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Antimicrobial Property of Photocatalytic Nanoparticles-Coated Personal Protective Equipment (PPE) on Bacteria and Fungi

(Peralatan Pelindung Diri (PPE) bersalut Nanozarah Fotopemangkin yang Bersifat antimikrob terhadap Bakteria dan Kulat)

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ABSTRACT

Photocatalytic nanoparticles are new applications that can be used as coatings on surfaces through a photocatalytic process that reacts in the presence of chemical catalysts and light. The resulting reactive oxygen species (ROS) would damage pathogenic components and result in antimicrobial effects. This study was conducted to evaluate the antimicrobial properties of photocatalytic nanoparticles on personal protective equipment (PPE), namely surgical gowns and masks. Antimicrobial testing of photocatalytic nanoparticles against PPE inoculated with pathogens was carried out. The growth log reduction of isolates tested on the photocatalytic nanoparticles-coated PPE showed 100% with growth reduction exceeding 4 log against *Escherichia coli* ATCC 25922 as well as *Staphylococcus aureus* ATCC 25923 but less than 50% reduction against *Candida albicans* ATCC 10231. For 20 h incubation periods, both bacteria showed growth reduction of at least 4 log with 99.99% of reduction. The 5 and 20 times washing effects showed an overall reduction of 99.99%-100% against both bacteria but less than 99.99% against *C. albicans*. Photocatalytic nanocoating produces an antimicrobial effect that helps to kill the tested pathogens and reduce the attachment of bacteria but not fungi, on the surface of PPE. This nanoparticle is capable of continuous self-disinfection to reduce the number of pathogens. The number of washing cycle also does not affect its function to reduce the growth of pathogens.

Keywords: Antimicrobial; nanoparticle; personal protective equipment (PPE); photocatalytic

ABSTRAK

Nanozarah fotopemangkin adalah aplikasi baharu yang boleh digunakan sebagai salutan pada permukaan di mana proses fotopemangkin akan bertindak balas dengan kehadiran bahan pemangkin kimia dan cahaya. Spesies oksigen reaktif (ROS) yang terhasil akan merosakkan komponen patogen dan menghasilkan kesan antimikrob. Kajian ini dijalankan untuk menilai sifat antimikrob nanozarah fotokatalitik pada peralatan pelindung diri (PPE), iaitu gaun pembedahan dan pelitup muka. Ujian antimikrob nanozarah fotopemangkin pada PPE yang diinokulasi dengan patogen telah dijalankan. Pengurangan log pertumbuhan bagi pencilan yang diuji pada PPE bersalut nanozarah fotopemangkin menunjukkan 100% dengan pengurangan pertumbuhan melebihi 4 log terhadap *Escherichia coli* ATCC 25922 serta *Staphylococcus aureus* ATCC 25923 tetapi pengurangan kurang daripada 50% terhadap *Candida albicans* ATCC 10231. Bagi tempoh pengeraman 20 jam, kedua-dua bakteria menunjukkan pengurangan pertumbuhan sekurang-kurangnya 4 log dengan pengurangan 99.99%. Kesan 5 dan 20 kali basuhan menunjukkan pengurangan keseluruhan 99.99%-100% terhadap kedua-dua bakteria tetapi kurang daripada 99.99% terhadap *C. albicans*. Salutan nanozarah menghasilkan kesan antimikrob yang membantu membunuh pathogen yang diuji dan mengurangkan perlekatan bakteria tetapi bukan kulat pada permukaan PPE. Nanozarah ini mampu melakukan pembasmian tersendiri secara berterusan untuk mengurangkan bilangan patogen. Bilangan basuhan juga tidak menjejaskan fungsinya untuk mengurangkan pertumbuhan patogen tersebut.

Kata kunci: Antimikrob; fotopemangkin; nanozarah; peralatan perlindungan diri (PPE)

Introduction

An increase in hospital admissions increased the risk of infections such as hospital-acquired diseases or nosocomial infections. A complete personal protective equipment (PPE) kit included a face shield, goggles, face mask, gloves, gown or cloth, headgear, and shoe covers helping prevent the spread of germs in hospitals (WHO 2020). Emergency measures for the reuse and continued use of PPE were being considered to address the shortage of PPE.

Reuse referred to the practice of using the same PPE for multiple patient treatments but disposing of it between treatments. Continuous use referred to using the same equipment for repeated treatment with patients without removing PPE. Continuous use was preferred over reuse to reduce the risk of self-contamination from repeated use and disposal of the same equipment (Chu, Ponce de Leon & Hoopman 2022). The use of nanoparticles had proven well in the field of microbiology including antimicrobial activity against bacteria, fungi, viruses, and protozoa (Kobayashi & Nakazato 2020).

