Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Major Article

Pretreated household materials carry similar filtration protection against pathogens when compared with surgical masks

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Objective: The past 4 months, the emergence and spread of novel 2019 SARS-Cov-2 (COVID-19) has led to a global pandemic which is rapidly depleting supplies of personal protective equipment worldwide. There are currently over 1.6 million confirmed cases of COVID-19 worldwide which has resulted in more the 100,000 deaths. As these numbers grow daily, hospitals are being forced to reuse surgical masks in hopes of conserving their dwindling supply. Since COVID-19 will most likely have effects that last for many months, our nationwide shortage of masks poses a long term issue that must be addressed immediately.

Methods: Based on a previous study by Quan et al., a salt-based soaking strategy has been reported to enhance the filtration ability of surgical masks. We propose a similar soaking process which uses materials widely available in anyone's household. We tested this method of pretreating a variety of materials with a salt-based solution by a droplet test using fluorescently stained nanoparticles similar in size to the COVID-19 virus.

Results: In this study, we found that paper towels and surgical masks pretreated with the salt-based solution showed a noticeable increase in filtration of nanoparticles similar in size to the COVID-19 virus. We also show that the TWEEN20 used by Quan et al. is not a critical component for the solution, and using salt alone in solution still provides a dramatically increased level of protection.

Conclusions: We believe this method will allow for healthcare workers to create a disposable added layer of protection to their surgical masks, N95s, or homemade masks by using household available products. Adoption of this method may play an essential role in ensuring the safety of healthcare workers during the COVID-19 pandemic and any pandemics that may arise in the future.

BACKGROUND

Since December 2019, the novel 2019 SARS-Cov-2 (COVID-19) has rapidly spread leading to a global pandemic.[1] According to the World Health Organization, globally, as of April 14, 2020, there are been more than 1.6 million confirmed cases of COVID-19 and more than 100,000 reported deaths.[2] During this time, the World Health Organization has called upon industry to increase production of PPE equipment by 40% to relieve the shortage that is currently putting healthcare providers worldwide at risk of contracting the illness.[3] Despite manufacturers working at full capacity to make masks, production across the country is unable to match the number of disposable masks needed each day.[4] The Centers for Disease Control and Prevention recently released guidelines advising the public to wear masks in public settings, such as supermarkets, where following social distancing may be more difficult.[5] Therefore, the need for masks in the community is robust and cannot be met. However, there is no quick solution and relying on foreign countries to manufacture personal protective equipment (PPE) for the US raises significant risk for national safety and financial burden.[6] It is not practical to increase mask manufacturing in the US as the needs of PPE is not consistent each year, its periodical. In addition to this, the current disruption in transportation poses a problem for the distribution of masks and other PPE.[7] As supply rapidly dwindles, hospitals have begun requiring their workers to reuse their masks throughout a shift, leading to the Centers for Disease Control and Prevention releasing recommended guidance on the reuse of N95s and surgical masks.[8] Providers are now at increased risk of contracting COVID-19 because these masks are made for 1 time use and offer inferior protection when reused.[9] New York hospitals have reported growing numbers of hospital workers contracting COVID-19, and many dying from the

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illness.[10] As more hospital employees fall ill and cannot work, hospital's capacity to accept and treat new patients severely decreases which puts the public at an even greater risk.[11] Some have begun making homemade masks to wear over their reused surgical mask in hopes it will make them effective for longer,[12] however no studies have confirmed if this, or if the reuse of N95s and surgical masks is effective. Therefore, our thoughts are to find a material that is used in daily life, is cheap, and can easily be transformed into an effective PPE usable by anyone. The overall purpose of this work is to compare and analyze the efficacy of protection by a variety of household materials, specifically testing both viral particle penetration and pathogen killing. Our group proposes a simple concept for healthcare providers to adopt which enables them to make their own extra layer of protection that will increase the strength and effectiveness of reused surgical masks and non-N95 masks. This material can also add an additional layer outside of an N-95 mask in order to preserve it for multiple uses.

Previously, Quan et al. described and tested a similar pretreatment of salt-based solution on the filter of surgical masks by exposing it to various strains of the flu virus.[8] Our report advances this approach by finding a readily available household material which can be used, simplifying the protocol, and making it easily replaceable and disposable.

