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IN-VITRO EVALUATION OF THE EFFECTS OF STERILIZATION PROCEDURES ON THE ANTIBACTERIAL CHARACTERISTICS OF ZINC OXIDE NANOPARTICLES COATED ORTHODONTIC MINI SCREWS

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ABSTRACT

Background: There is a lack of data in regard to sterilization effects on titanium alloy mini screws and subsequent changes in their mechanical properties and clinical function. However none of these researches investigated the effects of repeated sterilization on antibacterial characteristics of coated orthodontic mini screws with different agents. Accordingly, the aim of this study was to evaluate the effects of sterilization procedures on the antibacterial characteristics of zinc oxide nanoparticles coated mini screws.

Methods: A steam autoclave was used to sterilize a group of fifteen zinc oxide nano coated Self-drilling Ti6 Al4 V alloy mini screws. SEM (Scanning electron microscope) and EDAX (Energy-dispersive X-ray analysis) analysis were used to analyze the ZnO nanoparticles morphology and chemical composition of the particles after sterilization. Antibacterial activity of the sterilized and non-sterilized coated mini screws was compared against Streptococcus mutans (Gram-positive), Staphylococcus aureus (S.aureus) (Gram-positive) and Escherichia coli (E. coli) (Gram-negative).

Results: According to measurements of the zone of inhibition of the test samples for quantitative assessment, the area of the zone of inhibition for S.aureus around (25 ± 4.88 mm) was the largest with non-sterilized ZnO NP coated mini screws, followed by that of S. mutans ; no zone of inhibition was observed for E.coli. The sterilized mini screws showed no antibacterial effects on both S. mutans and E-coli test bacteria, only S.aureus showed smallest zone of inhibition.

Conclusion: Sterilization of coated ZnO mini screws has weak effect on antibacterial properties, making it possible to sterilize it before screw insertion.

KEYWORDS: Orthodontic mini screw; Scanning electron microscope; Sterilization

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INTRODUCTION

One of the main problems faced by clinicians has been resolved with the introduction of temporary anchoring devices (TADs), such as plates and screws, which have given rise to a system that is almost entirely anchored during orthodontic movement of teeth. Mini screws have been employed in a wide range of orthodontic mechanic setups and applications in the brief time since their inception, making treatment options for both basic and complex situations almost endless. [1]

Mini screws used in orthodontic therapy may succeed or fail depending on several variables, including osseointegration at the bone-screw interface and the level of bacterial colonization surrounding the screws.[2] Failure of mini screws is related to peri-implantitis causing inflammatory disease and bone loss, particularly in the area surrounding the screws' necks.[3] An inflammatory lesion known as peri-screw mucositis was observed in up to 50% of patients,[4] that not only harms the supporting bone but also has an impact on the soft tissues.[5] Limiting the degree of inflammation and preventing the attachment of microorganisms to the surface of the implanted devices are therefore necessary for the long-term stability and effectiveness of the screw.[6]

It was discovered that Staphylococcus aureus, Streptococcus sanguinis, and Streptococcus mutans are the primary colonizers that stick to the surfaces of teeth and screws. [7] A gram-negative bacteria known as E. coli is also fundamentally recognized to be the cause of several periodontal and peri-screw disorders. The bacterial aggregation surrounding titanium-based prostheses has been reduced or eliminated using a variety of techniques, including polishing and other surface treatments that alter surface-free energy. [8-11]

Particularly well-known for their wide range of antibacterial and anti-inflammatory capabilities are zinc oxide nanoparticles (NPs), the antibacterial properties of coated orthodontic mini-screws with various agents, including zinc oxide NPs, have been the subject of several prior researches. [12-15]

There are a lot of data in the literature about how sterilization affects other orthodontic products, like nickel-titanium arch wires and different types of pliers, but not much about how sterilization affects titanium alloy mini screws and how that affects their mechanical characteristics and clinical utility.[12-14] Previous studies[1] have established the use of insertion torque and lateral displacement from applied forces as reliable parameters to compare the effects of sterilization based on mini screw stability.

This study focused on effects of sterilization on titanium mini screws coated with zinc oxide NPs, aiming to test the null hypothesis that there was no difference in the antibacterial effects of nano coated mini screws following sterilization.

