Biosynthesised Silver Nanoparticles from Fungi as Antimicrobial Agents for Endo-Perio Lesions – A Review

Kiran R. Halkai1*, Jayashree A. Mudda2, Vasundhara Shivanna3, Vandana Rathod4 and Rahul Halkai5

1Department of Conservative Dentistry and Endodontics, HKES’S SN Institute of Dental Sciences and Research, Kalaburgi, Karnataka, India.
2Department of Periodontics, HKES’S SN Institute of Dental Sciences and Research, Kalaburgi, Karnataka, India.
3Department of Conservative Dentistry and Endodontics, College of Dental Sciences, Davangere, Karnataka, India.
4Department of Microbiology, Gulbarga University, Kalaburgi, Karnataka, India.
5Department of Conservative Dentistry and Endodontics, Al-Badar Rural Dental College and Hospital, Kalaburgi, Karnataka, India.

Authors’ contributions

This work was carried out in collaboration between all authors. Authors KRH and RH designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JAM, VS, VR and RH managed the analyses of the study. Author RH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Even after rapid development in the treatment modalities and contemporary dental practice, still we encounter the failures due to endodontic, periodontal or combined lesions. Complete elimination of bacteria is the key for successful treatment. The emerging new microorganisms, increase in the...
number of resistant microbial strains and complexity of the tooth system demands the development of new treatment strategies to control the disease process and to achieve high success rate. Silver nanoparticles (AgNps) are considered to be the best antimicrobial agents because of their unique mode of action, broad spectrum of activity and biocompatibility. Concern has been raised on the toxicity of chemical agents used in AgNps synthesis. Compared with the synthetic methods, biological methods provide a novel idea for the production of nano-materials. Therefore, nanotechnology rather biosynthesized nanoparticles is the field of interest for research. This review article highlights the application of biosynthesised AgNps produced from fungi as antimicrobial agents for endodontic, periodontal or combined lesions.

Keywords: Biosynthesis; silver nanoparticles (AgNps); fungi; antimicrobial agents; endo-perio lesions.

1. INTRODUCTION

The interrelationship of pulp-periodontal tissues is unique and can be considered as a single biological system with several paths of communication for exchange of infectious elements and irritants from the pulp to periodontium or vice versa [1]. Both tissues have embryonic, anatomic and functional interrelationship; influencing each other during health, disease and function. They get affected individually or combined; when both tissues are involved they are called as endo-perio lesions consisting of the inflammatory products in varying degrees in both the tissues [2,3]. Today, more than 50% of tooth mortality is due to these lesions [4].

The main etiological factors for endodontic, periodontal or combined lesions are the microorganisms [5]. The microorganisms of endodontic and periodontal diseases are similar to some aspect. Proteolytic bacteria predominate in the root canal flora which changes over time to anaerobic microbiota. According to Kerekes and Olsen [6] similarities exist between the microflora of both the tissues of an intact tooth such as Fusobacterium, Eubacterium, spirochetes, Selenomonas, Peptostreptococcus and Wolinella. Zehnder [5] confirmed the microflora within root canals and the periodontal pocket such as P. gingivalis, P. intermedia, P. nigrescens, A. actinomycetemcomitans, Cochracea, A. naeslundii and S. sanguis. However, the periodontal pocket presents a greater variety of microorganisms. E. faecalis is the most frequently detected species in root-filled teeth with persistent apical or lateral periodontitis [7].

The main objective of the treatment is to control the bacterial infection. Periodontal and endodontic therapies can be grouped into three broad categories: mechanical debridement and cleaning to eliminate bacteria; treatment aimed at killing the bacteria by antiseptics and antimicrobial agents and treatment affecting the environment of the infectious organism [8]. However, complete eradication of the bacteria is not possible because of the complex anatomy of the tooth system and increase in the number of resistant strains resulting in persistent or refractory infections [9].

Long-term antibiotic therapy often results in occurrence of antibiotic resistance. Recent microbiological studies have focused on developing novel antimicrobial agents with higher efficacy while being non-toxic, non-invasive and without drug resistance [10,11]. Use of nanoparticles (NPs) with antimicrobial properties is an efficient method to overcome these limitations [12].

Nano technology broadly refers to a field of applied science and technology whose unifying theme is to control the matter on atomic and molecular scale [13]. NPs are usually clusters of atoms in the size range of 1–100 nm. Various NPs have gained popularity as antimicrobial agents owing to their unique mode of action. [14,15]. Among the nobel metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine [16]. AgNps are considered to be the best antimicrobial agents because of their broad spectrum of activity and biocompatibility [17]. Therefore, offers an attractive alternative to conventional antimicrobial agents [12,18].

AgNps can be synthesized by different approaches; chemical methods like reduction in solutions, sol-gel method, thermal decomposition, radiation, microwave assisted, pyrolysis, chemical vapour deposition and bio based protocols etc. [19]. Concern has been raised on the toxicity of chemical agents used in AgNps synthesis. Thus, it is essential to develop
bio-mimetic approaches without using hazardous substances to the human health and environment. Compared with the traditional synthetic methods, biological methods provide a novel idea for the production of nano-materials [18]. Therefore, biosynthesis of NPs is the field of interest for research today.

