NOTE

Removal of viruses from their cocktail solution by liquid-crystalline water-treatment membranes

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Abstract

Liquid-crystalline (LC) water-treatment membranes obtained by in situ photopolymerization of ionic mesogenic monomers have been shown to efficiently remove viruses. In our previous works, bicontinuous cubic (Cub_{bi}) and smectic (Sm) LC membranes prepared from ionic taper- and rod-shaped polymerizable mesogens, respectively, were used for this purpose. Here, we report the results of virus removal by columnar (Col) LC water-treatment membranes having ionic nanochannels obtained from ionic taper-shaped mesogens. These effects are compared with those obtained for Cub_{bi} membranes. The effects of these Col and Cub_{bi} LC ionic membranes on the removal of several viruses from their cocktail solution are also examined.

Introduction

The United Nations set goals to ensure a safe and affordable water supply for all by 2030. Viruses are among the most concerning pathogens because the presence of even the slightest amount in water can result in a large-scale public health crisis [1]. Disinfection technologies such as thermal treatment, ultraviolet (UV) light, and chlorination are effective in inactivating most viruses [2]. However, these disinfection technologies may not guarantee complete removal of pathogens. In contrast, filtration technologies using membranes form a physical barrier to prevent pathogens from entering treated effluent [3, 4].

Filtration using polymer membranes is an essential technology for water purification and for supplying safe water at low cost and with low energy consumption [3–5]. Aromatic polyamide thin films have been used as the separation layer of membranes. Recently, advanced water filtration membranes [5] with materials such as liquid-crystalline (LC) polymers [6–14], carbon nanotubes [15], and block polymers [16] have been proposed. For example, we developed nanostructured membranes with three-dimensional (3D) nano ionic channels from an ionic bicontinuous cubic (Cub_{bi}) LC monomer with a taper-shaped mesogen [10]. 1D nanochannels of diol moieties were obtained from supramolecular columnar (Col) LC mixtures [11], and 2D ionic channels were prepared from ionic smectic (Sm) LC monomers [12, 13]. These LC membranes show high levels of rejection of bacteriophage Qβ (Qβ).

In the present study, nanostructured membranes have been prepared from ionic LC monomers with taper-shaped mesogens forming Col structures. The virus removal properties of the ionic 1D channels for Qβ have been examined. As the first approach for LC membranes, the removal of several viruses from their cocktail solution by these Col and Cub_{bi} ionic membranes was also studied.

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Experimental section

The virus rejection for all membranes was tested using 25 mm membrane filtration units with agitation (Advantec® UHP-25K, Tokyo, Japan) connected to a reservoir

containing a feed solution of Milli-Q water (Advantec® RP-2) [10–13]. Details for the preparation of feed virus solution are presented in the Supporting Information. The membranes were submerged in Milli-Q water for 15 min prior to the filtration of viruses. The prepared virus stock was inoculated into the feed solution and mixed to obtain a concentration of approximately 10^7 copies mL^{-1}. Pure air containing less than 0.1 ppm CO_2 was used to provide a pressure of 0.3 MPa for the feed solution entering the stirred cell. Filtrate samples were collected over time periods from 0 to 1, 1 to 2, 2 to 4, and 4 to 6 h.

The viruses were quantified by reverse transcription–quantitative polymerase chain reaction (RT–qPCR). In this study, cocktail solutions containing Qβ, bacteriophage MS2 (MS2), enteric virus Aichi virus (AiV) and poliovirus (PV) were used to check the simultaneous removal of different viruses. The plaque assay method used in the previous reports was not applicable in this study since Qβ and MS2 shared the same host bacteria. The collected water samples were subjected to RNA extraction and one-step RT–qPCR. Specifically, 5 μL of sample was heated at 95 °C for 5 min to extract RNA with a thermal cycler (PCR Thermal Cycler Dice Gradient, Takara Bio Inc., Kusatsu, Japan). Note that this method can extract DNA/RNA with an efficiency comparable to that of a commercially available DNA/RNA extraction kit (Qiagen, Hilden, Germany) in pure water [17]. Then, the extracts were mixed with 15 μL of reaction mix, composed of 10 μL of 2× QuantiTect Probe RT–PCR master mix (Qiagen), 0.2 μL of QuantiTect RT mix (Qiagen), 2.3 μL of Milli-Q, 1 μL each of forward and reverse primer (10 μM each) and 0.5 μL of probe (5 μM). The primers for MS2 and Qβ [18], AiV [19] and PV [20] were prepared according to the literature. RT–qPCR was performed in duplicate with an ABI StepOnePlus thermocycler (Thermo Fisher, USA) under the temperature conditions suggested by the abovementioned studies depending on the virus type. Plasmid DNA was serially diluted tenfold (10^0 to 10^5 copies) and used to generate the standard curve. Samples with cycle thresholds (Ct values) below 40 were considered quantifiable. The quality of the qPCR was guaranteed by an amplification efficiency of 93% and a correlation rate of 0.997. The log reduction value was calculated from the equation below:

