cellular association, membrane penetration, endosomal escape, virion uncoating, and genome replication, will be instrumental for improvement of therapeutic outcomes.4 Attachment of SARS-CoV-2 to the cell membrane is the initial step in the pathogenesis of COVID-19. This process is attributable to the viral spike (S) protein, which can be degraded into S1 and S2 subunits by proteases.5 S1 is known to be responsible for recognizing host receptors, while S2 mediates viral fusion into the cytoplasm.6 Angiotensin-converting enzyme-2 (ACE2), a negative regulator of the renin-angiotensin system, is abundant in the kidneys, lungs, and intestines.7 S1 binds to ACE2 with a high affinity of 8.02 nM.8 Cells insensitive to SARS-CoV-2 become susceptible to the virus after transfection with ACE2. Additionally, anti-ACE2 serum and ACE2 blocking peptide efficiently inhibit viral invasion,6,8 indicating that ACE2 is a critical receptor for SARS-CoV-2.

Similar to the extracellular part of the coxsackievirus receptor, which is used to inhibit Coxsackie-B viruses,9 recombinant ACE2 markedly reduces the early infection of SARS-CoV-2 and protects human kidney organoids.10 Because of the natural location of ACE2 on the cell membrane and because membranes with active ingredients are potential drug candidates,11 we attempted to use the membranes of human cells with abundant ACE2 to combat SARS-CoV-2. Cell membrane-based nanoparticles (CMBNPs) were designed to overcome the shortcomings of membranes with uneven sizes.12 Taking advantage of...
functional elements such as inflammatory factor receptors, Toll-like receptors, and viral receptors on cell membranes, CMBNPs have been employed to suppress inflammation and microbial infections.\textsuperscript{13−16}

Herein, we developed CMBNPs against SARS-CoV-2 by extruding the membranes of human embryonic kidney (HEK)-293T cells highly expressing ACE2 (HEK-293T-ACE2, Figure 1). The nanomaterials, termed ACE2-nanoparticles (NPs), act

Figure 1. Diagram depicting the preparation and function of ACE2-NPs.

Figure 2. ACE2 screening in human cells and the characterization of ACE2-NPs. (a) Western blotting detection of ACE2 in five cell lines. β-actin was used as the reference. (b) Immunofluorescence microscopy showing the location of ACE2 (red) in HEK-293T-ACE2 cells. Nuclei were stained with DAPI (blue). The scale bar indicates 20 μm. (c) Immunofluorescence microscopy showing the location of ACE2 (red) and the adherence of S1 (green) on ACE2-NPs. The scale bar indicates 500 nm. (d) TEM image of ACE2-NPs. The scale bar indicates 200 nm. (e) Hydrodynamic diameters and surface charges of NPs. The results are shown as the means ± standard deviations (SDs). (f) ELISA results showing the ACE2 levels in NPs and cell lysates. *** $P < 0.001$, relative to the total cell lysate group. (g) Comparison of the amounts of ACE2 antibody bound to ACE2-NPs and HEK-293T-ACE2 cells containing equal amounts of membrane content. n.s., not significant.
as baits to trap S1 and efficiently block SARS-CoV-2 S pseudovirion infection in vitro and in vivo and thus are promising drug candidates for COVID-19 treatment.

RESULTS AND DISCUSSION

Preparation and Characterization of ACE2-NPs. ACE2 levels in human cell lines, including HEK-293T cells, HEK-293T-ACE2 cells, HK-2 proximal tubular cells, Caco-2 enterocytes, and A549 type II pneumocytes, were analyzed by Western blotting. HEK-293T-ACE2 was superior to the other cell lines for carrying the viral receptor (Figure 2a). Consistent with the location of ACE2 in enterocytes,8 ACE2 was mostly located in the membranes of HEK-293T-ACE2 cells (Figure 2b). The cells were then processed by repeated freezing and thawing to separate the membranes, which were further broken by sonication and used to fabricate ACE2-NPs using a classic extrusion method.17 The prepared NPs carried ACE2 and did not contain residual nucleic acids (Figure 2c). Transmission electron microscopy (TEM) showed that like red blood cell (RBC)-derived NPs prepared using the same size of polycarbonate membrane,18 ACE2-NPs were approximately 100 nm in size with a preferable dispersity in solution (Figure 2d). The average diameter of the negatively charged nanomaterial was 169 nm, as detected by dynamic light scattering (DLS) (Figure 2e, polydispersity index [PDI]: 0.188, Supporting Information (SI) Figure S1). HEK-293T-derived NPs (293T-NPs) prepared using the same method had ζ-potentials and diameters comparable to those of ACE2-NPs. The prepared NPs carried much more ACE2 than the total cell lysates (Figure 2f), indicating the successful removal of irrelevant proteins from the cytosol. 293T-NPs were less rich in ACE2 than ACE2-NPs (Figure 2f). The content of ACE2 in ACE2-NPs was 265.1 ng mg⁻¹, 3.2-fold higher than that in 293T-NPs. Different batches of ACE2-NPs had comparable levels of ACE2 (SI Figure S2). ACE2-NPs were stable in phosphate-buffered saline (PBS) (SI Figure S3) and presented a right-side-out ACE2 orientation (Figure 2g), which was a prerequisite for further functional assessment.

