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RESEARCH ARTICLE **OPEN ACCESS**

THE ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF COPPER MATERIAL

Flores-Encarnación, M.[1,5*], Aguilar-Gutiérrez G.R.[2], Valentin-Aguilar I.[1], Flores-Encarnación S.E.[3], Cabrera-Maldonado C.[4] and García-García S.C.[5]

[1]Laboratorio de Microbiología Molecular y Celular. Biomedicina, Facultad de Medicina, Benemérita Universidad Autónoma de Puebla. Puebla. Puebla, México
[2]CISEI, Instituto Nacional de Salud Pública, Cuernavaca, Morelos. México
[3]Hospital Regional ISSSTE, Puebla. Puebla, México
[4]Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Puebla, Puebla, México
[5]Centro de Investigaciones Microbiológicas, Benemérita Universidad Autónoma de Puebla, Puebla, Puebla, México
[6]Programa de Microbiología. Grupo de Académicos de Puebla S.C. Puebla, Puebla, México

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ABSTRACT

The biofilm-forming bacteria are asocciated in communities of organisms which are strongly attached to the biotic and abiotic surfaces. They cause infectious diseases among the population and are the source of contamination of medical devices or surfaces within hospitals or in different areas of industrial production. Bacterial biofilm can be formed in various materials such as glass, stainless steel, polyvinyl chloride, polystyrene, etc. Therefore, it is necessary to search materials that prevent the formation of biofilm on the surface of them.

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INTRODUCTION

Bacteria have mechanisms that allow them to develop and survive in different environments. Some of these mechanisms occur individually, such as the spore formation, the capsule, production of toxins, and exopolysaccharide synthesis (Flores-Encarnación *et al*., 2019; Kramer and Assadian, 2014). In other cases, survival mechanisms include bacterial community association, such is the case of biofilm formation (Costerton *et al*., 1999). The bacteria forming biofilms are asocciated in communities of organisms which are strongly attached to the biotic and abiotic surfaces (Costerton *et al*., 1999; Chauhan *et al*., 2012; Donlan *et al*., 2002; Flores-Encarnación *et al*., 2014; Kostakioti *et al*., 2013).

Corresponding author: *Flores-Encarnación, M.,
Laboratorio de Microbiología Molecular y Celular. Biomedicina, Facultad de Medicina, Benemérita Universidad Autónoma de Puebla. Puebla. Puebla, México.

Bacteria in biofilms have a matrix of exopolysaccharides and other molecules of excretion like proteins, nucleic acids, bacterial lysates, water, etc, which facilitate the adherence to different surfaces or materials (Decho, 2013; Flemming and Wingender, 2010; Mohammed *et al*., 2013). In relation to this, the contamination of surfaces by microorganisms forming biofilm is a frequent problem in the world, especially in developing countries where disinfection measures are deficient. So, in healthcare settings, the areas adjacent to patients and frequently touched surfaces by hands ("high-touch surfaces") is correlated with a higher risk of contamination of surrounding surfaces through direct or indirect contact with hands (Kramer and Assadian, 2014). It has been reported that the bacterial biofims can form on surfaces of various materials, such as stainless steel, PVC, glass, ceramic, plastic, etc. (Morvay *et al*., 2011). Therefore, it is necessary to search for materials that naturally prevent the formation of biofilm by pathogenic bacteria.

In the present study, the antibacterial and antibiofilm activity of copper material using uropathogenic *Escherichia coli* as a biological model was studied.

MATERIAL AND METHODS

Copper material: In this study, the copper material (commercial product) was used. The material consisted of half inch copper couplings without groove. It was obtained from a hardware store at Puebla, México.

Bacterial strain: A strain of uropathogenic *Escherichia coli* CFT073 was used. Bacterial strain was stored in cryovials at -40°C until analysis.

Culture conditions: The trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md) was used for bacterial culture. Test strain that had been cultured at 37°C for 48 to 72 hours in trypticase soy broth were seeded crosswise in a Petri dish containing trypticase soy agar, and the plate was incubated at 37°C for 24 hours.

Antimicrobial activity: The antimicrobial activity of copper material was determined placing the sterile copper couplings in tubes contaning 25 mL of the trypticase soy broth. Each inoculum contained approximately 10^6 CFU mL^{-1}. Then, the tubes were incubated at 37°C for 48 to 72 hours. After time, the copper couplings were removed from the culture tubes. The effect of copper material on uropathogenic *E. coli* growth was determined by observing the formation of biofilm on the surface of copper couplings, while the bactericidal or bacteriostatic effect was determined by passing the bacteriological handle on surface of copper couplings and fresh tripticasein soy agar plates was inoculated by cross-streak. The plate was incubated at 37°C for 24 hours. The analyses were conducted in triplicate.

