The Inhibitory Action Of (Ag-Nanoparticles) On Microorganism In The Root Canal Necrosis.

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Abstract:
Our current research includes the manufacture of silver nanoparticles in a biological way, and their use in treating one of the diseases that affects teeth, which is the root canal necrosis. These nanoparticles act as an antibiotic that prevents the growth of bacteria and fungi at the site of caries that occurred in the dead root canal, and thus acts as a treatment for such and other pathological conditions caused by microbes to the teeth, which consequently lead to periodontitis. Infections on periodontal tissue due to root canal decay represent a natural response to biological defense, which is affected by the presence of microbial activity at the site of infection. The inflammatory response model is the same in any other part of the human body. Therefore, this inflammation is able to cause harmful consequences in the area around the decayed root canal that has not been properly treated when the filling is imprecise. The microorganisms that were diagnosed from the isolates that were taken from the place of decay are (Streptococcus sp., Staphylococcus aureus sp., Candida sp., Actinomyces sp., Actinobacillus sp., Bacillus sp.), in different proportions (37.6 %, 18 %, 16.4 %, 12.8 %, 9.5 %, 5.7 %), respectively. And after the process of producing silver nanoparticles, it was used in vitro to inhibit the growth and kill of these types of microorganisms, and
thus eliminate inflammation originating in the root canal through the validity of using these nanoparticles as an irrigation agent or sealer in endodontics and an oral antiseptic in the form of an oral rinse, or any other method chosen by specialists in preventing the growth of microbes at the site of infection.

**Key word:** Ag-Nanoparticles, microorganism in root canal necrosis, inhibitory action

**Introduction.**

Periodontitis is an inflammatory reaction in the surrounding tissues due to the presence of bacteria in the necrotic root canal system. Clearly, the affected root canal system is the place considered suitable for selective types of microorganisms. The microbial action of infected root canals has been the focus of much research over the years. The results conducted in the studies clearly identified the microbial differences between the pulp duct and its treatment methods. Continuous periodontitis after root canal treatment provides a more complex causes and treatment. Often, the patient does not see a dentist unless his health condition worsens and worsens due to the inflammation caused by the action of microorganisms, which is the main cause of inflammation in the periodontium, as it caused caries of the root canal and the development of that decay and its extension to the depth of the root canal, which caused necrosis over there.

Nanomaterial denotes a natural or manufactured material containing unbound particles in which half or more of the particles in number and size is in the size range of 1-100 nm. These materials present unique physicochemical properties, such as large surface area/mass ratio, and increased chemical reactivity. The increased number of atoms and increased surface to volume ratio compared with micro/macro-structures are suggested to contribute to the distinctly different properties of nanomaterials. These advantages may be exploited to design highly specific materials and devices to interact with at the subcellular and molecular level of the human body in order to achieve maximal therapeutic efficacy with minimal side effects.
Silver nanoparticles (AgNPs) is the most important nanomaterials proposed for applications in the biomedical field due to excellent physicochemical and biological properties. AgNPs are the most common metallic nanomaterials used for the control of several types of microorganisms due to their very well-known antimicrobial properties even in drug-resistant microorganisms 7.

On the other hand, silver has been used in various forms, metallic silver, silver nitrate, and silver sulfadiazine, for treating burns and severe bacterial infected wounds and injuries, but, due to the discovery and increase of antibiotics, these silver compounds were no longer used. Silver in the form of nanoparticles has reappeared as a potential antimicrobial agent because of the greatly developed microbial resistance of a large variety of microorganisms. In this scenario the materials in nanoscale have reappeared as a new antimicrobial organism agent due to their physical and chemical properties 7.

The objective of this study was to determine the reduction effect of silver nanoparticles, it was used in vitro to inhibit the growth and kill of these types of microorganisms.

**Diagnosis of periodontitis due to necrosis of the root canal using X-ray.**

The accurate diagnosis was performed using X-rays, for a group of patients with root canal caries, which led to periodontitis. This diagnosis was made at Manipoli Hospital / Department of Oral and Dental Health, in Andhra Pradesh, Hyderabad, India. During (March / April 2015).

The dentist must perform the diagnosis directly through the use of X-rays to identify the type of injury and the extent and severity and exact location. In this case, after the diagnosis of the condition and the conclusion that the caries reached the root canal, and that it was he who caused the inflammation due to the growth of microorganisms at the site of the infection, the case would be under control. The use of the filling as a treatment for such a condition is the appropriate treatment, but if the injury resulted in damage to the tooth tissue, the dentist will have to extract the tooth. But the risk of infection is that after the diagnosis the status and treatment using the permanent filling after the temporary, then after a few days the case will worsen and the inflammation will be accompanied by more severe pain from the previous pain, because the caries
remained in the deep of root canal even after the filling, which led to the occurrence of inflammation at the site of injury in the deep of root canal, and this inflammation expanded and extended to lead to inflammation of the periodontium completely as shown in figure (1). All this process because of the presence of microorganisms in the place of caries inside the root canal. This inflammatory reactions are initiated by the bacterial, and fungal antigens interacting with the local immune system (1, 2, 3).

