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Antibacterial Effectiveness of a Silver-Copper Nano-Coating to Improve Personal Protective Equipment Used in the Medical and Dental Fields

Velia Mariana Pérez-Bucio[1], Alejandro Lee-Colín[1], Lorena Reyes-Carmona[2], Gina Prado-Prone[2], Sandra E. Rodil[3], Argelia Almaguer-Flores[2]

[1] Especialidad en Periodoncia e implantología, División de estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de México.

[2] Laboratorio de Biointerfases, División de Estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de México.

[3] Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México.

Correspondence author:
Dra. Argelia Almaguer-Flores
E-mail: aalmaguer@comunidad.unam.mx

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ABSTRACT

Introduction: Personal protective equipment is used to protect healthcare personnel, such as doctors and dentists, from contact with potentially pathogenic microorganisms that may be present in aerosols generated by fluids like blood and saliva. **Objective:** To evaluate the antibacterial effect of the silver-copper nano-coating (SakCu®) deposited on polypropylene textiles on bacterial species frequently associated with nosocomial infections and microorganisms from

subgingival biofilm samples of patients with periodontitis, simulating contact with contaminated droplets generated during dental care. **Materials and Methods:** The *in vitro* experimental study was carried out by means of tests with nosocomial strains of the following bacterial species: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis*. For the clinical trials, subgingival biofilm samples were taken from five patients diagnosed with periodontitis. All tests consisted of maintaining contact of the microorganisms with the nano-coating for 24 hours. **Results:** The results showed a decrease in the cell viability of nosocomial strains when they were in contact with the nano-coating, with *P. aeruginosa* being the bacteria that presented the greatest sensitivity to contact, followed by *S. epidermidis* and *S. aureus*. However, *E. coli* appeared to be unaffected by the nano-coating. Regarding the evaluation of bacteria from the subgingival biofilm samples, there was a decrease of between 32.9% and 80% in the number of microorganisms that were exposed to the nano-coating, compared to the number of bacteria present on the uncoated polypropylene textiles. **Conclusions:** The results demonstrate the potential of silver-copper nano-coating for the use in personal protective equipment made from polypropylene textiles, such as gowns, caps, and face masks, commonly used in clinical settings.

Keywords: personal protective equipment, nano-coating, antimicrobial, silver, copper.

INTRODUCTION

During dental care, patients and dentists are exposed to bacteria, viruses, and fungi, among other pathogenic microorganisms that may be present in aerosols generated by fluids such as blood and saliva, and the use of rotating handpieces and ultrasonic equipment. That is why the main objective of personal protective equipment (PPE) is to protect healthcare professionals and patients from these splashes or aerosols. One of the first documented reports of PPE use dates back to the 14th century, when doctors protected themselves from contracting the Black Death through contact with sick patients[1]. It wasn't until the 1980s, in the wake of the HIV-AIDS pandemic and more recently, the SARS-CoV-2 (COVID-19) pandemic, that the use of PPE for infection control in the medical and dental fields became essential[2,3].

The oral cavity is home to a large number of microorganisms that normally coexist homeostatically with their host[4]. However, there are diseases such as periodontitis that disrupt this homeostasis and whose treatment includes various procedures using ultrasonic equipment[5]. These cause the generation of droplets and aerosols that may be contaminated with various microorganisms present in the oral cavity[6]. Such microorganisms can remain viable for several minutes and even hours, contaminating dental clinic air[7,8], PPE, and dental office surfaces[9,10].

The American Dental Association (ADA) has recognised dental aerosols as potential sources of transmission for diseases such as tuberculosis, influenza, *Legionella* sp. infections, and severe acute respiratory syndrome (SARS)[11]. During the COVID-19 pandemic, a study published estimates of exposure risk and PPE use by dental professionals during various clinical procedures, with the highest-risk procedures being those involving ultrasound equipment[12]. The potential for contamination of various PPE components following aerosol-producing treatments has also been investigated. The results showed that face masks were among the most contaminated items, with the most frequently detected bacteria being: *Staphylococcus aureus, Streptococcus* spp., *Pseudomonas* spp., and *Escherichia coli*[11].

