Application Note

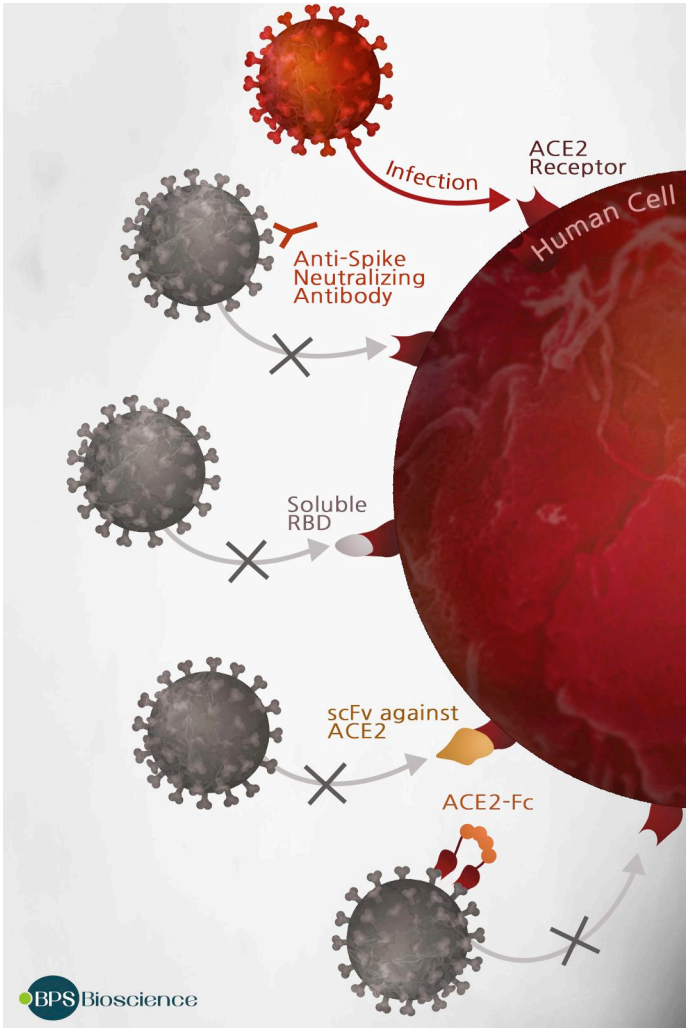
SARS-CoV-2 Spike pseudoviruses

Spike pseudotyped lentiviruses are particularly useful to safely study the interaction between the SARS-CoV-2 Spike protein and its human receptor ACE2 in cellular models.

Background

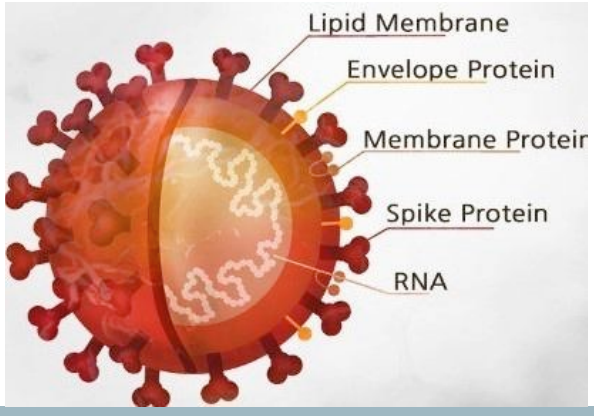
The SARS-CoV-2 virus responsible for COVID-19 infects human cells through the binding of its protein Spike to human ACE2 (Angiotensin Converting Enzyme 2), a protein present on the surface of cells in the respiratory system, arteries, heart, kidney, neurological and digestive systems. Most of the Spike protein protrudes outside the virion, making it a prominent target for our immune system. Therefore, patients with COVID-19 usually develop antibodies against Spike, including antibodies against the RBD (Receptor Binding Domain). These antibodies block the interaction between RBD and ACE2, prevent virus entry and neutralize the infection.

