The Effect of Hypochlorous Acid on Clean and Contaminated N95 Respirators

J. Patrick Brooks, MD[1], Christopher Lupfer, PhD[2], Wang Yang, PhD[3,4], Weixing Hao[3], and Kashala Fabrice Kapiamba[3]

[1] Department of Biomedical Science and School of Anesthesia, Missouri State University

[2] Department of Biology, Missouri State University

[3] Department of Civil, Architectural, and Environmental Engineering, Missouri University of Science and Technology

[4] Department of Chemical, Environmental and Materials Engineering, University of Miami

Summary:

Because N95 Respirators frequently see extended use, we evaluated the use of hypochlorous acid (HOCl) spray that might allow decontamination of the respirators between patient encounters at the point-of-care. HOCl had no effect on the N95 fabric's filtration functions, which is important when considering the increasing use of this product in hand cleaners and hospital disinfectants. However, HOCl did not provide N95 fabric decontamination, likely due to the hydrophobic outer layer of these devices.

Background:

The COVID-19 pandemic resulted in worldwide, long lasting shortages of N95 filtering facepiece respirators (FFR). Strategies to increase the life cycle of these masks, originally intended for single use, have included numerous sterilization techniques allowing reuse[1], and novel designs to create alternative masks.[2-4] Extended use, "the practice of wearing the same N95 respirator for repeated close contact encounters with several different patients, without removing the respirator between patient encounters''[5] has become commonplace. Due to concerns that extended use of N95 respirators might contribute to microbial transmission between patient rooms, this study aims to evaluate a decontamination technique using stabilized 150 ppm hypochlorous acid (HOCl) that might be feasible at the point-of-care. In addition, fumes from alcohol-based hand sanitizers and disinfectants are known to decrease the filtration effectiveness of N95 respirators by reducing the electrostatic charge of filter material, especially if not protected by an outer, thick hydrophobic layer.[6] Hypochlorous Acid is increasingly used as a disinfectant in heavily contaminated hospital settings[7-9], and has been used for patient decontamination[10], yet there is no data regarding its effect on N95 respirators.

Hypochlorous Acid is an Environmental Protection Agency approved disinfectant and Food and Drug Administration approved antiseptic with wide microbicidal activity. Several HOCl formulations are listed on the EPA N-list of disinfectants effective against COVID-19.[11] Hypochlorous Acid was originally used in the form of "superoxidized water" but is now commercially available in several stabilized forms. It has very wide bactericidal, fungicidal, and viricidal coverage. Unlike alcohol solutions, HOCl is an effective sporicidal agent, nontoxic to biologic tissues, and effective in low concentrations.[12]

Methods:

Due to the shortage of N95 respirators and the difficulty obtaining them during the COVID-19 pandemic, commercially available 3M^{TM} 1860 Health Care Particulate N95 Respirators were cut into quarters to test fabric swatches. Swatches were treated with 1, 4, 8, 16, or 32 sprays of stabilized 150 ppm Hypochlorous acid (Pure&Clean, Nixa, MO), producing 4 test swatches at each level of treatment. The HOCl product was delivered directly onto the swatches from a uniform distance, using the 2-ounce hand cleanser spray bottle as a point-of-care model of mask decontamination.

Filtration performance of HOCl-treated N95 swatches was tested using standardized aerosol sizing instruments and filtration setup, as shown in **Figure 1**. Similar to our previous work (Hao et al. 2020; Hao, Xu and Wang 2021; Hao et al. 2022), the experimental setup includes the aerosol generation and filtration assessment sections. The test aerosols were generated by a constant output atomizer (Model 3076, TSI Inc., Shoreview, MN) nebulizing a NaCl-water solution with a mass concentration of 0.1%. The atomizer generated aerosols at a flow rate of 3.0 liters per minute (lpm). The aerosols were first diluted by an inline diluter and then dried by a custom build diffusion dryer. Afterward, the aerosols, together with a stream of filtered make-up air, were introduced to a mixing chamber. The homogeneous aerosols were then directed into a 37-mm filter cassette (Air Sampling Cassette, Zefon International Inc., Ocala, FL), where the cut HOCl-treated N95 fabric swatches were firmly pressed onto a mesh support and sealed at the edge. The flow rate of the mixed aerosol through these swatches was maintained at 6.6 lpm (where the airflow velocity is 10 cm s^{-1}) using a critical orifice (In-Line Orifice Restrictor Kits, Orange Coast Pneumatics, Yorba Linda, CA) installed between the filter cassette and the vacuum. The flow resistance across the filter material was measured as an indicator of the breathability of the material.

