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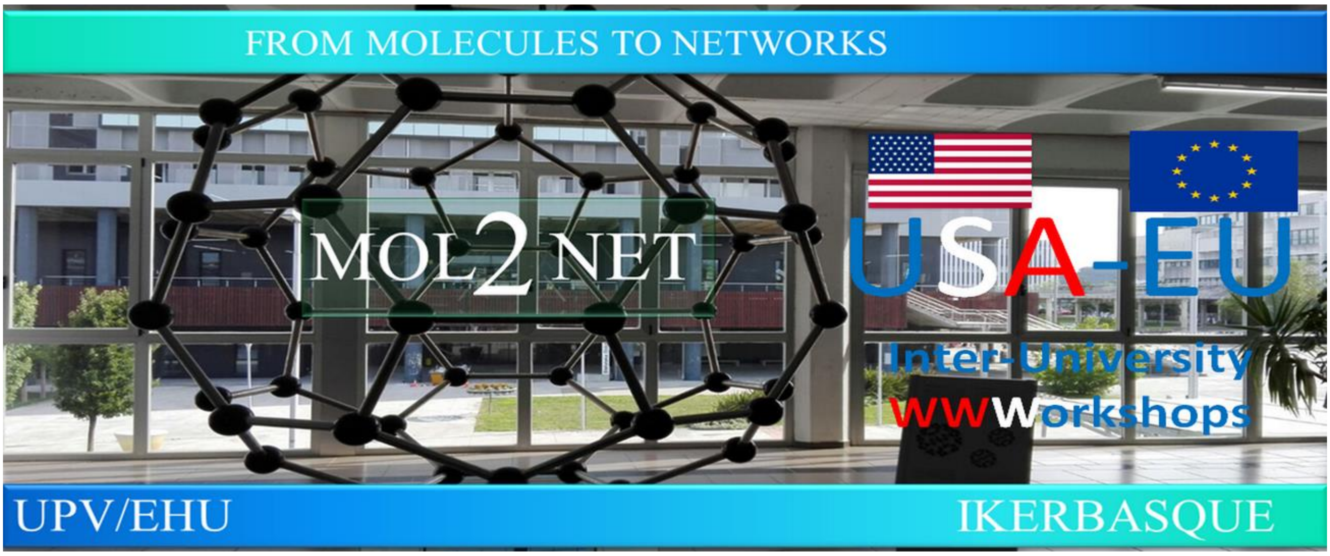

Protective infection prevention clothing and transparent equipment capable of inactivating SARS-CoV-2 and multidrug-resistant bacteria

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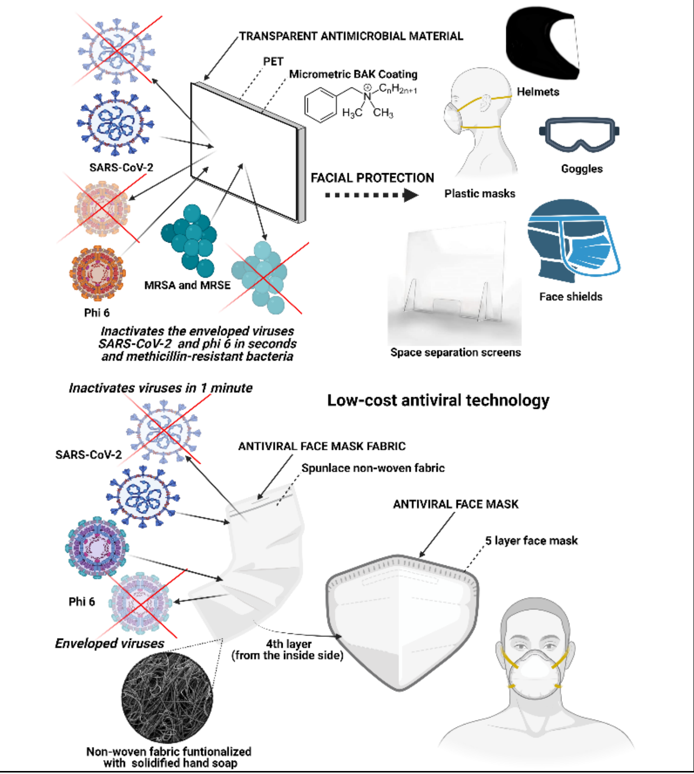