Nanoparticle is a form of nanotechnology made up of various types of compounds such as metals, metal oxides, polymers, organic carbon as well as biomolecules (Nagarajan 2008). The characteristic features of photocatalytic nanoparticle used involves the presence of photon reacting together with a specific compound such as metal oxide to change the rate of chemical reaction (Ameta et al. 2018). One study has proposed that the pathway of nanoparticles was by the neutralization of the surface electric charge present on the bacterial membrane affecting cell permeability (Ramalingam, Parandhaman & Das 2016).

Photocatalytic coatings were used for self-disinfection by reducing the risk of transmission of infection through environmental surfaces as well as decontamination and disinfection of medical devices (Tsendzughul & Ogwu 2019). The photocatalytic coating, along with the light within it, destroyed viruses, bacteria, mold spores and other volatile organic compounds. This coating disinfected surfaces when exposed to light (Biospectrum 2021). Nanoparticles could be used in textile materials to impart properties such as surface self-cleaning, stain resistance, water repellent, electrical conductivity, antimicrobial resistance, hydrophilicity or controlled hydrophobicity, wrinkle resistance, antistatic, anti-odour, flame retardant, UV protection, abrasion resistance, shrinkage and resistance (Jeevani 2011).

Materials and MethodS

Two types of surgical gown, blue (spun bound non-woven polypropylene) and white (spun bound polypropylene with polypropylene fabric) as well as three-ply face masks (melt blown non-woven spun bound polypropylene) were used for this study. All the PPE used in this study sterilized with UVC for 15 min before being coated once or twice with photocatalytic nanoparticles AnanoCoat™ formulated by Anano Sphere Sdn. Bhd. (Rawang, Selangor) to undergo antimicrobial tests. The process of coating the PPE involves hanging the PPEs on a wire to expose the largest surface area before being sprayed with AnanoCoat using electrostatic gun with a nozzle size of 0.1 mm. For PPEs that were coated twice, the second layer of coating were applied after 30 min once the first coating was checked to be fully dried.

AnanoCoat COMPOSITION AND CHEMICAL STRUCTURE

AnanoCoat™ is a trademarked product that is purely made of zinc oxide, ZnO. The size of the nanoparticles of ZnO used in AnanoCoat is less than 10 nm which is smaller than most microbes (Siddiqi et al. 2018). Of all the metal oxide nanoparticles studied thus far, zinc oxide nanoparticles exhibited the highest toxicity against microorganisms (Hu et al. 2009). It has also been demonstrated from SEM and TEM images that ZnO nanoparticles first damage the bacterial cell wall, then penetrate, and finally accumulate in the cell membrane. They interfere with metabolic functions of the microbes causing their death. All the characteristics of the zinc oxide nanoparticles depend on their particle size, shape, concentration, and exposure time to the bacterial cell (Siddiqi et al. 2018).

BACTERIA INOCULUM PREPARATION AND SUBCULTURE

Four colonies from both subcultures, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were inoculated into nutrient broth (NB) and supplemented with 1% (v/v) Tween 80 and incubated. Serial dilution and spread plate were carried out and incubated for 24 h. Colony count was carried out and it was observed that after the fourth hour, growth showed a decline indicating log phase. Hence, both strains were incubated in an orbital shaker for 4 h. The concentration of the bacteria inoculum was read at 625 nm and was fixed according to a concentration of 10^8 or optical density (OD) reading of 0.08-0.1.

FUNGUS INOCULUM PREPARATION AND SUBCULTURE

C. albicans was cultured on potato dextrose agar and incubated for 24 h. Bovine serum albumin (BSA) was prepared by dissolving 0.15 g of the serum with 50 mL of distilled water. 10-12 colonies were inoculated into potato dextrose broth. The concentration of the fungus inoculum was then read using a spectrophotometer at 530 nm and standardized according to the McFarland standard of 1 - 5 × 10^6 CFU/mL or OD reading of 0.11-0.14.

ANTIBACTERIAL TEST ON PPE GOWN AND FACE MASK COATED WITH PHOTOCATALYTIC NANOPARTICLE

The antibacterial test carried out in this study was based on ISO 27447:2019 with modifications to the room lighting (Tanner 2022). Negative controls were set by using a non-coated PPE for comparison. Both negative control and samples were cut into 50 mm × 50 mm dimensions and sterilized under UVC for 15 min. Antibacterial test was carried out by setting all sterilized PPE gown and mask into petri dishes followed by 200 µL of bacteria inoculum that had been standardized to a concentration of 10^8 CFU/mL and slip glass were used to fix the PPE in place. The samples were incubated under room lighting for 4 h before the PPE and slip glass were placed in zipped bags filled with 0.9% normal saline. The zipped bags were then rubbed gently to suspend the bacteria halting the antimicrobial activity. Serial dilutions were then carried out once on bijou bottles with concentrations of 10^2 and 10^4. Spread plates on agar count plates on all negative controls and samples were carried out and incubated at 37 °C for 24 h followed by colonies count (CFU/mL).

