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Evaluation of the Cell Viability Effects of Orthodontic Mini-Implants Coated with Zinc Oxide Nanoparticles: An In Vitro Study

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ABSTRACT
Background: Success and long-term stability of orthodontic mini-implants rely on limiting the inflammation amount and inhibiting the microbes adhesion on the surface of the implanted devices. Zinc Oxide nanoparticles were known to be used as a coating on orthodontic mini-implants to decrease colonization and plaque formation. The mini-implants should be biocompatible because they are in contact with the mucosa and fluid of the oral cavity for a long time. This study aimed to assess the cell viability around orthodontic mini-implants coated with zinc oxide (ZnO) nanoparticles.
Methods: Forty orthodontic mini-implants were separated into two groups, control group (uncoated mini-implants), and ZnO group (zinc oxide-coated mini-implants). The magnetron sputtering technique was used to cover orthodontic implants with nano-ZnO. In both groups, electron microscope and EDAX (Energy-dispersive X-ray analysis) analysis were used to examine the morphology of ZnO nanoparticles and the chemical composition of them after coating procedure and to assess and visualize the coated mini-implants surface morphology. The coated mini-implants were assessed for cytotoxicity in human lung fibroblast (HLF) using the viable cells yield which was determined by a colorimetric method to evaluate biocompatibility of zincoxide nanoparticles as implantable material. Optical density and percentage of viable cells were compared using one-way analysis of variance with a post-hoc test.
Results: In the ZnO group, a homogenous layer of nanoparticles on the mini-implant was demonstrated by Scanning electron microscope (SEM). At lower concentrations of ZnO nanoparticles (12.5 mg/ml to 0.475 mg/ml), the cell viability in the ZnNPs group was generally comparable to the second group. This implies that at lower concentrations, ZnNPs did not significantly affect cell viability.
Conclusions: Zinc oxide nanoparticles coated mini-implants generally exhibited insignificant cytotoxicity to human fibroblasts. They can be safely used as a coating of orthodontic mini-implants.

Keywords: Orthodontic mini-implants; ;Coating ; Nanoparticles ;Cytotoxicity.

INTRODUCTION

Surgical stainless steel, titanium alloys, and commercially pure titanium are frequently used in orthodontic mini-implants production. Due to its superior tissue compatibility, bacteriostatic action, corrosion resistance, and mechanical strength, titanium alloy is favored over stainless steel. This is due to the titanium dioxide (TiO2) coating that develops on the alloy surface. (1)
Mini-implants used in orthodontic therapy may succeed or fail depending on several variables such as the level of bacterial colonization surrounding the implants and osseointegration at the bone-implant interface. (2) Failure of mini-implants is related to peri-implantitis causing inflammatory disease and bone loss, particularly in the area surrounding the implant's neck. (3) Peri-implant mucositis is an inflammatory lesion observed in 50% of patients. (4) It 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 implanted devices' surfaces is, therefore, necessary for the long-term stability and effectiveness of the implant. (6)
It was discovered that Staphylococcus aureus, Streptococcus sanguinis, and Streptococcus mutans are the primary colonizers that stick to the surfaces of teeth and implants. A gram-negative bacteria known as E. coli is also fundamentally recognized to be the cause of several periodontal and peri-implant disorders. (7) 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.
The antibacterial properties of coated orthodontic mini-implants with various elements such as silver and copper oxide nanoparticles, have been the subject of several prior research. (12) This procedure involved using straightforward electrolysis in a solution with the necessary metal ion or its chemicals to deposit a thin and securely adherent layer of metal oxide on the surface of a conductor substrate.
Other inexpensive method is, physical vapor deposition, also may be used that offers coated metals corrosion resistance, may enhance coated metals' mechanical properties, and can offer a fresh approach to treating and preventing dental diseases. (13) Additionally, NPs coated on biomaterials or coupled with polymers show improved antibacterial capabilities in the oral cavity. (14)
The biocompatibility of orthodontic brackets and wires coated with nano-ZnO was investigated (15) but research on orthodontic mini-implants are lacking.
In light of the aforementioned data, the goal of this work was to evaluate cytotoxicity of ZnO nanoparticles as an implantable material, aiming to test the null hypothesis that both coated and non- coated mini-implants have similar effects on cell viability.