Inhibitory Effect of ACE2-NPs on S1 Recruitment to Sensitive Cells. S1, which contains a receptor-binding domain (RBD), is the ligand of ACE2.19 We immobilized biotinylated RBD on streptavidin (SA) biosensors and determined the interaction with NPs by biolayer interferometry (BLI). At equivalent concentrations, more ACE2-NPs than 293T-NPs...
coated the RBD (Figure 3a). As S1 adheres to the surfaces of sensitive cells, including HK-2 cells, we incubated the cells with 10 μg mL\(^{-1}\) S1 in the absence and presence of NPs. ACE2-NPs markedly decreased S1 recruitment at 2.5 mg mL\(^{-1}\) (based on the membrane proteins) and were more efficient than 293T-NPs (Figure 3b). ACE2-NPs bound to the RBD in a dose-dependent manner (Figure 3a), in line with the dose-dependent S1 neutralization (SI Figure S4). Immuno-fluorescence revealed that S1 was adsorbed onto ACE2-NPs (Figure 2c). Accordingly, few S1 subunits adhered to HK-2 cells after ACE2-NP treatment (Figure 3c).

With viral mutation, an S1 variant in which Asp\(^{614}\) is replaced with Gly (D614G) has become globally prevalent. This single site mutation makes the mutant SARS-CoV-2 much more infectious than the wild type. Our data showed that D614G-S1 bound to ACE2 with an affinity of 11.6 nM (Figure 3d), which is comparable to the affinity of wild-type S1 binding to ACE2 (8.02 nM). This finding supports the idea that the stronger contagiousity of the D614G mutant virus than the ancestral form is not due to enhanced receptor recruitment. The D614G mutation did not alter the dose-dependent binding of ACE2-NPs to S1 (SI Figure S5). ACE2-NPs were still more efficient
than 293T-NPs at binding to D614G-S1 (SI Figure S5) and blocking the recruitment of D614G-S1 (Figure 3e).

**Suppressive Effect of ACE2-NPs on S1-Induced Apoptosis.** S1 was located not only on the cell membranes but also in the cytoplasm of HK-2 cells (Figure 3c). A recent multilevel proteomics analysis has shown that individual proteins of SARS-CoV-2 reshape the central pathways of host cells, causing metabolic disorders, including mitochondrial dysfunction. SARS-CoV-2 S1 might affect cellular metabolism beyond the function of receptor recognition. A tandem mass tag (TMT) proteomics study was conducted to test this possibility. A total of 1269 proteins were uniformly altered in HK-2 cells independently exposed to 100 ng mL$^{-1}$ S1 and D614G-S1 for 24 h (SI Table S1), and mitochondrion organization and the apoptotic signaling pathway were determined to be enriched via Gene Ontology (GO) analysis (Figure 4a).

Dynamic alterations in mitochondrion organization are correlated with apoptosis. Optic atrophy 1 (OPA1), a dynamin-related protein located in the mitochondrial inner membrane, controls apoptosis through cristae remodeling independent of mitochondrial fusion. Loss of OPA1 induces cytochrome c release and caspase-dependent apoptotic nuclear events. Conversely, OPA1 overexpression protects cells from apoptosis by preventing the release of cytochrome c and activation of caspase 3. In the current study, S1 and D614G-S1 stimulations both reduced OPA1 expression and promoted cytochrome c release and caspase 3 activation, as revealed by Western blotting (Figure 4b) and quantitative real-time polymerase chain reaction (q-PCR, Figure 4c,d). Flow cytometry verified that S1 and D614G-S1 significantly increased apoptosis in HK-2 cells (Figure 4e). ACE2-NP treatment (100 μg mL$^{-1}$) elevated cellular OPA1 levels and attenuated the activity of the subsequent signaling cascade, reducing the apoptosis caused by S1 and D614G-S1 in a dose-dependent manner (SI Figure S6a). In line with the discrepancy in S1 recruitment, ACE2-NPs were superior to 293T-NPs in inhibiting apoptosis (SI Figure S6b).

