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Synergistic Polymer Coatings with Antibacterial and Antiviral Properties for Healthcare Applications

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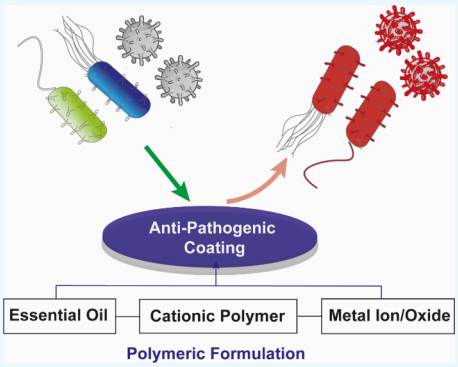
ABSTRACT: The role of frequently touched surfaces in the transmission of infectious diseases is well-documented, and the urgent need for effective surface technologies with antipathogen activity has been highlighted by the recent global pandemic and rise in antimicrobial resistance. Here, we have explored combinations of up to 3 different classes of compounds within a polymeric matrix to enable the fabrication of coatings with broad-spectrum activity. Compounds were either based on metals or metal oxides, namely, copper, silver, and copper oxide, essential oils, namely, cinnamaldehyde, tea tree oil, and carvacrol oil, or cationic polymers, namely, poly(ε-lysine) and poly(hexamethylene biguanide). These compounds were mixed into a polymer matrix, coated, and dried to yield durable coatings. Coatings containing up to 7.5% (w/w) of the compounds were assessed in the zone of inhibition and biofilm assays using *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as infectivity assays using human coronavirus OC43. Our data demonstrate that a selected combination of additives was able to provide a 5-log reduction in the colony-forming units of both bacteria and a 4-log reduction in viral infectivity. This simple but highly effective technology is expected to find applications in environments such as hospitals, aged care facilities, or public transport.



1. INTRODUCTION

Frequently touched surfaces are known to be an important source of pathogen transmission.[1,2] Particularly in environments where vulnerable people are present, such as hospital or aged care environments, the prevention of bacterial and viral pathogen transmission via this path has received increased attention over the past few years.[3,4] Moreover, frequently touched surfaces associated with public transport, schools, and publicly accessible buildings have also become a focal point for prevention measures, such as frequent cleaning with antimicrobial agents.[5,6]

To address the risk of pathogen transmission on these surfaces, a broad range of technologies have been proposed, and some of these have been translated into commercial products. Examples range from frequent cleaning with solutions containing antimicrobial agents to the exposure of surfaces to UV light.[7−10] Here, surface coatings providing effective protection over an extended period are an attractive solution, often requiring the following properties:

- The coating is highly effective against a broad spectrum of pathogens, including Gram-positive and Gram-negative bacteria as well as viral pathogens.
- The coating can be easily applied, for example, by spray coating or using a brush.
- The coating is durable for an extended period when frequently touched.
- The coating can be easily removed without the use of toxic solvents.
- The polymer coating formulation has a suitable shelf life after incorporation of all components

However, this list is not comprehensive, and additional factors such as the use of sustainable materials also need to be considered.[11]

While the focus related to the transmission of pathogens in the context of frequently touched surfaces has been traditionally on bacteria, this changed during the global pandemic. The need for preventive technologies to reduce the spread of the highly infective coronavirus SARS-CoV-2[12] also shifted the attention to viral surface transmission. The ability of the SARS-CoV-2 virus in particular to remain infective on surfaces such as stainless steel and plastic for hours has been documented.[13] Therefore, antipathogen surface technologies are now expected

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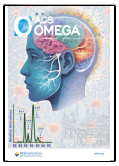

to provide effectiveness against a broad range of pathogens, including bacteria and viruses.

While routine manual sanitization and disinfection protocols of surfaces can be effective to reduce the spread of bacterial and viral pathogens in, e.g., hospital environments, the increased use of common disinfectants containing quaternary ammonium compounds, hydrogen peroxide, bleach, and alcohols may also cause detrimental effects on human health and the environment, which need to be considered.[14] Moreover, the costs associated with (typically manual) routine surface cleaning protocols are high and do not guarantee complete decontamination.[15] Effective antimicrobial agents over an extended period reduce the cost of sanitization and provide more effective protection.

The effectiveness of antimicrobial surface technologies is related to the mechanism of action of the antimicrobial compound and the characteristics of the outer membranes of the microorganisms. For example, virus adsorption has been proposed to be regulated by the spike protein, which is an important component of the outer layer of the SARS-CoV-2 virion.[16] Once in contact with surfaces, the ability to maintain their viability differs between viruses and bacteria. On one hand, the ability of a virus to remain infectious is a function of environmental factors such as humidity and temperature.[17] On the other hand, bacteria affected by surrounding environmental conditions can survive through their ability to organize and thrive in biofilms. These biofilms are communities of microorganisms enclosed in a 3D structural matrix composed of extracellular polymeric substances (EPS). The matrix provides a structural support and protects embedded bacteria from external environmental challenges including antimicrobial agents.[18]

The increased use of antibiotics in humans and animals, industrialization, and the greater movement of people are some factors influencing the development of antimicrobial resistance (AMR).[19] As defined by the World Health Organization (WHO), AMR is developed when pathogens (bacteria, viruses, fungi, and parasites) change over time, reducing their response to treatment or medicine. This increases the risk of morbidity and mortality as their infections become hard to treat.[20] Gram-negative strains such as *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Enterobacteriaceae* are of great concern due to their high level of resistance to commercially available drugs and are therefore included in the priority pathogens published by the WHO.[21] They represent two of the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) organisms with the ability to evade antibiotics and represent a leading cause of healthcare-acquired infections worldwide.[22]

Among antibacterial and antiviral agents, metal ions and metal oxides are of particular interest including silver-, copper-, and zinc-based materials. Silver-based nanomaterials are the most established metal antimicrobials.[23] Silver nanoparticles have the ability to interact with disulfide bonds of the proteins in viruses, bacteria, and fungi. By interfering with the S−S bond, silver nanoparticles and silver ions can modify the structure of the proteins and disrupt the functionality.[24] Compared with silver nanoparticles, other metal species such as copper oxide-based nanoparticles can be more cost-effective and can be easily synthesized by green technologies, representing promising candidates.[25] Other antipathogenic compounds are essential oils (EO). Importantly, their natural origin has promoted a better perception for consumers and a high demand for their use in various industries, including food applications.[26] Alternatively, cationic polymers with their two functional components, the cationic part that facilitates electrostatic interactions with the bacterial negatively charged membrane and the hydrophobic groups that are expected to disrupt them, have also found a wide range of applications as antipathogenic agents.[27] The majority of the studies where these compounds have been employed focused on the fabrication of antimicrobial technologies.[28−30] However, some reports have also highlighted their antiviral activity. For example, Hodek et al.[31] evaluated coatings containing silver, copper, and zinc cations against different viruses including nonenveloped, double-stranded DNA and negative and positive single-stranded RNA viruses. Virucidal activity at diverse exposure times was observed against most enveloped viruses with better performance against human immunodeficiency virus type 1 (HIV-1). The antiviral activity of other widely used antimicrobial compounds such as cationic polymers, e.g., poly(hexamethylene biguanide) (PHMB), has also been evaluated with concerns about cytotoxicity. Despite the moderate *in vitro* activity of PHMB against HIV-1 virus, high cytotoxicity toward cervicovaginal epithelial cells was reported.[32] In another study, PHMB was incorporated into a synthetic polymeric fabric, and its antiviral activity against feline coronavirus, a surrogate virus of SARS-CoV-2, was evaluated.[33] The fabric killed 99% of the virus within 2 h of contact in addition to displaying antibacterial activity against *S. aureus*. Similarly, when the antiviral activity of the essential oil carvacrol was evaluated against the nonenveloped murine norovirus, inactivation of the virus after 1 h of exposure was reported acting directly on the viral capsid and RNA of the virus.[34]

Importantly, synergistic effects between different classes of antimicrobial as well as antiviral compounds have also been observed across a broad range of examples. If the mechanism of action of 2 or more compounds is different, this combination is often more difficult to overcome for pathogens compared with single compounds. For example, the use of antimicrobial peptides in combination with antibiotics has been reported to provide significantly enhanced antibacterial efficacy compared to the single components.[35] In this particular combination, the antimicrobial peptide is more resistant to the development of antimicrobial resistance, opening the door to extending the lifetime of antibiotics. Other examples where this general concept of synergistic effects has been exploited range from the combination of silver with antibiotics[36] to combining different types of metal nanoparticles.[37] Overall, the exploitation of synergistic effects has emerged as an important concept, particularly in the fight against antimicrobial resistance. However, efforts to explore this concept in the context of frequently touched surfaces have only emerged recently.[38]

Here, we report the fabrication of broad-spectrum polymer coatings using a blending method. Three categories of established compounds with reported antibacterial and antiviral activity were selected for screening experiments using Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria, as well as the human coronavirus OC43.

Compounds based on metals or metal oxides, essential oils, and cationic polymers were mixed into a polymeric matrix, coated onto substrate materials, and dried to yield a durable coating.

A commercial nail polish formulation was used as the polymeric matrix to provide a coating that can be easily applied to a range of substrate materials, that is durable when touched frequently, that is nontoxic, and that can be easily removed again.

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Selected combinations of additives representing all 3 different classes of compounds were able to provide more than a 5-log reduction in the colony-forming units of both Gram-positive and Gram-negative bacteria and a 4-log reduction in the infectivity of virus. The coatings are versatile and can be applied to a variety of surfaces where antipathogenic activity is desired, including but not limited to frequently touched surfaces in healthcare settings.