MATERIALSANDMETHODS
Forty titanium alloy (Ti6Al4V) mini-implants (Dentaurum, Turnstr. 31 I 75228 Ispringen I Germany) were divided into two equal groups of, control (non-coated) group and experimental group (coated) to be ZnO nanoparticles coated . Computer generated program for randomization (Random Allocation Software 2.0, Informer Technologies.Inc.) was used to allocate eligible samples to intervention and control groups with allocation ratio 1:1.
For sample size calculation, the power analysis was performed using G power soft-ware by T test ,the criteria for significance was set at 0.05 (type I error) and total power of 80%, the effect size was set at 0.94 depend on the difference in mean cell viability between studied groups.(16) (0.45±0.02 for ZnO group and 0.48±0.04 for control group), the sample size calculated was 38, divided into 2 equal groups with 19 in each group.
This study was conducted at Faculty of Dentistry, Minia University, Faculty of Nanotechnology For postgraduate Studies, Cairo University, El-Sheikh Zayed and Campus and Central Laboratory for Microanalysis and Nanotechnology, Minia University.
Ethical committee No 107-2024, Faculty of Dentistry- Minia university, was considered before starting this study.
In the coated group, the magnetron sputtering of zinc oxide nano-coating was used on Self-drilling titanium alloy mini-implants (Tomas pins SD, Dentaurum, Germany),which had 1.6 mm diameter and 8.0 mm length.
This was carried out by the use of PROTO-FLEX model 1600physical vapor deposition platform of ANGSTROM ENGINEERING INC., Faculty of Nanotechnology For postgraduate studies, Cairo University, Cairo, Egypt. (Figure 1)
Orthodontic mini-implant substrates were covered with pure porous Zn using direct current (DC) magnetron sputtering. These substrates were initially washed for 1-2 hours at room temperature with a mixture of chromium (7gK2Cr2O7-10ml H2O-100ml H2SO4). A consistent vacuum pressure of $5 \times 10{-}3$ Torr was maintained by carefully controlling the pressure of the argon (Ar) working gas. The deposition duration was 9 to 22 minutes, and the DC utilized was 0.12 to 0.15 A. 210°C or thereabouts was the steady temperature for the substrate.

Coating verification of ZnO nanoparticles on mini-implants:
SEM (ZEISS EVO 10, CARL ZEISS AG Göttingen, Germany) (Figure 3) and EDAX analysis were used to assess the surface morphology of the mini-implants and determine the chemical content and morphology of the ZnO nanoparticles.

Cytotoxicity of the ZnO nanoparticles coated mini-implants:
Cell culture.
Normal human lung fibroblast cell line (HLF) (WI0-38, CCL 75) was kindly supplied from the International Center for Training and Advanced Research, Cairo, Egypt. Cells were cultured in Minimum Essential Medium (MEM-E) containing 10% fetal bovine serum (GIBCO, Thermo Fisher Scientific, Waltham, MA, USA) in an atmosphere of 5% CO2 at 37°C (Jouan SA, Saint-Herblain, Pays de la Loire, France). Depending on the manufacturing protocol, cells were maintained where the growth medium was decanted, and they were treated with phosphate buffer saline (Adwia Pharmaceuticals, Sharqia, Egypt). Cells were treated with 0.05% (v/v) EDTA (GIBCO) and 0.25% trypsin enzyme for five minutes at 37°C. Finally, the detached cells were spitted depending on the need.