ANTIFUNGAL TEST ON PPE GOWN AND FACE MASK COATED WITH PHOTOCATALYTIC NANOPARTICLE

The antifungal test was carried out based on the protocol of EN 14562:2006 which was the quantitative evaluation of infectious fungus and yeast activity for a preventative instrument in the medical industry. BSA was used as a simulation test for the possibility of microbial contamination. BSA was used 15 min after it was prepared and vortexed with the fungus inoculum until fully mixed. The antifungal test was carried out by setting all the sterilized PPEs into petri dishes followed by 200µL of fungus inoculum that had been standardized to the McFarland standard of 1.5 × 10^6 CFU/mL and slip glass was used to fix the PPE in place. The samples were incubated under room lighting for 1 h. After 1 h, the PPE and slip glass were placed in zipped bags filled with 0.9% normal saline. The zipped bags were then rubbed gently to suspend the fungus on the PPE to halt the antimicrobial activity. Sample neutralization solution was then obtained, and serial dilution was not carried out spread plates on agar count plates on all negative controls and samples were carried out and incubated at 37 °C for 48 h followed by colonies count (CFU/mL).

TIME KILLED STUDY AND DURABILITY AND EFFICACY STUDY

Antibacterial and antifungal tests were also carried out by comparing the different times of incubation to study the effectiveness of PPE coated with the photocatalytic nanoparticle. Both tests were carried out according to their respective protocols. Furthermore, a washing test was also conducted to study the durability of the nanoparticle's attachment on the surface of the PPE. Both tests were carried out according to similar ISO protocols for antibacterial and antifungal study.

DATA ANALYSIS

Data analysis from the colony forming unit (CFU/mL) counted for all samples used percentage of reduction where the number of microbial growths on coated PPE either once or twice were compared with the number of microbial growths from negative control samples. Moreover, $\log_{10}$ reduction was also used to illustrate the effectivity of the antimicrobial property of photocatalytic nanoparticle coatings. The term '$\log_{10}$' is defined as the power to which 10 must be raised to generate a particular number. In general terms, a decrease of 1 log corresponds to a 90% reduction, a reduction of 2 logs to a 99% reduction and so on (Biologicalprep 2021). Table 1 summarized the relationship between the $\log_{10}$ reduction and the percentage of reduction together with the estimated number of microbes.

For the calculation of percentage reduction and log reduction were based on the formula as stated herewith:

$$\text{Percentage of reduction, \%} = \frac{A-B}{A} \times 100\%$$

$$\text{Log}_{10} \text{ of reduction} = \text{Log}_{10} A - \text{Log}_{10} B$$

where A is the microbes colony count of non-coated PPE; and B is the microbes colony count on coated PPE.

TABLE 1. Relationship between $\log_{10}$ reduction, percentage reduction and number of microbes

Starting number = 1 million colony		
$\log_{10}$ reduction	% reduction	Bacteria remaining
0	0	1000000
1	90	100000
2	99	10000
3	99.9	1000
4	99.99	100
5	99.999 / 100	0-10

(Source: Biologicalprep 2021)

Results and Discussions

Antimicrobial test on PPE gown and face mask coated with photocatalytic nanoparticle

For *E. coli* inoculated samples that were coated once and twice, they showed a growth reduction of more than 4 log for all types of PPE which correlates to more than 99.99% reduction in bacteria growth. For *S. aureus* inoculated samples that were coated once and twice, they showed growth reduction of more than 4 log for all types of PPE which correlates to more than 99.99% reduction in bacteria growth. For all samples, samples that were coated twice have almost similar reduction capabilities. This result coincides with a previous study where the bacterial reduction of cotton fabrics treated with zinc oxide nanoparticles (ZnONPs) reaches a sufficient value of 94.9% when the cotton fabrics were treated with the lowest concentration, and it was also discovered that the bacterial reduction of cotton fabrics increased slightly with increasing ZnONPs, whereas the bacterial reduction of untreated cotton fabrics was zero (Shaheen et al. 2016). Another study found that ZnONPs loaded starch-coated HDPE sheets were biocidal against *E. coli*. The co-antimicrobial effect of chitosan and ZnONPs could result in a chain reaction, beginning with adherence of nanofibers to cell membranes through electrostatic attraction, which causes changes in microbial cell membranes, followed by disruption of cell membranes by reactive oxygen species (ROS) produced by ZnONPs, which causes leakage of intercellular contents, and finally interference in cell metabolism by ZnONPs and chitosan disrupting DNA and RNA replications (Panáček et al. 2006).

