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Evaluation of the Anti-Bacterial Effect of Selenium Nanoparticles in Peri-Implantitis Patients

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ABSTRACT

Aim: To determine the concentration of selenium nanoparticles that inhibit the growth of anaerobic bacteria with signs and symptoms of implant failure increasing around implants.

Material and methods: It included 10 partly edentulous subjects (five females, five males) aged 30-73 years with one or more periimplantitis implants. Peri-implantitis was defined as: I the existence of seeping and additional decay on testing and (i) Radiographic images showed negligible bone misfortune >1.8 mm after 1 year of ability." The integration models were: incompletely edentulous patients with one implant determined to have peri-implantitis in any case; (ii) No anti-microbial therapy for half a year prior to clinical evaluation.' A total of fifteen implants were diagnosed with peri-implantitis following this description. Subgingival bacterial specimens were collected from infected implants of each person using sterile paper points.

Results: Revealed that selenium nanoparticles' minimum inhibition concentration for Total anaerobic bacteria was (5 percent), this concentration showed growth on plain BHI-A media after re-culturing

Conclusion: The antibacterial activity increased from 5 percent and above with the rise in the concentration of selenium nanoparticles, and selenium nanoparticles had no impact at 0.5 percent, 1 percent, 2 percent concentration.

Key words:
Minimum inhibition concentration, Selenium, Nanoparticles, Anaerobic bacteria

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INTRODUCTION

Selenium, a significant trace ingredient, is essential for good health and should be consumed on a daily basis. The ability of covalently bound selenium to catalyse the formation of superoxide radicals (O2-), which can prevent bacterial attachment to solid surfaces, has been demonstrated [1]. In humans, selenium is needed for the synthesis of 25 selenoproteins (such as glutathione peroxidase). Cancers of the lungs, liver, colorectal, kidney, oesophageal, gastric cardia, thyroid, and bladder are also covered by it [2].

New methods to fight bacterial drug resistance and biofilm formation have been established in the last few decades.

The two main categories of these strategies are controlled antibiotic delivery and the development of non-drug antimicrobial materials. Techniques for improving drug penetration into bacterial biofilms are also being investigated.

Selenium nanoparticles (Se NPs)–a trace, essential metalloid ingredient–have recently emerged as a promising antimicrobial material in both suspension and immobilized types. Importantly, these particles have been shown to be extremely low in mammalian cell toxicity, making them a potential antimicrobial agent [3].

The bacterial relation is crucial in determining the performance and outcome of a Ti-based implant system. Surface alteration of titanium appears to be an effective way of improving the advantage of clinical treatment by coating or adding antibacterial properties of metals or amalgams to reduce the amount of microscopic organisms and microbial grasp [4].

In vitro and in vivo experiments were used to investigate microbial colonization and antibacterial movement in metallic and ceramic embedded materials. Titanium does not have antibacterial properties, but titanium inserts can cause plaque to form. Since these are the sections that are brushed as a method of plaque control, the adapted surfaces can withstand wear. Dry cycle surface modifications have been used in the medical and dental fields to provide excellent wear resistance as well as to create dainty and glue fine pottery [5].

To determine the concentration of selenium nanoparticles that inhibit the growth of anaerobic bacteria with signs and symptoms of implant failure increasing around implants.

PREPARATION OF CULTURE MEDIA

Brain heart infusion agar (BHI.A)

By the guidance of Oxoid Laboratories Company, the readiness of this media was suspended by 47 gm of powder in 1000 ml of refined water at that point blended well using attractive stirrer to ensure the entire amount of the powder disintegrated. The medium was sanitized by the Headings of the Creator. Autoclave at 121 oC for fifteen minutes after it has been poured into sanitized petri dishes can be used for sanitized media, and then set aside to cool until used [6].

Brain heart infusion – blood agar (BHIB.A)

This media arrangement was guided by the Oxoid Company. This medium allowed cooling at 45 oC after cleansing and then added 5-7 percent ml of blood and the medium was poured into sterilized petri dishes to cool at room temperature, set and then placed in the refrigerator until used [7].

