Electronic Supplementary Information

A water-soluble membrane for SARS-CoV-2 viral nucleic acid sampling and detection

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Experimental details

1. Materials

Chitosan (MW: 100 kDa) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Urea (ACS, ≥99.5%), Glycerin, tert-butyl alcohol and Tween 80 were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Ethanol was bought from Sinopharm Chemical Reagents Co. Ltd (Shanghai, China). An RNA fragment from the N gene of SARS-CoV-2 was obtained by *in vitro* transcription (New England Biolabs, USA). The COVID-19 Real-Time PCR Kit was provided by Kaipu Biotechnology Co., Ltd. (Guangzhou, China).

2. Preparation of chitosan hydrochloride (CSH)

The preparation method of CSH was adopted from reported work[1]. 4 g of chitosan were dissolved in 280 ml of hydrochloric acid under stirring at 700 rpm at 40°C. The solution was filtered and the white and yellowish CSH was rinsed with ethanol 3 times.

3. Fabrication of the CSH membrane

2.5 g of CSH were dissolved and stirred in 50 mL of an aqueous solution containing 0.1% urea, 2% Tween 80, 7% glycerin, and 88.9% DI water (wt%). The solution was centrifuged at 7,500 rpm to eliminate gas bubbles and then casted on a glass plate with a casting knife on the AT-TB-2100 machine (ANMT, China). Hydrogel was formed on the glass plate and the glass plate with the hydrogel was transferred and soaked in ethanol for more than 8 hours. The hydrogel became white and then was detached from the glass plate and soaked in tert-butyl alcohol for 12 hours. Finally, the CSH membrane was obtained by freeze-drying the hydrogel.

4. Characterization

The topography of the CSH membrane was examined by scanning electron microscopy (SEM) on the ZEISS SUPRA 55 (Germany). X-ray photoelectron

spectroscopy (XPS) was performed on the ESCALAB 250Xi (Thermo Fisher, USA) and Fourier transform infrared (FTIR) spectroscopy was conducted on the Lambda 750s (PerkinElmer, US) equipped with a multi-reflection Smart Performer ATR accessory. The pore size distribution and flow rate of the membrane were determined using a BSD-PBL membrane analyzer (BeishiDe Instrument Ltd., China). The SARS-CoV-2 viral nucleic acids were analyzed on the Light Cycler Nano PCR Detection System (Roche, Germany).

5. SARS-CoV-2 viral nucleic acids sampling

Before collecting the SARS-CoV-2 viral nucleic acids, all the instruments are sterilized with ethanol. The CSH membrane was installed on a filtration adapter connected to a suction pump (VMSTR, Shenzhen, China). The filtration adapter and suction pump were transferred to an acrylic chamber (10 L). Before viral sampling, the SARS-CoV-2 viral nucleic acid sample was prepared with a certain concentration and aerosolized in the chamber by a compressor (PARI TurboBOY N, Germany). The system was operated for 30 mins to sample the SARS-CoV-2 viral nucleic acids and after collection, the CSH membrane was dissolved in DI water rapidly for further testing.

6. SARS-CoV-2 detection

The SARS-CoV-2 viral nucleic acids concentration of the CSH membrane dissolved in DI water was tested by PCR test kits purchased from Kaipu Biotechnology Co., Ltd. (Guangzhou, China). To further concentrate viral nucleic acids and eliminate possible inhibition of subsequent qPCR detection due to contaminants in aerosol samples, CSH membrane solution was subject to viral nucleic acids extraction. To purify the nuclear acid, 300 μL of the virus-captured CSH membrane aqueous solution was added to a mixture of magnetic beads and binding buffer in the PCR test kits. The negative sample containing 300 μL of DI water without the CSH membrane was designed to confirm that there was no viral contamination in both the PCR kits and

experimental environment. After incubation at room temperature for 13 min, the solutions with and without SARS-CoV-2 viral nucleic acids were subject to magnetic beads-based nucleic acids extraction (MagAttract Viral RNA Kit, Qiagen, Germany) by following the manufacturer's instruction. Finally, a certain amount of the elution buffer from different samples was added to the PCR buffer and tested on the Light Cycler Nano PCR Detection System (Roche, Germany) following the instructions of the PCR kit. In real scenarios, the major potential risk of the filtering membrane for real viral particle detection is the risk of virus passage through membrane without being efficiently captured. This risk will give false negative results, and thus pose serious dangers to users without triggering effective alarm when they are exposing to potential infectious risks. To solve such risk, future investigation will be focused on optimizing membrane fabrication strategies to produce water soluble membranes with better mechanical properties, higher filtering performances and less defects.

