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ORIGINAL ARTICLE

The study of anticariogenic effect of Silver nanoparticles for dental applications

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Abstract

Dental caries is one of the most prevalent chronic diseases of people worldwide. In the development of caries a major role play bacteria of the *Streptococcus* and *Lactobacillus* genera which are acidogenic and aciduric. In this work, the antibacterial effect of silver nanoparticles was investigated on *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus casei* and *Lactobacillus acidophilus* for inhibition and inactivation of cell growth. We successfully synthesized uniformly dispersed silver nanoparticles with a uniform size and shape using *salmonella typhirium*. The formation of silver nanoparticles was confirmed by UV-Visible spectrophotometer, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Silver nanoparticles synthesized were in size ranging 4–11 nm with an average size of 7.5 nm. Silver nanoparticles exhibited high antibacterial activity against *S. mutans, S. sobrinus, L casei* and *L. acidophilus* and guided towards the fact that silver nanoparticles have emerged as a promising anticariogenic agent to treat dental diseases.

Keywords: *Anticariogenic; Dental; Dynamic Light Scattering (DLS); Silver nanoparticles; Synthesis.*

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INTRODUCTION

Dental caries is a multifactorial, chronic bacterial disease that causes demineralization and destruction of the hard tissues, usually by production of acid by bacterial fermentation of the food debris accumulated on the tooth surface. Today, caries remain one of the most common diseases of people worldwide. Individuals are susceptible to this disease throughout their lifetime. Worldwide, approximately 36% of the population have dental caries in their permanent teeth. In baby teeth, it affects about 9% of the population [1]. Risk of caries includes physical, biological, environmental, behavioural and lifestyle-related factors [2]. About 700 different bacteria species have been identified from the human oral microbiome. In the development of caries a major role play bacteria of the *Streptococcus* and *Lactobacillus* genera which are acidogenic and aciduric [3].

Nanotechnology is an interesting field now days, involving in synthesis, design, characterization, production and application of structures, by controlling shape and size at the nanometer scale. Various methods for the preparation of nanoparticles are employed such as plasma synthesis, chemical vapour deposition, micro emulsion processing, combustion synthesis, sol-gel processing, hydrothermal techniques etc. Recent efforts for the preparation of nanoparticles are focused to control size, morphology and surface reactivity of nanoparticles [4]. Among the synthesized nanoparticles; silver nanoparticles can be used in applications that include spectrally selective coating for solar energy absorption and intercalation material for electrical batteries, as optical receptors, catalysts in chemical reactions, biomedical and antibacterial agents owing to their unique properties. Nanoparticles due to their smaller size offer advantage by improving biocompatibility. It seems that bacteria are less likely to develop resistance against nanoparticles

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because nanoparticles have broad spectrum antibacterial activity and for bacteria to get resistant many mutations must occur. Studies on action of silver nanoparticles have shown that positive charge on metal ion is critical for its antimicrobial activity as it allows electrostatic interaction between negatively charged bacterial cell membranes and positively charged nanoparticles [5]. It has also been proposed that there can be release of silver ions by the nanoparticles, and these ions can interact with the thiol groups of many vital enzymes and inactivate them [6]. Silver ions contact with bacterial cell which inhibit the different functions in the cell and damages the cells. During this process generation of reactive oxygen species, this is responsible for produced possibly through the inhibition of a respiratory enzyme by silver ions and damages to cells. This cell presents the nanoparticles cause the reaction take place and finally lead to cell death. One another reason that the DNA has phosphorus and sulfur is main components; the nanoparticles react with soft bases and destroy the DNA and finally lead to cell death [7].

We describe an easy route for silver nanoparticles synthesis using salmonella typhirium. Then the nanoparticles were characterized by various techniques and studied antibacterial activity against *S. mutans, S. sobrinus, L casei* and *L. acidophilus* as dental caries bacteria.

