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How does breathing frequency affect the performance of an N95 filtering facepiece respirator and a surgical mask against surrogates of viral particles?

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Abstract

Background: Breathing frequency (breaths/min) differs among individuals and levels of physical activity. Particles enter respirators through two principle penetration pathways: faceseal leakage and filter penetration. However, it is unknown how breathing frequency affects the overall performance of N95 filtering facepiece respirators (FFRs) and surgical masks (SMs) against viral particles, as well as other health-relevant submicrometer particles.

Methods: A FFR and SM were tested on a breathing manikin at four mean inspiratory flows (MIFs) (15, 30, 55 and 85 L/min) and five breathing frequencies (10, 15, 20, 25 and 30 breaths/min). Filter penetration (P_{filter}) and total inward leakage (TIL) were determined for the tested respiratory protection devices against sodium chloride (NaCl) aerosol particles in the size range of 20 to 500 nm. “Faceseal leakage-to-filter” (*FLTF*) penetration ratios were calculated.

Results: Both MIF and breathing frequency showed significant effects ($p<0.05$) on P_{filter} and TIL. Increasing breathing frequency increased TIL for the N95 FFR whereas no clear trends were observed for the SM. Increasing MIF increased P_{filter} and decreased TIL resulting in decreasing *FLTF* ratio. Most of *FLTF* ratios were >1, suggesting that the faceseal leakage was the primary particle penetration pathway at various breathing frequencies.

Conclusions: Breathing frequency is another factor (besides MIF) that can significantly affect the performance of N95 FFRs, with higher breathing frequencies increasing TIL. No consistent trend of increase or decrease of TIL with either MIF or breathing frequency was observed for the tested SM. To potentially extend these findings beyond the manikin/breathing system used, future studies are needed to fully understand the mechanism causing the breathing frequency effect on the performance of respiratory protection devices on human subjects.

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Keywords

breathing frequency; N95; respirator; surgical mask; manikin

INTRODUCTION

The National Institute for Occupational Safety and Health (NIOSH) certified N95 filtering facepiece respirators (FFRs) are widely used in various occupational environments to reduce the workers' exposure to hazardous aerosols. In healthcare environments, N95 FFRs and surgical masks (SMs) are the most commonly used devices to prevent transmission of infectious diseases.[1, 2] N95 FFRs are certified by NIOSH in accordance with Title 42 of the Code of Federal Regulations.[3] The letter 'N' stands for non-oil-resistance, and the number '95' denotes the filter efficiency of at least 95% when the filter is challenged with NaCl aerosols having a mass median aerodynamic particle diameter of 300 nm (the most penetrating particle size, MPPS, for mechanical filters) at a constant flow of 85 L/min.[4] Presently, the vast majority of FFRs are manufactured utilizing electrostatic fibers, which feature much smaller MPPS: 30 to 100 nm.[5–12] The latter range includes many viral species. SMs are not subject to NIOSH filter certification approval; instead they are regulated by the US Food and Drug Administration (FDA). Previous studies have shown that the filter efficiency for SMs is much lower than that for N95 FFRs.[5, 13–15]

Besides filter penetration, faceseal leakage can have a significant impact on the performance of N95 FFRs and SMs. One study showed that the efficiency of N95 FFRs was high when sealed to a manikin headform but decreased significantly due to faceseal leakage when the same respirators were tested on human subjects.[16] NIOSH has proposed total inward leakage (TIL) testing to assess respirator performance since it takes into account both penetration pathways.[17] Grinshpun and colleagues quantified the relative contributions of the two pathways for an N95 FFR and a SM by determining the filter penetration (P_{filter}) and faceseal leakage penetration ($P_{leakage}$) using manikin-based and human subject-based experimental protocols.[13] The "faceseal leakage-to-filter" ratio ($FLTF = P_{leakage}/P_{filter}$) was > 1, indicating a greater number of particles penetrated through faceseal leaks than the filter media.[13] While the quoted study addressed a wide range of particle sizes (30 – 1000 nm), it did not examine breathing frequency.

