Comparison of spike and aerosol challenge tests for the recovery of viable influenza virus from non-woven fabrics

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Background To experimentally determine the survival kinetics of influenza virus on personal protective equipment (PPE) and to evaluate the risk of virus transfer from PPE, it is important to compare the effects on virus recovery of the method used to contaminate the PPE with virus and the type of eluent used to recover it.

Methods Avian influenza virus (AIV) was applied as a liquid suspension (spike test) and as an aerosol to three types of non-woven fabrics [polypropylene (PP), polyester (PET), and polyamide (Nylon)] that are commonly used in the manufacture of PPE. This was followed by virus recovery using eight different eluents (phosphate-buffered saline, minimum essential medium, and 1·5% or 3·0% beef extract at pH 7, 8, or 9).

Results For spike tests, no statistically significant difference was found in virus recovery using any of the eluents tested. Hydrophobic surfaces (PP and PET) yielded higher spiked virus recovery than hydrophilic Nylon. From all materials, the virus recovery was much lower in aerosol challenge tests than in spike tests.

Conclusions Significant differences were found in the recovery of viable AIV from non-woven fabrics between spike and aerosol challenge tests. The findings of this study demonstrate the need for realistic aerosol challenge tests rather than liquid spike tests in studies of virus survival on surfaces where airborne transmission of influenza virus may get involved.

Keywords Aerosol, influenza virus, non-woven fabrics, personal protective equipment, spike.

Introduction

The spread of influenza is of increasing concern due to its pandemic potential. Influenza virus can be transmitted via contact, large droplets, and aerosol routes. The relative contribution of different routes is still unknown and is under debate. Some researchers[1] believe that large droplet transmission is the dominant route, while others[2,3] argue in favor of aerosols. That the aerosol route is an important mode of transmission is clear from recent studies.[4–9]

One common way to reduce the spread of influenza is to use personal protective equipment (PPE) such as face masks, respirators, and garments, which are often made of non-woven fabrics. However, there is concern that influenza virus may survive on PPE long enough to render used PPE into vehicles for virus transmission. The virus could be easily transferred from contaminated PPE to the skin when the PPE is removed from healthcare workers,[10] which may greatly increase the risk of contact transmission. It has also been documented that influenza virus can survive on a wide variety of surfaces including non-woven fabrics.[11–15]

The above-mentioned studies utilized spiking with virus as a challenge method; that is to apply a known concentration of virus suspension onto surfaces of interest followed by virus elution. The comparative efficacy of different eluents used in these studies has not been investigated. One reason for poor virus recovery from porous surfaces is believed to be inefficient elution of the virus.[12] Therefore, in studies on virus survival on PPE and their subsequent transfer from PPE, an optimum eluent for virus recovery should be found by comparing the recovery efficiency of different eluents.

Another limitation of the previous studies is that the test methods relying on virus spike tests may not sufficiently mimic the real-life situation of aerosol or large droplet transmission of influenza, although they may serve as good proxies to simulate contamination of fomites. As reviewed by Gralton *et al.*,[16] breathing, coughing, sneezing, and talking can easily generate particles of a wide size range, and influenza virus has been detected in the particles generated by human respiratory activities.[8,17,18] Therefore, we investigated the difference in viable influenza virus recovery from PPE

after liquid suspension spike tests versus aerosol challenge tests.

The objectives of this study were (i) to evaluate the efficiency of eight eluents for recovering influenza virus from three non-woven fabrics commonly used to manufacture PPE and (ii) to compare influenza virus recovery from non-woven fabrics spiked with virus suspension versus loaded with virus aerosol.

Methods

Non-woven fabrics

Three non-woven fabrics were tested (Table 1), for example, polypropylene (PP), polyester (PET), and polyamide (Nylon) manufactured by First Quality Enterprises (style SB3396018), Fiberweb (style Reemay 2214), and Midwest Filtration (style PBN-II 100), respectively. All three non-wovens were made by a spunbond process with basis weight from 33·9 to 46 g/m^2. Spunbond non-woven fabrics in this basis weight range are typically used as the outer layer of respirators where a significant amount of aerosolized virus can deposit.[19] Both hydrophobic (PP and PET) and hydrophilic surfaces (Nylon) were included in this study.