$$\text{Log reduction value(LRV)} = \log_{10}\left(\frac{C_{\text{feed}}}{C_{\text{effluent}}}\right)$$

where C_{feed} and C_{effluent} represent the virus concentration in the feed and the effluent solution, respectively.

The average and standard deviation of the data, which included nondetected results (i.e., left-censored data), were calculated by the Kaplan–Meirer method using an R package (NADA package) [21]. Left-censored data of the concentration in the permeated solution gave right-censored LRV data.

Results and discussion

Nanostructured membranes formed by in situ polymerization of **1** and **2** were used for virus removal experiments. Taper-shaped compound **1** exhibits a Col structure with 1D ionic channels from 60 to −12 °C upon cooling, although taper-shaped compound **2**, similar to **1**, gives a Cub$_{bi}$ structure with 3D channels below approximately 20 °C and shows an LC-crystal transition at −30 °C (Fig. 1) [14]. Rod-shaped compound **3** with a biphenyl moiety shows two different 2D Sm structures from approximately 100 to 85 °C and from 85 to 30 °C upon cooling [12].

Water-permeable membranes for virus filtration were fabricated by a previously reported photopolymerization method [14]. Films of monomers **1** and **2** containing photoinitiator were prepared by spin-coating on a poly (vinyl alcohol) (PVA)/poly(ethylene terephthalate) (PET) substrate. The thicknesses of spin-coated samples of **1** and **2** were approximately 100 nm [14]. Film **3** was approximately 400 nm thick [12]. The thicker film was made for **3** to enhance mechanical stability. We expected that due to the smaller number of crosslinking groups per molecule than those for **1** and **2**, a film of **3** with the same thickness would have lower mechanical stability than **1** and **2**. The monomer film with the PVA/PET substrate was laminated to a polysulfone membrane composed of 40 μm thick polysulfone and 90 μm thick PET fabric layers. The LC monomers sandwiched by the polymers were heated to a temperature 20 °C higher than the isotropization temperatures of each compound and then cooled to 10 °C. After cooling to the LC state, the monomer films on the polymer substrate were irradiated with UV light (365 nm) for 10 min at 10 °C. The diene groups reacted to form networks of covalent bonds [10]. After polymerization, the PVA layer was removed by immersing the composite sample in water. Details for the membrane preparation are described in the Supporting Information.