7. Calculation of recovery rate of viral RNA by the CSH membrane

The standard curve of viral nucleic acids with concentrations ranging from 10^1 copies/mL to 10^4 copies/mL was firstly calculated in three experimental replicates. Afterwards, 1.5 mL of the PBS solution containing a 10^3 copies/mL of the viral RNA fragments was prepared and 1 mL was nebulized for 10 min in a 10 L chamber giving a theoretical air viral nucleic acid concentration of 10^2 copies/L. Afterwards, the filtration system with the CSH membrane was operated for 30 min to filter the aerosol in the chamber. After collection, the CSH membrane was dissolved in 10 mL of DI water due to the viscosity of the dissolved solution and 300 μL of the solution (with a theoretical air viral nucleic acid concentration of 10^2 copies/mL) was subjected to viral nucleic acid extraction and RT-PCR detection using commercialized kits. The recovery rate of viral RNA by the CSH membrane was calculated as:

$$Recovery\ Rate\ (\%) = \frac{1}{2^{\Delta Ct}} \times 100\%$$

in which: $\Delta Ct = Ct(CSH\ membrane) - Ct(10^{2copies}/_{mL} in\ standard\ curve)$

References

1 P. B. Shelke, S. N. Mali, H. K. Chaudhari and A. P. Pratap, *J Heterocycl. Chem.*, 2019, **56**, 3048-3054.

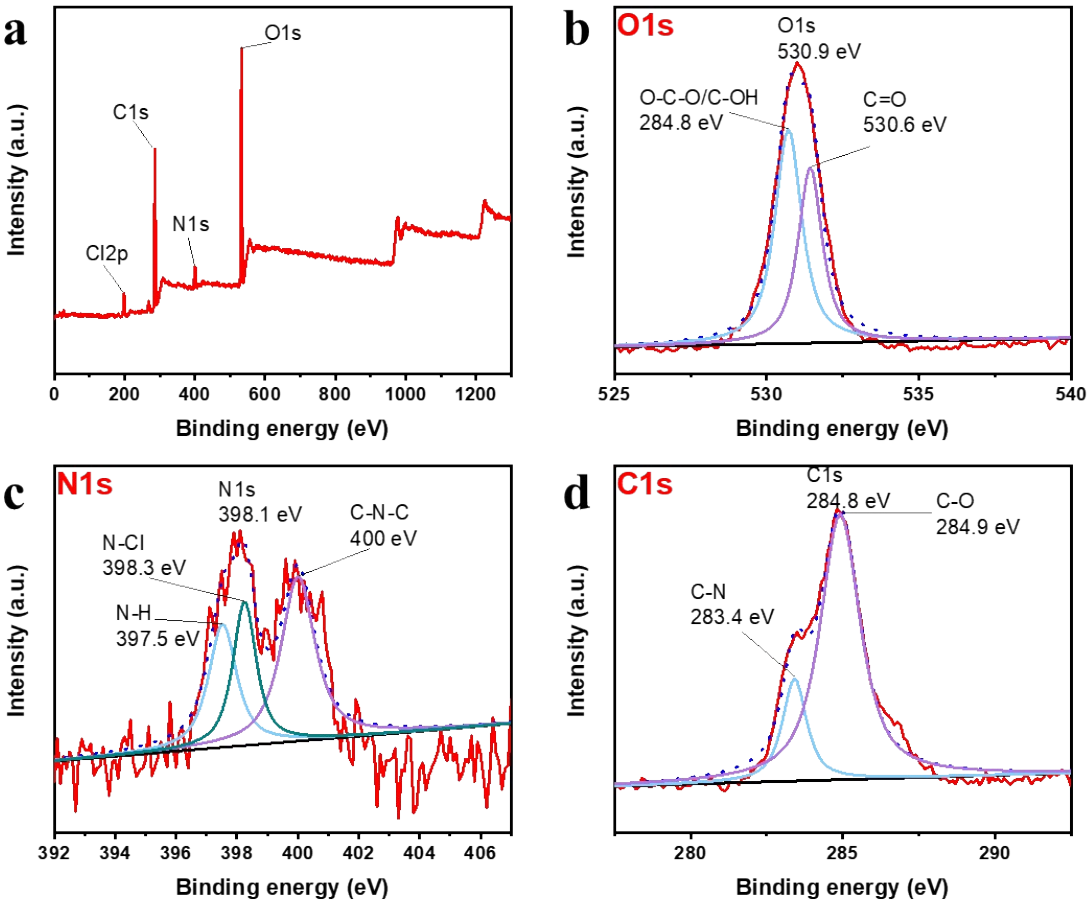

Fig. S1. XPS spectra of the CSH membrane: (a) Survey spectrum, (b) O 1s spectrum,

c) N 1s spectrum, and (d) C 1s spectrum.

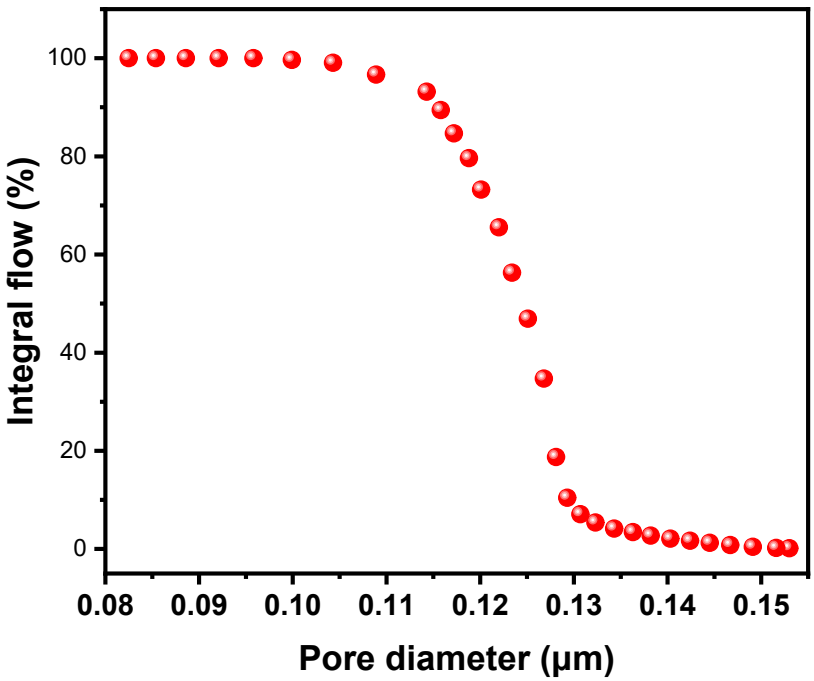

Fig. S2. Integral flow pore diameter distribution of the CSH membrane.