Cells and virus

Subtype H9N9 [A/chicken/Maryland/2007(H9N9)] of avian influenza virus (AIV) was grown in Madin-Darby canine kidney (MDCK) cells. Although this virus may not be very close to current circulating viruses, it is not pathogenic to humans and shares many characteristics of human influenza viruses. Therefore, it was selected as a surrogate in this study. The cells were grown in minimal essential medium (MEM) supplemented with 8% fetal bovine serum (FBS), gentamicin (50 µl/ml), neomycin sulfate (15 000 units/ml), penicillin G (75757 i.u./ml), streptomycin sulfate (455 µg/ml), and amphotericin B (5·6 µg/ml). Cell monolayers were washed three times with Hanks' balanced salt solution (HBSS) containing trypsin followed by inoculation with AIV sus-

pension at a multiplicity of infection of 0·1. After incubation for 60 min at 37°C in a CO_2 incubator, maintenance medium (MEM with trypsin) was added followed by incubation for five more days. After three freeze-thaw cycles, the infected cell culture fluid was centrifuged at 2000 × g for 30 min, and the partially purified virus stock was aliquoted and stored at −80°C until used.

Liquid suspension spike tests

For the spike tests, each sample of non-woven fabric was cut into 1 cm by 1 cm squares using ethanol-treated scissors. The pieces were then placed in a sterile 24-well plate with one square per well. Each square was contaminated with 20 µl of AIV suspension applied as a single droplet. The contaminated fabrics were then placed in a biosafety cabinet to be air-dried for different time periods (drying time) before the virus was eluted and recovered using different eluents.

Three drying times were used for each type of fabric. The first drying time was 0 min, which means AIV was eluted immediately after the virus suspension was applied. The second drying time represented the time when the applied virus suspension droplets evaporated and became air-dried, which depends on the surface characteristics of the non-woven fabrics, ambient temperature, and humidity. The hydrophobic PP required ~95 min. For the less hydrophobic PET and the hydrophilic Nylon, the time was about 75 and 30 min, respectively. The third drying time was set to be 30 min after the second drying time, which was 125, 105, and 60 min for PP, PET, and Nylon, respectively. The second and third drying times were chosen to mimic a) the environmental conditions experienced by airborne virus in an aerosol challenge test where the virus is believed to be attached and/or encased in droplet nuclei with less surrounding water than the virus in a liquid suspension and b) the real situation when virus recovery may be performed one hour after PPE is contaminated through hand contact and/or by aerosolized particles.

A total of eight eluents were compared for their performance in recovering AIV from non-woven fabrics: phosphate-buffered saline (PBS), Eagle's minimum essential medium (MEM), and 1·5% beef extract-0·05 M glycine solution (BE) at pH 7, 8, or 9, and 3·0% BE at pH 7, 8, or 9. PBS, MEM, and BE were chosen because all have been used to recover influenza virus from various surfaces including PPE and other fabrics.[12–15] In addition, the effect of concentration and pH of BE on virus recovery efficiency was evaluated because organic matter and alkaline pH tend to reduce virus adsorption to surfaces,[20] thus assisting virus recovery.

