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Major Article

The effect of hypochlorous acid on the filtration performance and bacterial decontamination of N95 filtering facemask respirators

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Filtration efficiency
Alcohol

Background: Stabilized hypochlorous acid (HOCl) is increasingly used as a hospital disinfectant and antiseptic, yet its effect on N95 filtration facemask respirators (FFR) is unknown. These FFRs could also contribute to fomite-based transmission of nosocomial infections if worn for extended use between patient rooms.
Methods: Filtration performance of N95 FFR fabric swatches was assessed after various levels of HOCl exposure. N95 swatches were then contaminated with 10^8 *Escherichia coli* or 10^8 *Staphylococci aureus* and treated with HOCl solution, 70% ethyl alcohol, or normal saline. Surviving bacterial numbers were assessed by plate counts.
Results: The size-dependent filtration efficiency of HOCl-sprayed N95 FFR fabric ranged from 96% to 100%, showing no significant change. Flow resistance testing revealed almost no change compared to control. Submersion in HOCl, but not spraying, had an excellent bactericidal effect on contaminated swatches.
Discussion: The role of the outer hydrophobic layer of N95 FFRs is discussed regarding the effects of HOCl on filtration and bacterial decontamination.
Conclusions: N95 material, sprayed with or briefly submerged in HOCl, maintained its filtration function. HOCl delivery by spray pump, however, would not accomplish decontamination of extended use FFRs between patient encounters. HOCl submersion of intact FFRs, contaminated with various hospital pathogens, is worth further study.

Hypochlorous acid (HOCl) is a potent antimicrobial agent formed by the oxidative burst within neutrophils as part of the innate immune system. HOCl has been used since World War I as a disinfectant and antiseptic, although feasibility was limited by its short shelf life measured in days. Stabilized HOCl with a shelf life of greater than one year is now commercially available as an Environmental Protection Agency approved disinfectant and Food and Drug Administration approved antiseptic. It has a wide microbicidal activity against bacteria, fungi, and viruses. Several HOCl formulations are listed on the EPA N-list of disinfectants effective against COVID-19.[1] Unlike alcohol solutions, the CDC lists commercially available solutions of HOCl as effective sporicidal agents, effective in low concentrations, and nontoxic to human tissues.[2] HOCl is increasingly used as a disinfectant in heavily contaminated hospital settings[3] and has been used for wound management,[4] patient decolonization,[5] and an ever-growing list of other clinical applications.

Despite increasing use of HOCl solutions in close proximity to health care providers, the effects of HOCl on the filtration performance of N95 filtering facemask respirators (FFR) are unknown. Vapors from alcohol-based hospital disinfection agents have been shown to decrease the effective filtration of N95 FFRs worn by subjects performing surface decontamination.[6] Multiple other hospital disinfectant solutions affect FFR performance as well.[7-9]

The ability of N95 FFRs to withstand disinfectants is also of importance when considering the worldwide shortage of these disposable

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respirators during the COVID-19 pandemic. Strategies to increase the life cycle of these masks, originally intended for single use, have included numerous sterilization techniques allowing reuse.[10] 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"[11] has become commonplace. If an FFR is not disposed of between patient encounters; however, it is a potential fomite,[8,9] as there are no established protocols to immediately decontaminate the extended-use N95 FFRs at the point of care. Ideally, health care providers could quickly minimize the microbial burden on extended-use N95 FFRs upon exit from the patient room to decrease the risks of fomite-based transmission.

This study will determine if stabilized HOCl affects the filtration properties of N95 FFR material. This study will also determine if HOCl delivered from a 2-ounce spray bottle can effectively disinfect bacterially contaminated N95 FFRs. If successful, this could serve as a point-of-care model for quick N95 FFR decontamination between patient rooms.

