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Development of polyurethane antimicrobial coatings by composition with phenolic-, ionic- and copper-based agents

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ABSTRACT

Microorganisms can be found in almost all environments with high-touch surfaces being an important fomite for microbial growth. Considering the health issues associated to acquired infection from inanimate surfaces, as well as the raising hygienic concerns, the incorporation of antimicrobial compounds in high-touch surfaces emerges as an effective solution for biomedical and common daily applications.

In this work, we incorporated different antimicrobial agents (phenolic-, ionic- and copper-based compounds) into polyurethane commercial formulations to produce antimicrobial lacquer-films and evaluated not only their physical/chemical properties, but also their antimicrobial activity against bacteria (*Staphylococcus aureus*, *Escherichia coli*), fungi (*Candida albicans*), and virus (SARS-CoV-2).

The incorporation of antimicrobial agents did not affect the performance of lacquer-films and the main properties were maintained, specifically the visual aspect, gloss values, optical properties and chemical stability.

Among the different compounds tested, copper-based lacquer-films exhibited the strongest antibacterial and antifungal activity, with a >4 log reduction, but not against virus. Importantly, copper-based lacquer-films maintained their cytocompatibility, even at high concentrations. Regarding the ionic lacquer-films, the highest tested concentration also showed a strong antimicrobial action (5 log reduction) against fungi and gram-positive bacteria, but not against gram-negative bacteria and virus. However, at this concentration, the ionic-containing lacquer-films presented cytotoxic potential. The phenolic-based compounds were not associated with antimicrobial activity, regardless the concentrations tested.

Collectively, these results highlight the potential of incorporating antimicrobial agents in polyurethane surface coatings as a promising strategy to avoid the microbial

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colonization on inanimate surfaces and, ultimately, prevent the spreading of potentially harmful pathogens among humans.

1. Introduction

Inanimate surfaces have an important role in the transmission of pathogens. These surfaces can act as secondary reservoirs for microorganisms and offer important opportunities for the transmission and dissemination of pathogens among humans [1,2]. This situation is particularly relevant in indoor environments, in which humans spend almost 90% of their lives [3–5]. These indoor environments are characterized as multi-shared and confined spaces in which air (e.g. ventilation systems, air conditioning devices, airborne particles), high-touch surfaces (e.g. door handles, faucets, light switches) and water systems are the most common routes for microbial transmission [6].

Several studies have been published related to the persistence of pathogens on inanimate surfaces, including door handles of public service cars [7], automobile indoor surfaces [8], and frequently touched objects in healthcare facilities [9]. These studies identified Streptococci, Enterococci, *Klebsiella pneumoniae*, *Salmonella* ssp., *Escherichia coli*, and Staphylococci as the most common contaminating bacteria [10]. Among Staphylococci, methicillin-resistant *S. aureus* (MRSA) have been a rising concern given its generalized dissemination, increased persistence, and resistance to β-lactam antibiotics [11]. Other common group of microorganisms potentially pathogenic for humans are fungi [12,13]. The most common fungi identified on inanimate surfaces include *Aspergillus* spp. [14], *Cryptococcus* spp. [15], *Candida* spp. [1,16], and *Torulopsis glabrata* [1]. Importantly, the concern about viral persistence on inanimate surfaces has been highlighted in the last years, in particular after the COVID-19 pandemic, as an increasing concern for public health [17] and include hepatitis A virus (HAV), rhinoviruses, corona-, noro- and rotaviruses [16].

The persistence of microorganisms on inanimate surface varies according to the type and surface properties, type(s) of pathogen, microbial burden, and environmental conditions [18]. *S. aureus* can persist in low humidity conditions over time. On the contrary, gram-negative bacteria, such as *Pseudomonas* spp., *Klebsiella* spp. and *E. coli*, improve their survival rates in high relative humidity [1]. Furthermore, bacteria and fungi show different survival rates depending on the type of material surface they encounter, i.e. glass, stainless steel, aluminum, wood and cloth [18–20]. In addition, viruses, such as corona, coxsackie, influenza, or rhino virus, can persist in inanimate surfaces, such as surgical gloves, aluminum, glass, silicon, ceramic, metal or wood [21]. Specifically, *Human Coronavirus* 229E survives on polymer substrate up to 5 days at 21°C [22]. Biofilm formation is also an important factor for survival rate. In a biofilm state, bacteria are at least 500 times more resistant to antimicrobial agents. The biofilm matrix contains water, nutrients and extracellular substances like polysaccharides and proteins, that not only provide a nutritional source but also a certain level of humidity that protects the microorganisms against environmental influences [1].

Considering the ability of microorganisms to persist and survive for long periods of time in inanimate surfaces, the use of antimicrobial compounds emerges as an attractive solution for biomedical and common daily applications. Some examples include anti-fouling materials [23], industrial water systems [24], electronic materials (e.g. keyboards [25]) and medical devices (e.g. catheters, endotracheal tubes [26] and implants [27]).

Polymer materials can be modified to enhance their antimicrobial performance by following different strategies: (i) modification of the polymer surface properties without an antimicrobial agent; (ii) deposition of an antimicrobial compound directly on the polymer surface or after a chemical surface modification; and (iii) incorporation of the antimicrobial compound in the polymer matrix. The first strategy is based on polymer modification of the chemical or physical properties of the surface, including its free energy, polarity, or topography. The second approach involves the immobilization of an antimicrobial agent at the surface material by surface deposition techniques. The last strategy can be carried out by incorporating the antimicrobial agent in the polymer solution or by dispersing the antimicrobial agent in the polymer bulk [28]. Although the development of polymeric coatings with antimicrobial agents is an important strategy to avoid the colonization of microorganisms on inanimate surfaces [29], the selection of antimicrobial compounds is a critical step to produce antimicrobial coatings, since the intrinsic properties of antimicrobial agents can impact the properties of the final product [29] and, ultimately its functionality. Moreover, manufacturing process parameters are a key factor when producing antimicrobial polymeric coatings since materials must withstand extreme pressure and temperature conditions while keeping antimicrobial activity.

The most frequent antimicrobial agents incorporated in polymer materials are quaternary ammonium compounds [30], metal based-compounds [31], antimicrobial peptides [32], antibiotics [33], as well as other organic (such as chlorhexidine, povidine-iodine or triclosan) [34,35], and inorganic compounds (graphene based-compounds) [36,37]. Phenolic-based compounds are a large group of molecules based on a hydroxyl group attached to one or more aromatic benzene ring(s) [38]. Due to their antioxidant properties, these compounds represent an important group of metabolites in plants with a wide range of biological properties [39,40]. In materials science, phenolic-based materials have been used as additives in the food packaging industry [41]; as films stabilizers when incorporated in polymeric matrices [42]; in tissue engineering [43]; and as a commercial form as epoxy phenolic resins/compounds [44]. Phenols can be classified in different groups according to the phenol units and the structural elements

linked to aromatic groups. The phenol-based compounds chosen for this study were [1,1′-Biphenyl]-2-ol, mostly know as 2-phenylphenol or o-phenylphenol (OPP), which is a class of hydroxybiphenyl groups in which a biphenyl is substituted by a hydroxyl group at position 2; and 2-phenoxyethanol (PE). PE is not a pure phenol, but rather a derivative from a phenolic compound with a 2-hydroxyethyl group substituting the aromatic ether on oxygen. Both OPP and PE are used as preservatives for surface disinfection, wood treatment, vegetable packaging, textiles, and cosmetics, due to their large spectrum of action against gram-negative and gram-positive bacteria as well as against fungi [45].

In addition to the antibacterial activity associated to phenol-based-compounds [46], antiviral (against rabies virus) [47] and antifungal activity (against *Candida albicans* and *Trichophyton rubrum*) [48] has also been described. In particular, it has been shown that OPP and other derivatives of phenolic compounds have potential effect over different types of virus, such as human immunodeficiency virus (HIV), herpes simplex virus (HSV) or hepatitis B viruses [49].

Ionic-based liquids (IL) are a new class of compounds that have been gaining more attention since the beginning of the century, not only as an alternative green approach to organic solvents in industrial processes [50], but also in detergents and cosmetics [51]. The possibility to physically, chemically, and biologically modify each constituent of IL independently (cationic head and tail, and anion) makes these compounds interesting molecules for biomedical and industrial applications as antimicrobial agents [52–54]. In fact, it is estimated that there are 10^{18} possible combinations [55]. 1,3-didecyl-2-methylimidazolium chloride (DMIC) is one of those combinations and comprises a heterocyclic group with a chloride anion. Antimicrobial properties of IL depend on their structure, in particular the cationic organic head group, the tail (usually an alkyl linear chain with a variable length) and an organic/inorganic or biobased anion [55].

Finally, the use of metal based-compound materials has gained great interest not only in the biomedical field but also in physical and materials science due to their intrinsic properties, including good compatibility with biological tissues and bioactivity, high density, high melting point and thermal conductivity [56]. The most common metal compounds used as antimicrobial agents are silver, titanium, gold, calcium, magnesium, and copper-based compounds [57]. The Environmental Protection Agency (EPA) of the United States has approved copper's ability to inactivate or kill 99.9% pathogenic bacteria within 2 h [2]. Importantly, copper's antimicrobial properties are maintained regardless the mode of presentation, whether that be in bulk form [58]; associated to nanoparticles [59–62]; incorporated in coating material alone or combined with other compounds (e.g. with lactic acid) [63]; or associated to metal-organic frameworks [64].

In this work, antimicrobial polyurethane (PUR) lacquer-films were produced with the incorporation of different antimicrobial agents to avoid the colonization by microorganisms on inanimate surfaces and ultimately prevent the spreading of potentially harmful pathogens among humans. The antimicrobial lacquer-film was obtained by adding phenolic-based compounds such as: 2-phenylphenol (OPP) or 2-phenoxyethanol (PE); an ionic-based compound: 1,3-didecyl-2-methylimidazolium chloride (DMIC); or a metal-based compound: copper (II) sulphate pentahydrate (CuSP) to a PUR-based lacquer formulation. The antimicrobial materials were firstly characterized optically, chemically, and physically. The antimicrobial analysis was performed using the most abundant microorganisms that contaminate indoor surfaces, while the biocompatibility assays of the produced materials were performed with a representative cell line from the skin.

2. Materials and methods

2.1. Production of PUR-modified antimicrobial films

The base lacquer formulation was obtained by mixing different commercial raw materials, such as waterborne PUR dispersion, rheology modifiers, defoamers, levelling agents, hand modifiers, matting agents (silicone) and a crosslinker agent (polyisocyanate). The isocyanate functional groups (NCO) of the crosslinker react with free amine or hydroxyl groups of urethanes increasing the molecular weight of the pre-polymer. Finally, the antimicrobial stock solutions were added to the base lacquer formulation and mixed to create a homogeneous solution. The films were obtained by solvent casting. Briefly, the lacquer formulation was spread, with a 30 μm thickness, on polyethene inert surface and was submitted to two heating cycles: 4 min at 50°C on a digital hotplate (Stuart SD300, R330000718) following 1 min at 140°C in a laboratory hot air oven type (Werner Mathis AG, LTF-ST 119988). The polyethene surface acts only as a substrate to film deposition. All raw materials were made available by TMG Automotive. Antimicrobial compounds OPP 99% ($C_{12}H_{10}O$, CAS 90-43-7), PE ≥ 99% ($C_6H_5OCH_2CH_2OH$, CAS 122-99-6) and DMIC 96% ($C_{24}H_{47}ClN_2$, CAS 70862-65-6) and CuSP 99% ($CuO_4S \cdot 5H_2O$, CAS 7758-99-8) were purchased from Sigma-Aldrich.