To recover the spiked virus, 1 ml eluent was placed on each piece of fabric in the same plate at the given drying time followed by orbital shaking at 125 RPM for 2 min (Hybri-tech, San Diego, CA, USA). Immediately after elution, the

Table 1. Non-woven fabrics used for influenza virus recovery

Fabric material	Basis weight (g/m^2)	Isoelectric point	Reference(s)	Surface characteristics
Polypropylene (PP)	33·9	2·9–3·8	[36]	Hydrophobic
Polyester (PET)	46	2·3–2·5	[37]	Hydrophobic
Polyamide (Nylon)	34	5·2–6·9	[37,38]	Hydrophilic and can hydrogen bond

eluates were transferred to 1·5-ml Eppendorf tubes and were stored at −80°C until virus infectivity assays. As positive control, AIV was spiked into the eluent without the presence of a non-woven fabric and recovered in the same way. Briefly, 20 μl AIV was spiked into 1 ml eluent, which was contained in one well of the 24-well plate. After the specified drying time, the virus sample was agitated at 125 RPM for 2 min and frozen at −80°C until infectivity assay. The "non-fabric" positive control took into account the natural decay of virus viability at room temperature, the potential virus inactivation by the agitation method and the high pH of the eluents. Negative control was also maintained by adding 1 ml of each eluent to a sample of each non-woven fabric. All tests were performed in triplicate.

Aerosol challenge tests

The test tunnel (Figure 1) as well as the methods for virus aerosol generation and deposition are similar to the protocol described in ASTM E2720-10 standard test method.[21] Pieces of each non-woven fabric (7 cm by 7 cm square) were cut and sealed using adhesive tape to a plexiglas plate with a circular hole (diameter of 5·64 cm) in the center, which was then inserted into the tunnel and held by two gasketed chucks controlled by a pneumatic motor.

Avian influenza virus was aerosolized using a 6-jet Collison nebulizer (BGI, Waltham, MA, USA) operated at 10 psig. The nebulizer suspension consisted of 49·5 ml undiluted virus stock, 0·1 ml antifoam (Sigma Chemical, St. Louis, MO, USA), and 0·5 ml uranine dye (0·025 g/ml), which is commonly used as a fluorescent particle tracer.[22] The generated virus aerosol had a count median diameter of ~60 nm with a geometric standard deviation of 2·2, as measured by a scanning mobility particle sizer (TSI, Shoreview, MN, USA). The initial titer of the virus used in the nebulizer ranged from $6·31 \times 10^4$ to $6·76 \times 10^5$ $TCID_{50}$/ml.

The generated virus aerosol was mixed and diluted with HEPA-filtered and relative humidity-controlled room air before challenging the non-woven fabric. For each test, the non-woven fabric was exposed to AIV aerosol for 15 min at a standard flow rate of 12·5 LPM. Meanwhile, an AGI-30 liquid impinger (Ace Glass, Vineland, NJ, USA) loaded with 20 mL collection liquid (the same liquid as used to elute aerosolized virus from the non-woven fabric) was used to sample the virus-laden particles upstream from the non-woven fabric at 12·5 LPM for 15 min. The temperature and relative humidity inside the test tunnel was 23–25°C and 30–40%, respectively.

To recover the challenged virus, contaminated non-woven fabric was removed from the plexiglas plate, cut into pieces, and submerged in 5 ml of eluent placed in a 15-ml centrifuge tube followed by vortexing (American Scientific Products, McGaw Park, IL, USA) for 1 min. Two samples were drawn from the non-woven fabric eluate and the collection liquid in the impinger, respectively. One was stored at −80°C until virus infectivity assay. The other was diluted in 0·01 mol/L NaOH, and the fluorescence signal was measured using a fluorometer (Sequoia-Turner, Mountain View, CA, USA).

The above tests were repeated at least in triplicate for each type of non-woven fabric using PBS and MEM and twice for 1·5% BE at pH of 7·0.