Biosynthesis offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications over chemical and physical methods. It is cost effective, environment friendly, can be scaled up for large-scale synthesis and no need of using high-pressure, energy, temperature and toxic chemicals [20].

Current research has shown the microorganisms such as bacteria and fungi play an important role in the remediation of toxic metals through the reduction of the metal ions. The ability of such microorganisms to control the synthesis of metallic NPs should be employed in the search for new materials [21]. Holmes et al. [22] reported the bacteria *Klebsiella aerogenes* when exposed to cadmium ions resulted in intracellular formation of CdS particles in the range of 20–200 nm. According to Klaus et al. [23] *Pseudomonas stutzeri* AG259, isolated from silver mine when placed in a concentrated solution of silver nitrate produced AgNps. Sastry et al. [24] reported the fungi *Verticilium sp.* and *Fusarium oxysporum* when exposed to gold and silver ions, reduced the metal ion rapidly and formed respective metallic NPs. A novel biological method using *Verticilium* *sp.* for AgNps synthesis was proposed by Mukherjee et al. [25] using two-step mechanism. The first step involves trapping of the Ag+ ions at the surface of the fungal cells. In the second step, the enzymes present in the cell reduce silver ions. The fungi and bacteria produce the inorganic materials either intra- or extracellularly through biological pathways and thus potentially utilized as eco-friendly nanofactories [26].

The filamentous fungi posses certain advantages over the bacteria for AgNps synthesis; as most of the fungi are easy to handle, require simple nutrients, possess high wall-binding capacity, intracellular metal uptake capabilities, secrete higher amounts of bioactive substances which makes suitable for large-scale production. Extracellular biosynthesis using fungi also makes downstream processing much easier [25]. Previous reports have shown that the large number of active substances secreted by fungi plays an important role in biosynthesis of AgNps. Enzymes such as nitrate reductase secreted by fungi act as reducing agents and proteins as stabilizing and capping agents for AgNps. Thus, fungi act as naturally occurring silver nano-factories opening the exciting possibilities for biosynthesis of AgNps without using the chemicals [15,26].

Production of the AgNps using fungi includes the isolation of the fungi from different sources such as leaves, soil samples etc. Culture techniques and media vary depending upon the requirements of the fungal isolate involved. However, the general procedure consists of the following: Isolated fungal hyphae are placed in liquid growth media and in shake culture until increase in the fungal biomass. The biomass obtained is then filtered, washed with distilled water to remove the growth media, placed in distilled water and incubated on shake culture for 24 to 48 hours. An aliquot of fungal filtrate is added to 1.0 mM ion solution. The ion solution is then monitored for 2 to 3 days for the formation of NPs. Another common culture technique is to add washed fungal hyphae directly into 1.0 mM ion solution instead of utilizing the fungal filtrate. The biological reaction takes place between the fungal filtrate and the silver ion solution. Silver nitrate is the most widely used source of silver ions, but silver sulphate can also be used. [14,15,27]. The silver ions were reduced in the presence of enzyme nitrate reductase secreted by the fungi leading to the formation of AgNps stabilized by the capping peptide [28]. The reaction can be visually observed by color change. For externally synthesized AgNps, the silver ion solution generally becomes brownish in color [29,30]. This browning reaction may be absent for fungi that synthesize intracellular AgNps, the hyphae darken to a brownish color while the solution remains clear [27]. In both cases the color change occurs due to the excitation of surface plasmon resonance in the silver metal nanoparticle [12,31].

Fundamental of the nanotechnology lies in the fact that properties of materials change dramatically when their size is reduced to the nanometer range (1-100 nm). But measuring this nano dimension is not an easy task. Characterizing these nano sized materials is also an emerging field posing many challenges to scientists and technologists. Thus, nanotechnology has motivated increase in research activities on the discovery and invention of the sophisticated nano characterization
techniques to allow the better control of morphology, size and dimensions of the materials in nano range. The AgNps are characterised by scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive analysis of X-ray (EDX), UV-visible spectroscopy and X-ray diffraction [14,15,32]. Both SEM and TEM can be used to visualize the location, size and morphology of the NPs, while UV-visible spectroscopy confirms the metallic nature, size and aggregation level. Energy dispersive analysis of X-ray determines the elemental composition. X-ray diffraction determines the chemical composition and crystallographic structure. Fourier Transform Infrared Spectroscopy (FTIR) determines the functional groups of proteins and poly peptides.