For *C. albicans* inoculated samples, all the samples tested showed growth reduction of less than 90% with less than 1 log growth reduction with blue surgical showing the least growth reduction with less 30% and less than 0.5 log of growth reduction. No further test was conducted to study the reasoning behind the low effectivity against *C. albicans* on blue surgical gowns. However, other results showed that by increasing the number of coatings to two layers, the antifungal property showed a better outcome. Previous study which investigated the turbidity and colony counts showed a dose-dependent inhibitory effect of ZnONPs on *C. albicans*, with increasing ZnONPs concentration causing incremental antifungal effects (Lipovsky et al. 2011). As the exact composition of AnanoCoat was not disclosed, it could not be determined whether the dosage applied was enough to exert maximum amount of antifungal property. The cell wall of *C. albicans* is made up of glycoproteins, polysaccharides, particularly glucan and chitin), hydrophobins, and amphipathic proteins, which are proteins in fungus that interact with their surroundings. Using energy dispersive X-ray (EDX) analysis, dynamic physicochemical interactions, kinetic and thermodynamic exchanges, and attractive van der Waals forces occur between the surface of the ZnONPs and the surface of the biological component, such as the membrane and proteins, when fungal cells are exposed

to ZnO. All these interactions are thought to result in an interaction between ZnONPs and the cell membrane of fungal cells, resulting in NP buildup on the cell membrane and subsequent membrane breakdown (Jiang et al. 2010; Min et al. 2008; Omoike & Chorover 2004). Table 2 and Figure 1 showed the log reduction of *E. coli*, *S. aureus* and *C. albicans* on different types of PPE with different number of coatings.

TIME KILLED STUDY FOR ANTIMICROBIAL PROPERTY

The PPE that had been coated was incubated for 4 h and 20 h, respectively, to see the differences in incubation time on the antibacterial activity by comparing them to negative control which was samples that were not coated with photocatalytic nanoparticle. For *E. coli* samples that have been incubated for 4 h, white surgical gowns that have been coated once and twice showed growth reduction of more than 4 log with more than 99.99% reduction. In comparison, the white surgical gowns that have been coated once and twice that undergone 20 h incubation showed lower growth reduction of more than 3 log with 99.99% reduction. For blue surgical gowns that have been incubated for 4 h and coated once and twice, a growth reduction of more than 5 log with 100% reduction

TABLE 2. Log reduction of tested isolates on the photocatalytic nanoparticles-coated PPE. 100% with more than 4 log reduction for both bacteria but less than 90% reduction for *C. albicans*

Tested isolates	*S. aureus* ATCC 25923		*E. coli* ATCC 25922		*C. albicans* ATCC 10231	
	Log reduction (% reduction)					
Types of PPE	1x coated	2x coated	1x coated	2x coated	1x coated	2x coated
White surgical gown	4.70 (100)	4.70 (100)	5.23 (100)	4.03 (99.99)	0.48 (60)	0.48 (60)
Blue surgical gown	4.70 (100)	4.40 (99.99)	5.18 (100)	5.18 (100)	0.05 (12)	0.12 (24.44)
3-ply surgical mask	4.70 (100)	4.70 (100)	5.65 (100)	5.65 (100)	0.53 (70.5)	0.85 (86.10)

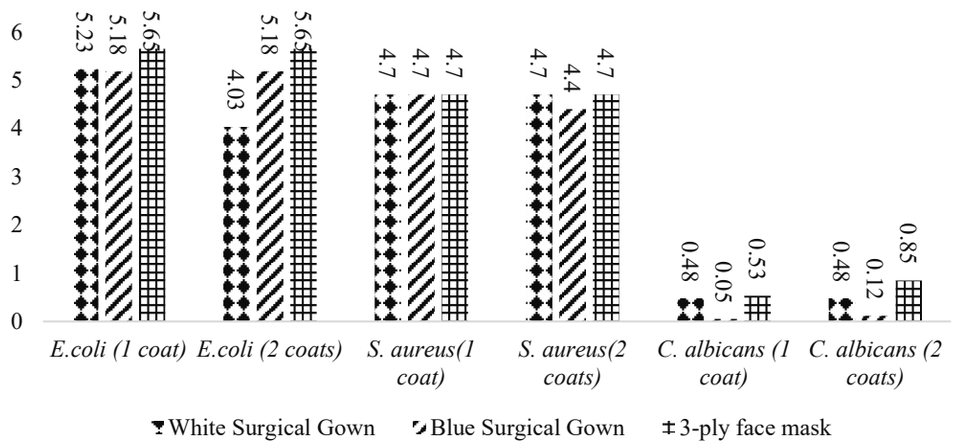

FIGURE 1. Log10 reduction of E. coli, S. aureus and C. albicans on PPE coated once and twice against negative control (0 coating)

was observed while blue surgical gowns that have been coated once and twice and incubated for 20 h showed growth reduction of more than 2 log with more than 99% reduction. For 3-ply face masks that have been coated and twice and incubated for 4 hours, they showed growth reduction of 5.65 each with 100% reduction. However, the same samples showed a lower growth reduction of 4.33 and 3.83 each and 99.99% reduction.

