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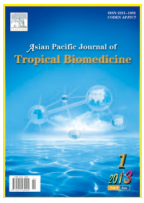

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Antimicrobial effects of silver zeolite, silver zirconium phosphate silicate and silver zirconium phosphate against oral microorganisms

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PEER REVIEW

Peer reviewer

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Comments

This is a good preliminary study that investigates the potential antimicrobial action of AgNPs: AgZ, AgZrPSi and AgZrP on oral microorganisms. The results showed that AgNPs were effective even at low concentrations and suggests the implication of AgNPs in dental materials to reduce the risk of opportunistic infections such as dental caries and candidiasis.
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ABSTRACT

Objective: To evaluate the antimicrobial activities of silver inorganic materials, including silver zeolite (AgZ), silver zirconium phosphate silicate (AgZrPSi) and silver zirconium phosphate (AgZrP), against oral microorganisms. In line with this objective, the morphology and structure of each type of silver based powders were also investigated. **Methods:** The antimicrobial activities of AgZ, AgZrPSi and AgZrP were tested against *Streptococcus mutans, Lactobacillus casei, Candida albicans* and *Staphylococcus aureus* using disk diffusion assay as a screening test. The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) were determined using the modified membrane method. Scanning electron microscope and X-ray diffraction were used to investigate the morphology and structure of these silver materials. **Results:** All forms of silver inorganic materials could inhibit the growth of all test microorganisms. The MIC of AgZ, AgZrPSi and AgZrP was 10.0 g/L whereas MLC ranged between 10.0-60.0 g/L. In terms of morphology and structure, AgZrPSi and AgZrP had smaller sized particles (1.5-3.0 μm) and more uniformly shaped than AgZ. **Conclusions:** Silver inorganic materials in the form of AgZ, AgZrPSi and AgZrP had antimicrobial effects against all test oral microorganisms and those activities may be influenced by the crystal structure of carriers. These results suggest that these silver materials may be useful metals applied to oral hygiene products to provide antimicrobial activity against oral infection.

KEYWORDS

Silver zeolite, Silver zirconium phosphate silicate, Silver zirconium phosphate, Oral microorganisms

1. Introduction

The bioactivity of noble metals and their uses are research areas of growing interest due to their high reactivity, high compatibility and non-toxicity on eukaryotic cells. Among various kinds of noble metals, silver seems popular to use in biomedical field due to its attractive physiochemical properties and lower cost price.

Silver has long been extensively used in medical fields to prevent and treat a variety of diseases, most notably infections. It has been described as being "oligodynamic" as a result of its ability to exhibit bactericidal activity at minute concentrations[1,2]. Consequently, a large number of oral healthcare products nowadays contain silver, principally due to its broad-spectrum antimicrobial effects and low toxicity to humans.

Several kinds of inorganic materials containing silver including antimicrobial agents using different

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inorganic carriers such as zeolite, phosphate, titanium dioxide, activated carbon, montmorillonite, water-soluble glass and mesoporous silica, were developed to extend lifetime of silver . The prolonged constant dissociation of silver ions into the surrounding environment provides a higher clinical efficacy of these silver containing materials. In addition, inorganic materials containing silver do not disturb the color of the products[3,4].

The antimicrobial activities of silver depend on the silver cation Ag^+, which binds strongly to electron donor groups in biological molecules containing sulphur, oxygen or nitrogen. These silver-based materials have to release Ag^+ to a pathogenic environment to be effective.