Cell Viability

The viable fibroblasts' percentage was measured by the colorimetric MTT assay, which assessed the capability of mitochondrial succinate dehydrogenase to convert the yellow color tetrazolium dye into purple color formazan. After that, these cells were divided into three groups (each group contains 10 well plates) and then incubated. Following the confluence of the cells, Zno NPs-coated mini-implants were put into the cell plates and incubated at 37°C with 5% CO2 in the air for two days. Culture media containing ZnO nanoparticles and cells were incubated under similar conditions as a second group. Culture media containing cells without mini-implants were also incubated under identical conditions as a control. After the incubation, MTT dye (200 µl) for every ml of culture was added to each well and then the plates were incubated for 4 hours at 37°C with 5% CO2 in the air. The preparation of MTT solution was through dissolving the dye (5 mg) in Phosphate buffered saline PBS (1 ml) and then filtering the mixture through a filter (0.2 µm). Following intubation, dimethyl sulfoxide (300 µl) was used for each culture well and incubated for about 30 minutes to lyse cells and provide a uniform color. Finally, the solution was centrifuged for two minutes to sediment the cells and then 100 µl from each well was put into a new plate for measuring the optical density. (17-18-19-20)

The assay depended on the ability of metabolically active fibroblasts to convert the yellow-colored MTT salt to purple formazan crystals. The obtained purple color intensity was proportional to the viable cells quantity and was calculated as the absorbance degree or optical density (OD) at 570 nm by using an enzyme-linked immunosorbent assay reader (U2000, Hitachi, and Tokyo, Japan). The cell viability was measured as the ratio between the optical densities of the experimental wells and that of the control ones.

The mean optical density and the percentage of cell viability were calculated.

The cell viability percentage was calculated using the following formula:

Viability percentage (%) = Mean OD (optical density) of test dilution × 100/Mean OD of control wells. (21)

The viability percentage, mean optical density (MOD), and standard deviation (SD) were reported for each group and concentration. The viability percentage indicates the percentage of cells that remained viable after exposure to the respective treatment. The MOD is a measure of the absorbance of the dye used to assess cell viability, with higher values indicating higher viability.

Statistical analysis:

Shapiro- wilk test was used to test normality of the data (the cell viability effects of ZnONPs at different concentrations)

one-way analysis of variance (ANOVA) comparing the cell viability effects of ZnONPs at different concentrations was done Simple linear regression model analyses for the optical density and cell viability percentage means of orthodontic mini-implants coated with zinc oxide nanoparticles (ZnONPs) at different concentrations were done. These analyses aimed to investigate the relationship between the concentration of ZnONPs and the respective dependent variables (optical density and cell viability percentage).

Comparison of the percentage of cell viability and optical densities between both groups was assessed with ANOVA and Post hoc Tuckey HSD. The P-value was considered statistically significant as it was ≤ 0.05.

Figure 1: PROTOFLEX physical vapor deposition platform, Faculty of Nanotechnology For postgraduate studies, Cairo University, Cairo, Egypt.

Figure 2: Bel model M214a balance for accurate weighing of mini-implants before and after coating.

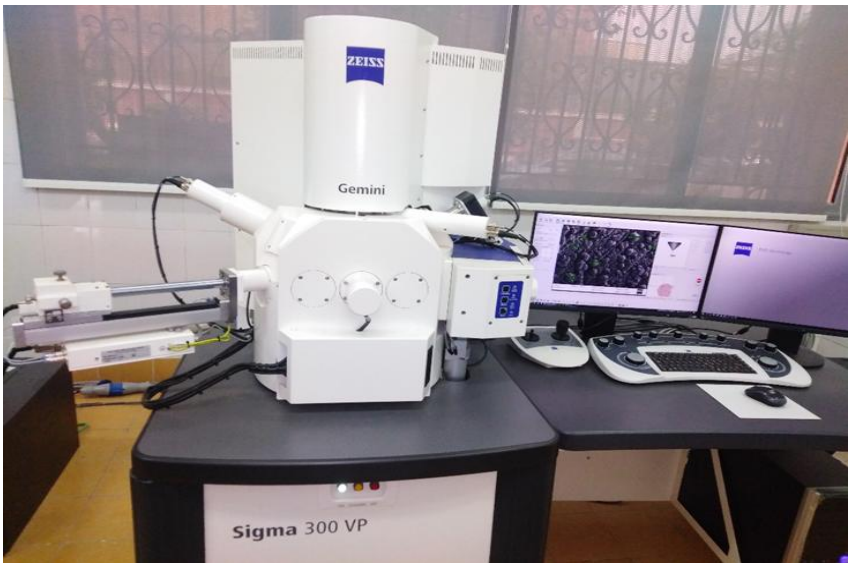

Figure 3: ZEISS EVO 10, CARL ZEISS AG Göttingen, Germanyused for SEM and EDAX analysis.