For *S. aureus* samples that have been incubated for 4 h, white surgical gowns that have been coated once and twice showed a growth reduction of 4.70 with 100% reduction. In comparison, white surgical gowns that have been coated once and twice showed growth reduction of more than 3 log with more than 99.9% reduction. For blue surgical gowns that have been coated once and twice and incubated for 4 h, the growth reduction observed was more than 4 log and more than 99.99% reduction. When the samples of blue surgical gown were incubated for 20 h, the growth reduction for one coating was 4.60 and 99.99% while the one coated twice showed growth reduction of 5.44 and 100% reduction. For 3-ply face mask that have been coated once and twice and incubated at 4 h, the growth reduction was 4.70 corresponding to 100% reduction while similar percentage reduction of 100% was also observed for 20 h incubation period while 1 coating showed log reduction of 5.22 and 2 coating showed 5.16.

Overall, incubation period of 4 h showed a higher growth reduction compared to 20 h of incubation period for both bacteria. The differences between different types of PPE were indistinguishable for 4 h and 20 h of incubation period. The slight reduction in the 20 h period could be attributed to the leaching out of nanoparticles overtime after long exposure to photon, especially on certain fabrics. Previous study of ZnO nanoparticles showed that overtime the photocatalytic activity decreased that could be explained by the presence of intermediates on its surface, which impede its adsorption and light absorption overtime (Sudrajat 2018). Another study showed that the highest intensity in antibacterial activity occurs during the first 2 h in ZnO nanoparticles and more effective against Gram-positive bacteria (Chen et al. 2015; Reddy et al. 2007). Table 3 and Figure 2 showed the log reduction and percentage of reductions for all samples.

Table 3. Log reduction of tested isolates after 4 h and 20 h incubation. The 4 h incubation showed above 99.99% reduction for both bacteria but not for 20 h. For *C. albicans*, the growth study was not determined

Tested isolates		*S. aureus* ATCC 25923	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*E. coli* ATCC 25922
Log reduction (% reduction)					
Types of PPE	Incubation hours	1x coated	2x coated	1x coated	2x coated
White surgical gown	4 h	4.7 (100)	4.7 (100)	5.23 (100)	4.03 (99.99)
	20 h	3.17 (99.94)	3.87 (99.99)	3.93 (99.99)	3.71 (99.98)
Blue surgical gown	4 h	4.7 (100)	4.4 (99.99)	5.18 (100)	5.18 (100)
	20 h	4.6 (99.99)	5.44 (100)	2.33 (99.54)	2.74 (99.82)
3-ply surgical mask	4 h	4.7 (100)	4.7 (100)	5.65 (100)	5.65 (100)
	20 h	5.22 (100)	5.16 (100)	4.33 (99.99)	3.83 (99.99)

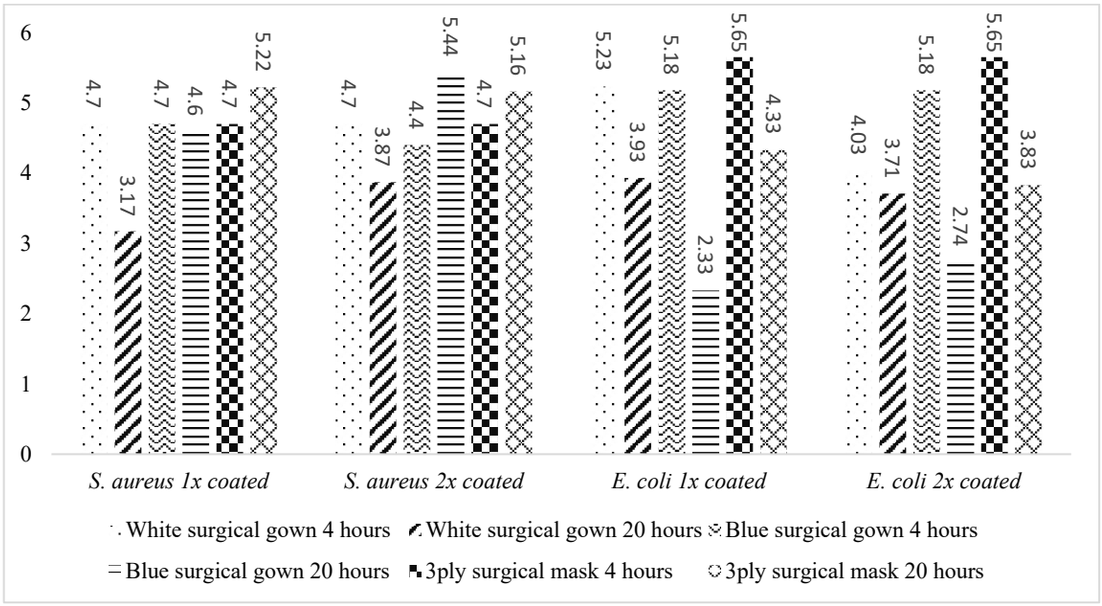

FIGURE 2. Log reduction of E. coli and S. aureus on PPE coated once and twice against negative control (0 coating) for time killed study