Knowledge of this mechanism has allowed scientists to design a variety of therapeutic approaches meant to compete with and inhibit the interaction between SARS-CoV-2 Spike and human ACE2. Potential drugs include soluble forms of ACE2 or antibodies directed against either ACE2 or Spike RBD. Neutralizing antibodies identified from convalescent COVID-19 patients have been deployed as new therapeutic options by pharmaceutical companies, while forced expression of the Spike protein to induce antibody production by the immune system is the basis of several vaccines.



Pseudotyped SARS-CoV-2 lentiviruses

BPS Bioscience has developed off-the-shelf, HIV-based lentiviruses, ready for infection and capable of transducing most types of dividing or non-dividing mammalian cells. Efficient gene transduction leads to robust expression of reporter proteins. Importantly, none of the HIV genes (gag, pol, rev) are expressed in the infected cells. Our lentiviruses are produced from packaging cells and are replication-incompetent; they **can be used in a Biosafety Level 2 facility**, which makes them accessible to many research laboratories.



Pseudotyped SARS-CoV-2 lentiviruses contain the SARS-CoV-2 protein Spike (wild-type or variant) as an envelope protein instead of the commonly used VSV-G protein, and work by infecting a target cell expressing human ACE2. Upon infection, the reporter gene (luciferase, eGFP, or both) under the control of a constitutive mammalian promoter is transduced into the cells. The resulting eGFP fluorescence or luciferase activity is directly proportional to the amount of Spike:ACE2 binding.

SARS-CoV-2 Spike pseudoviruses are designed to support COVID-19 research using biologically-relevant cellular models such as ACE2-HeLa, ACE2-CHO and ACE2-HEK293 cells.

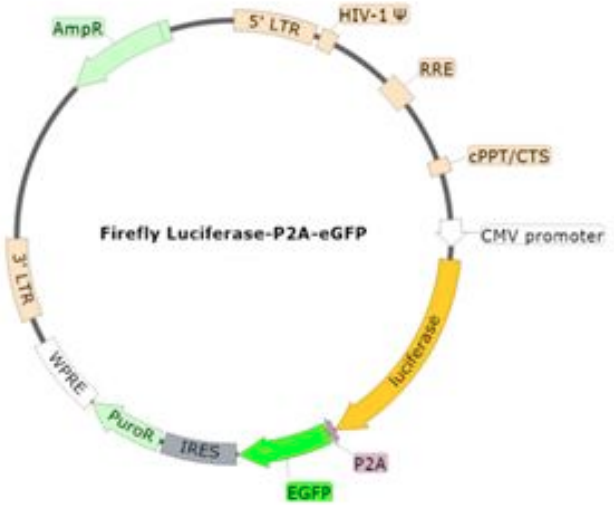
Other good models include Vero E6 cells, commonly used for coronavirus studies and expressing ACE2 endogenously. In this instance, the TMPRSS2-Vero E6 cells express serine protease TMPRSS2, which promotes membrane fusion and optimizes viral entry into the cells. Our pseudotyped viruses do not carry the risk of using live coronavirus and are useful to screen, validate or otherwise study the effect of a candidate drug on Spike:ACE2 binding.

Principle

The Spike pseudotyped viral particle binds to human ACE2 on the surface of ACE2-expressing cells, allowing the virus to infect the cells and transduce its genome. These virions contain a gene encoding reporter protein luciferase, so that expression of luciferase is observed approximately 48-72 hours after transduction in most cell types. The resulting enzymatic activity of luciferase can be measured and is directly proportional to Spike:ACE2 binding and subsequent transduction of the reporter gene. Optimal experimental conditions such as MOI (Multiplicity Of Infection), concentration of polybrene, and timing should be optimized for each cell line. Typically, inhibitors acting against Spike are pre-incubated with the pseudovirus before adding the mix to the cells, whereas inhibitors acting against ACE2 are pre-incubated with the cells before the addition of the pseudovirus.