RESULTS

Surface characteristics of the ZnO nanoparticles

ZnO nanoparticle-coated mini-implants (group 2) viewed under a scanning electron microscope showed that the nanoparticles were spherical and homogenous in shape, with a visually acceptable distribution. (Figure 4).

According to figure 4 and table 1, ZnO nanoparticles formed on the surface of the mini-implant (Zn = 69.02 percent by weight, O = 21.36 percent by weight, Ti = 7.91 percent by weight, and AL = 1.71 percent by weight), as evidenced by EDAX analysis. Table 2 displays the results of an EDAX study of a non-coated mini-implant with the following composition: AL = 8.66% by weight, Ti = 87.6% by weight, and V = 3.70% by weight.

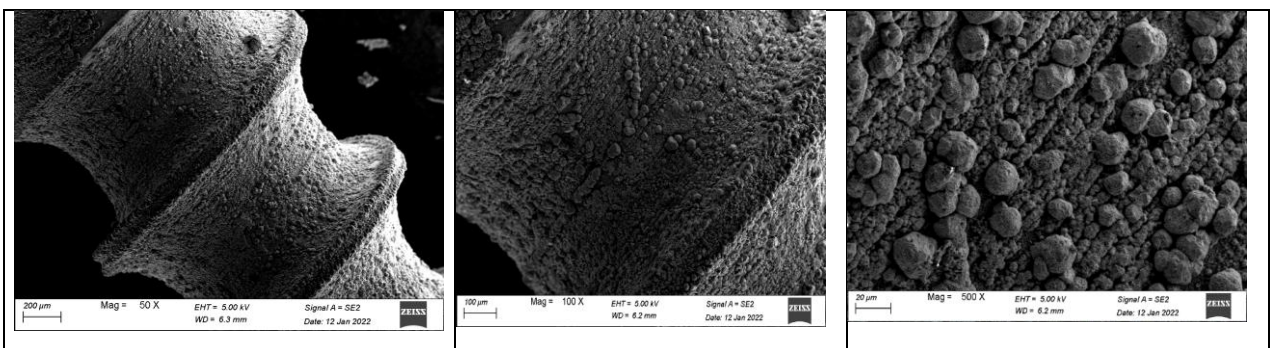

Figure 4: ZnO nanoparticles homogenous layer on mini-implants surface.

Figure 5: Colour change of mini-implants after nanoparticles coating process**.**

Table 1: Atomic% and weight% of the elements of nano-coated mini-implants.

Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	R	A	F
O K	21.36	50.97	541.10	10.54	0.8156	0.0558	1.0000
AlK	1.71	2.42	77.61	11.97	0.8423	0.1035	1.0041
TiK	7.91	6.31	1293.53	3.47	0.8804	0.8092	1.1074
ZnK	69.02	40.30	5571.97	1.67	0.9100	0.9571	1.0484

Table 2: Atomic% and weight% of the elements of uncoated mini-implants.

Element	Weight %	Atomic %	Net Int.	Error %	R	A	F
AlK	8.66	14.43	1678.85	8.80	0.8559	0.2111	1.0122
TiK	87.64	82.30	29599.22	2.02	0.8924	0.8835	1.0135
V K	3.70	3.27	1065.39	3.12	0.8959	0.8699	1.0165

Evaluation of cytotoxicity against HLF cell line

With a control sample containing one layer of HLF cells having normal morphology, indirect contact assay (figure 6) revealed that the cells' morphology remained unchanged upon contact with the mini-implants coated with nano-ZnO.