Virus infectivity assay

Each virus sample was briefly vortexed and serially diluted ten-fold in a 96-well plate using MEM with antibiotics,
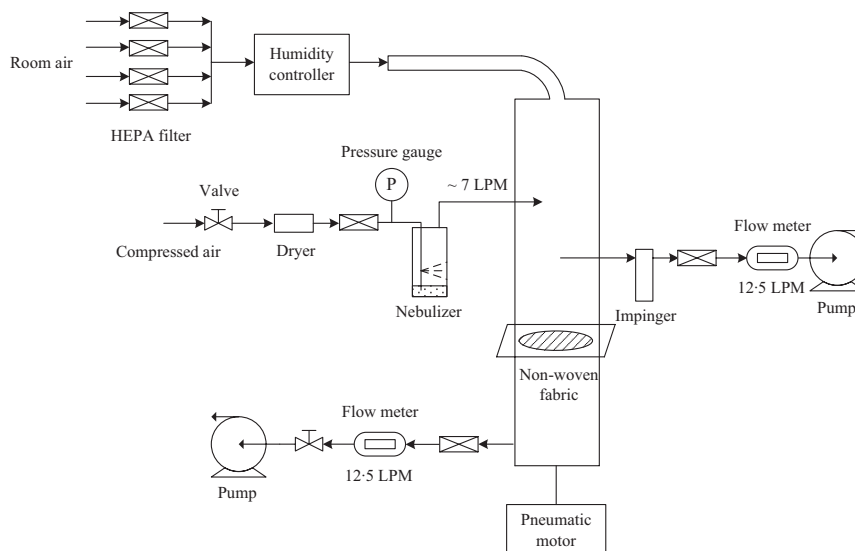

Figure 1. Schematic diagram of the experimental setup for virus aerosol challenge tests.

bovine serum albumin, and trypsin. 100 µl from each dilution was transferred to a 96-well plate with confluent MDCK monolayer cells, which were previously washed three times with HBSS containing trypsin and antibiotics. Plates were then incubated at 37°C in a humid chamber with 5% CO_2 for 5 days. After incubation, the plates were evaluated for cytopathic effects, and virus titers were calculated as 50% tissue culture infective dose ($TCID_{50}$/ml) using the Karber method.[23]

Data analysis

To determine the efficiency of different eluents for virus recovery, virus titers from the spike test, C_{spike}, were compared with the positive control, $C_{positive\ control}$, and the recovery efficiency was calculated as:

$$\text{Recovery efficiency}(\%) = 100 \times \frac{C_{spike}}{C_{positive\ control}} \qquad (1)$$

Similar recovery efficiency for the aerosol tests is not as straightforward to determine, because it is difficult to quantify the amount of virus captured by the non-woven fabric. To solve this problem, relative recovery was used as an alternative, which is defined as:

$$\text{Relative recovery}(\%) = 100 \times \frac{C_{fab}}{\frac{C_{imp}}{FS_{imp}} \times FS_{fab}}$$
$$= 100 \times \frac{C_{fab}/FS_{fab}}{C_{imp}/FS_{imp}} \qquad (2)$$

where the numerator is the ratio of virus titer, C_{fab} to fluorescence signal, FS_{fab} of the non-woven fabric eluate, while the denominator is the ratio of virus titer, C_{imp} to fluorescence signal, FS_{imp} in the collection liquid of the impinger. Relative recovery has been used to determine the biological collection efficiency of virus aerosol samplers.[24] Here, relative recovery is calculated as the ratio of the virus recovered from the non-woven fabric to the estimated amount of virus applied, assuming the amount of virus carried by an aerosol particle is proportional to the amount of fluorescence carried by the particle. As a particle tracer, the fluorescence signal represents the total particle volume collected by the liquid impinger/non-woven fabric. Therefore, the amount of virus captured by the non-woven fabric could be estimated based on a) the relative amount of particles collected by the non-woven fabric/liquid impinger and b) the relationship between the airborne virus infectivity and the amount of particles (e.g., fluorescence signal) provided by the liquid impinger.

Data of virus recovery efficiency and relative recovery were statistically analyzed using multiple-way analysis of variance (ANOVA) in MATLAB 7·1 (MathWorks, Natick, MA, USA).