Detection of biofilm: The biofilm formed by uropathogenic *E. coli* on surface of copper couplings was detected using the crystal violet staining. For it, the sterile copper couplings were placed in tubes contaning 25 mL of the trypticase soy broth. Each culture tube contained approximately 10^6 CFU mL^{-1} and tubes were incubated at 37°C for 48 to 72 hours. After time, the copper couplings were removed from the culture tubes and they were stained with 0.1% violet crystal for 20 min at room temperature. The analyses were conducted in triplicate. As reference of biofilm formation other materials were used. So, uropathogenic *E. coli* was grown on the 96-well sterile polystyrene plates and 250 µL of trypticase soy broth were used.

A medium without bacteria incubated under the same conditions was treated as a negative control. The plate was incubated at 37°C for 48 to 72 hours. After that time, the culture broth was removed and each well was it was dyed with 0.1% violet crystal for 20 min at room temperature. The analyses were conducted in quadrupled. A glass test was also performed to test biofilm formation by uropathogenic *E. coli* . For this, a coverslip was placed in a petri dishes containing 10 mL of the trypticase soy broth and 10^6 CFU mL^{-1}. The petri dishes were incubated at 37°C for 48 to 72 hours. After time, coverslip was removed with the help of sterile forceps and stained with0.1% violet crystal for 20 min at room temperature. The analyses were conducted in triplicate.

RESULTS

In this study, the antibacterial and antibiofilm activity of copper material was determined using a strain of uropathogenic *E. coli* and half inch copper couplings without groove. In the first case, the sterile copper couplings were placed in steril tubes contaning 25 mL of trypticase soy brothas in Materials and Methods was described. The results are shown in Fig. 1. The Fig. 1A and B show the sterile copper couplings that were placed in tubes containing trypticase soy broth. As can be seen in Fig. 1C, uropathogenic *E. coli* showed growth in the liquid culture medium, with turbidity in the trypticase soy broth. Therefore, growth of uropathogenic *E. coli* was observed in the surroundings of the copper couplings. Fig. 1D shows the results obtained whenthe copper couplings were removed from the culture tube as in Materials and Methods was described. As shown in this figure, the copper couplings did not present a biofilm observable to the naked eye, both inside and outside the tested coupling. From this, ascraping was made with a bacteriological loop on the surface of copper coupling and it was seeded in atrypticase soy agar plate. The results obtained indicated a bactericidal effect, since no growth was observed on the agar plate after being incubated at 37°C for 24 hours (data not shown). To determine the biofilm formation by uropathogenic *E. coli* on the surface of copper couplings, 0.1% violet cristal was used (Fig. 1E). As seen in Fig. 1E, the copper couplings were slightly stained with crystal violet, which suggested the formation of a biofilm by uropathogenic *E. coli* in some areas of the copper material. However, as can be seen in Fig. 1F, the crystal violet staining of the copper coupling that was incubated at 37°C for 48 to 72 hours in trypticase soy broth in absence of uropathogenic *E. coli*, showed the appearance of light spots in different portions of the coupling. This suggested that the violet crystal bound nonspecifically to the copper coupling and that it was not a biofilm formation. As indicated above, the biofilm formation was detected in other materials. As shown in Fig. 1G, the wells of the polystyrene plate were intensely stained with 0.1% crystal violet after incubating uropathogenic *E. coli* at 37°C for 48 to 72 hours. The results showed that uropathogenic *E. coli* can easily form a biofilm on polystyrene material. As shown in Fig. 1H, surfaces of glass coverslips were also stained with 0.1% crystal violet after incubating uropathogenic *E. coli* at 37°C for 48 to 72 hours. The results showed that uropathogenic *E. coli* can easily form a biofilm on glassmaterial.

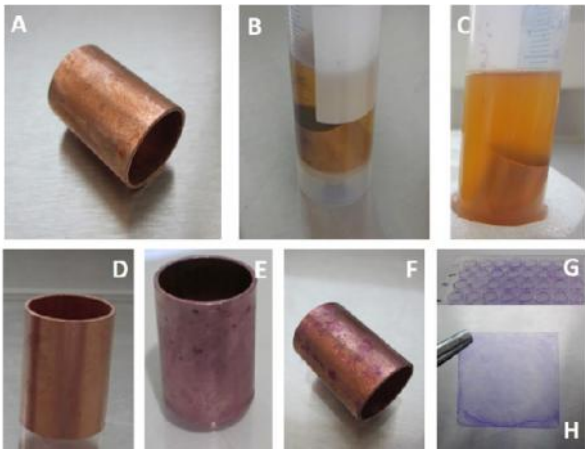

Fig. 1 The antibacterial and antibiofilm activity of copper material. A. Copper coupling after autoclaving. B. Copper coupling immersed in trypticasein soy broth. C. Growth of uropathogenic *E. coli* in trypticasein soy broth. D. Copper coupling after extraction from uropathogenic *E. coli* culture. E. Copper coupling stained with violet crystal. F. Control condition. G. Polystyrene plate stained with violet crystal. H. Glass coverslip stained with violet crystal

