Chlorhexidine-filled porous ceramic coating fabricated by the aerosol deposition method for immediate and long-term enveloped virus inactivation

Taku Goto*1, Yoichi Yamada2b, Takeji Ueda3, Sumiko Shiota4, Mitsugu Sohma5 and Jun Akedo3

*Advanced Coating Technology Research Center, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan;
1School of Pharmacy, Shijitsu University, Okayama, Japan

ABSTRACT
We developed an antiviral ceramic coating for immediate and long-term enveloped virus inactivation. The coating consisted of a porous ceramic coating fabricated by aerosol deposition and filled with chlorhexidine (CHX). The coating immediately inactivated enveloped viruses by releasing CHX. Furthermore, after the coating was repeatedly washed to simulate practical use, it still showed antiviral activity because CHX leakage was suppressed by the nanopores in the coating. We expect our antiviral coating to exhibit antiviral activity under conditions similar to practical use.

KEYWORDS
Porous ceramic coating; aerosol deposition method; anti-viral coating

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1. Introduction
The COVID-19 pandemic has led to people adopting mitigation measures, such as wearing masks, washing hands, and avoiding crowded places and close contact. These behavioral patterns, except for hand washing, are intended to avoid the risk of infection from the scattering of virus-containing droplets and direct inhalation. Simulations of the effects of humidity and masks on droplet scattering have shown that droplet scattering is the main infection route [1].

The activity retention of virus particles on surfaces depends on temperature and humidity, and infectivity is maintained for as long as 1 week at low temperature and humidity [2]. Indirect infection through contamination of solid surfaces can be prevented by hand washing with alcohol or dilute detergent and cleaning of solid surfaces. However, indirect infection has been treated as an irreducible risk because it has not been possible to rule out droplet infection; however, there have been cases in which indirect infection may have occurred [3,4]. Hygiene and disinfection in hospitals, schools, colleges, and industrial and public buildings are becoming increasingly important for public health. To prevent the contact transmission of viruses via surfaces that are touched by an unspecified number of people, such as faucets and doorknobs, there is a strong need for a method of inactivating viruses both immediately and in the long-term. Chemical compounds, such as alcohols [5], sodium hypochlorite [6], hydrogen peroxides [7], and chlorhexidine gluconate [8], are used as disinfectants and antiviral agents. Although these chemical compounds immediately inactivate the viruses, the antiviral effect is not sustained because the compounds evaporate or dissipate.

A variety of antiviral coatings have been developed, for example, photocatalytic antiviral coatings [9] and various antiviral nanoparticle coatings, such as copper [10–12] and silver [12–14]. These coatings or nanoparticles have a long-term antiviral effect; however, photocatalytic antiviral coatings do not exhibit sufficient antiviral efficiency in the dark and nanoparticles with strong bactericidal and antiviral properties may be cytotoxic [15]. Furthermore, it takes a long time for these coatings to sufficiently inactivate the virus [10–15].

CHX is a hydrophobic surfactant, and the related compound, chlorhexidine gluconate, shows an antiviral effect. Surfactants inactivate enveloped viruses, such as SARS-CoV-2 and the influenza A virus, by disrupting the lipid bilayer of the envelope, and thus CHX should also inactivate enveloped viruses. Moreover, the low solubility of CHX in water (0.08% (w/v) at 298 K) is expected to suppress the undesirable leakage of CHX during washing.

In this work, we developed an antiviral ceramic coating for immediate and long-term enveloped virus inactivation. The coating consisted of a porous ceramic coating fabricated by aerosol deposition (AD) [16–18] filled with chlorhexidine (CHX) (Figure 1). The AD process developed by J. Akedo can be used to grow highly dense or porous ceramic coatings at room temperature by controlling the starting powders [19]. AD coatings are formed by impact adhesion of fine particles. AD coatings can be applied to a variety of substrates, such as plastic, metal, and glass, and exhibit mechanical properties close to that of the bulk material [16–18].
This coating was expected to achieve immediate inactivation of viruses in microdroplets (e.g., saliva) on the antiviral surface by directly inactivating the viruses in contact with the coating surface (Figure 1) and indirectly inactivating the viruses in droplets by releasing CHX into the droplets (Figure 1). Moreover, to achieve a long-term antiviral effect, the nanopores in the ceramic layer were filled with CHX to prevent the undesirable leakage and peeling of CHX after repeated washing and rubbing. To simulate practical use, we also measured antiviral activity after repeated washing with water.