DURABILITY AND EFFICACY STUDY

The durability and efficacy tests were carried out to study the strength of attachment of the photocatalytic nanoparticles on the PPE that have been coated after several washing cycles and the effect on the antimicrobial property. For this test, the PPE that have been coated once and twice as well as negative control samples were subjected to 5 times and 20 times washing cycles.

For the samples that have been inoculated by *E. coli*, white surgical gowns that have been coated once and subjected to 5 times and 20 times washing cycles showed a 99.99% reduction with growth reduction of more than 4 log. For the white surgical gown samples that have been coated twice and subjected to 5 times and 20 times washing cycles, they both showed 99.98% reduction with growth reduction of more than 3 log. For blue surgical gowns that have been coated once and twice and subjected to 5 times washing cycles showed 99.99% reduction with growth reduction of more than 4 log. In comparison, the blue surgical gowns that have been coated once and subjected to 20 times washing cycles showed log reduction of 3.80 with 99.98% reduction while blue surgical that have been coated twice showed higher reduction in microbial growths of 99.99% with 4.20 log reduction. For 3-ply face masks that have been coated once and twice that were subjected to 5 times washing cycles showed a log reduction of more than 3 log with 99.9% growth reduction. For 3-ply face masks that have been coated once and subjected to 20 times washing cycles showed log reduction of 4.01 with 99.99% reduction while 3-ply face masks that have been coated twice showed log reduction of 5.11 and 100% reduction.

For the samples that have been inoculated by *S. aureus*, white surgical gowns that have been coated once and twice that were subjected to 5 times washing cycles showed log reduction of more than 4 log with and 99.99% growth reduction. In comparison, white surgical gowns that have been coated once and twice and subjected to 20 times washing cycles show growth reduction of more than 4 log with more than 99.99% reduction. For blue surgical gowns that have been coated once twice that were subjected to 5 times and 20 times washing cycles, growth reduction of more than 4 log with more than 99.99% reduction. For 3-ply face masks that have been coated once and twice treated to 5 times and 20 times washing cycles both showed more than 4 log of growth reduction with more than 99.99% reduction.

Overall, the percentage of reduction for *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 on surgical gowns and face masks coated with photocatalytic nanoparticle after 5 times and 20 times of washing overall was 99.99%

and showed growth reduction of more than 4 log. The washing durability for the coated PPE was still effective and showed little to no reduction in antimicrobial property after several washing cycles and showed a high reduction towards both bacteria. The slight decrease in the growth reductions of both bacteria coincided with the outcome of previous research which showed that after 5 to 10 washing cycles, the zone of growth inhibitions decreased slightly (Anita et al. 2011). Even after 20 times washing cycles, the antibacterial effectivity was still above 99.99% which was similar to another study that used AgNP as a coating and after 10 washing cycles, it still retained above 99.99% effectivity. Further morphology analysis showed that the percentage of nanoparticle loss during washing cycles was 14.2% (Xing et al. 2020).

Next, for the antifungal test of the durability and efficacy test. Similar procedures and parameter of 5 times and 20 times washing cycles was applied. For samples that have been inoculated with *C. albicans*, white surgical gowns that have been coated once and subjected to 5 times washing cycles showed log reduction of 0 with 0% reduction meaning there were no difference when compared to negative controls while white surgical gowns that have been coated twice showed 7.5% reduction with log reduction of 0 meaning the differences against negative control was almost indistinguishable. In comparison, white surgical gowns that have been coated once and subjected to 20 times washing cycles showed log reduction of 0.78 with 82.20% reduction while white surgical gowns that have been coated twice showed log reduction of 0.60 with 78% reduction. For blue surgical gowns that have been coated once and subjected to 5 times washing cycles, log reduction of 0 with 4.50% reduction was observed while blue surgical gowns that have been coated twice showed log reduction of 0.1 with 19.10% reduction. In comparison, blue surgical gowns that have been coated once and subjected to 20 times washing cycles showed log reduction of 0.05 with 13.3% reduction while blue surgical that have been coated twice showed log reduction of 0.25 with 45% reduction. For 3-ply face masks that have been coated once and subjected to 5 times washing cycles showed log reduction of 0.4 with 58% while 3-ply face masks that have been coated twice showed log reduction of 0.6 with 71.3% reduction. In comparison, the 3-ply face masks that have been coated once and twice and subjected to 20 times washing cycles both showed reduction of -0.47 with -179.8% reduction meaning both samples showed higher growths of *C. albicans* compared to negative control.