Guangquan Li et al. reported the biosynthesis of AgNps using the fungi Aspergillus terreus. The AgNps were characterized using ultraviolet-visible spectroscopy, TEM and X-ray diffraction. The synthesized AgNps were polydispersed spherical particles ranging in size from 1 to 20 nm and stabilized in the solution. The formation of AgNps might be an enzyme-mediated extracellular reaction process [32]. Ninganagouda S et al. [14] reported the isolation of the fungi Aspergillus flavus from soil samples, extracellular biosynthesis of AgNps, characterised by UV visible spectroscopy, FTIR and X-ray diffraction and determined antibacterial activity against the gram negative MDR strains and concluded fungi have the ability to reduce metals, displayed considerable antibacterial activity hence, provides a novel method for synthesising the antimicrobial agents. In a study, AgNps were synthesized by both C. tropicun and F. oxysporum. The study concluded the size of AgNps increased as the pH of the ion solution decreased and a lower temperature resulted in smaller nanoparticles while higher temperatures produced larger nanoparticles [33,34].

The mechanism of the antibacterial activity of metal NPs has yet to be fully elucidated. However, evidence shows that the reaction between the positively charged metal particles and negatively charged membrane of the microorganisms plays an important role in this aspect [35]. Metal NPs may be used in oxidized form. In this state, particles have high antimicrobial properties owing to their higher surface area and unconventional shape of crystals causes numerous highly reactive corners [36]. AgNps have higher affinity for Gram-negative [37] and anaerobic bacteria [38] which are related to the lower peptidoglycan content in Gram-negative bacteria. The difference in affinity for aerobic and anaerobic bacteria is attributed to the ability of AgNps for binding to the enzymes containing sulfhydryl (SH) groups. The mechanism for antibacterial activity of AgNps includes the release of Ag ions, disruption of bacterial cell membrane, DNA damage; other factors such as the concentration, [39] size of particles [40] and their shape [41] also affect the efficacy against the microorganisms. Further, decrease in size of NPs increases their antimicrobial efficacy due to increased surface area and biocompatibility [40]. On the other hand, using AgNps in inadequately low concentrations can cause bacterial resistance [39]. The antimicrobial efficacy of metal NPs is variable and silver has higher efficacy than copper, zinc, titanium, magnesium and gold in lower concentrations [42].

Even after rapid development in the treatment modalities and contemporary dental practice still, we encounter the failures due to endodontic, periodontal or combined lesions. The biosynthesis of AgNps has received increasing attention due to the growing need to develop safe, cost-effective and environment friendly technologies. The ability to synthesize AgNps as potential anti-microbial agents using fungi is highly promising for the green, sustainable production and also enhances its widespread application as an important strategy in the treatment of endodontic, periodontal or combined lesions.

Recently in a study, the AgNps were synthesized from the fungi Aspergillus terreus, incorporated in Mineral Trioxide aggregate (MTA) and tested for antimicrobial activity against P. gingivalis. The study concluded NS-IMTA completely inhibits the proliferation of P. gingivalis in a dose-dependent manner which has significant effect in prognosis for root perforation leading to the periodontal diseases [43].

The aim of the root canal treatment is to completely eliminate the bacteria, bacterial products and debris from the root canal system. Bacteria present within the dentinal tubules are inaccessible to the commonly used irrigants. AgNps can penetrate the tissues owing to their extremely small size, high surface area and antibacterial activity. Some NPs have recently been incorporated into the formulation of the root canal cements and irrigants highlighting their antimicrobial properties [44].
Biosynthesized AgNps shows an insight for research in different areas of endodontic and periodontal therapy; can be used as root canal irrigants, intracanal medicaments [ICM], incorporated with other ICMs for synergetic action, root canal sealers etc. [45]. Pagonis et al. [46] studied the effects of PLGA NPs against E. faecalis in both plankotitic and biofilm form and concluded as promising adjunct in antimicrobial endodontic treatment. According to Sapra P et al. [47] nano-particulate system has more advantages compared to other delivery systems in periodontal therapy such as increased stability, controlled release rate, high dispersibility in an aqueous medium. Penetrate deeper regions that may be inaccessible to other delivery system and reduce the frequency of administration and further provide a uniform distribution of the active agents. Therefore, compared to the traditional synthetic methods, biosynthesised AgNps can be used alternative to topical antiseptics and antimicrobial agents and for local drug delivery during periodontal therapy increasing the success rate.

2. CONCLUSION

Even after rapid development in treatment modalities and contemporary dental practice, still we encounter the failures due to endodontic, periodontal or the combined lesions. Therefore, new treatment strategies have to be developed to control the disease progress and to achieve high success rate. Use of synthetic NPs are at large, However literature is scarce regarding the biosynthesised AgNps used as antimicrobial agents. No reports or few reports are available on the application of biosynthesised AgNps as antibacterial agents in the treatment of endo-perio lesions.

The present review article shows an insight for the use of biosynthesised AgNps as antibacterial agents in the treatment of endodontic, periodontal and the combined lesions. Biosynthesised AgNps offers an attractive alternative as antimicrobial agents. However, further in-vivo and in-vitro studies should be conducted for the effective use of these particles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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