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Dual functional membrane capable of both visual sensing and blocking of waterborne virus

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ABSTRACT

Norovirus, which is transmitted by contact with a contaminated object or person who has a norovirus infection, eating contaminated food, or drinking contaminated water, is a highly contagious virus for humans and animals. Among many infection routes, water supply systems from reservoirs or dams become very important due to the possibility of mass infection. Because only a small amount of norovirus (10–100 particles forming unit) can cause the infection, a rapid and easy detection of the virus is required. Once norovirus is detected, the water supply should be quickly shut down.

Here, we introduce a dual functional membrane capable of both easy detecting with the naked eye and blocking noroviruses. We employed an anodized aluminum oxide (AAO) membrane with uniform pores. The top and bottom parts of the membrane were grafted with polydiacetylene (PDA) and poly(N-isopropylacrylamide) (PNIPAM), respectively. The top part was further bioconjugated by antibody for feline calicivirus (FCV), which is a model virus whose structure is similar to norovirus. The membrane showed color change from blue to red when the FCVs were attached to the antibodies. Water flux was controlled (turned on and off) by changing water temperature by using the well-known thermosensitive property of PNIPAM chains. On and off control of water flow was quickly established (less than 5 s) when a laser with near infrared (NIR) wavelength was turned on and off, respectively. In addition, the membrane completely blocked the virus influx into the water system by antibody binding before visual sensing followed by flow control.

1. Introduction

Waterborne viruses such as norovirus are highly contagious for humans and animals [1]. Because of their high contagiousness [2], the delivery of safe water [3,4] is the most crucial challenge against biohazards such as waterborne viruses according to the World Health Organization (WHO) [5,6]. Among many waterborne viruses, norovirus is the most common cause of viral gastroenteritis in humans worldwide. It is known that only a small amount of norovirus (10–100 particle forming unit) can cause the infection, and there has not been a remedy or vaccine available for the norovirus [7–14].

To date, most public health and clinical virology laboratories have detected for norovirus by using real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays [15,16]. But, they demand expensive equipment and a time-consuming process. Although some methods of the norovirus inactivation or removal using ultraviolet (UV), heat, and chlorine treatments, have been individually studied, the detection of the virus has not been investigated [17–19]. Thus, a single system which could sequentially perform detection, removal (or blocking) of viruses is most preferred. For instance, once the norovirus is detected, the water supply should be quickly shut down in a highly controlled manner. For this purpose, a membranous system with multifunctionalities including both rapid detection and shut down water flow should be developed. In addition, any virus should not influx into the water system before the detection followed by shut down water flow.

To address this issue, Li et al. [20] fabricated a hierarchically structured titanate nanotube membrane (TNM) by directly growing nanotubes on a porous membrane, and the TNM successfully isolated E. Coli. Although this system shows the potential for effective detection and removal of viruses or pathogen in water, the control of the water flow still remains unclear.

To govern both detecting norovirus and controlling the water flow, a special membrane with dual functions should be designed: One for virus sensing and the other for on-demand blocking of the virus.

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Previously, we reported a membrane having double gates by modifying differently the top and bottom parts of the membrane, and showed that each gate was independently controlled depending on different stimuli [21].

Inspired by our previous study [21], we introduce herein a novel dual function membranous system capable of easy detection of a waterborne virus and flow controllability. We employed an anodized aluminum oxide (AAO) membrane with uniform pores. The top and bottom parts of the membrane are modified with polydiacetylene (PDA) and poly(*N*-isopropylacrylamide) (PNIPAM), respectively. PDA has been a well-known polymer showing color change from blue to red depending on external stimuli, including mechanical strength, specific ligand-receptor interactions [22, 23]. The color change of PDA facilitates a simple and prompt detection with the naked eye when the viruses are approaching the membrane. On the other hand, PNIPAM exhibits thermosensitive property whose volume changes with temperature [24–27]. Thus, the PNIPAM chains could be used as a gate of defense against propagation of the viruses.

Because norovirus handling is very dangerous and cannot be cultivated in living cells or organ culture [28, 29], in this study, we used feline calicivirus (FCV) as a model virus for norovirus. It is noted that FCV is easily cultivable and quite similar to norovirus in genetic, morphological, biochemical properties [28, 29]. We easily recognized the color change of the membrane visually when FCV was adsorbed on the membrane. After the virus was detected, the water flow containing the FCV was successfully blocked by changing temperature with a laser. Furthermore, the membrane completely blocked the virus influx to the water system by antibody binding before visual sensing followed by shut down water flow.

2. Experimental

2.1. Materials

A highly pure aluminum plate (99.999%, 1 mm thickness) was purchased from Aldrich. Ethanol (≥ 99.8%), n-hexane, methanol, chloroform, 11-amino-1-undecanethiol (AUT, 99%), 10, 12-pentacosadiynoic acid (PCDA, ≥ 97.0%), 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC, ≥ 99.0%), N-hydroxysuccinimide (NHS, 98%), phosphate buffered saline (PBS), copper(I) bromide (99.999% trace metals basis), copper(II) bromide (99.999% trace metals basis), N,N,N′,N′′,N′′-pentamethyldiethylenetriamine (99%), Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), non-essential amino acid (NEAA) and hydroxyethyl-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich. Feline calicivirus antibody (FCV1-43), Crandell Rees feline kidney (CRFK) cell were purchased from Thermo Fisher Scientific. N-isopropylacrylamide (NIPAM) was purchased from Wako Pure Chemicals. FCV was purchased from ATCC (U.S.A.). Crystal violet was purchased from Junsei.