Exposure to viral particles is best characterized by the number or volume of inhaled particles rather than mass concentration[18, 19] Viral airborne particles (known as virions) are generally much smaller than airborne bacteria. Most naked viruses referenced in the literature are between 20 and 300 nm in diameter.[20] For instance, coronavirus, the causative agent of severe acute respiratory syndrome (SARS), has a primary physical size ranging from 80 to 140 nm; the avian influenza virus (H5N1 and H1N1) is between 80 to 120 nm.[21, 22] The size range of aerosol particles containing viruses (often referred to as "carriers") found in an occupational setting is substantially larger as they may consist of respiratory secretions, dead cells, mucous, etc., in addition to single viruses or virus aggregates.[23–25] However, particles < 500 nm are capable of penetrating deeper in the respiratory tract during inhalation; this range also includes particles featuring the highest penetration through most of respiratory protection devices and filters. In addition, the differences in filtration performance between surgical masks and FFRs are less noticeable for the particles of around 1 μm or larger.[5, 13, 14] Therefore, this study was focused on particle sizes of < 500 nm.

The Institute of Medicine estimates that during an influenza pandemic, more than 13 million healthcare workers, patients, family members and friends may need respiratory protection devices to protect them from receiving or spreading infectious illness.[26] Among population groups (e.g., young vs. old, small vs. large, healthy vs. sick), breathing frequency (breaths/min) differs and will differ significantly with level of physical activity (e.g., at rest vs. active).[27, 28] In addition, studies examined the physiological impact of respirators on healthcare workers, reported that wearing a FFR did not impose any important physiological burden during one hour of use, at realistic clinical work rates (16 – 27 breaths/min at low to moderate work rates); the use of a surgical mask over the same period at a low-to-moderate work rate was not associated with clinically significant physiological impact or significant subjective perceptions of exertion or heat.[29–31] Human breathing has a cyclic flow pattern, which is primarily determined by mean inspiratory flow (MIF, L/min) and breathing frequency (breaths/min). Unlike the constant flow regime with a fixed flow rate, the cyclic regime features a constantly changing flow that depends on the level of the breathing frequency. Various studies have addressed the effect of flow rate on filter efficiency and faceseal leakage.[32–35] However, with the exception of our recent study in which an elastomeric half-mask respirator was tested,[36] no published study has evaluated the effect of breathing frequency on the performance of N95 FFRs and SMs when both filter and faceseal leakage penetration pathways are present.

Although the nature of an inert aerosol (e.g., NaCl) differs from that of bioaerosols, several studies have confirmed that filter performance against biological particles is consistent with that determined using non-biological particles of the same size.[5, 7, 16] This suggests that inert aerosol surrogates such as NaCl particles may generally be appropriate for predicting penetration of similarly sized virions. The present manikin-based study addresses the effects of breathing frequency and flow rate on the filter efficiency and faceseal leakage of an N95 FFR and a SM challenged with NaCl particles (20 – 500 nm), which represent many viral species, as well as other health-relevant particles (e.g., combustion generated or engineered nanoparticles). The tested N95 FFR/SM was sealed to plastic manikin headform to investigate filter performance. It was also donned without sealing to a different advanced manikin headform to quantify TIL. The advanced manikin utilized in this study was recently developed to mimic the properties of the human face.[37, 38] Faceseal leakage represents the difference between TIL and filter penetration. The hypothesis of this study was that the P_{filter} as well as the TIL of FFRs and SMs are generally affected by MIF and breathing frequency.

MATERIALS AND METHODS

Tested N95 FFR and Surgical Mask

One N95 FFR and one SM were chosen for the study. Both models are commercially available and widely used in healthcare environments. The model of the N95 FFR was identical to the one tested in our previous studies.[5, 6] It has three principle layers with the middle layer composed of electrically charged polypropylene fibers to enhance filter capture efficiency.[5] The selected SM, according to the manufacturer, is fluid resistant and capable of providing at least 95% filter efficiency for 100 nm particles (not charge-neutralized).

For filter efficiency testing, the FFR/SM was sealed to the face of a hard plastic manikin headform. For the TIL tests, it was donned on an advanced manikin headform according to the FFR/SM manufacturer's user instruction. After 20 tests, the tested FFR/SM was removed from the manikin and replaced with a new one to minimize the effect of NaCl loading on the filter media.