A scanning mobility particle sizer (SMPS, Model 3936, TSI Inc., Shoreview, MN) measures the mobility size distributions of aerosols upstream and downstream of the filter media. The size distribution (30 nm – 500 nm) of aerosols ($n(D_P)$) is obtained by scanning the voltage that is applied to the differential mobility analyzer (DMA, Model 3081, TSI Inc., Shoreview, MN). Similar to our previous work (Hao et al. 2020; Li et al. 2018), the size-dependent filtration efficiency ($\eta(D_p)$) was calculated by Eq. (1),

$$\eta(D_p) = 1 - \frac{n_o(D_p)}{n_i(D_p)} \tag{1}$$

where $n_o(D_p)$ and $n_i(D_p)$ are the particle number concentrations for each particle size measured at the outlet (downstream) and inlet (upstream) of the filter cassette. Size-dependent particle number concentrations were measured for a minimum of three times, and the standard deviation (σ) of the filtration efficiency was calculated by

$$\sigma = \frac{n_o(D_p)}{n_i(D_p)} \sqrt{\left(\left(\frac{\sigma_o}{n_o(D_p)}\right)^2 + \left(\frac{\sigma_i}{n_i(D_p)}\right)^2\right)} \tag{2}$$

where σ_o and σ_i are the standard deviations of the size distributions upstream and downstream of the filter media.

N95 fabric swatches were then contaminated with 10^8 E. coli or 10^8 Staph aureus (three swatches each) delivered in phosphate buffered saline. They were then sprayed with three sprays of either stabilized 150 ppm HOCl via 2-ounce hand pump, 70% ethyl alcohol, or normal saline. The swatches were allowed to sit for 10 minutes. Swatch material was then vortexed in phosphate buffered saline. Surviving bacterial numbers were then determined by applying the solution onto LB agar for standard plate counts.

Results:

The size-dependent filtration efficiency of HOCl-sprayed N95 respirator fabric under 1, 4, 8, 16, and 32 sprays ranges from 96 to 100% for particle sizes of 30 – 500 nm at the tested face velocities of 10 cm s-1, showing no significant effect on the material's filtration efficiency for these particle sizes. See **Figure 2**. The flow resistance of the HOCl-sprayed N95 respirators material under 1, 4, 8, 16, and 32 sprays shows pressure drops of 0.17 ± 0.01, 0.17 ± 0.01, 0.17 ± 0.01, 0.16 ± 0.01, and 0.16 ± 0.01 kPa, showing almost no change in flow resistance (breathability). According to the NIOSH standards, the maximum inhalation resistance is 0.34 kPa with an airflow velocity of 9.44 cm s^{-1} for N95 FFRs. These results show that the tested HOCl-sprayed N95 respirators materials satisfy this standard.

The HOCl treatment had no bactericidal effect on either E. coli or S. aureus contaminated swatches when compared to saline control. Ethyl alcohol had a profound treatment effect, resulting in minimal to no surviving bacteria of either species. See **Figure 3**. One additional N95 swatch contaminated with E. coli and one with S. aureus were completely submerged in HOCl solution for one minute. This complete submersion into HOCl solution also eliminated all bacterial growth.

Discussion:

Hypochlorous acid, in up to 32 spray treatments, does not diminish the filtration effects of N95 respirator material. The strong resistance of the N95 respirator to HOCl spray is most likely due to its thick hydrophobic outermost layer. The inner layers of N95 respirators, made of charged melt-blown, non-woven polypropylene, are responsible for most of the particle capture by electrostatic attraction. Unable to reach these inner layers, sprayed HOCl does not affect their filtration and electrostatic properties under current experimental conditions. This is contrasted against prior evidence showing the deleterious effect of alcohol fumes on in-use N95 respirator function.[6] Although ethyl alcohol is hydrophilic, it is highly volatile and known to reach the polypropylene fibers of N95 masks and neutralize their surface charges, whereas chlorine-based solutions less easily penetrate the outer hydrophobic fabric layer.[15]

E. coli is reported to have both hydrophilic and hydrophobic properties in various studies, and may be motile by use of flagella.[16] Staphylococci aureus is hydrophobic[17], and although previously considered non-motile, has confirmed spreading motility.[18] Bacterial movement through the hydrophobic outer layer of N95 respirators may limit the ability of HOCl spray to reach the microorganisms. Complete soaking of the material with HOCl did provide excellent decontamination, yet this is not a useful point-of-care model.

Conclusions:

Although alcohol fumes are known to damage N95 respirator filtration[6], this study has shown that up to 32 rounds of HOCl sprayed directly onto N95 respirator material does not affect its filtration efficiency or breathability. We should also note that this study evaluates the filter efficiency and pressure drop only. The remaining fit tests for respirators will be examined in our future studies. The application of HOCl by spray bottle, however, is not an effective method to decontaminate these respirators.

