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RESEARCH PAPER

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Zinc Oxide Nanoparticles from Internal Cavity of Dental Implant and Natural Teeth (*in vitro* study)

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ABSTRACT

Nanotechnology characterizes a new knowledge that potentials to deliver a wide range of uses and improved technologies for biological and biomedical applications. Nanotechnology permits synthesis of materials that have structure is less than 100 nanometers. The present work revealed the determination minimum inhibitory concentration and maximum bactericidal concentration of zinc oxide nanoparticles for aerobic and anaerobic bacteria isolated from internal cavity of the implant and sulcus of the natural teeth. Bacteria were isolated from internal hole of the implant after 90 days from surgical placement for 16 dental implant and sulcus of natural teeth for 6 female age range 30-44years. Different concentrations of ZnO NPs were prepared in solvent (water 3:1 ethanol) and mixed with brain heart infusion agar all the experiments were conducted *in vitro*. Agar dilution method was used to study the minimum inhibitory concentration and minimum bactericidal concentration and MBC for tested bacteria. The MIC for aerobic and anaerobic bacteria isolated from internal cavity of the implant and the sulcus of the natural teeth maximum plate was (0.05mg/ml) and (0.08mg/ml) respectively while the MBC for both bacterial groups were (0.08mg/ml) and (0.1mg/ml) respectively. This study exposed that zinc oxide nanoparticles were able to inhibit and kill the aerobic and anaerobic bacteria.

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INTRODUCTION

Oral biofilms, also identified as dental plaque, are defined as "matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces"[1].the formation of the pellicle on the surfaces of the teeth is first step to initiate the formation of the dental plaque this provide favorable receptor for primary colonizer [2].then secondary colonizer attached to primary colonizer this shifting in the composition of microorganism is representative of a clinical transformation from a healthy to disease of periodontium [3, 4].

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Primary studies recommended that Gram-positive bacterial population is predominant bacteria for two weeks from the placement of a trans-mucosal implant in patient loss tooth with no history of the periodontitis these bacterial population similar to those related with gingival health or gingivitis was detected in the peri-implant sulcus [5, 6].

The periodontal pathogen found in residual periodontal pockets furthermore existing in the tissue around the implant (peri implant tissue) after three months from implant placement

[6] and for more recent date recommended the colonization happen faster and pathogen existing could be after implant placement in 10- 14 days [7, 8].

The capability of the microorganisms to attached to the surface of the dental implant is not only affected by the roughness of the surface, but the surfaces of the implant is free energy also assists in biofilm formation [9, 10].

within minutes of implant placement, the colonization of the peri-implant environment can be occurred [11]. As well as the periimplant tissue, the bacteria may seepage inside the implant-abutment interface [12, 13] or stay confined in the internal cavity of the implant because of exposing to bacteria in the oral cavity at the time of surgical placement [14-16] that is cause inflammation of the area at the implant-abutment-bone junction because the bacteria at this site [17-19] the bacterial leakage increase when functional loading increased also into the implant abutment interfere [20, 21].

The internal part of the dental implant contaminated by microorganism has been approved for years. Many studies were conducted the bacterial leakage can move in both direction towards the internal cavity of the implant from the peri-implant tissue or as a result of being trapped on the inside where they proliferate and leak towards the peri-implant tissue. In order to study the possibility of microbial “outward” leakage from inside the implant to its outer surface and surrounding [15].

Nanotechnology is a developing technology including production or application of nanosized structures or materials [22]. Nanotechnology is considered as the production, categorization, and exploration of materials in the nanometer. the materials that relevant in this technology are those whose structures display new and significantly enhanced biological and physicochemical properties in addition to functionalities as a effect of the nanoscale size [23]. The combination between nanotechnology and biotechnology for emerging biosynthetic and ecological friendly technology for creation of nanomaterials it is called Bionanotechnology [24].