2. MATERIALS AND METHODS

2.1. Materials. A commercial polymer formulation was used to incorporate compounds with antipathogen properties and to produce coatings (gel-like nail polish, Sinful Colors). The cationic polymers poly ε-L-lysine HCl (MW, 3500−5000 Da) (purity, 98%) and polyhexamethylene guanidine (purity, 94%) were obtained from Biosynth. Essential oils including cinnamaldehyde (purity, 95%) and carvacrol (purity, 98%) were obtained from Sigma-Aldrich. Tea tree oil was purchased from Thursday Plantation. Silver and copper nanoparticles as well as copper(II) oxide (nanopowder, <50 nm particle size (TEM)) were acquired from Sigma-Aldrich. Silver-coated nanoparticles were obtained from Nanoshel containing 80% copper and 20% silver (size, 80−100 nm; purity of 99.9%). For bacterial and viral testing, nutrient agar, Mueller−Hinton broth, and sterile filter paper were purchased from Thermo Fisher Scientific (Oxoid). Eagles' minimal essential medium (EMEM) and 1−5−10% fetal calf serum were acquired from Sigma-Aldrich. Penicillin/streptomycin (100 μg/uL), sodium pyruvate (1 mM), nonessential amino acids (0.1 mM), sodium bicarbonate (0.18%), L-glutamine (2 mM), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (20 mM) were purchased from Life Technologies. Dulbecco's modified eagle medium high glucose (DMEM) was obtained from Hyclone. Aluminum discs were laser-cut to various sizes and used as substrates in the different assays.

2.2. Pathogens. *2.2.1. Bacteria.* Gram-negative *Pseudomonas aeruginosa* ATCC 27853 and Gram-positive *Staphylococcus aureus* ATCC 25923 strains were employed. Bacterial stocks were prepared in 60% glycerol and stored at −80 °C until needed. All surfaces were sterilized with UV light prior to bacterial assays.

2.2.2. Viruses. The antiviral activity of the materials was tested using human coronavirus (OC43) according to ISO 21702:2019. OC43 virus was obtained from ATCC (ATCC number VR-1558), and a working stock was generated by passaging the virus three times in an MRC-5 fetal lung fibroblast cell line. MRC-5 cell lines were used to propagate and assay viral infectious titers. MRC-5 cells were seeded at a final concentration of 1×10^6 cells/10 mL in 10% EMEM. All surfaces were sterilized with UV prior to viral assays.

2.3. Coating Fabrication. *2.3.1. Single-Component Coatings.* Suspensions containing 5% (v/w) of each antimicrobial additive were created by adding 50 mg of the antimicrobial agent and 200 μL of ethyl acetate to a glass vial per mL of polymer paint. This solution was then mixed thoroughly in each case using a spatula. Substrate materials used in this study included filter paper, aluminum discs, and individual wells (bottom and walls) in 96-well plates. Samples were painted within a biohazard cabinet to reduce the level of contamination. After allowing the samples to dry for at least 3 h, these were UV treated within the cabinet for 20 min on each side to sterilize the surface.

2.3.2. Multiple-Component Coatings. Suspensions containing 2.5% (v/w) of each antimicrobial additive were created for two-component or three-component polymer coatings by adding 25 mg of each component and 300 μL of ethyl acetate to a glass vial per mL of the polymer paint. This solution was mixed thoroughly in each case using a spatula. These suspensions were then used to paint the substrate materials using a small brush. Substrate materials used in this study included filter paper, aluminum discs, and individual wells (bottom and walls) in 96-well plates. Samples were painted within a biohazard cabinet to reduce contamination. After allowing the samples to dry for at least 3 h, these were UV treated within the cabinet for 20 min on each side to sterilize the surface.

2.4. Coating Thickness Measurements. Coating thickness measurements were carried out using a micrometer (Mitutoyo Absolute model ID S112x). The commercial nail polish polymer formulation and single-component coatings and multiple-component coatings were applied on aluminum substrates and dried overnight. After the overall thickness was measured, the paint was removed, and the thickness was measured again in the same location. The coating thickness was the difference of the 2 thickness measurements. A minimum of 3 thickness measurements were carried out for each coating type.

2.5. X-ray Photoelectron Spectroscopy (XPS). X-ray photoelectron spectroscopy (XPS) analysis was used to characterize the surface chemistry (surface elemental composition) of the coatings. XPS analysis was performed using an AXIS Ultra-DLD spectrometer (Kratos Analytical Ltd., UK) equipped with a monochromated Al Kα X-ray source at a power of 180 W (12 mA, 15 kV). An internal electron flood gun was used to compensate for sample charging during irradiation. All of the elements presented were identified from low-resolution survey spectra (acquired at a pass energy of 160 eV). The atomic concentrations of the detected elements were calculated using integral peak intensities and sensitivity factors supplied by the manufacturer. Data processing was performed using CasaXPS processing software, version 2.3.21 (Casa Software Ltd., Teignmouth, United Kingdom).

2.6. Antimicrobial Testing. *2.6.1. Zone of Inhibition Assay.* Bacterial stocks were streaked onto nutrient agar plates to be used as a working stock. After 24 h of incubation at 37 °C, a single colony from the streaked plate was used and inoculated in 10 mL of Mueller−Hinton broth (MHB), which was incubated overnight (180 rpm) at 37 °C. This culture was further diluted in MHB to obtain 10^6 colony-forming units per mL (cfu mL^{-1}). Cultures were spread evenly onto agar plates by using a sterile swab. Sterile filter paper discs (6 mm Ø, Oxoid antimicrobial susceptibility test discs) previously coated with mixtures of the polymeric formulation and individual antimicrobial compounds at a 5% (v/w) concentration were gently pressed onto the surface of the agar plates. Discs coated with the polymeric film alone were used as the controls. Plates were then inverted and incubated for approximately 24 h at 37 °C, and the diameter of the inhibition zones was measured in millimeters, including the diameter of the disc.

2.6.2. Biofilm Experiments. Bacterial stocks were streaked onto nutrient agar plates to be used as a working stock. After 24 h of incubation at 37 °C, a single colony from the streaked plate was used and inoculated in 10 mL of MHB, which was incubated overnight (180 rpm) at 37 °C. For the biofilm test, aluminum discs (6 mm-diameter laser-cut aluminum discs) with both sides coated with specific antimicrobial polymer combinations were incubated for 24 h at 37 °C with 50 μL of bacteria solutions (10^6 cfu mL^{-1}). After incubation, samples were gently washed 3 times using sterile phosphate-buffered saline (PBS) to remove any planktonic cells. The samples were then individually transferred

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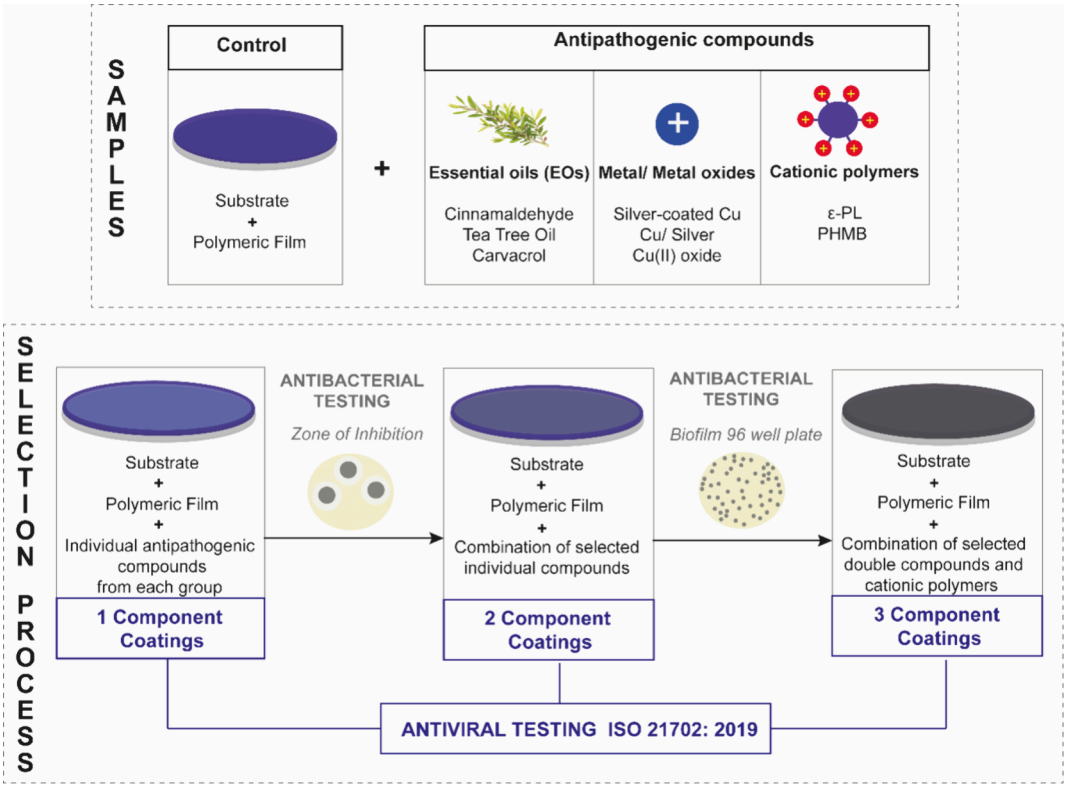

Figure 1. Schematic representation of the polymer coating fabrication and selection process. Substrates representing the unmodified polymer were used as the controls. Coatings representing 1 antimicrobial component were then fabricated and tested in a zone of inhibition test. The lead candidates were then selected for a second set of experiments, in which coatings with 2 components were tested using a biofilm 96-well plate assay. Based on these results, samples with 3 components were fabricated and tested in the biofilm 96-well plate assay. In parallel, selected samples containing 1, 2, and 3 components underwent the antiviral ISO 21702:2019 test.

to sterile Eppendorf tubes containing 1 mL of sterile PBS. Samples were vortexed (3 times for 30 s) and sonicated for 10 min to detach the bacterial biofilm from the surface. After sonication, samples were vortexed again for 30 s. The suspensions obtained were serially diluted, and aliquots of 20 μL were plated on nutrient agar plates for viable counts.