In this work, the highest concentration produced for OPP and PE were 224 μg/cm^2 and 235 μg/cm^2, respectively. These values were obtained after an optimization process to maximize antimicrobial agent incorporation. The phenolic concentrations in lacquer-films were in the upper limit of compatibility between antimicrobial agent and PUR waterborne dispersion. Efforts were made to increase the phenolic concentration in lacquer-films; however, above the mentioned concentrations a rheological modification of lacquer liquid solution was observed (mainly an increase in viscosity), which compromised the production of lacquer-films and their visual appearance.

2.2. Visual inspection and optical analyses of lacquer-films

A macroscopic inspection of lacquer-films incorporating antimicrobial agents was performed to analyse the surface topography and homogeneity, while gloss analysis was done to evaluate the optical and surface properties using a single angle glossmeter with an incidence angle of 60° (Micro-gloss 60° supplied by BYK Instruments).

2.3. Transmittance spectroscopy analysis of lacquer-films

Transmittance spectra were collected from 360 to 700 nm, with a resolution of 10 nm, from 3 different lacquer-films for each compound in a Datacolor 650™ spectrophotometer. Only the transmittance analysis of lacquer-films produced with highest concentration of antimicrobial agents are presented.

2.4. Fourier transform infrared (FTIR) spectroscopy analysis of lacquer-films

FTIR analyses were performed on a FT-IR Spectrum Frontier. This technique is based on an interaction between an infrared beam and a diamond or a crystal with a high refractive index. As a result, an evanescent wave is generated, which penetrates a few microns on the surface of the sample and is attenuated by interactions with material, affecting energy absorption. Peak position and intensity are dependent on the various vibrational modes of molecules that comprise the test sample [65]. In this study, FTIR analyses were done on lacquer-films with and without antimicrobial compounds to characterize the chemical influence of these additivities on lacquer-films. Spectra were collected at wavenumber range from 400 to 4000 cm^{-1}, with a resolution of 1 cm^{-1}. Only the FTIR analysis of lacquer-films with highest concentration of the antimicrobial compound are presented.

2.5. Evaluation of antibacterial and antifungal activity of lacquer-films

The antibacterial and antifungal assays were based on the protocol of the International Organization for Standardization (ISO) 22196(E):2011 - Measurement of antibacterial activity on plastics and other non-porous surfaces [66]. Briefly, lacquer-films of 5 × 5 cm^2 were inoculated with 0.4 mL of a bacterial or antifungal suspension (*Staphylococcus aureus* ATCC 6538 P, *Escherichia coli* ATCC 8739 and *Candida albicans* SC5314/ATCC MYA-2876) prepared in a suspension medium (6 × 10^5 bacteria/mL). An inert cover film (4 × 4 cm^2) was placed over the material surface to ensure the contact between inoculum and lacquer-film. Test samples were incubated for 24 h at 35°C (or 32°C for antifungal assays) in an atmosphere with high relative humidity. To validate the initial inoculum dose, and consequently validate the assay, samples of untreated material (lacquer-control films) are processed immediately after inoculation. After incubation, the materials were washed with a wash solution composed by soybean casein, lecithin and polyoxyethylene sorbitan monooleate. Ten-fold serial dilutions of washing medium were plated on agar plate medium (based on yeast extract, tryptone and glucose) for bacterial assays and YPD agar medium (composed by peptone, glucose and yeast extract) for antifungal assays. The plates were incubated for 40–48 h at 35°C or 32°C, for bacteria or fingi, respectively. Following this incubation period, the number of colonies forming units (CFU) were counted. The antibacterial activity (R) was calculated comparing the average of common logarithm of number of viable colonies recovered from untreated materials 24 h after inoculation and the average of common logarithm of number of viable colonies recovered from treated materials after 24 h of inoculation. In addition, a microbial ratio (MR) for treated materials from untreated materials was also analysed.

2.6. Evaluation of antiviral activity of lacquer-films

The antiviral activity were based on the SARS-CoV-2 ribonucleic acid (RNA) degradation assay using a highly sensitive reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method [67]. SARS-CoV-2 samples derived from excess human swabs were used. Samples were stored at the University of Minho diagnostic laboratory, approved by the competent Institutional Review Board, Comissão de Ética para an Investigação em Ciências da Vida e da Saúde (CEICVS), with the reference CEICVS008/2022. Briefly, 0.3 mL of a SARS-CoV-2 sample (with viral loads from 1000 to 3000 copies per mL) was inoculated on lacquer-film surface (3 × 3 cm^2). As control, viral suspensions were also inoculated directly in the polyethene inert surface (where the lacquer-film is deposited). An inert cover film (2 × 2 cm^2) was placed on the surface of inoculum for all the conditions tested and incubated for 24 h at room temperature in an atmosphere with high relative humidity. After incubation, 150 μL of the viral suspension was collected and inactivated. RNA was further extracted using a semi-automated magnetic-particle processor (KingFisher Flex Purification System; Thermo Fisher Scientific, Waltham, Massachusetts, USA), according to the manufacturer instructions. For RT-qPCR, 10 μL of RNA were mixed with 20 μL of a NZY-Supreme One Step RT-qPCR Probe Master Mix, MB414 (©2020 NZYTech, Lda, Lisbon, Portugal). RT-qPCR assays were performed on a QuantStudio™ 6 Pro (Applied Biosystem by Thermo Fisher Scientific) following the protocol previously described [67]. For the antiviral assays, only the highest concentration of OPP, PE, DMIC and CuSP incorporated compounds in the lacquer-films were analysed. The percentage of viral elimination was calculated using Cq values and respective viral quantification load obtained from the control samples (viral sample diluted in water) minus the viral load obtained in the functionalized lacquer-films as previously described [68,69].

2.7. Cytocompatibility of antimicrobial lacquer-films

Indirect cytocompatibility assays were performed using a colorimetric assay based on resazurin reduction and an adaption of protocol of the International Organization for Standardization (ISO) 10993–5:2009 - Biological evaluation of medical devices, Part 5: Tests for in vitro cytotoxicity [70]. Resazurin is a non-fluorescent dye used to analyse bacterial or cell viability [71]. This assay is based on the resazurin reduction (blue dye) by reductase or diaphorase-type enzymes from mitochondria of living cells to resorufin (pink color). The color change is an indicator of metabolically viable cells [72]. Briefly, lacquer-films (0.31 cm^2) were incubated with cell culture media (Dulbecco's Modified Eagle's Medium) supplemented with 10% of fetal bovine serum (FBS) and 1% L-glutamine at room temperature for 24 h under orbital shaking (100 rpm), to obtain supernatant with lacquer extract. The L929 fibroblast cell line (1 × 10^4 cells/well) was incubated in a 96-well plate for 24 h plate at 37°C in a CO_2 incubator with atmosphere of 5% of

CO_2 (Heraeus HERAcell 150 Incubator, Thermo). After reaching 90% of confluence, cell culture media was replaced with the lacquer-extract medium. Plates were incubated for an additional 24 h. A positive control (cells cultured in normal cell culture medium), a negative control (only cell culture medium) and a positive control of cytotoxicity (1% Triton X-100) were also included. After incubation, 0.02% resazurin dye was added. The plates were further incubated for 3 h to allow for resazurin reduction. The optical density was read spectrophotometrically at 575 and 610 nm. The results were expressed as percentage of cell viability from positive control, by absorbance ratio between at 575 and 610 nm for the different conditions, according to the following equation:

$$\%\ cell\ viability = \frac{Abs\ sample - Abs\ negative\ control}{Abs\ positive\ control - Abs\ negative\ control} \times 100$$

According to ISO10993–5:2009 protocol, a cell viability below 70% of the positive control is defined as a potential cytotoxic result [70].

2.8. Statistical analysis

Data was reported as mean ± standard deviation. Statistical analysis was done using GraphPad Prism (version 8.0.2) by testing the normality and the significance values. Bonferroni's multiple comparisons test was used for antibacterial and antifungal lacquer-films analysis and Dunn's multiple comparisons test was used for antiviral lacquer-films analysis. Data was considered statistically significant for $p < 0.05$.

3. Results and discussion

PUR is one of the most important polymer materials used in coatings, due to its intrinsic properties, high absorptivity, mechanical stability, softness, and flexibility. In addition, the possibility to functionalize PUR-based materials to improve specific properties has made it an attractive material for many biomedical and common daily applications, including hospital environments, packaging, automotive industry, home appliances and infant care [73]. The selection of antimicrobial agents to be incorporated in PUR coating formulations should take into consideration some key criteria: (i) compatibility with processing parameters of the PUR lacquers (thermal stability); (ii) chemical compatibility between antimicrobial compound and the lacquer formulation used, and (iii) spectrum of bioactivity (bacteria, fungi, and virus). With these three criteria in mind, two phenolic-based compounds (OPP and PE); an ionic based-compound (DMIC); and a metal based-compound (CuSP) were selected.

Given that the production process of lacquer-films for surface coatings requires successive cycles of high temperatures to facilitate solvent evaporation, trials were conducted to confirm that the selected compounds maintained their antimicrobial activity, even after being subjected to two consecutive cycles with a minimum temperature of 140 °C. OPP, PE, and CuSP are reported to be thermally stable at high temperatures, given their boiling points of 286°C [45], 247°C (according to supplier), and >500°C [74], respectively. Accordingly, these compounds maintained their half-maximal inhibitory concentration values (IC50), even after being submitted to cycles of high temperatures. In contrast, the IC50 of DMIC after two cycles of high temperatures increased; however still presented antimicrobial activity against the strains tested (Supplementary Material). In fact IL, such as DMIC, are reported to be stable between 100 and 125 °C [75], but above these temperatures, a partial degradation of the alkyl chain can occur, which consequently impact their properties [75].

3.1. Evaluation of physical, optical, and chemical properties of lacquer-films incorporated with selected compounds

An important aspect evaluated in this study was the maintenance of the optical, physical, and chemical properties of the lacquer-films after composition with the above-mentioned compounds. The goal of this analysis was to ascertain whether the incorporation of these compounds affected the physical and chemical properties of lacquer-films commonly used as surface coatings. In that sense, antimicrobial compounds were incorporated in a PUR waterborne resin by solvent evaporation to produce antimicrobial lacquer-films. As shown in Fig. 1A, macroscopic observation of the lacquer-films with antimicrobial compounds revealed a white hue with a continuous surface. In some conditions it was possible to identify a few white aggregates, most likely due to poor solubility in water or in the PUR resin/formulation. This situation was more visible in lacquer-films with increasing concentrations of OPP (dashed line in Fig. 1A). However, some minor clusters could also be identified in other lacquer-films (p.e. lacquer-films of CuSP or DMIC), but in these cases, the unintended visual impact was lower.