METHODS

Due to the shortage of N95 respirators and the difficulty obtaining them during the COVID-19 pandemic, commercially available 3M 1860 Health Care Particulate N95 Respirators were cut into quarters to test fabric swatches. The swatches were placed on a horizontal surface and treated with 1, 4, 8, 16, or 32 sprays of stabilized 0.15% HOCl (Pure&Clean, Nixa, MO), producing 4 test swatches at each level of treatment. The HOCl product was delivered vertically onto the swatches using the product's 2-ounce fingertip spray bottle from a distance of 25 cm above. This pocket-sized delivery system was chosen for its feasibility at the point of patient care. Output from this spray bottle measured roughly 0.15 ml of HOCl solution per spray, with droplet sizes averaging 4-5 μm as measured by an Aerodynamic Particle Sizer (APS, Model 3321, TSI Inc, Shoreview, MN). This device was used for delivery of all disinfectants on both clean and contaminated FFR swatches during the study.

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, the experimental setup includes the aerosol generation and filtration assessment sections.[12-14] 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-built 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) 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) measured the mobility size distributions of aerosols upstream and downstream of the filter media. The size distribution (30-500 nm) of aerosols ($n(D_P)$) was obtained by scanning the voltage applied to the differential mobility analyzer (DMA, Model 3081, TSI Inc, Shoreview, MN). Similar to our previous work,[12,15] the size-dependent filtration efficiency ($\eta(D_\mathrm{p})$) was calculated by Eq. (1),

$$\eta(D_\mathrm{p}) = 1 - \frac{n_\mathrm{o}(D_\mathrm{p})}{n_\mathrm{i}(D_\mathrm{p})} \quad (1)$$

where $n_\mathrm{o}(D_\mathrm{p})$ and $n_\mathrm{i}(D_\mathrm{p})$ 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

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

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 *Escherichia coli* or 10^8 *Staphylococci aureus* (3 swatches each) delivered in phosphate buffered saline. The swatches were then placed on a horizontal surface and treated from a distance of 25 cm with three sprays of either stabilized 0.15% HOCl, 70% ethyl alcohol, or normal saline. Each solution was delivered by the 2-ounce fingertip spray bottle. The swatches were allowed to sit for 10 minutes. Swatch material was then vortexed in phosphate buffered saline. Surviving bacterial

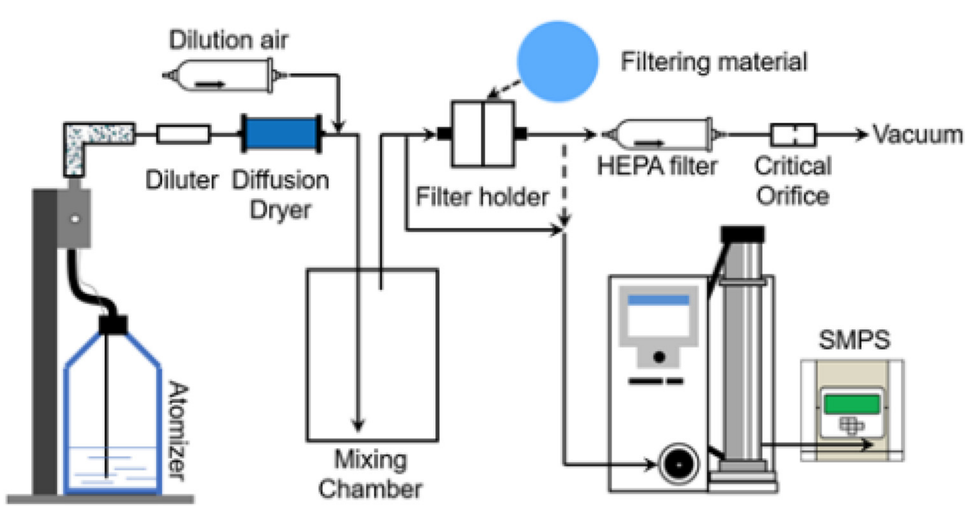

Fig 1. The experimental setup.

numbers were then determined by applying the solution onto LB agar for standard plate counts. Prism 6 software was used for the microbiology statistical evaluation.

RESULTS

The size-dependent filtration efficiency of HOCl-sprayed N95 respirator fabric under 1, 4, 8, 16, and 32 sprays ranged from 96% to 100% for particle sizes of 30-500 nm at the tested face velocities of 10 cm/s, showing no significant effect on the material's filtration efficiency for these particle sizes (Fig 2).

