Entreciencias
DIÁLOGOS EN LA SOCIEDAD DEL CONOCIMIENTO

Entreciencias 3 (7): 133-142, Ago. 2015
ISSN: 2007–8064

Ciencias de la Salud, Biológicas y Químicas

www.entreciencias.enes.unam.mx

Silver nanoparticles for the inhibition of *Staphylococcus aureus*

Las nanopartículas de plata inhiben el desarrollo de *Staphylococcus aureus*

Recibido: 18 de octubre de 2014; aceptado: 17 de abril de 2015

Miguel Ángel Ortiz-Gila[a1] , Rosa Elvira Nuñez-Anita[b2] , Ma. Concepción Arenas-Arrocena[c3], Omar Martínez-Álvarez[d4], Joel Emiliano-Ramírez[a5], Javier de la Fuente-Hernández[c6] , Laura Susana Acosta-Torres[c7]

[a]Universidad de Guanajuato, [b]Universidad Michoacana de San Nicolás de Hidalgo, [c]Escuela Nacional de Estudios Superiores Unidad León, [d]Universidad Politécnica de Guanajuato.

Resumen

Existe un gran ecosistema microbiano en la cavidad oral donde *Staphylococcus aureus* (*S. aureus*) se puede encontrar, causando patologías orales tales como quelitis angular, las paperas y la mucositis estafilocócica. Estas enfermedades producidas por *S. aureus* en la cavidad oral son consecuencia de los factores de virulencia, toxinas y multiresistencia a los antibióticos, lo que contribuye a la infección. La colonización en la cavidad oral por *S. aureus* en pacientes sanos es de 24% a 36%. Sin embargo, la incidencia aumenta a 48% en pacientes con prótesis debido a la formación de biofilms en la superficie de las dentaduras postizas. Actualmente, no existe ningún tratamiento para infecciones orales sin el uso de antibióticos. Investigaciones recientes indican que las nanopartículas de plata (AgNPs) son un material o estrategia para eliminar *S. aureus* debido a su efecto antibacteriano. Sin embargo, el mecanismo del efecto inhibidor de los iones de Ag sobre *S. aureus* es sólo parcialmente conocida y muy poco se ha informado. Por lo tanto, el propósito de la presente revisión sistemática es determinar las estrategias y retos de la utilización de biomateriales antimicrobianos con AgNPs frente a las infecciones orales de *S. aureus*.

Palabras clave: bacteria, nanoparticulas de plata, actividad antimicrobiana, resistencia a los antimicrobianos

Abstract

A large microbial ecosystem exists in the oral cavity where *Staphylococcus aureus* (*S. aureus*) can be found, causing oral pathologies such as angular cheilitis, mumps and staphylococcal mucositis. This pathogenicity is due to the virulence factors, toxins and multi-resistance to antibiotics in the oral cavity, which contributes to its colonization. Microbial colonization in the oral cavity by *S. aureus* has been isolated from healthy patients in a range of 24% to 36%. However, the incidence has increased to 48% in patients with dentures due to the formation of biofilm on the surface of the dentures. Currently, there is no treatment for oral infectious without the use of antibiotics. Recent investigations indicate that silver nanoparticles (AgNPs) are a promising material or strategy to eliminate *S. aureus* because of their well-known antibacterial effect. However, the mechanism of the inhibitory effect of Ag ions on *S. aureus* is only partially known and very little has been reported. Therefore, the purpose of the present systematic review is to determine the strategies and challenges of the use of antimicrobial biomaterials with AgNPs versus oral *S. aureus* infections.

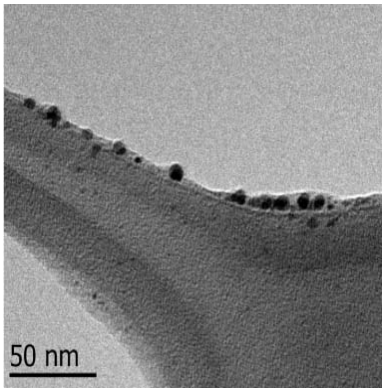
Keywords: bacteria, particle size, antimicrobial activity, antimicrobial resistance

[1] Departamento de Ciencias Médicas, Universidad de Guanajuato, Campus León. Maestría en Ciencias de la Salud. Líneas de investigación: genética bacteriana, con especial interés sobre las áreas de la epidemiología molecular, infecciones nosocomiales, resistencia bacteriana, inmunología, bioquímica y vacunología. Correo electrónico: maog86@gmail.com.
[2] Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán. Doctorado en Ciencias. Líneas de Investigación: evaluación de la eficacia antimicrobiana y biocompatibilidad de nanocompuestos. Correo electrónico: qfbrena@hotmail.com.
[3] Escuela Nacional de Estudios Superiores, Unidad León, UNAM. Doctorado en Ciencias de la Energía. Líneas de Investigación: desarrollo de biomateriales y nanocompuestos. Correo electrónico: mcaa05@gmail.com.
[4] Departamento de Ingeniería en Energía, Universidad Politécnica de Guanajuato, Guanajuato. Doctorado en Energía. Líneas de investigación: energía y nanotecnología. Correo electrónico: omartinez@upgto.edu.mx.
[5] Departamento de Ciencias Médicas, Universidad de Guanajuato, Campus León. Líneas de Investigación: colonización bacteriana y susceptibilidad antibacteriana. Correo electrónico: joelre@ugto.mx.
[6] Escuela Nacional de Estudios Superiores, Unidad León, UNAM. Maestría en Salud Pública. Líneas de Investigación: calidad de vida asociada a salud bucal. Correo electrónico: fuente@unam.mx
[7] Corresponding author. Escuela Nacional de Estudios Superiores, Unidad León, UNAM. Doctorado en Ciencias Odontológicas; Biomateriales. Líneas de Investigación: nanotecnología y desarrollo de biomateriales antimicrobianos. Correo electrónico: lacosta.enes@gmail.com