2.7. Antiviral Efficacy According to ISO 21702:2019. *2.7.1. Human Coronavirus Inactivation Assay.* Twenty-five μL of OC43 at 4×10^7 TCID50/mL (diluted in EMEM 1% FCS to give 1×10^6 TCID50/25 μL) was applied to selected coatings on 15 mm aluminum discs containing one, two, or three antimicrobial components placed into sterile 24-well tissue culture plates. Samples were then incubated for 1 h in a class II biosafety cabinet (lid of the plate left on), with each sample being tested in triplicates. Similarly, the final selection of coatings representing 3 different antimicrobial components were incubated for 30 min with 25 μL of OC43 at 4×10^7 TCID50/mL (diluted in EMEM 1% FCS to give 1×10^6 TCID50/25 μL), and each sample was tested in quadruplicates. As controls, 25 μL of 1% EMEM or 1% DMEM was added to assess cell toxicity of the materials. Samples were assayed in MRC-5 cells. One h post virus/media addition, samples were collected in 975 μL of virus infectivity assay media (EMEM 1% FCS) and immediately transferred to a sterile 1.7 mL tube. All samples and virus inoculums were stored at −80 °C upon harvest, until the infectivity assay was performed. The cell toxicity of the components was verified by adding 25 μL of media for 1 h. Data were analyzed by ordinary one-way ANOVA.

2.7.2. Measure of Viral Infectivity using the TCID50 Assay. Human coronavirus (OC43) infectivity following exposure to different materials was determined by using a TCID50 assay in MRC-5 cells. Cells were seeded at a final concentration of 1×10^6 cells/10 mL in growth media and incubated overnight at 37 °C/5% CO_2 or until 30−40% confluence was achieved. Samples/viruses were then serially diluted in the required amount of 1% EMEM. A 200 μL portion of each sample was pipetted into a 96-well plate. Samples were analyzed in quadruplicates. Plates were incubated for 5 days at 35 °C/5% CO_2. Finally, the cytopathogenic effect (CPE) was observed using light microscopy, and the tissue culture infectious dose (TCID50) was calculated using the Karber method.[39]

3. RESULTS AND DISCUSSION

The work described in this study is based on the hypothesis that the combination of agents with known antimicrobial activity in a polymer coating can achieve much more potent antimicrobial activity compared to the individual components against both bacterial and viral pathogens. To test this hypothesis, different antimicrobial agents representing 3 different categories of antimicrobial agents were employed, including the following:

1. Metal/metal oxide nanoparticles (Cu(II)O, silver, copper, and silver-coated copper)
2. Essential oils (cinnamaldehyde, tea tree oil, and carvacrol)
3. Cationic polymers (ε-PL and PHMB)

A schematic representation of the process employed to select the best antipathogenic combinations is presented in Figure 1. Antibacterial testing was attempted for all sample types using representative Gram-positive and Gram-negative bacteria. Starting with screening experiments for polymer formulations

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Table 1. XPS Analysis of Polymer Coatings Representing Unmodified Controls and Combinations of 1, 2, and 3 Antimicrobial Components

	O%		N%		C%		Si%	
samples	mean	std	mean	std	mean	std	mean	std
polymeric film	32.6	0.2	5.1	0.2	62.1	0.2	0.1	0.0
Individual components (5%)								
cinnamaldehyde	31.3	0.2	4.5	0.2	64.0	0.4	0.3	0.1
Cu(II)O	32.7	0.2	5.0	0.1	62.0	0.3	0.2	0.0
ε-PL	32.9	0.1	5.1	0.2	61.8	0.2	0.2	0.1
PHMB	32.9	0.2	5.1	0.1	61.8	0.2	0.2	0.0
Two-component combination (2.5% each)								
cinnamaldehyde/Cu(II)O	31.3	0.1	4.6	0.1	63.9	0.2	0.3	0.1
cinnamaldehyde/PHMB	31.1	0.3	4.5	0.2	64.2	0.3	0.2	0.1
cinnamaldehyde/ε-PL	31.8	0.1	4.8	0.2	63.1	0.2	0.3	0.1
Cu(II)O/PHMB	32.9	0.4	5.1	0.1	61.7	0.4	0.3	0.1
Cu(II)O/ε-PL	32.8	0.2	5.1	0.3	61.8	0.5	0.2	0.1
ε-PL/PHMB	32.7	0.1	5.1	0.1	62.0	0.1	0.3	0.1
Three-component combination (2.5% each)								
Cu(II)O/Cinnamaldehyde/ε-PL (LY)	31.5	0.3	4.7	0.2	63.5	0.4	0.3	0.1
Cu(II)O/Cinnamaldehyde/PHMB (PB)	30.6	0.5	4.5	0.1	64.7	0.5	0.2	0.1

containing 1 antimicrobial component, subsequent selection experiments using 2- and 3-component combinations were tested to identify lead candidates containing 3 components. In parallel, selected samples representing 1, 2, and 3 components were also tested for antiviral efficacy. These experiments were used to verify 3-component coating candidates with broad antipathogen activity.

3.1. Surface Analysis. Coating thickness measurements were carried out using a micrometer after application of the commercial nail polish polymer formulation as well as combinations of 1, 2, and 3 antimicrobial components on aluminum substrates. The coating thickness measured across the samples varied between 55 and 98 μm. However, the average coating thickness determined for the different formulations remained constant at 75 μm.

To confirm the composition of the surface of the selected coatings used in this study, X-ray photoelectron spectroscopy (XPS) analysis was employed (Table 1). As the XPS method provides an information depth of approximately 10 nm, the method is often used in studies related to biointerfacial interactions, including pathogen−material interactions. Here, a first observation is that the elemental composition of all samples is very similar to the unmodified commercial nail polish polymer control sample, emphasizing the fact that the antimicrobial components are incorporated in the polymer bulk (rather than being present at the surface) as expected. This observation is further supported by the fact that the element copper was not detected at the surface of any of the samples. However, a slight change in the elemental composition was observed for all samples containing the small molecule cinnamaldehyde. The observed small decrease in the oxygen content and the corresponding small increase in the carbon content on these samples are consistent with some of this compound migrating to the surface in a hydrophobic environment such as air. Only a small amount of silicon, a common contamination in XPS experiments, was observed on all of the coatings.

3.2. Bacterial Testing. *3.2.1. Single-Component Coatings.* Homogeneous mixtures representing single antimicrobial components within the polymer formulation were first fabricated. Filter papers were coated with each individual formulation, and their *in vitro* antibacterial activity against both Gram-positive and Gram-negative bacteria was assessed via the zone of inhibition test as shown in Figure 2. Across the compounds tested, more compounds were active against the Gram-positive *S. aureus* bacteria compared to the Gram-negative *P. aeruginosa*. The cationic polymers PHMB and ε-PL were the only compounds that showed antibacterial effects on both types of bacteria (Figure 2G,H). Based on these results, both PHMB and ε-PL were included in further screening experiments. Electrostatic interactions between these polymers and the bacterial cell membranes and, for the specific case of PHMB, DNA binding have been proposed as the mechanism of action.[40,41] It is known that the structural characteristics of the external membrane of Gram-negative bacteria, particularly its thickness and composition, confer more protection against antimicrobial agents when compared to Gram-positive bacteria.

Screening of the essential oils (EOs) showed activity of cinnamaldehyde and carvacrol against *S. aureus*. Figure 2D shows cinnamaldehyde being particularly effective with the biggest zone of inhibition of all compounds (∼25 mm) observed. Here, the antimicrobial activity of EOs is suspected to be related to their lipophilic characteristics. EOs may interfere with DNA and RNA synthesis, cell membranes, transport of electrons, ionic gradients, protein translocation, phosphorylation, and other enzyme-dependent reactions.[42,43] Again, the higher susceptibility of Gram-positive bacteria to EOs compared to Gram-negative bacteria has been attributed to the different characteristics of their cell membranes, where the outer membrane of Gram-negative bacteria may limit the diffusion of hydrophobic compounds.[25,29] Based on the results, only cinnamaldehyde was included in the next round of testing.