We determined the antiviral activity of the proposed antiviral coating for an influenza A virus as the enveloped virus. The coating inactivated 83% of the enveloped viruses in 10 min and 99.95% of the viruses in 120 min. Furthermore, to investigate the practical coating performance, we measured its antiviral activity after repeated washing with water. Even after repeated washing, the coating showed the antiviral activity. These results indicate that the coating exhibits immediate and long-term antiviral effects for the enveloped virus.

2. Experimental procedure

2.1. Fabrication of anti-viral coating

The porous ceramic layer was fabricated by AD method on a SUS403 stainless-steel substrate using an equal mixture of alpha-alumina (Al₂O₃) and alumina hydroxide (Al(OH)₃) powder. In the AD method, submicrometer ceramic particles are accelerated by a gas flow in a nozzle to a velocity of several hundred meters per second and sprayed onto a substrate, and thick ceramic layers are formed via impact adhesion of fine particles. More information about the AD method is available in Refs. [16–18]. The experimental parameters for the AD method are shown in Table 1. The pores of the ceramic layer were then filled with an ethyl acetate CHX solution, and the ethyl acetate was allowed to evaporate at room temperature.

2.2. Determination of the antiviral activity of samples

The antiviral activity of the samples was tested following ISO 21702 as follows. An influenza virus A/PR/8/34 (H1N1) suspension containing 10⁶–10⁷ plaque forming units (pfu)/mL was dropped onto the test sample. The sample was covered with a film and left at 25°C for 10 min, 2 h, or 24 h. The viruses on the sample were collected by washing with soybean casein digest lecithin polysorbate (SCDLP) medium (10 mL), and the solution containing the viruses was diluted with SCDLP medium. The solution was applied to a confluent six-well plate containing Madin-Darby canine kidney cells at 37°C for 1 h under 5% CO₂. The supernatant was replaced with 0.8% o xo d agar and plates were incubated for 24 to 48 h at 37°C under 5% CO₂. The cells were fixed with 5% glutaraldehyde and stained with methylene blue. The virus infection titer was calculated from the number of counts of plaques and the dilution factor. The virus inactivation ratio was calculated by dividing the virus infection titer of samples by the virus infection titer of the control.

![Figure 1. Schematic of anti-viral mechanism of porous AD coating filled with CHX.](image-url)
2.2.1. Time-kill test
CHX solution (1.8 mL) and influenza virus A/PR/8/34 (H1N1) suspension (0.2 mL) containing $1 \times 10^6$ to $5 \times 10^6$ pfu/mL were mixed and incubated at room temperature. The mixture (0.12 mL) was diluted with SCDLP medium. The virus inactivation ratio was obtained in the same way as described in Section 2.2.

2.3. Sample characterization
The microstructures and morphology of the samples were measured using scanning electron microscopy (SEM; JEOL Ltd., JSM-6700 F), annular dark field scanning transmission electron microscope (ADF-STEM; Hitachi Ltd., HD-2700), and energy dispersive X-ray spectroscopy (EDX, AMETEK Octane T Ultra W 100mm2SDD). Structural characterizations of the films and starting powders were performed by X-ray diffraction using CuKα radiation (XRD; Rigaku, RINT-2100 V). The Raman signals were obtained using NR5-7100, Jasco. The CHX concentration in water was determined by colorimetric analysis using ultraviolet-visible absorption spectroscopy (UV-Vis; Jasco, V-570). The ball-on-disk (BoD) test was performed at 60 rpm with 50 g using 2 mm ball.

3. Results and discussion
3.1. Morphology and structure of the coating
SEM images of the starting powders and the porous AD layer on the SUS403 substrate are shown in Figure 2.Figure 2(a,b) show the SEM images of the raw Al₂O₃ and Al(OH)₃ powders, respectively. The Al₂O₃ particles were 0.2–1.0 μm in size and the Al(OH)₃ powder consisted of nanoparticle aggregates 1 μm in size. The porous ceramic layer was fabricated on a SUS403 substrate using an equal mixture of Al₂O₃ and Al(OH)₃ powder. Figure 1(c) shows a photograph of the SUS403 substrate and the porous AD coating on the SUS403 substrate. The porous AD coating was opaque due to the pores. A top-view SEM image of the porous AD coating is shown in Figure 1(d).