Results

Recovery of AIV after spike tests

Titers of AIV used as "non-fabric" positive control for the spike tests ranged from $2\cdot63 \times 10^4$ to $2\cdot45 \times 10^5$ $TCID_{50}$/ml, which were found independent of the type of eluent ($P = 0\cdot625$) and drying time ($P = 0\cdot549$) by two-way ANOVA using the type of eluent and drying time as two factors. These data suggest no virucidal effects are caused by the eluents (e.g., high pH) within the time tested. No live virus was detected from negative control as expected.

Figure 2 represents the recovery efficiency of AIV after spike tests as a function of eluent, non-woven fabric, and drying time. In most cases, recovery efficiency was less than 100% and decreased with increasing drying time. Generally, virus recovery was the highest for PP, followed by PET, and then Nylon. Three-way ANOVA using the type of eluents, type of non-woven fabrics, and drying time as three factors indicated that there was no statistically significant difference in virus recoveries from any of the eight eluents ($P = 0\cdot232$). However, both drying time ($P < 0\cdot001$) and the type of non-woven material ($P < 0\cdot001$) had a significant effect on virus recovery.

Recovery of AIV after aerosol challenge tests

Three eluents with relatively high recovery efficiency in the spike tests (PBS, MEM, and 1·5% BE at pH 7) were selected for recovering AIV after aerosol challenge tests. As shown in Figure 3, relative recovery of AIV varied widely (0·22–4·42%) with 1·5% BE at pH 7 giving the highest average recovery, followed by MEM and PBS. Two-way ANOVA using the type of eluent and non-woven material as two factors suggested that the latter ($P = 0\cdot651$) did not significantly affect the recovery of AIV, while eluent ($P = 0\cdot053$) had a borderline effect, probably due to the relatively low recovery by PBS.

Virus recovery in the spike tests was not reproduced by the aerosol tests. As shown in Table 2, the average recovery of AIV after the aerosol challenge tests was much lower than after the spike tests at all three drying times for each pair of tested eluent and non-woven fabric. Note the wide variation between replicates, indicated by the large standard deviations. Generally, except for some cases where large data variability existed, one-way ANOVA suggests the recovery after aerosol tests versus spike tests was significantly different ($P < 0\cdot05$).

Discussion

The recovery efficiency of frequently used eluents[12–15] was systematically evaluated using spike tests. Although no statistically significant differences were observed in the
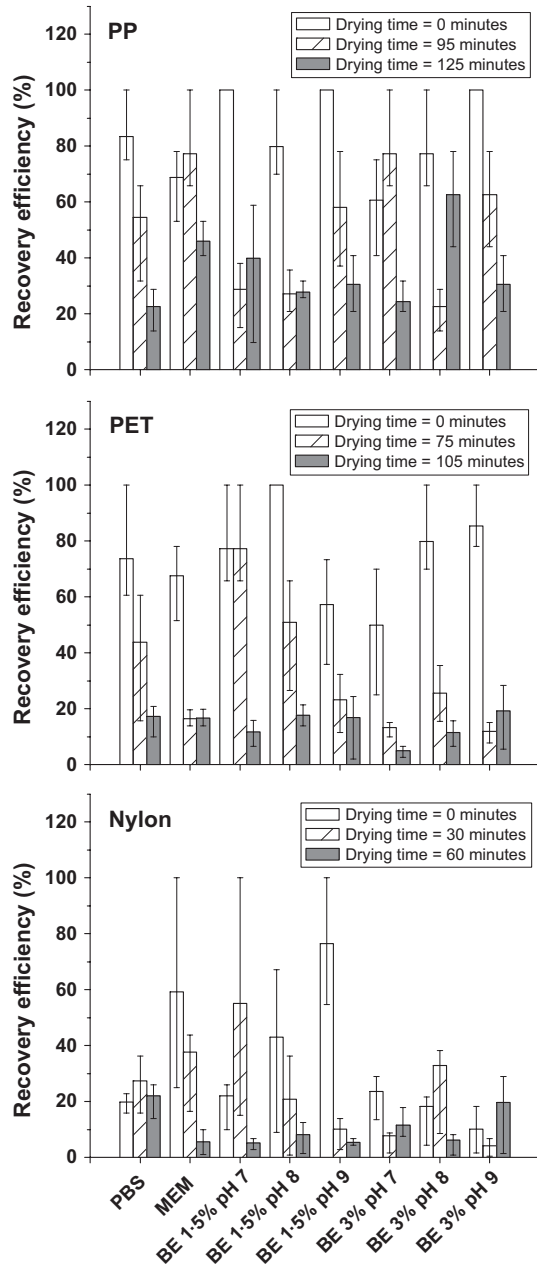
Figure 2. Recovery efficiency of avian influenza virus (AIV) after spike tests for polypropylene (PP), polyester (PET), and Nylon as a function of eluent type and drying time. Each bar represents the mean with 25th and 75th percentiles for three experimental runs.