Challenge Aerosol

To produce the challenge agent (NaCl), a liquid salt solution was aerosolized using a particle generator (Model: 8026, TSI Inc., Shoreview, MN) and charge-equilibrated by passing through a ^{85}Kr electrical charge equilibrator (Model: 3054, TSI Inc., Shoreview, MN) prior to being released inside the test chamber. Before each experiment, the particle generator operated for at least one hour to achieve a uniform NaCl concentration in the chamber; it continued operating during the testing to maintain a stable particle concentration level. The challenge aerosol was log normally distributed with a size range of 20 – 500 nm, a count geometric mean of 125.4 nm, and a geometric standard deviation of 1.68 as measured with a Nanoparticle Spectrometer (Nano-ID NPS500, Naneum Ltd., Kent, UK). This size range covers the size of individual and aggregate virus particles. The NaCl concentration inside the challenge chamber ranged from 30,000 to 60,000 particles/cm^3 (such high ambient level was chosen to assure that enough particles would be detected inside the respirator).

Experimental Design and Test Conditions

Experiments were carried out in a room-size (24.3 m^3) test chamber described in recent studies.[36, 39] Temperature and relative humidity inside the chamber were kept at 17–22 °C and 30–60 %, respectively. The headform was connected to a Breathing Recording and Simulation System (BRSS, Koken Ltd., Tokyo, Japan) with a HEPA filter placed in-between to keep particles from re-entering the respirator cavity during exhalation cycles. Details regarding the BRSS are described in our previous studies.[34, 36, 39, 40]

The experiments were conducted at four cyclic breathing flows (MIF =15, 30, 55 and 85 L/min) and five breathing frequencies (10, 15, 20, 25 and 30 breaths/min). Completely randomized factorial design was implemented for the breathing frequency and flow rate with three replicates. Particle size-independent (overall) concentrations inside and outside the FFR/SM were obtained using a condensation particle counter (Model: 3007, TSI Inc., Shoreview, MN) having a total sampling time of 3 min with a time resolution of 1 sec.

Filter Penetration (P_{filter}) Test—The filter penetration (P_{filter}) was determined as the ratio of concentrations inside [$C_{in\_(Sealed)}$] and outside [$C_{out\_(Sealed)}$] of the FFR/SM sealed to the plastic headform:

$$P_{filter} = \frac{C_{in\_(Sealed)}}{C_{out\_(Sealed)}} \times 100\% \quad (1)$$

Total Inward Leakage (TIL) Test—For TIL, the same experimental protocol and test conditions were used except the FFR/SM was not sealed onto the advanced manikin headform. TIL values were determined as the ratio of concentrations inside [$C_{in\_(Donned)}$] and outside [$C_{out\_(Donned)}$] of the FFR/SM:

$$TIL = \frac{C_{in\_(Donned)}}{C_{out\_(Donned)}} \times 100\% \qquad (2)$$

Faceseal Leakage to Filter (FLTF) Ratio—The TIL test measures total penetration through the filter and faceseal leakage (TIL = P_{filter} + $P_{leakage}$). The *FLTF* ratio represents the relative contribution for each and was calculated as:

$$FLTF = \frac{P_{leakage}}{P_{filter}} = \frac{TIL - P_{filter}}{P_{filter}} \qquad (3)$$

In this study, the *FLTF* ratio was calculated using the average TIL and P_{filter} values over three replicates in order to identify the primary penetration pathway (leakage or filter penetration) for the entire particle size range of interest.

Data analysis

SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for data analysis. Normality of the data was checked prior to performing any statistical analyses. Two-way Analysis of Variance (ANOVA) was performed to analyze the effect of breathing frequency and flow rate on the filter penetration and TIL. All pairwise comparisons were conducted using Tukey's range test. *P*-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

1. N95 Filtering Facepiece Respirator

N95 Filter Penetration (P_{filter})—Filter penetration results for the N95 FFR are presented in Figure 1A. Filter penetration (P_{filter}) consistently increased with increasing MIF. This result can be explained by the differences in linear air velocities. Penetration of very small particles, which deposit on filter fibers primarily due to diffusion, increases with a decreasing residence time (also known as removal time). Thus, small particles are more likely to penetrate the filter at higher breathing flows. At the higher flows (MIF = 55 and 85 L/min), the P_{filter} curves are not flat, in contrast to those at the 15 and 30 L/min, suggesting that the effect of lower breathing frequencies are more pronounced at higher MIFs.

