Review on the Bio-Synthesis of Silver Nanoparticles and its Use as an Endodontics Disinfectant

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Abstract

Root canal treatment can only be considered successful if endodontic biofilms are completely removed from the canals. Silver nanoparticles, which have significant antibacterial activity, are increasingly being evaluated for use in dental applications, particularly endodontics. This review is a comprehensive contribution to the area of environmentally friendly silver nanoparticle synthesis, antibacterial activity and characterisation, which can be used as an effective endodontic disinfectant. Additionally, this review provides insights into the problems related to current root canal irrigant.

Keywords: *Biosynthesised, silver nanoparticles, endodontics, biofilm, disinfectant*

1.Introduction

Endodontics is a sub specialty of dentistry that focuses on the anatomy and physiology of the dental pulp, also known as the endodontia. Endodontic therapy, also known as root canal therapy, deals with the tooth's smooth pulp tissue. The Greek words for "inside" and "tooth" are "endo" and "odont," respectively(1).

Endodontic therapy usually employs an antimicrobial agent such as sodium hypochlorite (NaOCl) to combat microbial biofilms, despite some adverse reactions. Likewise, chlorhexidine, MTAD (a tetracycline, acid, and detergent mixture), iodine, and chelators are also used. Due to the complex anatomy of the root canal, the bacteria that enter the dentinal tubules cause a significant challenge for root canal disinfection. The proportion of failure treatments has not gone below 18–26% despite efforts over the past few decades to produce brand-new irrigation technologies. There have been numerous attempts to introduce new irrigants. Despite the fact that several decades' worth of work has been put into it, this result has been achieved. Nanoparticles are a good alternative because they can deliver active compounds to the appropriate place and make the chemicals more effective. The ever-increasing focus on how nanotechnology may be utilised in the dental industry has paved for the development of a brand-new discipline known as "nano dentistry"(2).

Over the period, the field of nanotechnology has seen a significant amount of development. As a result of the advancements that have been made in nanotechnology, nanoparticles (NPs) are now regarded as one of the most promising carriers for the delivery of pharmaceuticals. Nanoparticles are an appropriate option for effectively delivering irrigant to the intended site, increasing their therapeutic efficiency(3). When it comes to the series of metals that have been utilised as antibacterial agents from the beginning of time, silver have a prominent position(4). In the treatment of endodontic conditions, intracanal irrigation with silver nanoparticles may be utilized. Despite the fact that cariogenic bacteria play a substantial role in the formation of biofilms, silver nanoparticles used as an antimicrobial agent to kill the bacteria(5).

The synthesis of nanoparticles may often be broken down into three categories: physical, chemical, or biological. Temperature, pressure, and energy levels are going to be kept at the same levels throughout the physical procedure of producing nanoparticles. Laser pyrolysis, atomic or molecular condensation, sol-gel procedures, chemical etching, sputtering, and spray pyrolysis are some of the chemical methods that may be used to produce nanoparticles(6). Nanoparticles frequently undergo shape and size parameter changes as a result of the varying amounts of chemicals and reaction conditions that are applied. When the manufactured nanoparticles are used for a particular purpose, they will be confronted with the difficulties of bioaccumulation, hazardous nature, modelling variables, regeneration, reuse, and recycling(7).Bio-directed silver nanoparticles are a convenient, affordable, easily scaled-up, and ecologically sound alternative to chemically synthesised metal nanoparticles(8).In order to biosynthesize silver nanoparticles, bacteria, fungi, yeast, actinomycetes, and plant extracts are all required(9).In order to synthesis green nanoparticle production to proceed, Ag^{+} ions must be treated with a biological reducing agent(10).In recent years, bacteria such as *Capsicum annum*, *Escherichia coli*, and *Bacillus subtilis* have been utilised in the manufacturing of silver nanoparticles(11).

The biosynthesis of silver nanoparticles (NPs) and their particular applications as root canal irrigants will be covered in this review. In addition, an overview will be given of the use of irrigation in root canal procedures, as well as the silver nanoparticle synthesis from plants and other micro-organism like bacteria and fungi which will be specified based on their characterization of these specimens. In addition, the use of silver nanoparticles as a disinfectant used in endodontics will be discussed.