MATERIALS AND METHODS:

This study included twenty-four ZnO NPs coated titanium alloy mini screws (Dentaurum, Turnstr. 31 I 75228 Ispringen I Germany), which were randomly divided into serilized group and non-sterilized group. The study was carried out at Faculty of Dentistry, Minia University.

Ethical committee No 107, Faculty of Dentistry-Minia university, in 28/5/2024 considered before starting our study.

In this work, the magnetron sputtering method was used for coating of zinc oxide nano coating on Self-drilling mini screws **(Tomas pins SD, Dentaurum , Germany)** which had 8.0 mm length and 1.6 mm diameter.

This was carried out by the use of PROTOFLEX model 1600physical vapor deposition platform of ANGSTROM ENGINEERING INC., Faculty **of Nanotechnology For postgraduate studies, Cairo University, Cairo, Egypt**. (Figure 1)

Fig. (1) PROTOFLEX physical vapor deposition platform, Faculty of Nanotechnology for postgraduate studies, Cairo University, Cairo, Egypt., used for coating mini screws with zinc oxide NPs.

The results of a power analysis were presented for a test comparing two proportions. The analysis is focused on determining the sample size needed to achieve a target power of 0.9 when comparing a baseline proportion of 0.1 to a comparison proportion of 0.7, using a two-sided test with a significance level (α) of 0.05.

The results indicate that a sample size of 12 per group is required to achieve the desired power. With this sample size, the actual power achieved is 0.905838, which slightly exceeds the target power of 0.9. This means that if the true difference between the proportions is as large as specified (0.1 vs 0.7), the study has a 90.6% chance of detecting this difference as statistically significant.

Twenty-four mini screws were divided into two groups of 12 each. One group was exposed to one cycle of sterilization and compared with the other non-sterilized group. Computer generated program for randomization will be used to allocate eligible samples to intervention and control groups with allocation ratio 1:1.

The first group was sterilized using the Statim 5000 autoclave equipment (SciCan USA, Canonsburg, Pa.) and steam sterilization in sealed bags in accordance with the American Dental Association's standards for important instruments. For six minutes, cycles at 132°C were conducted. In order to guarantee adequate sterilization, chemical and biological indicator tests were incorporated.

Characterization of ZnO nanoparticles:

Scanning electron microscope (SEM) (ZEISS EVO 10, CARL ZEISS AG Göttingen, Germany) (figure 2) and energy-dispersive x-ray spectroscopy (EDAX) analysis were used to examine the surface morphology of the mini screws and determine the chemical content and morphology of the ZnO NPs.

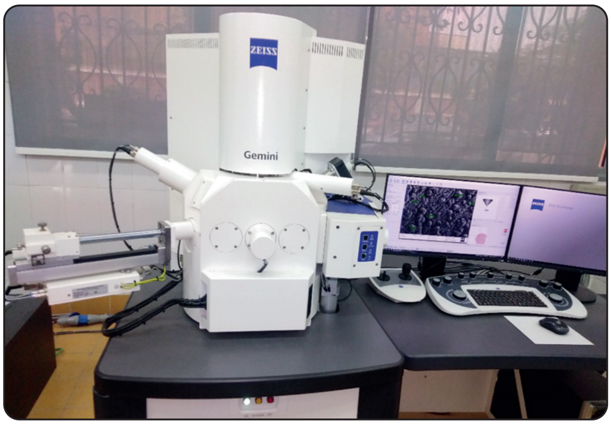

Fig. (2) Scanning electron microscope (SEM) and energy-dispersive x-ray spectroscopy (EDAX) analysis

Antibacterial activity of the ZnO NPs coated mini screws:

The antibacterial properties of the coated mini screws were evaluated against Gram-negative E. coli, Gram-positive Staphylococcus aureus, and Streptococcus mutants. Twelve aseptic plates, each holding two cubic centimeters (cc) of nutritional agar, were prepared. After that, the plates were incubated at 37 °C for 24 hours. The antibacterial activity of coated groups that had been sterilized and those that had not was assessed. The plates received the transfer of twenty-four Mini screws. Thus, measuring the area surrounding the test mini plates was assessed for bacterial growth inhibition.