**Antiviral Action of ACE2-NPs.** To obtain insights into the antiviral properties of ACE2-NPs in vitro, we infected HK-2 cells with SARS-CoV-2 S pseudovirions containing a luciferase reporter system. SARS-CoV-2 binds to ACE2 and enters host cells after proteolysis of transmembrane protease serine 2 (TMPRSS2) at the S1/S2 boundary. The intracellular proprotein convertase furin also promotes SARS-CoV-2 entry
Figure 6. Inhibitory effect of ACE2-NPs on S pseudovirion infection in mice. (a) Diagram depicting the establishment of the pseudovirion-based mouse infection model. Adenovirus, Adv; pseudovirions, Pv. (b) Protein bands of Flag-tag and His-tag in mouse lungs. β-actin was used as the reference. (c) EGFP mRNA expression relative to β-actin expression in mouse livers, (d) lungs, and (e) kidneys. The results are shown as the means ± SDs. *** P < 0.001. (f) Immunofluorescence microscopy revealing the inhibitory effect of ACE2-NPs on pseudovirion infection in mouse lungs. Flag-tag and His-tag are shown in red and green fluorescence, respectively. The scale bar indicates 20 μm.
by preactivating the viral S protein. Because human kidneys abundantly express ACE2, TMPRSS2, and furin, the S pseudovirions were largely endocytosed by HK-2 cells after 1 h of coincubation (Figure 5a). ACE2- and 293T-NP treatment both reduced viral entry. TEM showed that the S pseudovirions were adsorbed onto ACE2- and 293T-NPs (Figure 5b) to form a coronavirus-like complex. The luciferase assay revealed the dose-dependent antiviral activity of ACE2-NPs (Figure 5c). The half-maximal inhibitory concentration (IC50) of ACE2-NPs was 431.2 μg mL−1, lower than that of 293T-NPs (546.9 μg mL−1, Figure 5d). As ACE2-NPs contained 265.1 ng mg−1 ACE2 on the surface, the ACE2 content in ACE2-NPs at the IC50 was calculated to be 0.114 μg mL−1, comparable to the IC50 of ACE2 (0.1 μg mL−1) for blockade of SARS-CoV-2 S pseudovirions.

Figure 7. Distribution and toxicity analysis of ACE2-NPs. (a) ELISA results showing the ACE2 content in mouse serum at 1, 3, 6, and 12 h post injection of ACE2-NPs. (b) In vitro imaging of DiO-labeled ACE2-NPs in mouse kidneys, lungs, spleens, livers, and hearts, at 0.5, 1, 3, 6, 12, and 24 h post intravenous administration. (c) Counts of RBCs, WBCs, and PLTs in mouse blood at 1, 3, and 5 days post injection of ACE2-NPs. The results are presented as the means ± SDs. (d) HE staining of the organs of mice treated with sterile PBS and ACE2-NPs. The scale bar indicates 200 μm.

The in vivo antiviral properties of the NPs were evaluated using a pseudovirion-based mouse infection model that was developed to assess the capabilities of monoclonal antibodies and vaccines against Ebola virus and Lassa virus, respectively, outside of BSL-4 laboratories. Adenoviruses expressing ACE2 with a Flag-tag were intravenously administered to C57 mice; the mice were then injected with S pseudovirions expressing enhanced green fluorescent protein (EGFP) with a His-tag and NPs (Figure 6a). The mice infected by the adenovirus produced ACE2-Flag, resulting in susceptibility to S pseudovirions (Figure 6b). Pseudovirion infection was measured by detecting EGFP and His-tag. We found EGFP expression in the livers (Figure 6c), lungs (Figure 6d), kidneys (Figure 6e), and spleens (SI Figure S7) of the mice. NP
treatment significantly lowered viral invasion in all organs. ACE2-NPs were more efficient than 293T-NPs at blocking the virus, which was further confirmed by detection of the His-tag in mouse lungs via Western blotting (Figure 6b) and immunofluorescence (Figure 6f) analyses.