2.Teeth Anatomy

Enamel, dentin, and cementum are three distinct hard tissues that comprise the teeth. Dentin is the major component of the tooth, whereas enamel and cementum cover the surface of the tooth crown and root, respectively(12).Enamel covers the top 1-2 mm of the tooth crown and has a high mineral content, making it strong. Dentin, which makes up most of the tooth(13).A thick dentin layer forms the majority of dental mineralized tissues. Dentin is topped by a crown of highly mineralized and protective enamel, and it is covered in the root by cementum, a substance involved in tooth attachment to the bone socket. Dental pulps, which are generally non-mineralized, are located in the centre portions of teeth(14). The cross-sectional morphology of root canals in maxillary first premolars are complicated, comprising of complex structures in the form of round, oval, long oval, flat canal, and irregular canal morphologies. The morphology of root canals indicated a vast range of complex variation structures(15). The cell-rich layer forms the central pulp zone, this mass of tissue mass comprises vast arteries and nerves that reach out into the periphery region of root canal and serve as the primary support system for the peripheral region. It is the cell-rich zones, is densely packed with fibroblasts(16)[figure 1].

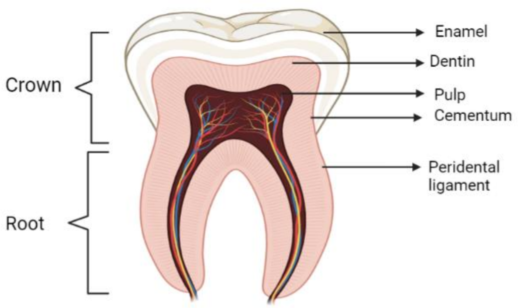


Figure 1: Anatomy of teeth

3.Infection in the root canals

The development of microbial biofilm is commonly observed on the walls of the root canal, however certain species of microbes are capable of entering the dentine tubules to various depths. The infections may last a long time and become complex, or when there is a possibility that they will spread(17).

The bacterial biofilm matrix, which is usually made of polysaccharides and may contain one or more anionic uronic acids, is densely packed around the microcolonies of cells that made the polymers and less densely packed in the large spaces between these microcolonies. This is possible due to the fact that the polysaccharides that comprise the matrix have the ability to cling to the surfaces of the microcolonies(18).

The microcolony is the fundamental building block of the biofilm. When cells inside a microcolony or between microcolonies are close to one another, this creates an environment that is optimal for the formation of nutritional gradients, the exchange of genes, and quorum sensing. It is possible for redox reactions to easily take place in aquatic and soil biofilms due to the fact that microcolonies can be composed of more than one species. This allows for the cycling of numerous nutrients, such as nitrogen, sulphur, and carbon(19).

Oral biofilm formation consists of three steps:

Biofilm formation takes place at the three different temperatures listed below.

Stage 1: The beginning of the conditioning layer is mostly dependent upon the adsorption of organic and inorganic molecules to the durable surface.

Stage 2: Microbial cell adhesion to the prepared layer pH, temperature, substrate floor energy, food availability, bacterial contact duration, bacterial mobile floor cost, and floor hydrophobicity all impact bacterial attachment. The interaction between the microbe and the substrate really occurs in three stages.

- First stage: During the first stage, extracellular polysaccharides, pili, flagella, and fimbriae are responsible for mediating the migration of the microbe to the floor of the substrate (glycocalyx).
- Second stage: Initial, non-specific microbial-substrate adhesion may be affected by electrostatic attraction, covalent and hydrogen bonding, dipole-dipole and hydrophobicity and other processes.
- The third stage is called the adhesion of native bacterial substrates. During this stage of the process, the adhesins or ligands found on the bacterial cell wall engage with the receptors found on the substrate.

Stage 3: Growth and development of oral biofilm

At this step, a monolayer of bacteria colonies forms a microcolony, and the succession of microcolonies shapes the biofilm's ultimate morphology(20),(21),(22),(23),(24).

Polymicrobial infections are common in primary endodontic infections. *Bacteroides, Prophyromonas, Prevotella, Fusobacterium, Treponema, Peptostreptococcos, Eubacterium, and Camphylobacter* are the most common microbes that cause endodontic infection(25) .Stages of biofilm formation is shown in (Figure 2)

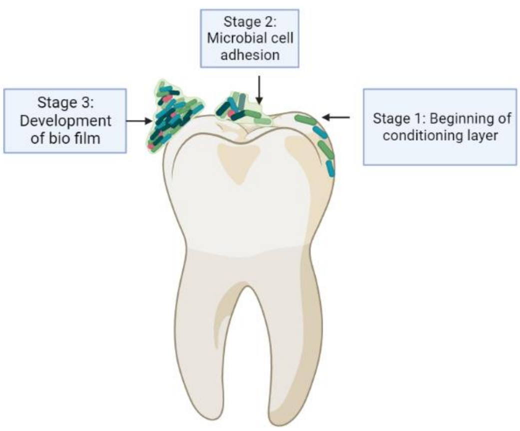


Figure 2: Formation of biofilm

4.Root canal treatment

Endodontic therapy is a treatment sequence for the pulp of a tooth that results in infection removal and protection of the decontaminated tooth from future microbial invasion. This group of operations is also referred to as "root canal therapy" (26). A summary of stages of root canal can be found in *Table 1*.