DISCUSSION

As is known, bacteria are capable of living in various environments. Microbes are omnipresent in the biosphere, and their presence invariably affects the environment in which they grow (Gupta *et al*., 2017). They have developed mechanisms that guarantee their survival even under adverse environmental conditions. In addition to resistance to antibiotics, bacteria have shown resistance to multiple disinfectants (Bridiera *et al.*, 2011; Odonkor and Addo, 2011). As a survival strategy, the biofilm formation on medical devices is a serious problem associated with deaths, resulting from nosocomial infections (Krishnan, 2015). In nosocomial environments, bacteria, bacterial spores, viruses and yeasts are mainly transmitted from infected and/or colonized patients, but also from staff, and in some situations from visitors to the inanimate hospital environment, particularly to areas adjacent to patients and frequently touched surfaces by hands (Kramer and Assadian, 2014). In this context, it is important that nosocomial areas and other environments, for example industrial production areas, have materials that prevent the formation of biofilms by bacteria. So, in the present study the antibacterial and antibiofilm activity of copper material was determined. The results obtained shown that uropathogenic *E. coli* grew around the copper couplings in trypticase soy broth (Fig. 1C). Once copper couplings were removed from the culture tube, the coupling surfaces (both inside and outside) did not show adhering uropathogenic *E. coli* cells (Fig. 1D). This was verified when the copper couplings were stained with crystal violet and the formation of biofilm was not observed (Fig. 1E). Uropathogenic *E. coli* also did not grow on tryptic soy agar plates after scraping the surface of the copper coupling with a bacteriological loop (data not shown). It has been reported that adhered cells correspond to the first stages of biofilm formation. After adhering on a surface, microbial cells start producing a matrix of extracellular polymeric substances, where they are embedded and protected from external stressful conditions (Costerton *et al*., 1999; Flores-Encarnación *et al.*, 2014; Garrett *et al*., 2008; Gomes *et al.*, 2020). *E. coli* forms biofilms on different materials such as glass, polyvinylchloride (PVC), stainless steel, polystyrene (Flores-Encarnación *et al*., 2017; Lajhar *et al.*, 2018; Lianhua *et al*., 2013).

In this study, the results obtained were in accordance with that reported by other authors, in relation to the formation of biofilm in materials different than copper (Fig. 1G and Fig. 1H). It has been reported that copper surfaces can eliminate bacteria, such as meticillin-resistant *Staphylococcus aureus, Salmonella enterica, Campylobacter jejuni, Enterobacter aerogenes*, *Pseudomonas aeruginosa, E. coli* O157:H7, as well as viruses and fungi (Faúndez *et al*., 2004; Noyce *et al.*, 2006; Reyes-Jara *et al*., 2016; Grass *et al*., 2011; Santo *et al*., 2010). That is very important because the presence of sessile microorganisms on abiotic surfaces can cause significant problems, representing considerable costs and negative effects in many different areas, such as health and medical care units, water transport systems, ships and marine industry, heat exchangers and cooling systems (Flores-Encarnación *et al*., 2016; Di Pippo *et al*., 2018; Gomes *et al.*, 2020; Teng *et al*., 2008). It has been reported that in biofilms, bacteria are protected by a matrix of extracellular polymeric substances. Some authors reported that the presence of copper might reduce the production of extracellular polymeric substances by bacteria (Chari *et al*., 2017; Gomes *et al*., 2020). Some mechanisms have been described to explain the antimicrobial action of copper: 1. Damaging the microbial DNA; 2. Altering bacterial protein synthesis; 3. Altering membrane integrity (Grass *et al*., 2011; Warnes *et al*., 2010). As can be seen, the copper material offers some important advantages for the control and inhibition of bacterial biofilm formation on surfaces. As is known, fortunately the copper material has been used in drinking water systems worldwide (Gomes *et al*., 2019). This material seems to be safer than others materials, due to its antimicrobial and antibiofilm properties. Therefore, the use of medical devices containing copper material is proposed in order to reduce the permanence and transmission of pathogenic bacteria in nosocomial environments and some other areas that are of interest.

CONCLUSION

Biofilms are ubiquitous in nature and are worldwide. They represent a bacterial survival strategy. However, bacterial biofilms are a frequent problem polluting devices or surfaces in health units, water transport systems, cooling systems, etc. The study provided evidence on the antibacterial and antibiofilm activity of the copper material tested.

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