Additional optical analysis of the lacquer-films was performed by gloss measurements and transmittance studies. Specular gloss can be defined as a measure between a specular reflectance of a surface relative to the specular intensity reflected at an angle of incidence θ [76]. Gloss is a simple and useful parameter that can be used as an indicator of material durability by monitoring the chemical bonds and functional groups existent in materials [77]; the impact of additives, including pigments [77]; and the surface roughness [78]. Specifically, there is a correlation between surface roughness and gloss, in which the increase of roughness decreases the gloss of the surface for the same angle of incidence [76,79]. In the present study, gloss analysis showed a value of 8.66 ± 0.59 for control lacquer-films (Fig. 1B), which was similar to the gloss of lacquer-films with the highest concentrations of PE (8.60 ± 0.45), DMIC (9.16 ± 0.41) and CuSP (9.09 ± 0.41). In contrast, lacquer-films with the highest concentration of OPP presented an increase of 1.5 in gloss values (10.13 ± 0.27), when compared to control lacquer-films, which could be related to intrinsic fluorescent properties of phenolic-based compounds [80]. The fluorescence can be defined as a radiation emission at lower energy by an object after exposure to a light source. Indeed, in OPP-based materials, this emitted radiation could be detected by the glossmeter [81], explaining the increased values. This increase was not visible in the other tested phenolic-based compound, PE, since its chemical

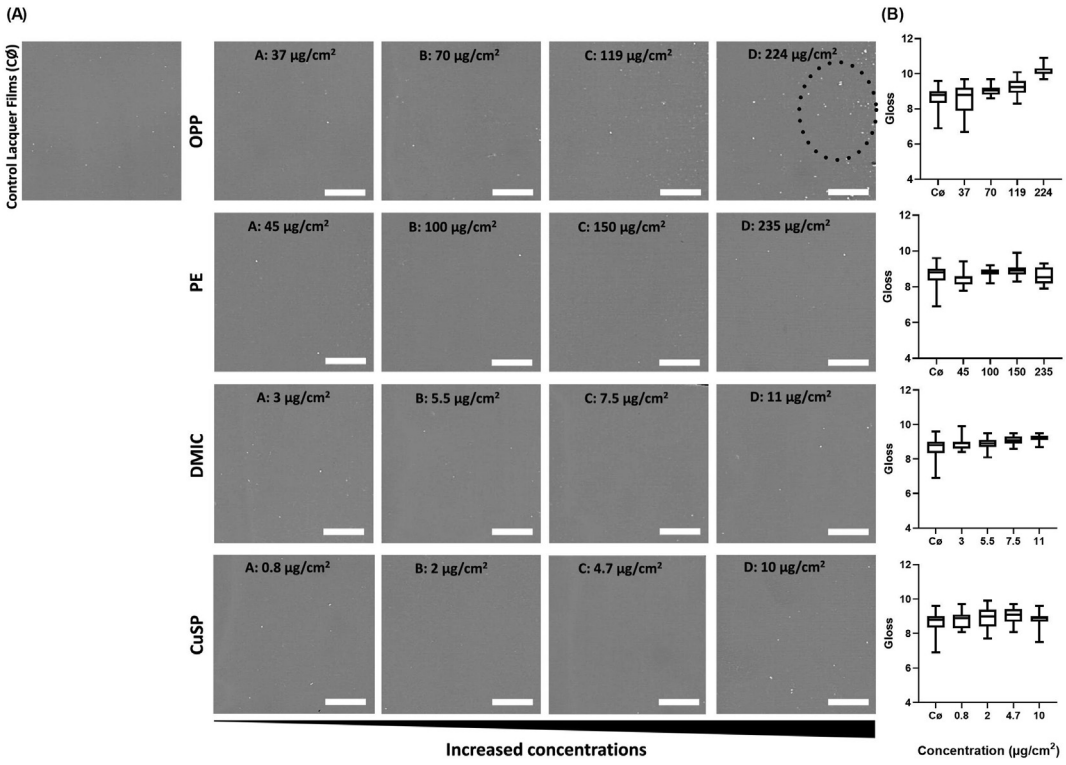

Fig. 1 – Analysis of lacquer-films produced: (A) macroscopic view and (B) gloss measurements of lacquer-films incorporated with different concentrations of antimicrobial compounds. The gloss values were obtained in three different lacquer-films for each condition, with 12 reads in different locations across each film length. Scale bar = 1 cm.

structure is not a pure phenolic compound, as described above.

Transmittance measurements were performed to analyse the optical integrity of the lacquer-films after incorporation of antimicrobial agents. The results showed that the incorporation of antimicrobial agents does not affect the optical properties of lacquer-films produced, since the differences between control and samples are less than 1.5% (Fig. 2A). Indeed, the transmittance value at 580 nm for control lacquer-films is 97.5%, while for lacquer-films with OPP, PE, DMIC and CuSP the transmittance values are 98.4%, 97.4%, 98.1% and 98.0%, respectively.

To assess any possible changes in the chemical bonds of produced lacquer-films induced by the presence of antimicrobial agents, we performed FTIR analysis comparing the normalized FTIR spectrum obtained from the control lacquer-films with lacquer-films with the highest concentration of antimicrobial compounds. Firstly, the impact of the incorporation of antimicrobial compounds in the polymerization process of PUR was investigated. It has been well described in

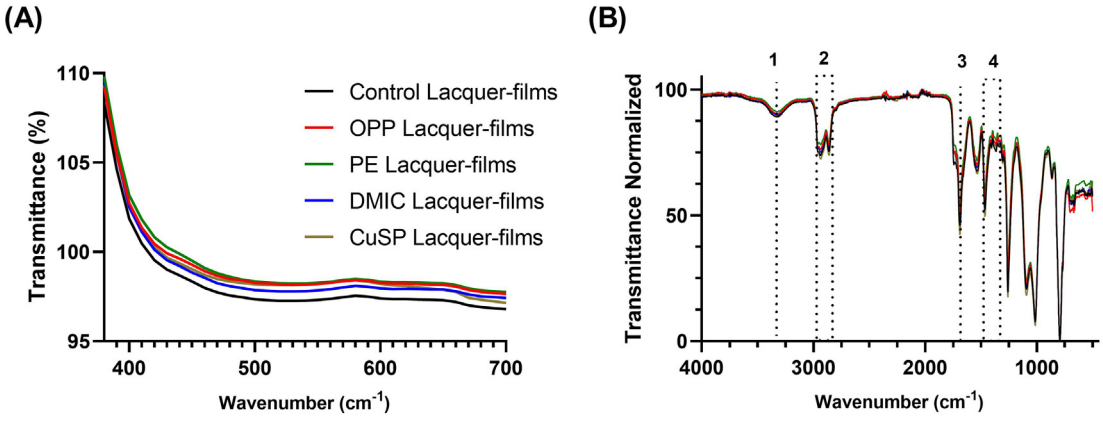

Fig. 2 – (A) Transmittance and (B) FTIR analysis of lacquer-films produced with antimicrobial compounds. The lines correspond to the lacquer-films with the highest concentration of antimicrobial compounds produced. Numbers represent specific bands used to analyse the FTIR spectrum.

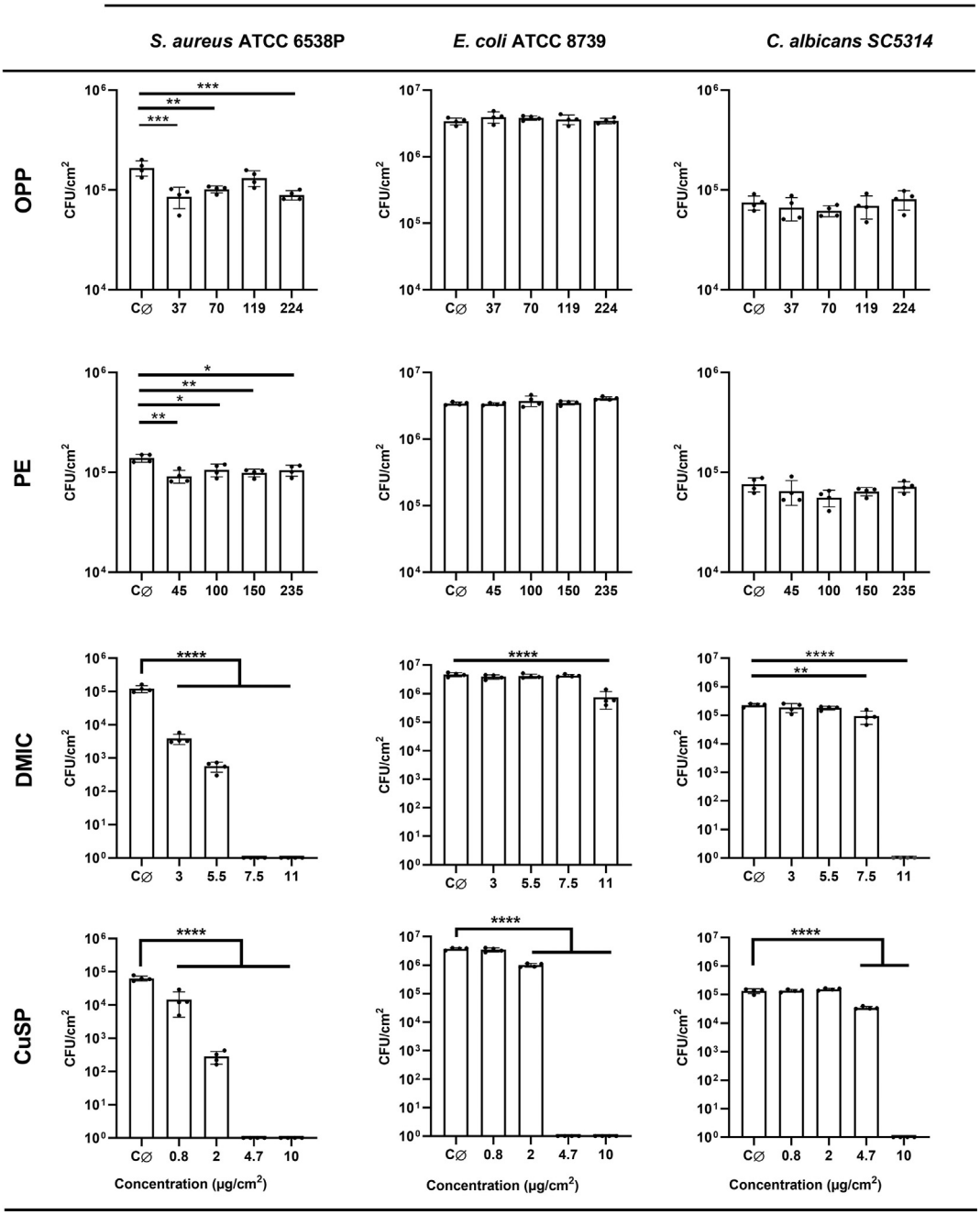

Fig. 3 – Antimicrobial activity for OPP, PE, DMIC and CuSP lacquer-films against *S. aureus*, *E. coli* and C. albicans. CØ represents the lacquer-films without antimicrobial compound (control lacquer-film). Error bars represent the standard deviation (n = 4). Bonferroni's multiple comparisons test: *p < 0.05; **p < 0.01; ***p < 0.001 and ****p < 0.0001. Each condition was tested in quadruplicate in two independent assays.

the literature that PUR formation can be monitored by measuring the isocyanate (NCO) content, since it reacts with diols being consumed [82,83]. As shown in Fig. 2B, the FTIR spectrum of the lacquer-films, regardless of the addition of antimicrobial compounds, revealed the absence of the characteristic band of NCO (asymmetric isocyanate stretching band at approximately between 2250 and 2270 cm^{-1}), confirming a reduction of NCO bands and consequent PUR synthesis.