**Biocompatibility and Biodistribution of ACE2-NPs.** Overexpression of membrane receptors does not affect the biocompatibility of CMBNPs.55 We incubated increasing concentrations of ACE2-NPs with human umbilical vein endothelial cells (HUVECs) for 24 h. ACE2-NPs did not influence cell survival at doses up to 500 μg mL⁻¹ (based on the membrane proteins, SI Figure S8a). The negligible hemolysis additionally indicated the biosafety of these nanomaterials in vivo (Figure S8b). For in vivo evaluation, C57 mice were given 25 mg kg⁻¹ ACE2-NPs by intravenous injection. The NPs were almost eliminated from blood after 3 h (Figure 7a). Fluorescence imaging showed that ACE2-NPs, which were probed by 3,3‘-diododecylxacarbocyanine perchlorate (DiO), were mainly distributed in the liver and lungs (Figure 7b). ACE2-NP treatment had minor effects on the RBC, white blood cell (WBC), and platelet (PLT) counts (Figure 7c) and on the hemoglobin content (SI Figure S9) in the blood. The major organs, including the heart, liver, spleen, lungs, and kidneys, were collected 7 days after the initial injection. Hematoxylin and eosin (HE) staining revealed no pathological changes in the tissues (Figure 7d), suggesting nontoxicity in vivo and thus laying a foundation for the application of ACE2-NPs as nanaontagonists against SARS-CoV-2.

**CONCLUSION**

Membrane nanomaterials derived from HEK-293T-ACE2 cells with activity against SARS-CoV-2 were prepared and evaluated in the present study. The ACE2-NPs contained abundant ACE2, a critical receptor of SARS-CoV-2 S1. Through competitive inhibition, the ACE2-NPs bound to S1 and blocked the viral ligand from adhering to human renal tubular epithelial cells in a dose-dependent manner. Owing to the S1 recruitment, the ACE2-NPs adsorbed S pseudovirions onto their surfaces and blocked viral entry into the cytoplasm, thus protecting the host cells from viral infection. The ACE2-NPs also attenuated the apoptosis induced by S1 by elevating OPA1 expression and decreasing cytochrome c release. As there is a shortage of effective measures for COVID-19 treatment, we believe that these biocompatible and easy-to-produce nanoantagonists may be useful therapeutic candidates for COVID-19 treatment.

**METHODS**

**Cell Culture.** HEK-293T and A549 cells were purchased from Beyotime (Shanghai, CHN). HK-2 cells, Caco-2 cells, and HUVECs were obtained from the cell bank of the Chinese Academy of Sciences (CAS, Shanghai, CHN). HEK-293T-ACE2 cells were obtained from Prof. Lilin Ye.56 The cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, 11995065, Gibco, Thermo Fisher Scientific, Shanghai, CHN) containing 10% fetal bovine serum (FBS, 10100147, Gibco). Geneticin (400 μg mL⁻¹, ST081, Beyotime) was added to the culture medium of HEK-293T-ACE2 cells for stress screening.

**Western Blotting.** Cells were collected and lysed for protein extraction. A total of 25 μg of each protein sample were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A primary rabbit polyclonal antibody (1:1000, 10108-T26, Sino Biological, Beijing, CHN) and a goat anti-rabbit secondary antibody (1:1000, A0208, Beyotime) were employed to detect ACE2. To determine the amounts of S1 and D614G-S1 recruited to HK-2 cells, cells were seeded into a 6-well plate at a density of 1 x 10⁶ cells per well. Recombinant S1 (20 μg mL⁻¹, 40591-V08H, Sino Biological) and D614G-S1 (20 μg mL⁻¹, 40591-V08H3, Sino Biological) containing His-tags were pretreated with 2.5 mg mL⁻¹ NPs at 37 °C for 1 h and then added to cells; the cells were then incubated for another 1 h. A primary mouse monoclonal antibody (1:1000, AF5060, Beyotime) and a goat antimagouse secondary antibody (1:1000, A0216, Beyotime) were employed to detect S1. To elucidate the underlying mechanism of apoptosis, HK-2 cells were coincubated with 100 ng mL⁻¹ S1 and D614G-S1 at 37 °C for 24 h. A primary anti-OPA1 rabbit monoclonal antibody (1:1000, ab157457, Abcam, Shanghai, CHN), a primary anticytochrome c rabbit monoclonal antibody (1:1000, AF2047, Beyotime), and a primary anti-c-caspase 3 rabbit monoclonal antibody (1:1000, 9664S, Cell Signaling Technology, Shanghai, CHN) were used to detect target proteins. The cells in the sham group were treated with sterile PBS.