Figure (1): shows the inflammatory conditions that occurred due to the development of microbiological activity in the root canal again, even after treatment with the filling.

Materials and Method:

A-Isolation and diagnostic processes of microorganism:

1. Samples collection:
The total of (20 swabs) of the root canal caries, are collected from patients, in the dental section, of manepuolei hospital, in Andrapradesh city, Hyderabad State, India, during (March \ April, of 2015)

2. The colonies isolation, and diagnosis:
All samples were grown in prepared media, and laboratory examinations were completed for diagnoses the microorganisms, according to the following diagnostics steps:

3. **Cellular Diagnosis:**

This diagnoses is done by examination of the gram stain, for the shapes, and assembly determination of the microbial cells, and their interactions with dye.

4. **Culture Diagnosis:**

It included the diagnoses of the sizes, and shapes of the microbial colonies, and its growing in culture media.

5. **Biochemical examination:**

This type of the microorganisms diagnosis are based on their ability for producing enzymes, and biochemical effects.

**B. Preparation of Silver Nanoparticles:**

1. **Methods.**

   - The leaves of (*Plectranthus stockssi*) was prepared and cleaned thoroughly with water, then by distilled water.

   - The leaves cut into small pieces, then boiled in (ddH2O) sterile deionized water (1.9 g/ml), at about (60°C) for 10 mins.

   - Then filter through (0.4 µm) membrane filter, and stored in dark containers at (5°C).

2. **Biological Syntheses of Silver Nanoparticles.**

   - Using standard methods, (1mM AgNO3) solution was added to extracted leaves at ratio 1:5, then put them in rotary shaker at (110 rpm) at (37°C) for 1 hr.

   - Purifying of silver nanoparticles by centrifugation at (10,000 rpm) for (15 mins).

   - The purified silver nanoparticles were freeze dried, then silver nanoparticles were characterized by spectroscopy methods.
Reduction of (Ag-ions to Ag-NPs), followed by a color change, and (U.V-Vis) spectroscopy.

The Ag-NPs were characterized through (U.V-Vis) spectroscopy. SEM, and TEM were performed on the samples.

3. UV–Vis Spectroscopy.

The production of Ag-NPs was observed by monitoring color change of the samples.

The bio-reduction of Ag-ions was monitored with (UV-visible spectra) of the solutions after diluted a small aliquot (0.2ml) of sample for (10-munits) with d.d.H2O.

(UV–visible) spectra were decumanted with (Schimadzu) double beams spectrophotometer, (Schimadzu, Japan), (300-700 nm) wavelength at the room temperature.

4. (D.L.S) Dynamic light-Scattering.

Particles distribution, and Zeta potential of bioreduced Ag-NPs are measured using DLS (Zetasizer-Nano ZS-Ns, ZEN3600, and Malvern, U.K).

The dynamic light-scattering (D.L.S) provides insight into the dynamic characteristics of smooth materials by the measure of single scattering events.

5. (TEM) Transmission Electron Microscope.

TEM (Philips CM-10) is a microscopically technology, whereby a beams of electrons are interacted with the sample when it passes through, then image formed from the interaction of the electrons transmitted in the sample.

The images of TEM in the transmission of a focused beam of electrons in the specimen, forming an images like the light microscope images.

6. (SEM) Scanning Electron Microscopy.

It experiments were performed for characterization of the shape and size through bioreducing of Ag Nps.
Purified Ag Nps were sonicated at (20 minutes) for making it uniform distribution, and a drop of the solution was loaded on carbon coated copper grids, and solvent was allowed to evaporate at the Infrared light during (35 minutes). The Scanning Electron Microscope (SEM) image of the Ag NPs synthesized.

Result:
The ratios of germs were isolated from the root canal which suffered of caries showed Streptococcus sp. about (37.6 %), this percentage followed by (18 %) Staphylococcus aureus sp., and which followed by (16.2 %) and (12.8 %) of Candida sp. and Actinomyces sp., respectively, while the Actinobacillus sp., represented by (9.4 %). The other ratios of microorganisms and the ratios of microorganisms after reduced by action of Ag Nps, are recorded various percentages as shown in a table: (1), figure: (1), and figure: (2) below:

| No. | Types microorganisms. | The ratios of isolated microorganisms from the decayed root canal. | The ratios of microorganisms after reduced by action of Ag Nps |
|-----|-----------------------|---------------------------------------------------------------|-------------------------------------------------------------|
| 1-  | Streptococcus sp.     | 37.6 %                                                        | 2%                                                          |
| 2-  | Staphylococcus aureus sp. | 18 %                                                      | 1%                                                          |
| 3-  | Candida sp.           | 16.4 %                                                        | 0.97%                                                       |
| 4-  | Actinomyces sp.       | 12.8 %                                                        | 0.78%                                                       |
| 5-  | Actinobacillus sp.    | 9.5 %                                                         | 0.73%                                                       |
| 6-  | Bacillus sp.          | 5.7 %                                                         | 0.5%                                                        |
figure: (1) The ratios of isolated microorganisms from the decayed root canal.

figure: (2) The ratios of microorganisms after reduced by action of Ag Nps

- The purified silver nanoparticles were freeze dried, then silver nanoparticles were characterized by spectroscopy methods. Reduction of (Ag-ions to Ag-NPs), followed by a color change, and (U.V-Vis) spectroscopy.

