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Research Article

Keywords: surface coating materials, Photocatalytic nanoparticles, infectious diseases, COVID-19, global pandemic

Posted Date: October 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-951098/v1

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Development of a high-throughput method to screen novel antiviral materials

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Abstract

Respiratory infectious diseases pose a serious threat worldwide, and novel antiviral materials are highly demanded. Photocatalytic nanoparticles have been developed to inhibit indirect transmission of pathogens by acting as surface coating materials. During development of such antiviral materials, researchers use bacteriophages as model viruses due to their safety and experimental efficiency. Screening methods are used to identify potential antiviral materials, and better screening technologies will accelerate the discovery of antiviral treatments. In this study, we constructed a novel platform to evaluate antiviral activity of surface coating materials using the M13 bacteriophage and phagemid system derived from phage display technology. The evaluation results generated by this system for the two tested antiviral materials were comparable to those for the materials tested on the Qβ bacteriophage and influenza virus using traditional screening methods. The experimental system developed in this study provides rapid and effective screening and can be applied to the development of novel antiviral materials.

Introduction

Infectious diseases pose a serious threat worldwide, and respiratory viruses in particular can cause rapid and explosive infections. Influenza viruses were responsible for global pandemics in 1918, 1957, 1968, and 2009. More recently, coronavirus 2 caused the COVID-19 global pandemic, which resulted in more than 4.7 million deaths and 230 million cases as of 29 September 2021. In addition
to human health impacts, pandemics have significant negative economic and social impacts due to measures introduced to reduce the spread of infection. Therefore, developing methods to suppress respiratory infections requires urgent attention.

Respiratory viruses can be transmitted via direct contact, indirect contact, droplets, or aerosols, and a variety of nanotechnology-based materials have been developed to inhibit each transmission pathway. Among them, photocatalytic nanoparticles such as titanium dioxide (TiO$_2$)-based photocatalysts have been used for surface disinfection, resulting in inhibition of indirect transmission. In particular, Cu(II) nanocluster-grafted TiO$_2$ has been shown to effectively inactivate viruses under visible light and is now being used as an antiviral surface coating material in indoor environments.

During development of antiviral materials, bacteriophages are often used to evaluate antiviral activity. Bacteriophages specifically infect bacteria and archaea and therefore do not pose the risk of human infection. In addition, high-throughput techniques can be used in bacteriophage-based experiments because bacteria grow more rapidly than mammalian cells, thus the evaluation process is faster and more effective. Further improvements in experimental efficiency will accelerate the development of novel antiviral materials.

Phage display technology enable peptides and proteins to be displayed on the surface of bacteriophages, and it is a robust platform for screening antibody fragments as antigen binders. Although a variety of bacteriophages have been used in this system, the M13 filamentous phage is
the one most commonly utilized \cite{17,18}. In the system, a phagemid vector containing the genes for the displayed proteins is introduced into *Escherichia coli* (*E. coli*) upon infection, and the vector provides antibiotic resistance to the *E. coli* cells, thereby allowing the antibiotic-based selection (Fig. 1).

In this study, we constructed a novel platform to evaluate the antiviral activity of materials using the M13 bacteriophage and phagemid system derived from the phage display system. *E. coli* cells were infected with the M13 bacteriophage treated with antiviral materials, and active M13 phages were evaluated by colony counting. Our platform enables more effective screening than previous bacteriophage-based evaluation systems that rely on plaque formation assays, and it should be applied to the development of novel antiviral materials.

**Results**

**Estimation of inactivation of the M13 phage by treatment with antiviral materials**

To evaluate the screening system using bacteriophage M13 developed in this study, we assessed anti-viral effect of treatments with commercially available photocatalytic particle-based anti-viral reagents, PROTECTON VK-500 and PROTECTON BARRIERX Spray on M13 phage. The anti-viral effect of those anti-viral reagents were validated by officially approved third parties following Industrial Standards (JIS 1756R for Qb phage and ISO 21702 for influenza virus) (Fig. S1).

Schematic of the experiment using M13 bacteriophage is shown in Fig. 2. We inoculated M13 phage suspension onto glass slides coated with the anti-viral reagents or a blank glass slide as control,
followed by covering with transparent films. After overnight incubation at room temperature in bright light, we collected M13 phage by rinsing the glass slides with PBS and made a series of 1/10 dilution. Subsequently, we infected *E.coli* cells with serially diluted M13 phage and spotted 5 μl of each *E.coli* cells into grids on TYE plates containing ampicillin. Following overnight incubation at 37 degree, we counted the number of colonies in a grid.