All the tested viruses, AiV, PV, Qβ, and MS2, for removal by the membranes have sizes of approximately 30 nm in diameter with icosahedral symmetry. They are smaller than *Coronaviridae* (the family of coronaviruses), whose diameter is approximately 100 nm. The isoelectric point of PV ranges from 3.8 to 8.3, while Qβ and MS2 have values in the lower range of 1.9–5.3 and 2.2–4.0, respectively [22]. Under the neutral pH used in this study, it is possible that Qβ and MS2 were negatively charged, while PV might be less negatively or even positively charged. The isoelectric point of AiV is not yet available. In general, the surface charge and hydrophilicity of viruses also influence their rejection efficiency by membranes [23]. In this study, cocktail solutions were used to examine whether viruses with different properties could be removed simultaneously by the nanostructured LC membranes.
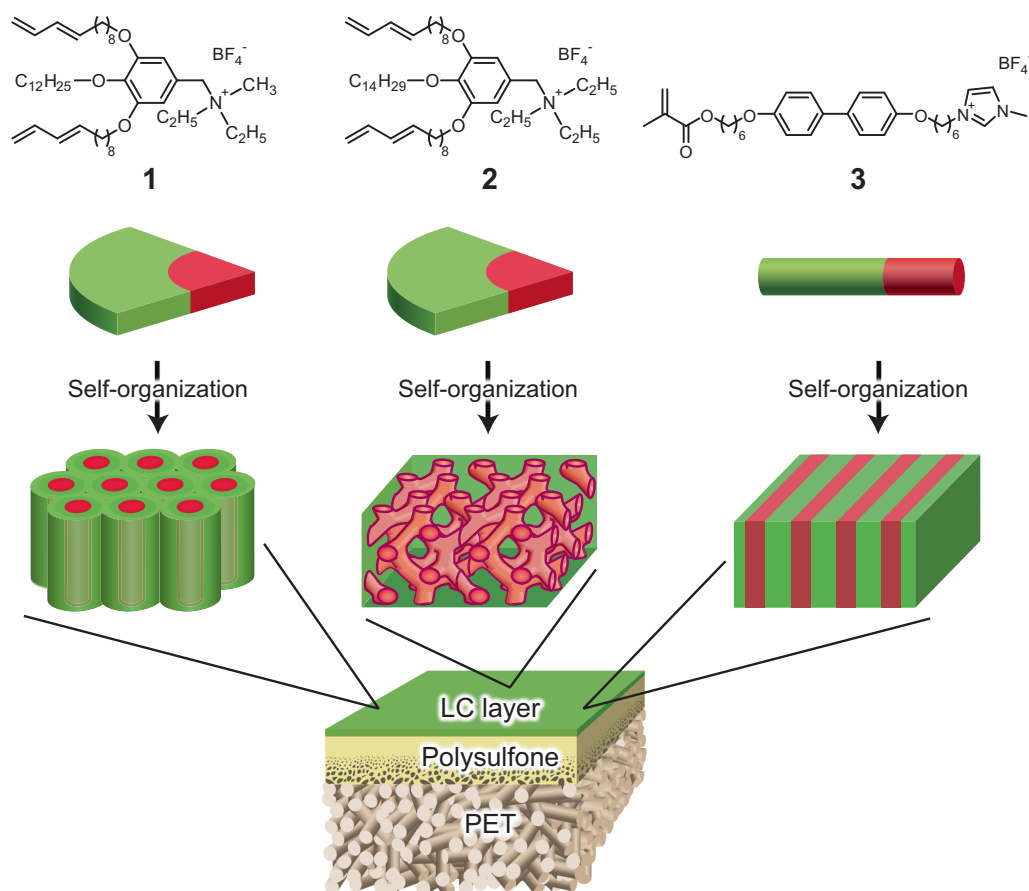
Fig. 1 Schematic illustration of the membrane and the molecular structures of LC monomers **1–3**

AiV is representative for the evaluation of virus removal in full-scale wastewater treatment [24]. Moreover, AiV may be an appropriate model virus for water quality assessment as an alternative to the conventional enteric model virus PV, which is under the World Health Organization (WHO) polio eradication program. To understand whether AiV serves as an appropriate replacement for PV, both viruses should be examined together in a virus cocktail in conjunction with other commonly used indicator viruses in water treatment, such as Qβ and MS2.

Rejection of the selected viruses with nanostructured membranes prepared from **1** and **2** was studied. The results of Qβ filtration with the Col membrane based on **1** and the Cub$_{bi}$ membrane based on **2** are shown in Fig. 2. The membrane of **1** showed similar reduction behavior and a higher water flux compared to those of membrane **2**. The flux of the membrane of **1** at 0–2 h was $3.7 \, \text{L m}^{-2} \, \text{h}^{-1}$, although the value for the membrane of **2** was $0.44 \, \text{L m}^{-2} \, \text{h}^{-1}$. The virus rejection values for the membrane of **1** met the criteria of the WHO for drinking water (LRV > 3) during filtration. The ionic Col nanostructured membranes based on **1** showed a high virus rejection value, as did the Cub$_{bi}$ membrane of **2** and the Col membrane having nanochannels of diol moieties [11].
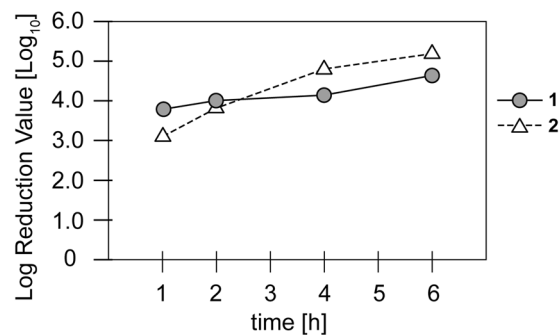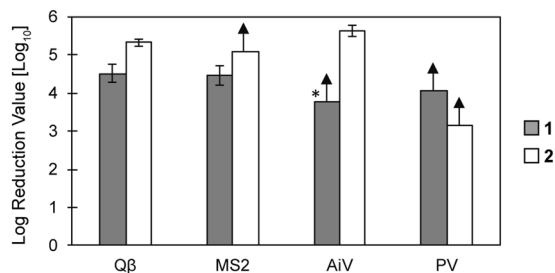