ZnO is described as a strategic, promising, functional, and Valuable white inorganic material with a wide range of applications ZnO have a chemical sensing, unique optical, electric conductivity semiconducting, and piezoelectric properties [25] ZnO to have significant uses in varied fields [26] ZnO have a wide band gap this feature has significant effect on its properties, such as the optical absorption and electrical conductivity. Zinc oxide has very strong ionic bonding in the Zn–O. Its longer durability, higher selectivity, and heat resistance are preceded than organic and inorganic materials [27].

The synthesis of nano-sized ZnO has led to the investigation of its use as new antibacterial agent. In addition to its unique antibacterial and antifungal properties, ZnO-NPs possess high catalytic and high photochemical activities and demonstrated that ZnO with different morphologies such as flowers and rods can be controllable obtained by simply varying the basicity in the solution. [28].

MATERIALS AND METHODS

Characterization and preparation of ZnO NPs

Surgical implant placement

Flapless surgical technique used for placement of the dental implant (Root®) for 6 female ages between 30-44 years and used sixteen implants. When complete drilling to suitable size and implant placed Before the placement of the healing abutment, all the internal holes of implant rinsed with about 25 ml of sterile saline solution by disposable syringe and dried using surgical suction, thus preventing further contamination.

Determination of the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of zinc oxide nanoparticles (agar dilution method)

After 3 months from implant placement healing abutment remove and samples were collected by small sterile brush from implant internal hole of the fixture in Heart Infusion Broth (BHI-B) and from sulcus of natural teeth. Antimicrobial

activities of the ZnO NPs nanoparticles were performed against both aerobic and aerobic. The antibacterial activity was done by well diffusion method. (previous study)

the method conducted was the preparation of the different concentrations of ZnO NPs incorporated with BHI-A to get 25 ml BHI-A of different concentrations of the ZnO NPs (agar dilution method) to find the minimum bactericidal concentration of the ZnO NPs. Sixteen isolates from each of aerobic and anaerobic bacteria from internal cavity of implant and six isolates from each of aerobic and anaerobic bacteria natural teeth were used in this experiment.

Final concentrations of (1, 0.5, 0.25, 0.1, 0.08, 0.05and 0.02) mg/ml were prepared from ZnO NPs and combined with sterile brain heart infusion agar to get 25ml of agar and ZnO NPs.

The experimental bottles 25 ml were poured into sterile petridishes and wait to become hard then inoculated with 0.1ml from the activated isolates of anaerobic and aerobic bacteria and spread it by microbiological sterile spreader equally.

All these petridishes were incubated for 24 hrs at 37°C including the control plate negative control which contained BHI-A with microbial inoculum without the addition of the ZnO NPs and the positive control plates which contained BHI-A and different concentrations of ZnO NPs without microbial inoculum. Each Petridish was checked and examined for microbial growth. The MBC was determined as the lowest concentration of ZnO NPs killed the microorganisms. While the MIC was determined as the lowest concentration of ZnO NPs inhibit microbial growth. And after that swap was taken from MBC plate for aerobic and anaerobic bacterial strain and re culture it in plain BHI agar to ensure from that concentration is give no growth.

Table 1. MIC of zinc oxide nanoparticles against aerobic bacterial strain from implant hole.

Numbers Of The isolates	No. of isolates within MIC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
16		10	5	1			