Statistical analysis:

Several normality tests were performed, including Shapiro-Wilk, Anderson-Darling. All tests consistently failed to reject the null hypothesis of normality at the 5% significance level. The Shapiro-Wilk test, often considered one of the most powerful tests for normality, yielded a p-value of 0.6074. The D'Agostino Omnibus test, which combines skewness and kurtosis measures, resulted in a p-value of 0.8362.

The statistical analysis in this study primarily used paired t-tests to compare various properties between non-sterilized and sterilized-coated mini-implants. Paired t-tests were employed to assess differences in the atomic and weight percentages of elements (carbon, oxygen, titanium, and zinc), with significant differences ($p<0.05$) reported for all elements. The same test was used to compare particle sizes between the two types of mini-implants, revealing a significant difference ($p<0.0001$). Additionally, paired t-tests were utilized to evaluate differences in inhibition zones against Staphylococcus aureus and Streptococcus mutans, again showing significant differences ($p < 0.0001$) between non-sterilized and sterilized-coated mini-implants. The consistent use of paired t-tests suggests that the study design involved matched samples, allowing for direct comparisons between the two conditions (non-sterilized vs. sterilized) across various measurements.

RESULTS

Characteristics of the ZnO nanoparticles:

Sterilized ZnO-coated mini screws viewed under a scanning electron microscope showed that the NPs were characterized by relative homogeneity with regard to of particle size and topographical dispersion. (Figure 3).

SEM images for the sterilized ZnO coated mini screws revealed nanoparticles of spherical shape with perfect homogenous distribution. The images showed agglomeration as non-sterilized one, meaning that the sterilization of coated mini screws have a good dispersion of nanoparticles (Fig. 4). The particle size was 138-450 nm to aggregates of size of 13 micron.

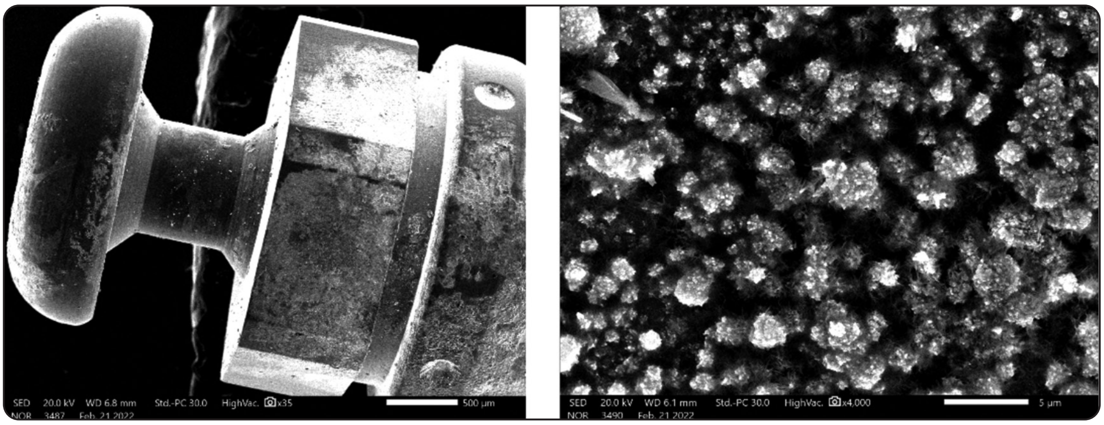

Fig. (3) Scanning electron microscope views of head of sterile coted mini screw (left side magnified 35 times, right view after 4000 times magnification).