Two-way ANOVA performed on the P_{filter} data revealed that both the MIF and breathing frequency had a significant effect on filter penetration (p <0.0001, see Table I). Pairwise multiple comparison results (see Table I) show that the four MIFs produced four different P_{filter} groups with the highest mean P_{filter} (0.72%, Tukey grouping "A") occurring at the highest MIF of 85 L/min, and the lowest mean P_{filter} (0.05%, Tukey grouping "D") occurring at the lowest MIF of 15 L/min. The breathing frequency comparisons show that 10 and 15 breaths/min produced higher values of P_{filter} (0.39% and 0.38%, Tukey grouping "I") than those observed at 20, 25 and 30 breaths/min (0.25%, 0.23% and 0.26%, respectively, Tukey grouping "II").

N95 Total Inward Leakage (TIL)—Figure 1B presents the results obtained from the TIL measurements for the tested N95 FFR. It is seen that the MIF of 15 L/min produced the highest TILs. Interestingly, the TIL increased with increasing the breathing frequency, especially at MIF = 15 L/min. The exhaled particle-free air dilutes the aerosol in the respirator cavity. At a higher breathing frequency (given the same MIF), the dilution air volume per breathing cycle is lower, which results in a less efficient dilution and consequently increases the aerosol concentration inside the respirator. This explains why a higher breathing frequency produced a higher TIL.

Statistical analysis revealed significant effects of MIF (p =0.0019) and breathing frequency (p =0.0025) on TIL (see Table II). The pairwise multiple comparison results presented in Table II show that the lowest MIF (15 L/min) was associated with the highest mean TIL (1.93%, Tukey grouping "A"). The mean TIL values among the three higher MIFs (30, 55 and 85 L/min) were not significantly different from each other (1.37%, 1.31% and 1.29%, Tukey grouping "B"). The highest breathing frequency (30 breaths/min) produced the highest mean TIL (1.73%, Tukey grouping "I") compared to the lowest mean TIL (1.22%, Tukey grouping "II") with the lowest breathing frequency (10 breaths/min). The highest and lowest breathing frequencies were significantly different (Tukey groups I & II). As was pointed out in our previous study on elastomeric respirators,[36] higher MIF may create a higher sucking force that made a tighter contact between the respirator and the soft skin of the headform, possibly reducing the leak size. We anticipate that the quoted effect showed up when MIF increased to 30 L/min. The finding is consistent with previous FFR performance studies conducted using hard manikins and challenge aerosol particles above 500 nm.[32, 41, 42]

N95 Faceseal Leakage-to-Filter (FLTF) Ratio—The size-independent (overall) *FLTF* ratios calculated by Eq. (3) are presented in Figure 1C as a function of the breathing frequency and MIF. Except for MIF = 85 L/min, all the *FLTF* ratios were > 1, which suggests that overall particle penetration through faceseal leaks exceeded N95 filter penetration at lower breathing rates. Remarkably, at the lowest MIF (15 L/min) the *FLTF* ratios ranged from 25 to 47, suggesting that the absolute majority of the measured virus-size aerosol particles penetrated through faceseal leaks. At MIF =15 L/min, increase in breathing frequency was generally associated with increase in *FLTF* ratio. However, the breathing frequency effect was not clearly seen for the three higher MIFs (30, 55 and 85 L/min).

It is seen that increasing MIF resulted in decreasing *FLTF* ratio. This finding agrees with two other N95 FFR studies.[13, 43] Grinshpun *et al* tested an N95 FFR using 25 human subjects, and reported that "deep breathing" produced higher *FLTF* ratios compared to "normal breathing".[13] Rengasamy and Eimer also reported higher *FLTF* ratios occurred at higher flow rates when testing the N95 FFRs with artificially created leaks.[43] In both quoted studies, all the *FLTF* ratios exceeded the unity, indicating that the faceseal leakage was the primary penetration pathway for N95 FFRs.