The flow resistance of the HOCl-sprayed N95 respirator material under control, 1, 4, 8, 16, and 32 sprays, and 1-minute and 5-minute submersion in HOCl revealed pressure drops of 0.16 ± 0.01, 0.17 ± 0.01, 0.17 ± 0.01, 0.17 ± 0.01, 0.16 ± 0.01, 0.16 ± 0.01 0.18 ± 0.02, and 0.18 ± 0.02 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 for N95 FFRs.[16] These results show that the tested HOCl-sprayed N95 respirators materials satisfy this standard.

The HOCl spray treatment had no bactericidal effect on either *E coli* or *S aureus* contaminated swatches when compared to saline control. Ethyl alcohol spray treatment had a profound effect, resulting in minimal to no surviving bacteria of either species (Fig 3). One additional N95 swatch contaminated with *E coli* and one with *S aureus* were completely submerged in HOCl solution for 1 minute. This complete submersion into HOCl solution eliminated all bacterial growth. Filtration performance was therefore evaluated on N95 respirator material after 1 minute of submersion in HOCl, revealing no decrease in filtration performance. Five minutes of submersion in HOCl reduced the filtration efficiency of N95 material by 2%-4% (Fig 2).

DISCUSSION

The filtration performance of N95 FFR material is not diminished by spray treatment (up to 32 rounds) of 0.15% HOCl or by brief submersion in this disinfectant. This is notable given health care workers' increasing exposure to HOCl products in hospitals and patient care clinics. The strong resistance of the N95 respirator to HOCl spray is due to its thick hydrophobic outermost layer. The inner layers of N95 respirators, made of charged melt-blown, nonwoven polypropylene, are responsible for most of the particle capture by electrostatic attraction. In this model, HOCl solutions do not appear to reach the polypropylene layer and affect its electrostatic charge unless the N95 FFR material is completely submerged in HOCl for longer than 1 minute.

To our knowledge, this is the first report evaluating the effects of HOCl on N95 FFR material filtration. Although sodium hypochlorite (NaOCl), commonly known as bleach, has been evaluated for this purpose, NaOCl has different chemical, microbicidal, and toxic properties than HOCl. NaOCl dissociates in alkaline water (pH > 8) into hypochlorite ion (OCl-), sodium hydroxide, and small amounts of HOCl, yet this HOCl is the active microbicidal agent in bleach solutions. HOCl solutions therefore have a wider microbicidal spectrum at much lower concentrations than NaOCl solutions.[17] Acidification (pH 4-6.5) of a hypochlorite solution makes HOCl the predominate molecule in solution, with proprietary techniques now used to keep HOCl shelf stable for a year or longer.

Submersion of FFRs in 0.5% bleach can decrease their filtration performance, an effect attributed to loss of electrostatic charge.[7] In other studies bleach did not have this affect, but the results were concerning due to the respirators persistent chlorine odor even after drying.[18] Since hypochlorite vapors and solutions are irritating and corrosive to the eyes, skin, and respiratory tract, the author does not