Metal ions are expected to translocate cell membranes, interrupting DNA duplication and degrading intracellular ATP. They may also lead to cellular damage through the generation of reactive oxygen species (ROS) and electrostatic interactions.[23,44,45] Figure 2B,C shows the lack of a clear zone of growth inhibition around the metal and metal oxide samples, which could be attributed to the limited diffusivity of the ions and/or other species released by the nanoparticle-containing samples into the agar plate. This effect may be closely related to the ion releasing mechanism intrinsic to each material used in this study.[46] However, a secondary blurred zone around the

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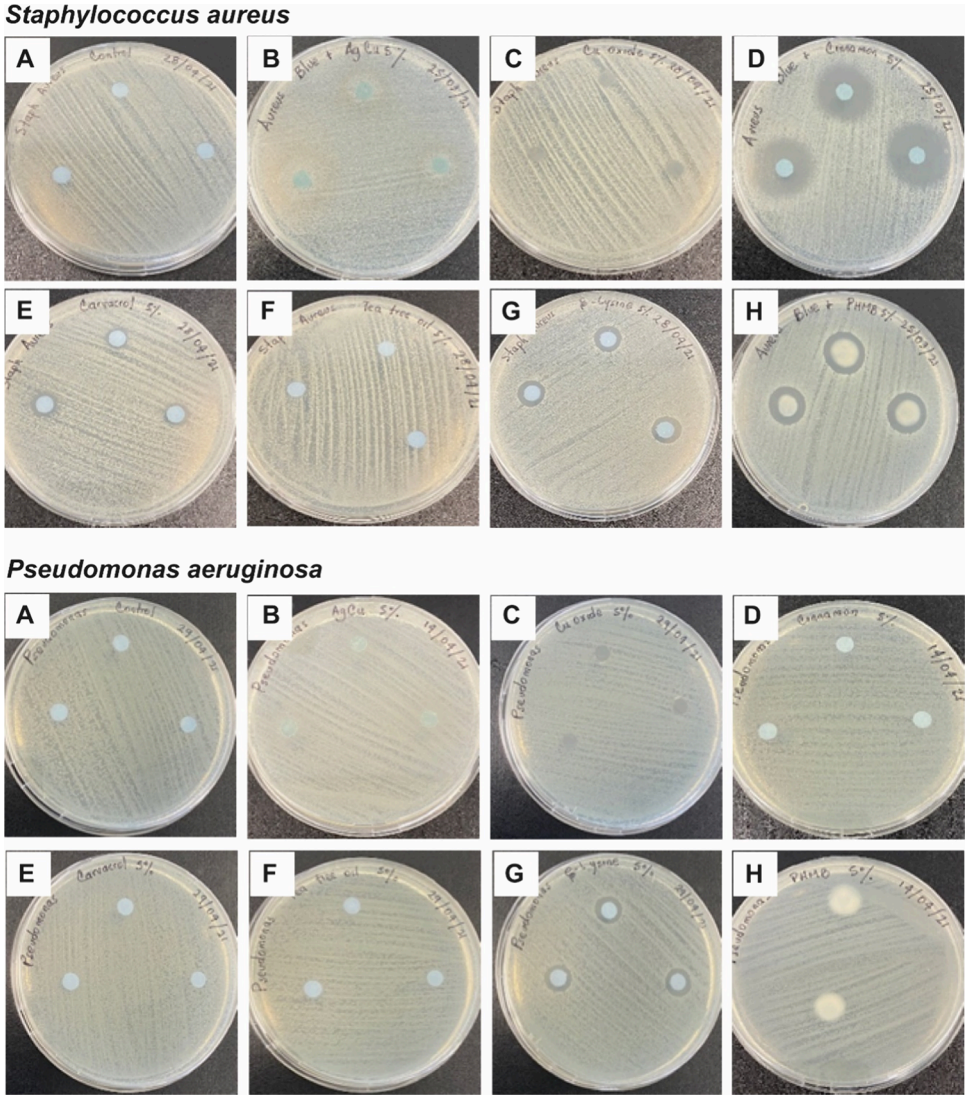

Figure 2. Antibacterial activity of different coating samples representing a single antimicrobial component evaluated using the zone of inhibition test. The concentration of compounds was set at 5% (w/v) and tested using *S. aureus* and *P. aeruginosa* with (A) unmodified polymer (control). (B) AgCu nanoparticles. (C) Copper dioxide (Cu(II)O). (D) Cinnamaldehyde. (E) Carvacrol. (F) Tea tree oil. (G) ε-PL. (H) PHMB.

silver-coated nanoparticle samples (Figure 2B top) was observed, when tested against *S. aureus*. This zone likely is associated with a bacteriostatic effect where ions released from the nanoparticles prevent bacteria from growing rather than killing them.[47] We concluded that this specific test may not be an appropriate method to confirm the antibacterial activity of the metal and metal oxide nanoparticles included in this study. Therefore, both Cu(II)O and silver-coated copper particles were included in the next round of testing based on their widely recognized antibacterial activity. Moreover, based on their widely recognized antibacterial activity, silver and copper nanoparticles were also added to the analysis.

3.2.2. Two-Component Coatings. In the second round of antimicrobial testing, the activity of combinations of two of the selected compounds, one from each class (metal/metal oxide nanoparticles and essential oils), against Gram-positive and Gram-negative bacteria was evaluated. Cationic polymers were not included in this set of experiments as their ability to inhibit the growth of both *S. aureus* and *P. aeruginosa* was previously demonstrated via the zone of inhibition test. Coated samples were subjected to a quantitative 96-well plate biofilm experiment where viable counts were assessed, and the results are presented in Figure 3. Aluminum substrates with a diameter of 6 mm were painted on both sides with the corresponding combinations. Samples were incubated with both Gram-positive and Gram-negative bacteria for 24 h. Then, samples were washed to remove any nonadherent planktonic bacteria followed by the detachment of the bacterial biofilm in PBS. Aliquots of the solution were diluted and plated on agar to evaluate bacterial viability. Only combinations containing cinnamaldehyde showed a reduction in *S. aureus* bacterial viability. The highest activity with a 5-log reduction of *S. aureus* colony counting was observed when cinnamaldehyde was combined with either copper oxide or silver-coated copper nanoparticles. Based on these results, cinnamaldehyde, copper oxide, and silver-coated copper nanoparticles (as the only metals) were included in the next round of testing.

The higher antibacterial activity of cinnamaldehyde against *S. aureus* compared to other bacterial strains including Gram-negative *Escherichia coli* has previously been reported.[48] A study using methicillin-resistant *S. aureus* (MRSA)[49] showed how the presence of cinnamaldehyde at subminimum inhibitory

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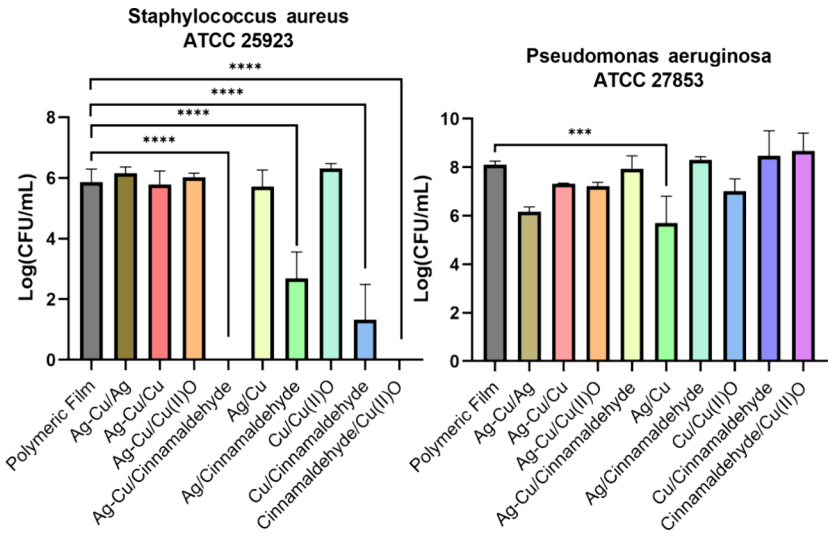

Figure 3. Biofilm experiment using aluminum substrates. Logarithmic reduction of cfu/mL was obtained from the viable counting of the samples. Both *S. aureus* and *P. aeruginosa* were evaluated. Data were analyzed by ordinary one-way ANOVA using multiple comparisons to the baseline (****$p <$ 0.0001, ***$p = 0.0003$).

concentrations drastically affected the expression of the gene SarA, a regulator of the biofilm-associated protein (Bap), reported to be crucial for the adherence and biofilm formation by this strain.[50]

Despite the promising result toward Gram-positive bacteria, none of the combinations promoted a sufficient reduction in the viability of the Gram-negative *P. aeruginosa* biofilm as seen in Figure 3.

3.2.3. Three-Component Coatings. Combinations of either copper oxide or silver-coated copper nanoparticles with cinnamaldehyde from the two-compound coatings showed a significant reduction in *S. aureus* viability. The lack of activity of these 2-component combinations against *P. aeruginosa* contrasted the effectiveness displayed by the cationic polymers in the zone of inhibition test. In order to ensure activity of the coatings against Gram-negative bacteria and, therefore, accomplish broad-spectrum coatings, PHMB and ε-PL cationic polymers were incorporated into a three-compound coating system. The details of the combinations fabricated for this set of experiments are presented in Table 2.

Table 2. Three-Component Combination Samples Used in This Study

sample	metals/metal oxide	essential oil	cationic polymer
A	silver-coated copper nanoparticles	cinnamaldehyde	ε-PL
B	silver-coated copper nanoparticles	cinnamaldehyde	PHMB
C	Cu(II)O	cinnamaldehyde	ε-PL
D	Cu(II)O	cinnamaldehyde	PHMB

The three-component coatings were evaluated via a biofilm experiment similar to what was previously conducted with the two-component coatings. All combinations (A−D) showed significant activity against both *S. aureus* and *P. aeruginosa*, with the absence of bacterial growth after 24 h of incubation being observed in Figure 4. However, despite the effectiveness of all combinations, samples A and B containing silver-coated nanoparticles showed instability of the polymer formulation over time after the addition of all components, limiting the use of these formulations due to an insufficient shelf life. Therefore, these formulations were discarded for further experiments, and only the Cu(II)O/cinnamaldehyde/ε-PL (LY) and Cu(II)O/cinnamaldehyde/PHMB (PB) combinations were employed in further experiments.