Further analysis of the FTIR spectrum revealed a spectral overlap among the different samples indicating the absence of chemical alterations in lacquer-films, even after the incorporation of antimicrobial compounds. In Fig. 2B, it is possible to confirm some characteristic bands of PUR: (i) band 1 is a large absorption band identified at 3322 cm^{-1}, probably resulting from the overlap of two peaks corresponding to NH stretching (at higher wavenumber) and OH group (at smaller wavenumber) [84–86]; (ii) band 2 is attributed to –CH2 stretching

Table 1 – Antimicrobial activity of produced waterborne polyurethane-based lacquer-films formulated with different concentrations of antimicrobial compounds.

Concentration (μg/cm²)		R value[a]/Microbial Ratio (%)[b]			
		S. aureus	*E. coli*	*C. albicans*	SAR-CoV-2[c]
OPP	37	0.295/48.53	−0.060/−15.75	0.059/11.35	–
	70	0.210/38.90	−0.051/−12.06	0.082/17.53	–
	119	0.070/13.54	−0.024/−6.23	0.042/7.68	–
	224	0.269/46.50	−0.007/−1.47	−0.027/−7.51	n.d./41.17
PE	45	0.130/24.78	−0.075/−18.98	0.075/14.52	–
	100	0.154/29.17	−0.009/−2.19	0.135/26.40	–
	150	0.124/24.15	−0.035/−9.49	0.069/15.18	–
	235	0.190/34.47	0.006/1.46	0.022/5.28	n.d./53.17
DMIC	3	1.506/96.83	0.083/17.11	0.101/16.87	–
	5.5	2.350/99.54	0.053/11.50	0.104/21.14	–
	7.5	5.077/100	0.038/8.82	0.425/59.09	–
	11	5.077/100	0.854/84.41	5.355/100	n.d./19.51
CuSP	0.8	0.716/76.02	0.038/7.67	−0.001/0.27	–
	2	2.374/99.54	0.579/73.50	−0.051/−11.80	–
	4.7	4.799/100	6.573/100	0.599/74.95	–
	10	4.799/100	6.573/100	5.135/100	n.d./13.67

[a] Antibacterial and antifungal activity (R) is calculated according to ISO 22196:2011, by using the following formula: **R = (Ut−U0) − (At−U0) = Ut−At** in which **U0**, **Ut** and **At** are the average of the common logarithm of the number of viable bacteria recovered immediately after inoculation for untreated samples, 24 h of incubation for untreated samples and 24 h of incubation of test samples, respectively.

[b] Microbial Ratio (MR) is the percentage of viable bacteria or fungi recovered from treated samples compared to untreated samples after 24 h of incubation. MR were calculated by the next formula: **MR = 100 − (Ts x 100)/Cs**, in which Ts and Cs is the average of viable bacteria or fungi recovered from treated and untreated samples, respectively. Negative values indicated an increase of microbial burden comparing to control group and positive values indicates a microbial reduction to control samples.

[c] Antiviral activity is calculated by percentage of viral reduction according to control group (polyethene inert surface).

[84]; (iii) band 3 (1688 cm^{-1}) is assigned by a C=O groups of PUR linkage [85] and (iv) bands in region 4 can be attributed to other modes of vibration of –CH2 group [84–86]. This lack of specific bands associated to the antimicrobial compounds was expected, given that OPP and PE exhibit the same chemical groups already present in lacquer control formulations and that in the DMIC and CuSP lacquer-films the antimicrobial compounds are present at relatively low concentrations (≤11 μg/cm^2 and, ≤10 μg/cm^2, respectively), which makes it difficult to identify specific bands. Collectively, these results suggest that the addition of these antimicrobial compounds did not significantly change the chemical nature of the lacquer, with exception of OPP in which high concentrations impacted the gloss values and the surface homogeneity.

3.2. *Antimicrobial performance of lacquer-films modified with the selected compounds*

Following the validation of the chemical and physical properties of the lacquer-films with the addition of antimicrobial agents, we next evaluated their antimicrobial activity against a representative gram-positive and gram-negative bacterial strains and a fungal strain, following the ISO 22196(E):2011 protocol. According to this protocol, antimicrobial activity is determined by the R value, which is expressed as the difference between the common logarithm of the number of CFU collected in control lacquer-films and the common logarithm of the number of CFU collected in the samples. R values ≤ 0.5 are classified as having no antimicrobial activity; 0.5–1 moderate microbial reduction; 1–2 medium microbial reduction; and ≥3 good antimicrobial reduction [87].

Regarding the phenolic-based compounds, although OPP and PE lacquer-films were responsible for a significant CFU log reduction of *S. aureus* in all concentrations tested (Fig. 3), this translated to an R value below 0.5, indicating no antimicrobial activity (Table 1). Additionally, these phenolic compounds showed no antibacterial effect on *E. coli*, as well as no antifungal activity for *C. albicans*, as the microbial load was similar to the control condition. Nevertheless, our data suggests that gram-positive bacteria are more susceptible to phenolic-based compounds incorporated in polymer materials. These results are aligned with Gaikwad, K et al. (2018), which reported an antimicrobial effect of the phenolic compound pyrogallol incorporated in low-density polyethylene (LDPE) against *S. aureus*, but only moderate activity against *E. coli* [88]. A similar result has been reported, showing that films with higher phenolic content (gallic acid equivalent) have an increased bacterial inhibition (>1 log reduction) against *B. subtilis* and *S. aureus* in comparison with gram-negative strains [89]. In fact, the presence of an outer membrane in the cell wall of gram-negative bacteria plays an important protective role against environmental conditions by stabilizing its inner membrane and protecting its thin peptidoglycan layer [90]. This complex composition of the cell wall in gram-negative strains seems to contribute to their increased resistance to membrane active agents, such as phenolic-based compounds [91]. Despite the high range of applications of phenolic-based compounds for cosmetic [40] and biomedical purposes [38,92,93] their mechanisms of action have not been well described. Their action have been mainly attributed to the inhibition of microbial enzymes, toxins and other virulence factors, by directly altering bacterial metabolism; changing bacterial polarity

through alteration of surface electron receptors; and impacting the production of substrates required for growth [94,95]. Most importantly, phenolic-based compounds have been described to act by compromising bacterial cell wall permeability, suggesting that the presence of the outer membrane of gram-negative bacteria provides protection against phenolic compounds [96]. Indeed, it is suggested that OPP's biocidal effect is associated to the disruption of the diaminopimelate and lysine biosynthesis pathway, leading to the down-regulation of amino acid synthesis essential for the peptidoglycan cell wall [97]. PE can also act as a bacteriostatic agent by changing the permeability of the cytoplasmatic membrane through inhibition of DNA and RNA synthesis [98]. In in this work, increased antimicrobial effect of phenolic compounds could not be obtained, since higher concentrations of OPP and PE in lacquer formulations, would significantly change the lacquer properties, specifically viscosity.

On the other hand, DMIC lacquer-films exhibited an intermediate antibacterial activity against *S. aureus* for the lowest concentration tested (3 μg/cm^2) with a 1.51 CFU log reduction, and a good antibacterial reduction for the intermediate concentration (5.5 μg/cm^2) with 2.35 CFU log reduction. These values represent a bacterial reduction of 96.8 and 99.5%, respectively. For the highest concentrations tested (7.5 and 11 μg/cm^2) no bacteria were detected, representing a 100% bacterial reduction. Regarding DMIC's antibacterial activity against *E. coli*, only the highest concentration tested (11 μg/cm^2) was able to control bacterial proliferation (0.85 CFU log reduction and 84.4% of bacterial reduction). These results are in accordance to those reported previously, which describe that gram-positive bacteria seem to be more susceptible to IL than gram-negative bacteria or fungi [50,99,100], specifically 1-ethylpyridinium docusate and tributyl(2-hydroxyethyl)phosphonium docusate [101]; tetrahexyl-ammonium bromide [102] and methylimidazolium chloride and pyridinium chloride based compounds [103]. Gram-negative bacteria have an additional outer layer which is negatively charged. This layer can act as a barrier for neutral or negatively charged compounds, while cationic compounds can bind to the outer membrane of the cell wall and cause bacterial damage [104]. In that sense, the limited effect of DMIC on gram-negative bacteria could be related to the presence of chloride. Similar results are reported by He et al. (2013), in which an IL with a fumarate anion presents increased antimicrobial properties against *B. subtilis* when compared to *E. coli* [105]. Regarding its antifungal properties, DMIC exhibited a great control on fungal proliferation of *C. albicans*. Specifically, the two highest concentrations of lacquer-films with DMIC had a significant log reduction. Indeed, 7.5 μg/cm^2 and 11 μg/cm^2 DMIC lacquer-films showed a 0.43 and 5.36 CFU log reduction, respectively, representing 59% and 100% fungal reduction (Fig. 3 and Table 1). DMIC has also been described to control different fungi strains, such as *A. padwickii*, *A. dauci*, and *A. linicola* [106]. Although the mechanism of antimicrobial control by IL is not completely understood it has been related to the interaction of cations or anions on the cell surface that promotes structural and dynamic changes causing pathogen disruption [107]. IL antimicrobial activity is also potentiated by extending the number of alkyl groups present in chain length rather than the anion/cation combination [50,55].

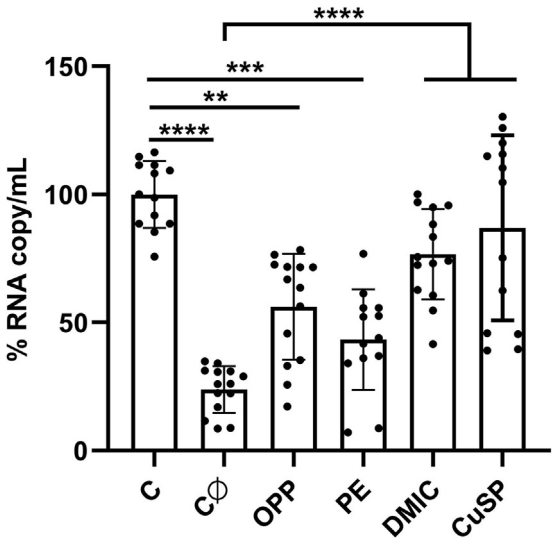

Fig. 4 – Antiviral activity of lacquer-films with OPP, PE, DMIC and CuSP, (with 224, 235, 11 and 10 μg/cm^2) against SAR-CoV-2. C represents the control condition (polyethene inert surface where lacquer-films are deposited) and CØ represents the lacquer-films without antimicrobial compounds (control lacquer-film). Error bars represent the standard deviation of four independent essays with replicates. Dunn's multiple comparisons test: **p < 0.01, ***p < 0.001 and ****p < 0.0001.