- The Ag-NPs were characterized through (U.V-Vis) spectroscopy. SEM, and TEM were performed on the samples.
Discussion:

The clinical isolates of microorganisms recovered from infections of the root canal offer the opportunity to do studies in vitro in which the mechanics of virulence, genetic structure, and defense are not altered. This allows us to efficiently study the bactericidal effect of components that need to be evaluated.

In this study demonstrates a positive bacteriostatic and bactericidal effect from the silver nanoparticles solution on endodontic microorganisms (bacteria and fungi).

Current study is agreement with researchs in the endodontic field have focused their efforts in developing a new nanomaterial-based irrigant. Wu et al. obtained similar results when they evaluated the antibacterial efficacy of silver nanoparticles against E. faecalis; Javidi et al. evaluated the bactericide effect of Ca(OH)2 with and without silver nanoparticles on E. faecalis from root canals. They found that the number of CFUs observed after the use of Ca(OH)2 plus silver nanoparticles suspension was significantly less than the number observed with Ca(OH)2 alone.

The laboratory work examined the effect of the size of nanosilver particles on antimicrobial activity revealed that the fine particles have stronger effect comparing to coarse particles. The inhibitory zones surrounded the experimental materials had not any collapse or reduction in size. Even after full set of the root-canal sealer, the inhibitory zone progressed to the full capacity representing antimicrobial activity. The sustainable antibacterial effect on the experimental plate may have a role in the significance of result.

This study is agreement with a previous study, the antibacterial activities of nanosilver particles and ions were tested. A suspension from Ag/SiO2 are examined at the beginning the suspension was centrifuged and the particles were removed. The remnant ions in the solution had a positive antimicrobial effect in advance. (Sotiriou & Pratsinis, 2010). The same conclusion was illustrated by Lok et al. (Lok et al., 2007) Thus, in addition to direct toxicity, the different diffusion rates of the different experimental specimens may influence the results (Sotiriou & Pratsinis, 2010).
Endodontic disease, different microbes are the primary predisposing agents. Anaerobic bacteria has the capacity to adapt and survive on the necrotic tissues which has limitation in nutrient and oxygen supplies. The interaction between the facultative anaerobic microorganisms with other anaerobes, may alter the nutrition and cause potential oxygen tension, which determine microbial-survival relationship. The antibacterial effect of the commercial sealers. From the clinical findings, the antimicrobial effect for the sealer needs to inhibit bacterial growth over long period. After aging the nanosilver particles to the experimented root canal sealer powder, there was a significant antibacterial effect. There is a rational thought regarding the role of freeing Ag+ ions from nanosilver and its toxicity towards microorganisms. Current study is agreement with Tolaymat et al., 2010 The potential effect of nanosilver its role as a source of Ag+ ions. Navarro et al., 2008 Miao et al. (Miao et al., 2009) elaborated the effect of nanosilver to the dissolved Ag+ ions toxicity.

Further in vitro experiments with single and multi-species biofilms demonstrated for the first time that AgNPs@SiO2 based irrigation solutions possess excellent antimicrobial activities for at least 7 days, whereas the bare AgNPs lose the activity almost immediately and do not show any antibacterial activity after 2 days. The long term antimicrobial activity exhibited by AgNPs@SiO2 solutions can be attributed to the sustainable availability of soluble silver, even after 7 days.

AgNPs attracting increased attention in the biomedical field due to their bactericidal activity against some bacteria and fungi. The physicochemical properties of AgNPs have developed to analyze biocompatibility, biodegradability, bacteriostatic and bactericidal activity, aggregation capability, solubility, and precipitation range. To analyze some of these properties, nanoparticles size have potential properties are important issues to provide insights into the different applications. Nowadays, AgNPs are involved in biomedical applications such as antibacterial agents, drug delivery vectors, the ragnostic agents, and cancer treatment.

Thus, it is very important to provide information about physicochemical properties to know the pharmaceutical and adverse effects for the different applications in the biomedical field. It has been reported the importance of the antibacterial
properties of root canal in vitro studies against microorganisms. Also, some sealers and irrigants have significantly demonstrated antimicrobial efficacy against E. faecalis, Candida albicans, and Staphylococcus aureus. This antibacterial property could prevent residual and recurrent infections and contribute to endodontic therapy.

Conclusion:

the producing silver nanoparticles, it was used in vitro to inhibit the growth and kill of certain types of microorganisms (bacteria and fungi), and thus eliminate inflammation originating in the root canal through the validity of using these nanoparticles as an irrigation agent or sealer in endodontics and as an oral antiseptic in the form of an oral rinse, or any other method chosen by specialists in preventing the growth of microbes at the site of infection.

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