Table 1: Stages of root canal therapy

Stage 1:	Diagnosing the infected Pulp	In this stage root canal treatment is required usually when the pulp has become infected. Digital imaging and digital x-rays to confirm that root canal treatment is required for the tooth. (94)
Stage2:	Extirpation	In this stage the tooth is numbed and clamped with a rubber dam. Small aperture to clean the root canal using irrigants. (94)
Stage 3:	Instrumentation	In this step the root canal is thoroughly clean to remove any bacterial infection and clamps are placed .(94)
Stage 4:	Obturation: Filling of root canal	In this step Gutta percha is used to seal root canals after therapy.(94)

5.Irrigants in root canal treatment

Irrigation is essential to root canal therapy because it removes small fragments of dentin from the canals. Therefore, they do not become entangled near the root canal. Because dry canals don't have enough lubrication, instruments can't work right. Using lubricants like RC prep, REDTAC, Glyde, etc, makes instrumentation simpler and smoother but lubricant alone is not enough so along with it irrigants are used which cleans the canal thoroughly and also removes the smear layer and kill the microbes which is very important because it cause root canal infection not remove properly. When the canals are wet, instrumentation works better. Irrigants work like a solvent for dead tissue, getting rid of debris, pulp tissue, and germs that are stuck on uneven dentinal walls. They help clear out debris from auxiliary and lateral channels where tools can't reach(27),(15).

5.1. Characteristics of root canal irrigants.

1. It should have broad antimicrobial activity and be effective against both anaerobic and aerobic microorganisms in biofilms.
2. It should dissolve the remains of necrotic pulp tissue.
3. It should Prevent the formation of a smear layer during instrumentation or dissolve it once it has formed.
4. It should not be toxic.
5. It should not burn periodontal tissues.
6. It should not be likely to cause an anaphylactic reaction.

Normal saline, sodium hypochlorite, chlorhexidine, EDTA (ethylenediaminetetraacetic acid), citric acid, and MTAD (a mixture of tetracycline isomer, acid, and detergent) are some of the root canal irrigates that are used now(28),(29).

Table 2: Types of irrigation solutions in the root canal with their advantages and disadvantages

Irrigants	Advantage	Disadvantage
Normal Saline	It cleans and lubricates the root canal.(95)	It has moderate antimicrobial activity. It has to be used in conjunction with chemical irrigants.(95)
Sodium Hypochlorite	It has excellent anti-microbial activity. It is commonly employed as an oxidizing and hydrolyzing agent.(95)	It is toxic. Smear layers are not removed effectively. It cause discoloration.(95)
Chlorhexidine	It has good antibacterial properties.(95)	It doesn't dissolve in tissue. It may cause tooth and oral discoloration, desquamative gingivitis, and a foul taste.(95)
EDTA (Ethylenediaminetetraacetic Acid)	Remove any debris or smear layer from the root canal system.(96)	Has no antibacterial effect but works very effectively with sodium hypochlorite(96)
Citric Acid	Citric Acid worked well as a last irrigation solution for eliminating smear layers.(96)	Removes Enterococcus faecalis biofilms poorly.(96)
MTAD (Mixture of Tetracycline Isomer, Acid, and Detergent)	Have a strong antibacterial effect against biofilm.(96)	It has an effect on the dentine degeneration Effect the sealing properties of root-end filling materials.(96)

5.2. Problems with the application of root canal irrigant

The primary issues associated with the use of irrigant solutions include their inability to penetrate the final third of the apex as well as the anatomical structures that are the most complex (isthmi and anastomoses), the presence of sick organic and inorganic waste, the clinical use period, and the toxicity to the tissues around the periapex(30).