Polypropylene (PP) is one of the most widely used materials in the manufacture of PPE due to its physical filtration properties. It is a lightweight, nonwoven synthetic textile resulting from the polymerisation of propylene monomer[13]. Examples of PPE made from PP textiles include face masks, gowns, caps, surgical drapes, and others[14]. Despite its qualities as a filter material, PP textiles do not have inherent antimicrobial properties, so the accumulation of different microorganisms over time could become a fomite or source of infection.

To increase the effectiveness of face masks and PPE, the incorporation of metallic elements such as copper (Cu)[15] and silver (Ag) nanoparticles has been studied[16]. Recently, a 30 nm thick nano-coating of Ag and Cu deposited on PP textiles called SakCu® was developed. This nano-coating was shown to have virucidal properties against SARS-CoV-2 and pathogenic bacteria from the ESKAPE group[17].

The purpose of the present study was to evaluate the antibacterial effect of the SakCu® nano-coating deposited on PP textiles through two challenges: The first consisted of the use of strains of bacterial species frequently associated with nosocomial infections such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis*. In the second, we proposed to evaluate the antimicrobial capacity of said nano-coating using microorganisms from subgingival biofilm samples of patients with periodontitis, simulating contact with contaminated droplets generated during dental care.

MATERIALS AND METHODS

This study was an *in vitro* experimental study. The Ag-Cu bimetallic nano-coating was deposited on the PP textiles using the cathodic sputtering technique, or magnetron sputtering, at the Materials Research Institute of the Universidad Nacional Autónoma de México (UNAM). The deposition conditions and the physicochemical characterisation of the nano-coating have been previously published[17,18]. Once the nano-coating was done, 1 cm diameter discs were cut from both the Ag-Cu coated PP and the uncoated PP (control).

For tests with bacterial species associated with nosocomial infections, the following strains were used: *Escherichia coli* (ATCC® 33780), *Pseudomonas aeruginosa* (ATCC® 43636), *Staphylococcus aureus* (ATCC® 25923) and *Staphylococcus epidermidis* (ATCC® 14990). The strains were grown on trypticase soy agar (TSA) (BD BBL™ Trypticase™ Soy Agar, Becton, Dickinson and Company, Franklin Lakes, USA) and incubated at 35°C for 24 hours and then transferred to obtain pure cultures. A suspension in trypticase soy broth (TSB) (BD BBL™ Trypticase™ Soy Broth, Becton, Dickinson and Company, Franklin Lakes, USA) adjusted to a concentration of $1x10^7$ cells/mL was obtained from each bacterium. From this suspension a 40 µL drop was placed on each of the previously sterilised discs of uncoated PP and PP with Ag-Cu, and left to incubate for 24 hours at 35°C under aerobic conditions. The tests were performed in triplicate, placing an extra disc in each experiment so that it could be observed and analysed qualitatively by optical microscopy.

The antimicrobial evaluation was accomplished using the colorimetric cell viability assay with MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) (Cell Proliferation Kit I (MTT), Roche Diagnostics GmbH, Mannheim, Germany), which indirectly evaluates the cell viability of metabolically active bacteria[19]. After incubation, each disc was washed twice with TSB and transferred to a well of a new culture plate. Later, TSB and MTT were added. The samples were incubated for 3 hours in the dark at 35°C and then processed to obtain aliquots to be analysed in a spectrophotometer (FilterMax™ F5 Multi-Mode Microplate Readers, Molecular Devices, LLC., San Jose, USA) at a wavelength of λ = 570 nm.

With the absorbances obtained from the MTT assay, the percentage of cell viability (CV%) was determined using the following formula:

$$CV\% = \left(\frac{Sample\ O.D - Blank\ O.D}{Control\ O.D - Blank\ O.D}\right) \times 100$$

Where:

Sample O.D.: corresponds to the absorbance of the aliquots of the bacteria samples that were in contact with the nano-coating.

