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Letter

Cellular Nanosponges Inhibit SARS-CoV-2 Infectivity

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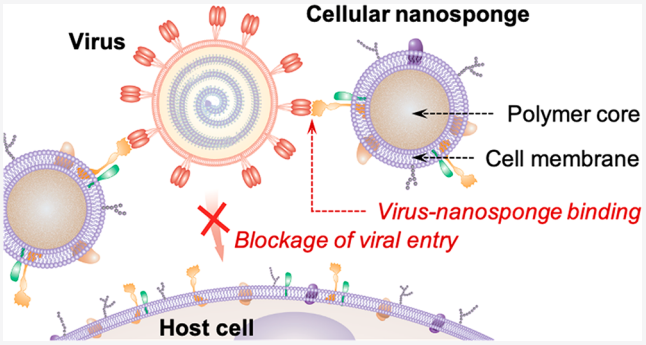
ABSTRACT: We report cellular nanosponges as an effective medical countermeasure to the SARS-CoV-2 virus. Two types of cellular nanosponges are made of the plasma membranes derived from human lung epithelial type II cells or human macrophages. These nanosponges display the same protein receptors, both identified and unidentified, required by SARS-CoV-2 for cellular entry. It is shown that, following incubation with the nanosponges, SARS-CoV-2 is neutralized and unable to infect cells. Crucially, the nanosponge platform is agnostic to viral mutations and potentially viral species, as well. As long as the target of the virus remains the identified host cell, the nanosponges will be able to neutralize the virus.



KEYWORDS: *COVID-19, coronavirus, nanosponge, cell membrane, broad spectrum*

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an outbreak of coronavirus disease (COVID-19), and the pandemic has unfolded into a severe global public health crisis.[1,2] Remdesivir is currently the most advanced antiviral drug for COVID-19 treatment, which received an emergency-use authorization in the United States for patients with severe disease, but the mortality benefit is unproven.[3] The search for new drugs requires a clear understanding of the underlying molecular mechanisms of viral infection, which is a particular challenge with emerging viruses such as SARS-CoV-2.[4,5] Moreover, antiviral medicine often targets a specific viral species that cannot be deployed across different species or families of viruses and may be rendered ineffective as the virus accumulates mutations and escapes treatments.[6] Therefore, an effective therapeutic agent to inhibit SARS-CoV-2 infectivity, as well as its potential mutated species, would be a significant game changer in the battle against this public health crisis.

Early understanding of the clinical manifestation of COVID-19 is severe viral pneumonia. Emerging data are clear that SARS-CoV-2 elicits significant damage on other organ systems either directly or indirectly through downstream immunological effects.[7] Up to 75% of COVID-19 patients present with some renal involvement, with a significant portion of patients developing acute kidney injury.[8] Acute respiratory distress syndrome (ARDS) is a common and deadly manifestation of COVID-19 and is associated with prolonged intubation and high mortality.[9] Typically, COVID-19 patients initially present mild symptoms, yet a subset of patients rapidly develop complications such as ARDS and multiorgan failure and ultimately death. The rapid clinical deterioration is thought to be closely related to the cytokine storm.[10] Recently, coagulopathy has been described as a critical morbidity in COVID-19 patients and is associated with worse outcomes.[11] All of these clinical complications speak to the complexity of this disease and that the consequence of immune response to the viral infection may be the main driver of morbidity and mortality of COVID-19.