The root canal system is susceptible to bacterial colonisation because of the enormous surface area covered by dentinal tubule apertures. The smear layer is created during the shaping phase of endodontic treatment in the anastomosis, isthmus regions, and over tubule holes made by the blades of endodontic tools(31). Irrigation of the root canal is unlikely to eradicate the bacterial biofilm and inorganic waste that build in these areas. The irrigation fluid must come into contact with both the tissues being treated and the diseases being removed to be effective inside the canal system(32).

6.Nanoparticles

Nanoparticles are small particles that range in size from 1 to 100 nanometres. They can have a variety of important physical and chemical properties(33).According to The European Commission the definition of nanoparticles is the particle size of nanoparticles is said to be in the range of 100 nm or less. The majority of nanoparticles are made up of a few hundred atoms(34).

Nanotechnology's goals are to enable the analysis of nanoscale structures, to comprehend the physical properties of structures at the nanoscale dimension, to manufacture nanoscale structures, to develop nano-precision devices, and to invent methods to connect the nanoscopic and macroscopic universes(35).

The core of nanotechnology is the idea that functional structures may be made by individually manipulating atoms and molecules. Nanoparticles are intriguing and have special features since they are smaller than the critical lengths determining many physical phenomena. A popular translation of nanotechnology is "the science of the tiny." The development of materials, tools, and systems having distinct physical, chemical, and biological characteristics from those of large-scale structures is also a part of nanotechnology, in addition to the production of tiny structures(36).

6.1. Nanoparticles in endodontics

Nanoparticles generated from substances such as, biologically active glass species, zirconia, polymeric chitosan, hydroxyapatite, nano synthesized silver particles and zinc oxide have been shown to improve durability, tissue regeneration, and antibacterial properties(37). Because biofilm is the cause of many root canal treatment failures, nanotechnology can be used to create materials with stronger bactericidal properties. Even in the last two decades, the field of nanotechnology has advanced at a breakneck pace(38).

Because of advancements in nanotechnology, nanoparticles (NPs) are now one of the most promising pharmaceutical delivery systems. As a result, nanotechnology has been used to create environmentally friendly materials as well as to identify and treat a variety of disorders, with truly fascinating results. However, one of the most advantageous applications of NPs is as carriers for the delivery of active ingredients in therapeutic and imaging medicines like the bio-molecules containing protein moieties, peptide molecules and nucleic acid species. The main advantages of nanoparticles are their selectivity, reduced toxicity levels, active/ passive targeting, and biocompatibility parameters of the synthesized molecules. Nanoparticles are made up of a variety of elements, such as lipids, metals, silicon, silica, polymers, proteins, and carbon(39),(40).

Silver nanoparticles may be used in intracanal irrigation in lieu of sodium hypochlorite during root canal treatment. It has been shown that gutta-percha coated with silver nanoparticles works well as an antibacterial obturator for root canal obturation. Silver nanoparticles are added to mineral trioxide aggregate as an antimicrobial agent to increase the strength of pulp capping, apexification, and filling gaps in teeth(41),(42).

6.2. Silver nanoparticles in endodontic

In addition to being an effective irrigant for root canals, silver nanoparticle have been demonstrated to render excellent antibacterial properties against a wide range of bacterial strains(43),(44). Inadvertently conflating the chemical approach with "green synthesis," some publications have employed it. , silver nanoparticle are ecologically favourable due to their usage in electronics, catalysis, medicines, and regulating the growth of microorganisms in biological systems(44). Plant extracts, actinomycetes, yeast, bacteria, and fungi are all used in the biogenic production of , silver nanoparticle. The manufacture of gold and silver nanoparticles has lately utilised a range of plant components, besides enzymes, such as flowers, leaves, and fruits. The technique of production, solvent type, concentration, potency of the reducing agent, temperature, and stability all have an impact on the size, shape, and stability of nanoparticles(45),(46).

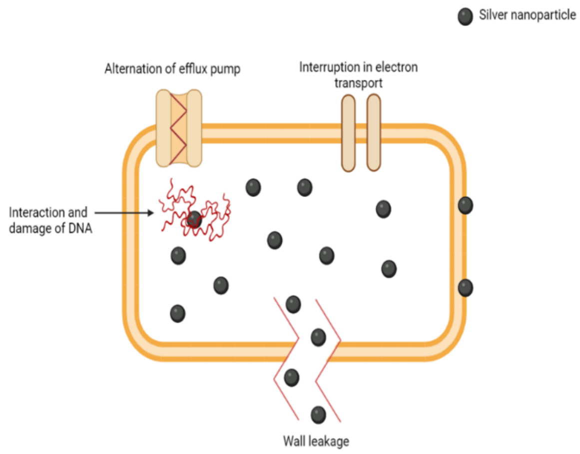
7. Antimicrobial activity of silver nanoparticles

Silver compound itself is used as an antibacterial agent, with the developments of nano technologies. The ability of anti-bacterial growth has been incorporated in the form of silver nitrate and silver sulfadiazine for burns, ulcers dressings and also in food packaging to prevent contamination, also used at homes in appliances like refrigerators, washing machines, as well to establish several industrial applications (52),(53).With nanotechnology development, towards silver nanoparticles applications for antibacterial capacity was a logical step to broad the knowledge of silver's antibacterial activity (54).