Introduction

The most prevalent odontogenic anaerobic bacteria includes species of the genera *Peptostreptococcus, Prevotella, Fusobacterium, Gemella, Porphyromonas* and *Bacteroides*. These microorganisms can be isolated throughout the oral cavity although there is a predilection for certain niches. Aerobic gram-positive bacteria have developed a number of specific receptors that allows their anchoring to certain structures of the oral cavity (Prieto-Prieto, and Calvo, 2004). The health of the mouth can be an indicator of the health of the body. Many diseases, such as diabetes, HIV, cancer, and eating disorders can cause oral health problems. *S. aureus* may produce mixed lesions in the oral mucosa, such as angular cheilitis, mumps and staphylococcal mucositis. Biomedical materials can be colonized by microorganisms that form a biofilm adhesive on their surface. Most bacterial biofilms are produced by *S. aureus* and *Staphylococcus epidermidis* (Baena-Monroy *et al.,* 2005). Some antimicrobial agents such as silver nanoparticles (AgNPs) have been used for the inhibition of *S. aureus*. Spherical AgNPs with an approximate size of 12-16 nm (Figure 1), are usually reported for this purpose, with a minimum inhibitory concentration (MIC) with different concentrations of silver nanoparticles (0.0, 6.25, 12.5 and 50 mM) (Bindhu and Umadevi, 2014; Ajitha, Reddy and Reddy, 2014; Lara *et al.,* 2010). The present article is a systematic review intending to highlight new information concerning the antibacterial effect of silver nanoparticles against *S. aureus*, including main reports about the mechanism of action.

Figure 1. Representative TEM image of spherical silver nanoparticles



Source: own elaboration

Characteristics of *S. aureus*

Virtually, any organ is prone to infection by *S. aureus* (Kanafani; and Fowler, 2006). Before the use of antibiotics, a bacteremia caused by *S. aureus* produced a mortality rate of approximately 82% of the patients. Currently, this percentage remains high, between 25% and 63% (Howe, Brown and Spencer, 1996). This is because *S. aureus* produces a wide variety of exoproteins that contribute to its ability to colonize and cause diseases in humans (Dinges, Orwin and Schlievert, 2000). *S. aureus* that lives in the human nose is carried either on the skin or inside the nose of healthy people; however, 25% - 30% of the population is inhabited in the nose. The optimum pH for the *Staphylococcus* to live in is 7.0-7.5, and the optimum temperature is 30-37 degree Celsius (Gluck and Gebbers, 2003). Members of the genus *Staphylococcus* are Gram-positive cocci 0.5 to 1.5 µm in diameter and disposed individually or singularly, and form couples, tetrads, short chains or irregular groups in cluster mode. These organisms are immobile, non-sporing, catalase-positive, and often capped or partially capped. Most of the *Staphylococcus* species are facultative anaerobes; the genus *Staphylococcus* grouped 32 species, 16 of them are in the human being (Mandell *et al.,* 2009).

Pathogenesis and antimicrobial resistance of *S. aureus*

S. aureus secretes enzymes and cytotoxins, including four hemolysins (alpha, beta, gamma and delta), nucleases, proteases, lipases, hyaluronidase and collagenase. The primary function of these proteins may be to help in the degradation of local host tissues to serve as nutrients for bacterial growth (Dinges *et al.,* 2000). A compendium of different virulence factors of *S. aureus* is mentioned in Table 1. Bacteria may be intrinsically resistant to one or more antimicrobials; they can acquire resistance by de novo mutations or resistance genes from other organisms, as well as metabolic adaptations to the drug (Becerra *et al.,* 2009). In 1945, the first strain of penicillin-resistant *S. aureus* was detected. In 1958 a new antibiotic, methicillin, was introduced by adding side chains of isoxazolyl. In 1961, the first strain of methicillin-resistant *S. aureus* (MRSA) was detected in England; soon after

glycopeptide (Vancomycin) appeared as a new antibiotic. The first report on the Vancomycin-resistant *S. aureus* (VRSA) was published in June 2002 in the United States (Dissemond, 2009).

S. aureus presents four mechanisms of resistance to beta-lactam antibiotics: 1) the β- lactam enzymes or β-lactamases; 2) the mecA gene that encodes the protein PBP2a (penicillin binding protein), which has low affinity for β-lactam antibiotics; (3) the flow pumps, which expel the antibiotic from the cell to cytoplasm, helping to maintain the intracellular levels below a lethal concentration and promote resistance to multiple antibiotics; and (4), the decrease in the permeability of the membrane, caused by a thickening of the cell wall of *S. aureus*, which prevents the entry into the cytoplasm of the antibiotic (Fuchs et al., 1994). The origin of the mecA gene is unknown, but it was probably transposed from coagulase-negative to *Staphylococcus* (*S. sciuri*) or resulted from an event of recombination that merged around 300 pairs of bases of a β-lactamase gene *Staphylococcus* and part of a gene that encodes the PBP2a of an unknown organism, perhaps *E. coli* (Monica-Gil, 2000). Fragments of approximately 30-50 kb of additional chromosomal DNA make up the mecA gene, which is found in methicillin-resistant strains. The antibacterial activity of β-lactam antibiotics is the covalent binding to active sites of the PBP2a. The PBP2as are transpeptidase enzymes that catalyze the peptidoglycan crosslinking (Chambers, 1997). MRSA strains are characterized by the presence of a mobile element called heterologous genetic, staphylococcal chromosomal cassette mec (SCCmec), which includes the mecA gene, and which is the central element of resistance to methicillin (Milheirico, Oliveira and Lencastre, 2007). Under the methodology of a previous study, different SCCmecs have been identified in the structural differences as well as in the genes complex of SCCmec recombination (Nübel et al., 2008). The mecA gene measures 2.1 kb in length and is located on a genomic island. So far, seven main types of SCCmec (type I-VII) have been identified measuring 209 to 66.9 kb in (Deurenberg and Stobberingh, 2008). For *S. aureus*, the SCCmec has its own evolutionary history and is able to offer varying determinants of virulence or antibiotics resistance (De Lencastre, Oliveira and Tomasz, 2007).