In dentistry, dental caries and candidiasis are common infections found in the oral cavity and create health problems for people in many countries. These conditions are caused by bacteria and yeast residing in the oral cavity. Dental caries is the destruction of hard tissue of teeth due to acids produced by bacteria. *Streptococcus mutans* (*S. mutans*)and *Lactobacillus* spp. have been shown to have cariogenic potential owing to their acidogenic and aciduric abilities[5]. *S. mutans* appears to be important in the initiation of dental caries since its activities lead to colonization of the tooth surface, dental plaque formation and demineralization. *Lactobacilli*, mainly found in carious lesions, have been suspected to be secondary invaders that contribute to the progression of lesions. Apart from dental caries, oral candidiasis is a common opportunistic infection of the oral cavity caused by *Candida* spp. In the debilitated, compromised host, oral candidal infection may spread to the gastrointestinal tract, trachea, lungs, liver and central nervous system, capable of causing septicemia, meningitis and endocarditis[6-8]. The most frequent *Candida* spp. isolated from oral cavity is *Candida. albicans* (*C. albicans*) with the ability to adhere and colonize oral surfaces in large numbers[9]. In addition, yeast cells can co-aggregate and make synergistic relationships with pathogenic bacteria such as *Staphylococcus aureus* (*S. aureus*) leading to a mixed infection. These include angular cheilitis, osteomyelitis of the jaw, parotitis and some endodontic infections[10].

To our knowledge, no information exists regarding the comparison of antimicrobial activities of these different kinds of silver materials on the oral microorganism. The objective of this study was to evaluate the antimicrobial activity of silver inorganic materials, including silver zeolite (AgZ), silver zirconium phosphate silicate (AgZrPSi) and silver zirconium phosphate (AgZrP), against oral microorganisms. The morphology and structure of each type of silver-based powder were also investigated.

2. Materials and methods

2.1. Silver based materials

AgZ, AgZrP and AgZrPSi used in the present study contained 3 000 mg/kg silver ions. All test materials were supplied by the National Direct Network Company, Thailand and made into suspension in sterile distilled water at concentrations ranging from 1.0-20.0 g/L before testing.

2.2. Materials characterization

The original silver-based materials in powder form were used in the investigation. The morphology and crystal structure of the silver-based materials were characterized by scanning electron microscopy (SEM, 15 kV, S-2500 Hitachi, Japan) and X-ray diffraction (XRD) technique (30 kV, 30 mA, Cu Kα, λ=0.15418 nm, Bruker D8 with Ni Kβ filtered), respectively.

2.3. Microorganisms

Oral microorganisms, *S. mutans* KPSK2, *L. casei* ATCC6363, *C. albicans* ATCC13803 and *S. aureus* ATCC 5638, were obtained from the culture collection of the Department of Oral Microbiology, Faculty of Dentistry, Mahidol University, Thailand, They were maintained on brain heart infusion agar BBL, USA). Overnight cultures were prepared by inoculating approximately 2 mL Mueller Hinton broth (Difco, USA) with 2-3 colonies of each organism taken from BHI agar. Broth was incubated overnight at 37 °C for 48 h. Inocula were prepared by diluting cultures in saline solution to approximately 10^8 CFU/mL for bacteria and 10^7 CFU/mL for yeast. These suspensions were further used for antimicrobial tests.

2.4. Antimicrobial activity

2.4.1 Agar diffusion

Agar cup diffusion method was employed to obtain the susceptibility pattern of microorganisms against each silver-based material. Briefly, 20 mL of Mueller Hinton agar was poured into a 80 mm-petri dish. The medium was allowed to cool and plates were then inoculated with 10^8 CFU/mL of bacteria or 10^7 CFU/mL of yeast. By punching the agar container with a sterile cork borer and scooping out the punched part, agar cups of 5 mm diameter were made.

Each of the silver material suspensions (100 μL) at concentration of 10.0, 100.0 and 200.0 g/L was placed in each cup. Suspensions of carriers (zeolite, ZrP and ZrPSi) were used as controls. The plates were left to

stay for an hour to facilitate the diffusion of the drug solution. Then the plates were incubated at 37 °C for 24 h. The antimicrobial activity was evaluated based on zones of growth inhibition (mm).

2.4.2. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC)

The MIC and MLC were determined using the membrane method described by Tantaoui-Elaraki *et al*[11]. Briefly, serial dilutions of silver material and carrier suspensions, ranging from 1.0 to 200.0 g/L were prepared in Mueller Hinton agar. Cellulose acetate membrane filters (0.45 μm porosity) (Sartorius, Germany) were placed on the surface of the agar plate and 25 μL of each microbial suspension (10^8 CFU/mL for bacteria and 10^7 CFU/mL for yeast) was dropped onto each filter. The plates were incubated at 37 °C for 24 to 48 h. The MIC values were determined as the lowest concentration of suspension inhibiting the visible growth of each organism on the membranes.