2. Surgical Mask

SM Filter Penetration (P_{filter})—The data on filter penetrations (P_{filter}) for the tested SM are shown in Figure 2A. Compared to the N95 FFR, the SM had a much higher filter penetration. This is not surprising given the less stringent filter penetration test requirements for SMs. In fact, previous studies have reported SMs providing much lower levels of respiratory protection than N95 FFRs when challenged with biological or non-biological particles.[5, 13–15] Increasing MIF frequently resulted in an increase in filter penetration, especially at the lowest breathing frequency. Statistical analysis suggests that the effects of MIF and breathing frequency on the P_{filter} were both significant (p <0.05; see Table III). The pairwise multiple comparisons (see Table III) show the highest MIF (85 L/min) produced the highest mean P_{filter} (9.65%, Tukey grouping "A"), whereas the lowest P_{filter} (5.41%, Tukey grouping "C") occurred at the lowest MIF (15 L/min). Table III also shows that the mean P_{filter} = 7.81% (Tukey grouping "I") obtained at 30 breaths/min was significantly higher (p <0.05) than P_{filter} = 6.67% (Tukey grouping "II") obtained at 20 breaths/min. However, no consistent trend was identified throughout the frequency scale.

SM Total Inward Leakage (TIL)—TIL results for the SM are presented in Figure 2B. The average TIL values ranged from 17% to 35% compared to filter penetrations of 3 – 12%, suggesting that faceseal leakage had a greater effect on the mask performance. Increasing MIF caused the mean TIL to decrease (see Table IV), which is in agreement with the finding reported for the N95.

While ANOVA revealed that both MIF and breathing frequency had a significant effect on the TIL (p <0.05; see Table IV), no consistent trend of increase or decrease of TIL with breathing frequency was observed. For instance, increasing the breathing frequency from 10 to 15 breaths/min was associated with a decrease in TIL, whereas changing the frequency from 10 to 30 breaths/min at MIF = 55 or 85 L/min resulted in essentially no change in TIL. When comparing the mean TIL values among the five breathing frequencies, the highest mean TIL (25.7%, Tukey grouping "I") occurred at 30 breaths/min, and the lowest mean TIL (22.2%, Tukey grouping "II") at 15 breaths/min.

At the same time, the data produced by a pairwise comparison presented in Table IV demonstrate that increasing MIF indeed decreased the TIL with MIF = 15 L/min generating the highest mean TIL (30%, Tukey grouping "A") and 85 L/min producing the lowest mean TIL (19.9%, Tukey grouping "C").

SM Faceseal Leakage-To-Filter (FLTF) Ratio—The *FLTF* ratios calculated from the overall P_{filter} and the TIL data are presented in Figure 2C. It is seen that increasing MIF from 15 to 55 L/min resulted in a decrease in *FLTF*, with most of the *FLTF* ratios were > 1 (which means $P_{leakage}$ > P_{filter}). Increasing MIF from 55 to 85 L/min had less effect, with FLTF ratios < 2 and even < 1 at the highest MIF (85 L/min) for the two lowest frequencies of 10 and 15 breaths/min. The results had a similar pattern to those presented for the N95 FFR. However, the *FLTF* ratios for the SM were lower. For example, at MIF =15 L/min, the

FLTF ratios for the SM were between 4 and 7 while those found for the N95 FFR ranged from 24 to 50. This difference is attributed to much higher filter penetration of the SM as compared to the N95 FFR.[13]

No clear trend was identified between the breathing frequency and the *FLTF* ratio for the three highest flows (MIF = 30, 55 and 85 L/min), where the curves are relatively flat (Figure 2C). For the lowest flow rate (15 L/min), increasing breathing frequency initially decreased *FLTF*, but this too leveled off.