Table 1. Classification of *Staphylococcus aureus* virulence factors

Category	Virulence factor	Physiology
Adhesion to cell guest or extracellular matrix	Coagulase	This complex transforms Fibrinogen into insoluble fibrin and this causes the agglomeration of staphylococci, localizing the infection and protecting the bacterium from phagocytosis (Bustos-Martínez, Hamdan-Partida and Gutiérrez-Cárdenas, 2006).
Evasion of host defenses	Enterotoxins (SEs) Staphylococcal	Superantigens and pyrogenic toxins that stimulate proliferative, non-specific T cells, stimulate intestinal peristalsis and exert an effect on the central nervous system, manifested by vomiting, which accompanies gastrointestinal illness (Balaban and Rasooly, 2000).
	Toxin (TSST) toxic shock syndrome 1	Stimulates increased expression of cytokines and suppresses the processing of antigens in the major histocompatibility (MHC) complex (Lappin and Ferguson, 2009).
	Capsular polysaccharides	Capsule increases the resistance to phagocytosis (O'Riordan and Lee, 2004)
Invasion of the cell host and penetration of tissues	Exfoliative toxins (ETA and ETB)	Serine proteases that induce intraepidermal division through the granular layer, or epidermal necrosis (Yamaguchi *et al.*, 2002)
	Panton Valentine (PVL) of multidrug	Produces pores in the membranes of white blood cells, which leads to increased permeability of the membranes of the cells and lysis (Karahan *et al.*, 2008).

Source: own elaboration

Epidemiology and clones of MRSA

In 2005, MRSA caused more than 94,000 life-threatening infections and almost 19,000 deaths in the United States (Klevens *et al.,* 2007). Data between 2004 and 2005 obtained from the Antimicrobial European Resistance Surveillance System (EARSS), SENTRY program, and national programme of bacterial resistance, show MRSA rates in different regions: Canada 5%, USA 38%, Mexico 36%, Latin America 36%, Europe 1-40%, Asia (Japan) 60% (Sifuentes-Osornio and Pérez-Patrigeon, 2006).

Data from 479 U.S. patients were analyzed to assess the economic impact on patients with surgical site infection by MRSA. Infected patients with MRSA experienced increased hospitalization time after infection. The median of the hospital expenses was near $ 29,455 US dollars for control subjects, $ 52,791 for patients with MSSA-SSI (methicillin-susceptible *S. aureus*-surgical site infections), and $ 92,363 for patients with MRSA-SSI. In addition, resistance to methicillin is associated independently with an increase in mortality and hospitalization rates (Engemann *et al.,* 2003). The molecular epidemiology of infectious disease aims to determine the clonal relationship among several isolates of the same species (Fernández-Cuenca, 2004). Molecular typing is applied in MRSA epidemiology studies to determine whether a group of strains is clonal; that is, from a common precursor (Vivoni and Moreira, 2005). Multiple MRSA clone lineages are consequences of the success of the horizontal transfer of MecA (Humphreys, 2004). From 1990 to 1997, an analysis was carried out in three hospitals in Portugal of 210 MRSA isolated by PFGE (pulsed-field gel electrophoresis) revealing the sudden emergence and wide intrahospital spread of the brazilian clone, suggesting the intercontinental transfer of this strain from Brazil to Portugal (Aires de Sousa *et al.,* 1998).

In a study conducted from 1996 to 1998 in 22 hospitals throughout five Latin American countries, Argentina, Brazil, Chile, Uruguay, and Mexico, 499 MRSA strains isolated from clinical samples were analyzed. The prevalence and persistence of Brazilian clones was reported as follows: Brazil 97%, Argentina 86%, Chile 53%, massive dissemination to Uruguay 100%. In addition, a single clonal type was detected in the pediatric isolates in Mexico named M. clone (Aires de Sousa et al., 2001). In a study conducted between 1997 and 2003, in the Pediatric Hospital of the National Medical Center, also named XXI Century-IMSS (Instituto Mexicano del Seguro Social) in Mexico City, 659 different samples of *S. aureus* strains were analyzed. The results indicate that the frequency of MRSA ranged from 17% to 23% in 2001; the frequency decreased to 4% in 2002 and to 0% in 2003. Thus far, M clones have not been detected in other Latin American countries (Velázquez- Meza *et al.,* 2004).