A novel approach to drug development is to place the focus on the affected host cells instead of targeting the causative agent. Inspired by the fact that the infectivity of SARS-CoV-2 relies on its binding with the protein receptors, either known or unknown, on the target cells, we create cellular nanosponges as a medical countermeasure to the coronavirus. These nanosponges are made of human-cell-derived membranes, which are sourced from cells that are naturally targeted by SARS-CoV-2 (Figure 1A). The nanosponges display the same receptors that the viruses depend on for cellular entry. We hypothesize that, upon binding with nanosponges, the coronaviruses are unable to infect their usual cellular targets. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) and CD147 expressed on the host cells, such as human alveolar epithelial type II cells, as receptors for cellular entry.[12] Human

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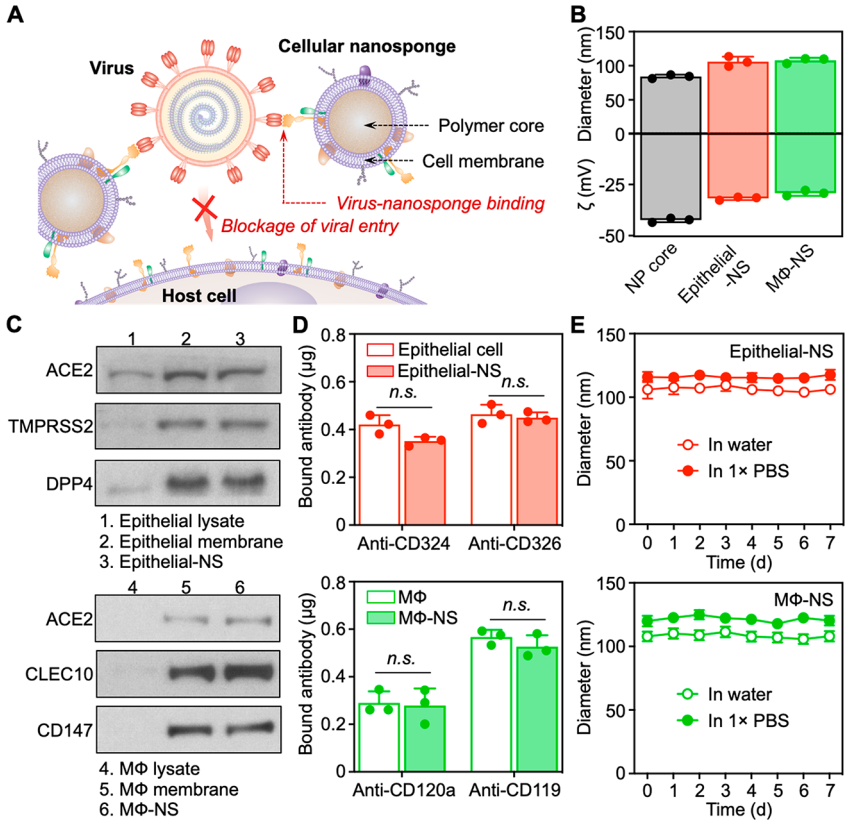

Figure 1. Fabrication and characterization of cellular nanosponges. (A) Schematic mechanism of cellular nanosponges inhibiting SARS-CoV-2 infectivity. The nanosponges were constructed by wrapping polymeric nanoparticle (NP) cores with natural cell membranes from target cells such as lung epithelial cells and macrophages (MΦs). The resulting nanosponges (denoted "Epithelial-NS" and "MΦ-NS", respectively) inherit the surface antigen profiles of the source cells and serve as decoys to bind with SARS-CoV-2. Such binding interaction blocks viral entry and inhibits viral infectivity. (B) Dynamic light scattering measurements of hydrodynamic size (diameter, nm) and surface zeta-potential (ζ, mV) of polymeric NP cores before and after coating with cell membranes (n = 3; mean + standard deviation). (C) Selective protein bands of cell lysate, cell membrane vesicles, and cellular nanosponges resolved with Western blotting analysis. (D) Comparison of the fluorescence intensity measured from cellular nanosponges (100 μL, 0.5 mg/mL membrane protein concentration) or source cells (100 μL, approximately 2.5 × 10^6 cells) containing equal amounts of membrane content and stained with fluorescently labeled antibodies (n = 3; mean + standard deviation; n.s.: not significant; statistical analysis was performed with paired two-tailed t-test). (E) Stability of cellular nanosponges in 1× phosphate-buffered saline determined by monitoring particle size (diameter, nm) over a span of 7 days (n = 3; mean ± standard deviation).