Prepared of Brain Heart Infusion Broth BHI.B

According to processing, 34.5 gm of it was used to prepare the media, dispersed in one thousand ml of distilled water and locked with caped bottles. Autoclave cleaning was done at a pressure of 15 Ibs, 121 °C for 15 minutes [8].

Prepare of Muller Hinton agar

By following the manufacturer's instructions, 35 agar powder was used in 1 liter of water refining until the complete agar powder was disintegrated to sanitize it by autoclaving it [9].

Method of sterilization

Media, water, and phosphate buffer solution (PBS) were used in sterilization by autoclave at 121 C and pressing factor of 15 pounds/inch2 for 15 minutes, but mouth reflection, kidney dishes, and all spotless glasses were handled for 60 minutes by dry air broiler at 180 Co. Dettol's clean arrangement sanitized the chairs and floor of the laboratory [10].

Sample and culture

In 10 patients at the dental school, anaerobic microbes were extracted from the mouth with signs of embedded deceit and bone loss. A swab and sterile paper points were collected from the mouth by anaerobic microorganisms [11].

This method involves delicate scouring of pockets about 11 mm with a sterile q-tip, and then immunizing an essential separation medium along these lines, such as Sabouraud dextrose agar (SDA) [12]. Swabs were distilled on Sabouraud dextrose agar at 37°C for 24-48 hours and forcibly brooded, then stored at 4 ° C for additional analysis [13].

CULTURE AND ANALYSIS OF MICROBES

A study of anaerobic bacteria mixed in a vortex for 2-3 minutes in glass universal tubes containing 5 ml of Phosphate buffer solution Bacteria were absorbed and traced by the sterile loop, then incubated in blood agar for 96 hours. At 37oC, plan tubes containing 10 millilitres of BHI broth were also inoculated and incubated at 37oC for 48 hours. All plates and tubes were used gas pack to incubate it in anaerobic condition.

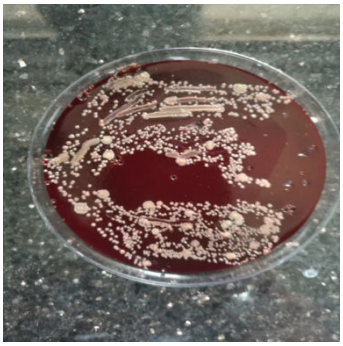

Figure 1: Incubated bacteria.

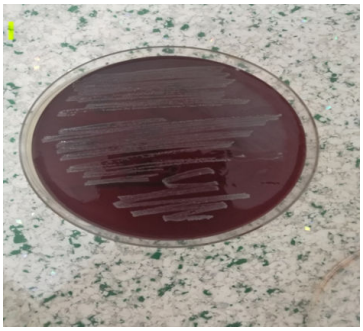

Figure 2: Colonies of Anaerobic bacteria.

Maintenance of bacterial isolate

States were collected from the bacterial agar media from bacterial confines and transferred to 10 ml of sterile BHI stock and hatched at 37 C in anaerobic for 24 hours. These supplies were kept cooler once used, and twice a month to month this technique was rehashed [14].

Activation of isolated anaerobic microorganisms

Bacterial inoculums were performed by extending unadulterated disconnects to ten millilitres of sterile BHI stock that were vigorously brooded for 24 hours at 37 oC and anaerobically for anaerobic microscopic species [15].

Determination of viable count

Fair actual search using sequential weakening with PBS, CFU/ml was completed and 0.1 ml of 10-3-10-5 was vaccinated with BHI. A brooded anaerobically at 37 oC for 24 hrs. [16].

Gram's stain

Gram's stain was put on the distraction components of blood agar in the sterilization state. A little inoculum was

taken from a disconnected area, emulsified on a glass slide in a drop of traditional saline to frame a suspension, spread, dried and heat-fixed. Gram's staining was done for 1 moment starting with gem violet and then washed with water, stained with iodine and, dried, extracted colors for half an hour with ethanol oil. The counter was stained for an extra moment with safranin, cleaned and dried. The slides were examined under a light magnifying lens with a 100X amplification for staining properties, cell morphology, and course of action.