Overall, for 5 times and 20 times washing cycles, the colony growth for the fungus on the agar plate was more than 60-200 CFU/mL. The percentage of reduction on the coated PPE coated once and twice with the photocatalytic nanoparticle was overall at 21% and 33%, respectively, for 5 times washing while -28.1% and -19%, respectively, for 20 times washing. The washing durability test on the antifungal property was moderate at best where the antifungal property was still present, but the activity observed was low. The loss of nanoparticles during the washing cycles showed a negative effect in terms of number of fungal growth reduction. As explained by previous study where *C. albicans* was very resistant to photocatalytic degradation due to its thick eukaryotic cell wall. Despite the negative results, AnanoCoat still showed promising signs by being able to reduce the number of *C. albicans* by almost 50% in some samples. Further analysis on the mechanisms of action of the photocatalytic nanoparticles towards *C. albicans* should be carried out to come up with an inference as to the low and inconsistent growth reduction in the antifungal property of the nanoparticles. Figures 5, 6 and Table 6 show the durability and efficacy tests results for the antibacterial property.

ANTIMICROBIAL PROPERTY OF PHOTOCATALYTIC NANOPARTICLE

Nanoparticle was a good choice as an additional antimicrobial supplement as its size was almost identical to most bacterial cells and could enter the cell membrane at ease. The main mechanism of the nanoparticle is by its toxicity through oxidative pressure which damaged the lipid, carbohydrate, protein, and DNA of the cells. Lipid peroxidation was considered the most dangerous due the changes it caused in the properties of the cell membrane (Jašková, Hochmannová & Vytřasová 2013). The antimicrobial coatings consisting of nanoparticle semiconductors block or at least halt the progression of microorganism on surfaces of materials.

Antimicrobial property test on the PPE coated with the photocatalytic nanoparticle was based on number of coatings and type of PPE materials was carried out on bacteria and fungi of interest. Overall, the reduction for all antibacterial test showed more than 99.99% reduction with log of reduction of 4 log. No distinguishable differences were observed between the reduction in Gram-positive and Gram-negative bacteria for all the PPE with different number of coatings. The overall reduction for the antifungal test was only 50% with log of reduction of 1 log.

TABLE 4. Log reduction based on 5 times and 20 times washing effect. 5- and 20-times washing effect showed an overall of 100% reduction, whereas 20 times washing effect shows an overall of 99.99% reduction for both bacteria but for *C. albicans*, it less than 99.99%

Tested isolates		*S. aureus* ATCC 25923		*E. coli* ATCC 25922		*C. albicans* ATCC 10231	
		Log reduction (% Reduction)					
Types of PPE	Washing Cycles	1x coated	2x coated	1x coated	2x coated	1x coated	2x coated
White surgical gown	5X	4.01 (99.99)	5.11 (100)	4.5 (99.99)	3.66 (99.98)	0 (0)	0 (7.5)
	20X	4.5 (99.99)	4.5 (99.99)	4.37 (99.99)	3.1 (99.98)	0.78 (82.2)	0.6 (78)
Blue surgical gown	5X	4.81 (99.99)	5.11 (100)	4.05 (99.99)	4.8 (99.99)	0 (4.5)	0.10 (19.1)
	20X	4.18 (99.99)	4.58 (99.99)	3.8 (99.98)	4.2 (99.99)	0.05 (13.3)	0.25 (45)
3ply surgical mask	5X	4.6 (100)	4.6 (99.99)	3.41 (99.96)	3.52 (99.97)	0.4 (58)	0.25 (45)
	20X	4 (99.99)	4.5 (100)	4.02 (99.99)	4.12 (9.99)	-0.47 (-179.8)	-0.47 (-179.8)