Figure 5 shows the logarithmic reduction of the cfu/mL obtained from the viable counting of samples C (LY) and D (PB). Significant differences ($p < 0.0001$) between the control, polymeric film samples, and the three-component coatings were observed. Samples LY and PB showed a 5-log and more than 6-log reduction in the viable counting of *S. aureus* and *P. aeruginosa*, respectively.

PHMB and its proteolysis resistance properties[51] have been suggested as a candidate for the treatment of chronic ulcers where *P. aeruginosa* is present. This cationic polymer has been widely employed in hospitals and a range of industries as a result of its antibacterial and antiviral properties;[41] however, its inherent toxicity against mammalian cells must be considered. The ability of PHMB to penetrate bacterial membranes leading to bacterial death has commonly been accepted as its mechanism of action.[52] PHMB has been incorporated into for example polydimethylsiloxane (PDMS) films where inhibition of *E. coli*, *S. aureus*, and *Staphylococcus epidermis* among other strains has been reported at different loading concentrations (0.1, 0.3, and 0.5% (w/w)).[53] Despite the described low cytotoxicity against L929 cells of the PHMB-loaded samples at a concentration of 0.1%, another study has highlighted the likelihood of PHMB entering mammalian cells.[41] Chindera et al.[41] also suggested a mechanism of action of PHMB, which involves DNA binding. This mechanism of chromosome condensation, apart from resulting in toxicity, may also represent a path for bacteria to develop resistance against this compound.

In the case of ε-PL, electrostatic adsorption as a result of its cationic nature has been recognized as the mechanism of action.[54] ε-PL has been widely used in food applications,[55] but recently, its uses have been expanded into medical research and industrial applications. For biomedical applications, ε-PL has been grafted to methacrylamide for the fabrication of hydrogels, which showed a 3-log reduction of *P. aeruginosa in vitro*.[56]

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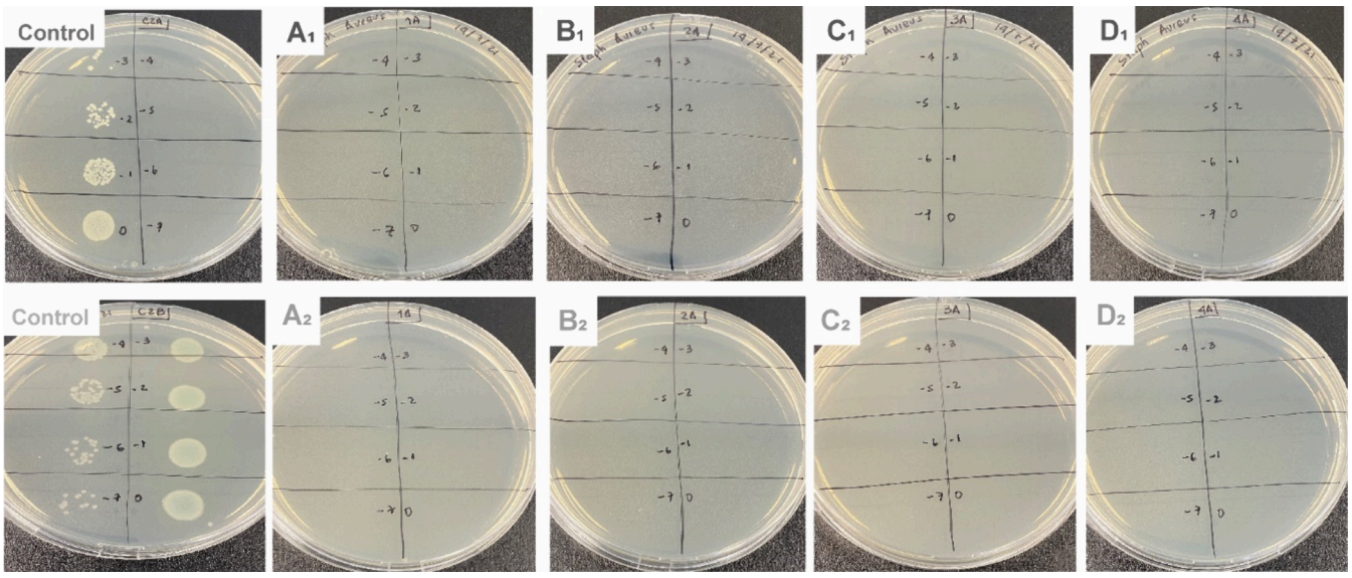

Figure 4. 96-Well plate biofilm experiment using a coating representing three different antimicrobial components. Agar plates A1−E1 used *S. aureus*, while plates A2−E2 used *P. aeruginosa*. The different coatings tested were the control, unmodified polymeric matrix control samples, (A_{1-2}) Ag−Cu/cinnamaldehyde/ε-PL combination coatings, (B_{1-2}) Ag−Cu/cinnamaldehyde/PHMB combination coatings, (C_{1-2}) Cu(II)O/cinnamaldehyde/ε-PL (LY) combination coatings, and (D_{1-2}) Cu(II)O/cinnamaldehyde/PHMB (PB) combination coatings.

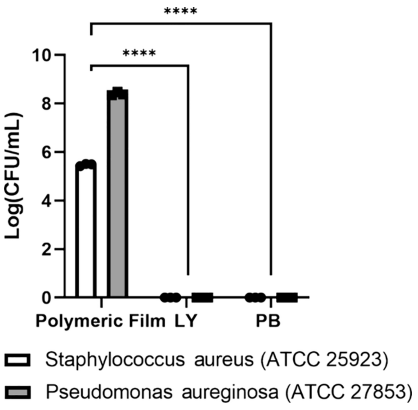

Figure 5. 96-Well plate biofilm experiment using a coating representing three different antimicrobial components. Logarithmic reduction of the cfu/mL obtained from the viable counting of Cu(II)O/cinnamaldehyde/ε-PL (LY) and Cu(II)O/cinnamaldehyde/PHMB (PB) combination samples. Data were analyzed by ordinary one-way ANOVA using multiple comparisons to the baseline (****$p < 0.0001$).

Similarly, in another study, ε-PL was cross-linked with catechol for the fabrication of antibacterial coatings for medical devices. These coatings at the highest concentration of ε-PL reported a 99.99% reduction in both Gram-positive and Gram-negative bacteria as well as the ability to inhibit biofilm formation for 1 week.[57] When the antimicrobial properties of ε-PL were evaluated against different strains of *P. aeruginosa*, a 3-log reduction in the viability was reported when concentrations greater than two times the minimum inhibitory concentrations were employed. ε-PL was reported to hold the highest microbial selectivity over mammalian cells compared to other cationic polymers such as linear polyethylenimine (LPEI), α-poly-D-lysine (PDL), and α-poly-L-lysine (PLL), among others.[58] Therefore, toxicity issues are more likely to be dismissed using ε-PL instead of PHMB.

3.3. Viral Infectivity Assay. Two viral infectivity assays were performed. The first assay examined the antiviral activity of 1-, 2-, and 3-component coatings on OC43 after 1 h of incubation. The second assay evaluated the antiviral activity of the final 3-component coatings Cu(II)O/cinnamaldehyde/ε-

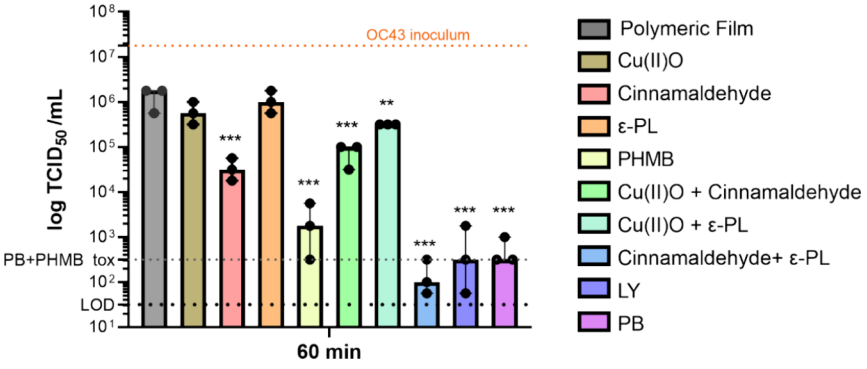

Figure 6. Viral infectivity assay of coatings representing 1, 2, or 3 components. The cell toxicity of the components was verified by adding 25 μL of media for 1 h. The initial inoculum, limit of detection of the TCID50 assay (LOD), and cell toxicity for samples containing the PHMB component (PB +PHMB tox) are shown on the graph (dotted lines). Data were analyzed by ordinary one-way ANOVA using multiple comparisons to the baseline (***$p < 0.0005$; **$p < 0.005$).

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PL (LY) and Cu(II)O/cinnamaldehyde/PHMB (PB) and the control material on the infectious OC43 virus at 30 min and 1 h of incubation.