Control O.D.: corresponds to the absorbance of the bacterial samples that were in contact with the uncoated PP.

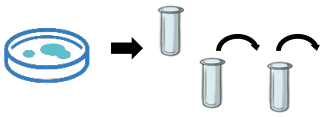
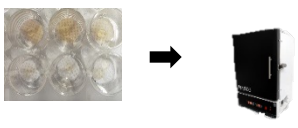
Blank O.D.: corresponds to the absorbance of the culture medium without bacteria after incubation with the PP with and without the nano-coating.

Qualitative analysis by optical microscopy was carried out using an Eclipse Ni series microscope (Nikon Instruments Inc., Tokyo, Japan). Table 1 presents the methodological sequence of this assay.

Regarding the assay with subgingival biofilm microorganisms, this part of the study involved taking subgingival biofilm samples from patients diagnosed with stage III or IV periodontitis, treated at the Periodontology and Implantology Clinic of the Division of Postgraduate Studies and Research, Faculty of Dentistry, UNAM. The study was conducted with the approval of the Ethics and Research Committee of the Faculty of Dentistry (CIE/0102/08/2022), and all patients agreed to sign the Informed Consent to participate. Five patients were included who had not received any type of periodontal treatment above prophylaxis, who did not have systemic involvement and who had one of the first molars with probing depth ≥5 mm. Each patient received a complete periodontal evaluation performed by an experienced specialist according to the procedure previously described in the literature[20-22].

After clinical evaluation, subgingival biofilm samples were collected from the mesiobuccal site of any first molars present in each patient, using sterile 11/12 Gracey curettes (Harmony™

Table 1.
Methodological sequence of the assay with strains associated with nosocomial infections.

Step	Description	
I	Pure cultures of bacterial strains and obtaining a solution with 1x10^7 cells/mL of each strain.	
II	Inoculation of each strain onto uncoated PP discs and PP with Ag-Cu, and incubation for 24 hours.	
III	MTT cell viability assay.	
IV	Quantitative analysis (MTT, absorbance) and qualitative analysis (optical microscopy).	

Handle, Hu-Friedy Mfg. Co., Illinois, USA). Each sample was placed in a microcentrifuge tube with *Mycoplasma* culture broth, (BBL™ Mycoplasma Broth Base, Becton, Dickinson and Company, Franklin Lakes, USA) enriched with 0.3 µg/mL of vitamin K (Menadione, Sigma-Aldrich Inc., St. Louis, USA) and 5 µg/mL of hemin (Hemin, Sigma-Aldrich Inc., St. Louis, USA). Next, each sample was dispersed by vortex to obtain a homogeneous bacterial suspension and a 40 µL drop of this was placed on the PP discs with and without the nano-coating. The samples were incubated for 24 h at 35°C in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, USA) with an atmosphere of 80% N_2, 10% CO_2 and 10% H_2 (Figure 1. A), after the incubation time, each disc was processed to remove the bacteria present in each one (Figure 1. B). Five serial dilutions of 1:10 were made from each sample and 5 µL of each of them were seeded in Petri dishes with enriched agar (TSA, Brain Heart Infusion Agar, Yeast extract, supplemented with 25 mL of defibrinated sheep blood (5%), 5 mL of vitamin K (0.3 µg/mL) and 5 mL of hemin (5 µg/mL)). Once all the dilutions were seeded, the Petri dishes were incubated at 35°C under anaerobic conditions for 5 days. After incubation, colony forming units (CFUs) were counted for each of the seeded dilutions (Figure 1. C). The antibacterial effectiveness of the Ag-Cu nano-coating on the cultivable microorganisms of each of the subgingival biofilm samples was expressed as percentage of inhibition according to the following equation:

$$\% \, \boldsymbol{inhibition} = \left(\frac{\boldsymbol{A1 - A2}}{\boldsymbol{A1}}\right) \mathbf{X\ 100}$$

Where:

A1: number of CFUs present in the uncoated PP (negative control).

