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Arc discharge-mediated disassembly of viral particles in water

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ABSTRACT

In this study, we investigated the inactivation effects on murine norovirus (MNV-1) with/without purification in water using a submerged plasma reactor of arc discharge (underwater arc), which produced a shockwave, UV light, reactive oxygen species and reactive nitrogen species. Underwater arc treatments of 3 and 6 Hz at 12 kV resulted in 2.6- and 4.2-log reductions in the virus titer of non-purified MNV-1 after 1 min of treatment, respectively. The reduction of purified MNV-1 was higher than that of non-purified MNV-1 after underwater arc treatment for all applied conditions (12 or 15 kV and 3 or 6 Hz). One of the viral capsid proteins (VP1) was not detectable after underwater arc treatment, when its integrity was assessed by western blot analysis. Transmission electron microscopy analysis also revealed that MNV-1 particles were completely dissembled by the treatment. This study demonstrates that underwater arc treatment, which was capable of disintegrating the MNV-1 virion structure and the viral capsid protein, can be an effective disinfection process for the inactivation of water-borne noroviruses.

1. Introduction

Human norovirus is a leading microbial agent that causes most common outbreaks of non-bacterial gastroenteritis worldwide. Human infection by norovirus is based on fecal-oral transmission and is mainly mediated by the consumption of contaminated food and water (Jaykus et al., 2013). Waterborne transmission of norovirus is a serious public health problem because the high infection rate associated with norovirus may be due to a low infectious dose in humans (≥18 viral particles) (Artmar et al., 2014) and the virus's high resistance to various environmental stresses, such as the manipulation of temperature, water activity, and pH (Jaykus et al., 2013; Marshall and Bruggink, 2011). Thus, an efficient disinfection method is necessary to reduce the incidence of illness associated with norovirus.

Despite the public health risk due to human norovirus, information on the effectiveness of disinfection methods for human norovirus is limited because there are no conventional cultivation methods available for this virus. For various disinfection studies, murine norovirus 1 (MNV-1) is generally used as a cultivable surrogate of the viral pathogen due to its biochemical and genetic similarities with human norovirus (Wobus et al., 2006).

For water disinfection, chlorine is the most widely used due to its low cost (Rodriguez and Sérodes, 2001). However, the resistance of norovirus to chlorine disinfection has been reported (Keswick et al., 1985; Duizer et al., 2004), and the use of chlorine is associated with the concern that undesirable by-products can be generated, such as trihalomethanes (Morris et al., 1992; Rook, 1974). Therefore, various water disinfection methods have been investigated as alternatives to chlorine, and the effects of disinfection attempts on enteric viruses have been reported (Shin and Sobsey, 2008; Ko et al., 2005; Lee and Ko, 2013). UV radiation does not affect water quality, and UV-C is an efficient disinfection method for norovirus inactivation due to its high energy (Ko et al., 2005). Water disinfection efficacies using UV-B or UV-A with TiO_2 have been reported to inactivate norovirus and the MS2 bacteriophage (Lee and Ko, 2013). However, investigation of novel alternative disinfection processes for water is continuously required to develop disinfection technologies for water and various related applications, such as water disinfection in a pipe network.

Recently, plasma, a gas ionized by electrical discharge, has

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drawn attention as an emerging method for biological inactivation due to the availability of environmentally friendly technology without residual toxicity for water treatment (Chang, 2001; Jiang et al., 2014). Three types of plasma are suitable for water treatment, including remote plasma (e.g., downstream plasma), indirect plasma (e.g., high- and low-pressure gas discharge lamps), and direct plasma methods (e.g., pulsed arc and/or pulsed corona discharge in water) (Chang, 2001). Among these plasma types for water treatment, direct plasma, like a submerged plasma reactor for arc discharge (underwater arc), can be used to treat water directly along with UV radiation, radical species, and shock waves (Lee et al., 2003). Underwater arc treatment is considered to have a higher capability of biological inactivation (Chang, 2001; Kang et al., 2015). However, the utility of underwater arc treatment in the inactivation of enteric viruses such as norovirus remains poorly understood.

The effects of biological inactivation in water by disinfection treatment can be affected by the preparation of the test microorganism (Sobsey, 1989). Using a virus stock without purification (crude) is considered more resistant than a purified virus for a disinfection treatment, while using a purified virus could significantly reduce the required dose of the disinfectant (Shin and Sobsey, 2008). Therefore, in this study, an underwater arc was applied to crude and purified MNV-1 to characterize the inactivation achieved with the device depending on the preparation conditions. After underwater arc treatment of water containing MNV-1, plaque assays and quantitative real-time RT-PCR were performed to detect infectious virus and viral RNA, respectively. To characterize the inactivation mechanism, one of the viral capsid proteins, VP1, was investigated by SDS-PAGE and western blot analysis. Purified MNV-1 treated with an underwater arc was also observed by transmission electron microscopy (TEM).