CONCLUSIONS

Breathing frequency was found to be another factor (in addition to MIF) that can significantly affect the performance of N95 FFRs and SMs. However, the filter mechanism causing P_{filter} to change as a function of breathing frequency is complex and not fully understood at this time. For the tested N95 FFR, the increase of breathing frequency caused an increase in TIL. No consistent trend of increase or decrease of TIL with either MIF or breathing frequency was observed for the tested SM. To potentially extend these findings beyond the manikin/breathing system used, future studies are needed to fully understand the mechanism causing the breathing frequency effect on the performance of FFRs and SMs on human subjects. The *FLTF* ratios obtained for the N95 FFR were generally higher than those for the SM for all tested breathing frequencies and MIFs. It is primarily because of the higher efficiency of the N95 filter. Increasing MIF was also generally associated with decreasing *FLTF* ratio for the tested FFR/SM. Except for MIF = 85 L/min, all the calculated *FLTF* ratios were > 1, suggesting that the faceseal leakage was the primary particle penetration pathway for the tested FFR/SM at various breathing frequencies.

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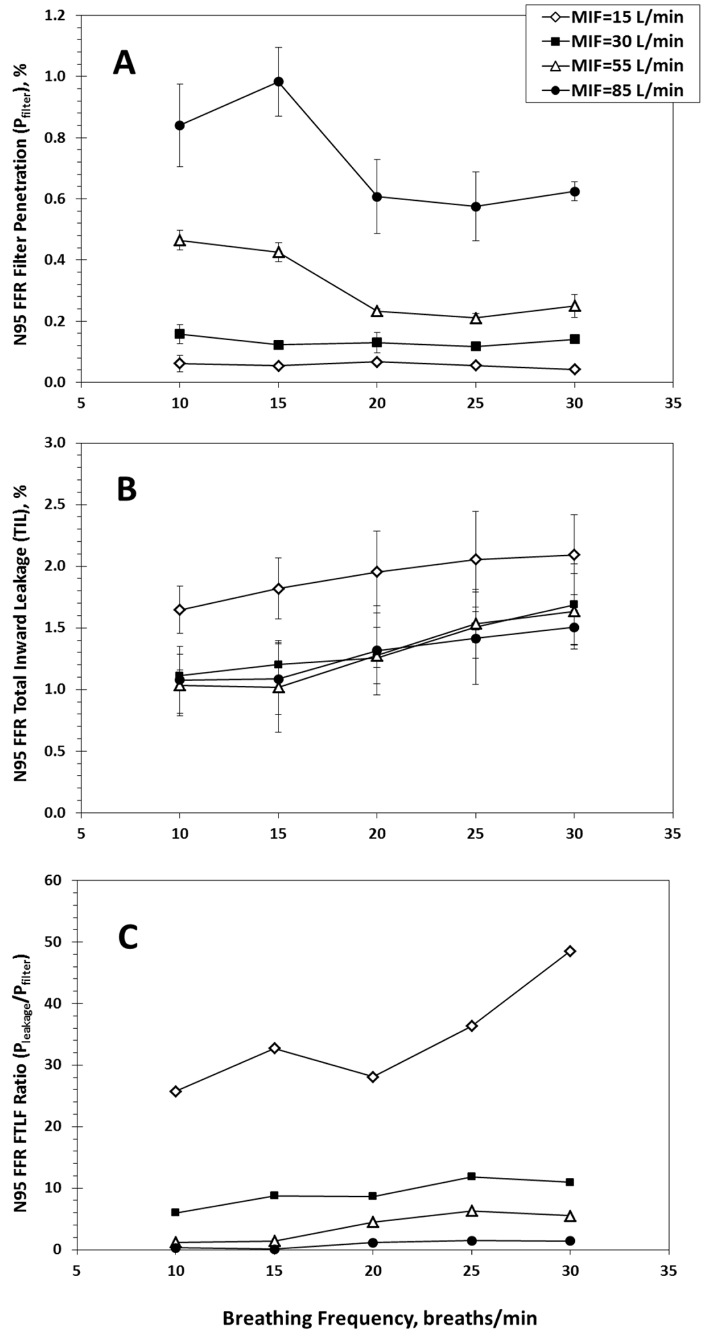

Fig 1.