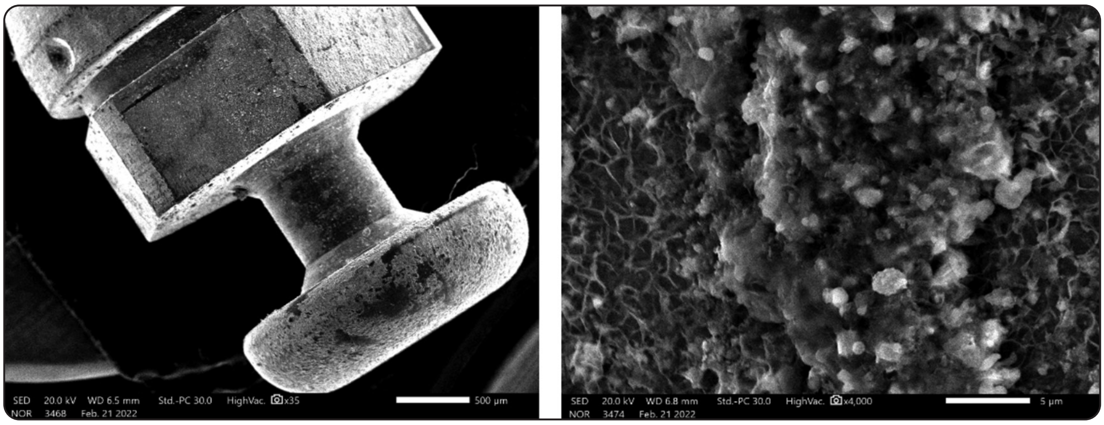

Fig. (4) Scanning electron microscope views of head of non-sterile coted mini screw (lleft side magnified 35 times, right view after 4000 times magnification).

Table 1 compares the particle size (in nanometers) of the non-sterilized and sterilized-coated mini screws. The results show a significant difference ($p < 0.0001$) in particle size, with sterilized-coated mini screws having a much larger average particle size (272.4 ± 60.03 nm) compared to non-sterilized-coated mini screws (62.64 ± 27.88 nm). The bar chart (figure 5) illustrates this difference clearly, with the bar representing sterilized-coated mini screws being significantly longer than the bar for non-sterilized-coated mini screws.

Table 2 show that ZnO NPs formed on the surface of the non-sterilized mini screw (Zn(zinc)= 50.00±0.84 percent by weight, O(oxygen) = 32.99±0.43 percent by weight, Ti (titanium)= 9.52±0.20 percent by weight, and C(carbon) = 7.49±0.19 percent by weight), as evidenced by EDAX analysis.

TABLE (1) Comparison of Particle size (nm) of non-sterilized and sterilized-coated mini screws.

Particle size (nm) (M±SD)		P-value
Non-Sterilized-Coated Mini screws	Sterilized Coated Mini screws	
62.64±27.88	272.4±60.03	<0.0001*

M; Mean, SD; Standard Deviation, P; Probability Level
****; significant difference using Paired t-test***

Table 3 displays the results of an EDAX study of a sterilized coated mini screw with the following composition: Zn=40.29±0.69, O=29.54±0.45 by weight, Ti=24.76±0.29 by weight, and C=5.41±0.14% by weight.

The given table (Table 4) presented a comparison of the atomic and weight percentages of various elements present in non-sterilized and sterilized-coated mini screws. The elements analyzed are carbon (C), oxygen (O), titanium (Ti), and zinc (Zn).

The data in the table reveals significant differences between the non-sterilized-coated and sterilized-coated mini screws for all the elements

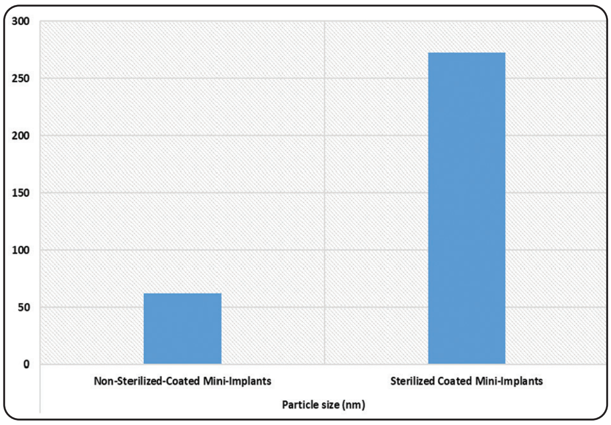

Fig. (5) Bar chart of comparison of particle size (nm) of non-sterilized and sterilized-coated mini screws.

TABLE (2) Atomic% and weight% of the elements of non-sterilized-coated mini screws.

Element	Line	Mass%	Atom%
C	K	7.49±0.19	17.10±0.43
0	K	32.99±0.43	5650±0.74
Ti	K	9.5.2±0.20	S.4S±0.12
Zn	K	50.00±0.84	20.96±0.35
Total		100.00	100.00
Spc_001		Fittin ratio 0.0282	

TABLE (3) Atomic% and weight% of the elements of sterilized coated mini screws.