Possible Applications

- Screening of compounds inhibiting Spike:ACE2 interaction, such as neutralizing antibodies, soluble ACE2, or ACE2 fragments and peptides
- Determining the IC_{50} of neutralizing antibodies using WT or variant Spike; identifying the best antibody or the best drug candidate in a direct comparison assay
- Examining the effect of SARS-CoV-2 strain variation or mutation on the efficacy of a neutralizing antibody or drug
- Exploring how various experimental parameters (e.g. buffer components, pH) affect Spike:ACE2 interaction or affect the efficacy of an antibody or drug of interest
- Assessing vaccines against wild-type and variant SARS-CoV-2



Dual eGFP/luciferase reporter construct

Application Example #1: Assessment of candidate ACE2 decoys for drug development purposes

The following study entitled "**Designed variants of ACE2-Fc that decouple anti-SARS-CoV-2 activities from unwanted cardiovascular effects",** was published by Liu P, Xie X, Gao L, Jin J. in *Int. J. Biol. Macromol.* (2020) 165(Pt B): 1626-1633; PMCID: PMC7568492.

Goal of the study

The authors designed a recombinant ACE2 decoy consisting of an ACE2-Fc fusion protein, to use as a therapeutic approach for the treatment of COVID-19. The addition of an Fc fragment to ACE2 is expected to increase the half-life of the protein *in vivo* and may facilitate virus clearance. However, the cardiovascular functions of ACE2 mediated by its carboxypeptidase activity, notably on vasoactive peptides, raise the concern of potentially harmful side effects of ACE2 decoys. This was addressed by engineering a series of mutants of the ACE2-Fc decoy, which were then tested for their intrinsic enzymatic activity and their capacity to neutralize SARS-CoV-2 infection. The ideal candidate would be an ACE2-Fc decoy that has lost its enzymatic activity but retains neutralizing efficacy against Spike:ACE2 binding and viral infection. The experiment described here was designed to compare the EC_{50} of the various ACE2-Fc mutants on Spike/ACE2-mediated infection and transduction of a reporter gene.

Methods

ACE2-HEK293 cells were seeded at a density of 10,000 cells per well into white 96-well microplates the day before infection. On the day of the experiment, 5 µl of wild-type Spike (SARS-CoV-2) pseudotyped lentivirus luciferase reporter was pre-incubated with 5 µl of vehicle or serially diluted ACE2-Fc variants at 37°C for 1 hour, then added to the cells for an overnight incubation after which the cell culture medium was replaced with fresh medium. After 36 hours, luciferase activity was measured using the ONE-Glo™ luciferase assay system.

Results

Of the nine ACE2-Fc mutants tested, three mutants: R273A, H378A and E402A, completely lost their enzymatic activity. All mutants were tested for their SARS-CoV-2 neutralizing activity using varying concentrations of recombinant ACE2-Fc decoy to determine the EC_{50}. As shown in Figure 1, all mutants retained their ability to bind the SARS-CoV-2 Spike protein and inhibit the transduction of the reporter gene luciferase in cell culture by competing with cellular ACE2, as demonstrated by decreases in the resulting luciferase activity in the targeted cells. Enzymatically inactive mutants R273A, H378A and E402A showed lesser efficacy than the wild-type decoy of reference, however mutant R273A displayed acceptable neutralizing efficacy with an EC_{50} of 16.7 ng/ml and is the best enzymatically inactive candidate of the three.

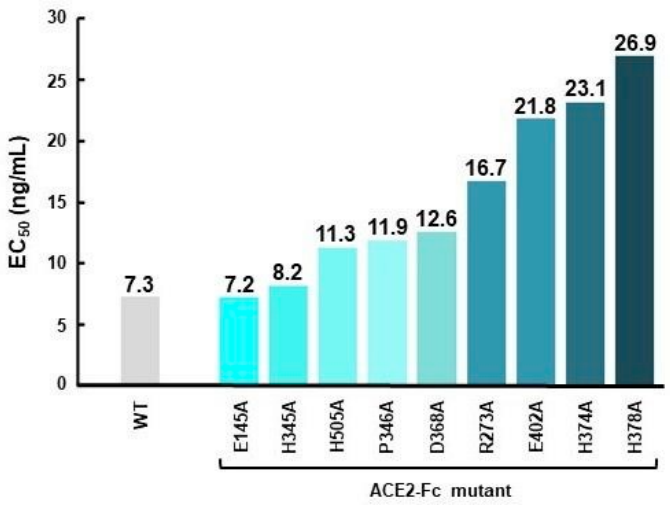


Figure 1: Inhibition of Spike-mediated infection of ACE2-expressing cells by wild-type (WT) and mutant recombinant ACE-Fc. The IC_{50} was determined for each soluble ACE2-Fc candidate.