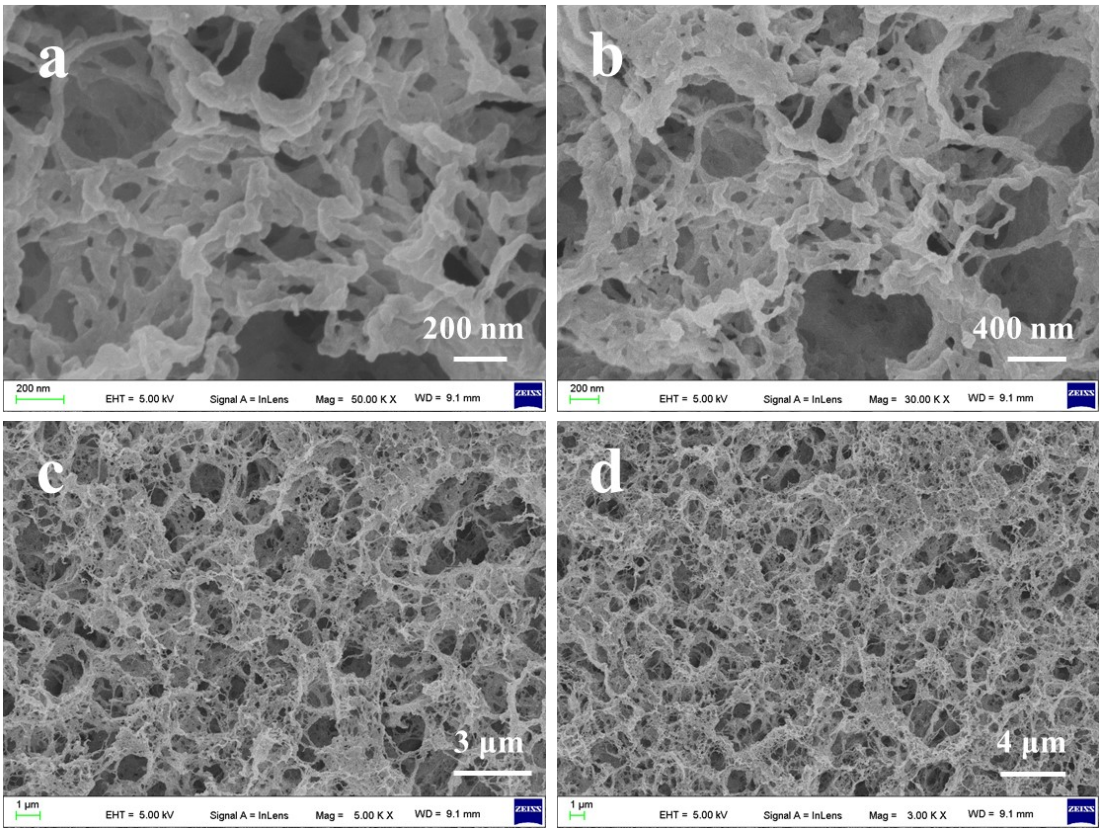

Fig. S3. Top surface morphology of the CSH membrane at different magnifications: (a) 50 kX, (b) 30 kX, (c) 5 kX, and (d) 3 kX.