Element	Line	Mass%	Atom%
C	K	5.41 ±0.14	13.14±0.33
O	K	.29.54±0.45	53.83±0.82
Ti	K	.24.76±0.29	15:07 ±0.18
Zn	K	40.29±0.69	17.96±0.31
Total		100.00	100.00
Spc_006			Fittin ratio 0.0263

TABLE (4) Comparisons of Atomic% and weight% of the elements of non-sterilized and sterilized-coated mini screws.

Element	Line	Mass % (M±SD)		P-value	Atom % (M±SD)		P-value
		Non-Sterilized-Coated Mini screws	Sterilized Coated Mini screws		Non-Sterilized-Coated Mini screws	Sterilized Coated Mini screws	
C	K	7.49±0.19	5.41±0.14	< 0.0001*	17.10±0.43	13.14±0.33	< 0.0001*
O	K	32.99±0.43	29.54±0.45	< 0.0001*	56.50±0.74	53.83±0.82	< 0.0001*
Ti	K	9.52±0.20	24.76±0.29	< 0.0001*	5.45±0.12	15.07±0.18	< 0.0001*
Zn	K	50.00±0.84	40.29±0.69	< 0.0001*	20.96±0.35	17.96±0.31	< 0.0001*

M; Mean, SD; Standard Deviation, P; Probability Level ****; significant difference using Paired t-test***

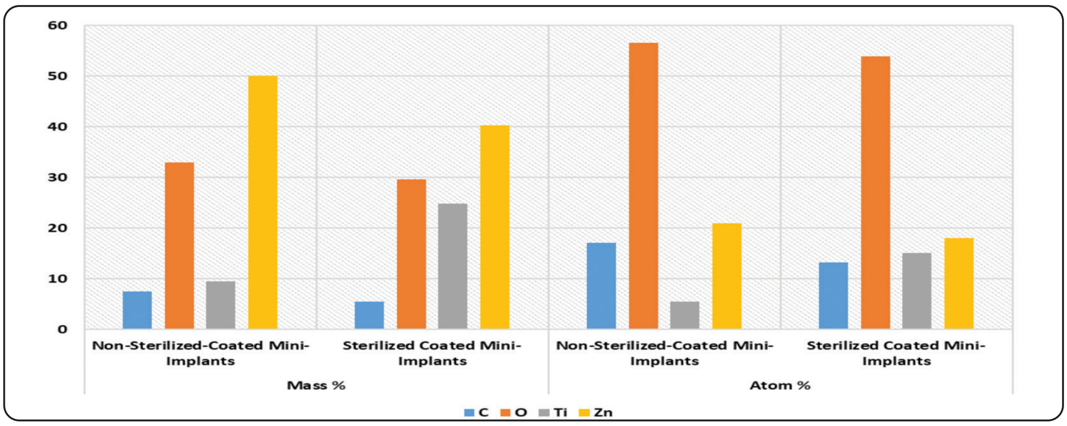

Fig. (6) Bar chart of comparisons of Atomic% and weight% of the elements of non-sterilized and sterilized-coated mini screws.

analyzed, as indicated by the P-values (marked with an asterisk) obtained from the paired t-test.

Specifically, the sterilized-coated mini screws exhibit lower percentages of carbon (5.41%±0.14% by weight, 13.14% ± 0.33% by atom) and oxygen (29.54%±0.45% by weight, 53.83%±0.82% by atom) compared to the non-sterilized-coated mini screws (carbon: 7.49%±0.19% by weight, 17.10%±0.43% by atom; oxygen: 32.99%±0.43% by weight, 56.50%±0.74% by atom).

Conversely, the sterilized-coated mini screws show higher percentages of titanium (24.76%±0.29% by weight, 15.07%±0.18% by atom) compared to the non-sterilized-coated mini screws (9.52%±0.20% by weight, 5.45% ± 0.12% by atom).

For zinc, the non-sterilized-coated mini screws exhibit a higher percentage (50.00% ± 0.84% by weight, 20.96% ± 0.35% by atom) compared to the sterilized-coated mini screws (40.29% ± 0.69% by weight, 17.96% ± 0.31% by atom).