CuSP lacquer-films exhibited a complete clearance (>4 CFU log reduction) for the highest concentrations tested (4.7 and 10 μg/cm^2) for both bacterial strains. For the fungal strain, the highest concentration tested (10 μg/cm^2) resulted in >5 CFU log reduction, while lacquer-films incorporated with 4.7 μg/cm^2 of CuSP showed only a moderate microbial reduction (0.57 log reduction, representing an almost 75% reduction in the fungal burden). At lower concentrations (0.8 and 2 μg/cm^2), gram-positive bacteria presented a CFU log reduction of 0.72 and 2.37, respectively (representing a 76.0 and 99.5% bacterial reduction); while for gram-negative bacteria only the 2 μg/cm^2 condition displayed antibacterial activity with a 0.58 CFU log reduction (73.5% bacterial reduction). Lacquer-films incorporated with 0.8 μg/cm^2 or 2 μg/cm^2 of CuSP showed no antifungal activity. According to this, CuSP lacquer-films showed to be highly effective in limiting both bacterial and fungal growth. These results are aligned with other authors which describe the antimicrobial activity of copper-based compounds against *S. aureus*, *E. coli* [108] and MRSA [109]. Although the associated antimicrobial mechanisms are not fully elucidated, it is thought that the release of ions from copper plays an important role, leading to reactive oxygen species (ROS) production [110,111]. Others have suggested that intracellular copper ions can selectively bind to sulfhydryl groups of respiratory enzymes and disrupt some essential proteins [112]. Copper's proposed mechanism of action justifies its broad spectrum, ranging from several bacteria, such as *Staphylococcus* spp., *E. coli*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, and *S. tryphimurium* [111], to fungi, such as *C. albicans*, *A.niger*,

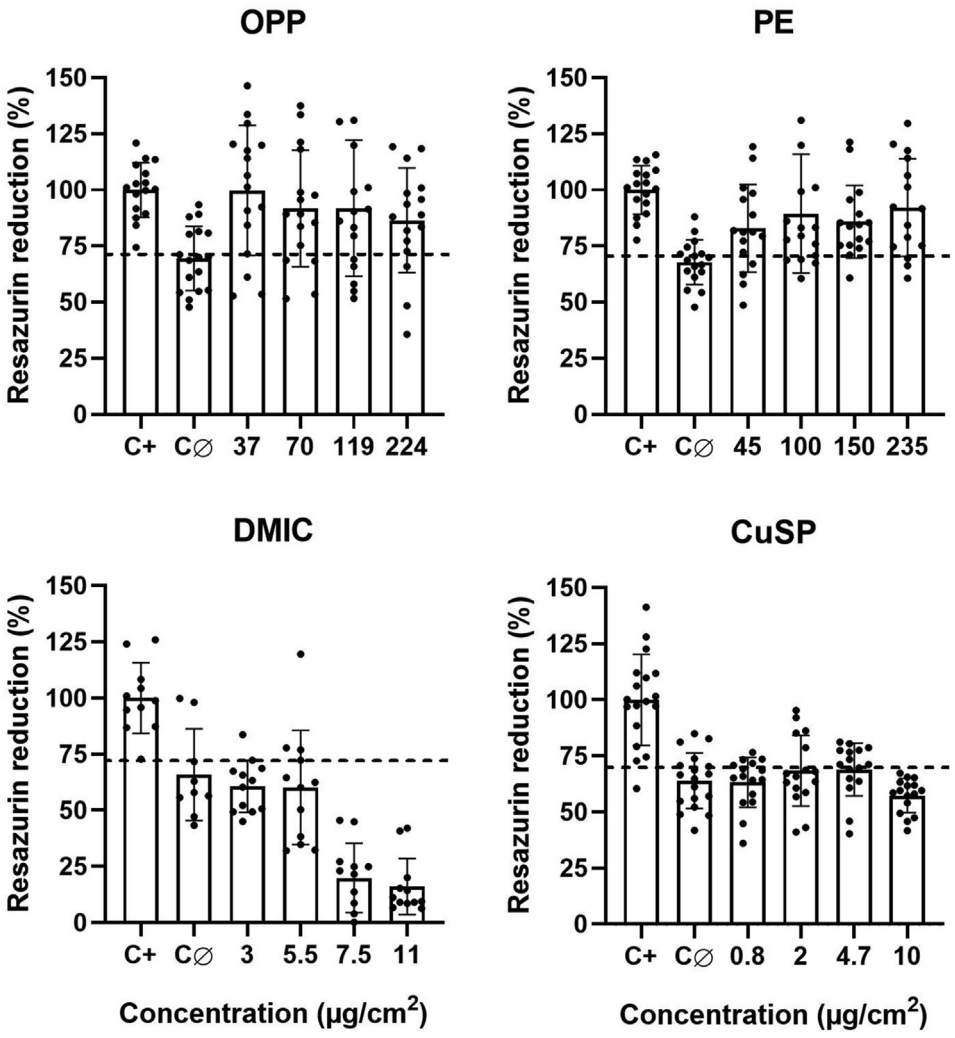

Fig. 5 – Viability assay of lacquer-films with antimicrobial compounds against L-929 mouse fibroblast cell line using an indirect exposure method. C+ represents the positive control (only cells + medium) and CØ represents the control of lacquer-films without antimicrobial compounds (control lacquer-film). Dashed line represents 70% of resazurin reduction and error bars represent the standard deviation. Results are from three independent assays with quadruplicates in each assay.

A.oryzae, *Fusarium oxysporum* and *Alternaria alternata* [64,113,114]. Regarding the difference in susceptibility between gram-positive and negative strains, we observed that CuSP based lacquer-films are more effective against *S. aureus* than *E. coli*, which is aligned with what reported by Ude. Z. et al. (2019) [115]. In the same work, the authors showed an antifungal activity of copper-based compounds against filamentous and non-filamentous fungi (such as different *Candida* strains, *T. asahii*, *A. fumigatus*, *A. corymbifera* and *T. mentagrophytes*) [115].

The lacquer-films with and without addition of antimicrobial agents were tested for antiviral activity by evaluating the percentage of SARS-CoV-2 deoxyribonucleic acid degradation. The exposure of SARS-CoV-2 samples to the lacquer-films *per se* (without addition of antimicrobial compounds), when compared with the control condition (polyethene inert surface), resulted in the elimination of 75.7% of the virus RNA. However, the lacquer-films incorporated with the different antimicrobial compounds did not enhance viral elimination. In fact, the incorporation of DMIC and CuSP in lacquer-films reduced the initial antiviral activity of the lacquer-film to 19.5% and 13.7%, respectively (Fig. 4 and Table 1). This effect could be related to potential interactions with stabilizers of the lacquer formulation. Considering that the waterborne PUR lacquer formulation results from a mixture of different commercial raw materials, it is difficult to pinpoint which compound is responsible for the antiviral activity. The protocol used in this work (RT-qPCR-based detection method) only targets the RNA content by measuring the difference of the copy numbers of nucleic acids in each sample after exposure to viral inoculum. Although the measurement of RNA degradation clearly testifies to virus elimination, the lacquer, as well as the antimicrobial agents tested, could target virus viability through the disruption of alternative biological processes. An alternative methodology to test the antiviral activity could be the measurement of number of plaque forming units (PFU).

Collectively, these results highlight the antimicrobial performance of several compounds, with CuSP and DMIC presenting the highest potential and the broadest spectrum.

3.3. *Cytocompatibility performance of lacquer-films*

An important parameter in processing materials with antimicrobial activity is the selective action against pathogens of interest, while maintaining cytocompatibility with mammalian cells, in particular for surfaces exposed to human contact. To assess the cytocompatibility of the materials produced, L929 fibroblasts were incubated for 24 h with medium previously exposed to PUR lacquer-films or PUR lacquer-films incorporated with antimicrobial compounds. Fibroblasts exposed to PUR lacquer alone exhibited 67.2 ± 13.5% of cell viability, indicating a potential cytotoxic effect against these cells (Fig. 5). Importantly, OPP, PE and CuSP lacquer-films, regardless the concentration tested, exhibited a similar or an increased cytocompatibility, when compared to control films. Regarding the phenolic-based compounds, the increased cytocompatibility can be explained by their protective antioxidant activity [116]. Indeed, phenols, specifically their catechol group, can provide protective effect against ROS produced during oxidative stress through its free radical scavenging activity [116,117]. In contrast, lacquer-films with the highest concentrations of DMIC (7.5 and 11 $\mu g/cm^2$) significantly decreased cytocompatibility, with only 31.79 ± 21.1 and 25.9 ± 15.6% of cell viability, respectively. The length of alkyl chain and the pair anion/cation seem to play an important role in the cytotoxicity of IL. IL with longer alkyl chain length (n > 4) are more lipophilic and therefore, tend to be more easily incorporated in the phospholipid bilayers [118]. This incorporation further increases the lipophilic nature of the biological membranes, and results in delocalized charges affecting the physiological function of the cell membrane. Regarding the impact of the anion/cation pair, IL may contain either bromide [Br]-, chloride [Cl]-, tetrafluoroborato [BF4]- or hexafluorofosfato [PF6]-, which have also been associated with cytotoxic effect [118]. This information is aligned with our data, since DMIC possesses a longer alkyl chain, as well as the [Cl]- anion, and for this reason it may have contributed for its cytotoxicity at higher concentrations. Although the extracts of PUR lacquer were associated with relevant cytotoxicity, cell viability is still within acceptable parameters established in ISO 10993–5. In fact, PUR are described as polymers with good cytocompatibility in what regards L929 mouse fibroblasts, MRC-5 human fibroblast [119], and HeLa cell line [120]; and have been used in medical devices [121], chronic wound dressings [122], and water filters [123]. In this work, the lacquer-films with OPP, PE, and CuSP, independently of the concentration tested, did not alter the cytocompatibility of the lacquer-films. In contrast, lacquer-films incorporated with the highest concentrations of DMIC presented a cytotoxic effect [70].

4. Conclusion

Commercial PUR resins are a versatile material used in many domains of society. In this study, selected antimicrobial agents, OPP, PE, DMIC and CuSP, were incorporated in PUR-based lacquer-films aiming to obtain antimicrobial properties. Transmittance and FTIR analyses showed that the incorporation of the antimicrobial agents did not have an impact on the visual and chemical properties of the lacquer-films. Among the different compounds tested, CuSP lacquer-films exhibited the most promising antimicrobial properties, with strong antibacterial activity against both gram-positive and gram-negative bacteria, and antifungal properties, while maintaining cytocompatibility. On the other hand, DMIC lacquer-films, although exhibiting good antimicrobial activity against *S. aureus* and *C. albicans*, display reduced cytocompatibility at higher concentrations. Regarding the OPP and PE lacquer-films, they present a null or lower antibacterial and antifungal activity. Furthermore, none of the developed lacquer-films incorporated with antimicrobial agents showed a significant effect on viral load. In future studies, it would be important to evaluate whether the selected compounds maintain their antimicrobial properties when tested against other microorganisms commonly present on inanimate surfaces, such as *P. aureuginosa, A. niger* or other representative viruses. In summary, the results reported in this study highlight the potential of incorporating antimicrobial agents in surface coatings as a promising strategy to avoid the attachment and colonization of microorganisms on inanimate surfaces, and reduce the exposure of potentially harmful pathogens to humans.

Credit author statement

Tiago Costa: Conceptualization; Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Maria I. Veiga:** Formal analysis, Funding acquisition, Investigation, Resources, Writing – review and editing. **Nuno S. Osório:** Formal analysis, Funding acquisition, Resources, Writing – review and editing. **Nuno M. Neves:** Conceptualization, Formal analysis, Funding acquisition, Resources, Supervision, Validation, Writing – review and editing. **Helena Aguilar:** Conceptualization; Formal analysis, Funding acquisition, Resources, Supervision, Validation, Writing – review and editing. **Alexandra G. Fraga:** Conceptualization; Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – review and editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmrt.2023.04.243.