To determine pore size and surface area distribution in the porous AD coating, a mercury porosimetry test was performed on the coating. However, the measured data was almost no different from the blank (data not shown), and almost no measurable pores (100 μm–30 nm) were confirmed by the mercury porosimetry test. Thus, to observe the pores under several tens of nm, the pore morphology in the coating was observed using the cross-sectional ADF-STEM technique. Figure 3(a) shows the cross-sectional ADF-STEM images of the upper side of porous AD coating. As shown in Figure 3(a), the dark areas (low contrast areas) with a range from 30 nm to several nm were observed. Since these dark areas coincided with the region without Al in the EDX mapping as shown in Figure 3(b), these dark areas should be pores and the size of pores in the porous AD coating ranged from 30 nm to several nm. The molecule dimensions of CHX is ca. 34 × 7 × 6 Å [20], the CHX molecules were small for the pores in the coating. To observe the distribution of the CHX in the porous AD layer, EDX mapping of the Cl element was performed. The Cl is included only in

![Figure 2](image-url) SEM images of raw (a) Al₂O₃ and (b) Al(OH)₃ powder. (c) Photograph of the SUS403 substrate and porous AD coating on the SUS403 substrate. (d) SEM image of the porous AD coating (top view).
the CHX(C_{22}H_{30}Cl_{2}N_{10}) in this study. Figure 3(c) shows the EDX mapping image of the Cl element of the same region as Figure 3(a). The almost Cl distributed in the pores in the coating, the CHX filled the pore in the coating. Moreover, the Cl was also distributed near the substrate of the coating as shown in Figure 3(d–f), and the CHX penetrated the entire coating.

Figure 4 shows the EDX spectra and composition ratios of N, O, Al, and Cl for various regions. The average of the Cl/Al in the three regions (top (Figure 4(b)), middle(Figure 4(c)), and bottom region (Figure 4(d)) in the coating) were 0.67 wt.%, thus the CHX /ceramics(50 wt.% Al_{2}O_{3} + 50 wt.%Al(OH)_{3}) was 0.20 wt.%.
3.2. Antiviral activity of coatings

To determine the antiviral activity of CHX, a time-kill test was performed for CHX solution (128 μg/mL) against the influenza A virus (Figure 6). In the water control sample, the viral titer was almost constant until 10 min, and then decreased slightly at 30 min, which may have been spontaneous weakening of the virus. In contrast, in the CHX solution, the viral titer of the influenza A virus decreased dramatically up to 10 min, and decreased to 1/2000 of its initial value at 30 min. The CHX solution inactivated 99.993% of the influenza A virus in 30 min, demonstrating its antiviral activity against enveloped viruses. This immediate inactivation of viruses is a feature of disinfectants.

To determine the antiviral activity of the coating, the inactivation ratio of the influenza A virus on the coating was measured. We measured the time required for virus inactivation on the coating by incubating the virus on the coating for various times and measuring the inactivation ratio. Figure 7(a) shows the virus inactivation ratio on the coating with 128 μg/cm² CHX and without CHX as a function of incubation time. Without CHX, the virus inactivation ratio was 19% after 2 h incubation, indicating no antiviral activity, whereas with CHX, the inactivation ratio was 83% after 10 min and 99.994% after 2 h, indicating that the coating with CHX had antiviral activity against enveloped viruses. The time required to inactivate more than 99.994% of the virus (antiviral activity value > 4) was incompatible or shorter than that of antiviral coatings that did not contain disinfectants in previous studies [11,14,21,22], and our coating showed an immediate virus inactivation effect.

The amount of CHX on the coating required for virus inactivation was examined. Figure 7(b) shows the inactivation ratio after 2 h of incubation as a function of the amount of CHX on the coating. Even for 16 μg/cm² of CHX, the coating inactivated 99.93% of the virus, demonstrating that a small amount of CHX was sufficient for achieving antiviral activity. Therefore, the coating should show sufficient antiviral activity over the long term.