macrophages both express CD147 and have been reported to play a significant role in the infection by frequent interactions with virus-targeted cells through chemokines and phagocytosis signaling pathways.[13]

Based upon the current knowledge of SARS-CoV-2, we fabricated two types of cellular nanosponges, human lung epithelial type II cell nanosponge (denoted "Epithelial-NS") and human macrophage nanosponge (denoted "MΦ-NS"). The resulting cellular nanosponges were thoroughly characterized for their physicochemical and biological properties, followed by *in vivo* evaluation of their safety in the lungs. Then, these samples were independently tested in a biosafety level 4 (BSL-4) laboratory for inhibitory effects on human SARS-CoV-2 virus and demonstrated clear antiviral efficacy *in vitro*.

To prepare cellular nanosponges, cell membranes of human lung epithelial cells and macrophages were derived with a differential centrifugation method and verified for purity. The membranes were then coated onto polymeric nanoparticle cores made from poly(lactic-*co*-glycolic acid) (PLGA) with a sonication method to form Epithelial-NS and MΦ-NS, respectively. When examined with dynamic light scattering, both Epithelial-NS and MΦ-NS showed hydrodynamic diameters larger than that of the uncoated PLGA cores (Figure 1B). The surface zeta-potential of the nanosponges was less negative than that of the PLGA cores but comparable to that of the source cells (Table S1). These changes are consistent with the addition of a bilayer cell membrane. Cell membrane coating allows nanosponges to inherit the viral receptors related to coronavirus entry into the host cells. For verification, Western blot analysis showed the presence of viral receptors such as ACE2, transmembrane serine protease 2 (TMPRSS2), and dipeptidyl peptidase IV (DPP4) on the Epithelial-NS, and ACE2, C-type lectin domain family 10 (CLEC10), and CD147 on the MΦ-NS (Figure 1C).[12,13] The results also showed that the nanosponge preparation facilitated membrane protein retention and enrichment on the nanosponges, without contamination from intracellular proteins (Figure S1). For viral neutralization, right-side-out membrane orientation, driven by the asymmetric repulsion between the cores and the extracellular membrane versus the intracellular membrane, is essential.[14] To examine the membrane sidedness, we stained cellular nanosponges and their source cells containing equal amounts of membrane content using fluorescently labeled antibodies against select membrane antigens. After the removal of free antibodies, cellular nanosponge samples showed fluorescence intensities compa-

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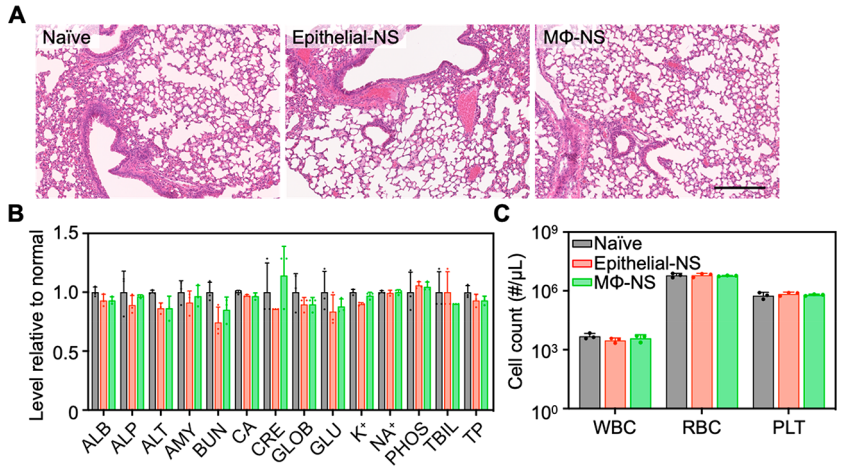

Figure 2. *In vivo* safety of cellular nanosponges. (A) Hematoxylin and eosin (H&E) staining of representative lung sections taken 3 days after intratracheal administration of the cellular nanosponges (scale bar: 250 μm). (B) Comprehensive serum chemistry panel performed 3 days after intratracheal administration of the cellular nanosponges (n = 3; mean + standard deviation). ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; BUN, urea nitrogen; CA, calcium; CRE, creatinine; GLOB, globulin (calculated); GLU, glucose; K^+, potassium; NA^+, sodium; PHOS, phosphorus; TBIL, total bilirubin; TP, total protein. (C) Blood cell counts 3 days after intratracheal administration of cellular nanosponges (n = 3; mean + standard deviation).