For the determination of MLC, cellulose acetate filters without any microbial growth were transferred into brain heart infusion (BHI) broth and incubated at 37 °C for 72 h. The lowest concentration of silver materials or carriers showing no visible growth of the organisms in the tube was considered as the MLC. All the tests were performed in triplicate on three separate occasions.

3. Results

Table 1

Inhibition zone of silver inorganic materials against test microorganisms.

Concentration (g/L)	Materials	Zone of inhibition (mm)			
		S. mutans	*L. casei*	*C. albicans*	*S. aureus*
1.0	AgZ	–	–	–	–
	Ag ZrPSi	–	–	–	–
	AgZrP	–	–	–	–
	Zeolite	–	–	–	–
	ZrPSi	–	–	–	–
10.0	AgZ	10	7	7	11
	Ag ZrPSi	17	9	9	8
	AgZrP	21	10	8	14
	Zeolite	–	–	–	–
	ZrPSi	–	–	–	–
100.0	AgZ	15	17	17	13
	Ag ZrPSi	11	15	14	11
	AgZrP	9	15	12	12
	Zeolite	–	–	–	–
	ZrPSi	–	–	–	–
200.0	AgZ	14	16	18	14
	Ag ZrPSi	11	15	15	12
	AgZrP	10	17	14	13
	Zeolite	–	–	–	–
	ZrPSi	–	–	–	–

Using the agar cup diffusion method as a screening test, all of silver materials showed antimicrobial effects against all of test organisms with a zone of inhibition ranging from 7 to 21 mm (Table 1). No growth inhibition was observed when exposed to the carriers without silver (zeolite, zirconium phosphate silicate and zirconium phosphate). The MICs and MLCs of silver materials are shown in Table 2. All the test microorganisms were inhibited and killed at >10.0 g/L of silver materials except for that of AgZ against *S. aureus*, which exhibited the MLC value of 60.0 g/L.

Table 2

MIC and MLC of silver inorganic materials against test ed microorganisms.

Materials	Concentration (g/L)							
	S. mutans		*L. casei*		*C. albicans*		*S. aureus*	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
AgZ	10.0	10.0	10.0	10.0	10.0	10.0	10.0	60.0
Ag ZrPSi	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
AgZrP	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

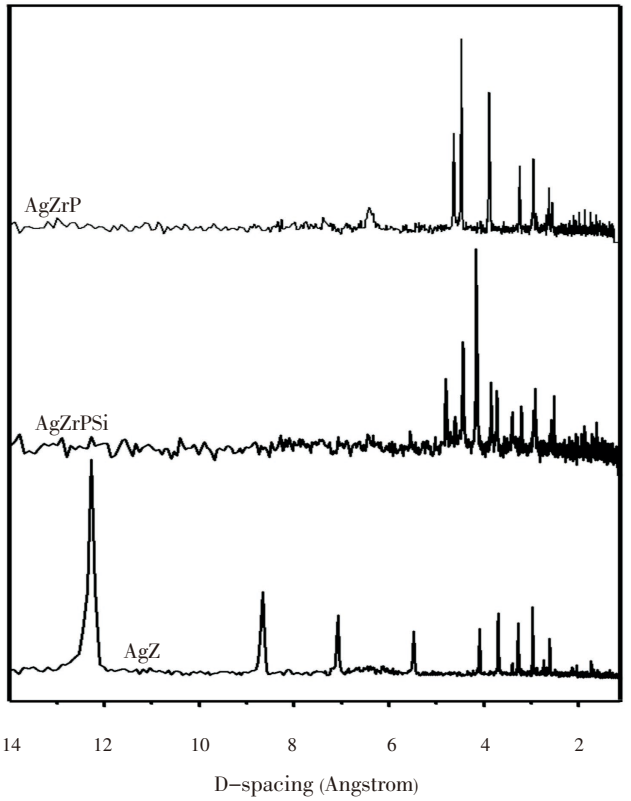

Figure 1. X-ray diffraction spectra of silver inorganic materials. AgZ shows 9 diffraction peaks consisting of D-spacing=12.278, 8.672, 7.081, 5.488, 4.095, 3.708, 3.284, 2.983 and 2.622 Å. AgZrPSi presents 7 diffraction peaks of crystal structure (D-spacing=6.326, 4.564, 4.396, 3.802, 3.165, 2.876 and 2.543 Å); the other 2 peaks (D-spacing=3.650 and 3.341 Å) are impure. For AgZrP, 7 diffraction peaks are observed including d-spacing=6.326, 4.552, 4.396, 3.810, 3.165, 2.876 and 2.539 Å.