Oral infections caused by *S. aureus*

The main dental infections caused by *S. aureus* include angular cheilitis, parotitis and staphylococcal mucositis, since biofilm formation by *Candida albicans (C. albicans), S. aureus* and *Streptococcus mutans* are the main species colonizing surface and oral mucosa of dentures (Queiroz et al., 2013). Epidemiological reports indicate a prevalence of 36 % of *S. aureus* isolated from the oral cavity in healthy patients, whereas this prevalence increases to 48 % when the patient has a dental prosthesis. A three-year study in the United Kingdom found 5% of oral prostheses samples containing *S. aureus*. In another separate study, MRSA was recovered from 10% of patients with dentures in Japan and 19% of mouths from a group of hospitalized elders (Lee *et al.,* 2009).

Silver nanoparticles (AgNPs) in dentistry

The use of silver has been severely limited by the cytotoxicity of silver ions; however, nanotechnology has enabled the production of small silver particles with higher surface area by volume, showing greater efficacy against bacteria and lower toxicity (Rai, Yadav and Gade, 2009). In the dentistry field, silver compounds have been used since the 1840s, when silver nitrate was used for the reduction of tooth decay. It was subsequently used as a preventive agent in permanent molar caries. In the 1960's, silver was combined with fluoride as an anticaries product (Peng, Botelho and Martinlinna, 2012).

From the biomedical perspective, because silver nanoparticles have low cytotoxicity in mammal cells and are easily synthesized, they offer a promising alternative for the indiscriminate use of antibiotics, as this cause the development of antimicrobial resistance. In our and

other recent investigation into the toxic effect of nanoparticles (Nuñez-Anita *et al*., 2014), we found that AgNPs, specifically, seem to induce toxicity for different reasons. These may include: 1) size (5 nm was more toxic than 20 or 50 nm), data which showed that smaller nanoparticles enter cells easier than larger ones, which in turn may be the cause of higher toxic effects; 2) doses (starting from 10 µg/mL doses exhibit toxicity) (Gliga *et al*. 2014; Liu *et al*., 2010); and, 3) the AgNPs solution was more toxic than AgNPs-coated materials and agglomeration was determinant for toxicity. Moreover, some cellular types showed more susceptibility to damage caused by AgNPs than others. For example, AgNPs induce DNA damage in human lung cells, but no cellular ROS increase upon exposure to AgNPs; this suggests a mechanism independent of oxidative stress (Gliga *et al*., 2014). In this sense Liu *et al*. (2010) showed that the proliferation of some human cell lines was inhibited after treatment with AgNPs, in lung (A549), Hepatic (HepG2), mammary gland (MCF-7) and Gastric (SGC-7901) treatments. Conversely, acrylic resins covered with AgNPs were biocompatible, with no evident genotoxic or cytotoxic effect in normal fibroblast and Leucocytes from peripheral blood (Acosta-Torres *et al*., 2012). Also, minimal citotoxicity was reported in undifferentiated and differentiated human adipose-derived and osteogenic-derived stem cells exposed to AgNPs at 10 µg/mL (Samberg *et al*., 2012)

Silver nanoparticles have the ability to inhibit bacterial growth, and therefore AgNPs are used in the area of health for coating dental biomaterials (Rai *et al*., 2009). Nanomaterials have important physical and chemical properties, such as small size, large surface areas and high reactivity, which are different from polymeric to ceramic or metal nanoparticles used as vehicles in the pharmaceutical and medical fields (Moreno-Vega *et al*., 2012). In dentistry, some research is focused on the development of acrylic resins with silver nanoparticles for dentures, which exhibit antimicrobial properties and reduction *of C. albicans* adherence (Acosta-Torres *et al*., 2012). Removable dentures are made usually of acrylic resin, and when they come into contact with the oral mucosa, act as a reservoir for the adhesion and proliferation of *S. aureus*, inviting opportunistic pathogen of important infectious diseases in the oral cavity (Quintero *et al*., 2012).

Antibacterial properties of AgNPs and the inhibition of *S. aureus*

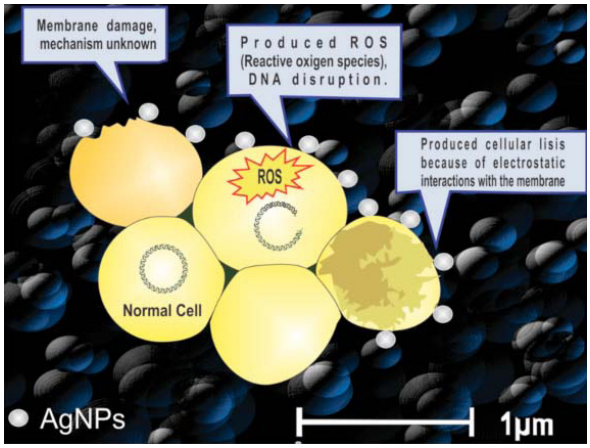
The mechanism of the inhibitory effects of silver ions (Ag+) in organisms is partially known. Some studies have reported that the positive charge of Ag+ is crucial for its antimicrobial activity, because electrostatic attraction of negatively loaded cell membranes of microorganisms can cause cell damage (Kim *et al*., 2007). Comparative studies of *S. aureus*, opposite AgNPs and Gram-negative bacteria have shown that silver ion is less effective against *S. aureus*; this is possibly due to the thickness of the layer of Peptidoglycan, which can prevent the action of silver ions through the wall cell (Pal, Tak and Song, 2007). When *S. aureus* is in contact with AgNPs the cell division is stopped in its initial stages and this provokes morphological changes that cause lysis (Jung *et al*., 2008). An existing proposal states that AgNPs in aqueous solution release silver ions exhibiting a bactericidal effect. Silver nanoparticles showed potent antibacterial activity in contrast to free silver ions. The mechanism proposed involves AgNPs-membrane cell interactions, which lead to cellular lysis (Choi *et al*., 2008).