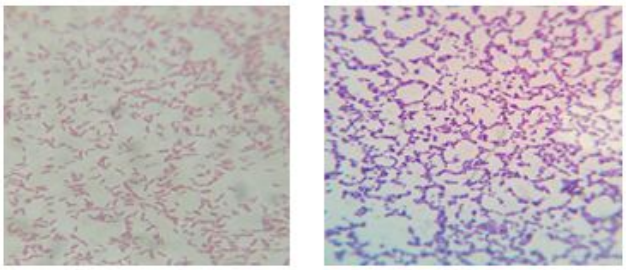

Figure 3: Colonies of anaerobic bacteria under light microscope (gram negative and gram positive).

Calculate the activity of nanoparticles selenium against anaerobic bacteria

In this research, the antimicrobial effect of selenium NPs on total anaerobic bacteria using the agar diffusion method was described using ten isolated numbers.

PROCEDURE

Around twenty-five millilitres of Muller Hinton agar media were placed in a separate partition of the sterile Petri dishes and waited until set.

Brooded for 24hrs at 370C. to ensure that the media are sterilized. Approximately 0.1 ml of all-out anaerobic (dilution 10-1) cell forming unit/ml confines were triggered and spread on MH agar plates and left at room temperature for twenty minutes, at that point equal size and depth wells made with clean hardened steel Cork drill were set up in the agar in the MH agar 6 mm in diameter.

In each plate, three holes. In different fixations, 0.5,1, ,3,5,7 percent, each very much was loaded up with selenium nanoparticles Plates were left at room temperature f or 10 minutes and subsequently brooded at 37 oC in an anaerobic container with a 24-hour gas pack.

There were restriction zones around the width of each hole. Under aseptic conditions, the breadth of restraint around the wells containing the test materials was measured and reported after hatching.

The area of the inhibition zone can be determined by the Vernia calipers.

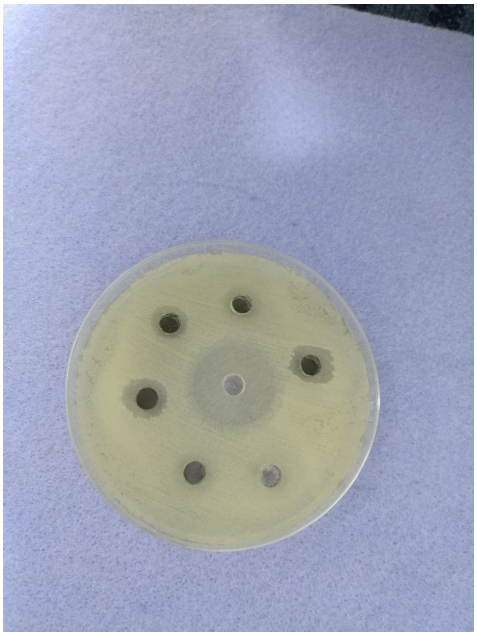

Figure 4: Minimum inhibition concentration determination.

Determination of minimum inhibition concentration (MIC) for anaerobic bacteria

After pouring it into sufficient Petri dishes, all selected concentrations were dispersed separately with BHI-A to obtain 25 millilitres of agar to become strong, then brooded with 0.1 millilitre of separately activated anaerobic bacteria. Incubated for one day at 37°C including control plates (negative control containing BHI-A with microbial inoculum without the addition of selenium nanoparticles and BHI-A containing plates and different concentrations of selenium nanoparticles without microbial inoculum) Examination of all prepared Petri dishes in order to notice bacterial growth. The minimum inhibition concentration should be considered to be the lowest concentration destroyed by microorganisms.

Figure 5: Bacterial growth before the lowest concentration of selenium that killed microorganism.

RESULTS AND DISCUSSIONS

Antibacterial activity of selenium nanoparticles on microorganisms (agar well diffusion method). Results revealed that Minimum Inhibition Concentration of

selenium nanoparticles for Total anaerobic bacteria was (5%), this concentration showed growth after re-culturing on plain BHI–A media (there was no effect of selenium nanoparticles at concentration 0.5%,1% ,2% and 3%) number of isolates within the MIC and the concentrations of the selenium nanoparticles as shown in Table 1.

Table 1: Total viable of anaerobic bacteria.

Concentration of selenium	Total viable of bacteria
0.50%	growth
0.80%	growth
1%	growth
2%	growth
3%	growth
5%	growth
7%	No growth
8%	No growth
10%	No growth

Mean of MIC for 10 samples of each 5, 7, and 10 % of selenium as shown in Table 2.