EXPERIMENTAL

Biosynthesis of silver nanoparticles

Silver nanoparticles solution was prepared using the following previously described technique [8]. Trypti casein Soy Broth (TSB) was prepared, sterilized and inoculated with a fresh growth of Salmonella typhirium. The culture was centrifuged at 5,000 rpm for 15 min, and the supernatant was used for the synthesis of silver nanoparticles. 1 ml supernatant was added separately to the reaction vessel containing 100 ml aqueous silver nitrate solution (0.001 M). After 5 min, the colourless solution of silver nitrate in the vessel turns into brown colour. The obtained suspension was characterized by UV–Vis spectra and transmission electron microscopy (TEM). Distribution of the particles of various sizes was determined using dynamic light scattering (DLS).

Antibacterial Activity

A group of bacteria known to cause tooth decay were selected and freeze dried. These bacteria were *Streptococcus mutans, Streptococcus sobrinus, Lactobacillus casei* and *Lactobacillus acidophilus*. The antimicrobial activity was studied by agar well diffusion method. A fresh microbial culture was spreader on agar plates with glass spreader. A well was punched off in pertiplates and then silver nanoparticle solution and control sample (supernatant) were loaded. Plates were incubated at 37 ˚C for 24 hours. The zone of inhibition was measured as a property of antimicrobial activity.

RESULTS AND DISCUSSIONS

Characterization of silver nanoparticles

One of the useful techniques for structural characterization and stability of the silver nanoparticles in the aqueous solution is UV-Vis spectroscopy [9, 10]. The samples were scanned at the wavelength between 350-600 nm. A single peak was observed at 421 nm which was due to surface plasmon resonance of free electrons present in the silver nanoparticles (Fig. 1). By UV-visible spectroscopy we got the λ_{max} at 421 nm which is strong evidence for the formation of silver nanoparticles. Particle size can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution. This technique is commonly known as dynamic light scattering (DLS). Dynamic light scattering is a used method for the determination of nanoparticle size. The size distribution of silver nanoparticles shows that the nanoparticles size is 4-11 nm, with a mean size of 7.5 nm (Fig. 2). The shape, size and morphology of the synthesized silver nanoparticles were elucidated by transmission electron microscopy (TEM). As shown in Fig. 3, some silver nanoparticles were obviously spherical in shape and well dispersed, with a mean size of 7.5 nm.

Anticariogenic Activity

Dental caries is one of the globally affecting diseases of oral cavity. The species that are most prevalent includes *S. mutans, S. sobrinus, L. casei* and *L. acidophilus*. The anticariogenic activity of silver nanoparticles was investigated against these bacteria. The zone of inhibition of silver nanoparticles against these bacteria was measured. The results are tabulated in Table 1. *S. mutans* was shown to have highest zone of inhibition in compare with *S. sobrinus, L.*

acidophilus and *L. casei* by silver nanoparticles. The reports on the inhibitory action of silver ions on microorganisms showed that upon silver ion treatment, DNA loses its replication ability and expression of ribosomal subunits proteins as well as some other cellular proteins, and enzymes essential to ATP production becomes inactivated [11, 12].

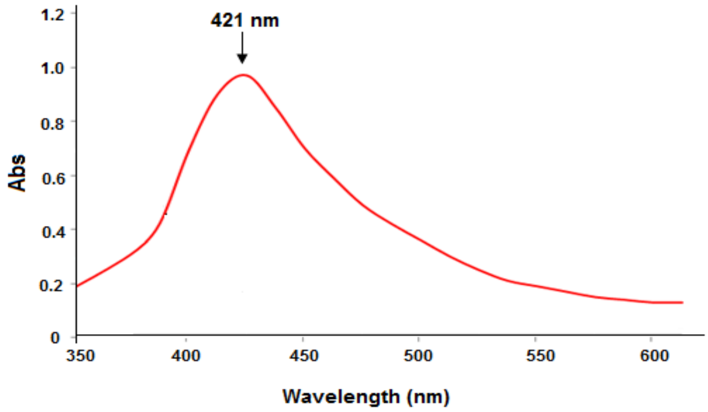

Fig. 1: UV–Visible spectrum of silver nanoparticles.