Abstract: Face masks, face shields and other personal protective equipment (PPE) have been accepted to be an effective tool in order to avoid bacterial and viral transmission, especially against indoor aerosol transmission. However, commercial PPE are made of materials that are not capable of inactivating pathogenic particles such as SARS-CoV-2 or multidrug-resistant bacteria. In this context, we describe here the development of new antimicrobial materials that can be used in PPE manufacturing, which include composite materials with a biofunctional coating of benzalkonium chloride (BAK) or solidified hand soap. These coatings were capable of inactivating SARS-CoV-2 in less than 1 minute of viral contact. Moreover, the BAK coating was also effective against the life-threatening methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. These novel protective materials will be useful to combat the current COVID-19 pandemic in the current bacterial-resistant era.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first reported in Wuhan, Hubei province, China, in December 2019[1] has rapidly spread all around the globe and has caused the current COVID-19 pandemic, putting in high risk the health and economy of most developed and underdeveloped countries and causing a still increasing number of deaths in most countries. SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus[2], belonging to Baltimore group IV[3]. It is similar to other enveloped virus such as influenza A virus (H1N1) that can be inactivated by quaternary ammonium compounds such as benzalkonium chloride (BAK)[4]. Moreover, the oral rinse Dequonal, which contains BAK, has shown antiviral activity against SARS-CoV-2 supporting the idea that oral rinsing might help to reduce a possible viral load in saliva and become an advantage in order to reduce SARS-CoV-2 transmission[5]. The Centers for Disease Control and Prevention has repeatedly recommended hand washing with hand soap and water as an efficient strategy of preventing SARS-CoV-2 spread[6].

Face masks and other facial protective equipment such as face shields have been accepted as effective protective tools by blocking the pass of viral and bacterial particles[7,8]. However, they are made of materials that only impede the pass of the pathogenic particles and are not able to inactivate them. In this regard, we propose here the use of functionalized materials that are able to inactivate pathogens. Thus, physical absorption of BAK or hand soap using the dip coating method[9] onto the surface of a commercial non-woven fabric filters or polyethylene terephthalate (PET) plastic, have shown to be a low-cost antimicrobial strategy to produce protective equipment capable of preventing SARS-CoV-2 and/or multidrug-resistant bacteria infections[8,10,11].

Bacterial resistance is becoming a real-life threat to humans. In fact, the World Health Organization (WHO) has reported that deaths caused by this kind of pathogens could be higher than deaths caused by other relevant diseases including cancer by the year 2050[12].

Materials and Methods

Antiviral tests using SARS-CoV-2

A volume of 50 µL of a viral suspension in phosphate-buffered saline (PBS) was added up to each filter at a titer dose of 1.3 x 10^5 median tissue culture infectious dose per material sample (TCID50/sample). Incubation for 1 minute at room temperature was carried out after virus addition. After this, 1 mL of PBS was added together with the sample material and then vortexed for 5 minutes at room temperature.

Viral titers were concluded through the TCID50 assays inside Biosafety Level 3 laboratory at Kyoto University. TMPRSS2/Vero cells[13] (JCRB1818, JCRB Cell Bank) cultured with the minimum essential media (MEM, Sigma-Aldrich) supplemented with 5% fetal bovine serum (FBS), 1% penicillin/streptomycin, were seeded into 96-well plates (Thermo Fisher Scientific). Samples were serially diluted 10-fold from 10^{-1} to 10^{-8} in the culture medium. Dilutions were placed onto the TMPRSS2/Vero cells in triplicated and incubated at 37ºC for 96h. Cytopathic effect was evaluated under a microscope. TCID50/mL was calculated using the Reed-Muench method[14].