MATERIALS AND METHODS

E. coli culture and treatment

Bacteria cells (*E. coli*) were cultured overnight in LB broth at 37 °C with shaking (180 rpm) until the desired optical density (OD) was reached. For treatment of filter materials with *E. coli*, first, filter paper was cut into small squares to fit into 150 mm cell culture dishes (ThermoFisher, Waltham, MA). One filter paper was added to each culture dish, and one square of control or treated filter material was placed on top of the filter paper. Each sample material was treated with a 50 μL drop of *E. coli* (1×10^7 CFU/mL). After 1.5 hours of treatment, the tested filter material was removed and the remaining filter papers were placed in 50 mL sample tubes containing 10 mL of LB broth to incubate for 8 hours at 37 °C with shaking (180 rpm). Following incubation, growth of *E. coli* was measured using an Implen OD600 DiluPhotometer.

Outer-membrane vesicles (OMV) isolation

Bacteria cells (*E. coli*) were cultured overnight in LB broth at 37 °C with shaking (180 rpm) until the desired OD was reached. The cultured cells were pelleted twice at 5,000 x g for 20 min at 4 °C. The supernatant was filtered through a 0.45 μm pore size filter. This supernatant was then further filtered through a 0.22 μm pore size filter to remove any bacteria or cell debris. The OMVs were purified from the filtered supernatant using ExoBacteria OMV Isolation Kits (SBI, Palo Alto, CA) according to the manufacturer's protocol. Bradford assay (Bio-Rad, Hercules, CA) was used to determine protein content of isolated OMVs. The sample was aliquoted and stored at −80 °C until use.

OMV fluorescence staining and washing

OMVs were treated with 1 v/v% of Red Fluorescent Cell Membrane Label (MINI26-1KT, Sigma), incubated in the dark for 10 minutes. The tagged OMVs were then concentrated and washed using an Amicon Ultra-0.5 Centrifugal Filter Device. Briefly, labeled OMVs were pipetted into the filter device and centrifuged at 14,000 x g for 10 minutes to concentrate the OMVs within the device. The concentrated OMVs then were washed with 500 μL of PBS and centrifuged at 14,000 x g for 5 minutes. This washing process was repeated 3x total. To recover the labeled OMVs from the filter device, the device was inverted into a new sample tube provided by the manufacturer and centrifuged at 1,000 x g for 2 minutes. Labeled OMVs were then diluted to the desired concentrations of 10 mg/mL and 1 mg/mL.

Preparation of treated filter materials

Three materials were tested: kitchen paper towel (Kirkland brand, Costco), laboratory paper towel (Scott C-Fold), and the middle filter layer of a standard surgical mask (VWR, Advanced Protection Mask). For each time point, 2 samples were used for each of the three materials tested. A modified version of the protocol used by Quan et al. was used to prepare the soaking solution.[13] For the NaCl + TWEEN20 solution, 30 grams of NaCl (Sigma Aldrich, St. Louis, MO) was placed into a clean 500 mL beaker, followed by the addition of 100 mL of distilled water. This mixture was stirred at 90 °C and 400 rpm until all of the NaCl was visibly dissolved into the solution. The heat was turned off and 1 mL of TWEEN20 (Fisher Scientific) was stirred in at 400 rpm until completely incorporated into the solution. For the NaCl only solution, the same protocol was followed as described but no TWEEN20 was added after the NaCl was completely dissolved into solution. Each material tested was then soaked in the solution for 5 minutes while being simultaneously mixed by hand to be sure all surfaces were coated. The soaked material was then removed and lightly squeezed to remove excess liquid dripping off of the material. The soaked material was then placed on a flat surface and dried overnight at room temperature.

Treatment of material with labeled nanoparticles

After drying, materials were cut into small rectangles equal in size of a microscope slide, and placed on glass microscope slides. To ensure complete contact with the microscope slide, a light book was placed on top of the slide for 1 hour to flatten the dried material. Next, 20 μL of solution containing labeled nanoparticles was pipetted onto the material surface. After 10 minutes or 2 hours of treatment the material was removed from the microscope slide. In preliminary experiments, after 1.5 hours of treatment it was observed that the treated material was completely dry. Therefore, our longest time point is 2 hours which we believe represents the maximum particle penetration possible using the volume and concentration of OMVs we used for this study. To analyze the penetration of the labeled nanoparticles, immunofluorescence images were captured of each microscope slide using a fluorescence microscope (Eclipse TS100, Nikon) at × 10 magnification and analyzed using ImageJ software.