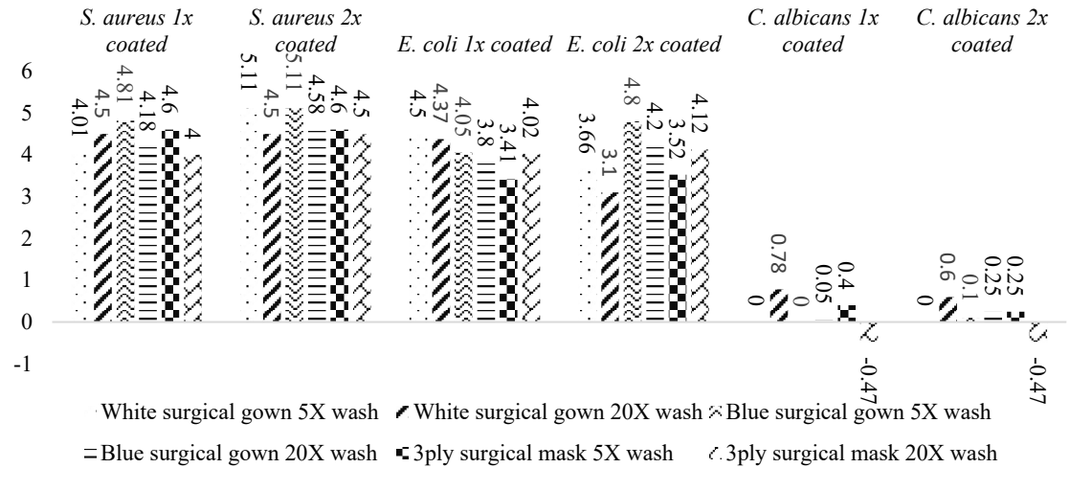

FIGURE 3. Log reduction of *E. coli* and *S. aureus* and *C. albicans* on PPE coated once and twice against negative control (0 coating) for durability and efficacy tests

The reduction of all types of PPE with 1 or 2 coatings showed a slight difference in the reduction of fungus. The reduction between both bacteria was higher compared to the fungus on all PPE with 1 or 2 coatings. *C. albicans* showed a better resistance towards the nanoparticle compared to the bacteria. The interaction between the nanoparticle coatings like NP metal that was positively charged and the negatively charged cell wall disrupted the cell membrane and disrupted the cell components increasing the reactive oxygen species (ROS) which caused cell damage and eventually cell death. Bacteria cell wall which consisted of peptidoglycan while the fungal cell wall consisted of chitin. However, no specific study was carried out that focused on the differences between the interaction of the nanoparticles and the cell wall of bacteria and fungus (Garcia-Rubio et al. 2020; Slavin et al. 2017). Further studies on the effect of the photocatalytic nanoparticles needed to be carried out as the inconsistencies in terms of results for different types of PPEs and different number of coatings needed to be understood. In previous study using silver nanoparticles, it had shown that silver nanoparticles could exert antifungal activity by destroying cell membrane of *C. albicans* and suppressing the cell divisions (Kim et al. 2009; Rozhin et al. 2021). It was found that the antifungal activity occurred as a result of formation of insoluble compounds with sulfhydryl groups that were present in the cell wall of fungi causing altercation of membrane-bound enzymes with lipids eventually leading to cell lysis (Dorau, Arango & Green 2004; Rozhin et al. 2021).

The reduction of bacteria tested on this study showed a correlation with the study conducted previously where cotton fabric that was coated with Ti showed antibacterial activity under light source where a reduction could be analyzed on both *E. coli* and *S. aureus* up to 99% (Zhang et al. 2019). Another study showed that face mask coated with low concentration nanoparticle was effective against *S. aureus* and *E. coli* where the bacteria present on the surface of the face mask was fully eradicated (Li et al. 2006). Antifungal test study previously showed that the number of *C. albicans* was effectively reduced after 60 min of UV light exposure (Akiba et al. 2006).

Antimicrobial property of the photocatalytic nanoparticle based on the time of death time test was carried out with the overall reduction of 99.99% with log of reduction of 4 $\log_{10}$ on bacteria while less than 1 $\log_{10}$ for the duration of 4 h and 20 h. A correlating study previously carried out showed that unlike conventional germicide which had a non-continual and ritualistic characteristic which limited the effectivity, antimicrobial photocatalyst had a continuous effect (Ramsden 2015). The washing durability test carried out on the photocatalytic nanoparticle show an overall 99.99% and log of reduction of 4 $\log_{10}$ for antibacterial property while less than 99.99% reduction with a log of reduction of 1 log for both 5 times and 20 times washing. The antimicrobial property remained on the surfaces of coated photocatalyst nanoparticle inoculated with bacteria and fungus after several washings. Previous study showed that the effectivity of antimicrobial property of Ag-NP coated surfaces was not affected after several washes (Deshmukh et al. 2019).

Conclusions

In conclusion, the reduction showed more than 99.99% reduction with the log of reduction of 4 log for both bacteria but less than 90% reduction for the fungus of interest. The total number of coatings and the different types of PPE materials exerted the same effectivity for both bacteria but not for the fungus. The 4 h incubation period showed a 99.99% reduction for both bacteria but not for 20 h incubation while for the fungus, the reduction was less than 99.99% for all samples. The effect of 5 times washing and 20 times washing showed an overall reduction of almost 100% while the effect of 20 times washing showed a reduction of almost 99.99% for both bacteria but not for the fungus. The nanoparticle coatings assisted in the antimicrobial activity and reduced the presence of microbes on the surfaces of PPE while having a continuous effect after several washing. Overall, the nanoparticle coating was less effective against the fungus, *C. albicans* ATCC 10231.

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