The literature now supports three mechanisms, which may function alone or in combination, through which silver nanoparticles exert their antibacterial activity. The first theory involves silver nanoparticles function at the membrane level because they can pass through the outer membrane and accumulate in the inner membrane, where their adhesion to the cell results in cell destabilisation and damage, increasing membrane permeability, and causing leakage of cellular content, which ultimately leads to cell death (55). Additionally, it has been shown that silver nanoparticles bind to proteins that contain sulphur in bacterial cell walls, which may lead to structural damage and cell wall rupture (56).

According to the second method, nanoparticles may enter into the cells and pass via cell membrane with surface modification of its composition and permeability enhancing modifiers. Upon conjugation the synthesized silver nanoparticles will be drawn by the sulphate functional groups and phosphorus groups present in intracellular components bound with proteins and DNA molecules. They have the tendency to alter the respiratory chain by interfering with thiol groups coupled with enzymatic action thus generating reactive oxygen species and their corresponding free radicals by inciting apoptosis. The first two techniques are believed to cooperate with the discharge of silver ions from nanoparticles, which, because of their size and charge, may interact with biological elements and affect metabolic pathways, membranes, and maybe genetic material (57), (58). In [Figure 3] antimicrobial activity of silver nanoparticles in bacteria is shown.

Figure 3: Antimicrobial activity of silver nanoparticles in bacteria



8. Synthesis of silver nanoparticles

Generally, there are two different approaches for the synthesis of metallic nanoparticles, top-down method that can be reduced in different forms such as, atom-atom reduction, molecule-molecule synthesis or cluster-cluster agglomeration) whereas, the other method is known as bottom-up followed by the similar process of cluster formation of nanoparticles by slicing or successive cutting of a bulk material to obtain nano-sized particle. The "bottom-up" approach is frequently used to generate nanoparticles because it involves a homogeneous system in which catalysts used as reducing agents and enzymes synthesis of nanostructures that are controlled by the catalyst. On the other hand, the "top-down" approach often uses materials in their bulk state, and size reduction to nanoscale measurement is done by the use of thermal decomposition method, mechanical grinding, cutting, and sputtering(47).The fundamental drawback of the top-down strategy is the predominance of surface structural faults. These flaws significantly affect the surface chemistry and physical characteristics of metallic nanoparticles. There are several ways to synthesis silver nanoparticles, including chemical, physical, and biological approaches(48).There are four kinds of chemical synthesis methods: chemical reduction, electrochemical, irradiation-assisted chemical, and pyrolysis. The biggest drawback is the high energy costs(49).Due to their high cost and laborious process of chemical synthesis silver nanoparticle biological synthesis of silver nanoparticles from plant extracts or from bacteria has several benefits over chemical and physical synthesis processes, it has emerged as a viable alternative. It is also often assumed that these routes are simple. It is easily scalable for large yields and low-cost production. In the realm of nanotechnology, the creation of metal and metal oxide nanoparticles utilising biological agents such as bacteria, fungus, yeast, plant, and algae extracts has grown in popularity(50),(51).

8.1. Biosynthesis of silver nanoparticles

The biogenic production of silver nanoparticles is a simple, one-step process that does not produce harsh or poisonous compounds, making them cost-effective, economical, and environmentally safe. Both plants and bacteria have been widely studied in recent years for the biosynthesis of silver nanoparticles of varied size, shape, stability, and antimicrobial activity(59).

The employment of reducing agents in the production of nanoparticles has provided a critical route that ensures environmental sustainability and restricts the use of these noble materials to biological ones. Because of the use of hazardous chemicals and the high cost of the synthesis process, conventional techniques are no longer seen as being economically and environmentally benign(60).