RESULTS

Surgical masks do not adequately prevent the penetration of nanoparticles similar in size to COVID-19 and other viruses

COVID-19 has been reported to be 70-90 nm in diameter.[14] To test the effectiveness of standard surgical masks in protecting against the penetration of COVID-19 and nanoparticles of similar size, OMVs harvested from *E. coli* were used because of their similarity in size (20-200 nm).[15,16] Additionally, OMVs are also known to deliver virulence factors into host cells therefore it is useful to test their ability to penetrate a standard surgical mask.[16] We first tested control (untreated) kitchen paper towel, laboratory paper towel, and the middle filter layer of a standard surgical mask. The purpose of using 2 different paper towels was to test effects of using a thick, highly absorbent paper towel (kitchen paper towel) vs a thin, less absorbent paper towel (laboratory paper towel). Given the nationwide shortage of PPE, hospitals have been urging providers to conserve the supply of N95 masks and instead reuse surgical masks for lengthy shifts. Therefore,

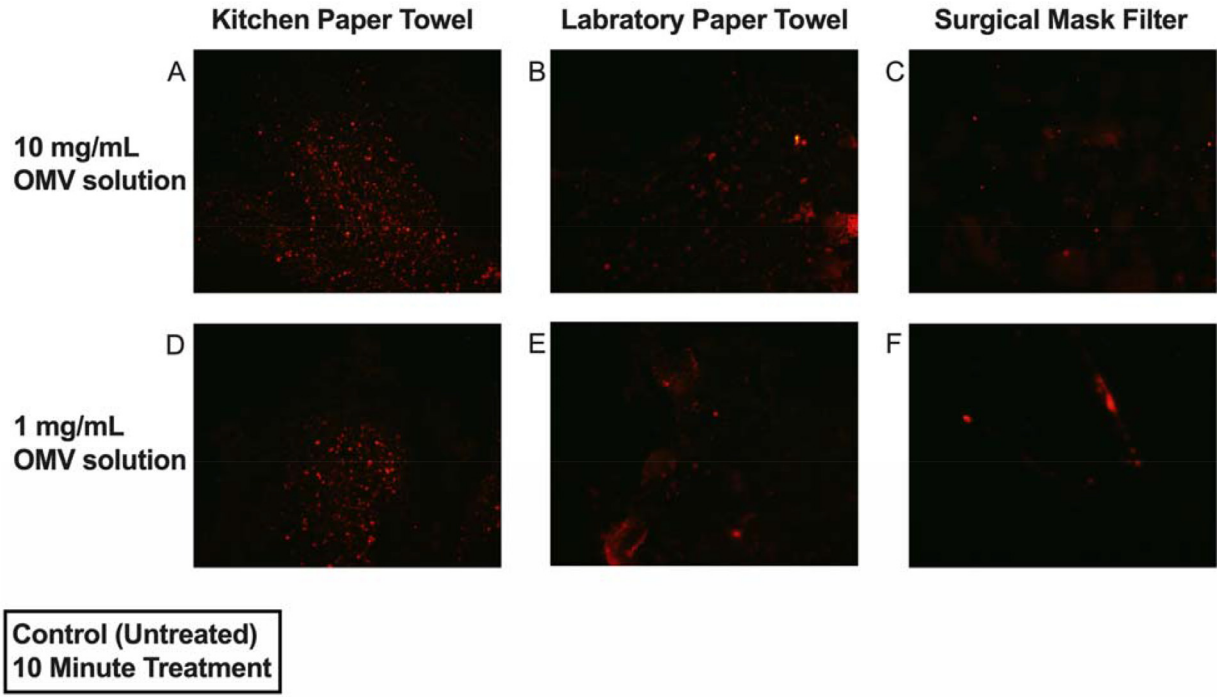

Fig 1. Untreated filters do not adequately protect providers from the penetration of nanoparticles. (A-F) Control (untreated) materials were treated with 20 μL of labeled nanoparticles at a concentration of 10 mg/mL (A-C) or 1 mg/mL (D-F) for 10 minutes. An image was captured in the microscope field containing the highest penetration of nanoparticles, and each sample was tested 3 times. The image included in the figure represents the closest level of average penetration determined by immunofluorescence microscopy.

we believed including a surgical mask was critical to test its ability to filter out nanoparticles of this size. We found all 3 materials did a poor job at filtering out OMVs when untreated (Fig 1) Most worrisome is the large amount of OMVs which penetrated a surgical masks filter when tested with only a 10 minute treatment (Fig 1C and F).