Conclusion

This study established a new recombinant, optimized ACE2-Fc candidate as a potential antiviral treatment for SARS-CoV-2 that may be able to circumvent the harmful side effects of an enzymatically active ACE2 on the human cardiovascular system.

Application Example #2: Determination of anti-Spike antibody IC_{50}

This study was performed by BPS scientists at BPS Bioscience, San Diego, USA.

Goal of the study

The experiments aimed at providing a proof-of-principle for the IC_{50} determination of neutralizing antibodies against SARS-CoV-2 Spike protein using the Spike pseudovirus luciferase reporter in intact cells.

Methods

Experiments were performed using BPS Bioscience's ACE2-HEK293 recombinant cell line, cell culture growth medium 1N, Spike (SARS-CoV-2) pseudotyped lentivirus luciferase reporter, and ONE-Step™ luciferase reagent. Recombinant human monoclonal antibodies against SARS-CoV-2 Spike correspond to clone 414-1 and clone 414-2. White, clear-bottom 96-well tissue culture plates were purchased from Corning (#3610).

ACE2-HEK293 cells were seeded at a density of 10,000 cells/well in white 96-well microplates the day before the experiment. Serial dilutions of anti-Spike antibody were pre-incubated with the pseudovirus in equal volumes of 5 µl each for 30 minutes before addition of the 10 µl mix to each well. The cell culture medium was changed 18 hours later. After 48 hours of transduction, 50 µl/well of ONE-Step™ luciferase assay reagent was added and incubated at room temperature for 15 to 30 minutes. Luminescence was measured using a luminometer. Results are expressed as percent of luciferase activity resulting from infection of ACE2-HEK293 cells with Spike pseudotyped lentivirus luciferase reporter in the absence of antibody. Results obtained with anti-Spike antibody clone 414-1 and clone 414-2 are shown on the upper panel and the lower panel of Figure 2, respectively.

Results

The addition of increasing amounts of neutralizing antibody to the pseudovirus prior to infection resulted in a dose-dependent decrease of luciferase activity compared to control, indicating that both antibodies block the binding of Spike pseudovirus to ACE2, thereby preventing ACE2-HEK293 infection and luciferase transduction. The IC_{50} was determined to be 1.3 nM for neutralizing antibody clone 414-1 and 1.8 nM for neutralizing antibody clone 414-2.

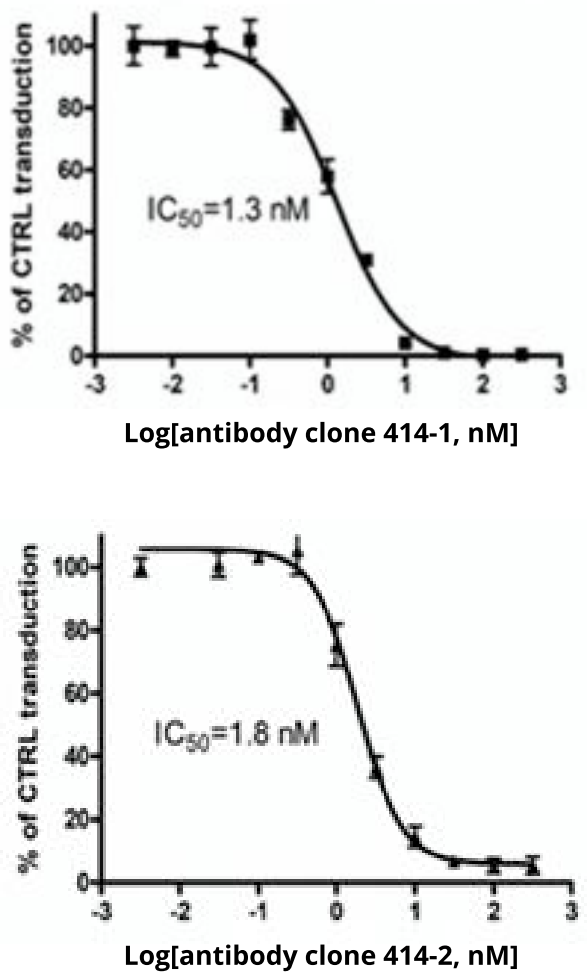


Figure 2: Neutralization of the interaction between Spike and ACE2 using antibodies against SARS-CoV-2 Spike.