A2: number of CFUs present in the PP with the Ag-Cu nano-coating (experimental).

For statistical analysis, all results were expressed as Mean ± SEM (standard error of the mean) values. Statistical significance was determined using the paired Student's *t*-test and differences were considered statistically significant from a $p < 0.05$, using GraphPad Prism version 8.0.1 software (GraphPad Software Inc., San Diego, USA).

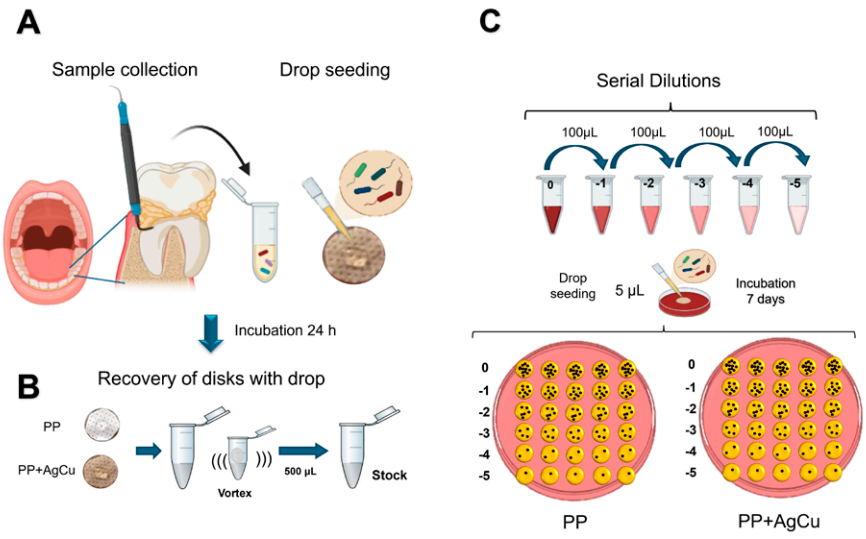


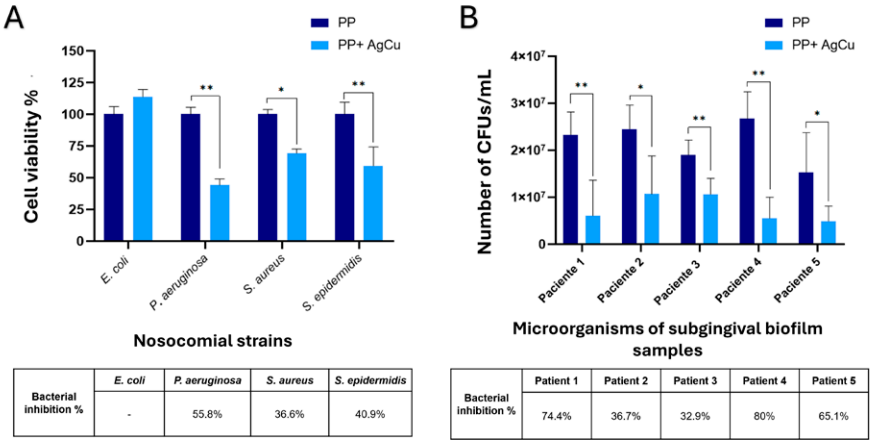
Figure 1. Biofilm sample processing. A. Biofilm sampling and inoculation onto PP textiles with and without the Ag-Cu nano-coating. B. Recovery of inoculated discs with microorganisms exposed for 24 hours to uncoated PP and PP with Ag-Cu. C. Serial dilutions and seeding in Petri dishes with agar for quantification of CFU.

RESULTS

The results of the quantitative analysis to determine the antibacterial capacity of the nano-coating using the MTT cell viability assay of the nosocomial bacteria used in this study are presented in Figure 2. A. It can be observed that there was a decrease in cell viability when the bacteria were in contact with the nano-coating, with *P. aeruginosa* being the most sensitive to contact with a viability of 44.2 ± 4.9%, followed by *S. epidermidis* and *S. aureus* with 59.4 ± 14.9 and 69.3 ± 3.4% viability, respectively. However, *E. coli* appeared to be unaffected by the nano-coating, as its cell viability percentage increased to 113.7 ± 6%.