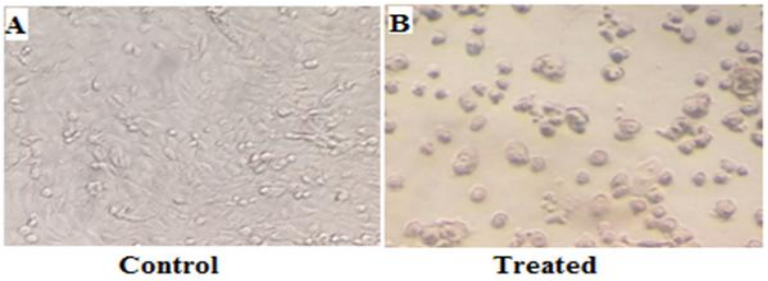

Figure 6: A, the control sample consists of a confluent layer of fibroblast cells. Most of the cells are spindle-shaped, which is considered normal. B, themini-implant coated with ZnO nanoparticles showed no change in cell morphology following contact with the HLF confluent layer.

Table (3) presents the results of a one-way analysis of variance (ANOVA) comparing the cell viability effects of orthodontic mini-implants coated with ZnONPs at different concentrations.
The table was divided into three main columns: "Free," "Coated," and "ZnONPs," representing the control group (without any coating), the orthodontic mini-implants coated with ZnONPs, and the ZnONPs alone, respectively. The rows represent different concentrations of ZnONPs, ranging from 100 mg/ml to 0.475 mg/ml.
At higher concentrations (100 mg/ml, 50 mg/ml, and 25 mg/ml), the ZnONPs group showed significantly lower cell viability compared to the "Free" and "Coated" groups, as indicated by the different letters in the same row.
However, at lower concentrations (12.5 mg/ml to 0.475 mg/ml), the cell viability in the ZnONPs group was generally comparable to the "Free" and "Coated" groups, as indicated by the same letters in the same row and the non-significant p-values (greater than 0.05).

Table 3: One-way analysis of variance comparisons of the cell viability effects of orthodontic mini-implants coated with zinc oxide nanoparticles of different concentrations:

Conc mg/ml	**Free**			**Coated**			**ZnNPs**			**P-value**
	Viabi %	**MOD**	**SD**	**Viabi %**	**MOD**	**SD**	**Viabi %**	**MOD**	**SD**	
100	100.00	0.330 A	0.015	25.15	0.083 B	0.004	13.43	0.044 C	0.001	**<0.0001***
50	100.61	0.332 A	0.018	66.46	0.219 B	0.005	14.75	0.049 C	0.004	**<0.0001***
25	101.52	0.335 A	0.003	98.99	0.327 B	0.004	44.55	0.147 C	0.005	**<0.0001***
12.5	100.71	0.332 A	0.017	101.82	0.336 A	0.010	92.83	0.306 B	0.016	**0.0009***
6.25	98.69	0.326 A	0.036	101.92	0.336 A	0.012	101.01	0.333 A	0.017	**0.6982(NS)**
3.125	100.20	0.331 A	0.013	100.61	0.332 A	0.004	101.82	0.336 A	0.010	**0.5635(NS)**
1.56	99.80	0.329 A	0.007	98.08	0.324 A	0.005	101.21	0.334 A	0.027	**0.4859(NS)**
0.78	101.41	0.335 A	0.023	98.48	0.325 A	0.030	100.71	0.332 A	0.018	**0.7015(NS)**
0.39	102.32	0.338 A	0.011	101.82	0.336 A	0.018	99.80	0.329 A	0.034	**0.7192(NS)**
0.195	101.21	0.334 A	0.021	99.29	0.328 A	0.007	101.01	0.333 A	0.015	**0.7109(NS)**
0.95	100.20	0.331 A	0.011	102.22	0.337 A	0.013	100.61	0.332 A	0.024	**0.7539(NS)**
0.475	100.81	0.333 A	0.010	101.82	0.336 A	0.010	101.52	0.335 A	0.014	**0.8689(NS)**