Antibacterial tests

Agar disk diffusion tests[15,16] were performed in order to determine the antibacterial activity of treated and untreated materials. Lawns of methicillin-resistant *Staphylococcus aureus*, COL[17], and methicillin-resistant *Staphylococcus epidermidis*, RP62A[18], in a concentration approximately of 1.5 x 10^8 CFU/mL in tryptic soy broth (TSB), were cultivated on trypticase soy agar plates. The sterilized disks were placed upon the lawns of bacteria to be incubated aerobically at 37ºC for 24 hours. The antibacterial activity of the tested filter disks was expressed according to the following equation (1)[15]:

$$nw_{halo} = \frac{\frac{d_{iz}-d}{2}}{d} \quad (1)$$

where nw_{halo} indicates the normalized width of the antimicrobial inhibition zone, d_{iz} is the inhibition zone diameter and d refers to the sample disk diameter. These diameters were measured by image software analysis (Image J, Wayne Rasband (NIH), Bethesda, MD, USA). The tests were carried out six times on different days to ensure reproducibility.

Results and Discussion

Face mask filter with a biofunctional coating of BAK

Results accomplished with the TCID50/mL method related to reduction of viral activity of SARS-CoV-2 after 1 minute of contact are shown in Figure 1[11].

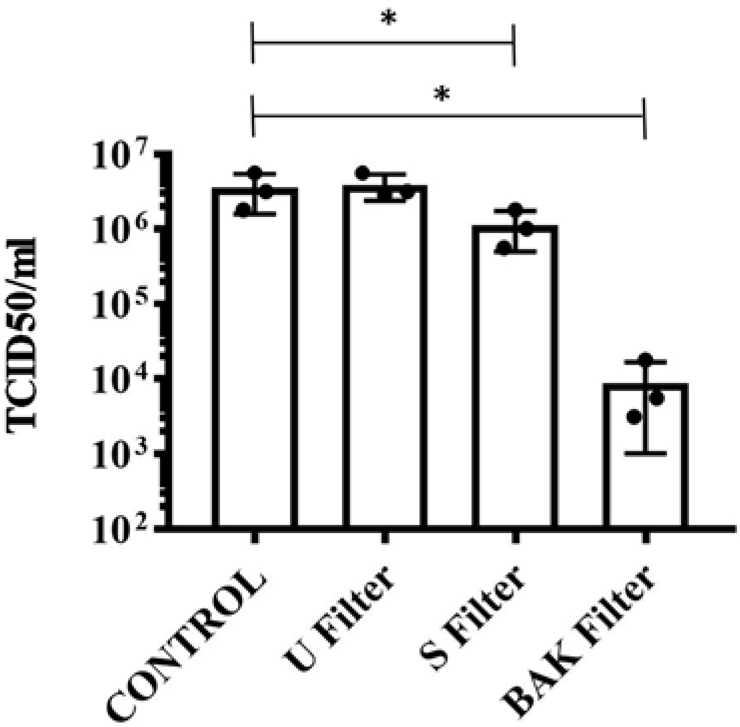

Figure 1. Reduction of infectious titers of SARS-CoV-2 after 1 minute of contact. Untreated filter (U filter), filter treated with the ethanol solvent (S filter), filter with the biofunctional BAK coating (BAK filter) and control (virus without being in contact with any material) via the TCID50/mL method. A dot plot is data set based on the value of each point[11].