Silver colloids generate a number of their chemical reactions through aggregation as storage duration increases. The manufacturing of metallic nanoparticles using green chemistry principles has been presented as a solution to the aforementioned concerns. Eco-friendly solvents, reducing agents, and stabilising agents are key components of green synthetic processes(61).

8.2. From plants

Preparation of plant extract by maceration, infusion, digestion, decoction, percolation, reflux, and Soxhlet extraction are examples of common conventional extraction techniques. The main disadvantages of these methods are the high extraction solvent requirements, lengthy extraction periods, and low extraction yields. Meanwhile, there has been significant progress in extraction methods as a result of the advent of ecologically friendly methods such as pressurised liquid extraction (PLE), supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE).

These methods necessitate less extraction solvent and shorter extraction times while providing excellent extraction yield. However, these procedures necessitate a sophisticated and costly experimental setup. There is a brief explanation of the different extraction methods(62),(63).

8.2.1. Making Silver Nanoparticles from Plant Extracts:

- Plant parts are chosen that are young, strong, and robust, and they are cleansed frequently with deionized water used after tap water to eliminate surface impurities.
- After cleaning, plant components are cut into small pieces and shade dried for a number of days or in a hot air oven at a specific temperature for a specific amount of time, then whirled into a powdery fineness.
- Plant material that has been ground up can then be obtained using either water or organic solvents or by utilising many methods, such as a simple few-minute boil, extraction techniques that use ultrasound and microwaves, etc.
- A rotary evaporator is used to dry the concentrate of the extract.
- A specified quantity of silver nitrate (AgNO3) solution is mixed in a 1:9 ratio with the plant extract. After that, the reaction mixture is left alone until the colourless AgNO3 solution takes on a reddish-brown hue. This demonstrates the synthesis of silver nanoparticles. Depending on these circumstances, this procedure can be performed at room temperature or at various temperatures.

- Centrifugation is used to settle down the silver colloids present in the solution.
- The silver colloids are washed with deionized water multiple times to eliminate excess silver ions.
- In addition, the lyophilized silver nanoparticles are stored in tightly packed vials at room temperature for characterization and use(64),(45).

8.3. From bacteria

The possibility of utilising microorganisms in the synthesis of silver nanoparticles has recently come to light. For instance, *Pseudomonas stutzeri* AG259, which was acquired from a silver mine, was used to induce Ag NP production inside the cells(65).*A. calcoaceticus, B. amyloliquefaciens, B. flexus, B. megaterium, and S. aureus* are just a few of the gram-positive and gram-negative bacteria that have been used to produce silver nanoparticles both extracellularly and intracellularly. Numerous shapes of silver nanoparticles such as triangles, cuboids, hexagons, and spheres, can be synthesised from these bacteria(66).They were produced using cells, aqueous cell-free extracts, or culture supernatants. have shown the extracellular biogenesis of silver nanoparticles (5–50 nm) by irradiating water with microwaves and *B. subtilis* culture supernatant(67).It has also shown the quick production of silver nanoparticles using the culture supernatants of *K. pneumoniae, E. coli,* and *Enterobacter cloacae* (within 5 min). We have previously shown the rapid extracellular synthesis of Ag NPs from B. megaterium culture supernatant in the presence of Ag+ ions in aqueous solutions(68). By combining a Bacillus flexus group bacterial strain S-27 with 1 mM $AgNO_3$ in an aqueous medium, the fast production of Ag NPs is now achievable. The colourless supernatant solution eventually changed from yellow to brown. The silver nanoparticles had a strong peak in the UV-vis spectrum at 420 nm due to their surface plasmon resonance (SPR). Even though anisotropic nanoparticles' gradual degradation cannot be prevented, they remained stable for five months at normal temperature. They were crystallized and had a face-centered cubic shape. These nanoparticles were shown to be effective against multidrug-resistant Gram-positive and Gram-negative bacteria. The concentration of the reacting elements determines the intensity of the colour and the speed of the reaction(69),(70).

According to reports, the Bacillus strain (CS11) produces silver nanoparticles extracellularly. The synthesis of nanoparticles took place within 24 hours after the interaction of 1 mM AgNO3 combining with bacteria at room temperature. The nanoparticles had a peak in the UV-vis spectrum at 450 nm. They were discovered to be between 42 and 92 nm in size, according to TEM examination(71).

8.4. From fungi

Numerous studies have been done on the biosynthesis of Ag NPs produced by both pathogenic and non-pathogenic fungi. According to reports, when fungi are present, silver ions were reduced extracellularly to produce stable Ag NPs in water(72).