REFERENCES

[1] Kramer A, Assadian O. Survival of microorganisms on inaminate surfaces. In: Borkow G, editor. Use of biocidal surfaces for reduction of healthcare acquired infections. Springer International Publishing; 2014. p. 6–26.
[2] Chang T, Sepati M, Herting G, Leygraf C, Rajarao GK, Butina K, et al. A novel methodology to study antimicrobial properties of high-touch surfaces used for indoor hygiene applications-A study on Cu metal. PLoS One 2021;16(2):e0247081. https://doi.org/10.1371/journal.pone.0247081.
[3] Nazaroff WW. Embracing microbes in exposure science. J Expo Sci Environ Epidemiol 2019;29:1–10. https://doi.org/10.1038/s41370-018-0075-4.
[4] Adams RI, Bhangar S, Dannemiller KC, Eisen JA, Fierer N, Gilbert JA, et al. Ten questions concerning the microbiomes of buildings. Build Environ 2019;109:224–34. https://doi.org/10.1016/j.buildenv.2016.09.001.
[5] Artiola JF, Reynolds KA, Brusseau ML. Urban and household pollution. In: Brusseau ML, Pepper IL, Gerba CP, editors. Environmental and pollution science. 3rd. ed. Elsevier Inc; 2019. p. 311–26.
[6] National Academies of Sciences. Engineering, and medicine. Microbiomes of the built environment: a research agenda for indoor microbiology, human health, and buildings. The National Academies Press; 2017. https://doi.org/10.17226/23647.
[7] Iskandar S, Saif A, Nawas T. Isolation of potentially pathogenic bacteria from public service cars door handles. Int. J. Curr. Microbiol. Appl. Sci. 2018;7(12):1154–9. https://doi.org/10.20546/ijcmas.2018.712.142.
[8] Stephenson RE, Gutierrez D, Peters C, Nichols M, Boles BR. Elucidation of bacteria found in car interiors and strategies to reduce the presence of potential pathogens. Biofouling 2014;30(3):337–46. https://doi.org/10.1080/08927014.2013.873418.
[9] Bhatta DR, Hamal D, Shrestha R, Subramanya SH, Baral N, Singh RK, et al. Bacterial contamination of frequently touched objects in a tertiary care hospital of Pokhara, Nepal: how safe are our hands? Antimicrob Resist Infect Control 2018;7:97. https://doi.org/10.1186/s13756-018-0385-2.
[10] Wißmann JE, Kirchhoff L, Brüggemann Y, Todt D, Steinmann J, Steinmann E. Persistence of pathogens on inanimate surfaces: a narrative review. Microorganisms 2021;9(2):343. https://doi.org/10.3390/microorganisms9020343.
[11] Stapleton PD, Taylor PW. Methicillin Resistance in *Staphylococcus aureus*: mechanisms and modulation. Sci Prog 2002;85(1):57–72. https://doi.org/10.3184/003685002783238870.
[12] Groza A, Ciobanu CS, Popa CL, Iconaru SL, Chapon P, Luculescu C, et al. Structural properties and antifungal activity against *Candida albicans* biofilm of different composite layers based on Ag/Zn doped hydroxyapatite-polydimethylsiloxanes. Polymers 2016;8(131):1–22. https://doi.org/10.3390/polym8040131.
[13] Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, et al. *Candida albicans*-the virulence factors and clinical manifestations of infection. J. Fungi 2021;7(79):1–19. https://doi.org/10.3390/jof7020079.
[14] Mousavi B, Hedayati MT, Hedayati N, Ilkit M, Syedmousavi S. *Aspergillus* species in indoor environments and their possible occupational and public health hazards. Curr Med Mycol 2016;2(1):36–42. https://doi.org/10.18869/acadpub.cmm.2.1.36.
[15] Camacho E, Casadevall A. Cryptococcal traits mediating adherence to biotic and abiotic surfaces. J. Fungi 2018;4(88):1–19. https://doi.org/10.3390/jof4030088.
[16] Jabłońska-Trypuć A, Makuła M, Włodarczyk-Makuła M, Wołejko E, Wydro U, Serra-Majem L, et al. Inanimate surfaces as a source of hospital infections caused by fungi, bacteria and viruses with particular emphasis on SARS-CoV-2. Int J Environ Res Publ Health 2022;19(9121):1–22. https://doi.org/10.3390/ijerph19138121.
[17] Rakowska PD, Tiddia M, Faruqui N, Bankier C, Pei Y, Pollard AJ, et al. Antiviral surfaces and coatings and their mechanisms of action. Commun. Mater. 2021;2(1):1–19. https://doi.org/10.1038/s43246-021-00153-y.
[18] Katzenberger RH, Rösel A, Vonberg R-P. Bacterial survival on inanimate surfaces: a field study. BMC Res Notes 2021;14(97):1–10. https://doi.org/10.1186/s13104-021-05492-0. 2021.
[19] Coughenour C, Stevens V, Stetzenbach LD. An evaluation of methicillin-resistant staphylococcus aureus survival on five environmental surfaces. Microb Drug Resist 2011;17(3):457–61. https://doi.org/10.1089/mdr.2011.0007.
[20] Ferreira TR, Costa SM, Souza JM, Castilho ACS, Eller LKW, Moris DV, et al. Viability of *Candida albicans* in different fomites and hospital surfaces under disinfectants and biological fluids influence. Res Soc Dev 2021;10(5):e38010515049. https://doi.org/10.33448/rsd-v10i5.15049.
[21] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6(130):1–18. https://doi.org/10.1186/1471-2334-6-130.
[22] Carraturo F, Guidice CD, Morelli M, Cerullo V, Libralato G, Galdiero E, et al. Persistence of SARS-CoV-2 in the environment and COVID-19 transmission risk from environmental matrices and surfaces. Environ Pollut J 2020;265:115010. https://doi.org/10.1016/j.envpol.2020.115010. 2020.
[23] Chapman J, Weir E, Regan F. Period four metal nanoparticles on the inhibition of biofouling. Colloids Surf B Biointerfaces 2010;78:208–16. https://doi.org/10.1016/j.colsurfb.2010.03.002. 2010.
[24] Alamri A, El-Newehy MH, Al-Deyab SS. Biocidal polymers: synthesis and antimicrobial properties of benzaldehyde derivatives immobilized onto amine-terminated

polyacrylonitrile. Chem Cent J 2012;6(111):1–13. https://doi.org/10.1186/1752-153X-6-111.
[25] D'Antonio NN, Rihs JD, Stout JE, Yu VL. Computer keyboard covers impregnated with a novel antimicrobial polymer significantly reduce microbial contamination. Am J Infect Control 2013;41:337–9. https://doi.org/10.1016/j.ajic.2012.03.030.
[26] Venkateswaran S, Santos ODD, Scholefield E, Lilienkampf A, Gwynne PJ, Swann DG, et al. Fortified interpenetrating polymers-bacteria resistant coatings for medical devices. J Mater Chem B 2016;4(2):5405–11. https://doi.org/10.1039/c6tb01110a.
[27] Skovdal SM, Jørgensen NP, Petersen R, Jensen-Fangel S, Ogaki R, Zeng G, et al. Ultra-dense polymer brush coating reduces *Staphylococcus epidermidis* biofilms on medical implants and improves antibiotic treatment outcome. Acta Biomater 2018;76:46–55. https://doi.org/10.1016/j.actbio.2018.07.00.
[28] Sedlarik V. Antimicrobial modifications of polymers. In: Chamy R, editor. Biodegradation - life of science. IntechOpen; 2013. p. 188–204. https://doi.org/10.5772/56222.
[29] Apicella A, Scarfato P, Maio LD, Incarnato L. Sustainable active PET films by functionalization with antimicrobial bio-coatings. Front. Mater. 2019;6(243):1–10. https://doi.org/10.3389/fmats.2019.00243.
[30] Howard L, Almousa R, Xie D. Polyurethane surface modified with a hydrophilic polymer containing quaternary ammonium bromide. Emergent Mater 2020;3(5):637–48. https://doi.org/10.1007/s42247-020-00104-2.
[31] Turner RJ. Metal-based antimicrobial strategies. Microb Biotechnol 2017;10(5):1062–5. https://doi.org/10.1111/1751-7915.12785.
[32] Yu K, Lo JCY, Yan M, Yang X, Brooks DE, Hancock REW, et al. Anti-adhesive antimicrobial peptide coating prevents catheter associated infection in a mouse urinary infection model. Biomaterials 2017;116:69–81. https://doi.org/10.1016/j.biomaterials.2016.11.047. 2017.
[33] Scaffaro, Botta L, Maio A, Gallo G. Incorporation of an antibiotic in poly(lactic acid) and polypropylene by melt processing. J Appl Biomater Funct Mater 2016;14(3):e240–7. https://doi.org/10.5301/jabfm.5000285.
[34] Greenhalgh R, Dempsey-Hibbert NC, Whitehead KA. Antimicrobial strategies to reduce polymer biomaterial infections and their economic implications and considerations. Int Biodeterior Biodegrad 2016;136:1–14. https://doi.org/10.1016/j.ibiod.2018.10.005. 2016.
[35] Marturano V, Cerruti P, Ambrogi V. Polymer additives. Phys. Sci. Rev. 2017:1–20. https://doi.org/10.1515/psr-2016-0130. 20160130.
[36] Kumar P, Huo P, Zhang R, Liu B. Antibacterial properties of graphene-based nanomaterials. Nanomaterials 2019;9(737):1–22. https://doi.org/10.3390/nano9050737.
[37] Mohammed E, Kumar A, Bekyarora E, Al-Hadeethi Y, Zhang X, Chen M, et al. Antimicrobial mechanisms and effectiveness of graphene and graphene-functionalized biomaterials. A scope review. Front Bioeng Biotechnol 2020;8(465):1–22. https://doi.org/10.3389/fbioe.2020.00465.
[38] Malta LG, Liu RH. Analyses of total phenolics, total flavonoids, and total antioxidant activities in foods and dietary supplements. In: Alfen NKV, editor. Encyclopedia of agriculture and food systems. Elsevier Ltd.; 2014. p. 305–14. https://doi.org/10.1016/b978-0-444-52512-3.00058-9.
[39] Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 2006;99:191–203. https://doi.org/10.1016/j.foodchem.2005.07.042.
[40] Panzella L. Natural phenolic compounds for health, food and cosmetic applications. Antioxidants 2020;9(427):1–5. https://doi.org/10.3390/antiox9050427.
[41] Martillanes S, Rocha-Pimienta J, Cabrera-Bañegil M, Martín-Vertedor D, Delgado-Adámez J. Application of phenolic compounds for food preservation: food additive and active packaging. In: Soto-Hernandez M, Palma-Tenango M, Garcia, editors. -Mateos MR. Phenolic compounds - biological activity. IntechOpen; 2017. p. 39–58.
[42] Shishatskiy S, Makrushin V, Levin I, Merten P, Matson S, Khotimskiy V. Effect of immobilization of phenolic antioxidant on thermo-oxidative stability and aging of poly(1-trimethylsilyl-1-propyne) in view of membrane application. Polymers 2022;14(462):1–14. https://doi.org/10.3390/polym14030462.
[43] Panzella L, Napolitano A. Natural phenol polymers: recent advances in food and health applications. Antioxidants 2017;6(30):1–24. https://doi.org/10.3390/antiox6020030.
[44] Jones FN, Nichols ME, Pappas SP. Epoxy and phenolic resins. In: Jones FN, Nichols ME, Pappas SP, editors. Organic coatings: science and Technology. fourth ed. John Wiley & Sons, Inc; 2017. p. 188–204.
[45] EPA - United States Environmental Protection Agency. Reregistration eligibility decision for 2-phenylphenol and salts (orthophenylphenol or OPP). United States of America; 2006.
[46] Kwon YI, Apostolidis E, Labbe RG, Shetty K. Inhibition of *Staphylococcus aureus* by phenolic phytochemicals of selected clonal herbs species of Lamiaceae family and likely mode of action through proline oxidation. Food Biotechnol 2007;21:71–89. https://doi.org/10.1080/08905430701191205.
[47] Chávez JH, Leal PC, Yunes RA, Nunes RJ, Barardi CRM, Pinto AR, et al. Evaluation of antiviral activity of phenolic compounds and derivatives against rabies virus. Vet Microbiol 2006;116:53–9. https://doi.org/10.1016/j.vetmic.2006.03.019.
[48] Simonetti G, Brasili E, Pasqua G. Antifungal activity of phenolic and polyphenolic compounds from different matrices of *Vitis vinifera* L. Against humam pathogens. Molecules 2020;25(3748):1–22. https://doi.org/10.3390/molecules25163748.
[49] Raja A, Pk S, Shekhar N, Singh H, Sarma P, Prakash A, et al. Pre-eminence and comparative analysis of skin and surface disinfectants: a systematic review to pave the way for SARS-CoV-2 prevention. Int. J. Clin. Virol. 2021;5:47–53. https://doi.org/10.29328/journal.ijcv.1001035.
[50] Carson L, Chau PKW, Earle MJ, Gilea MA, Gilmore BF, Gorman SP, et al. Antibiofilm activities of 1-alkyl-3-methylimidazolium chloride ionic liquids. Green Chem 2009;11(4):492–7. https://doi.org/10.1039/b821842k.
[51] Maximo GJ, Santos RJBN, Lopes-Da-Silva JA, Costa MC, Meirelles AJA, Coutinho JAP. Lipidic protic ionic liquid crystals. ACS Sustainable Chem Eng 2014;2(4):672–82. https://doi.org/10.1021/sc400365h.
[52] Cuppini M, Garcia IM, Souza VS, Zatta KC, Visioli F, Leitune VCB, Guterres SS, Scholten JD, Collares FM. Ionic liquid-loaded microcapsules doped into dental resin infiltrants. Bioact Mater 2021;6(9):2667–75. https://doi.org/10.1016/j.bioactmat.2021.02.002.
[53] Kumer A, Khan MW. Synthesis, characterization, antimicrobial activity and computational exploration of ortho toludinium carboxylate ionic liquids. J Mol Struct 2021;1245:131087. https://doi.org/10.1016/j.molstruc.2021.131087.
[54] Stenbæk J, Löf D, Falkman P, Jensen B, Cárdenas M. An alternative anionic bio-sustainable anti-fungal agent: Investigation of its mode of action on the fungal cell

