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Bismuth and Silver Nanoparticles as Antimicrobial Agent over Subgingival Bacterial and Nosocomial Strains

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Abstract: Bismuth compounds are used in the treatment of gastrointestinal infections due to its effectiveness against *Helicobacter pylori* as Gram-negative bacteria. Moreover, the nanometric materials show better biological properties that the materials in block. The aim of this study was to determine the MICs (minimal inhibitory concentrations) of three colloidal dispersions of bismuth nanoparticles compared with silver nanoparticles against oral and nosocomial bacteria. The nanoparticles were synthesized by chemical reduction in DMSO. The MICs of each colloidal dispersion were obtained on eight species representative of the subgingival biofilm, as well as in three species of medical importance: *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. All bismuth compounds showed antimicrobial effect on the tested bacterial species, with MICs between 37 to 329 μg/mL. On the other hand, AgNPs showed MICs from 16 to 32 μg/mL for the bacteria of subgingival biofilm and from 32 to 65 μg/mL for the species of medical importance. In accordance with this study, the different BiNPs had an antimicrobial effect in all the bacterial species, although with a potency lower than AgNPs.

Key words: Bismuth nanoparticles, silver nanoparticles, subgingival microbiota, *Streptococcus aureus, Escherichia coli, Pseudomona aeruginosa*.

Nomenclature

Bi	Bismuth
Bi_2O_3	Bismuth oxide
Bi_2S_3	Bismuth sulfide
Ag	Silver
NPs	Nanoparticles
DMSO	Dimethyl sulfoxide
MICs	Minimal inhibitory concentrations

1. Introduction

In the oral cavity there are approximately 400 bacterial species associated with the periodontal biofilm [1, 2]. This microbial ecology is described in three typical groups: [3, 4] the first settlers that adhere to the dental surface by means of specific molecules [5, 6], such as *Streptococcus* spp and *Actinomyces* spp. Then, the bridge settlers or secondary bacteria, which include species like *Fusobacterium nucleatum*, *Parvimonas micra* and *Prevotella intermedia*, that aggegate to the structure initially formed [7]. And, If the sequence of colonization of the dental plaque is not disrupted, the biofilm presents a third group of late settlers, these are Gram-negative bacteria such as *Treponema dentiloca* and *Porphyromonas gingivalis* [8]. Therefore, it is important to involve a large number of bacterial species in this study.

Bismuth compounds have been used in cosmetic and pharmaceutical industry for over than 250 years [9-11]. This metal is widely used like antimicrobial agent for the eradication of individual strains as

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Helicobacteri pylori and reduction of biofilm formation by *Pseudomonas aeruginosa* and Staphylococcus [12-16], in the treatment of general gastrointestinal disorders, syphilis and tumors, which has been discussed in several reviews [17, 18].

Nowadays, there is an interest for finding novel and better antimicrobial agents for medical and dental applications, and nanomaterials have shown to present higher biological properties using lower doses. Therefore, the aim of this work was to evaluate the antimicrobial effect of colloidal dispersions of zero valent bismuth, bismuth oxide, bismuth sulfide, and zero valent silver on eight bacterial strains typical of the dental badge and three pathogenic ones of medical importance.

2. Experimental Setup

2.1 Bismuth and Silver Nanoparticles

All the samples were provided through the Chemistry Laboratory from the Universidad Nacional Autónoma de México, in accordance with the synthesis and characterization of the nanoparticles obtained by optimized methods of chemical reduction, described by Velasco-Arias et al. [19].

For the synthesis of the bismuth-based nanoparticles, the precursors were: bismuth nitrate pentahydrate ($(Bi(NO_3)_3 \cdot 5H_2O)$, 99.99%, Sigma-Aldrich), sodium borohydride ($(NaBH_4)$, 96%, Baker), potassium hydroxide (KOH, 99%, Baker) and sodium sulfide (Na_2S, 99%, Sigma-Aldrich), depending on the sample. Also, sodium citrate dihydrated ($Na_3(C_6H_5O_7) \cdot 2H_2O$, 99% Sigma-Aldrich) and DMSO ((CH_3) 2SO, 99.9% J. T. Baker) were used as surfactants to obtain a monodispersed stabilized colloidal suspension [19].