Soaking filter materials in NaCl and TWEEN20 solution dramatically reduced the penetration of nanoparticles

In 2017, Quan et al. reported soaking the filter layer of surgical masks in a solution comprised of NaCl, TWEEN20, and filtered

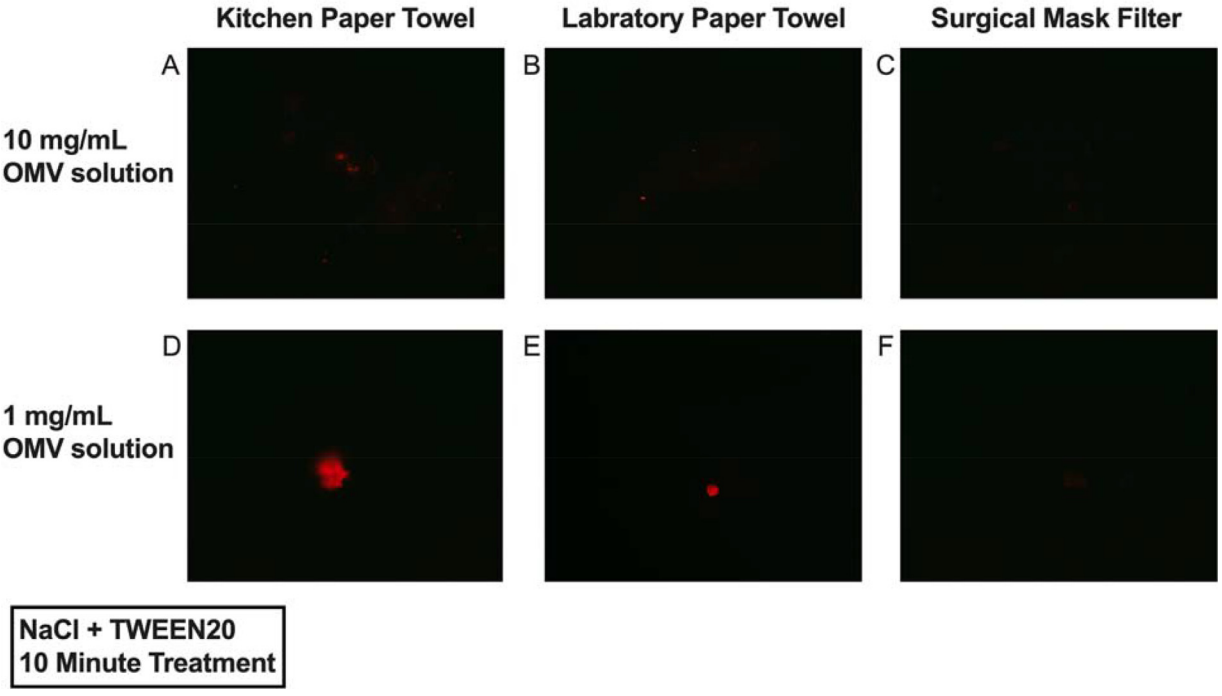

Fig 2. Materials treated with NaCl + TWEEN20 solution show dramatically decreased penetration of nanoparticles. (A-F) NaCl + TWEEN20 solution-soaked materials were treated with 20 μL of labeled nanoparticles at a concentration of 10 mg/mL (A-C) or 1 mg/mL (D-F) for 10 minutes. An image was captured in the microscope field containing the highest penetration of nanoparticles, and each sample was tested 3 times. The image included in the figure represents the closest level of average penetration determined by immunofluorescence microscopy.

