Electronic Supplementary Material (ESI) for Nanoscale Advances.

SARS-CoV-2 suppression depending on pH of graphene oxide nanosheet

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Experimental procedure:

Characterization of prepared samples

XPS was conducted on X-ray photoelectron spectroscopy (XPS, Theta Probe, Thermo Fisher Scientific) instrument. A monochromatized X-ray source (Al Kα, hν = 1486.6 eV) was used for the measurement, which was performed under vacuum at a pressure higher than 10^{-7} mbar. FT-IR spectra were measured using a fourier transform infrared spectroscopy (FT-IR, Spectrum two, Perkin Elmer) instrument. ^{13}C NMR was conducted using a solid State ^{13}C nuclear magnetic resonance apparatus (SS ^{13}C NMR , JNM-ECZ400R, JEOL).

Cell line

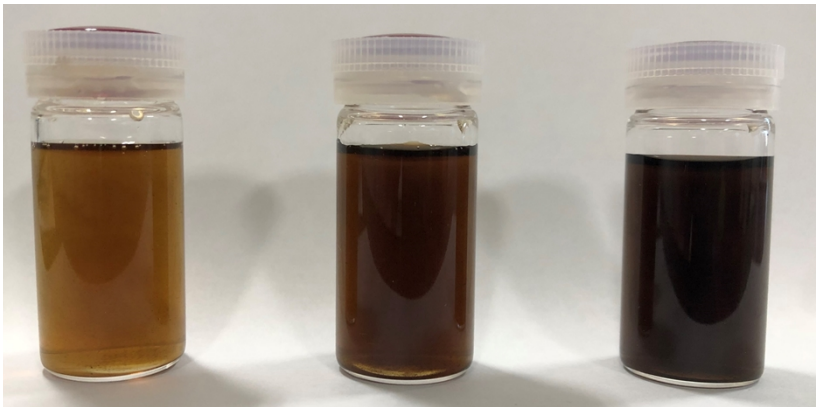
VeroE6/TMPRSS2 cells (JCRB1819)[1] were maintained in 10% heat-inactivated fetal bovine serum (NICHIREI, cat# 175012)/DMEM (Wako, cat# 041-29775) containing 1 mg/mL G418 (Wako, cat# 070-06803) and 1% penicillin/streptomycin (Wako cat# 168-23191).

Plaque assay

A plaque assay was performed as described previously.[2-7] The day before infection, 1×10^5 VeroE6/TMPRSS2 cells were seeded in a 24-well plate. Next day, 200 µl of each GO sample [GO (3), GO (7) and GO (11)] (1 mg/mL) were mixed in 200 µL of virus stock and 1.6 mL of serum-free virus dilution buffer [20 mM HEPES, nonessential amino acids (Thermo Fisher Scientific, cat# 11140-050) and antibiotics in 1 × DMEM] (final GO concentration; 100 µg/mL). After 1 h incubation at room temperature, the virus solution was centrifuged at 22,000 × g for 1 min and the supernatant was diluted with serum-free virus dilution buffer. Then, the cells were infected with 250 µl of each diluted virus solution at 37°C. At 1 h postinfection, 500 µL of mounting solution [1 × DMEM including 3% FBS and 1.5% carboxymethyl cellulose (Sigma, cat# C9481-500G)] was overlaid and the cells were incubated at 37 °C. After 3 days, the cells were washed with PBS three times, followed by fixation with 4% paraformaldehyde (Nacalai Tesque, cat# 09154-85). The fixed cells were washed with water, dried, and stained with 0.1% methylene blue (Nacalai Tesque, cat# 22412-14) in water. The stained cells were washed with water and dried, and the number of plaques was counted for PFU determination.

Quantification of N proteins

The quantification of SARS-CoV-2 N proteins was performed as described previously.[2] 1 mg/mL of GO solution was mixed in virus stock (final GO concentration; 100 µg/mL). At the indicated time points (0, 24, 48, and 72h), Triton X-100 (Nacalai Tesque, Cat# 35501-15)/PBS solution was added (final Triton X-100 concentration; 0.1 %) and the mixtures were frozen at −80 °C until sample preparation. Finally, samples were subjected to ELISA (Proteintech, cat# KE39997) for the quantification of N protein.



GO (3) **GO (7)** **GO (11)**

Figure S1: Optical photograph of GO (3), GO (7) and GO (11) dispersion.

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