Homogenates of mouse lung samples were prepared with a gentleMACS Dissociator (Miltenyi Biotec, Bergisch Gladbach, GER). The Flag-tag and His-tag were detected using a primary rabbit monoclonal antibody (1:1000, 14793S, Cell Signaling Technology) and a primary mouse monoclonal antibody (1:1000, AF5060, Beyotime), respectively. A BeyoECL Plus chemiluminescence kit (P0018S, Beyotime) was used to visualize the protein bands. β-actin was detected with a mouse monoclonal antibody (AA128, Beyotime, 1:1000) as a reference. These experiments were repeated three times on different days.

**Immunoﬂuorescence Microscopy.** HEK-293T-ACE2 and HK-2 cells were seeded into a 12-well plate with sterile glass slides at a density of 2 x 10⁵ cells per well. A total of 100 μL of ACE2-NPs (2.5 mg mL⁻¹, based on the membrane proteins) coincubated with 10 μg mL⁻¹ S1 at 37 °C for 1 h were dripped on sterile glass slides coated with polylysine. A primary rabbit polyclonal antibody (1:200, 10108-T26, Sino Biological) and a goat antirabbit secondary antibody (A0516, Beyotime) were employed to stain ACE2. Nuclei were stained with 2-(4-aminophenyl)-6-indolecarbamidine dihydrochloride (DAPI, C1002, Beyotime). Additionally, 10 μg mL⁻¹ S1 and 1.98 x 10⁵ TU mL⁻¹ SARS-CoV-2 S pseudovirions (Genewiz, Suzhou, Jiangsu Province, CHN) pretreated with 2.5 mg mL⁻¹ NPs were added to HK-2 cells. Coincubation was further performed at 37 °C for 1 h. The cells in the sham group were treated with sterile PBS and stained with normal rabbit IgG (A7016, Beyotime, 1:200). A primary rabbit monoclonal antibody (1:200, 40150-R007, Sino Biological) and a goat antirabbit secondary antibody (Alexa Fluor 488, Invitrogen, Thermo Fisher Scientific) were employed to stain S1.

Mouse lung samples were prepared into paraffin sections (3 μm), which were then processed with xylene, rehydrated with decreasing concentrations of ethanol, and subjected to antigen retrieval in citrate buffer at 95–100 °C. The sections were incubated with normal rabbit IgG, a primary anti-Flag-tag rabbit monoclonal antibody (1:200, 14793S, Cell Signaling Technology), and a primary anti-His-tag mouse monoclonal antibody (1:200, AF5060, Beyotime). A Cy3-labeled goat antirabbit secondary antibody (A0516, Beyotime) and a FITC-labeled goat antimouse secondary antibody (A0568, Beyotime) were employed to detect Flag-tag and His-tag, respectively. A Zeiss LSM 780 NLO confocal microscope was applied to observe the cells.