The crystal structure of tested materials investigated Agz and Agzrpsi particles have bigger sizes compare with Agzrp particles by X-diffractometer is shown in Figure 1. The XRD patterns of AgZ showed 9 diffraction peaks which were consistent with the determined patterns of sodium aluminum silicate hydrate with cubic system. AgZrPSi presented 9 diffraction peaks; however only 7 peaks were confirmed as having the sodium zirconium phosphate silicate pattern of a rhombohedral system. The other 2 diffraction peaks were identified as impure which may be contaminated during the synthesis process. For AgZrP, 7 diffraction peaks were observed confirming the structure of rhombohedral system. From the XRD data, it indicated that the structures of these silver inorganic materials and their carriers were almost identical. Small amount of silver ions adsorbed in the carriers did not change their crystal structures and no silver peak was found in the XRD patterns.

4. Discussion

The study demonstrated that silver inorganic materials including AgZ, AgZrPSi and AgZrP have antimicrobial effects on *S. mutans*, *L. casei*, *C. albican* and *S. aureus*. The MIC of AgZ, AgZrPSi and AgZrP was 10.0 g/L whereas MLC ranged between 10.0-60.0 g/L.

Several authors have reported that silver has a direct or indirect effect on microbial cells[12-15]. The antimicrobial activity exhibited by silver has resulted in the widespread use of silver particles in bedding, water purification, toothpastes, shampoos, fabrics, deodorants, filters, kitchen utensils and toys. Using silver in the form of small-sized particles shows efficient antimicrobial properties due to their extremely large surface area, producing an effective contact with microorganisms[15].

Even though the precise mechanisms of biocidal activity of silver against microorganisms is not fully understood, the proposed antimicrobial mechanisms are first that, silver ions can associate with the cell wall[16], cell membrane[17], and cell envelope of microorganisms[18,19]. The positive charge of a silver ion is critical for antimicrobial activity, allowing electrostatic attraction between the negative charges of the bacterial cell membrane and positively charged silver particles causing cell membrane rupturing[17,20-22]. Low concentrations of silver ions were demonstrated to have the ability to induce a massive proton leakage through the bacterial membrane causing subsequent cell death[23,24].

Second, silver ions can react with nucleophilic amino acid residues in proteins, attached to sulfhydryl, amino, imidazole, phosphate and carbonyl groups of membrane or enzyme proteins. A number of oxidative enzymes have been reported to be inhibited by silver ions such as yeast attached dehydrogenase, enzymes associated with the uptake of succinate by membrane vesicles and respiratory chains of bacteria. These resulted in the metabolites efflux, interfering with DNA replication, inactivation of ATP production and inhibition of growth[16,22,25].

Third, the antimicrobial action of silver particles is suggested to be related to the formation of free radicals and subsequent free radical-induced membrane damage[22]. As a result of the catalytic action of silver ions, oxygen is changed into oxygen radicals by the action of light energy and/or H_2O in the air or water only at polar surfaces and these oxygen radicals cause structural changes in microorganisms.