Numerous studies has shown that the thermophilic fungus *Humicola sp.* produces Ag NPs extracellularly. In an aqueous medium at room temperature, all procedures were carried out. Mycelia were suspended in 100 mL of a solution containing 1 mM AgNO3 and then put in an Erlenmeyer flask that was heated to 50 degrees Celsius. At a pH of 9, the mixture was stirred for 96 hours while being monitored for any colour shifts that could occur. The colour of the solution changed from yellow to brown as a result of the production of silver nanoparticles(73). After pulverising the dried basidiocarps, the liquid that was left over after straining was boiled in water and then freeze-dried. The lyophilized powder's hot water extract was mixed with various concentrations of 1 mM silver nitrate and then incubated at a temperature of 25 degrees Celsius(74).

The *Pleurotus ostreatus* also known as oyster mushroom is used in the biological production of silver nanoparticles (Ag NPs). Following the addition of dried aqueous mushroom extract at concentrations ranging from 1-6 mg/mL and 1 mM AgNO3, the mixture was left to incubate in the dark for 6–40 hours. As the colour progressed from pale yellow to brownish yellow with darker brown undertones, silver nanoparticles began to develop(75).

In a number of the research projects, the enzyme nitrate reductase was substituted by NADPH in order to generate nanoparticles. This is particularly exciting since it shows that alternate species may be utilised to synthesise nanoparticles without the necessity for reductase enzyme activity. This opens up a whole new world of possibilities. *Fusarium oxysporum* was used in the production of silver nanoparticles, and the reduction of silver ions was attributed to the activity of the nitrate reductase enzyme in conjunction with anthraquinones(76),(77).

8.4.1. Factors affecting the formulation of silver nanoparticles from fungus

a) Temperature

The rate at which the synthesis is carried out as well as the size and stability of the nanoparticles may all be impacted by the temperature that is employed during the synthesis of silver nanoparticles utilising fungus. Despite the fact that the vast majority of research has shown that higher temperatures result in faster rates of synthesis, it is critical to consider the quality of nanoparticles. Temperature may alter nanoparticle size and stability in addition to affecting the synthesis rate. At temperatures of 40, 20, and 60 C, nanoparticles with diameters of 2.86, 25.89, and 48.43 nm were produced, with the smallest size measured at the intermediate temperature(78).

b) Influence of pH

pH may be used to manipulate the properties of nanoparticles. The conformation of nitrate reductase enzymes might change depending on the number of protons in the reaction medium, causing the shape and size of the nanoparticles to change(79).

c) Amount of silver nitrate

The majority of the studies that made by the utilization of fungi for the extracellular production of silver nanoparticles made use of AgNO3 at a concentration of 1 mM (milli molar). In some instances, a decrease in the concentration of the metal precursor led to a reduction in the size of the nanoparticles as well as an enhancement in their dispersion(80).

d) Impact of the Medium Used for Culture

It is well known that various microorganisms will exhibit distinct reactions based on the culture medium and the environmental circumstances of the cultivation. Alterations to these circumstances lead to the production of a wide variety of metabolites and proteins(79).

9.The advantages of the Bio-Synthesis Route for silver nanoparticles

Use of an eco-friendly reducing agent and use of a non-hazardous stabilising agent are the primary methods for generating silver nanoparticles in a more ecologically friendly way. To be energy-efficient, the production procedures must be carried out at a neutral pH, ambient pressure, and temperature. (81) silver nanoparticles have been synthesised using a variety of biological methods throughout the past few decades. These biosynthesis techniques have been suggested as alternatives because they work well, are inexpensive, and are simple. The synthesis of nanoparticles may be done using a bio-based technique, which allows for more repeatability of the process as well as increased stability in the nanoparticles that are created. As a result, this environmentally benign method of synthesising nanoparticles is suitable for mass manufacturing, has a lower cost of investment, and is safe for use in human therapeutics. (82) In addition to being more consistent and stable, it has been shown that utilising biological agents results in a substantially quicker rate of bio-reduction of metal ions at ambient temperature and pressure. The bio reduction capability of the plant extracts is noticeably greater than that of the microbial culture(83).In addition, the waste products that are produced as a consequence of the process that is based on microorganisms have the potential to be more damaging to the environment, depending on the particular bacteria that were used in the synthesis. Because of this, plant-mediated synthesis results in less pollution, or nearly none at all, and hence has a less negative effect on the environment(84).