The TCID values for the OC43 virus after exposure to the tested samples for the first assay are presented in Figure 6. The PHMB polymer as a single component and as part of the 3-component coatings showed toxicity to MRC-5 cells, in line with a previously reported study.[41] The toxicity of all other components was negligible. Of the single components, the viral infectivity of OC43 was significantly lower in the cinnamaldehyde and PHMB-treated samples ($p < 0.0005$), in comparison to the baseline. There was no significant reduction of OC43 infectivity for Cu(II)O and ε-PL-treated samples after 1 h; however, the combination of Cu(II)O with ε-PL resulted in a significant reduction of infectious OC43 in comparison to polymeric film control samples ($p < 0.005$). Similarly, the infectious virus was reduced for samples treated with Cu(II)O combined with cinnamaldehyde ($p < 0.0005$); however, this reduction was comparable to cinnamaldehyde alone. A maximum reduction in the infectious virus was observed for the 2-component cinnamaldehyde and ε-PL coating, as well as the 3-component samples ($p < 0.0005$). For coatings representing 3 components (LY and PB), the reduction of infectious OC43 was greater than 99.9% in comparison to the baseline.

In order to extend the testing of the antiviral properties of the lead candidate samples, the LY and PB samples, which showed the best performance in the antimicrobial testing as well as in the first round of antiviral testing, were then tested against the infectious human coronavirus OC43. Figure 7 shows TCID

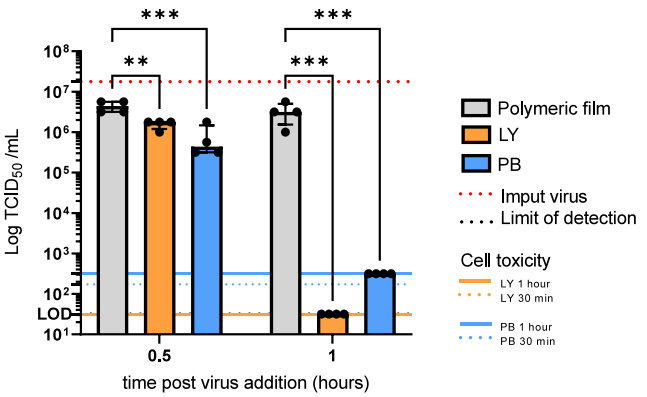

Figure 7. Component antiviral testing on OC43. Data were analyzed by two-way ANOVA using multiple comparisons to the baseline (***$p < 0.001$; **$p = 0.002$).

values for the OC43 infectious virus after exposure to the tested compounds. Again, cell toxicity was observed for PB-treated discs in MRC-5 cells due to the presence of the PHMB component. The toxicity of the LY component was equal to the limit of detection after 1 h, but not detectable at 30 min. There was a significant reduction in OC43 infectivity for every compound at both 30 min and 1 h time points. After 30 min, OC43 infectivity was reduced by 63.9 and 83% when exposed to LY and PB compounds, respectively ($p = 0.002$ for LY and $p < 0.001$ for PB). By 1 h, OC43 infectivity was reduced by 99.99% for the PB and LY compounds ($p < 0.001$ for both). The measured OC43 infectious virus after exposure to the compounds was equal to the cell toxicity observed for the LY and PB compounds in the absence of virus.

The ability of viral particles to retain infectivity in the environment and mainly on surfaces can be influenced by several factors such as the type of surface, environmental characteristics (humidity and temperature), inherent characteristics of the virus, their surroundings, and chemical properties.[59] In terms of structure, enveloped viruses are composed of structural proteins that perform essential functions including acting as viral antigens, protecting the viral genome, collaborating for attachment to cells, and facilitating transfer of viral nucleic acids.[60] Coronaviruses are enveloped single-stranded ribonucleic acid (ssRNA) viruses. SARS-CoV-2 encodes structural proteins including host−cell recognition spike (S) glycoprotein, as well as membrane (M) and envelope (E) glycoproteins and nucleocapsid (N) phosphoprotein.[61] Most of the studied antiviral surfaces have reported effectiveness for enveloped viruses due to the instability of their phospholipid layer against physical disruption.[62] Here, we have presented synergistic coatings with broad antipathogenic activity, including activity against enveloped human coronavirus OC43. This outstanding ability was exclusively achieved as a result of the combination of three different components, where each compound contributed in a specific fashion to the overall activity. Although the study of the mechanism of action of each compound is beyond the scope of this paper, other authors have studied their therapeutic potential in the specific context of SARS-CoV-2. Supporting the effectiveness of cinnamaldehyde observed here, Kulkarni et al.[63] evaluated the antiviral properties of a variety of essential oils using molecular docking and the conceptual density functional theory. Cinnamaldehyde is one of the best scoring phytochemicals considering its high docking score to the S1 subunit of the (S) glycoprotein and high electronegativity, both reassuring its high efficiency of inhibition and potential antiviral properties. In terms of polycationic compounds, they have been reported to inhibit the *in vitro* replication of enveloped viruses, e.g., retroviruses. Specifically, polylysines have attached to phospholipids at the cell membrane hindering viruses such as herpes simplex virus (HSV-1) from binding to cells.[64] Moreover, the interactions between ε-PL and negatively charged groups in cell membranes imply low toxicity against mammalian cells as well as susceptibility differences upon changes in membrane composition.[65] This low toxicity favors the use of ε-PL compared to its counterpart PHMB especially for applications in healthcare environments.

Finally, when using metal ions, evidence of the inactivation of the enveloped virus has been correlated to a reaction between those ions and the thiol and disulfide bonds of viruses' proteins and enzymes.[62] Moreover, the damage to the viral genome by metal ions could also be associated with reactive oxygen species (ROS).[66]

Overall, the combination of the selected three antipathogenic compounds led to the fabrication of broad-spectrum antipathogenic coatings, providing the potential to contain the surface-based transmission and spread of microorganisms. This simple but highly effective coating technology is expected to find applications in environments where surface pathogen transmission is particularly problematic, such as hospitals, aged care facilities, or public transport.

On the path to commercial translation, future work will be required to evaluate the long-term durability and effectiveness of the coatings, including under real-world conditions. Assessing the effectiveness of the antipathogen activity after repeated cleaning cycles or exposure to environmental factors will provide valuable information to potential users and will ultimately

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determine the instructions for the use of the coating. Here, it is expected that durability tests will be carried out by certified laboratories that are able to collect information according to relevant standards, such as ASTM E2149-20[67] and ISO 22196,[68] while testing under real-world conditions is expected to be carried out in controlled studies in healthcare environments.[69]

4. CONCLUSIONS

In this study, the combination of up to 3 different classes of compounds with documented antipathogen activity within a polymeric matrix was explored to enable the fabrication of coatings with broad-spectrum activity against bacterial and viral pathogens. Compounds were either based on metals or metal oxides, namely, copper, silver, and copper oxide, essential oils, namely, cinnamaldehyde, tea tree oil, and carvacrol oil, or cationic polymers, namely, poly(ε-lysine) (ε-PL) and poly(hexamethylene biguanide) (PHMB). These compounds were mixed into a commercial nail polish polymer matrix, coated onto substrate materials, and dried to yield a durable coating with a consistent thickness. Samples representing coatings containing up to 7.5% (w/w) of either 1, 2, or 3 different candidates from the different classes were assessed for their antibacterial activity in the zone of inhibition tests and biofilm formation assays using *S. aureus* and *P. aeruginosa*. Here, copper oxide and cinnamaldehyde displayed superior activity against Gram-positive bacteria after incorporation into the coatings. However, only the cationic polymers were effective toward the Gram-negative bacteria when incorporated into the polymer coatings. The screening of combinations representing three different additives resulted in the identification of a polymer coating formulation with broad-spectrum antibacterial properties.

Formulations comprising a metal-based component (Cu(II)-O), an essential oil (cinnamaldehyde oil), and a cationic polymer (either ε-PL or PHMB) within the polymeric base provided a 5-log reduction and more than a 6-log reduction in the colony-forming units of *S. aureus* and *P. aeruginosa* compared to controls. Moreover, the antiviral activity of the coatings was assessed using a viral infectivity assay using the human coronavirus OC43. Here, the selected combination of additives representing all 3 different classes of compounds was able to provide a 4-log reduction in infectivity.

Our data demonstrate that the concept of using multi-component antimicrobial coatings to provide broad-spectrum synergistic effects is suitable for exploitation in the context of frequently touched surfaces.

It is expected that this easy-to-apply but highly effective surface coating technology will find applications in environments where surface pathogen transmission is particularly problematic such as hospitals, aged care facilities, or public transport. Here, the reduction of the risk of transmission of both bacterial and viral infectious diseases that the technology is promising may provide significant benefits.

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Notes

The authors declare the following competing financial interest(s): The authors declare the following competing interest: Vishek Batra, Ghian Tjandaputra and Tony Tan have a financial interest in Coatd Pty Ltd, which is seeking to commercialize anti-pathogen surface technologies.

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REFERENCES

(1) Weber, D. J.; Anderson, D.; Rutala, W. A. The role of the surface environment in healthcare-associated infections. *Curr. Opin. Infect. Dis.* **2013**, *26* (4), 338−344, DOI: 10.1097/QCO.0b013e3283630f04.

(2) Lei, H.; Li, Y.; Xiao, S.; Yang, X.; Lin, C.; Norris, S. L.; Wei, D.; Hu, Z.; Ji, S. Logistic growth of a surface contamination network and its role in disease spread. *Scientific Reports.* **2017**, *7* (1), 14826.

(3) Cobrado, L.; Silva-Dias, A.; Azevedo, M. M.; Rodrigues, A. G. High-touch surfaces: microbial neighbours at hand. *European Journal of Clinical Microbiology & Infectious Diseases.* **2017**, *36* (11), 2053−2062.