New nanocompound waterborne polyetherurethane (PEU) ionomer embedding with AgNPs showed antibacterial activity against *Escherichia coli* (*E. coli*; 0.1-1 ppm doses), in contrast with *S. aureus* (1-10 ppm doses). On the other hand, cytotoxicity of AgNPs was observed at 10 ppm in fibroblast cells, which suggest cytocompatibility. These results demonstrated the strong bacteriostatic effect of AgNPs, which inhibited growth of *E. coli and S. aureus* (Liu *et al*., 2013). Past research suggests that the antimicrobial mechanism of the AgNPs is related to the formation of free radicals that induce damage in the cell membrane and provoke cell death. Formation of reactive oxygen species (ROS) correlated with the antimicrobial activity of hydrophilic polymer coated AgNPs against *E. coli* O157:H7, which is a pathogen microorganism. Xu *et al*., (2012) corroborated that antimicrobial activity was suppressed in presence of an antioxidant. Comparative studies of the antimicrobial effect of AgNPs carried out on both antibiotic resistant and susceptible bacteria established that AgNPs do not bind selectively to certain proteins; on the contrary, the targets of AgNPs include membrane, cytoplasmic proteins and plasmids (Rastogi *et al*., 2011). Silver (Ag) is an active antimicrobial against

gram-positive, Gram-negative bacteria and fungi. The silver ions attach to groups of donors of electrons in biological molecules containing sulfur or nitrogen, which results in defects in the cell membrane of bacteria and leads to the loss of their cellular contents and the death of the microorganism. In addition, the Ag+ ions may interact with DNA, preventing cell reproduction (Monteiro *et al.*, 2012).

A mechanism for the antimicrobial activity of AgNPs was proposed recently as concerns significant changes and damage to the bacteria membrane. The change in morphology increases permeability, leaving bacterial cells incapable of properly regulating transport through the plasma membrane, resulting in cell death. The increase in permeability of the cell membranes allows the AgNPs to penetrate the cell and cause cell death by breaking the DNA (Tamboli and Lee, 2013). Research on antibacterial AGNPS cytotoxic mechanism is speculative, some authors as: Marta *et al.*, 2014; Ajitha *et al.*, 2014; Roe *et al.*, 2008; Cao *et al.*, 2011; Zhao *et al.*, 2012; Sharma *et al.*, 2013; Mocanu *et al.*, 2014; Lu *et al.*, 2014 and Ahluwalia *et al.*, 2014. They have reported that more research needs to continue to make this subject to understand the mechanisms. In general, the antimicrobial mechanism of AgNPs proposed included: 1) Membrane damage, silver ions production, increase permeability of the cell membranes, and 2) Oxidative stress, free radical production, ROS production, oxidative released Ag+ from NPs surface, regulation of oxygen availability and mitochondrial dysfunction (Moritz and Geszke-Moritz, 2013) (Figure 2). Table 2 shows the antimicrobial/antibacterial activity of silver nanoparticles on *S. aureus* and other microorganisms reported in the literature.

Figure 2



Schematic representation of toxicity of silver nanoparticles (AgNPs) against *S. aureus*. AgNPs and their ions can produce reactive oxygen radicals, resulting in the onset of oxidative stress, as well as the production of cellular lysis due to the electrostatic interactions between the membrane and membrane damage by unknown mechanisms

Table 2. Antimicrobial activity of Ag nanoparticles on *Staphylococcus aureus*

Antimicrobial activity against	Morphology	Proportion of AgNPs	Size (nm)	Reference
MRSA	Nanoprisms (anisotropic nanoparticles)	6 - 61 µg/mL	30 - 50^{++} 4 - 6**	Marta *et al.*, 2014
S. aureus, Pseudomonas aeruginosa	Spherical	10 - 30 mL	12	Bindhu and Umadevi, 2014
E. coli, Pseudomonas spp., Bacillus spp., Staphylococcus spp., A. niger, A. flavus, Penicillium spp	Spherical nanoparticles	4 µl, 8µl,12µl and 16 µl obtained from aqueous 0.001M silver nitrate solution	16 - 18	Ajitha *et al.*, 2014
E. coli, Enterococcus, S. aureus, coagulase-negative staphylococci, Pseudomonas aeruginosa, C. albicans	Spherical nanoparticles	593 ± 2, 1019 ± 3.7 µg/g	7 - 10	Roe *et al.*, 2008
S. aureus, E. coli	<-Ti(AGNPs)		5 - 8	Cao *et al.*, 2011
E. coli	AgNPs/PVA/CM-chitosan	0.5 - 10 mmol/L	4 - 14	Zhao *et al.*, 2012
MRSA	Spherical	30 µl	28 - 50	Manikprabhu and Lingappa, 2013
Bacillus subtilis, S. aureus	AgNPs doped with ZnO	4 - 10 mg/mL	34 - 59	Sharma *et al.*, 2013
E. coli, S. aureus, Staphylococcus spp., Bacillus cereus, C. albicans	Semi-spherical	4.5 - 5.4 %	12.0 ± 5.0	Mocanu *et al.*, 2014
E. coli, S. aureus	Ag-SiO_2 nanoclusters	0.4 - 0.8 mg/mL	40	Lu *et al.*, 2014
Salmonella typhimurium, Pseudomonas aeruginosa, E. coli, S. aureus	Spherical	0.05 - 100 µg/mL	5-50	Tamboli and Sung, 2013
Klebsiella pneumonia, S. aureus	Spherical and irregular shape	3 - 15 µg/mL	51	Ahluwalia *et al.*, 2014

Source: own elaboration. Notes: *++ length; ** thickness*

Current and future perspectives

Over a span of a few years *S. aureus* increased resistance to a variety of antibiotic treatments. This, in turn, has led to the development of new antimicrobials (Chakraborty, Pramanik and Roy, 2012). The success of *S. aureus* colonization not only originates in different infection mechanisms, but also in the physiological conditions of the host organism. Growing evidence shows that AgNPs are an alternative to antibiotics because the doses that exhibit an antimicrobial effect are also cytocompatible.