Regarding the evaluation of the antibacterial effect of the Ag-Cu nano-coating against bacterial species from subgingival biofilm samples of patients, Figure 2. B shows the results of the CFU counts of bacteria exposed to textiles with and without the Ag-Cu nano-coating of each sample. These results show a decrease in the growth of microorganisms when they were exposed to PP with Ag-Cu, compared to the bacteria present in the uncoated PP, in all the samples evaluated. This decrease was statistically significant in the bacterial samples that were exposed to the nano-coating vs. those that were not exposed ($p < 0.05$ and $p < 0.001$).

Qualitative analysis of the samples using light microscopy (Figure 3) confirmed the findings from the MTT assay. Due to the nature of the MTT reagent, the greater the amount of purple staining, the greater the number of viable bacteria. In the micrographs it can be observed that the staining of the *E. coli* strain is very similar when comparing the uncoated PP and the PP with Ag-Cu. While in the other strains an antibacterial effect of the nano-coating can be distinguished. It is important to note that the topography of the PP fibers is maintained even after the nano-coating is deposited.



Bacterial inhibition %	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
	-	55.8%	36.6%	40.9%

Bacterial inhibition %	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
	74.4%	36.7%	32.9%	80%	65.1%

Figure 2. Cell viability of microorganisms exposed to PP textiles with and without the nano-coating. A. Results of the assay with nosocomial strains. B. Results of the CFU/mL count of microorganisms from subgingival biofilm samples from 5 patients with periodontitis. The percentage inhibition of each strain or microorganism obtained from the subgingival biofilm samples is presented at the bottom of each graph. *$p<0.05$, **$p<0.01$.

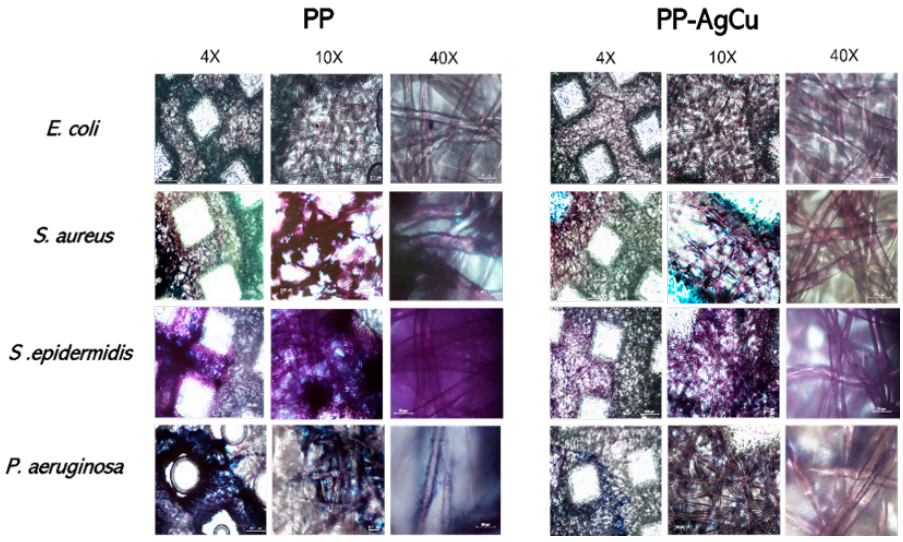


Figure 3. Qualitative analysis of nosocomial strains exposed to PP textiles with and without the nano-coating after performing the MTT assay. Optical microscopy images at 4X, 10X, and 40X magnifications.

DISCUSSION

The main objective of this research was to determine the antibacterial potential of a nanometre-thick silver-copper (Ag-Cu) coating deposited on polypropylene (PP) textiles, for incorporation into the manufacture of personal protective equipment (PPE), such as face masks, gowns, and caps, and to improve infection control in medicine and dentistry. The antimicrobial effectiveness of the nano-coating was examined using two tests: the first using strains of bacterial species frequently associated with nosocomial infections, and the second evaluating microorganisms from subgingival biofilm samples from patients with periodontitis.