(4) Costa, D. M.; Johani, K.; Melo, D. S.; Lopes, L. K. O.; Lima, L. K. O.; Tipple, A. F. V.; Hu, H.; Vickery, K. Biofilm contamination of high-touched surfaces in intensive care units: epidemiology and potential impacts. *Lett. Appl. Microbiol.* **2019**, *68* (4), 269−276.

(5) Lutz, J. K.; van Balen, J.; Mac Crawford, J.; Wilkins, J. R.; Lee, J.; Nava-Hoet, R. C.; Hoet, A. E. Methicillin-resistant Staphylococcus aureus in public transportation vehicles (buses): Another piece to the epidemiologic puzzle. *Am. J. Infect. Control* **2014**, *42* (12), 1285−1290.

(6) Patel, K. V.; Bailey, C. L.; Harding, A. H.; Biggin, M.; Crook, B. Background levels of micro-organisms in the busy urban environment of transport hubs. *J. Appl. Microbiol.* **2018**, *125* (5), 1541−1551.

(7) Nerandzic, M. M.; Cadnum, J. L.; Pultz, M. J.; Donskey, C. J. Evaluation of an automated ultraviolet radiation device for decontamination of Clostridium difficile and other healthcare-associated pathogens in hospital rooms. *BMC Infect Dis.* **2010**, *10*, 197.

(8) Casini, B.; Tuvo, B.; Cristina, M. L.; Spagnolo, A. M.; Totaro, M.; Baggiani, A.; Privitera, G. P. Evaluation of an Ultraviolet C (UVC) Light-Emitting Device for Disinfection of High Touch Surfaces in Hospital Critical Areas. *International Journal of Environmental Research and Public Health.* **2019**, *16* (19), 3572.

(9) Boyce, J. M. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. *Antimicrobial Resistance & Infection Control.* **2016**, *5* (1), 10.

(10) Nikmaram, A. The Application of Non-chloride Based Disinfectants in Inactivation of SARS-CoV-2 in Personal Protective

Equipment (PPE), Air and Surfaces of Hospitals. *Mediterranean J. Infect. Microbes Antimicrob.* **2022**, *11*, 90 DOI: 10.4274/mjima.galenos.2021.2021.12.
(11) Heydarian, S.; Ranjbar, Z.; Rastegar, S. Chitosan biopolymer as an antimicrobial agent in automotive electrocoatings: The effect of solid content. *Progress in Organic Coatings.* **2015**, *89*, 344−347.
(12) Tang, Z. M.; Kong, N.; Zhang, X. C.; Liu, Y.; Hu, P.; Mou, S.; Liljestrom, P.; Shi, J. L.; Tan, W. H.; Kim, J. S.; Cao, Y. H.; Langer, R.; Leong, K. W.; Farokhzad, O. C.; Tao, W. A materials-science perspective on tackling COVID-19. *Nature Reviews Materials.* **2020**, *5*, 847−860.
(13) van Doremalen, N.; Bushmaker, T.; Morris, D. H.; Holbrook, M. G.; Gamble, A.; Williamson, B. N.; Tamin, A.; Harcourt, J. L.; Thornburg, N. J.; Gerber, S. I.; Lloyd-Smith, J. O.; de Wit, E.; Munster, V. J. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *New England Journal of Medicine.* **2020**, *382* (16), 1564−1567.
(14) Dewey, H. M.; Jones, J. M.; Keating, M. R.; Budhathoki-Uprety, J. Increased Use of Disinfectants During the COVID-19 Pandemic and Its Potential Impacts on Health and Safety. *ACS Chemical Health & Safety.* **2022**, *29* (1), 27−38.
(15) Zhang, H. L.; Kelly, B. J.; David, M. Z.; Lautenbach, E.; Huang, E.; Bekele, S.; Tolomeo, P.; Reesey, E.; Loughrey, S.; Pegues, D.; Ziegler, M. J.; CDC Prevention Epicenters Program. SARS-CoV-2 RNA persists on surfaces following terminal disinfection of COVID-19 hospital isolation rooms. *Am. J. Infect. Control* **2022**, *50* (4), 462−464.
(16) Aydogdu, M. O.; Altun, E.; Chung, E.; Ren, G. G.; Homer-Vanniasinkam, S.; Chen, B. Q.; Edirisinghe, M. Surface interactions and viability of coronaviruses. *Journal of the Royal Society Interface.* **2021**, *18* (174), 20200798.
(17) Joonaki, E.; Hassanpouryouzband, A.; Heldt, C. L.; Areo, O. Surface Chemistry Can Unlock Drivers of Surface Stability of SARS-CoV-2 in a Variety of Environmental Conditions. *Chem.* **2020**, *6* (9), 2135−2146.
(18) Flemming, H. C.; Wingender, J.; Szewzyk, U.; Steinberg, P.; Rice, S. A.; Kjelleberg, S. Biofilms: an emergent form of bacterial life. *Nature Reviews Microbiology.* **2016**, *14* (9), 563−575.
(19) Hawkey, P. M.; Jones, A. M. The changing epidemiology of resistance. *J. Antimicrob. Chemother.* **2009**, *64* (suppl-1), i3−i10.
(20) World Health Organization *Antimicrobial Resistance*, 2020. https://www.who.int/health-topics/antimicrobial-resistance.
(21) World Health Organization *WHO publishes list of bacteria for which new antibiotics are urgently needed*, 2017. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.
(22) Zhen, X.; Lundborg, C. S.; Sun, X.; Hu, X.; Dong, H. Economic burden of antibiotic resistance in ESKAPE organisms: a systematic review. *Antimicrobial Resistance and Infection Control.* **2019**, *8* (1), 137.
(23) Makabenta, J. M. V.; Nabawy, A.; Li, C. H.; Schmidt-Malan, S.; Patel, R.; Rotello, V. M. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat. Rev. Microbiol.* **2020**, *19*, 23−36.
(24) Lara, H. H.; Garza-Treviño, E. N.; Ixtepan-Turrent, L.; Singh, D. K. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *Journal of Nanobiotechnology.* **2011**, *9* (1), 30.
(25) Makvandi, P.; Wang, C.-Y.; Zare, E. N.; Borzacchiello, A.; Niu, L.-N.; Tay, F. R. Metal-Based Nanomaterials in Biomedical Applications: Antimicrobial Activity and Cytotoxicity Aspects. *Adv. Funct. Mater.* **2020**, *30* (22), 1910021 DOI: 10.1002/adfm.201910021.
(26) Galié, S.; García-Gutiérrez, C.; Miguélez, E. M.; Villar, C. J.; Lombó, F. Biofilms in the Food Industry: Health Aspects and Control Methods. *Front. Microbiol.* **2018**, *9*, 898 DOI: 10.3389/fmicb.2018.00898.
(27) Yang, Y. C.; Cai, Z. G.; Huang, Z. H.; Tang, X. Y.; Zhang, X. Antimicrobial cationic polymers: from structural design to functional control. *Polymer Journal.* **2018**, *50* (1), 33−44.
(28) Dizaj, S. M.; Lotfipour, F.; Barzegar-Jalali, M.; Zarrintan, M. H.; Adibkia, K. Antimicrobial activity of the metals and metal oxide nanoparticles. *Materials Science & Engineering C-Materials for Biological Applications.* **2014**, *44*, 278−284.
(29) Chouhan, S.; Sharma, K.; Guleria, S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines* **2017**, *4* (3), 58.
(30) Carmona-Ribeiro, A.; De Melo Carrasco, L. Cationic Antimicrobial Polymers and Their Assemblies. *Int. J. Mol. Sci.* **2013**, *14* (5), 9906−9946.
(31) Hodek, J.; Zajícová, V.; Lovětinská-Šlamborová, I.; Stibor, I.; Müllerová, J.; Weber, J. Protective hybrid coating containing silver, copper and zinc cations effective against human immunodeficiency virus and other enveloped viruses. *BMC Microbiol.* **2016**, *16* (1), 56.
(32) Krebs, F. C.; Miller, S. R.; Ferguson, M. L.; Labib, M.; Rando, R. F.; Wigdahl, B. Polybiguanides, particularly polyethylene hexamethylene biguanide, have activity against human immunodeficiency virus type 1. *Biomedicine & Pharmacotherapy.* **2005**, *59* (8), 438−445.
(33) Wang, W. Y.; Yim, S. L.; Wong, C. H.; Kan, C. W. Study on the Development of Antiviral Spandex Fabric Coated with Poly-(Hexamethylene Biguanide) Hydrochloride (PHMB). *Polymers* **2021**, *13*, 2122 DOI: 10.3390/polym13132122.
(34) Gilling, D. H.; Kitajima, M.; Torrey, J. R.; Bright, K. R. Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *J. Appl. Microbiol.* **2014**, *116* (5), 1149−1163.
(35) Mhlongo, J. T.; Waddad, A. Y.; Albericio, F.; de la Torre, B. G. Antimicrobial peptide synergies for fighting infectious diseases. *Adv. Sci. (WeinhSep* **2023**, *10* (26), 2300472.
(36) Zou, L.; Wang, J.; Gao, Y.; Ren, X.; Rottenberg, M. E.; Lu, J.; Holmgren, A. Synergistic antibacterial activity of silver with antibiotics correlating with the upregulation of the ROS production. *Sci. Rep.* **2018**, *8*, 11131.
(37) Bankier, C.; Matharu, R. K.; Cheong, Y. K.; Ren, G. G.; Cloutman-Green, E.; Ciric, L. Synergistic antibacterial effects of metallic nanoparticle combinations. *Sci. Rep.* **2019**, *9*, 16074.
(38) Birkett, M.; Dover, L.; Lukose, C. C.; Zia, A. W.; Tambuwala, M. M.; Serrano-Aroca, Á. Recent advances in metal-based antimicrobial coatings for high-touch surfaces. *Int. J. Mol. Sci.* **2022**, *23* (3), 1162.
(39) Loo, S. L.; Wark, P. A. B.; Esneau, C.; Nichol, K. S.; Hsu, A. C. Y.; Bartlett, N. W. Human coronaviruses 229E and OC43 replicate and induce distinct antiviral responses in differentiated primary human bronchial epithelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology.* **2020**, *319* (6), L926−L931.
(40) Timofeeva, L.; Kleshcheva, N. Antimicrobial polymers: mechanism of action, factors of activity, and applications. *Appl. Microbiol. Biotechnol.* **2011**, *89* (3), 475−492.
(41) Chindera, K.; Mahato, M.; Kumar Sharma, A.; Horsley, H.; Kloc-Muniak, K.; Kamaruzzaman, N. F.; Kumar, S.; McFarlane, A.; Stach, J.; Bentin, T.; Good, L. The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. *Scientific Reports.* **2016**, *6* (1), 23121.
(42) Andrade-Ochoa, S.; Chacón-Vargas, K. F.; Sánchez-Torres, L. E.; Rivera-Chavira, B. E.; Nogueda-Torres, B.; Nevárez-Moorillón, G. V. Differential Antimicrobial Effect of Essential Oils and Their Main Components: Insights Based on the Cell Membrane and External Structure. *Membranes.* **2021**, *11* (6), 405.
(43) Kalemba, D.; Kunicka, A. Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry.* **2003**, *10* (10), 813−829.
(44) Singh, J.; Vishwakarma, K.; Ramawat, N.; Rai, P.; Singh, V. K.; Mishra, R. K.; Kumar, V.; Tripathi, D. K.; Sharma, S. Nanomaterials and microbes' interactions: a contemporary overview. *3 Biotech* **2019**, *9* (3), 68.
(45) Slavin, Y. N.; Asnis, J.; Häfeli, U. O.; Bach, H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *Journal of Nanobiotechnology.* **2017**, *15* (1), 65.
(46) Kourmouli, A.; Valenti, M.; van Rijn, E.; Beaumont, H. J. E.; Kalantzi, O.-I.; Schmidt-Ott, A.; Biskos, G. Can disc diffusion susceptibility tests assess the antimicrobial activity of engineered nanoparticles? *Journal of Nanoparticle Research.* **2018**, *20* (3), 62.
(47) Webster, T. J.; Seil, I. Antimicrobial applications of nanotechnology: methods and literature. *Int. J. Nanomed.* **2012**, *7*, 2767−2781.