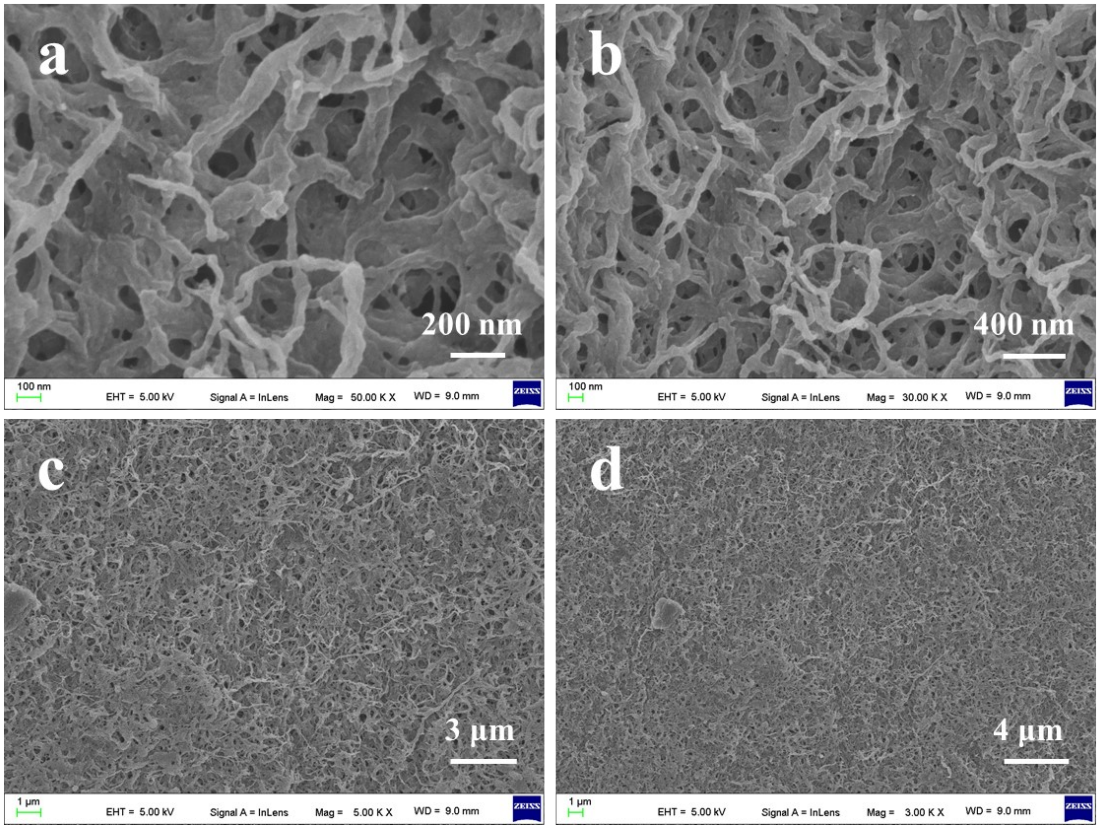

Fig. S4. Bottom surface morphology of the CSH membrane at different magnifications: (a) 50 kX, (b) 30 kX, (c) 5 kX, and (d) 3 kX.

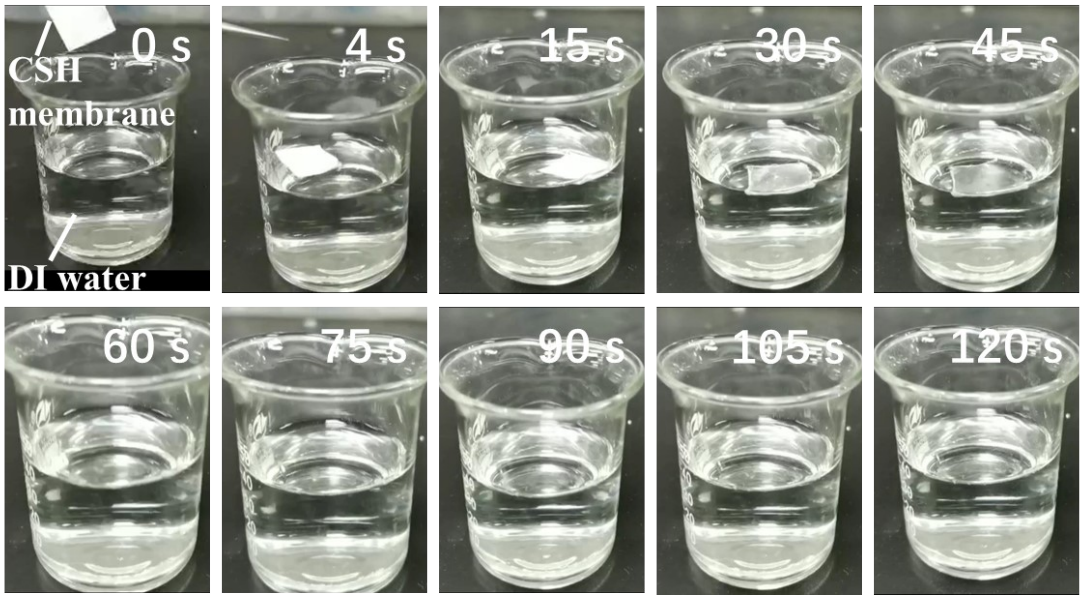

Fig. S5. Dissolution process of the CSH membrane in DI water during a period of 2 minutes.