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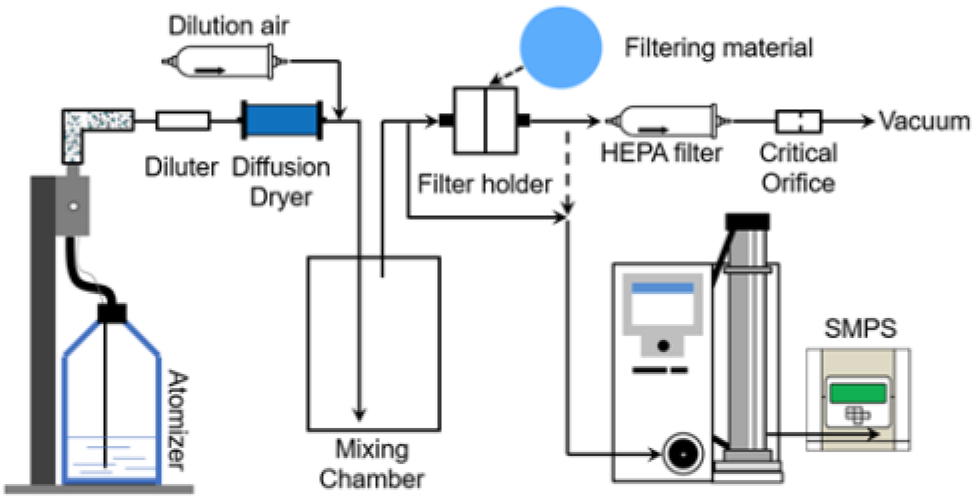

Fig. 1. The experimental setup.

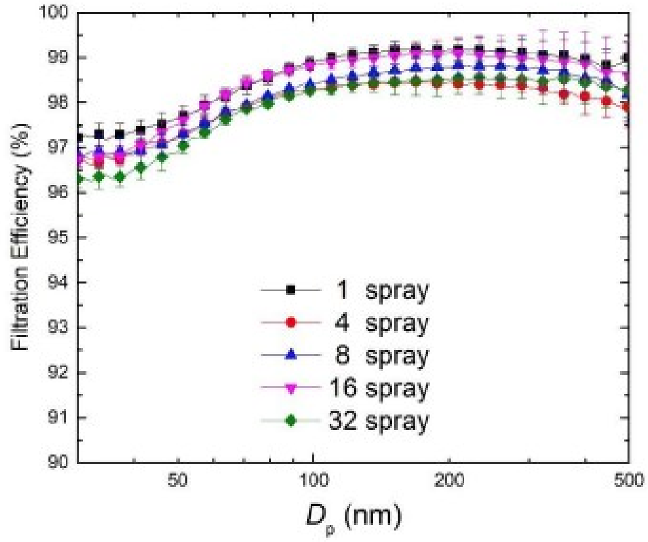

Fig. 2. Size-dependent filtration efficiency of HOCl sprayed N95 masks under 1 spray, 4 spray, 8 spray, 16 spray, 32 spray treatment at the face velocity of 10 cm s^{-1}.

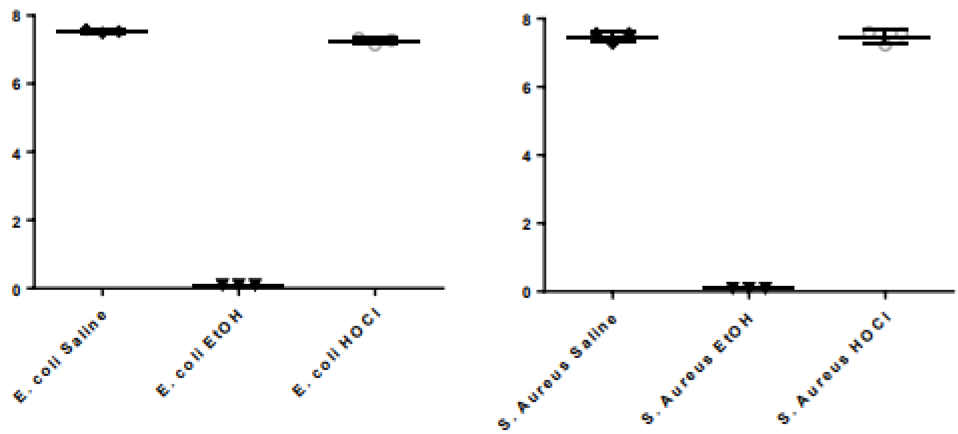

Fig. 3. Bacterial survival on N95 fabric treated with EtOH vs HOCl