On the other hand, silver nanoparticles were synthesized used silver 2-ethylhexanoate ($(C_8H_{15}AgO_2)$, Aldrich 99.9%) as starting salt and DMSO as surfactant, under controlled temperature [20]. The characterization will not be presented.

2.2 Minimal Inhibitory Concentrations

All the assays were realized in the Molecular Genetics Laboratory of the DEPeI (Division of Studies of Posgrado and Investigation) from the faculty of Odontology, UNAM.

Eight bacterial species representative of the subgingival microbiote and three of medical importance were tested. Lyophilized bacterial stocks (ATCC, Rockville, MD, USA) were rehydrated in *Mycoplasma* broth base (Becton Dickinson, Microbiology Systems, BBL®, Sparks, MD, USA). Eight strains were grown on *Mycoplasma* agar base (Becton Dickinson, Microbiology Systems, BBL®, Sparks, MD, USA), supplemented with 0.3 μg/mL menadione (Sigma-Aldrich), 5 μg/mL hemin (Sigma) and 5% defibrinated sheep blood (Microlab Laboratory, Mex.) at 34 °C under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) (Coy Laboratory Products Inc., Michigan USA). Three strains were grown on Tripticase soy agar (Bioxon, Becton Dickinson), supplemented with 0.3 μg/mL menadione (Sigma-Aldrich), 5 μg/mL hemin (Sigma) under aerobic conditions (Felisa® incubator) at 34 °C.

The growth of each strain was recolected of the agar surface and was suspended in a tube with broth HK (Mycoplasma Broth, Becton Dickinson), supplemented with 0.3 mg/mL of menadione and 5 ug/mL hemin or TSB enriched (Trypticase Soy broth base (Becton Dickinson), 5 ug/mL hemin), depending on the strain. The OD (optical density) of each tube was adjusted to 1 in a spectrophotometer at wavelength of 600 nm corresponding to 1×10^9 cells/mL. Then 20 mL of each tube was transferred to 96-well microplates. Each strain was tested in ten different concentrations, which were 2.50×10^{-6} to 1.28×10^{-3} M for BiNPs, Bi_2S_3NPS, Bi_2O_3NPS and 1.25×10^{-6} to 6.40×10^{-4} M for AgNPs.

The positive and negative controls were nutritive medium and 10 μg/mL of Ciprofloxacino ($C_{17}H_{18}FN_3O_3$) respectively. Also, the precursor

solutions were evaluated. Every bacterial strain was incubated at 34 °C in aerobio or anaerobio enviroment, with orbital shaking at 160 rpm for 24 hrs.

The test was performed in triplicate. All plates were incubated at 34 °C for 7 days under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) or 48 hrs in environment atmosphere. Following the incubation, the MICs of each sample tested were determined.

3. Experimental Results

In this study, the antimicrobial effect of three bismuth compounds in nanometric scale was compared with silver nanoparticles. The tests of antimicrobial susceptibility showed that the different bismuth nanoparticles and silver nanoparticles had antimicrobial effect for all bacterial strains. The minimum inhibitory concentrations determined for all antimicrobial agents are presented in Table 1.

The three bismuth compounds were effective in concentrations between 37 to 164 μg/mL for the oral strains and in concentrations between 164 to 329 μg/mL for the medical importance strains. The lability of the oral strains was evident when their susceptibilities were compared with *E. coli*, *S. aureus* and *P. aeruginosa* (Fig. 1). In general, no specific susceptibility between Gram-negative or Gram-positive bacteria for all nanoparticles was observed.

Table 1 Minimum inhibitory concentrations of bismuth NPS and AgNPS.