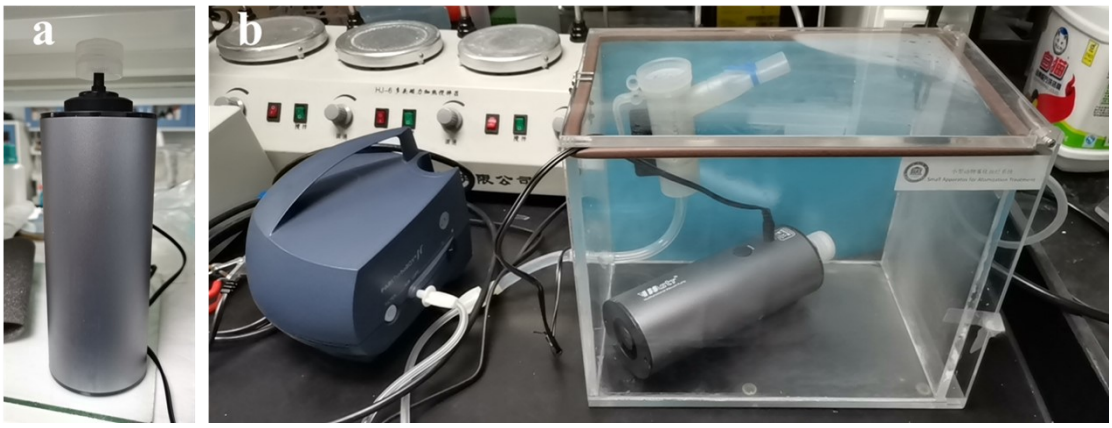

Fig. S6. (a) The suction pump with the membrane adaptor; (b) The picture of the viral sampling process.

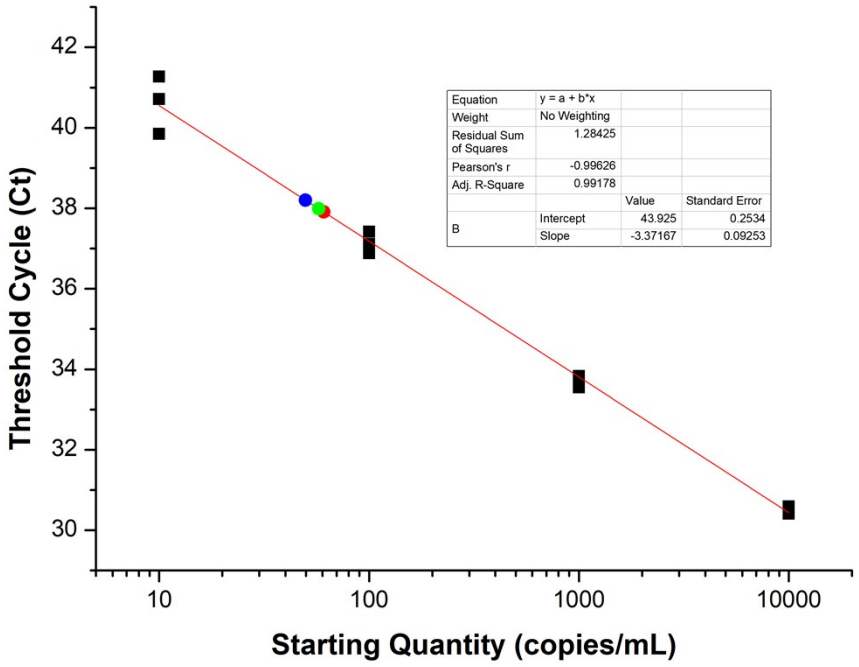

Fig. S7. The standard curve of viral nucleic acids with concentrations ranging from 10^1

copies/mL to 10^4 copies/mL in three experimental replicates (black squares, R-square: 0.99178), and viral capture and extraction with an air viral nucleic acid concentration of 10^2 copies/L in three experimental replicates (colored dots).

Table S1: Ct values and recovery rates of viral capture and extraction by CSH membrane with an air viral nucleic acid concentration of 10^2 copies/L in three experimental replicates

Experimental Replicates	Measured Ct	ΔCt	Recovery Rate (%)	Averaged Recovery Rate* (%)
1	37.91	0.77±0.22	58.64±8.94	54.03±4.77
2	37.99	0.85±0.22	55.48±8.46	
3	38.2	1.06±0.22	47.96±7.31	

* : error was calculated as Standard Deviation with Gaussian error propagation.