These results clearly show that BAK-treated filter is highly effective against SARS-CoV-2 even after 1 minute of contact. This is in good agreement too with antiviral tests performed with a biosafe viral model of SARS-CoV-2 (bacteriophage Φ6)[11]. The antibacterial results of the BAK-treated filters against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are shown in Figure 2[11]. The filter treated by the dip coating method[9] with 70% ethanol containing 0.1% BAK showed high antibacterial activity against both bacteria[11]. This antibacterial activity is attributed to the positively charged nitrogen atoms, which cause disruption of their phospholipid bilayer membrane[19], the glycoproteinaceous envelope and the associated spike protein interacting with the ACE2 receptor in the infection of host cells[20].

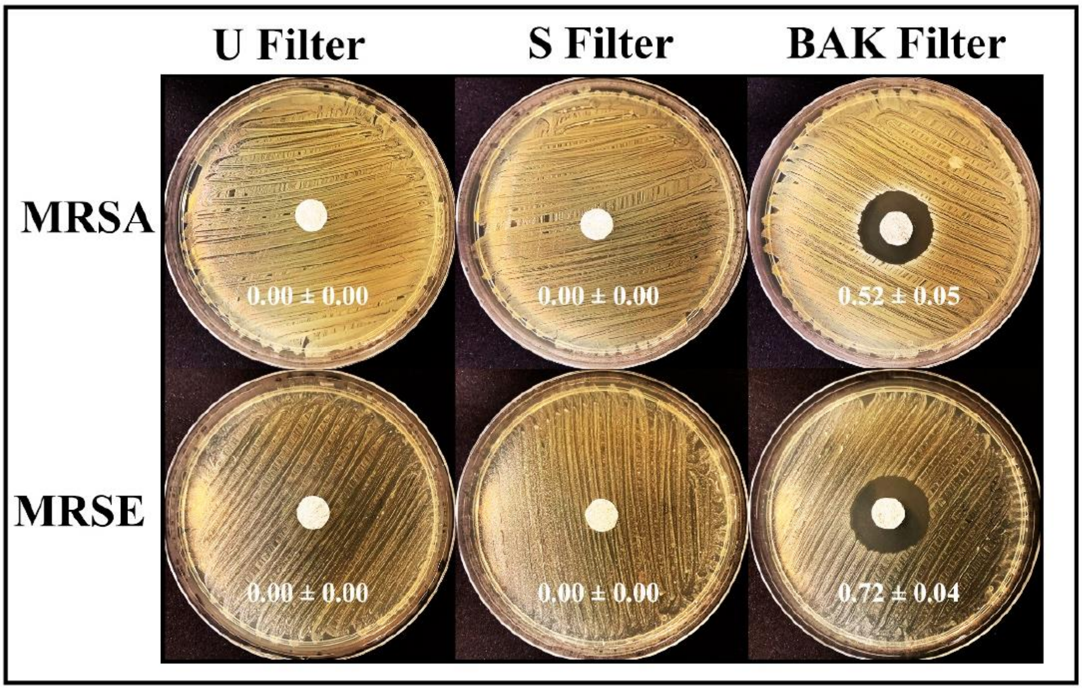

Figure 2. Antibacterial agar disk diffusion tests. Untreated filter (U filter), filter treated by dip coating with the ethanol-based solvent (S filter) and filter with the biofunctional BAK coating (BAK filter) after 24 hours of culture at 37ºC. The normalized widths of the antibacterial *halos*, expressed as mean ± standard deviation and calculated with equation (1), are shown in each image[11].

Antimicrobial face shield

A polyethylene terephthalate (PET) facial protective equipment was also functionalized with BAK to produce a composite with potent antimicrobial activity. This face shield showed to be very effective against SARS-CoV-2 after 1 minute of contact (Figure 3).