Figure 3. Relative recovery of AIV from PP, PET, and Nylon after aerosol challenge tests using three eluents. Each bar represents the mean ± one standard deviation for at least three experimental runs for PBS and MEM and two experimental runs for BE 1·5% pH 7.

recovery efficiency of the eight eluents, the type of non-woven material (e.g., hydrophobic or hydrophilic) was found to be a major factor influencing recovery of AIV, which should be noted in future studies involving the recovery of spiked virus from surfaces.

Virus recovery efficiency of spike tests can be best viewed by looking at recovery data at the drying time of 0 min with recovery less than 100% in most cases. Similar results were
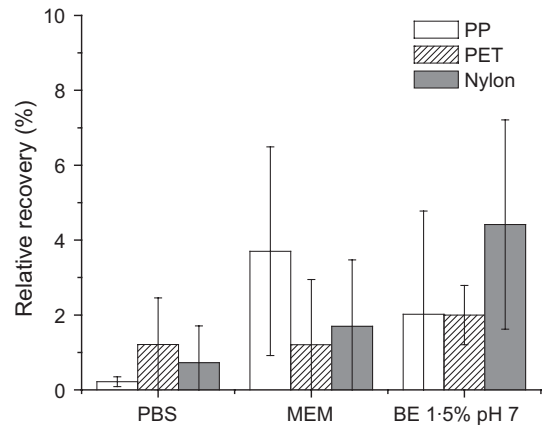
previously reported,[25] showing only 10% of the applied influenza virus recovered from a serge before drying took place. With minimum virus inactivation by desiccation at zero drying time, any decrease of virus titer could be attributed to virus adsorption onto the non-woven fabrics. One recent study[26] found that the recovery efficiency of H5N1 influenza virus spiked onto a polypropylene respirator was 70 ± 5% as determined by quantitative PCR, further suggesting that virus adsorption onto surfaces could affect virus recovery.

Virus adsorption onto surfaces can be reduced using electrostatic repulsion, which depends on the isoelectric point (IEP) of the virus and the surface as well as pH of the eluent.[20] The IEP for the three non-woven fabrics tested is listed in Table 1 and that of influenza virus ranges from 4·6 to 5·4.[27] Therefore, at eluent pH of 7·0 to 9·0, both the non-woven fabric and the influenza virus carried net negative surface charges and repelled each other, aiding virus recovery from the surfaces. Theoretically, there may be an increase in repulsive force with increased difference between IEP of virus or non-woven fabric and the eluent pH. However, the monotonic increase of recovery efficiency with increased pH was only observed for PP and PET using 3% BE and for Nylon using 1·5% BE at a drying time of 0 min. The high IEP of Nylon may partially explain the low recovery efficiency of spiked virus compared with PP and PET.