Table 2: The minimum inhibition zone.

	5 % of selenium	7% of selenium	10% of selenium
Sample 1	15 mm	16.5 mm	17 mm
Sample 2	16 mm	16.4 mm	17 mm
Sample 3	14 mm	15.8 mm	16.5 mm
Sample 4	15 mm	16 mm	18 mm
Sample 5	13 mm	17.5 mm	16.5mm
Sample 6	15 mm	18 mm	17 mm
Sample 7	17 mm	16.5 mm	18.3 mm
Sample 8	17 mm	15.3 mm	16.5 mm
Sample 9	13 mm	17.3 mm	17 mm
Sample 10	15 mm	16.7 mm	17.1 mm
mean	15	16.6	17.09
S.D.	1.34164	0.77588	0.58043

Both samples were diluted in standard saline at a 1:10 ratio (0.9 percent NaCl). In normal saline, serial dilutions (10-1 to 10-5) were made, and 100l were plated on blood agar. Using a gas bag, all plates were incubated at 37 C0 for 72 hours. The colonies were counted and registered as colony-forming units/ml (CFU/ml) at various dilutions. The results were calculated using 105 diluent, as shown in the table [3].

The antibacterial property of selenium was tested using the agar dilution process, which was obtained from the Biology Department's culture collection at Baghdad University. Dextrose Sabouraud was collected. Blood infusion broth (BHI) incorporation with agar that recommended the form of microorganisms to be examined, which was anaerobic bacteria, was done at concentrations of selenium ranging from 1% to 10%. To achieve homogeneity, the mixture was mixed in an ultrasonic bath. The investigated microbial strain was cultured overnight in the prepared assay media and then diluted before use. The number of cell forming units (CFU) was determined by dropping 20 lL drops of each diluted microbial culture on the surface of the respective assay medium (in triplicate), which was restricted to anaerobic bacteria and incubated at 37 C for one day.

The nanostructures were active against Gram-negative and Gram-positive bacteria at concentrations ranging from 0.5 to 25 ppm. As a result, SeNPs effectively inhibited bacteria at concentrations of less than 25 ppm. When SeNPs were used to inhibit the proliferation of both S. epidermidis and S. aureus when SeNPs were used as antimicrobial agents, MIC values of about 100 ppm were found when cultured with aerobic bacteria, while MIC values of 125 ppm were found when SeNPs were used to inhibit the proliferation of both S. epidermidis

and S. aureus when SeNPs were used as antimicrobial agents. This may be due to the Se NPs' naked surface coming into close contact with the bacteria's surface., The treatment with the nanostructures caused changes in both bacterial strains, including destruction of the outer cell membrane. Furthermore, after treatment with SeNPs, cell analysis is easily visible. As a result, visible cell damage was observed, like bacterial deformation and collapse, as well as many cracks in the cell membrane. However, other mechanisms, such as direct cell damage caused by nanostructure morphology, may also be hypothesized [16].

Table 3: The viable count of anaerobic bacteria (CFU/ml).

No. of samples	Viable count CFU/ml
Sample 1	88
Sample 2	71
Sample 3	70
Sample 4	83
Sample 5	90
Sample 6	83
Sample 7	80
Sample 8	71
Sample 9	84
Sample 10	75
mean	79.5
S.D.	6.9462

Coating external surgical equipment or surfaces that need to be sterilized with medium and higher concentrations of SeNPs could be beneficial [17-19]; the more Al particles in the design, the higher the basic intensity of the oxygen atoms that cause bacterial activity damage. The MIC was found to be the lowest at 5 percent selenium.

Conclusion

The action of NP selenium is essential in the inhibition of anaerobic bacteria. Antibacterial activity increased as selenium concentration increased from 5% to 10%, and selenium nanoparticles had no impact at concentrations of 0.5 percent, 0.8%, 1%, 2% and 3%, indicating that the MIC of selenium nanoparticles that obtained antibacterial properties was 5% of selenium in concentration.

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None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations for human use. The protocol will be submitted to ethics committee and Ethical approval has been obtained and attached.

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