Fig. 2 The log reduction values by the membranes based on **1** and **2** as a function of time against Qβ

The rejection of Qβ by the Col, Cub$_{bi}$, and Sm LC membranes forming ionic nanochannels is summarized in Fig. 3. All these membranes showed higher LRVs than the WHO criterion for drinking water. The diameters of nanopores in the ionic nanochannels in the membranes of **1** and **2** were estimated to be 0.6 nm [14, 25], which is much smaller than the diameter of the viruses, which have a diameter of approximately 25–30 nm. The width of the
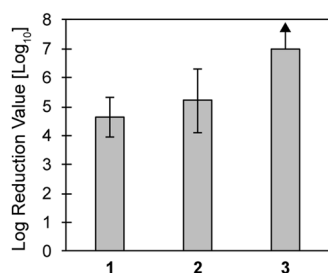
Fig. 3 Rejection of Qβ with the LC membranes based on **1**, **2**, and **3**. The results of **3** were reported in Ref. [12]. Error bars represent the standard deviation. Arrows show right-censored log reduction values

Fig. 4 The average log reduction values of different types of viruses at 4–6 h by the membranes based on **1** and **2** for the virus cocktail solution. Error bars represent the standard deviation. Asterisks indicate that the standard deviation is not available because the number of uncensored values is one. Arrows show right-censored log reduction values

hydrophilic 2D channels of **3** in the smectic membrane containing imidazolium moieties is expected to be approximately 1 nm [12, 13], which is also much smaller than viruses. The Sm membrane showed the highest LRV among the LC nanostructured membranes. The higher fluidity of Sm liquid crystals than other liquid crystals may have led to fewer surface defects and higher membrane performance.

The LRVs for the cocktail viruses (Qβ, MS2, AiV, and PV) with membranes **1** and **2** are shown in Fig. 4. The viruses before and after filtration were quantified with the RT–qPCR method. The LRVs of membranes **1** and **2** met the WHO criteria for all viruses. The nanostructured membranes rejected all viruses simultaneously at high levels. The apparent LRVs for PV with membranes **1** and **2** were slightly lower than those for other viruses; however, this result was due to the limitation of the LRV calculation. The concentrations of PV after filtration were reduced to below the limit for quantitative evaluation. The nanostructured membranes showed high virus removal despite their different surface charges since the pathways for water molecules in these membranes are much smaller than any virus.

In summary, the nanostructured membranes of ionic columnar liquid crystal **1** efficiently remove viruses from water as well as the membranes of ionic LC liquid crystals **2** and **3**. For the first time in the series of studies of our LC membranes, the removal of viruses from their cocktail

solution was examined. It was shown that the LC membranes efficiently remove viruses even from cocktail solutions consisting of four viruses. These membrane technologies should be useful for the removal of SARS-CoV-2, which has induced the pandemic disease COVID-19 since 2019 [26].