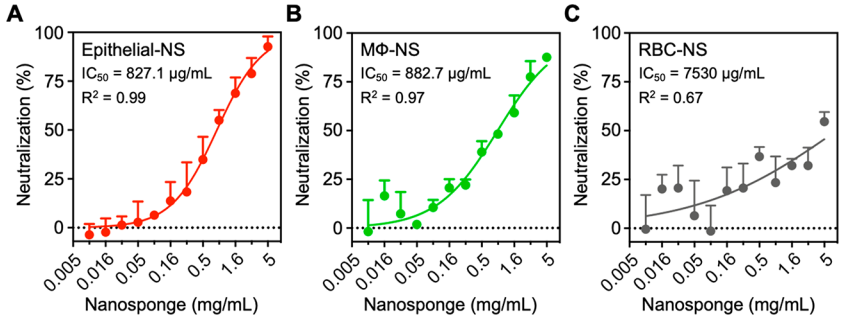

Figure 3. Cellular nanosponges neutralize SARS-CoV-2 infectivity. The neutralization against SARS-CoV-2 infection by (A) Epithelial-NS, (B) MΦ-NS, and (C) nanosponges made from red blood cell membranes (RBC-NS, used as a control) was tested using live SARS-CoV-2 viruses on Vero E6 cells. The IC_{50} values for Epithelial-NS and MΦ-NS were found to be 827.1 and 882.7 μg/mL (membrane protein concentration), respectively. In all data sets, n = 3. Data are presented as mean + standard deviation. Horizontal dashed lines mark the zero levels. IC_{50} values were derived from the variable slope model using Graphpad Prism 8.

rable with those of the cell samples (Figure 1D). This indicates that the nanosponges adopted a right-side-out membrane orientation because inside-out membrane coating would reduce antibody staining.[15] The membrane coating also provided cellular nanosponges with extended colloidal stability in 1× phosphate-buffered saline (Figure 1E).

After confirming the successful fabrication of Epithelial-NS and MΦ-NS, we sought to evaluate their acute toxicity after *in vivo* administration in mice. Given that our intended use is the deployment of cellular nanosponges for the treatment of coronavirus infections that predominantly affect the respiratory tract,[9] we elected to study the intratracheal route of administration using the highest feasible dose of Epithelial-NS or MΦ-NS (300 μg, based on membrane protein, in a suspension of 20 μL). Histopathological analysis of lung tissue 3 days after nanosponge administration revealed that immune infiltration was similar to baseline levels, and there was no evidence of lesion formation or tissue damage (Figure 2A). Furthermore, we examined multiple blood parameters, including a comprehensive serum chemistry panel and blood cell counts, 3 days after nanosponge administration (Figure 2B,C). All of the blood markers that were studied, in addition to red blood cells, platelets, and white blood cell counts, were consistent with baseline levels, confirming the short-term safety of the cellular nanosponges.