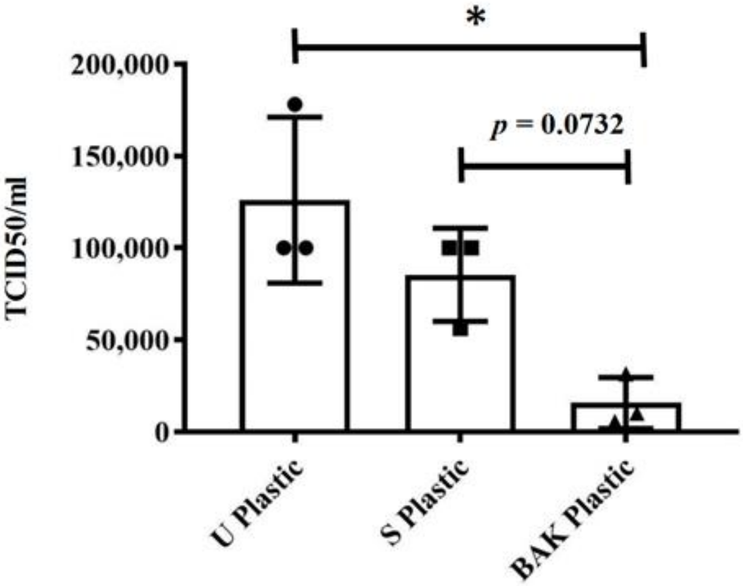

Figure 3. Reduction of infection titers in PFU/mL of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) after 1 minute of contact determined by the median tissue culture infectious dose per mL (TCID50/mL) method. Untreated PET (U plastic), PET treated with the ethanol solvent (S Plastic) and the PET with biofunctional BAK coating (BAK plastic). A dot, square and triangle plot is a data set based on the value of each point. *p<0.05[8].

These results were in good agreement with the antiviral results of the biosafe viral model, bacteriophage Φ6[8]. Figure 4 shows the potent antibacterial activity of the BAK-treated plastic against MRSA and MRSE with similar normalized antibacterial halos[8].

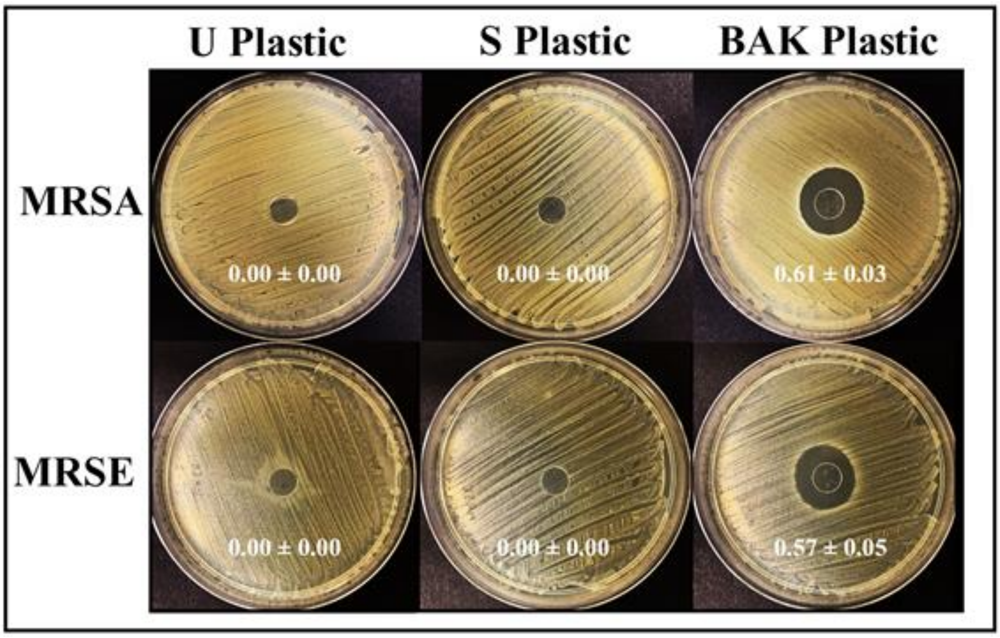

Figure 4. Antibacterial agar disk diffusion tests with two multidrug-resistant bacteria: MRSA and MRSE. Untreated PET (U Plastic), PET treated by dip coating with the ethanol-based solvent (S Plastic) and the PET with the biofunctional BAK coating (BAK Plastic) after 24 hours of incubation at 37ºC. The normalized widths of the antibacterial *halos*, expressed as mean ± standard deviation and calculated with equation (1), are shown in each image[8].