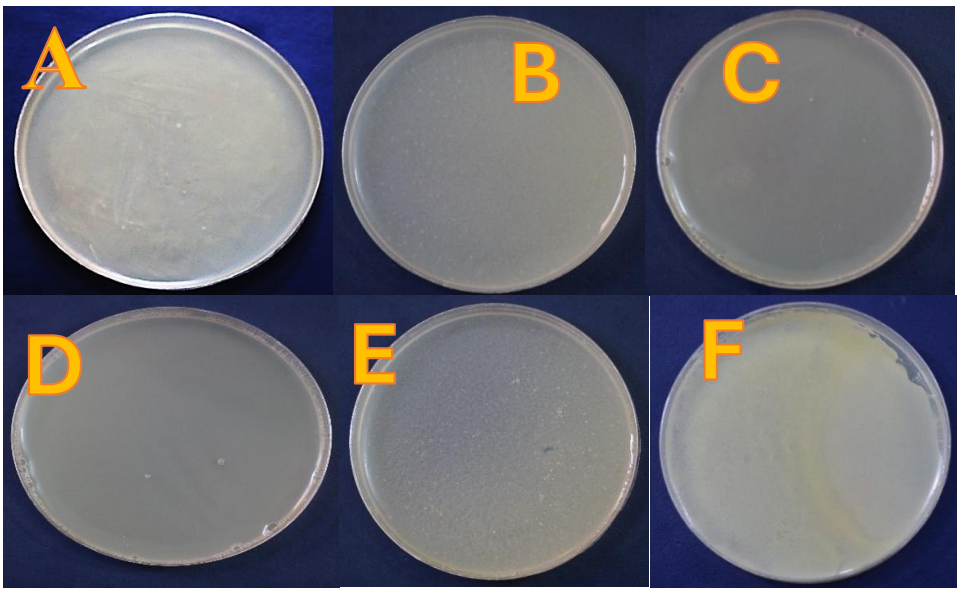

Fig. 1. A) plate with MIC 0.05mg/ml of Zno NPs weak growth B) plate with MBC 0.08mg/ml show no growth C, D, E) no growth for conc. 0.1, 0.25, 0.5 mg/ml F) Control plate heavy growth.

RESULTS AND DISCUSSION

Characteristics of ZnO-NPs

The absorption spectrum shows a sharp absorbance onset at 337nm andThe SEM image was taken at X 94,000 magnification. the size of the particles around 19.4-34.7 nm (previous study).

Determination of MIC and MBC for zinc oxide nanoparticles against aerobic bacterial strain from implant hole

Table 1 showed the highest number of isolates (10 agar plate with zinc oxide NPs) in concentration 0.05mg/ml with MIC lowest concentration to kill bacteria and then 4 agar for concentration0.08mg/ml and 2 agar plate for 0.1mg/ml .This means that (0.05, 0.08 and 0.1) mg/ml concentrations were able to inhibit the bacterial growth so the MIC for ZnO NPs against aerobic bacteria from implant hole is 0.05mg/ml as showed in Fig. 1.

0.05mg/ml concentration showed weak growth after re-culturing on plain BHI –A media while 0.08mg/ml concentration showed no growth. This mean that 0.08 mg/ml concentration had bactericidal effect and killed the bacteria, so that MBC of ZnO np that kill aerobic bacteria from implant hole was 0.08mg/ml concentration as shown Table 2.

Determination of MIC and MBC for zinc oxide nanoparticles against anaerobic bacterial strain from implant hole

Table 3 showed the highest number of isolates

Table 2. MBC of zinc oxide nanoparticles against aerobic bacterial strain from implant hole.

Numbers Of The isolates	No. of isolates within MBC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
16		4	9	3			

Table 3. MIC to anaerobic bacterial strain from implant hole.

Numbers Of The isolates	No. of isolates within MIC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
16		4	9	3			

Table 4. MBC to anaerobic bacterial strain from implant hole.

Numbers Of The isolates	No. of isolates within MBC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
16		2	6	8			

Table 5. MIC to aerobic bacterial strain from natural teeth.

Numbers Of The isolates	No. of isolates within MIC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
6		4	2				

(9 agar plate with zinc oxide NPs) in concentration 0.08mg/ml with MIC lowest concentration to kill bacteria and then 4 agar for concentration0.05mg/ml and 3 agar plate for 0.1mg/ml.

This means that (0.05, 0.08 and 0.1) mg/ml concentrations were able to inhibit the bacterial growth so the MIC for ZnO NPs against anaerobic bacteria from implant hole is 0.08mg/ml.

0.08mg/ml concentration showed growth after re-culturing on plain BHI –A media while 0.1mg/ml concentration showed no growth.

This mean that 0.1 mg/ml concentration had bactericidal effect and killed the bacteria, So that MBC of ZnO np that kill anaerobic bacteria from implant hole was 0.1mg/ml concentration as shown in Table 4.