Strains	MIC (Minimun inhibitory concentrations) (μg/mL ± SD)			
	Nanoparticles			
	Bi_2S_3NPs	BiNPs	Bi_2O_3NPs	AgNPs
A. *actinomycetemcomitans* (ATCC®43718™)	82 ± 0	66 ± 0	74 ± 0	16 ± 0
A. *israelii* (ATCC®12102™)	164 ± 0	133 ± 0	74 ± 0	32 ± 0
E. *corrodens* (ATCC®23834™)	41 ± 0	66 ± 0	37 ± 0	16 ± 0
F. *nucleatum* (ATCC®25586™)	41 ± 0	133 ± 0	149 ± 0	32 ± 0
P. *gingivalis* (ATCC®33277™)	164 ± 0	133 ± 0	74 ± 0	32 ± 0
P. *intermedia* (ATCC®25611™)	82 ± 0	66 ± 0	37 ± 0	32 ± 0
P. *micra* (ATCC®33270™)	82 ± 0	133 ± 0	74 ± 0	32 ± 0
S. *sanguinis* (ATCC®10556™)	164 ± 0	133 ± 0	149 ± 0	32 ± 0
E.*coli* (ATCC®11775™)	164 ± 0	267 ± 0	267 ± 0	65 ± 0
S. *aureus* (ATCC®25923™)	329 ± 0	267 ± 0	267 ± 0	65 ± 0
P. *aeruginosa* (ATCC®10752™)	164 ± 0	267 ± 0	149 ± 0	32 ± 0

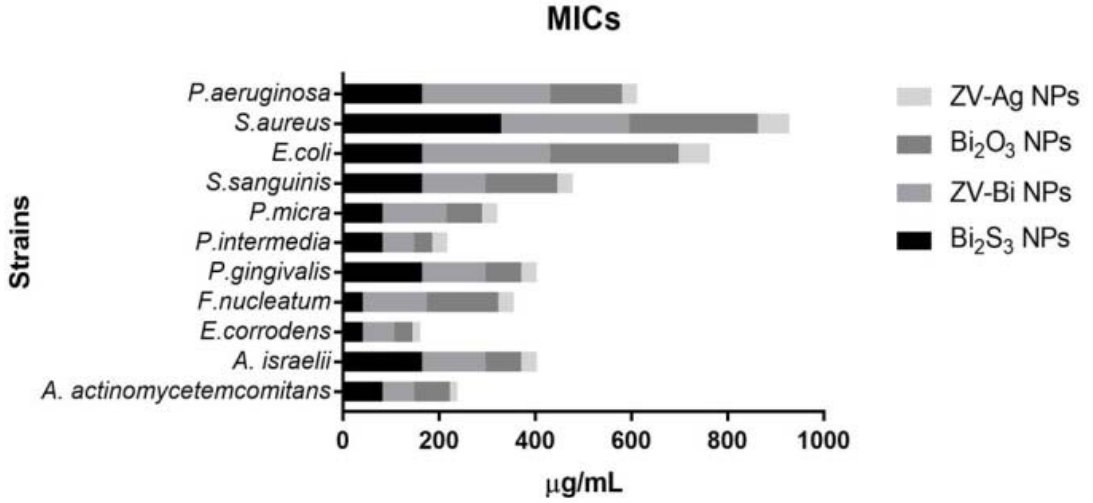

Fig. 1 Comparison of the effectiveness of each colloidal solution between the different bacterial.

Silver nanoparticles were more effective that the bismuth compounds, i. e., the Ag NPs showed lower MIC values. On the other hand, the putative bacterias *E. corrodens* and *P. intermedia* showed lower MICs with bismuth sulfide, metallic bismuth, and bismuth oxide compared with other oral strains.

4. Conclusions

The different bismuth nanoparticles showed antimicrobial effect for colonizing bacteria of the oral cavity, as well as on opportunistic pathogens, in the concentrations that were tested. We did not find differences in the antibacterial effect of bismuth nanoparticles between Gram-negative and Gram-positive bacteria. The Bi_2O_3NPs were the most effective nanometric bismuth compounds evaluated here, although with a lower potency when compared with AgNps.

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