Filter penetration (A), Total Inward Leakage (TIL) (B), and faceseal leakage-to-filter (*FLTF*) ratio (C) for an N95 FFR sealed to a plastic manikin's face. Each data point in plot C represents the *FLTF* ratio calculated as the mean $P_{leakage}$ (determined from 3 replicates) divided by the mean P_{filter} (also determined from 3 replicates). Consequently, no error bars are presented in plot C.

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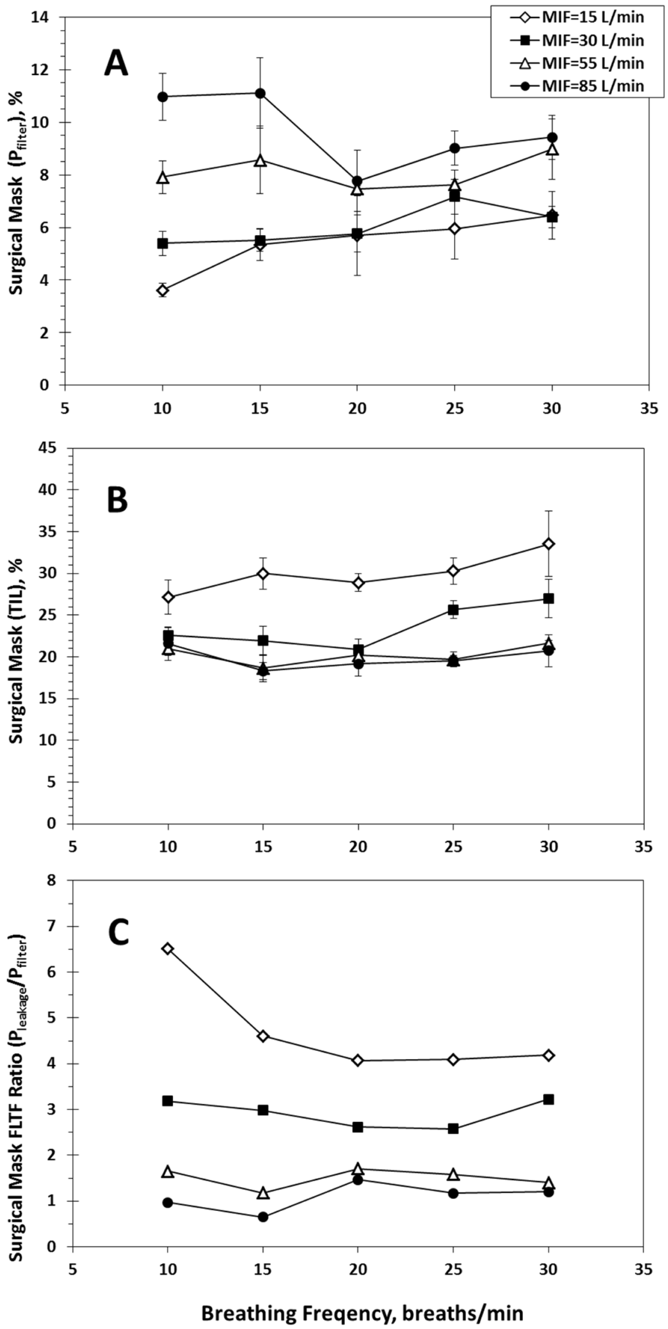

Fig 2.

Filter penetration (A), Total Inward Leakage TIL (B), and faceseal leakage-to-filter (*FLTF*) ratio (C) for a surgical mask sealed to a plastic manikin's face. Each data point in plot C represents the *FLTF* ratio calculated as the mean $P_{leakage}$ (determined from 3 replicates) divided by the mean P_{filter} (also determined from 3 replicates). Consequently, no error bars are presented in plot C.

TABLE I.