Conclusion

Quantification of the interaction between SARS-CoV-2 Spike protein and human ACE2 allowed by the Spike pseudovirus luciferase reporter system is useful to measure the efficacy of neutralizing molecules such as anti-Spike antibodies in a cellular context.

Application Example #3: Comparison of neutralizing antibodies effect on wild-type and variant Spike pseudoviruses

This study was performed by BPS scientists at BPS Bioscience, San Diego, USA.

Goal of the study

The goal of these experiments was to assess the effect of SARS-CoV-2 variation on the efficacy of two distinct neutralizing antibodies using wild-type and B.1.1.7 variant Spike pseudovirus luciferase reporter systems.

Methods

These experiments were performed using BPS Bioscience's ACE2-HEK293 recombinant cell line, cell culture growth medium 1N, wild-type Spike (SARS-CoV-2) pseudotyped lentivirus luciferase reporter, Spike B.1.1.7 variant (SARS-CoV-2) pseudotyped lentivirus luciferase reporter, Spike S1 neutralizing antibody (SARS-CoV-2) Clone: 414-1, Spike S1 neutralizing antibody (SARS-CoV-2) Clone: 414-2, and ONE-Step™ luciferase reagent. White, clear-bottom 96-well tissue culture plates were purchased from Corning (#3610).

ACE2-HEK293 cells were seeded at a density of 8,000 cells/well in white 96-well microplates the day before the experiment. Serial dilutions of anti-Spike antibodies clone 414-1 or clone 414-2 were pre-incubated with the pseudoviruses in equal volumes of 5 µl each for 30 minutes before addition of the 10 µl mix to each well. The cell culture medium was changed 18 hours later. After 48 hours of transduction, 50 µl/well of ONE-Step™ luciferase assay reagent was added and incubated at room temperature for 15 to 30 minutes. Luminescence was measured using a luminometer. Results are expressed as percent of luciferase activity resulting from infection of ACE2-HEK293 cells with each pseudovirus in the absence of a neutralizing antibody. Results obtained with anti-Spike antibody clone 414-1 and clone 414-2 are shown on the left panel and the right panel of Figure 3, respectively.

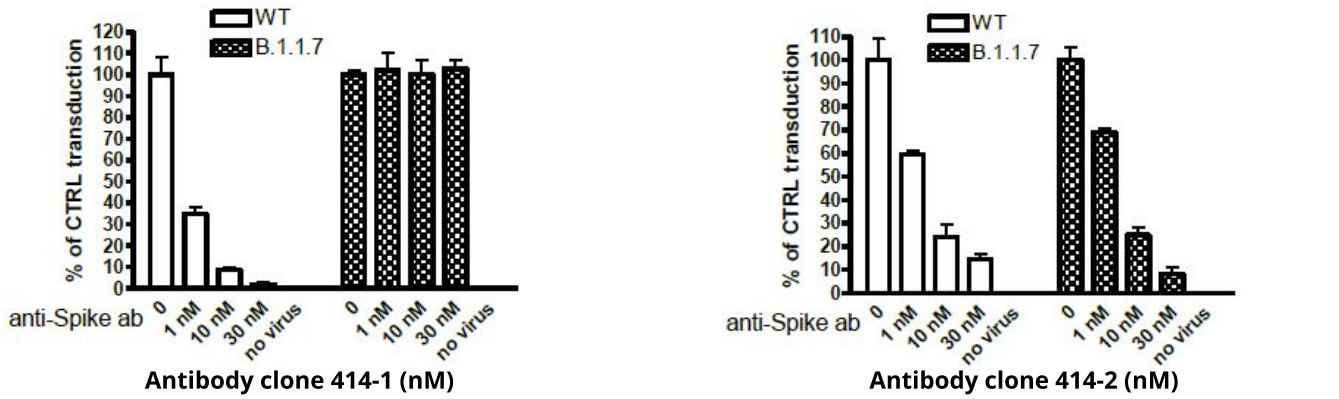


Figure 3: Effect of anti-Spike neutralizing antibodies against SARS-CoV-2 Spike wild-type or variant B.1.1.7.