The nano-coating was deposited using the cathodic sputtering technique or magnetron sputtering, a physical method that allows the deposition of nanometric films on surfaces of different nature[23, 24]. The antibacterial effectiveness of the nano-coating against the four bacterial strains associated with nosocomial infections tested[25,26], showed high effectiveness, especially against *P. aeruginosa*, *S. aureus* and *S. epidermidis*. Our results are consistent with other studies that have reported the sensitivity of these strains to silver and copper[27-29]. Furthermore, a study previously conducted by our working group reported an inhibition rate of 70 to 92% upon contact with aerosols contaminated with the same strains[18]. Although the results in the present study showed a lower percentage of inhibition (from ~37 to ~56%), it is important to clarify that the methodology used in this study was different, since the MTT reagent was used to measure cell viability and in the aforementioned study, direct quantification of colony forming units (CFU) was performed. An unexpected result was that the *E. coli* strain showed similar sensitivity to contact between the nano-coated PP and the uncoated PP. This may be explained by the low intrinsic capacity of *E. coli* to adhere to PP surfaces as previously described in the literature[30,31].

Regarding the results when microorganisms from subgingival dental biofilm of patients with periodontitis were used, it is interesting to observe the variability in sensitivity to contact with the nano-coating between the different samples from each of the patients. However, the variability between samples confirms the notion that the sensitivity of the same strain type

to an antimicrobial agent, whether antibiotic, antiseptic or, as in this case, to two metallic elements with biocidal potential, can vary due to factors such as intrinsic resistance or resistance acquired through previous exposure[32,33]. Although no identification of the microorganisms present in the samples was performed, it is understood that all samples contained anaerobic and facultative bacteria, both Gram-positive and Gram-negative[34]. It is important to mention that, although the number of clinical samples included was limited, the objective in this part of the research was to evaluate the antibacterial capacity against strains of clinical isolates in a complementary manner to the evaluations made using type strains (ATCC). This methodological approach allowed to analyse the behaviour of the Ag-Cu nano-coating against periodontal bacteria isolated from dental biofilm samples.

The importance of avoiding aerosol or droplet contamination during dental care is evident, since it has been proven that bacterial microaspiration is possible, causing lung damage, even in healthy individuals[35]. The presence of oral pathogens may be associated with severity or synergy with other systemic diseases. Airborne microorganisms and the oral microbiome are the primary sources of lung infections, and since the oral cavity is the entry point for the digestive system and part of the respiratory system, it is no surprise that some microorganisms from the microbiome of both systems are shared. Because the oropharynx and tracheobronchial tree are contiguous, continuous microaspiration is likely to inoculate the lungs with oral bacteria[36,37].

The use of small amounts of silver and copper in PP textiles represents an excellent option for infection control during dental care. Both metals in nanometric size have been reported as an alternative to the use of antibiotics with excellent results against a great diversity of oral bacteria, as well as in addition to mouthwashes and toothpastes in order to prevent the formation of dental biofilm, specifically against *Streptococcus* spp. in its planktonic state and against species such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, MRSA (methicillin-resistant *Staphylococcus aureus*), *Streptococcus mutans* and *Candida albicans*. Its use in adhesive systems and restorative materials has also been reported, improving their properties[38,39].

CONCLUSIONS

The results of this study demonstrate the potential of silver-copper nano-coating for use in personal protective equipment made from polypropylene textiles, such as gowns, caps, and face masks, in clinical environments. Such equipment could reduce healthcare personnel's exposure to these and other pathogens, and even reduce the risk of nosocomial infections in hospitalised patients. Similarly, the decrease in the viability of periodontopathogenic bacteria in contact with the Ag-Cu nano-coating opens the door for further research into broader clinical applications in the periodontal and implantology fields.

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