During practical use, the antiviral coating would be touched repeatedly by many people and washed to keep it clean. Therefore, we measured the antiviral activity of the coating after it was washed repeatedly in water. Figure 8 shows the inactivation ratio of the enveloped virus on the SUS403 substrate and on our coating (porous AD coating/SUS403 substrate) before and after washing with water. The substrate and the coating were filled with 128 μg/cm² of CHX before washing by immersion in clean water (15 mL) for 30 min five times. Before washing, the SUS403 substrate covered with CHX and the coating filled with the same amount of CHX showed similar antiviral activity, with inactivation ratios after 2 h of 99.98% and 99.994%, respectively. However, after washing, the SUS403 substrate did not show antiviral activity, with an inactivation ratio of 7.9%, suggesting that most of the CHX on the SUS403 substrate was washed off. In contrast, the porous AD coating/SUS403 substrate filled with
CHX had an inactivation ratio of 97.2%, indicating that the nanopores in the porous AD coating inhibited undesirable leakage of CHX during washing.

To evaluate the CHX retention on the coating, the elution of CHX from the coating into the water during ultrasonic washing was measured. Figure 9 shows the amount of CHX on the SUS403 substrate or the porous AD/SUS403 as a function of ultrasonication time. The CHX amount on the substrate or the coating was calculated from the CHX concentration in the water (60 mL). The CHX concentration was determined by UV-Vis colorimetric analysis. The amount of CHX on the porous AD coating was higher than that on the SUS403 substrate for all ultrasonication times. This result showed that the porous AD coating prevented CHX from leaking into the water. Further investigation of the adsorption mechanism of the CHX on ceramics is needed, however, since the CHX also eluted in small amounts into the water in case of using porous AD coating, we assume that CHX was physically adsorbed to the porous ceramic.

Finally, CHX retention during abrasion was measured by abrading the coating was using the BoD test, and the presence of CHX on the abraded area was evaluated by Raman spectroscopy. Figure 10 shows photographs of the coating after the BoD test and the Raman spectrum on the abraded area of the coating at various times during the BoD test. The Raman peaks were observed at 1603 cm\(^{-1}\), 1288 cm\(^{-1}\), 1252 cm\(^{-1}\), 1177 cm\(^{-1}\), 1093 cm\(^{-1}\), and 1012 cm\(^{-1}\). These peaks were consistent with the Raman peaks of CHX [23]. Although the intensity of the CHX Raman spectrum decreased as the abrasion time increased, the CHX Raman spectrum was visible throughout the test, indicating that CHX remained on the surface. Thus, we expect that our coating should show long-term antiviral activity on frequently touched surfaces.

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**Figure 7.** Virus inactivation by the CHX-filled porous AD coating. (a) Virus inactivation ratio as a function of time and (b) virus inactivation ratio after 2 h as a function of the amount of CHX on the porous AD coating.

**Figure 8.** Virus inactivation ratio after 2 h before and after the coatings were washed five times with water.

**Figure 9.** Amount of CHX on the substrates as a function of ultrasonic cleaning time.

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4. Conclusion

Pathogens, such as SARS-CoV-2, that mainly infect people through airborne droplets, can retain their activity after they adhere to the surface. To prevent contact infection via surfaces for which it is difficult to confirm that may or may not have been contaminated previously, antiviral surfaces are an important infection control measure that merits investigation.

We developed a porous ceramic AD coating filled with CHX with antiviral activity against an enveloped virus. The coating containing 128 μg/cm² of CHX inactivated 83%
of the influenza A virus after 10 min and 99.994% after 2 h. The coating containing even a small amount of CHX (16 μg/cm²) inactivated 99.93% of the virus after 2 h. To investigate the practical coating performance, we measured its antiviral activity after repeated washing with water, and the coating retained the CHX and showed antiviral activity owing its nanopores. The CHX on the coating was also retained in the long-term abrasion test.

Our coating showed excellent antiviral activity against an enveloped virus after tests simulating practical use, and the coating may prevent the contact transmission of viruses via surfaces touched by many people. The antiviral coating could be used on various surfaces due to the versatility of AD coating (Figure 11). Examples could include handrails, buttons, door handles, protective clothing, and car interior walls, steering wheels, and gear shifts for ambulances, car sharing, and so on. In future work, it is necessary to evaluate the antiviral activity of the coating in various applications, and to consider surface cleaning and CHX replenishment methods.

CHX is a widely used disinfectant and is used in a wide range of infection control as well as for virus control. Therefore, we believe that the this type of coating has great potential, and we would like to collaborate with governments and companies on examining various applications with the aim of rapid practical implementation.

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Disclosure statement

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