The use of microbes and biomolecules to generate NPs in bulk, including silver nanoparticles and other metallic NPs, is always regarded as an alternative to chemical approaches. Numerous studies that are biological, economical, eco-friendly, greener, and nontoxic have been described in relation to a more environmentally friendly synthesis of silver nanoparticles(85).

10.Characterization of silver nanoparticles

a) UV-Visible Spectroscopy

UV-vis spectroscopy is an effective approach for determining the initial characteristics of nanoparticles as well as for monitoring the development and stability of silver nanoparticles(86).

b) X-ray diffraction studies

X-ray diffraction, more commonly referred to as XRD, is a common analytical method that has been put to use for a wide variety of purposes, including the investigation of molecular and crystal structures, the qualitative identification of a variety of compounds, the quantitative resolution of chemical species, the measurement of the degree of crystallinity, the determination of isomorphous substitutions, particle sizes, and a great many other things. XRD has been put to use for these and many other purposes(87).

c) Dynamic light scattering

When using techniques that include radiation scattering, it is essential to first determine the physicochemical properties of the created nanomaterials before moving on to the examination of biological activities. Investigating the size distribution of microscopic particles in solution or suspension on a scale ranging from submicron to one nanometre requires the use of the dynamic light scattering (DLS) method(88).

d) Fourier Transform Infrared (FTIR)

The use of FTIR spectroscopy makes it possible to detect extremely minute variations in absorbance on a scale of 103, which is helpful in the execution of difference spectroscopy. Difference spectroscopy makes it possible to differentiate between the vast background absorption and the small absorption bands that are characteristic of functionally active residues(89).

e) X-ray photoelectron spectroscopy (XPS)

The X-ray Photoelectron Spectroscopy (XPS) technique is a quantitative spectroscopic surface chemical analysis methodology that is used to approximate empirical formulations. This method was developed in the 1970s. X-ray photoelectron spectroscopy, which is also known as electron spectroscopy for chemical analysis (ESCA), offers qualitative, quantitative, semi-quantitative, and speciation information that is exclusive to the surface of the sensor(90).

f) Scanning electron microscopy (SEM)

In recent years, the subfields of nanoscience and nanotechnology have been a major motivating factor in the development of a wide range of high-resolution microscopy methods. In order to explore things at a much smaller scale, these methods make use of a stream of very powerful electrons. These methods are being used with the end goal of acquiring a deeper understanding of nanomaterials. The scanning electron microscopy (SEM) technique is one of the many electron microscopy methods. It is a surface imaging strategy that is fully capable of differentiating between various particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the created particles at both the micro- and nanoscales(91).

g) Transmission Electron Microscopy

Transmission electron microscopy (TEM) is a technique that is advantageous, widely used, and essential when it comes to the characterization of nanomaterials. The transmission electron microscope (TEM) is a useful tool for gathering quantitative data on the size, size distribution, and form of particles(92).

h) Atomic force microscopy

In general, atomic force microscopy (AFM) is used in the investigation of nanomaterials' dispersion and aggregation, in addition to the examination of their size, shape, sorption, and structure. There are many other scanning modes that may be chosen from, such as the contact mode, the non-contact mode, and the intermittent sample contact mode(93).

11.Conclusion

In the subject of nanotechnology in dentistry, one of the most recent and innovative applications of nanotechnology may be discovered. Within the scope of this field of research, silver nanoparticles (NPs) with a concentration on dentistry, particularly dental disinfectants, are an important subject that should be investigated further. Nanomaterials provide a platform for the targeted dispersion of active chemicals and have the capacity to penetrate root canals while simultaneously combating biofilm resistance. The chemical and physical synthesis of silver nanoparticles is not easily scalable to large-scale manufacturing for a number of different reasons. These reasons include the presence of hazardous by-products and intermediate compounds, the generation of hazardous by-products, and the high amount of energy that is required. It is possible that this will result in an increase in the particles reactivity and toxicity, which may have a negative impact not only on human health but also on the environment. This may be the case because the composition is difficult to predict and there is a lack of clarity regarding the composition. In particular, it has been shown that biosynthesized silver nanoparticles are compatible with living organisms, have a reduced level of toxicity, and are also environment friendly. In addition, they do not pose any kind of danger to the human or domestic animal population in terms of their health. It has been demonstrated that employing silver nanoparticles as a root canal irrigant would have a remarkable impact however, only a limited number of studies in this sector have been published. As a result, additional research is required to generate effective silver nanomedicines in order to reduce the high failure risk associated with oral disinfection.

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