**Preparation of ACE2-NPs and 293T-NPs.** ACE2-NPs and 293T-NPs were prepared as previously described with minor modifications.57 Briefly, harvested HEK-293T-ACE2 and HEK-293T cells were frozen at ~80 °C and thawed at room temperature; this process was repeated three times. The cracked membranes were initially separated by centrifugation at 6000g at 4 °C for 10 min and washed with cold PBS containing protease inhibitors. The crude membranes were further fragmented by sonication on ice with a Sonics (Newtown, CT) Vibra-Cell VX250 ultrasonic processor for 10 min at a power of 120 W. High-speed centrifugation was performed at 100 000g for 10 min to collect the membrane pellet using a Beckman Coulter (Shanghai, CHN) Avanti JXN-30 centrifuge. CMBNPs were prepared by continuously extruding the cell membranes 13 times using a LiposoFast-Basic mini extruder (Avanti Polar Lipids, Alabaster, AL) equipped with a 200 nm porous membrane.57
Characterization Analysis. The morphology of ACE2-NPs was observed by TEM (JEM-1400 PLUS, JEOL, Shanghai, CHN). The results of the DLS and ζ-potential experiments were determined with a Nano-ZS instrument (Malvern, Worcestershire, UK) at room temperature. Protein concentrations were measured with a BCA kit (P0012, Beyotime). The location of ACE2 on ACE2-NPs was observed by immunofluorescence microscopy. The content of ACE2 in NPs was determined by ELISA according to the manufacturer’s instructions (ab235649, Abcam). The ACE2 orientation was analyzed by determining the extents of binding of a FITC-labeled anti-ACE2 antibody (10108-MM36-F, Sino Biological) to ACE2-NPs and HEK-293T-ACE2 cells, as recently described.\(^1\) Six ACE2-NPs (100 μL, 0.45 mg mL\(^{-1}\)) and HEK-293T-ACE2 cells (100 μL, 2.58 × 10\(^6\) cells) were blocked with 1% bovine serum albumin and incubated with 3 μg of antibody at 37 °C for 30 min. After centrifugation at 20 000g for 5 min, the supernatant was harvested. The fluorescence intensity of the unbound antibody was measured and used to calculate the amount of antibody bound to ACE2-NPs or HEK-293T-ACE2 cells. ELISA and ACE2 orientation analysis were conducted in triplicate and repeated twice.

BLI. The binding of NPs to the SARS-CoV-2 RBD (40592-V08H, Sino Biological) and D614G-S1 was measured using a ForteBio Octet Red 96 BLI platform (Sartorius BioAnalytical Instruments, Bohemia, NY, US). Biotinylated RBD and D614G-S1 were obtained with a G-MM-IGT biotinylation kit (Genemore, Shanghai, CHN) and immobilized on SA biosensors at 15 μg mL\(^{-1}\). NPs were prepared in PBS at concentrations of 1.1, 0.11, and 0.011 mg mL\(^{-1}\). In the sham group, immobilized RBD and D614G-S1 were immersed in PBS. Association was performed with shaking at 1,000 rpm for 300 s. To determine the affinity of ACE2 for D614G-S1, ACE2 was prepared in PBS at concentrations of 100, 75, 50, 25, and 10 nM. The running times for association and dissociation were both 300 s. The binding data were processed by interstep correction and Savitzky-Golay filtering using ForteBio Data Analysis 7.0 software. The equilibrium dissociation constant (K\(_d\)) and fit coefficient (R\(_f\)) were generated by global fitting using a 1:1 fitting model.

Proteomics Research. HK-2 cells were exposed to 100 ng mL\(^{-1}\) S1 and D614G-S1 at 37 °C for 24 h. The extracted proteins from three independent experiments were mixed and digested into peptides, which were then labeled with an amine-reactive TMTsixplex Isobaric Mass Tagging Kit (90064B, Thermo Fisher Scientific). Afterward, the samples were fractionated into 10 fractions, which were analyzed with a QExactive Orbitrap Mass Spectrometer (Thermo Fisher Scientific) at a voltage of 2.5 kV. Full scans ranging from 350 to 1500 m/z were acquired at a resolution of 60 000 (at 200 μm/z) with an automatic gain control (AGC) target value of 3 × 10\(^6\) and a maximum ion injection time of 20 ms. The MS scans were recorded in profile mode, while the MS/MS scan was recorded in centroid mode.

Three replicate injections were performed for each set of samples. The data were processed using Proteome Discoverer (PD) software (version 2.2, Thermo Fisher Scientific). Peptides with scores above 20 and significant below the threshold filter (0.05) were selected for analysis. Single peptide identification required a score equal to or above the identity threshold. Trypsin was specified as the protease, and a maximum of two missed cleavages was allowed. The MS and MS/MS mass tolerances were set to 10 ppm and 0.02 Da, respectively. A false discovery rate of 1% was set at the peptide-spectrum match (PSM) level as well as the protein level. LC-MS/MS testing was conducted by Novogene Co., Ltd. (Beijing, CHN). The gene names of the encoded proteins identified in the proteomics analysis were uploaded into the online Metascape database (https://metascape.org/) for GO analysis.