Results

Both antibodies neutralized the wild-type Spike pseudovirus as indicated by a dose-dependent decrease of luciferase activity compared to control. Antibody 414-2 neutralized both wild-type and variant B.1.1.7 with similar dose-effects (Figure 3, right panel). On the other hand, while antibody 414-1 was effective against the wild-type Spike pseudovirus, it did not neutralize variant B.1.1.7. (Figure 3, left panel), indicating that at least one of the modifications of the Spike protein interferes with antibody binding to Spike (i.e. deletion 69/70, deletion 144Y, mutations E484K, S494P, N501Y, A570D, D614G or P681H). Of note, Spike S1 neutralizing antibody Clone C-A11 differentiates between wild-type and P.1 variant (not shown).

Conclusion

The emergence of Spike variants in the human population has been shown to alter disease transmissibility and may eventually have worrisome consequences on mortality. Variant evolution may also decrease the efficacy of current vaccines and provide an escape mechanism for the virus. An expanding line of variant Spike pseudoviruses will be useful to probe the efficacy of anti-Spike therapeutics, research alternatives, or monitor vaccines.

Application Example #4: EC_{50} determination of a new drug candidate

The following study entitled "**Iota-carrageenan neutralizes SARS-CoV-2 and inhibits viral replication in vitro"**, was published by Morokutti-Kurz M, Fröba M, Graf P, Große M, Grassauer A, Auth J, Schubert U, and Prieschl-Grassauer E in *PLoS One.* (2021) 16(2): e0237480.

Goal of the study

Carrageenans are natural sulfated polysaccharides extracted from red seaweeds and widely used in the food industry for their thickening and stabilizing properties. They have emerged as formulation components in tissue engineering, wound repair and regenerative medicine because of their similarities with human glycosaminoglycans. Iota-carrageenan has two sulfate groups per disaccharide which makes it a polyanionic polysaccharide that binds strongly to proteins.

The authors hypothesized that iota-carrageenan may prevent SARS-CoV-2 infection by trapping the virus at the surface of host cells. The experiment described here was performed to measure the EC_{50} of Iota-carrageenan in cell culture.

Methods

Approximately 7,500 ACE2-HEK293 cells were infected with viral particles of wild-type Spike (SARS-CoV-2) pseudotyped lentivirus luciferase reporter at a MOI of 0.1. Increasing concentrations of iota-carrageenan (from 0.33 µg/ml to 100 µg/ml in 0.5% NaCl) were pre-incubated with the Spike pseudovirus for 30 minutes prior to infection. The carrageenan/virus mix was diluted with 5 volumes of cell culture medium and added to the cells for 4 hours before changing the medium (final concentrations ranging from 0.055 µg/ml to 17 µg/ml). After 48 hours cells were lysed by freeze/thaw and the luciferase reagent was added to measure luciferase activity. Results represent triplicates and are expressed as the percent of control defined by mock-treated infected cells. Luciferase data was corrected with metabolic data (Alamar blue) obtained from a parallel cell culture plate treated similarly.

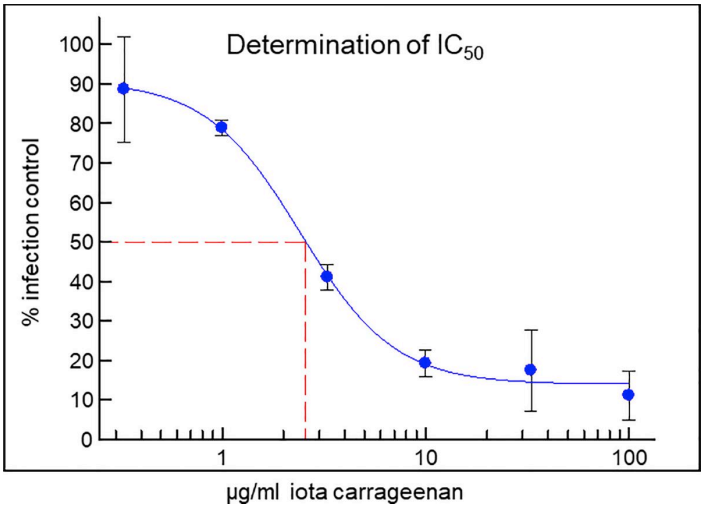


Figure 4: Inhibition of Spike-mediated infection of ACE2-HEK cells by iota-carrageenan. The IC_{50} was determined by measuring luciferase activity. Credit: Morokutti-Kurz *et al*, *PloS One* doi.org/10.1371/journal.pone.0237480,