The table is divided into three main columns: Element, Line, and two sub-columns for Mass % and Atom %. The Mass % column provides the mean and standard deviation values for the weight percentages of each element in non-sterilized-coated and sterilized-coated mini screws. Similarly, the Atom % column presents the mean and standard deviation values for the atomic percentages of the respective elements.

Antibacterial activity test

While non-sterilized coated mini screws showed antibacterial effect around them, sterilized coated mini screws showed less antibacterial effect. Following a 24-hour incubation period at 37°C for all strains of bacteria employed, including Gram-positive S. aureus, Gram-positive S. pyogenes, and Gram-negative E. coli,,sterilized ZnO-coated mini screws presented bacterial growth around both *S. pyogenes* and E-coli. Inhibition zone was formed only around ZnO NPs coated mini screws with *S. aureus*. (FIGURE 7).

The existence of a zone where S. aureus growth is inhibited validates the antibacterial action of the mini screws. No inhibition zone was observed around the sterilized ZnO NPs coated mini screws of *S. pyogenes* and E-coli.

Table 5 presents the comparison of the inhibition zones (a measure of antimicrobial activity) against Staphylococcus aureus and Streptococcus mutans for non-sterilized and sterilized-coated mini screws. The results show significant differences ($p < 0.0001$) in the inhibition zones for both bacterial species.

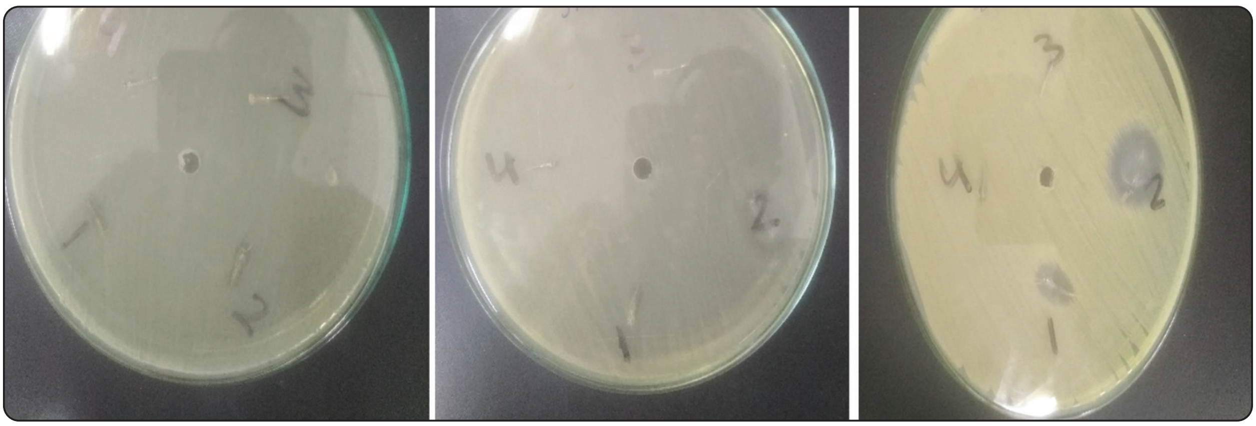

Fig. (7) A-no zone of inhibition around sterilized or non-sterilized coated mini screw, B-zone of inhibition around only non-sterilized Mini screw for S.aureus . C- zone of inhibition around non-sterilized coated mini screw around both for S. mutans and S.aureus.

TABLE (5) Comparison of inhibition zone of non-sterilized and sterilized-coated mini screws.

	Inhibition Zone (M±SD)		P-value
	Non-Sterilized-Coated Mini screws	Sterilized Coated Mini screws	
Staphylococcus Aureus	24.30±4.218(mm)	13.00±2.582(mm)	<0.0001*
Streptococcus Mutans	19.8±2.588(mm)	0±0(mm)	<0.0001*

M; Mean, SD; Standard Deviation, P; Probability Level ****; significant difference using Paired t-test***

For Staphylococcus aureus, non-sterilized-coated mini screws exhibited a larger average inhibition zone (24.30±4.218 mm) compared to sterilized-coated mini screws (13.00±2.582 mm). Similarly, for Streptococcus mutans, non-sterilized-coated mini screws showed an average inhibition zone of 19.8±2.588 mm, while no inhibition zone was observed for sterilized-coated mini screws (0 ± 0 mm). The bar chart shows these differences, with longer bars representing larger inhibition zones for non-sterilized-coated mini screws against both bacterial species. (Figure 8)