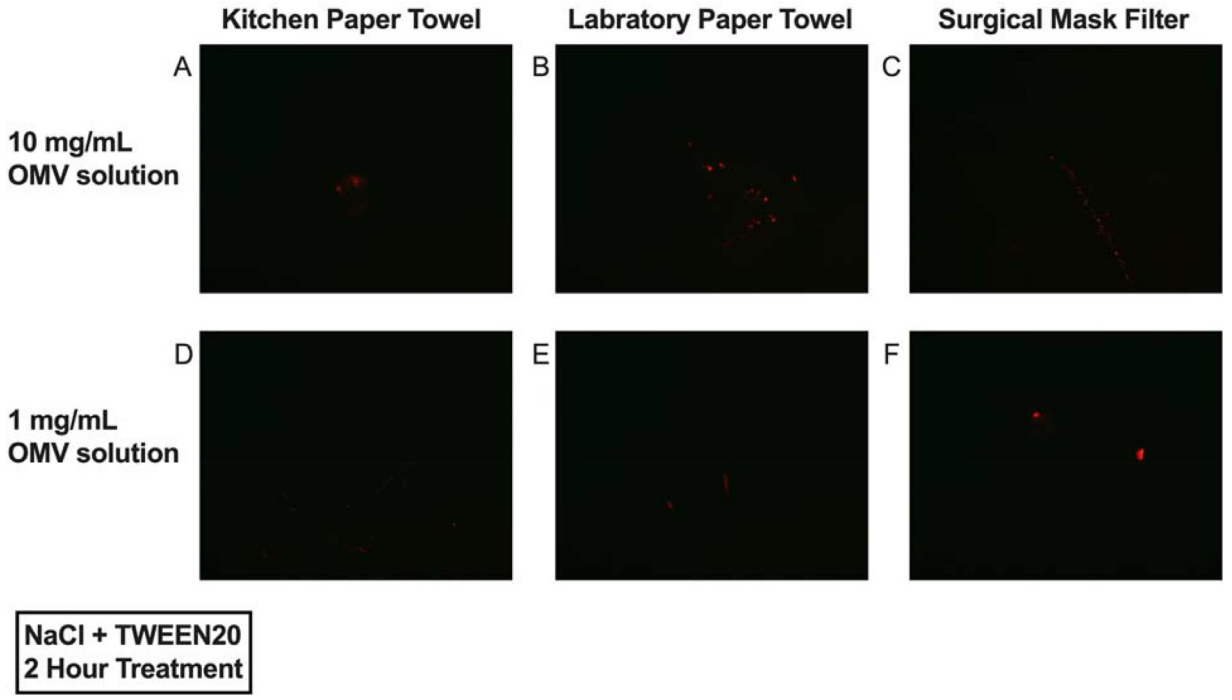

Fig 3. Materials treated with NaCl + TWEEN20 solution show dramatically decreased penetration of nanoparticles after an extended treatment time. (A-F) NaCl + TWEEN20 solution-soaked materials were treated with 20 μL of labeled nanoparticles at a concentration of 10 mg/mL (A-C) or 1 mg/mL (D-F) for 2 hours. An image was captured in the microscope field containing the highest penetration of nanoparticles, and each sample was tested 3 times. The image included in the figure represents the closest level of average penetration determined by immunofluorescence microscopy.