Acknowledgments

The authors wish to thank the academic projects DGAPA-UNAM: PAPIME- PE202214 and PAPIIT- TA200414.

References

Acosta-Torres, L.S., Mendieta, I., Nuñez-Anita, R.E., Cajero-Juárez, M., & Castaño, V.M. (2012). Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures. *Int J Nanomedicine*, 7, 4777-4786.

Ahluwalia, V., Kumar, J., Sisodia, R., Shakil, N.A., & Walia, S. (2014). Green synthesis of silver nanoparticles by Trichoderma harzianum and their bio-efficacy evaluation against Staphylococcus aureus and Klebsiella pneumonia. *Industrial Crops and Products*, 55, 202–206.

Aires de Sousa, M., Sanches, I.S., Ferro, M.L., Vaz, M.J,, Saraiva, Z., Tendeiro, T., Serra, J., & Lencastre, H. de (1998). Intercontinental spread of a multidrug-resistant methicillin-resistant Staphylococcus aureus clone. *J Clin Microbiol*, 36 (9), 2590-2596.

Aires De Sousa, M., Miragaia, M., Sanches, I.S., Ávila, S., Adamson, I., Casagrande, S.T., *et al.* (2001). Three-year assessment of methicillin-resistant Staphylococcus aureus clones in Latin America from 1996 to 1998. *J Clin Microbiol*, 39 (6), 2197-2205.

Ajitha B., Reddy, Y.A.K., & Reddy P.S. (2014). Biosynthesis of silver nanoparticles using Plectranthus amboinicus leaf extract and its antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 128, 257-262.

Baena-Monroy, T., Moreno-Maldonado, V., Franco-Martínez, F., Aldape-Barrios, B., Quindós, G., & Sánchez-Vargas, L.O. (2005). Candida albicans, Staphylococcus aureus and Streptococcus mutans colonization in patients wearing dental prosthesis. *Med Oral Patol Oral Cir Bucal*, 10 (Suppl l), E27-E39.

Balaban ,N., & Rasooly, A. (2006). Staphylococcal enterotoxins. *Int J Food Microbiol*, 61, 1-10.

Becerra, G., Plascencia, A., Luévanos, A., Domínguez, M., & Hernández, I. (2009). Mecanismo de resistencia a antimicrobianos en bacterias. *Enf Inf Microbiol*, 29, 70-76.

Bindhu, M.R., & Umadevi, M. (2014). Silver and gold nanoparticles for sensor and antibacterial applications. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 128, 37-45.

Bustos-Martínez, J.A., Hamdan-Partida, A., & Gutiérrez-Cárdenas, M. (2006). Staphylococcus aureus: la reemergencia de un patógeno en la comunidad. *Rev Biomed*, 17, 287-305.

Cao, H., Liu, X., Meng, F., & Chu, P.K. (2011). Biological actions of silver nanoparticles embedded in titanium controlled by micro-galvanic effects. *Biomaterials*, 32, 693-705.

Chakraborty, S.P., Pramanik, P., & Roy, S. (2012). A review on emergence of antibiotic resistant staphylococcus aureus and role of chitosan nanoparticle in drug delivery. *Int J Life Sci & Pharma Res*, 2, L96-L115.

Chambers, H.F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clin Microbiol Rev*, 10, 781-791.

Choi, O., Deng, K.K., Kim, N.J., Ross, L., Surampalli, R.Y., & Hu, Z. (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res*, 42, 3066-3074.

De Lencastre, H., Oliveira, D., & Tomasz, A. (2007). Antibiotic resistant Staphylococcus aureus: a paradigm of adaptive power. *Curr Opin Microbiol*, 10, 428-435.

Deurenberg, R.H., & Stobberingh, E.E. (2008). The evo-

lution of Staphylococcus aureus. *Infect Genet Evol*, 8, 747-63.

Dinges, M.M., Orwin, P.M., & Schlievert, P.M. (2000). Exotoxins of Staphylococcus aureus. *Clin Microbiol Rev*, 13, 16-34.

Dissemond, J. (2009). Methicillin resistant Staphylococcus aureus (MRSA): Diagnostic, clinical relevance and therapy. *J Dtsch Dermatol Ges*, 7, 544-553.

Engemann, J.J., Carmeli, Y., Cosgrove, S.E., Fowler, V.G., Bronstein, M.Z., Trivette, S.L., Briggs, J.P., Sexton, D.J. & Kaye, K.S. (2003). Adverse clinical and economic outcomes attributable to methicillin resistance among patients with Staphylococcus aureus surgical site infection. *Clin Infect Dis*, 36 (5), 592-598.

Fernández-Cuenca, F. (2004). Applications of PCR techniques for molecular epidemiology of infectious diseases. *Enferm Infecc Microbiol Clin*, 22, 355-360.