2.2. Fabrication of dual functional membrane

A dual functional membrane was prepared as schematically shown in Fig. 1. First, AAO membrane with a pore diameter of 200 nm and a thickness of 60 μm was fabricated by two step anodization [21]. Then, a thin Ti/Au layer was prepared by vacuum-evaporation of Ti and gold on the AAO membrane. A gold-deposited membrane was then immersed in an ethanol solution of AUT (0.1 mM) for 4 h to form AUT self-assembled monolayer (SAM) on the gold layer [30,31]. The membrane was thoroughly rinsed with ethanol to remove unadsorbed AUT molecules (step a).

Next, PCDA monomers in chloroform (6.72 mM) were spin coated for 30 s on the AAO membrane, and NHS/EDC coupling [32] was carried out in chloroform to form NHS-ester (step b). The membrane was dipped for 12 h into PBS (pH 7.2) solution containing feline calicivirus antibody (FCV1-43) (10 μg/ml), which allowed the bioconjugation of FCV1-43 on PCDA monomers. Then, the PCDA was irradiated with UV light with a wavelength of 254 nm for 2 h to form PDA. Unreacted PCDA monomers were completely removed by washing the membrane with deionized (DI) water (step c).

The opposite side of the membrane was also deposited by Ti/Au (step d). NIPAM monomer was purified twice by recrystallization in n-hexane, and dissolving it (0.795 g/ml) into a mixture of DI water (31 ml) and methanol (18 ml). Then, copper(I) bromide (0.04 g) and copper(II) bromide (0.0125 g) as a catalyst, and N,N,N′,N′′,N′′-pentamethyldiethylenetriamine (0.35 ml) as a ligand were added. The surface initiated atom transfer radical polymerization (SI-ATRP) was carried out at 50 °C for 6 h under nitrogen environment [21]. Ungrafted PNIPAM chains on AAO membrane were removed by rinsing with a mixed solvent of methanol and DI water (1:2 v/v) (step e).

2.3. Flux test

A flux test was performed with a home-made flux cell [21,33]. The membrane was placed at the bottom of the flux cell, and tightly fixed with O-ring to prevent any leakage. The fluid flows from the reservoir with a volume of 1 L to the flux cell and passes through the membrane under N_2 gas at 0.2 bar. The ejected fluid from the outlet of the membrane was dropped into an empty beaker on a balance connected to the computer to record the weight change with time. An 808 nm laser of NIR with a power density of 5 W cm^{-2} (JENOPTIK unique-mode Gmbh, Germany) was used for controlling temperature on the bottom part of AAO membrane. The laser was vertically irradiated on the bottom of the AAO membrane. After the laser was turned on (or off), the amount of ejected fluid was measured at a time interval of 2 s. Flux measurement was repeated by using five independently prepared membranes.

2.4. Virus test

CRFK cells were cultured in 5% CO_2 incubator (MCO-15AC, SANYO Electric Biomedical Co., Ltd) at 37 ℃, using DMEM including 10% FBS, 1% Penicillin-streptomycin, 1% NEAA. When CRFK cells formed the monolayer, FCV was inoculated onto the cells. Then, they were cultured in 5% CO_2 incubator at 37 ℃ for 1 h. After the culture, the medium containing DMEM liquid, 5% FBS, 1% NEAA and 1% penicillin-streptomycin was cultured in 5% CO_2 incubator at 37 ℃ for 4 days. When the adhered cells were detached, the freeze-thaw process was repeated three times. The supernatant obtained from the centrifugation at 2000 rpm for 8 min was divided into the 1 ml per vial. The virus stock was stored in liquid N_2 before being used.

The virus stock flowing out through the membrane was decimally diluted from 10^{-2} to 10^{-5} in HEPES buffer solution. For obtaining reliable PFU values, the assay tests were repeated with five independently prepared membranes. The viruses cultured in the tissue flasks were inoculated onto the monolayer-formed CRFK cells by 0.5 ml, and the adhesion process was conducted in 5% CO_2 incubator at 37 ℃ for 1 h. After the adhesion, 1:1 mixture of the 2x overlay medium containing DMEM liquid, 10% FBS, 1% NEAA, 1% penicillin-streptomycin and 1.5% agarose gel was dispensed to the flasks by 5 ml. After the plaques were formed, the cell immobilization was carried out in 18.5% formaldehyde for 20 min, and the cell layer was removed under running water, followed by crystal violet staining for 5 min.

3. Results and discussion

Fig. 2a gives FT-IR spectra of pristine AAO membrane, AAO membrane grafted with PDA chains bioconjugated with FCV1-43 antibody, and AAO membrane grafted with PNIPAM, respectively. The AAO membrane grafted with PDA chains shows the characteristic peaks of PDA at 2920 cm^{-1} (asymmetric CH_2 stretching), 2848 cm^{-1} (symmetric CH_2 stretching), 1693 cm^{-1} (carbonyl band hydrogen bonded carboxylic acid head groups), and 1466 cm^{-1} (scissoring band of the

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