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

References

1. World Health Organization. Guidelines for drinking water quality - 4th ed, Geneva, Switzerland: World Health Organization; 2011.
2. Hijnen WAM, Beerendonk EF, Medema GJ. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res. 2006;40:3–22.
3. Petersen RJ. Composite reverse osmosis and nanofiltration membranes. J Membr Sci. 1993;83:81–150.
4. Wickramasinghe SR, Stump ED, Grzenia DL, Husson SM, Pellegrino J. Understanding virus filtration membrane performance. J Membr Sci. 2010;365:160–9.
5. DuChanois RM, Porter CJ, Violet C, Verduzco R, Elimelech M. Membrane materials for selective ion separations at the water–energy nexus. Adv Mater. 2021;33:2101312–1/18.
6. Kato T, Uchida J, Ichikawa T, Soberats B. Functional liquid-crystalline polymers and supramolecular liquid crystals. Polym J. 2018;50:149–66.
7. Kato T, Uchida J, Ichikawa T, Sakamoto T. Functional liquid crystals towards the next generation of materials. Angew Chem Int Ed. 2018;57:4355–71.
8. Gin DL, Lu X, Nemade PR, Pecinovsky CS, Xu Y, Zhou M. Recent advances in the design of polymerizable lyotropic liquid-crystal assemblies for heterogeneous catalysis and selective separations. Adv Funct Mater. 2006;16:865–78.
9. Imran OQ, Li P, Kim NK, Gin DL, Osuji CO. Stable cross-linked lyotropic gyroid mesophases from single-head/single-tail cross-linkable monomers. Chem Commun. 2021;57:10931–4.
10. Marets N, Kuo D, Torrey JR, Sakamoto T, Henmi M, Katayama H, et al. Highly efficient virus rejection with self-organized membranes based on a crosslinked bicontinuous cubic liquid crystal. Adv Health Mater. 2017;6:1700252.
11. Hamaguchi K, Kuo D, Liu M, Sakamoto T, Yoshio M, Katayama H, et al. Nanostructured virus filtration membranes based on two-component columnar liquid crystals. ACS Macro Lett. 2019;8:24–30.
12. Kuo D, Liu M, Kumar KRS, Hamaguchi K, Gan KP, Sakamoto T, et al. High virus removal by self-organized nanostructured 2D liquid-crystalline smectic membranes for water treatment. Small. 2020;16:202001721.
13. Hamaguchi K, Ichikawa R, Kajiyama S, Torii S, Hayashi Y, Kumaki J, et al. Gemini thermotropic smectic liquid crystals for

two-dimensional nanostructured water-treatment membranes. ACS Appl Mater Interfaces. 2021;13:20598–605.

14. Sakamoto T, Ogawa T, Nada H, Nakatsuji K, Mitani M, Soberats B, et al. Development of nanostructured water treatment membranes based on thermotropic liquid crystals: molecular design of sub-nanoporous materials. Adv Sci 2018;5:1700405.

15. McGinnis RL, Reimund K, Ren J, Xia L, Chowdhury MR, Sun X, et al. Large-scale polymeric carbon nanotube membranes with sub–1.27-nm pores. Sci Adv. 2018;4:e1700938.

16. Yang SY, Ryu I, Kim HY, Kim JK, Jang SK, Russell TP. Nanoporous membranes with ultrahigh selectivity and flux for the filtration of viruses. Adv Mater. 2006;18:709–12.

17. Torii S, Hashimoto T, Do AT, Furumai H, Katayama H. Repeated pressurization as a potential cause of deterioration in virus removal by aged reverse osmosis membrane used in households. Sci Total Environ. 2019;695:133814.

18. Wolf S, Hewitt J, Greening GE. Viral multiplex quantitative PCR assays for tracking sources of fecal contamination. Appl Environ Microbiol. 2010;76:1388–94.

19. Kitajima M, Hata A, Yamashita T, Haramoto E, Minagawa H, Katayama H. Development of a reverse transcription-quantitative PCR system for detection and genotyping of Aichi viruses in clinical and environmental samples. Appl Environ Microbiol. 2013;79:3952–8.

20. Katayama H, Shimasaki A, Ohgaki S. Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. Appl Environ Microbiol. 2002;68:1033–9.

21. Lee S, Hata A, Yamashita N, Tanaka H. Evaluation of virus reduction by ultrafiltration with coagulation–sedimentation in water reclamation. Food Environ Virol. 2017;9:453–63.

22. Michen B, Graule G. Isoelectric points of viruses. J Appl Microbiol. 2010;109:388–97.

23. Elhadidy AM, Peldszus S, Van Dyke MI. An evaluation of virus removal mechanisms by ultrafiltration membranes using MS2 and phiX 174 bacteriophage. Sep Purif Technol. 2013;120:215–23.

24. Kitajima M, Iker BC, Pepper IL, Gerba CP. Relative abundance and treatment reduction of viruses during wastewater treatment processes – Identification of potential viral indicators. Sci Total Environ. 2014;488–489:290–6.

25. Nada H, Sakamoto T, Henmi M, Ogawa T, Kimura M, Kato T. Transport mechanisms of water molecules and ions in sub-nano channels of nanostructured water treatment liquid-crystalline membranes: a molecular dynamics simulation study. Environ Sci Water Res Technol. 2020;6:604–11.

26. Chen C, Guo L, Yanga Y, Oguma K, Hou L. Comparative effectiveness of membrane technologies and disinfection methods for virus elimination in water: a review. Sci Total Environ. 2021;801:149678.