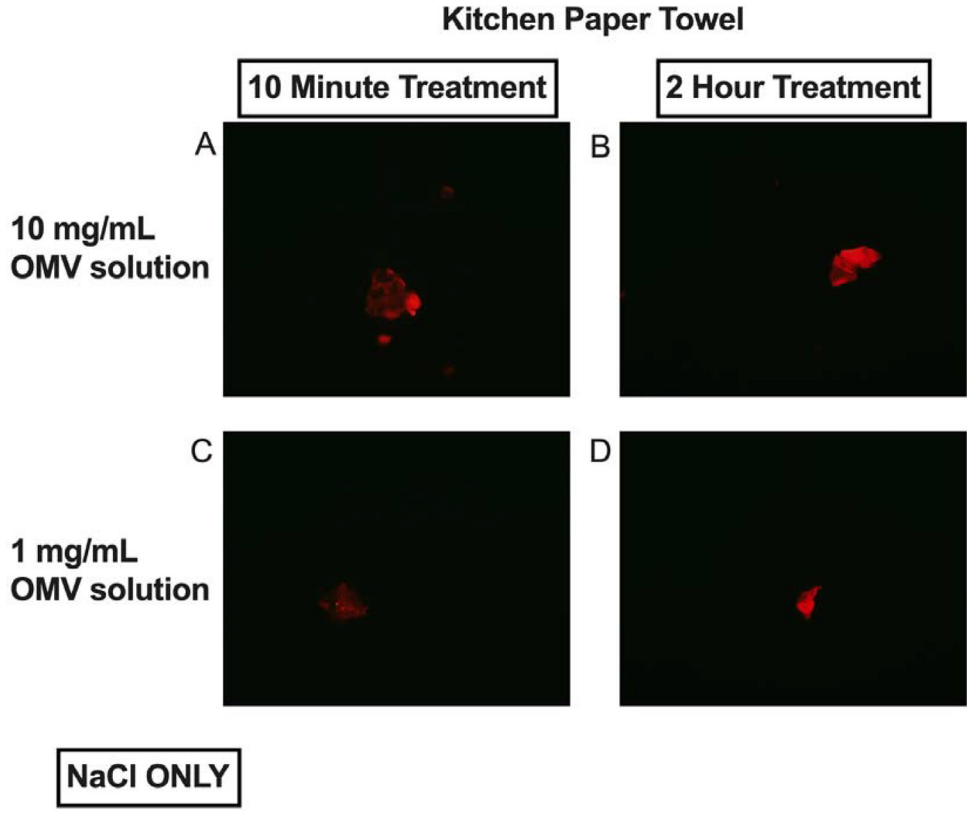

Fig 4. Kitchen paper towel treated with an NaCl only solution shows a notable decrease in penetration of nanoparticles. (A and B) NaCl solution-soaked kitchen paper towel was treated with 20 μL of labeled nanoparticles at a concentration of 10 mg/mL for (A) 10 minutes and (B) 2 hours. (C and D) NaCl solution-soaked kitchen paper towel was treated with 20 μL of labeled nanoparticles at a concentration of 1 mg/mL for (C) 10 minutes and (D) 2 hours. An image was captured in the microscope field containing the highest penetration of nanoparticles, and each sample was tested 3 times. The image included in the figure represents the closest level of average penetration determined by immunofluorescence microscopy.