Results

As shown in Figure 4, iota-carrageenan effectively neutralized infection of ACE2-HEK293 cells by the Spike pseudovirus with an IC_{50} of 2.6 µg/ml, supporting the hypothesis that the polysaccharide traps the virus particles, presumably by binding to viral glycoproteins.

Notably, iota-carrageenan displayed similar effects against various Rhino- and Coronaviruses. The drug did not adversely affect cell viability whereas it inhibited the infection of Vero B4 cells by a patient-derived SARS-CoV-2 isolate, which constitutes a clinically relevant model.

Notably, a nasal spray containing iota-carrageenan, used in a clinical setting, caused a reduction in the severity and duration of symptoms of common cold caused by several respiratory viruses in human patients, pointing to the potential therapeutic effectiveness of this naturally-derived candidate drug (Ludwig *et al*, PMID: 24219370).

Conclusion

Iota-carrageenan polysaccharide displays virus-trapping abilities with little toxicity, and reduces viral infection in cellular models of SAR-CoV-2 infection and in clinical cases of the common cold.

Conclusion

Wild-type and variant Spike pseudoviruses are useful to support the characterization and development of SARS-CoV-2 inhibitors targeting the interaction between SARS-CoV-2 Spike and human ACE2. The presence of a reporter gene facilitates the robust and reproducible quantification of virus entry under a variety of experimental conditions.

References

Liu P, Xie X, Gao L, Jin J. Designed variants of ACE2-Fc that decouple anti-SARS-CoV-2 activities from unwanted cardiovascular effects. *Int J Biol Macromol.* (2020) Dec 15;165(Pt B):1626-1633. PMCID: PMC7568492.

Morokutti-Kurz M, Fröba M, Graf P, Große M, Grassauer A, Auth J, Schubert U, Prieschl-Grassauer E. Iota-carrageenan neutralizes SARS-CoV-2 and inhibits viral replication in vitro. *PLoS One* (2021) 16(2):e0237480. PMCID: PMC7888609.

Table of Spike pseudotyped lentiviruses (as of July 2021)

Lineage	Known as	Country of identification	Spike mutations/deletions	Available reporter	Cat #
Wild-type	Wuhan reference strain	China	N/A	Firefly Luciferase	79942
D614G	D614G mutant	USA	D614G	eGFP	78035
K417T, E484K, N501Y	N/A	N/A	K417T, E484K, N501Y	Firefly Luciferase	78143
B.1.1.7	Alpha VOC 202012/01 20I/501Y.V1	UK	ΔH69/V70, ΔY144 N501Y, A570D, D614G, P681H, T716I, S982A,D1118H	eGFP Firefly Luciferase	78158 78112
B.1.351	Beta 501Y.V2 20H/501.V2	South Africa	L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, A701V	eGFP Firefly Luciferase	78160 78142
P.1	Gamma 20J/501Y.V3	Brazil	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	eGFP Firefly Luciferase	78159 78144
B.1.429	Epsilon CAL.20C 20C/S:452R	USA (CA)	S13I, W152C, L452R, D614G	Firefly Luciferase	78172
B.1.617	21A	India	L452R, E484Q, D614G, P681R	Firefly Luciferase	78204
B.1.617.1	Kappa 21A/S:154K	India	G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	Firefly Luciferase	78205
B.1.617.2	Delta 21A/S:478K	India	Δ156/157 T19R, G142D, R158G,L452R,T478K, D614G, P681R, D950N	Firefly Luciferase	78215
B.1.618	21A	India	Δ145/146 E484K, D614G	Firefly Luciferase	78206