Apoptosis Detection. Flow cytometry was performed as we have recently described.\(^6\) Briefly, HK-2 was seeded into 6-well plates at a density of 2 × 10\(^4\) cells per well and cultured overnight in DMEM containing 10% FBS. The cells were coincubated with 100 ng mL\(^{-1}\) S1 and D614G-S1 for 24 h in the absence and presence of NP (100, 50, and 10 μg mL\(^{-1}\), based on the membrane protein) pretreatment. The cells in the sham group were treated with sterile PBS. Apoptosis was measured with a Boyeetax Annexin V/PI detection kit (C1062M) and a BD C6 flow cytometry system (Franklin Lakes, NJ); 15 000 events per sample were obtained. The experiment was conducted in triplicate and repeated twice, and the data were processed using FlowJo software (version 7.6.1).

In Vitro Pseudovirion Infection Assay. The adherence of SARS-CoV-2 S pseudovirions to NPs was observed via TEM after 40 μL of pseudovirions (1.98 × 10\(^7\) TU mL\(^{-1}\)) were coincubated with 100 μL of NPs (2.5 mg mL\(^{-1}\), based on the concentration of membrane proteins) at 37 °C for 1 h. A luciferase assay was performed as we have recently described.\(^3\) Briefly, HEK-293T-ACE2 cells were seeded into a 96-well plate at a density of 5 × 10\(^4\) cells per well. S pseudovirions (20 μL, 1.98 × 10\(^7\) TU mL\(^{-1}\)) preincubated with increasing concentrations of ACE2-NPs (0.01, 0.05, 0.15, 0.2, 0.4, 0.6, 0.8, and 1 mg mL\(^{-1}\)) and 293T-NPs (0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mg mL\(^{-1}\)) at 37 °C for 1 h were added to HEK-293T-ACE2 cells, and the cells were incubated for 12 h. The plates were further cultured for 48 h in DMEM containing 10% FBS after removal of the pseudovirions. The luciferase activity was measured using a dual-luciferase reporter assay system (E1910, Promega, Beijing, CHN). The experiments were conducted in triplicate and repeated twice.

In Vivo Pseudovirion Infection Experiment. Thirty 8-week-old female C57 mice (18–22 g) were randomly divided into five groups (n = 6). The mice were infected with 2 × 10\(^7\) TU of adenovirus expressing ACE2 containing a Flag-tag (200 μL, AD-hACE2, Tsingke Biotechnology, Beijing, CHN) and exposed to 1 × 10\(^7\) TU of SARS-CoV-2 S pseudovirions expressing EGFP containing a His-tag (100 μL, LV-Spike-nCOV, Tsingke Biotechnology) 5 days later; the time for pseudovirions injection was selected according to the Flag-tag expression in mice livers, lungs, and kidneys (SI Figure S10). NP treatment (2.5 mg mL\(^{-1}\), 200 μL) was performed after 0.5 h. The pseudovirions and NPs were all intravenously administered. The mice in the sham group were treated with sterile PBS. After 24 h, the mice were sacrificed to obtain liver, spleen, lung, and kidney samples, which were then analyzed by q-PCR, Western blotting, and immunofluorescence microscopy. The animal experiment was approved by the Animal Experimental Ethics Committee of TMMU (AMUWEC202015SS). The mice were cared for and treated in accordance with the recommendations of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8523 Rev. 1985).

q-PCR. RNA extracted from HK-2 cells and mouse lungs with TaKaRa (Dalian, Liaoning, CHN) RNAsio Plus reagent was reverse-transcribed using a TaKaRa PrimeScript RT-PCR kit (DRR014A). The primers applied were as follows: OP01, 5′-CTGTTGGCCTGGATAGCACAGGA-3′, S′-CGGAGGCTGTTGACCCATTTACG-3′ (R); cytomeglovirus, 5′-TGCTGGCATTCTCCCAGTCG-3′ (F), 5′-AGGCACTGGAATTAAGTTAGCTC-3′ (R); EGFP, 5′-ACAGGGTAAACATTGCTCGACGC-3′ (F), S′-CGCGGATGTACTTCTTGCGAG-3′ (R); Flag-tag, 5′-ACACTGGAGAAGGAGAGAGACGC-3′ (F), 5′-ACGACAGCTGTTGTGCTCATCAC-3′ (R), and β-actin, 5′-AAGGATTCTATGTGGGCGAC-3′ (F), 5′-CTGGACAGGATGAGACCGC-3′ (R). The results were processed using a Bio-Rad iQ5 standard-edition optical system (version 2.1). This experiment was conducted in duplicate and repeated three times.