In dentistry, with the aim to reduce bacterial and fungal adhesion to oral materials and devices, silver particles have been developed and investigated for a range of possible applications, for example, incorporation into denture materials[26], dental implants[27], filling materials[28] and orthodontic adhesives[29]. However, little information is available regarding the antimicrobial effects of silver particles on oral species. Kawahara *et al*. evaluated the effect of AgZ on a range of obligate and facultative anaerobic oral microorganisms[30]. It was found that gram negative species (*Porphyromonas gingivalis, Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans*) were more susceptible than gram positive species (*S. mutans, Streptococcus sanguinis, S. aureus* and *Actinomyces viscosus*). It is speculated that the reduced amount of negatively charged peptidoglycans in the cell walls of gram negative species may account for the differences in susceptibility. In contrast, silver, in the form of titanium silver alloy, is often used as material for dental implants, orthodontic brackets, wires and screws, showing no inhibitory effect against *Lactobacillus acidophilus*[31]. With regard to oral yeast, silver particles have demonstrated a remarkable antifungal activity toward *C. albicans* by attacking the plasma membrane resulting in the formation of pores, disrupting the membrane potential, inhibiting the budding process, and causing subsequent cell death[32].

Considering the antimicrobial activity and structure of the test materials, AgZrPSi and AgZrP showed higher antimicrobial ability than AgZ. It seems that crystal structure, the rhombohedral structure, not particle size, influenced the antimicrobial activity.

In conclusion, silver inorganic materials in the form of AgZ, AgZrPSi and AgZrP had antimicrobial effects against all test oral microorganisms. The MIC of AgZ, AgZrPSi and AgZrP was 10.0 g/L whereas MLC

ranged between 10.0-60.0 g/L. It is possible that the activities were influenced by the crystal structures and sizes of carriers. These silver particles may be useful materials applied to oral hygiene products to provide antimicrobial activities against oral infection. However, further studies are needed to evaluate the proper concentrations for final products and clinical studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Dental caries and oral candidiasis are the most commonly seen opportunistic infections in the oral cavity. Recently there has been a surge in interest on the use of silver nano-particles (AgNPs) for its potential antimicrobial properties. It is based on incorporating AgNPs within minute voids found in crystalline structures of zeolites and inorganic carriers such as zirconium phosphate silicate and zirconium phosphate. Overtime these silver particles can get released or get dissociated into silver ions and exchanged with other cations in the environment. It has been postulated that upon release AgNPs and silver ions can come in contact with micro-organisms in the environment and cause suppression of their development. Various mechanisms have been purposed for antimicrobial action of AgNPs such as reduction in the production of vital microbial enzymes, interruption of RNA replication and alterations in cell wall permeability. This study evaluates the antimicrobial action of AgNPs on pathogenic oral micro-organisms primarily responsible for dental caries and oral candidiasis.

Research frontiers

Studies are being formed for the application of AgNPs in the field of biotechnology and bioengineering. The benefits are most sought in incorporating its antimicrobial properties in medical equipments such as surgical instruments, catheters, and wound dressings to reduce the risk of infection. Research is also being conducted to incorporate AgNPs in dental materials such as cements, liners and polymethyl methacrylate for a sustained antimicrobial action.

Related reports

This study concluded that the AgNPs had antimicrobial action against *S. mutans, Lactobacillus casei, S. aureus* and *C. albicans* at concentration of MLC concentration of 10.0-60.0 g/L. However the study could not explain the exact mechanism of action of AgNPs. Study by Xiu *et al.* (2012) have shown silver ions released from AgNPs had antimicrobial properties against *Escherichia coli* at concentrations of 120.0-310.0 g/L.

Innovations and breakthroughs

Data on the antimicrobial properties of AgNPs is limited. This study evaluates the minimum inhibitory concentration and minimum lethal concentration of silver zeolite, silver zirconium phosphate silicate and silver zirconium phosphate against oral micro-organisms. Furthermore this study also uses scanning electron microscope and X-ray diffusion to evaluate the structures of the silver particles.

Applications

It is beneficial to know the potential application of AgNPs in dentistry. The result of the study has shown that AgNPs are effective against opportunistic oral microorganisms even at low concentrations. However future studies on the sustainability of ion release from these nano-particles, changes in the physical properties of the incorporated material and the toxicity related to the oral tissues are necessary to justify its clinical use.

Peer review

This is a good preliminary study that investigates the potential antimicrobial action of AgNPs: silver zeolite, silver zirconium phosphate silicate and silver zirconium phosphate on oral microorganisms. The results showed that AgNPs were effective even at low concentrations and suggests the implication of AgNPs in dental materials to reduce the risk of opportunistic infections such as dental caries and candidiasis.

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