membrane. J Colloid Interface Sci 2017;497:242–8. https://doi.org/10.1016/j.jcis.2017.03.018.
[55] Simões M, Pereira AR, Simões LC, Cagide F, Borges F. Biofilm control by ionic liquids. Drug Discov Today 2021;26(6):1340–6. https://doi.org/10.1016/j.drudis.2021.01.031.
[56] Hermawan H, Ramdan D, Djuansjah JRP. Metals for biomedical applications. In: Fazel-Rezai R, editor. Biomedical engineering - from theory to applications. IntechOpen; 2011. p. 411–30.
[57] Javadhesari SM, Alipour S, Mohammadnejad S, Akbarpour MR. Antibacterial activity of ultra-small copper oxide (II) nanoparticles synthesized by mechanochemical processing against *S. aureus* and *E. coli*. Mater Sci Eng C 2019;105:110011. https://doi.org/10.1016/j.msec.2019.110011.
[58] Biagi M, Giachetti D, Miraldi E, Figura N. New non-alcoholic formulation for hand disinfection. J Chemother 2017;26(2):86–91. https://doi.org/10.1179/1973947813Y.0000000111.
[59] Essa AMM, Khallaf MK. Antimicrobial potential of consolidation polymers loaded with biological copper nanoparticles. BMC Microbiol 2016;16:144. https://doi.org/10.1186/s12866-016-0766-8.
[60] Sportelli MC, Picca RA, Ponco R, Bonerba E, Tantillo G, Pollini M, et al. Investigation of industrial polyurethane foams modified with antimicrobial copper nanoparticles. Materials 2016;9(7):1–13. https://doi.org/10.3390/ma9070544.
[61] Zhong T, Oporto GS, Jaczynski J, Jiang C. Nanofibrillated cellulose and copper nanoparticles embedded in polyvinyl alcohol films for antimicrobial applications. BioMed Res Int 2015;2015:1–8. https://doi.org/10.1155/2015/456834. 456834.
[62] Jayaramudu T, Varaprasad K, Reddy KK, Pyarasani RD, Akbari-Fakhrabadi A, Amalraj J. Chitosan-pluronic based Cu nanocomposite hydrogels for prototype antimicrobial applications. Int J Biol Macromol 2020;143:825–32. https://doi.org/10.1016/j.ijbiomac.2019.09.143.
[63] Gyawali R, Ibrahim SA, Hasfa SHA, Smqadri SQ, Haik Y. Antimicrobial activity of copper alone and in combination with lactic acid against *Escherichia coli* O157:H7 in laboratory medium and on the surface of lettuce and tomatoes. J Pathog 2011;2011(650968):1–9. https://doi.org/10.4061/2011/650968.
[64] Bouson S, Krittayavathananon A, Phattharasupakun N, Siwayaprahm P, Sawangphruk M. Antifungal activity of water - stable copper-containing metal-organic frameworks. R Soc Open Sci 2017;4(170654):1–9. https://doi.org/10.1098/rsos.170654. 4, pp. 1–9, 2017.
[65] Zhang J, Li B, Wang Q, Wei X, Feng W, Chen Y, et al. Application of Fourier transform infrared spectroscopy with chemometrics on postmortem interval estimation based on pericardial fluids. Sci Rep 2017;7(18013):1–8. https://doi.org/10.1038/s41598-017-18228-7.
[66] ISO22196:2011(E). Measurement of antibacterial activity on plastics and other non-porous surfaces. International Organization for Standardization; 2011.
[67] Carvalho-Correia E, et al. Omnisars2: a highly sensitive and specific rt-qpcr-based covid-19 diagnostic method designed to withstand sars-cov-2 lineage evolution. Biomedicines 2021;9(10). https://doi.org/10.3390/biomedicines9101314.
[68] Sousa VI, Parente JF, Marques JF, Calçada C, Veiga MI, Osório NS, et al. PMMA microcapsules for the inactivation of SARS-CoV-2. ACS Omega 2022;7(26):22383–93. https://doi.org/10.1021/acsomega.2c01446.
[69] Padrão J, Nicolau T, Felgueiras HP, Calçada C, Veiga MI, Osório N, et al. Development of an ultraviolet-C irradiation room in a public Portuguese hospital for safe Re-utilization of personal protective respirators. Int J Environ Res Publ Health 2022;19(4854):1–14. https://doi.org/10.3390/ijerph19084854.
[70] ISO10993-5. Biological evaluation of medical devices - parte 5: Tests for in vitro cytotoxicity. International Organization for Standardization; 2009.
[71] Schmitt DM, O'Dee DM, Cowan BN, Birch JW-M, Mazzella LK, Nau GJ, et al. The use of resazurin as a novel antimicrobial agent against *Francisella tularensis*. Front Cell Infect Microbiol 2013;3(93):1–6. https://doi.org/10.3389/fcimb.2013.00093.
[72] McGaw LJ, Elgorashi EE, Eloff JN. Cytotoxicity of african medicinal plants against normal animal and human cells. In: Kuete V, editor. Toxicological survey of african medicinal plants. Elsevier Inc.; 2014. p. 181–233.
[73] Udabe E, Isik M, Sardon H, Irusta L, Salsamendi M, Sun Z, et al. Antimicrobial polyurethane foams having cationic ammonium groups. J Appl Polym Sci 2017;45473:1–7. https://doi.org/10.1002/app.45646.
[74] Nafees M, Ikram M, Ali S. Thermal behavior and decomposition of copper sulfide nanomaterial synthesized by aqueous sol method. Dig J Nanomater Biostruct 2015;10(2):635–41.
[75] Williams ML, Dickmann JS, McCorkill ME, Hassler JC, Kiran E. The kinetics of thermal decomposition of 1-alkyl-3-methylimidazolium chloride ionic liquids under isothermal and non-isothermal conditions. Thermochim Acta 2020;685:178509. https://doi.org/10.1016/j.tca.2020.178509.
[76] Yong Q, Chang J, Liu Q, Jiang F, Wei D, Li H. Matt polyurethane coating: correlation of surface roughness on measurement length and gloss. Polymers 2020;12(326):1–12. https://doi.org/10.3390/polym12020326.
[77] Wypych G. Testing methods of weathered specimen. In: Wypych G, editor. Handbook of material weathering. 5th. Elsevier Inc; 2013. p. 277–350. https://doi.org/10.3390/polym12020326.
[78] McKeen L. Introduction to the physical, mechanical, and thermal properties of plastics and elastomers. In: McKeen L, editor. The effect of sterilization on plastics and elastomers. 3rd. ed. William Andrew Publishing; 2012. p. 57–84. https://doi.org/10.1016/B978-1-4557-2598-4.00003-4.
[79] Ariño I, Kleist U, Mattsson L, Rigdahl M. On the relation between surface texture and gloss of injection-molded pigmented plastics. Polym Eng Sci 2015;45(10):1343–56. https://doi.org/10.1002/pen.20393.
[80] Włodarska K, Pawlak-Lemańska K, Khmelinskii I, Sikorska E. Explorative study of apple juice fluorescence in relation to antioxidant properties. Food Chem 2016;210:593–9. https://doi.org/10.1016/j.foodchem.2016.05.007. 2016.
[81] Hanson AR. A national measurement good practice guide. Measurement good practice guide No.94 good - good practice guide for the measurement of gloss. 2006.
[82] Madra H, Tantekin-Ersolmaz SB, Guner FS. Monitoring of oil-based polyurethane synthesis by FTIR-ATR. Polym Test 2009;28:773–9. https://doi.org/10.1016/j.polymertesting.2009.05.013. 2009.
[83] Ling N, Hori N, Takemura A. Effect of postcure conditions on the dynamic mechanical behavior of water-based polymer-isocyanate adhesive for wood. J Wood Sci 2008;54(5):377–82. https://doi.org/10.1007/s10086-008-0966-4.
[84] Asefnejad A, Khorasani MT, Behnamghader A, Farsadzadeh B, Bonakdar S. Manufacturing of biodegradable polyurethane scaffolds based on polycaprolactone using a phase separation method: physical properties and in vitro assay. Int J Nanomed 2011;6:2375–84. https://doi.org/10.2147/ijn.s15586.
[85] Bahadur A, Shoaib M, Saeed A, Iqbal S. FT-IR spectroscopic and thermal study of waterborne polyurethane-acrylate