Hydrophobic interaction was expected to be dominant in the attachment of lipid-containing virus (e.g., influenza virus) onto hydrophobic surfaces (e.g., PP and PET). However, the hydrophobic fabrics gave a much higher virus recovery than hydrophilic Nylon, which agrees with the work of Sakaguchi et al.[15] The AIV suspension formed droplets when applied to hydrophobic PP and PET surfaces with a small contact area between the virus and fibers while it easily

Table 2. Comparison of avian influenza virus (AIV) recovery (%) from polypropylene (PP), polyester (PET), and Nylon using different eluents after aerosol challenge tests and spike tests with different drying times. Values are the means ± 1 standard deviation for at least three experimental runs for PBS and minimal essential medium (MEM) and two experimental runs for BE 1·5% pH 7. There is statistically significant difference between virus recovery after aerosol tests and spike tests ($P < 0.05$) unless asterisked

		PBS			MEM			BE 1·5% pH 7		
		PP	PET	Nylon	PP	PET	Nylon	PP	PET	Nylon
Aerosol test		0·22 ± 0·13	1·21 ± 1·25	0·72 ± 0·98	3·70 ± 2·79	1·21 ± 1·74	1·70 ± 1·77	2·0 ± 2·8	2·0 ± 0·8	4·4 ± 2·8
Spike test	Drying time 1	83·4 ± 28·8	73·8 ± 45·4	19·8 ± 8·94	68·8 ± 27·2	67·7 ± 28·4	59·2 ± 48·4	100·0 ± 0·0	77·2 ± 39·5	22·0 ± 19·1*
	Drying time 2	54·4 ± 39·5	43·8 ± 49·0	27·4 ± 17·6	77·2 ± 39·5	16·4 ± 5·8	37·7 ± 42·4	28·7 ± 24·3*	77·2 ± 39·5	55·1 ± 52·4*
	Drying time 3	22·5 ± 15·5	17·2 ± 12·5	22·0 ± 20·1	46·0 ± 12·8	16·6 ± 6·0	5·5 ± 5·2*	39·9 ± 52·7*	11·7 ± 9·5*	5·2 ± 3·5*

*No statistically significant difference between the recovery after aerosol tests and spike tests.

spread and soaked into the Nylon fabrics with a much larger contact area and a higher probability for virus adsorption onto the fibers, consequently lowering the recovery efficiency. Dissolved organic matter such as protein tends to reduce hydrophobic interaction.[20] However, the increase of BE from 1·5% to 3% did not increase virus recovery, especially for Nylon (Figure 2). Increased BE concentration was also found to yield a lower recovery of MS2 bacteriophage[28] and the mechanisms behind it are not clear.

A significant decrease in recovery with increased drying time might be due to a combined effect of inefficient virus removal from non-woven fabric and inactivation of virus by desiccation. The wind draughts in the biosafety cabinet might also result in virus loss from the fabric surface to the cabinet air, especially after long drying time. It is possible that virus was brought closer to the non-woven surface and became more easily adsorbed onto the fabric as the virus suspension evaporated (as in the case of Nylon at a drying time of 0 min), thus reducing virus recovery. On the other hand, inactivation of influenza virus on surfaces by desiccation[29] is well known. The stress-sensitive nature of influenza virus makes it difficult to determine whether the physical removal of viable virus from non-wovens is a function of drying time.

An optimum eluent to recover virus from PPE should dislodge virus effectively from the surface and help maintain virus viability once it gets removed. Optimum eluents could be designed for each specific virus-surface pair by adjusting the eluent pH and concentration of organic composition. Adding surfactants such as NaPP and Tween 80 may help by minimizing hydrophobic interactions.[30] In addition, inclusion of chaotropic and monovalent salts in the eluent can also promote virus recovery by decreasing the ordering of water molecules.[20] Certain PPE such as N95 respirators generally contains electrostatically charged (electret) fabrics, which may enhance virus adsorption onto surfaces. Ethanol has been used to recover virus from electret filter media,[17] because it can degrade the charge of fabrics and help elute more virus. However, it should be noted that electret-containing PPE may become less effective after ethanol treatment.