The number of colonies derived from M13 phage infection was drastically reduced by treatment with the antiviral reagents PROTECTON VK-500 and PROTECTON BARRIERX Spray (Fig. 3A). We calculated the number of colony forming units (cfus) of M13 phages for each sample and then evaluated the degree of M13 phage inactivation via comparison with control samples. The cfu values were decreased by more than hundred-fold, indicating that more than 99% of M13 phages were inactivated (Fig. 3B and C). These reductions agree with their antiviral effects on influenza virus and bacteriophage Qβ (Fig. S1), which validates the relevance of the novel method developed in this study to screen for antiviral materials.

We also evaluated the inactivation of M13 bacteriophages by PROTECTON BARRIERX Spray over time. The number of active phages gradually decreased over time (Fig. 4), showing that the antiviral material inactivated the bacteriophages in a time-dependent manner.

**Discussion**

In this study, we developed a novel screening method using the bacteriophage M13 to evaluate
antiviral materials. Utilization of a phagemid, which provided *E.coli* cells with antibiotic resistance upon infection with M13 phages harboring the phagemid, enabled efficient screening. Because the number of cfus can be calculated from only a 5 µl spot of phage-infected *E.coli* cells in a grid, the antiviral effect of materials under 36 test conditions can be assessed with a single TYE plate. Given that the typical plaque assay using bacteriophage Qβ require one dish for one condition \(^9\), our new screening method is 36 times more efficient.

Previous studies have shown that different antimicrobial materials have distinct antiviral activities\(^1\), likely due to differences in the composition of the viral surface. For example, Ly-Chatain et al. reported that the antiviral effect of cationic compounds on bacteriophages depended greatly on both the type of bacteriophages and the structure of antiviral compounds \(^11\). Therefore, it will be valuable to test the antiviral activity of novel antimicrobial materials against different kinds of viruses to develop materials that can protect against a broad spectrum a viruses.

In this study, we used the filamentous phage M13, which has five kinds of coat proteins. This filamentous phage has not been used to evaluate antimicrobial effects, perhaps because it does not cause bacteriolysis following infection and thus is not applicable to typical plaque assays. We took advantage of this property of M13 and used it together with a phagemid to develop an effective high-throughput system for screening potential antiviral materials. This new system enable more rapid screening than typical screening methods and thus should accelerate the development of novel antiviral
Materials.

Methods

Preparation of M13 bacteriophages

Phage stocks were prepared as described previously. Briefly, the *E. coli* stock containing an antibody library was grown in broth and infected with helper phages. After overnight culture, phages were purified by polyethylene glycol precipitation and dissolved in SM buffer (10 mM Tris-HCl pH 7.5 100 mM NaCl 8 mM MgSO$_4$). Purified phages were stored at 4°C.

Evaluation of phage inactivation by anti-viral materials

Figure 2 shows a schematic of the experiment using the M13 bacteriophage. We inoculated 50 µL of the M13 phage suspension onto glass slides (50 mm × 50 mm) coated with the antiviral reagents or a blank glass slide as the control and covered them with transparent polypropylene film (40 mm × 40 mm). After overnight incubation at room temperature under fluorescent lamp illumination (500 lx), we collected M13 phages by rinsing the glass slides with 5 ml of phosphate buffered saline and made a series of 1/10 dilutions. The eluted and serially diluted phages were mixed with *E.coli* strain XL-1 Blue cells at OD$_{600} = 0.4–0.6$ and incubated in water bath at 37°C for 30 min. Following incubation, 5 µL of each dilution were spotted onto grids on TYE plates containing ampicillin. Following overnight incubation at 37°C, we counted the number of colonies in grids and calculated the number
of active phages. For time course experiment, we collected M13 bacteriophage from the slides at each
time point followed by making serial dilution and *E.coli* infection.
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Author contributions

M.N. and K.T. conceived and designed this study. M.N, N. T., A. S., and N. M. performed the experiments and analyzed the data. M. N., N. T., and A. S wrote the manuscript and all authors approved it.

Competing of interests

This study was funded by Nippon Paint Holdings, and N. T., A. S., and N. M. are employees of this company.
**Figure 1** Schematic figure showing introduction of the phagemid upon infection. *E. coli* infected by M13 phages acquire antibiotic resistance derived from the phagemid vector.
Figure 2 Schematic of the experiment using the M13 bacteriophage to evaluate the effects of antiviral materials.
Figure 3 Validation of phage inactivation using commercially available antivirus products. (A) Representative picture of the TYE plate in which phage-infected *E. coli* cells were spotted. The dynamic range was set as $10^{-3}$ to $10^{-8}$ dilution in this experiment. (B, C) The calculated numbers of active phages after treatment with (B) PROTECTON VK-500 and (C) PROTECTON BARRIERX Spray.
Figure 4 Time course experiment using PROTECTON BARRIERX Spray. Phages were collected at 10, 30, 60, 180, and 1440 min time points and active phage number was calculated.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- M13basedscreeningsupplementary210930final.pdf