We next evaluated the neutralization of infectivity by authentic SARS-CoV-2 with a plaque reduction neutralization test. In the study, a low passage sample of SARS-CoV-2 (USA-WA1/2020, World Reference Center for Emerging Viruses and Arboviruses)[16] was amplified in Vero E6 cells to make a working stock of the virus. Vero E6 cells were seeded at 8 × 10^5 cells per well in 6-well plates the day prior to the experiment. Serial quarter-log dilutions of the nanosponges were mixed with 200 plaque-forming units (PFU) of SARS-CoV-2. The mixture was incubated at 37 °C for 1 h and then added to the cell monolayers followed by an additional 1 h of incubation. Mock-infected and diluent-only infected wells served as negative and positive controls, respectively. Monolayers were overlaid and incubated for 2 days followed by viral plaque enumeration. Following the incubation, cultures without adding Epithelial-NS showed a viral count comparable to that in the negative control, confirming viral entry and infection of the host cells. Inhibition of the infectivity increased as the concentration of Epithelial-NS increased, suggesting a dose-dependent neutralization effect (Figure 3A). Based on the results, a half-maximal inhibitory concentration (IC_{50}) value of

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827.1 μg/mL for Epithelial-NS was obtained. In parallel, a similar dose-dependent inhibition of the viral infectivity was observed with MΦ-NS (Figure 3B). In this case, an IC_{50} value of 882.7 μg/mL was obtained. These results indicate that the Epithelial-NS and MΦ-NS have comparable ability to inhibit viral infectivity of SARS-CoV-2. To further verify that the inhibition was indeed due to epithelial cell or macrophage membrane coating, control nanosponges made from membranes of red blood cells (denoted "RBC-NS") were also tested in parallel for viral inhibition but were not effective in neutralizing SARS-CoV-2 infection of Vero E6 cells (Figure 3C).

As a novel virus causing the current global pandemic, new information regarding SARS-CoV-2 is emerging on a daily basis. Since the first case that was reported at the end of 2019, it has been shown that the virus is mutating at a rapid rate.[17] This rapid rate of mutation will pose a major challenge to the development of therapeutics and preventive measures.[18] Both Epithelial-NS and MΦ-NS demonstrated the ability to neutralize SARS-CoV-2 in a concentration-dependent manner. The nanosponge platform offers a unique benefit over other therapies currently in development for COVID-19 in that the nanosponges are mutation and potentially virus agnostic. In principle, as long as the target of the virus remains the identified host cell, the nanosponges will be able to neutralize the infection, providing a broad-acting countermeasure resistant to mutations and protection against this and other emerging coronaviruses. The utility of the cellular nanosponges for the treatment of SARS-CoV-2 infection requires further validation in appropriate animal models, which is currently underway, and this will pave the way for human clinical trials in the future. Moreover, optimization of the lead formulation may further improve the antiviral efficacy of these nanosponges.

For the treatment of COVID-19, MΦ-NS may have some significant advantages over Epithelial-NS. The clinical manifestation of COVID-19 is partially driven by direct viral damage but primarily by the immune response to the infection. Previous studies on SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) demonstrated that macrophages play a significant role in the pathogenesis of those infections. Emerging data from SARS-CoV-2 also paint a similar picture, where macrophages play a central role either through direct viral entry *via* CD147 or downstream hyperinflammatory response to SARS-CoV-2.[19] Our previous work has demonstrated that MΦ-NS has a broad-spectrum neutralization capability, including against bacterial toxins and inflammatory cytokines.[20] Specific to COVID-19, MΦ-NS can neutralize the viral activity not only early on to reduce the viral load in the body but also even late in disease, and it will be able to address the fulminant inflammation associated with COVID-19. Given the central role that macrophages play in the immune system, the application of MΦ-NS extends beyond infections such as SARS-CoV-2 and may have significant roles in treating inflammatory diseases such as sepsis and other autoimmune diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.0c02278.

Materials and methods, Figure S1, and Table S1 (PDF)

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Author Contributions

Q.Z., A.H., and J.Z. contributed equally to this work. Q.Z., A.H., R.H.F., W.G., A.G., and L.Z. designed the study. Q.Z., A.H., J.Z., H.G., S.N.D., and J.H.V. performed the experiments. Q.Z., A.H., J.Z., R.H.F., and W.G. analyzed the data. Q.Z., A.H., R.H.F., W.G., A.G., and L.Z. wrote the paper.

Notes

The authors declare the following competing financial interest(s): L.Z. discloses financial interest in Cellics Therapeutics.

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