Conventional protocols that use spike tests as a virus challenge method may at best simulate fomite and contact transmission of influenza. In this study, AIV was applied to non-woven fabrics in the form of aerosols, which better simulated the airborne transmission of influenza. Although neither the type of eluent nor the non-woven fabric was found to significantly affect virus recovery, the results indicated that AIV recovery significantly depended on how the virus was applied (Table 2). Recovery of aerosolized virus was much more difficult than that of spiked virus, suggesting spike tests cannot be simply taken as an approximation for aerosol challenge tests in studies of virus survival on surfaces

where airborne transmission of influenza virus may get involved.

The low recovery of aerosolized influenza virus from non-woven fabrics is comparable to a 3·2% recovery from respirators found in another study.[31] Compared to spike tests, the lower recovery found in aerosol tests could be due to the poorer survivability of influenza virus in aerosol particles than in liquid suspension.[2,32] Another reason could be the inefficient physical removal of virus from non-wovens. The generated submicron virus aerosol particles could easily diffuse and be deposited deep into the non-woven fabric layer, making them difficult to recover. For example, electron microscopy analysis has shown that many particles remained on filter fibers after extraction by vortexing.[33] This may explain why there was no significant difference in recovery between hydrophobic and hydrophilic surfaces as seen in the spike tests.

One of the limitations of this study is that the agitation method used in the spike tests (orbital shaking) was different from that used in the aerosol challenge tests (vortexing). The different amplitude and frequency of the agitation method may cause different relative acceleration motion between eluent and non-woven fabrics, yielding different recovery results. Nevertheless, Fisher et al[19] found that sonication, vortexing, and shaking exhibited similar efficiency and repeatability for extracting aerosolized virus from respirator coupons, suggesting agitation methods gave minimal difference in virus recovery efficiency. Second, volume of the eluent and area of the non-woven fabric used in aerosol challenge tests were larger than spike tests (e.g., 49 cm^2 versus 1 cm^2 and 5 ml versus 1 ml), leading to different volume to area ratios in the two tests (~0·1 ml/cm^2 versus 1·0 ml/cm^2). A low volume to area ratio might result in insufficient mixing/elution and yield lower virus recovery in the aerosol challenge tests. Both the area of the fabric and the volume to area ratio should be standardized across the two tests to enable better comparison. Third, temperature and humidity during the spike tests should have been controlled, because they may affect the determination of drying time.

One should be cautious to generalize the experimental results to real-life situations. First, the nebulizer suspension tested was different from the suspending environment for the naturally aerosolized virus. In reality, virus may be encased within particles containing mucin and other respiratory excretion substances, the presence of which has been demonstrated to extend the survival of influenza virus[14] and probably gives higher virus recovery. Second, PPE in real life could be contaminated by both contact and aerosolized particles, and therefore, the actual virus recovery may fall between what determined from the spike and the aerosol tests. Third, only one layer of fabric was tested while PPE could be made of multiple layers of electret fabrics, which may significantly affect virus recovery. Fourth, the drying

time and challenge time tested might be short compared with clinical settings, where PPE may be worn for up to three hours. Therefore, the virus recovery at the time of PPE doffing (when virus transmission most likely to happen) may be different from what determined in this study. Last, although low virus recovery was found in the aerosol tests, in real life all used PPE should be regarded as contaminated and be removed appropriately.

For future studies, it will be helpful to use quantitative RT-PCR to measure total (both viable and non-viable) virus recovered from non-wovens, given the fact that infectious influenza virus is rarely found in natural environments due to its extremely low concentration. Although RT-PCR provides no indication of virus infectivity, it has been found to give a higher rate of virus detection than culture methods.[17,18,34,35] RT-PCR can be used combined with culture methods to help differentiate the relative contribution to recovery efficiency by the inefficient physical removal of virus from the non-wovens and by the natural decay of virus infectivity (e.g., desiccation). With the virus recovery efficiency issue sorted out, it will be interesting to investigate the potential difference in influenza virus survival kinetics on PPE when it is applied as a liquid suspension versus an aerosol.

Acknowledgements

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