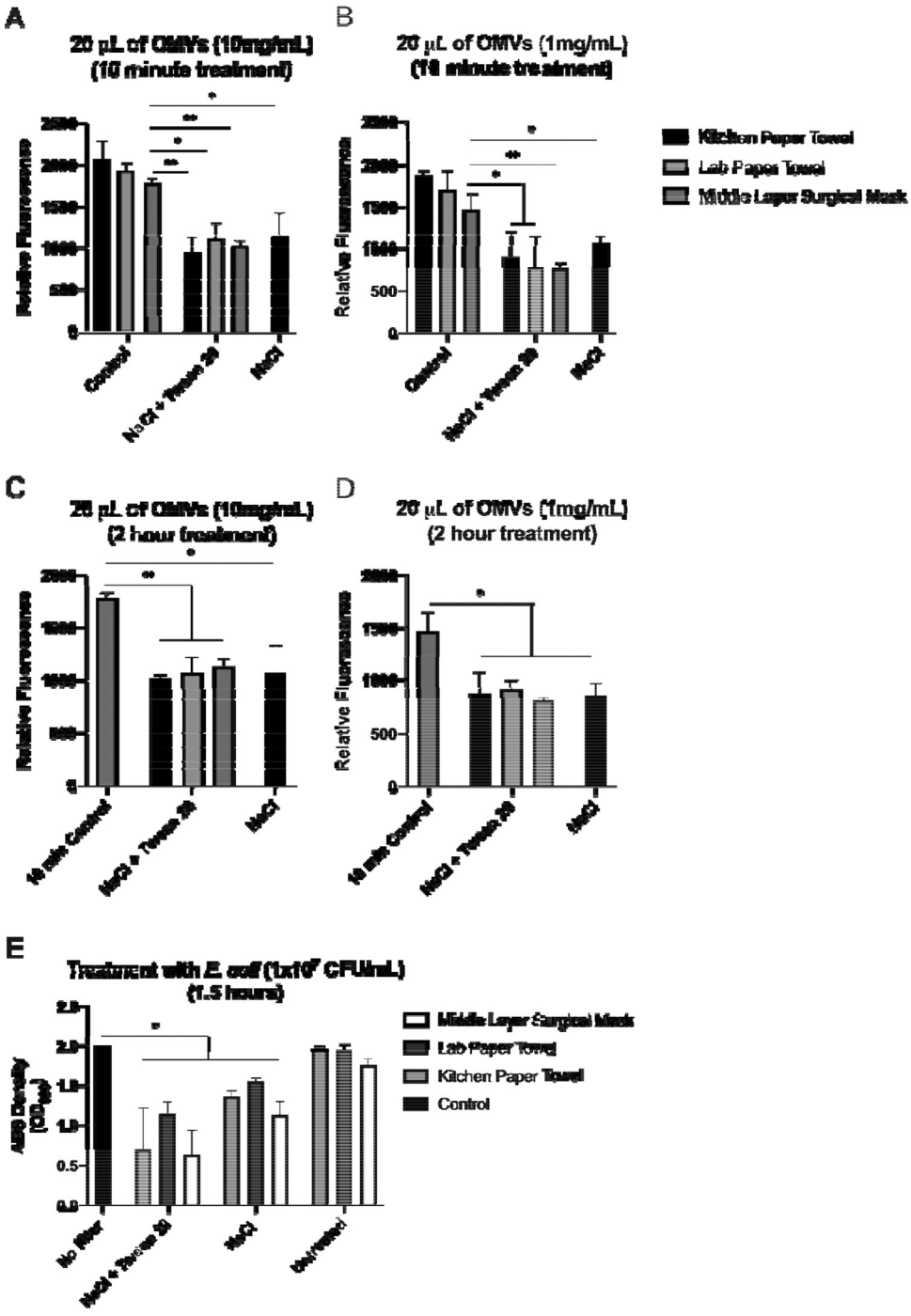

Fig 5. Analysis of microscopy images captured. Using ImageJ, microscopy images were analyzed. Materials treated with NaCl + TWEEN20 and NaCl only solution showed a statistically significant reduced penetration of nanoparticles when compared to the control (untreated) middle filter layer of a standard surgical mask. (A) control (untreated), NaCl + Tween solution-treated materials, and NaCl only solution-treated kitchen paper towel was treated with 20 μL of labeled nanoparticles at a concentration of 10 mg/mL for 10 minutes. (B) control (untreated), NaCl + Tween solution-treated materials, and NaCl only solution-treated kitchen paper towel was treated with 20 μL of labeled nanoparticles at a concentration of 1 mg/mL for 10 minutes. (C) NaCl + Tween solution-treated materials, and NaCl only solution-treated kitchen paper towel was treated with 20 μL of labeled nanoparticles at a concentration of 10 mg/mL for 2 hours. Statistical analysis compares these results to the control (untreated) middle filter layer of surgical mask after 10 minute treatment of nanoparticles at a concentration of 10 mg/mL. (D) NaCl + Tween solution-treated materials, and NaCl only solution-treated kitchen paper towel was treated with 20 μL of labeled nanoparticles at a concentration of 1 mg/mL for 2 hours. Statistical analysis compares these results to the control (untreated) middle filter layer of surgical mask after 10 minute treatment of nanoparticles at a concentration of 1 mg/mL. (E) control (no filter), NaCl + Tween solution-treated materials, NaCl only solution-treated kitchen paper towel, and untreated filter materials were treated with 50 μL *E. coli* (1.7×10^7 CFU/mL) for 1.5 hours and relative growth density was measured after 8 hours in incubation in LB media. Bars, mean ± SD; $^*P < .05$, $^{**}P < .01$.

deionized water prevented penetration of various flu viruses while also deactivating it on the surface.[13] Given the time-consuming and lack of practicality for this method to be used by providers themselves, we wanted to include household products in our study, specifically paper towels, to offer an alternative that may be more convenient. Both the kitchen and laboratory paper towels, as well as the surgical mask filter, when presoaked in a similar NaCl + TWEEN20 solution and left to dry overnight dramatically blocked the penetration of nanoparticles after 10 minutes of treatment (Fig. 2 and 5). The same effects were seen

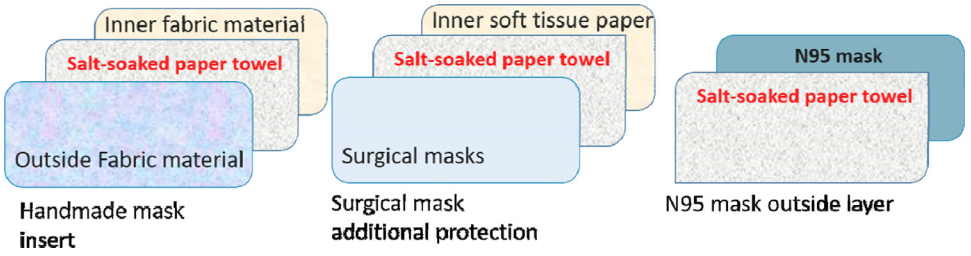

Fig 6. Schema showing potential application of the pretreated paper towel. Pretreated paper towel can be applied over a homemade mask, surgical mask, or N95 respirator to increase its filtration ability and lifespan for reuse.

when the presoaked materials were treated for 2 hours (Fig. 3 and 5), which shows the durability and longevity of this method of filtration.