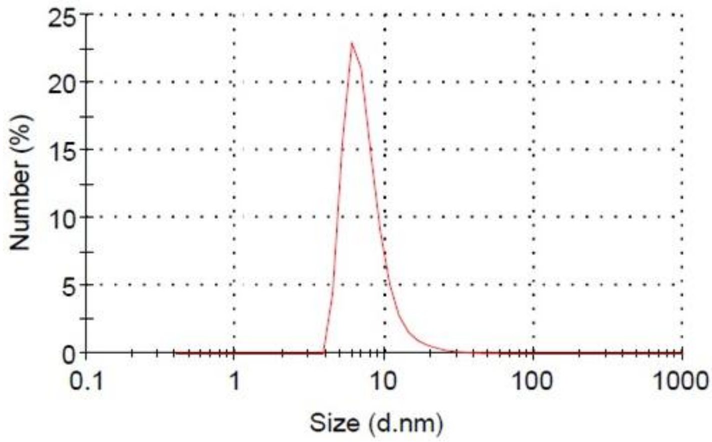

Fig. 2: The curve of size distribution silver nanoparticles by number.

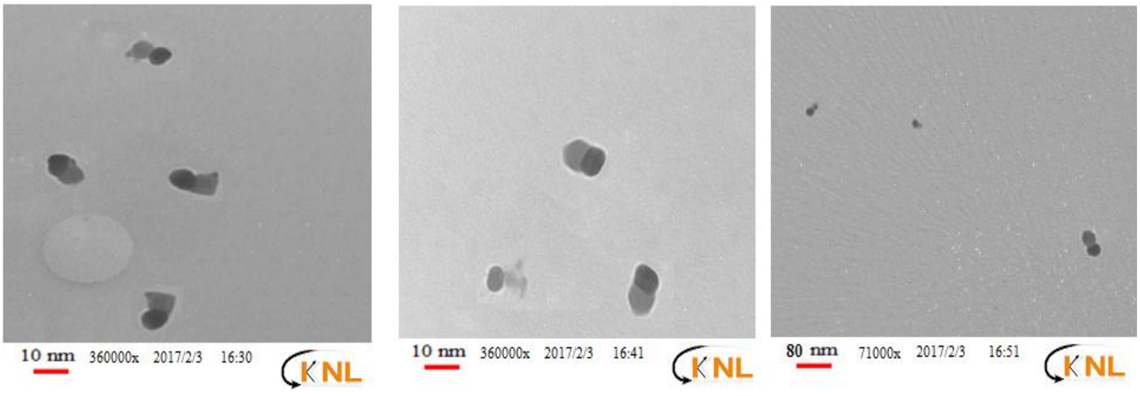

Fig. 3: TEM micrographs of silver nanoparticles.

Table 1: Anticariogenic activity of silver nanoparticles, Zone of Inhibition (mm).

Bacteria	*S. mutans*	*S. sobrinus*	*L. casei*	*L. acidophilus*
Silver nanoparticles	16	11	13	12
Control sample	0	0	0	0

CONCLUSIONS

Silver nanoparticles were successfully synthesized using *salmonella typhirium*. Synthesis of silver nanoparticles through this process was economical and rapid. UV–Vis spectroscopy confirmed silver nanoparticles formation. In addition, transmission electron microscopy (TEM) showed that silver nanoparticles are spherical in shape and well dispersed. Also, the average diameter of the silver nanoparticles was about 7.5 nm by DLS analysis. Silver nanoparticles exhibited antibacterial activity against *S. mutans, S. sobrinus, L. casei* and *L. acidophilus* as major bacteria of dental caries. Hence, silver nanoparticles have appeard as a favourable anticariogenic agent to treat dental diseases.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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