Face mask functionalized with solidified hand soap

An antiviral and non-cytotoxic fabric with a solidified hand soap coating was designed with the aim to fabricate an antiviral face mask. SARS-CoV-2 showed a significant reduction of infectious titers after being in contact with the hand soap-treated filter as shown in Figure 5[10].

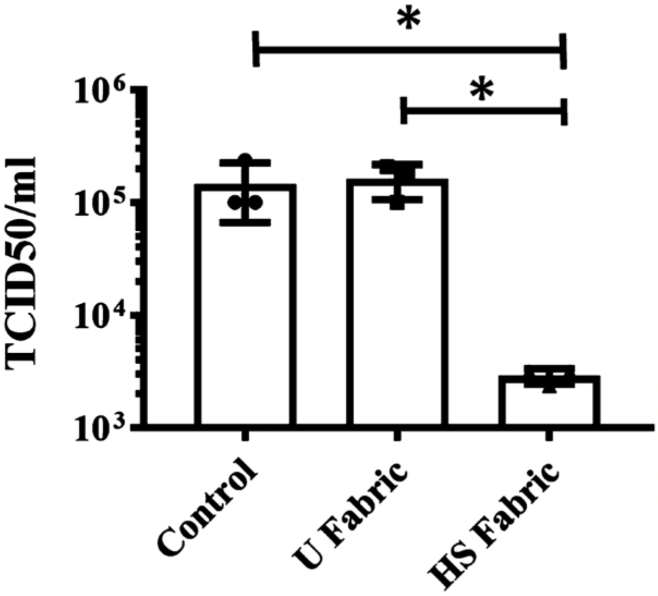

Figure 5. Infectious titers of SARS-CoV-2 after 1 minute of contact with the untreated fabric (U fabric) and the fabric functionalized with solidified commercial hand soap (HS Fabric) and control (without being in contact with any fabric) by the TCID50/mL method[10].

These tests were carried out also with a non-treated filter as a control which showed no reduction of viral activity as expected. These results clearly demonstrate the potent anti-SARS-CoV-2 activity of this next generation fabrics, in good agreement with the results obtained using another enveloped virus, the bacteriophage Φ6, which is usually used as biosafe viral model of SARS-CoV-2[10]. The hand soap-treated mask fabricated with these antiviral filters showed no toxic effects in human keratinocyte cells.

Conclusions

The first antimicrobial face mask filters and face shields capable of inactivating SARS-CoV-2 (>99% of viral inhibition) in one minute of viral contact and multidrug-resistant bacteria such as MRSA and MRSE are reported. These next generation antimicrobial developments are new promising tools to combat the COVID19 pandemic and future threats caused by pathogens and multidrug-resistant microorganisms. All these advanced developments were produced following an economic, reproducible, and rapid procedure using benzalkonium chloride or hand soap and the dip-coating method. This kind of protective equipment impedes viral and bacterial inhalation and entry into the body through the respiratory tract or by splashing, providing an extra biosafety due to its capacity of inactivating the infectious microorganisms as soon as they are in contact with the protective element. The applications of these antimicrobial protective equipment are immense in infection prevention clothing such as caps, scrubs, shirts, trousers, disposable gowns, overalls, hoods, aprons, and shoe covers, and for the fabrication of broad-spectrum antimicrobial transparent plastic-based facial protective tools such as face shields, goggles, helmets, plastic masks and space separation screens used for counters or vehicles. Therefore, these technologies could reduce significantly the COVID19 global spread and help in future pandemics in the current microbial resistant era.

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