Fuchs, L.Y., Chihu, L., Conde, C., González, V.M., Noguez, A.H., Calderón, E., Avonce, N., & Ovando, C. (1994). Mecanismos moleculares de la resistencia bacteriana. *Salud Pública de México*, 36 (4), 428-438.

Gluck, U. & Gebbers, J.-O. (2003). Local Pathogenic Bacteria in Allergic Rhinitis: A Novel Concept of Its Pathogenesis. *Institute of Pathology and Environmental Medicine*. 65, 202-205.

Gliga, A.R., Skoglound. S, Wallinder, I.O., Fadeel, B., & Karlsson, H.L. (2014). Size-dependent cytotocicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. *Particle and fibre toxicology*. 17; 11:11

Howe, R.A., Brown, N.M., Spencer, R.C. (1996). The new threats of Gram positive pathogens: re-emergence of things past. *J Clin Pathol*, 49, 444-449.

Humphreys, H. (2004). MRSA Current Perspectives. *J Antimicrob Chemother*, 53, 552-552.

Jung, W.K., Koo, H.C., Kim, K.W., Shin, S., Kim, S.H., & Park, Y.H. (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Appl Environ Microbiol*, 74 (7), 2171-2178.

Kanafani, Z.A., & Fowler, V.G. (2006). Staphylococcus aureus infections: new challenges from an old pathogen. *Enferm Infecc Microbiol Clin*, 24, 182-193.

Karahan, Z.C., Tekeli, A., Adaleti, R., Koyuncu, E., Dolapci, I., & Akan, O.A. (2008). Investigation of Panton-Valentine leukocidin genes and SCCmec types in clinical Staphylococcus aureus isolates from Turkey. *Microb Drug Resist*. 14, 203-210.

Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J., Kim, S.H., Park, Y.K., Park, Y.H., Hwang, C.Y., Kim, Y.K., Lee, Y.S., Jeong, D.H., & Cho, M.H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine*, 3 (1), 95-101.

Klevens, R.M., Morrison, M.A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L.H., Lynfield, R., Dumyato, G., Townes, J.M., Craig, A.S., Zell, E.R., Fosheim, G.E., McDougal, L.K., Carey, R.B., & Fridkin, S.K. (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *JAMA*, 298 (15), 1763-1771.

Lappin. E, & Ferguson, A.J. (2009). Gram-positive toxic shock syndromes. *Lancet Infect Dis*, 9, 281-290.

Lara, H. H., Ayala-Núnez, N. V., Turrent, L. D. C. I., & Padilla, C. R. (2010). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria.World *Journal of Microbiology and Biotechnology*, 26, 615-621.

Lee, D., Howlett, J., Pratten, J., Mordan, N., McDonald, A., Wilson, M., & Ready, D. (2009). Susceptibility of MRSA biofilms to denture-cleansing agents. *FEMS Microbiol Lett*, 291 (2), 241-246.

Liu, F., Wang, R., Shi, Y., Jiang, X., Sun, B., & Zhu, M. (2013). Novel Ag nanocrystals based dental resin composites with enhanced mechanical and antibacterial properties. *Prog Nat Sci: Materials International*, 23, 573–578.

Liu, W., Wu, Y., Wang, C., Li, H.C., Wang, T., Liao, C.Y., Zhou, Q.F., Yan, B., & Jiang, G.B. (2010). Impact of silver nanoparticles on human cells: effect of particle size. *Nanotoxicology*. 4(3), 319–330.

Lu, R., Zou, W., Du, H., Wang, J., & Zhang, S. (2014). Antimicrobial activity of Ag nanoclusters encapsulated in porous silica nanospheres. *Ceram Int*, 40, 3693–3698.

Mandell, G., Dolin, R., Bennett, J., Mandell, G. L., & Bennett, J. E. (2009). *Mandell, Douglas, and*

Bennett's Principles and Practice of Infectious Diseases (7th Edition). United States: Elsevier

Manikprabhu, D., & Lingappa, K. (2013). Antibacterial activity of silver nanoparticles against methicillin-resistant Staphylococcus aureus synthesized using model Streptomyces sp. pigment by photo-irradiation method. *J Pharm Res*, 6, 255-260.

Marta, B., Jakab, E., Potara, M., Simon, T., Imre-Lucaci, F., & Barbu-Tudoran, L., (2014). Pluronic-coated silver nanoprisms: Synthesis, characterization and their antibacterial activity. *Colloids Surf A: Physicochem Eng, Asp* 441, 77-83.

Milheirico, C., Oliveira, D.C., & de Lencastre, H. (2007). Update to the Multiplex PCR Strategy for Assignment of mec Element Types in Staphylococcus aureus. *Antimicrob Agents Chemother*, 51, 3374-3377.

Mocanu, A., Furtos, G., Rapuntean, S., Horovitz, O., Flore, Ch., & Garbo, C. (2014). Synthesis; characterization and antimicrobial effects of composites based on multi-substituted hydroxyapatite and silver nanoparticles. *Appl Surf Sci*, 298, 225-235

Monica-Gil, D.M. (2000). Staphylococcus aureus: Microbiology and molecular features of methicillin resistant Staphylococcus aureus. *Rev Chil Infect*, 17, 145-152.

Monteiro, D.R., Gorup, L.F., Takamiya, A.S., de Camargo, E.R., Filho, A.C.R., & Barbosa, D.B. (2012). Silver distribution and release from an antimicrobial denture base resin containing silver colloidal nanoparticles. *J Prosthodont*, 21, 7-15.