leather coatings using tartaric acid as an ionomer. E-Polymers 2016;16(6):463–74. https://doi.org/10.1515/epoly-2016-0154.
[86] Javidi M, Fathabadi FH, Jahromi SAJ, Khorram M. Investigating the interfacial synthesis of polyurethane microcapsules and optimization of the process using response surface method. Mater Res Express 2019;6(10):105302. https://doi.org/10.1088/2053-1591/ab3588.
[87] Scuri S, Petrelli F, Grappasonni I, Idemudia L, Marchetti F, Nicola CD. Evaluation of the antimicrobial activity of novel composite plastics containing two silver (I) additives, acyl pyrazolonate and acyl pyrazolone. Acta Biomed 2019;90(3):370–7. https://doi.org/10.23750/abm.v90i3.8561.
[88] Gaikwad KK, Singh S, Lee YS. Antimicrobial and improved barrier properties of natural phenolic compound-coated polymeric films for active packaging applications. J Coating Technol Res 2019;16:147–57. https://doi.org/10.1007/s11998-018-0109-9.
[89] Fawal GFE, Omer AM, Tamer TM. Evaluation of antimicrobial and antioxidant activities for cellulose acetate films incorporated with Rosemary and Aloe Vera essential oils. J Food Sci Technol 2019;56(3):1510–8. https://doi.org/10.1007/s13197-019-03642-8.
[90] Silhavy TS, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harbor Perspect Biol 2010;2:a000414. https://doi.org/10.1101/cshperspect.a000414.
[91] Park ES, Moon WS, Song MJ, Kim MN, Chung KH, Yoon JS. Antimicrobial activity of phenol and benzoic acid derivatives. Int Biodeterior Biodegrad 2001;47(4):209–14. https://doi.org/10.1016/S0964-8305(01)00058-0. 2001.
[92] Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microb Drug Resist 2013;19(4):256–65. https://doi.org/10.1089/mdr.2012.0244.
[93] Lopez-Corona AV, Valencia-Espinosa I, González-Sánchez FA, Sánchez-López AL, Garcia-Amezquita LE, Garcia-Varela R. Antioxidant, anti-inflammatory and cytotoxic activity of phenolic compound family extracted from raspberries (*Rubus idaeus*): a general review. Antioxidants 2022;11(6):1–20. https://doi.org/10.3390/antiox11061192.
[94] Liu J, Du C, Beaman HT, Monroe MBB. Characterization of phenolic acid antimicrobial and antioxidant structure–property relationships. Pharmaceutics 2019;12(419):1–17. https://doi.org/10.3390/pharmaceutics12050419.
[95] Miklasińska-Majdanik M, Kepa M, Wojtyczka RD, Idzik D, Wasik TJ. Phenolic compounds diminish antibiotic resistance of *Staphylococcus aureus* clinical strains. Int J Environ Res Publ Health 2018:1–18. https://doi.org/10.3390/ijerph15102321. 152390.
[96] Han Y, Wang L. Sodium alginate/carboxymethyl cellulose films containing pyrogallic acid: physical and antibacterial properties. J Sci Food Agric 2017;87(4):1295–301. https://doi.org/10.1002/jsfa.7863.
[97] Jang HJ, Nde C, Toghrol F, Bentley WE. Microarray analysis of toxicogenomic effects of Ortho-phenylphenol in *Staphylococcus aureus*. BMC Genom 2008;8:411. https://doi.org/10.1186/1471-2164-9-411.
[98] Dréno B, Zuberbier T, Gelmetti C, Gontijo G, Marinovich M. Safety review of phenoxyethanol when used as a preservative in cosmetics. J Eur Acad Dermatol Venereol 2019;7(S7):15–24. https://doi.org/10.1111/jdv.15944.
[99] Miskiewicz A, Ceranowicz P, Szymczak M, Bartuś K, Kowalczyk P. The use of liquids ionic fluids as pharmaceutically active substances helpful in combating nosocomial infections induced by *Klebsiella pneumoniae* New Delhi Strain, *Acinetobacter baumannii* and *Enterococcus* species. Int J Mol Sci 2018;19(9):2779. https://doi.org/10.3390/ijms19092779.
[100] Duman AN, Ozturk I, Tunçel A, Ocakoglu K, Colak SG, Hoşşgör-Limoncu M, et al. Synthesis of new water-soluble ionic liquids and their antibacterial profile against gram-positive and gram-negative bacteria. Heliyon 2019;5. https://doi.org/10.1016/j.heliyon.2019.e02607 (e02607):1-7.
[101] Choi SY, Rodrígues H, Mirjafari A, Gilpin DF, McGrath S, Malcolm KR, et al. Dual functional ionic liquids as plasticisers and antimicrobial agents for medical polymers. Green Chem 2011;13(6):1527–35. https://doi.org/10.1039/c1gc15132k.
[102] Popescu R, Filimon MN, Vlad Cd D, Verdes D, Moatar A, Moise G, et al. Antiproliferative and antibacterial potential of tetrahexylammonium bromide-based ionic liquids. Exp Ther Med 2021;22(672):1–9. https://doi.org/10.3892/etm.2021.10104.
[103] Gholami A, Shams MS, Abbaszadegan A, Nabavizadeh M. Ionic liquids as capping agents of silver nanoparticles. Part II: antimicrobial and cytotoxic study. Green Process Synth 2021;10:585–93. https://doi.org/10.1515/gps-2021-0054.
[104] Ferraz R, Silva D, Dias AR, Dias V, Santos MM, Pinheiro L, et al. Synthesis and antibacterial activity of ionic liquids and organic salts based on penicillin g and amoxicillin hydrolysate derivatives against resistant bacteria. Pharmaceutics 2020;12(3):221. https://doi.org/10.3390/pharmaceutics12030221.
[105] He B, Ou G, Zhou C, Wang M, Chen S. Antimicrobial ionic liquids with fumarate anion. J Chem 2013;2013:1–8. https://doi.org/10.1155/2013/473153. 473153.
[106] Karaman M, Vranes M, Tot A, Papoviç S, Miljakoviç D, Gadžuriç S, et al. Ionic liquids as potentially new antifungal agents against: *Alternaria* species. RSC Adv 2020;10:22318–23. https://doi.org/10.1039/d0ra02475a.
[107] Suchodolski J, Feder-Kubis J, Krasowska A. Antifungal activity of ionic liquids based on (–)-menthol: a mechanism study. Microbiol Res 2017;197:56–64. https://doi.org/10.1016/j.micres.2016.12.008.
[108] Miranda C, Castaño J, Valdebenito-Rolack E, Sanhueza F, Toro R, Bello-Toledo H, et al. Copper-polyurethane composite materials: particle size E ff ect on the physical-chemical and antibacterial properties. Polymers 2020;12. https://doi.org/10.3390/polym12091934. 1934.
[109] Ahmad Z, Vargas-Reus MA, Bakhshi R, Ryan F, Ren GG, Oktar F, et al. Antimicrobial properties of electrically formed elastomeric polyurethane-copper oxide nanocomposites for medical and dental applications. In: Düzgüneş N, editor. Methods in enzymology - nanomedicine, infectious diseases, immunotherapy, diagnostics, antifibrotics, toxicology and gene medicine. 1st. Elsevier Inc; 2012. p. 87–99. https://doi.org/10.1016/B978-0-12-391858-1.00005.
[110] Fowler L, Engqvist H, Öhman-Mägi C. Effect of copper ion concentration on bacteria and cells. Materials 2019;12(22):3798. https://doi.org/10.3390/ma12223798.
[111] Benhalima L, Amri S, Bensouilah M, Ouzrout R. Antibacterial effect of copper sulfate against multi-drug resistant nosocomial pathogens isolated from clinical samples. Pakistan J Med Sci 2018;35(5):1322–8. https://doi.org/10.12669/pjms.35.5.336.
[112] Evans A, Kavanagh KA. Evaluation of metal-based antimicrobial compounds for the treatment of bacterial pathogens. J Med Microbiol 2021;70:001363. https://doi.org/10.1099/JMM.0.001363.
[113] Oussou-Azo AF, Nakama T, Nakamura M, Futagami T, Vestergaard MCM. Antifungal potential of nanostructured crystalline copper and its oxide forms. Nanomaterials 2020;10:1003. https://doi.org/10.3390/nano10051003.

[114] Sharma AK, Sharma R, Saxena M. Biomedical and antifungal application of Cu (II) soaps and its urea complexes derived from various oils. Open Access J. Transl. Med. Res 2018;2(2):39–42. https://doi.org/10.15406/oajtmr.2018.02.00033.

[115] Ude Z, Kavanagh K, Twamley B, Pour M, Gathergood N, Kellett A, et al. A new class of prophylactic metallo-antibiotic possessing potent anti-cancer and anti-microbial properties. Dalton Trans 2019;48(24):8578–93. https://doi.org/10.1039/c9dt00250b.

[116] Carranza-Torres I, Viveros-Valdez E, Guzmán-Delgado N, García-Davis S, Morán-Martínez J, Betancourt-Martínez N, et al. Protective effects of phenolic acids on mercury-induced DNA damage in precision-cut kidney slices. Iran. J. Basic Med. Sci. 2019;22(4):367–75. https://doi.org/10.22038/ijbms.2019.30056.7242.

[117] Hernández-Rosas NA, García-Zebadúa JC, Hernández-Delgado N, Torres-Castillo S, Figueroa-Arredondo P, Mora-Escobedo R. Polyphenols profile, antioxidant capacity, and in vitro cytotoxic effect on human cancer cell lines of a hydro-alcoholic extract from *Calendula officinalis* L. petals. TIP. Rev Esp Cienc Quím Biol 2018;21(1):54–64. https://doi.org/10.22201/fesz.23958723e.2018.0.150.

[118] Musiał M, Zorębski E, Malarz K, Kuczak M, Mrozek-Wilczkiewicz A, Jacquemin J, et al. Cytotoxicity of ionic liquids on normal human dermal fibroblasts in the context of their present and future applications. ACS Sustainable Chem Eng 2021;9(21):7649–57. https://doi.org/10.1021/acssuschemeng.1c02277.

[119] Uscátegui YL, Díaz LE, Valero MF. *In vitro* and *in vivo* biocompatibility of polyurethanes synthesized with castor oil polyols for biomedical devices. J Mater Res 2019;34(4):519–31. https://doi.org/10.1557/jmr.2018.448.

[120] Xu C, Chen G, Tan Z, Hu Z, Qu Z, Zhang Q, et al. Evaluation of cytotoxicity *in vitro* and properties of polysiloxane-based polyurethane/lignin elastomers. React Funct Polym 2020;149:104514. https://doi.org/10.1016/j.reactfunctpolym.2020.104514.

[121] Davis FJ, Mitchell GR. Polyurethane based materials with applications in medical devices. In: Bártolo P, Bibanda B, editors. Bio-materials and prototyping applications in medicine. Spinger Cham; 2008. p. 27–48.

[122] Morena AG, Stefanov I, Ivanova K, Pérez-Rafael S, Sánchez-Soto M, Tzanov T. Antibacterial polyurethane foams with incorporated lignin-capped silver nanoparticles for chronic wound treatment. Ind Eng Chem Res 2020;59:4504–14. https://doi.org/10.1021/acs.iecr.9b06362.

[123] Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng 2005;90(1):59–63. https://doi.org/10.1002/bit.20368.