2. Materials and methods

2.1. Cell cultures and MNV-1 preparation

MNV-1.CW1 (GenBank: DQ285629) was used to generate viral stock and was propagated in RAW 264.7 cells as described previously (Wobus et al., 2004). RAW 264.7, a murine leukemia macrophage cell line, was purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea) and was maintained in DMEM (Lonza, MD, USA) supplemented with 10% fetal bovine serum (Lonza), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2.

2.2. Purification of MNV-1 particles

Purification of MNV-1 was performed as previously described with modifications (Huhti et al., 2010). Briefly, crude MNV-1 was pelleted by ultracentrifugation at 150,000*g* for 1 h at 4 °C. The crude pellet was gently suspended in a suspension buffer (50 mM of Tris-HCl [pH 7.5], 100 mM of NaCl) at 4 °C and was then pelleted again by ultracentrifugation at 150,000*g* for 1 h at 4 °C. After the supernatant was removed, the MNV-1 pellet was gently suspended again in a suspension buffer at 4 °C. The MNV-1 suspension was placed on a discontinuous sucrose density gradient (10–60%) and subjected to ultracentrifugation at 100,000*g* for 150 min at 4 °C in a SW41 Ti swinging-bucket rotor (Beckman Coulter, CA, USA). Fractions were separated in 1 ml aliquots from the top layer, and the fraction containing MNV-1 was determined by using SDS-PAGE to detect VP-1 (the MNV-1 capsid protein). MNV-1 in a sucrose buffer was pelleted by ultracentrifugation at 150,000*g* for 1 h at 4 °C, and the MNV-1 pellet was then suspended in PBS.

2.3. Underwater arc discharge apparatus

A schematic diagram of the experimental apparatus is shown in Fig. 1. The apparatus consisted of a DC power supply (Ksc, Seoul, Korea), a storage capacitor, an air gap switch and a reactor. The discharge frequency was controlled with an air gap switch with a distortion electrode, to which a high-voltage pulse was applied. This high-voltage pulse was applied to a stainless steel powered electrode placed inside a container with water. The other board electrode was located under the powered electrode with a 1 mm gap. Plasma was generated in the water by arcing between the two electrodes, discharged at 12–15 kV. The stable minimum voltage was 12 kV to generate an arc of this system, and 15 kV was chosen to evaluate the viral reduction based on the quantity of the voltage increase. The two different frequencies had a double gap to verify the differences in the viral reduction depending on the input energy. The voltage and current during arc discharge were monitored using an oscilloscope (Fig. 2A). Optical emission spectroscopy (OES) of the light created by generating an arc in water was performed using an optical emission spectrometer (HR4000, Ocean Optics Inc., FL, USA).

2.4. Sample treatment

MNV-1 with/without purification suspended in PBS was diluted with 4 °C distilled water (DW) for underwater arc treatment (5–6 log PFU/ml). A 50 ml sample was placed in the reactor container, and an arc discharge was then generated and applied to the MNV-1 suspension for the indicated time. After treatment, 10% (w/v) sterile sodium thiosulfate (0.5 ml) as a neutralizer was added to the 50 ml sample to arrest the virucidal activity at the end of the defined treatment time (Omidbakhsh and Sattar, 2005). As a blank inactivation test, the virus titer of MNV-1 in DW remained almost the same after incubation for 24 h at 4 °C. Hence, natural degradation can be ignored in the inactivation tests.

2.5. Plaque assay for MNV-1 infectivity

The plaque assay was performed as described previously (Gonzalez-Hernandez et al., 2012). Briefly, RAW 264.7 cells were seeded on 6-well plates (2×10^6 cells/well). On the next day, the cells were inoculated with the virus inoculum properly diluted in serum-free DMEM, and the plates were incubated for 1 h. The inoculum was then aspirated, and the fresh complete DMEM (high glucose) containing SeaPlaque® Agarose (1% w/v; Lonza) was overlaid. Plaque counting was performed after 48 h by visualization with neutral red staining.

2.6. Real-time quantitative RT-PCR for MNV-1 genomic RNA

MNV-1 genomic RNA in the pulsed plasma-treated samples was extracted with TRIzol LS reagent (Invitrogen, CA, USA) and purified according to the manufacturer's procedures. cDNAs were synthesized using ImProm-II reverse transcriptase (Promega, WI, USA), and real-time quantitative RT-PCR was performed using the iQ Supermix qRT-PCR kit (Bio-Rad, CA, USA). The TaqMan probe and the forward and reverse primer sequences specific for the MNV-1 genomic RNA were as follows: TaqMan probe, 5′-(6-FAM)-CGCTTTGGAACAATG-(BHQ-1)-3′; forward primer, 5′-CACGCCACC-GATCTGTTCTG-3′; reverse primer, 5′-GCGCTGCGCCATCACTC-3′ (Stals et al., 2009). The cDNAs were amplified by 30 cycles of 15 s at 95 °C, 15 s at 56 °C and 15 s at 72 °C, and fluorescence was detected by CHROMO4 continuous fluorescence detector (MJ Research, MA, USA). Standard RNA used for qRT-PCR was prepared by *in vitro* transcription of PCR products amplified using the forward primer