Biodistribution Analysis. Dio-labeled ACE2-NPs were prepared by extruding the membranes of HEK-293T-ACE2 cells stained with 10 μM Dio (C1038, Beyotime). Forty-two 8-week-old female C57 mice (18–22 g) were randomly divided into seven groups (n = 6). The NPs were intravenously administered at 20 mg kg\(^{-1}\). The mice in the sham group were treated with sterile PBS. The mice were sacrificed at 0, 0.5, 1, 3, 6, 12, and 24 h post injection. Whole blood and major organs, including the heart, liver, spleen, lungs, and kidneys, were obtained. The ACE2 level, flow cytometry assay at 1, 3, 6, and 12 h post injection of NPs, were detected by ELISA. The accumulation of Dio-labeled ACE2-NPs in major mouse organs was observed with an IVIS Spectrum Imaging System (PerkinElmer, Shanghai, CHN).

Toxicological Evaluation. HUVECs were seeded into a 96-well plate at a density of 5 × 10\(^4\) cells per well. ACE2-NPs were prepared in DMEM containing 10% FBS at concentrations of 100, 200, 300, 400, and 500 μg mL\(^{-1}\). After adherence, cells were exposed to ACE2-NPs at 37 °C for 24 h. Cell viability was measured using a Cell Counting Kit-8.
Mouse RBCs (300 μL) diluted in 0.9% NaCl solution were incubated with 1.2 mL of ACE2-NPs at 37 °C for 2 h. The absorbance of the supernatant was determined at 405 nm using a microplate reader. The toxicity of ACE2-NPs in vivo was evaluated with mouse experiments. ACE2-NPs (25 mg kg⁻¹) were administered by intravenous injection. Mouse blood obtained through the tail vein was analyzed on a Sysmex XT-2000i fully automatic hematology analyzer (Kobe, JPN) at 1, 3, and 5 days after injection. The mice were sacrificed on day 7. HE staining and an Olympus DXS1 optical microscope (Tokyo, Japan) were applied to observe the pathological changes in major organs. The cells and mice in the sham group were treated with sterile PBS.

**Statistical Analysis.** The significance (P) values of differences between groups were calculated using SPSS 25.0 software via paired two-tailed t tests and LSD multiple-comparison tests. A P value lower than 0.05 was considered to indicate statistically significant.

**ASSOCIATED CONTENT**

- **Supporting Information**
  The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.0c06836.

- **Size range, stability, and cytotoxicity of NPs; additional data showing the inhibition of NPs on S1 recruitment and S1-induced apoptosis; bindings of NPs to D614G-S1; EGFP and Flag-tag mRNA expressions in mice; hemoglobin contents in mouse blood (PDF)**

- **Table showing the result of proteomics study (XLSX)**

**AUTHOR INFORMATION**

**Corresponding Authors**

- **Jinghong Zhao** — Department of Nephrology, The Key Laboratory for The Prevention and Treatment of Chronic Kidney Disease of Chongqing, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China; Email: zhaojh@tmmu.edu.cn

- **Junping Wang** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China; Email: wangjunping@tmmu.edu.cn

**Authors**

- **Cheng Wang** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China; [orcid.org/0000-0002-6690-6433]

- **Shaobo Wang** — Department of Nephrology, The Key Laboratory for The Prevention and Treatment of Chronic Kidney Disease of Chongqing, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

- **Yin Chen** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

- **Jianqi Zhao** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

**Songling Han** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

**Gaomei Zhao** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

**Jing Kang** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

**Yong Liu** — Department of Nephrology, The Key Laboratory for The Prevention and Treatment of Chronic Kidney Disease of Chongqing, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

**Liting Wang** — Biomedical Analysis Center, Third Military Medical University, Chongqing 400038, China

**Xiaoyang Wang** — Biomedical Analysis Center, Third Military Medical University, Chongqing 400038, China

**Yang Xu** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

**Song Wang** — State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Combined Injury of PLA, Chongqing Engineering Research Center for Nanomedicine, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, China

**Yi Huang** — Biomedical Analysis Center, Third Military Medical University, Chongqing 400038, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsnano.0c06836

**Author Contributions**

*C Wang, S Wang, and Y Chen contributed equally to this work.

**Notes**

The authors declare no competing financial interest.

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