(48) Zhang, Y. B.; Liu, X. Y.; Wang, Y. F.; Jiang, P. P.; Quek, S. Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. *Food Control.* **2016**, *59*, 282−289.
(49) Jia, P.; Xue, Y. J.; Duan, X. J.; Shao, S. H. Effect of cinnamaldehyde on biofilm formation and sarA expression by methicillin-resistant Staphylococcus aureus. *Letters in Applied Microbiology.* **2011**, *53* (4), 409−416.
(50) Lasa, I.; Penades, J. R. Bap: A family of surface proteins involved in biofilm formation. *Research in Microbiology.* **2006**, *157* (2), 99−107.
(51) Werthén, M.; Davoudi, M.; Sonesson, A.; Nitsche, D. P.; Mörgelin, M.; Blom, K.; Schmidtchen, A. Pseudomonas aeruginosa-induced infection and degradation of human wound fluid and skin proteins ex vivo are eradicated by a synthetic cationic polymer. *J. Antimicrob. Chemother.* **2004**, *54* (4), 772−779.
(52) Sowlati-Hashjin, S.; Carbone, P.; Karttunen, M. Insights into the Polyhexamethylene Biguanide (PHMB) Mechanism of Action on Bacterial Membrane and DNA: A Molecular Dynamics Study. *J. Phys. Chem. B* **2020**, *124* (22), 4487−4497.
(53) Montemezzo, M.; Ferrari, M. D.; Kerstner, E.; dos Santos, V.; Lain, V. V.; Wollheim, C.; da Silva Frozza, C. O.; Roesch-Ely, M.; Baldo, G.; Brandalise, R. N. PHMB-loaded PDMS and its antimicrobial properties for biomedical applications. *J. Biomater. Appl.* **2021**, *36* (2), 252−263.
(54) Yoshida, T.; Nagasawa, T. epsilon-Poly-L-lysine: microbial production, biodegradation and application potential. *Appl. Microbiol. Biotechnol.* **2003**, *62* (1), 21−26.
(55) Li, S.; Mao, Y.; Zhang, L.; Wang, M.; Meng, J.; Liu, X.; Bai, Y.; Guo, Y. Recent advances in microbial ε-poly-L-lysine fermentation and its diverse applications. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 65 DOI: 10.1186/s13068-022-02166-2.
(56) Zhou, C. C.; Li, P.; Qi, X. B.; Sharif, A. R. M.; Poon, Y. F.; Cao, Y.; Chang, M. W.; Leong, S. S. J.; Chan-Park, M. B. A photopolymerized antimicrobial hydrogel coating derived from epsilon-poly-L-lysine. *Biomaterials.* **2011**, *32* (11), 2704−2712.
(57) Xu, M.; Song, Q.; Gao, L.; Liu, H.; Feng, W.; Huo, J.; Jin, H.; Huang, L.; Chai, J.; Pei, Y.; Qu, X.; Li, P.; Huang, W. Single-step fabrication of catechol-ε-poly-L-lysine antimicrobial paint that prevents superbug infection and promotes osteoconductivity of titanium implants. *Chemical Engineering Journal.* **2020**, *396*, No. 125240.
(58) Venkatesh, M.; Barathi, V. A.; Goh, E. T. L.; Anggara, R.; Fazil, M.; Ng, A. J. Y.; Harini, S.; Aung, T. T.; Fox, S. J.; Liu, S. P.; Yang, L.; Barkham, T. M. S.; Loh, X. J.; Verma, N. K.; Beuerman, R. W.; Lakshminarayanan, R. Antimicrobial activity and cell selectivity of synthetic and biosynthetic cationic polymers. *Antimicrobial Agents and Chemotherapy.* **2017**, *61* (10), e00469−17.
(59) Rakowska, P. D.; Tiddia, M.; Faruqui, N.; Bankier, C.; Pei, Y. W.; Pollard, A. J.; Zhang, J. T.; Gilmore, I. S. Antiviral surfaces and coatings and their mechanisms of action. *Communications Materials.* **2021**, *2* (1), 53.
(60) Chaturvedi, U. C.; Shrivastava, R. Interaction of viral proteins with metal ions: role in maintaining the structure and functions of viruses. *FEMS Immunol. Med. Microbiol.* **2005**, *43* (2), 105−114.
(61) Florindo, H. F.; Kleiner, R.; Vaskovich-Koubi, D.; Acurcio, R. C.; Carreira, B.; Yeini, E.; Tiram, G.; Liubomirski, Y.; Satchi-Fainaro, R. Immune-mediated approaches against COVID-19. *Nature Nanotechnology.* **2020**, *15* (8), 630−645.
(62) Wang, N.; Ferhan, A. R.; Yoon, B. K.; Jackman, J. A.; Cho, N. J.; Majima, T. Chemical design principles of next-generation antiviral surface coatings. *Chem. Soc. Rev.* **2021**, *50* (17), 9741−9765.
(63) Kulkarni, S. A.; Nagarajan, S. K.; Ramesh, V.; Palaniyandi, V.; Selvam, S. P.; Madhavan, T. Computational evaluation of major components from plant essential oils as potent inhibitors of SARS-CoV-2 spike protein. *Journal of molecular structure.* **2020**, *1221*, No. 128823.
(64) Hosoya, M.; Neyts, J.; Yamamoto, N.; Schols, D.; Smoeck, R.; Pauwels, R.; De Clercq, E. Inhibitory Effects of polycations on the replication of enveloped viruses (HIV, HSV, CMV, RSV, Influenza A Virus and Togaviruses) in vitro. *Antiviral Chem. Chemother.* **1991**, *2* (4), 243−248, DOI: 10.1177/095632029100200405.
(65) Hyldgaard, M.; Mygind, T.; Vad, B. S.; Stenvang, M.; Otzen, D. E.; Meyer, R. L. The Antimicrobial Mechanism of Action of Epsilon-Poly-L-Lysine. *Appl. Environ. Microbiol.* **2014**, *80* (24), 7758−7770.
(66) Govind, V.; Bharadwaj, S.; Ganesh, M. R. S.; Vishnu, J.; Shankar, K. V.; Shankar, B.; Rajesh, R. Antiviral properties of copper and its alloys to inactivate covid-19 virus: a review. *BioMetals* **2021**, *34* (6), 1217−1235.
(67) ASTM E2149−20 - *Standard Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions*. ASTM International, DOI: DOI: 10.1520/E2149-20.
(68) Cashera, A.; Porosa, L. *ISO 22196:2011 Measurement of antibacterial activity on plastics and other non-porous surfaces "Modified Large droplet Inoculation Method."*. 2016 https://www.iso.org/standard/54431.html.
(69) Bäumler, W.; Eckl, D.; Holzmann, T.; Schneider-Brachert, W. Antimicrobial coatings for environmental surfaces in hospitals: a potential new pillar for prevention strategies in hygiene. *Critical Reviews Microbiology* **2022**, *48* (5), 531−564.

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