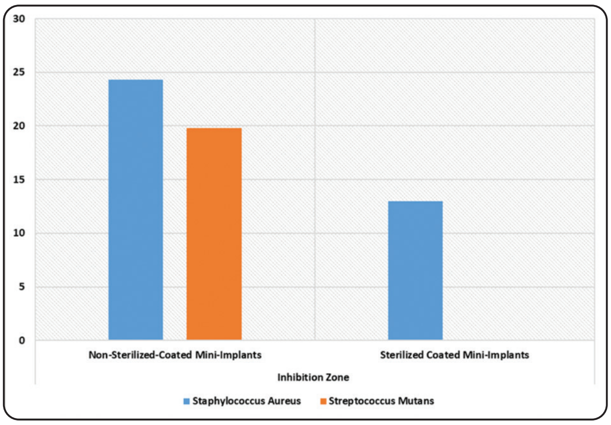

Fig. (8) Bar chat of comparison of inhibition zone of non-sterilized and sterilized-coated mini screws.

DISCUSSION

The titanium mini screws are good options for anchorage because they offer complete anchorage without damaging neighboring teeth in the dental arch. Due to their high failure rate [11], they do have significant drawbacks. In addition, surface roughness contributes to bacterial accumulation. ZnO NPs were employed in this work to coat titanium mini screws and then sterilized, their anti-bacterial characteristics were assessed**.**

Chemical analysis of the sterilized coated mini screws demonstrated the outstanding of ZnO NPs on the mini screw surface (Zn = 40.29±0.69 percent by weight, O = 29.54±0.45 percent by weight, Ti = 24.76±0.29 percent by weight, and C = 5.41±0.14 percent by weight). The EDAX analysis of the coated mini screws confirmed that the sterilized mini screws consisted of titanium, zinc and oxygen.

These differences in the elemental compositions between sterilized and non-sterilized coated mini screws suggest that the sterilization process may have influenced the surface characteristics of the coated mini screws, potentially affecting their properties and performance. The observed changes in the percentages of carbon, oxygen, titanium, and zinc could be attributed to various factors, such as oxidation, deposition, or removal of certain elements during the sterilization process.

The current results support the findings of Kachoei et al.[12], who found a stable and well-adhered ZnO coating on NiTi wires. The coated wires showed up to a 21% reduction in frictional forces and anti-bacterial activity against Streptococcus mutans. ZnO nanocoating dramatically enhanced the surface quality of NiTi wires. The EDS analysis of the coated wires verified that they are composed of nickel, titanium, zinc, and oxygen. The EDS results revealed that there were no other elemental contaminants in the ZnO NPs employed in this investigation.

The sterilized coated mini screw surface had ZnO NPss with a diameter of 138-450 nm to aggregates of size of 13 micron, which were sparsely spread in the SEM images. Many ZnO NPss still present as clusters or agglomerate as the non-sterilized screws (Figure 1D). These findings are consistent with those of Kachoei et al. [15], who reported spherical ZnO NPs ranging in size from 25 to 30nm.

In addition to evaluating the forces of friction that exist between the bracket and wire, Behroozian et al.[14] employed SEM to analyze the surfaces and patterns of ZnO nanoparticle deposition. These images clearly demonstrated an existence of ZnO NPs that are spherical on both the wire and the porcelain brackets. However, very few researches have investigated how sterilization affected orthodontic mini screws used in orthodontic applications. [1] The findings of this investigation showed that ZnO NPs significantly reduced the growth of several bacterial strains, including S. aureus and S. mutans (Gram-positive) and relatively sustain its antibacterial activity after sterilization. According to the observed inhibitory zones pattern, ZnO NPs have a stronger antibacterial effect on bacteria that are Gram-positive than on Gram-negative bacteria.