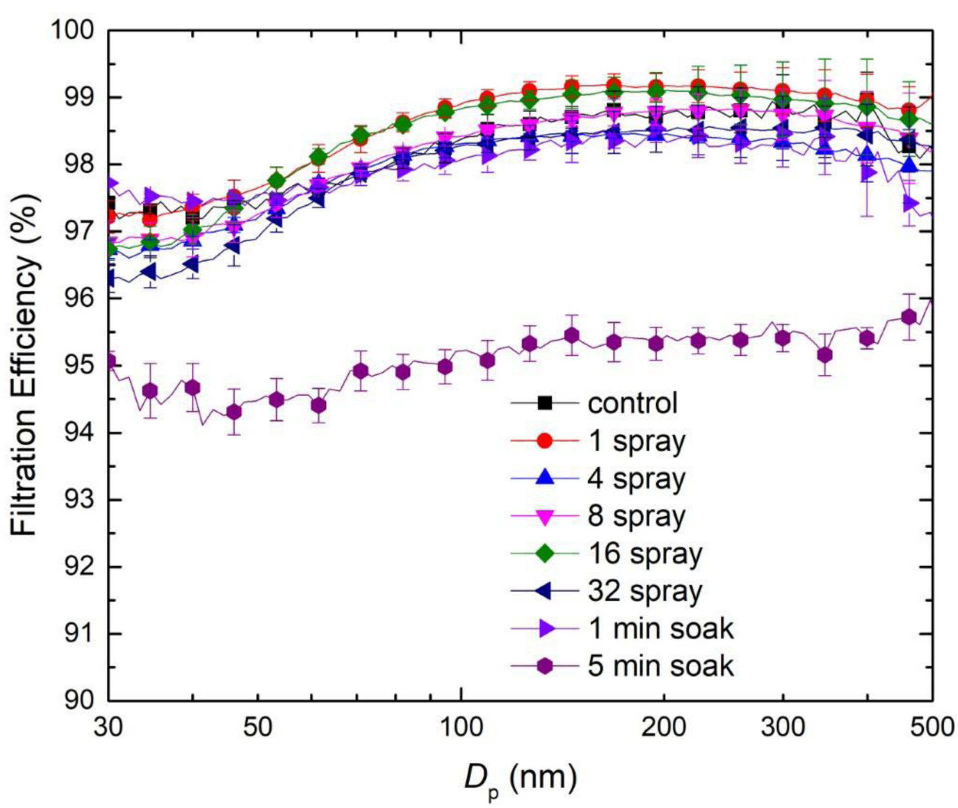

Fig 2. Size-dependent filtration efficiency of HOCl sprayed N95 respirator material at the face velocity of 10 cm/s. N95 material was tested after 1-32 sprays of 0.15% HOCl solution, or after submersion in HOCl solution for 1 or 5 minutes.

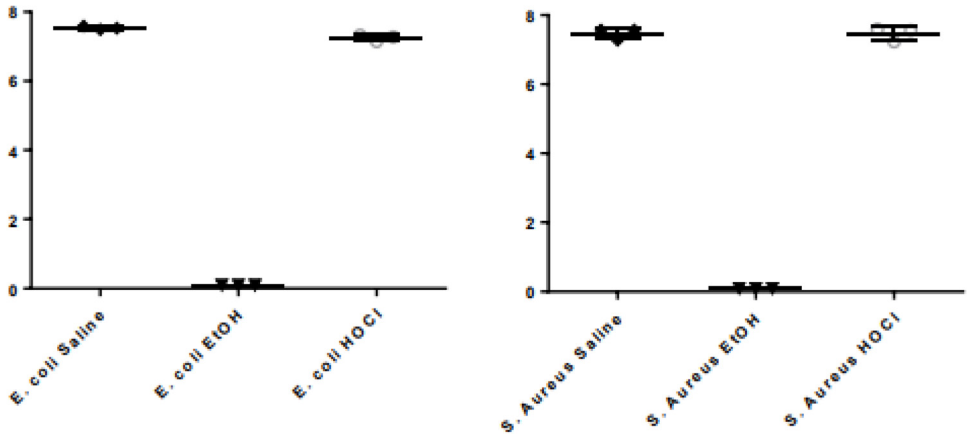

Fig 3. Bacterial survival on N95 fabric treated with 70% EtOH vs 0.15% HOCl.

recommend this technique. Unlike common bleach solutions, appropriately stabilized HOCl solutions do not off-gas a chlorine odor.

Alcohol-based disinfectants are highly volatile and require high concentrations for effectiveness, between 60% and 80%. The combination of high volatility and high concentration leads to significant off-gassing from alcohol-based hand sanitizers and disinfectants which are widely used in health care settings where N95 FFRs are worn. Although alcohols are hydrophilic, their volatility allows them to reach the inner-layer polypropylene fibers of N95 masks and neutralize their electrostatic charges.[19] In an experimental model, volunteers wore N95 respirators during intensive surface disinfection procedures with alcohol-based (60%-80% 2-propranol) sanitizers. Filtration efficiency decreased by nearly 9% after exposure to alcohol vapors during 30 minutes of cleaning.[6] Direct contact of alcohol solutions on N95 FFRs also decreases their filtration performance.[7,8] Like bleach vapors, alcohols can cause respiratory symptoms.[20] Quaternary ammonia compounds, the most widely used disinfectants, can have significant toxicities including pulmonary disease, infertility (in mice), and possible impairment of cholesterol biosynthesis.[20] Attempted decontamination of FFRs with quaternary ammonia compounds antibacterial wipes containing benzalkonium chloride resulted in decreased filtration performance by greater than 5% and yet achieved only partial decontamination.[9]