Filter materials soaked in an NaCl-only solution can also reduce the penetration of nanoparticles

Polysorbate 20, commercially mostly known as TWEEN20, is a polyoxyethylene sorbitol ester and nonionic surfactant commonly found in the laboratory setting which is added to buffers and reagents used in immunohistochemistry. Given the unlikeliness that most healthcare providers have TWEEN20 on hand or readily available, we wanted to test the effectiveness of soaking the filter materials in an NaCl-only solution. We found that both the kitchen and laboratory paper towels, as well as the surgical mask filter, were notably effective at reducing the amount of nanoparticle penetration after 10 minute and 2 hour treatments (Fig. 4 and 5) Interestingly, the few nanoparticles that penetrated the filter material in this method were relatively larger in size. These results suggest that providers unable to use TWEEN20 can use just NaCl to make a filter themselves, at home, that will reduce the penetration of nanoparticles. TWEEN20 most likely plays a role in evenly distributing the salt across the entire filter, preventing any gaps in the NaCl lattice that serves to filter out particles. Although it is probable that a filter made with solution of NaCl and TWEEN20 is more effective, an NaCl only solution will still offer providers more protection than a normal surgical mask would.

Bacterial growth is inhibited by treatment of filter materials

In addition to filtering out nanoparticles in the size range of viruses, it's critical for an effective mask to also prevent penetration of bacteria, which are larger in size. We found that after treating each presoaked filter material with a drop of *E. coli* for 1.5 hours there was an observed decrease in bacterial growth on the filter paper beneath it (Fig 5E). This was observed after presoaking the filter materials in both the NaCl + TWEEN20 solution or the NaCl-only solution. These results suggest presoaking the filter materials in either solution effectively prevents penetration of larger bacteria as well.

DISCUSSION

The purpose of our study is not to replace surgical or N95 masks. Our goal was to increase the protection of a surgical mask, add an additional layer outside of surgical and N95 masks to increase its lifespan, and give members of the community a convenient and economic option to increase the effectiveness of their home-made masks so surgical masks can be rationed for medical workers. These potential applications are illustrated in Figure 6.

Due to the urgency of addressing the shortage of PPE worldwide, our group only tested the filter materials by droplet test. Our results suggest that after 10 minutes of treatment with a droplet there is a no notable dose-dependency effect on the efficacy of the pretreated filter. At the 2 hour time point, there appears to be a slight effect on dose-dependency. Therefore, we recommend healthcare professionals using this method to increase the effectiveness of their PPE be cautious past this time point and preferably replace their pretreated filter after 2 hours of use.

One study conducted at the University of Hong Kong-Shenzhen Hospital found that kitchen paper towels have a similar layout of fibers as the filter layer in surgical masks, and that 2 paper towels stacked together have equal filtration ability as a surgical mask.[17] Therefore, we believe this is one underlying mechanism for the similar efficacy between paper towels and surgical mask filters shown in this study. In addition, small particles do not necessarily pass through the filter easier or faster than larger particles. Small particles are lighter in weight and sometimes charged, which in addition to Brownian motion, may explain our results which showed that larger particles more easily penetrate the paper towel treated in the salt-only solution.

Future studies will be required to address the performance of this solution in the setting of aerosol and splash. Also, it can be presumed that 2 layers of solution treated filter material will perform better than one, however this should also be tested in future studies. In addition, the potential mechanism that leads to the increased efficacy is likely driven by osmotic pressure and future studies should examine images under electron microscopy to understand this. It is unclear whether other substance, such as sugar which also generates osmotic pressure, can have the same effect as NaCl does in this study. This can be studied in the future work. Lastly, for definite results, a true SARS-Cov-2 viral particle should be used to repeat the above experiments in a BSL 3/4 laboratory.

CONCLUSIONS

In conclusion, we believe this method should be safe and convenient and can be adopted by healthcare professionals and community people during this pandemic. Although only tested by droplet, the observed effects are significant and could play a critical role in ensuring the safety of our healthcare workers and community people during the COVID-19 pandemic.

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