Although the precise mechanism of action is still unknown, nanoparticles' antibacterial mechanism can be broadly classified into three types. First, the compound interacts with the cell wall and membrane of peptidoglycan, leading to cell lysis; then, it disrupts protein synthesis by engaging with bacterial proteins; and lastly, it interacts with the bacterial (cytoplasmic) DNA, blocking DNA replication. [19-20-21]

A lot of conjecture surrounds ZnO NPss' antibacterial mechanism. Many explanations for this phenomenon focus on these nanoparticles capacity to release ionic zinc in an aqueous solution. [22] Others are connected to their capacity to create reactive oxygen species, while some are connected to their capacity to interact with the bacterial cell membrane. [24]

Most significantly, because of their wide surface area, which consequently gives for a broader area for microbial interaction, ZnO NPss are known to elicit antibacterial characteristics. According to certain research, zinc ions have a great affinity for the electron donor groups found in a variety of bacterial cells that include sulfur, oxygen, or nitrogen. [24] Streptococcus sp. And Staphylococcus sp., as the most prevalent bacteria in the oral microbiota,[25] were deemed suitable for carrying out the antimicrobial examinations for the coating technique in this investigation.

The results of this investigation correspond with those of Ramazanzadeh et al.'s [13] study towards the antimicrobial efficacy of brackets coated with ZnO and CuO (copper oxide) NPs towards S. mutans. In that study, bacterial suspension was introduced to the coated brackets, and 10μL of suspension was extracted from each tube at intervals of 0, 2, 4, 6, and 24 hours, and then cultured. The observation revealed an excellent antimicrobial effect of the coated brackets containing ZnO-CuO and ZnO NPs against S. mutans, as the bacterial count was eliminated after two hours of bacterial suspension addition to the coated brackets. The ZnO nanoparticle-coated brackets came in second place, but even after 24 hours, they were unable to completely eradicate S. mutans from the population, even though they significantly reduced it compared to the control group.

Azam et al. [22] When comparing the antibacterial activity of CuO, Fe_2O_3(ferric oxide) , and ZnO NPss against Gram-positive (S. aureus and P. aeruginosa) and Gram-negative (E. coli and Pseudomonas) bacteria, it was found that ZnO NPs had the greatest antibacterial effect and Fe2O3 NPs had the least activity. Cu NPs exhibit different chemical, physical and biological features and are inexpensive to prepare, but their use in orthodontics is limited because of their quick oxidation in the air.

To find out whether the sterilized ZnO NPss surfaces were still antibacterial, we employed the diffusion agar method. In all positive bacterial culture plates, the ZnO NPss coated mini screws demonstrated excellent antibacterial activities with distinct zones of inhibition while no zone of inhibition was seen surrounding the sterilized mini screw in S.mutans (Figure 7) . The quantity or concentration of ionic zinc that was leached from the test mini screws surface determined the results of our zone of inhibition testing. Moreover, in an aqueous environment, elemental zinc dissolves slowly.

Nanozinc is very reactive and easily interacts with surface oxygen to generate oxides, reducing the available free nanozinc for interaction and hence decreasing the antibacterial capabilities. [26]; accordingly, it was believed that the decreased concentration of zinc accounting for about 40.2 weight % of ZnO NPss on the mini screw surface after sterilization might explain why it was less antimicrobial than non-sterile coated mini screws.

In summary, the study found significant differences in the composition, particle size, and antimicrobial activity between non-sterilized and sterilized-coated mini screws. Non-sterilized-coated mini screws had higher percentages of oxygen, zinc, and carbon, smaller particle sizes, and larger inhibition zones against Staphylococcus aureus and Streptococcus mutans. In contrast, sterilized-coated mini screws had higher percentages of titanium, larger particle sizes, and smaller inhibition zones against the tested bacterial species.

This study's primary weakness is that it was conducted in a laboratory, making it impossible to apply directly to a strictly clinical scenario. The study's benefit in a lab environment, however, was the ability to regulate and homogenize a large number of factors for the most precise evaluation of the findings.

Further studies are recommended to detect the mechanical characteristics change, maximal insertion torque, primary stability, lateral displacement force, and modifications to the integrated material's physical characteristics, as well as the biopolymer coating's sustainability throughout the clinical use of zinc oxide nano-coated mini screws following sterilization.

CONCLUSION

The SEM photographs of the mini screws in the current investigation revealed that autoclave sterilization stimulates agglomeration of nanoparticles on coated mini screws, decreases percentage of zinc on the surface of mini screws. But the antibacterial evaluation revealed that sterilization of ZnO-NPs coated mini screws decreased its outstanding advantage of antibacterial effect.

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