Pairwise multiple comparisons for mean P_{filter} values among four MIFs and five breathing frequency groups (ANOVA with Tukey's range test) for an N95 FFR

Effect of MIF on P_{filter}				Effect of Breathing Frequency on P_{filter}			
MIF (L/min)	**Tukey Grouping**[a]	**Mean**[b] **P_{filter} (%)**	***p*-value**[c]	**Breathing Frequency (breaths/min)**	**Tukey Grouping**[a]	**Mean**[b] **P_{filter} (%)**	***p*-value**[d]
15	D	0.05	<0.0001	10	0.38	I	<0.0001
30	C	0.13		15	0.39	I	
55	B	0.31		20	0.25	II	
85	A	0.72		25	0.23	II	
				30	0.26	II	

a: Within each group of the MIF or the breathing frequency, means with the same letter are not significantly different (p-value > 0.05).

b: Calculated using the size-independent (overall) P_{filter} values.

c: P-values were obtained from the two-way ANOVA performed to examine the effect of the MIF on the filter penetration.

d: P-values were obtained from the two-way ANOVA performed to examine the effect of the breathing frequency on the filter penetration.

TABLE II.

Pairwise multiple comparisons for mean TIL values among four MIFs and five breathing frequency groups (ANOVA with Tukey's range test) for an N95 FFR

Effect of MIF on TIL				Effect of Breathing Frequency TIL			
MIF (L/min)	**Tukey Grouping**[a]	**Mean**[b] **TIL (%)**	***p*-value**[c]	**Breathing Frequency (breaths/min)**	**Tukey Grouping**[a]	**Mean**[b] **TIL (%)**	***p*-value**[d]
15	A	1.93	0.0019	10	II	1.22	0.0025
30	B	1.37		15	II	1.28	
55	B	1.31		20	I II	1.45	
85	B	1.29		25	I II	1.63	
				30	I	1.73	

a: Within each group of the MIF or the breathing frequency, means with the same letter are not significantly different (p-value > 0.05).

b: Calculated using the size-independent (overall) TIL values.

c: P-values were obtained from the two-way ANOVA performed to examine the effect of the MIF on the TIL.

d: P-values were obtained from the two-way ANOVA performed to examine the effect of the breathing frequency on the TIL.

TABLE III.

Pairwise multiple comparisons for mean P_{filter} values among four MIFs and five breathing frequency groups (ANOVA with Tukey's range test) for a surgical mask

Effect of MIF on P_{filter}				Effect of Breathing Frequency on P_{filter}			
MIF (L/min)	**Tukey Grouping**[a]	**Mean**[b] **P_{filter} (%)**	***p*-value**[c]	**Breathing Frequency (breaths/min)**	**Tukey Grouping**[a]	**Mean**[b] **P_{filter} (%)**	***p*-value**[d]
15	C	5.41	<0.0001	10	I II	6.97	<0.0143
30	C	6.04		15	I II	7.63	
55	B	8.11		20	II	6.67	
85	A	9.65		25	I II	7.43	
				30	I	7.81	

a: Within each group of the MIF or the breathing frequency, means with the same letter are not significantly different (*p*-value > 0.05).

b: Calculated using the size-independent (overall) P_{filter} values.

c: *P*-values were obtained from the two-way ANOVA performed to examine the effect of the MIF on the filter penetration.

d: *P*-values were obtained from the two-way ANOVA performed to examine the effect of the breathing frequency on the filter penetration.

TABLE IV.

Pairwise multiple comparisons for mean TIL values among four MIFs and five breathing frequency groups (ANOVA with Tukey's range test) for a surgical mask

Effect of MIF on TIL				Effect of Breathing Frequency TIL			
MIF (L/min)	**Tukey Grouping[a]**	**Mean[b] TIL (%)**	***p*-value[c]**	**Breathing Frequency (breaths/min)**	**Tukey Grouping[a]**	**Mean[b] TIL (%)**	***p*-value[d]**
15	A	1.93	0.0019	10	I II	23.1	0.0316
30	B	1.37		15	II	22.2	
55	B	1.31		20	I II	22.3	
85	B	1.29		25	I II	23.8	
				30	I	25.7	

[a:] Within each group of the MIF or the breathing frequency, means with the same letter are not significantly different (*p*-value > 0.05).

[b:] Calculated using the size-independent (overall) TIL values.

[c:] *P*-values were obtained from the two-way ANOVA performed to examine the effect of the MIF on the TIL.

[d:] *P*-values were obtained from the two-way ANOVA performed to examine the effect of the breathing frequency on the TIL.