The nontoxic nature of HOCl solutions, efficacy at very low concentrations, and the apparent lack of effect on N95 FFR material filtration performance make these solutions an intriguing choice for hospital disinfection. Some amount of toxicity can be experimentally identified with all antimicrobial wound and skin cleansers. In a 2014 evaluation of the effects of 18 such products on mouse fibroblasts in vitro, the least toxicity was seen with a 0.1% HOCl solution. It required only a 10-fold reduction in concentration to reach nontoxic levels. When all study solutions were diluted to non-toxic levels, HOCl was still able to achieve a 10^4-fold reduction in *S aureus* colony-forming units in one minute, while all HOCl-free products took longer than 24 hours to achieve the same bactericidal effect.[21] This model was highly sensitive to cellular toxicity, with Johnson & Johnson's Baby Shampoo requiring a 10^3-fold reduction in concentration to reach nontoxic levels against the cultured fibroblasts.

The safety of HOCl as compared to other disinfectants is further evident in its widespread clinical use as an antiseptic without reported side effects. The concentrations of these solutions for use as disinfectants or antiseptics range between 0.1% and 0.2% (100-200 ppm). At these low concentrations, HOCl is considered nontoxic by the CDC.[2] HOCl is safe for total body skin decolonization and use in chronic wounds where it decreases bacterial counts and improves healing rates.[4,5] Commercially available HOCl solutions are safe for nasal and oral mucosa.[22] At decreased concentrations of 0.01%, HOCl solutions have been used as eye drops without ophthalmologic toxicity.[23] Air purification by HOCl fogging of occupied rooms was reported as well tolerated in the 1930s,[24] yet no peer reviewed pulmonary toxicity studies have since been published. HOCl inhalation appears to be generally accepted as safe, with increased usage of commercially available air decontamination systems delivering HOCl mist into occupied rooms. Our group has tested the effects of 0.2% HOCl nebulized solution as well as nasally administered inhaled solution in a murine model and found that treated subjects gained or retained their weight better than control mice, demonstrating no overt toxicity (unpublished data).

Despite the highly bactericidal nature of HOCl, spraying this agent onto the surface of bacteria laden N95 FFR material did not accomplish any apparent decontamination. *E coli* is reported to have both hydrophilic and hydrophobic properties in various studies, and may be motile by use of flagella.[25] *S aureus* is hydrophobic,[26] and although previously considered non-motile, has confirmed spreading motility.[27] 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 as was sought in this study.

The limitations of this study include the use of a single brand of N95 FFR and the testing of material filtration without fit testing of intact FFRs. Low availability of these devices during the COVID-19 pandemic contributed to both limitations. This study only evaluated bacterial decontamination, as bacteria are by far the most common source of nosocomial infections. Without the ability to eliminate common hospital bacterial pathogens, fomite-based transmission cannot be significantly affected by spraying HOCl on N95 FFRs. In limited testing, complete submersion of FFRs in HOCl solution for one minute succeeded in bacterial decontamination without affecting filtration. Bacterial, fungal, and viral disinfection of FFRs by HOCl submersion may be worth further investigation.

CONCLUSIONS

Although alcohol vapors and direct contact with alcohol, bleach, and quaternary ammonium compounds are known to damage N95 FFR filtration,[6-9] this study has shown that up to 32 rounds of 0.15% HOCl sprayed directly onto N95 respirator material does not affect its filtration efficiency or breathability. The application of HOCl by fingertip spray bottle, however, is not an effective method to decontaminate these respirators. Limited data shows that bacterial decontamination of N95 FFR material can be achieved by submersion in HOCl solution for 1 minute without affecting filtration. HOCl submersion will be further examined in studies of fit testing, filtration testing, and decontamination of intact N95 FFRs contaminated with bacteria, fungi, and viruses.

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