Determination of MIC and MBC for zinc oxide nanoparticles against aerobic bacterial strain from natural teeth

Table 5 showed the highest number of isolates (4 agar plate with zinc oxide NPs) in concentration 0.05mg/ml with MIC lowest concentration to kill bacteria and then 2 agar for concentration0.08mg/ml.

This means that (0.05 and 0.08) mg/ml concentrations were able to inhibit the bacterial growth so the MIC for ZnO NPs against aerobic bacteria from natural tooth is 0.05mg/ml.

0.05mg/ml concentration showed growth after re-culturing on plain BHI –A media while 0.08mg/ml concentration showed no growth.

This mean that 0.08 mg/ml concentration had bactericidal effect and killed the bacteria, so that MBC of ZnO np that kill aerobic bacteria from natural tooth was 0.08mg/ml concentration as shown in Table 6.

Determination of MIC and MBC for zinc oxide nanoparticles against anaerobic bacterial strain from natural teeth

Table 7 showed the highest number of isolates (4 agar plate with zinc oxide NPs) in concentration 0.08mg/ml with MIC lowest concentration to inhibit bacteria and then 2 agar for concentration0.05mg/ml.

Table 6. showed MBC to aerobic bacterial strain from natural

Numbers Of The isolates	No. of isolates within MBC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
6		2	4				

Table 7. MIC to anaerobic bacterial strain from natural teeth.

Numbers Of The isolates	No. of isolates within MIC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
6		2	4				

Table 8. MBC to anaerobic bacterial strain from natural teeth.

Numbers Of The isolates	No. of isolates within MBC						
	Conc. Of the Zno NPs mg/ml						
	0.02	0.05	0.08	0.1	0.25	0.5	1
6		1	1	4			

This means that (0.05 and 0.08) mg/ml concentrations were able to inhibit the bacterial growth so the MIC for ZnO NPs against anaerobic bacteria from natural tooth is 0.08mg/ml.

0.08mg/ml concentration showed growth after re-culturing on plain BHI –A media while 0.1mg/ml concentration showed no growth.

This mean that 0.1 mg/ml concentration had bactericidal effect and killed the bacteria, So that MBC of ZnO np that kill anaerobic bacteria from natural tooth was 0.1mg/ml concentration as shown in Table 8.

Many microorganisms are emerging as crucial opportunistic pathogens of the oral cavity. Therefore, minimizing their population in the oral cavity will be of importance for maintaining good oral health and preventing periodontitis.

A wide variety of synthetic compounds exert antibacterial effect, but just some of them can be used as biocides to develop drugs or coatings. The primary impediment for their use is their toxicity compared with their bactericidal effect some of them are so toxic for eukaryotic cells that cannot be proposed as antibiotics. Among these materials ZnO nanoparticles compounds raise as potent antimicrobial agents. The advantage of using these inorganic oxides as antimicrobial agents is that they contain environmentally safe mineral elements essential to humans and exhibit strong activity even when administered in small amount. In our study were determined MIC and MBC against aerobic and anaerobic bacterial strain from implant hole and sulcus of natural teeth. The MIC of the ZnO NPs (10-30) nm at which concentration give weak growth in agar dilution test. The data shows that aerobic and anaerobic bacterial isolates from implant hole or from sulcus of natural teeth inhibited by concentration ranged from (0.05 - 0.08) mg/ml and this agree with Suha et al [29]

This means that the bacteria found in the sulcus of natural teeth also found in the implant [14-16]. The MBC determined on agar dilution test when the least concentration kill bacteria give no growth for aerobic and anaerobic bacterial isolates from internal cavity of implant fixture and sulcus of natural teeth (0.08-0.1) mg/ml.

CONCLUSION

Nanoparticulate zinc oxide have received particular care as a result of their antimicrobial activity, durability and commercially available. The study revealed that ZnO NPs were effective against aerobic and anaerobic bacteria that isolates from implant and sulcus of the natural teeth and MIC started from 0.05mg/ml and MBC started from 0.08mg/ml.

CONFLICT OF INTEREST

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

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