Moreno-Vega, A.I., Gómez-Quintero, T., Nuñez-Anita, R-E, Acosta-Torres, L.S., & Castaño, V.M. (2012). Polymeric and Ceramic Nanoparticles in Biomedical Applications. J *Nanotechno*, 2012, 1-10.

Moritz, M., & Geszke-Moritz, M. (2013). The newest achievements in synthesis, immobilization and practical applications of antibacterial nanoparticles. *Chem Eng* J, 228, 596-613.

Nuñez-Anita, R.E., Acosta-Torres, L.S., Vilar-Pineda, J., Martínez-Espinosa, J.C., de la Fuente-Hernández, J., & Castaño, V.M. (2014). Toxicology of antimicrobial nanoparticles for prosthetic devices. *International Journal of nanomedicine*. 20 (9) 3999-4006.

Nübel, U., Roumagnac, P., Feldkamp, M., Song, J.H., Ko, K.S., Huang, Y.C., Coombs, G., Ip, M., Westh, H., & Skov, R. (2008). Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. *Proc Natl Acad Sci USA*, 105, 14130-14135.

O'Riordan, K., & Lee, J.C. (2004). Staphylococcus aureus capsular polysaccharides. *Clin Microbiol Rev*, 17, 218-234.

Pal, S., Tak, Y.K., & Song, J.M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium *Escherichia coli*. *Appl Environ Microbiol*, 73, 1712-1720.

Peng, J.J.Y., Botelho, M.G., & Matinlinna, J.P. (2012). Silver compounds used in dentistry for caries management: a review. *J Dent*, 40, 531-541.

Prieto-Prieto, J., & Calvo, A. (2004). Microbiological basis of oral infections and sensitivity to antibiotics. *Med Oral Patol Oral Cir Bucal*, 15-8 (9 Suppl), 11-14.

Queiroz, J.R., Fissmer, S.F., Koga-Ito, C.Y., Salvia, A.C., Massi, M., Sobrinho, A.S., & Junior, L.N. (2013). Effect of diamond-like carbon thin film coated acrylic resin on Candida albicans biofilm formation. *J Prosthodont*, 22 (6), 451-455.

Quintero, T., Acosta-Torres, L.S., Hernández Padrón, G., Campos, P., de la Fuente-Hernández, J., & Castaño V.M. (2012). Nanopartículas con efecto antifúngico en prótesis dentales. *Ide@s CONCYTEG*, 7, 1101-1112.

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*, 27, 76-83.

Rastogi, S.K., Rutledge, V.J., Gibson, C., Newcombe, D.A., Branen, J.R., & Branen, A.L. (2011). Ag colloids and Ag clusters over EDAPTMS-coated silica nanoparticles: synthesis, characterization, and antibacterial activity against *Escherichia coli*. *Nanomedicine*, 7, 305-314.

Roe, D., Karandikar, B., Bonn-Savage, N., Gibbins, B., & Jean-Baptiste, R. (2008). Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *J Antimicrob Chemother*, 61, 869-876.

Samberg, M.E., Loboa, E.G., Oldenburg, S.J., & Monteiro-Riviere, N.A. (2012). Silver nanoparticles do not influence stem cell differentiation but cause minimal toxicity. *Nanomedicine,* 7(8),1197-1209.

Sharma, N., Kumar, J., Thakur, S., Sharma, S., & Shrivastava, V. (2013). Antibacterial study of silver doped zinc oxide nanoparticles against Staphylococcus aureus and Bacillus subtilis. *Drug Invention Today*, 5, 50-54

Sifuentes-Osornio, J., & Pérez-Patrigeon, S. (2006). Sepsis por *Staphylococcus aureus* resistente a meticilina: la sombra de una amenaza permanente. *Rev Inv Clin*, 58, 598-607.

Tamboli, D.P., & Lee, D.S. (2013). Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram negative bacteria. *J Hazard Mater*, 260, 878-884.

Velázquez-Meza, M.E., Aires de Sousa, M., Echaniz-Aviles, G., Solórzano-Santos, F., Miranda-Novales, G., Silva-Sanchez, J., & de Lencastre, H. (2004). Surveillance of methicillin-resistant *Staphylococcus aureus* in a pediatric hospital in Mexico City during a 7-year period (1997 to 2003): clonal evolution and impact of infection control. *J Clin Microbiol*, 42 (8), 3877-3880.

Vivoni, A.M., & Moreira, B.M. (2005). Application of molecular techniques in the study of Staphylococcus aureus clonal evolution-a review. *Mem Inst Oswaldo Cruz*, 100, 693-698.

Xu, H., Qu, F., Xu, H., Lai, W., Andrew Wang, Y., Aguilar, Z.P., & Wei, H. (2012). Role of reactive oxygen species in the antibacterial mechanism of silver nanoparticles on Escherichia coli O157:H7. *Biometals*, 25 (1), 45-53.

Yamaguchi, T., Nishifuji, K., Sasaki, M., Fudaba, Y., Aepfelbacher, M., Takata, T., Ohara, M., Komatsuzawa, H., Amagai, M., & Sugai, M. (2002). Identification of the Staphylococcus aureus etd pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. *Infect Immun*, 70, 5835-5845.

Zhao, Y., Zhou, Y., Wu, X., Wang, L., Xu, L., & Wei, S. (2012). A facile method for electrospinning of Ag nanoparticles/poly (vinylalcohol)/carboxymethyl-chitosan nanofibers. *Appl Surf Sci*, 258, 8867–8873.