%; Viability Percentage, MOD; Mean Optical Density, SD; Standard Deviation, P; Probability Level
Means with same letters in the same row were insignificant different using Tukey`s post hoc test
Means with different letters in the same row were significant different using Tukey`s post hoc test
NS; Insignificant Different using One Way ANOVA
*; Significant Different using One Way ANOVA
The p-values in the last column indicate the statistical significance of the differences between the groups for each concentration, as determined by the one-way ANOVA. A p-value less than the chosen significance level (typically 0.05) suggests that the differences between the groups are statistically significant.
Tables (4 and 5) display the results of simple linear regression model analyses for the optical density mean (Table 3) and cell viability percentage mean (Table 3) of orthodontic mini-implants coated with zinc oxide nanoparticles (ZnNPs) at different concentrations. These analyses aimed to investigate the relationship between the concentration of ZnONPs and the respective dependent variables (optical density and cell viability percentage).

Table 4: Simple linear regression model analysis of the optical density means of orthodontic mini-implants coated with zinc oxide nanoparticles along different concentrations:

	Free	Coated	ZnNPs
Slope	-2.000e-005	-0.002459	-0.003178
Y-intercept	0.3325	0.3428	0.3028
X-intercept	16621	139.4	95.27
1/slope	-49989	-406.7	-314.6
F	0.3772	163.0	47.78
DFn, DFd	1, 10	1, 10	1, 10
P value	0.5528	<0.0001	<0.0001
Deviation from zero?	Not Significant	Significant	Significant
Equation	Y = -2.000e-005*X + 0.3325	Y = -0.002459*X + 0.3428	Y = -0.003178*X + 0.3028

Table 4 presents the linear regression model parameters for the optical density mean. The slope values indicate the rate of change in optical density with respect to the concentration of ZnONPs. The "Free" group (without coating) has a near-zero slope, suggesting minimal change in optical density across different concentrations. In contrast, the "Coated" and "ZnONPs" groups have negative slopes, indicating a decrease in optical density as the concentration of ZnONPs increases.

The p-values in Table 4 demonstrate that the deviation from zero is statistically significant for the "Coated" and "ZnONPs" groups ($p < 0.0001$), but not for the "Free" group ($p = 0.5528$). This implies that the relationship between optical density and concentration is significant for the coated samples but not for the uncoated control group.

Table 5: Simple linear regression model analysis of the cell viability percentage mean of orthodontic mini-implants coated with zinc oxide nanoparticles along different concentrations:

	Free	Coated	ZnNPs
Slope	-0.005333	-0.7448	-1.062
Y-intercept	100.7	103.9	98.91
X-intercept	18886	139.5	93.14
1/slope	-187.5	-1.343	-0.9417
F	0.2960	163.1	47.18
DFn, DFd	1, 10	1, 10	1, 10
P value	0.5983	<0.0001	<0.0001
Deviation from zero?	Not Significant	Significant	Significant
Equation	Y = -0.005333*X + 100.7	Y = -0.7448*X + 103.9	Y = -1.062*X + 98.91

Table 5 focuses on the linear regression model parameters for the cell viability percentage mean. Similar to Table 4, the "Free" group exhibits a near-zero slopes, indicating minimal change in cell viability percentage across different concentrations. However, the "Coated" and "ZnONPs" groups have negative slopes, suggesting a decrease in cell viability percentage as the concentration of ZnONPs increases.

The p-values in Table 5 also showed that the deviation from zero is statistically significant for the "Coated" and "ZnONPs" groups ($p < 0.0001$), but not for the "Free" group ($p = 0.5983$). This finding aligns with the observations from Table 4, further confirming the significant relationship between cell viability percentage and concentration for the coated samples.

The equations provided in both tables allowed for predicting the optical density mean and cell viability percentage mean based on the concentration of ZnONPs in the respective groups.

DISCUSSION

The best option for anchorage is the titanium mini-implants because they offer absolute anchorage without damaging neighboring teeth in the dental arch. Due to their high failure rate, they do have significant drawbacks. In addition, surface roughness contributes to bacterial accumulation (2) and Coating orthodontic materials with nano-materials has enhanced surface texture by decreasing surface flaws and irregularities. (22) ZnO nanoparticles were employed in this work to coat titanium mini-implants, their potential to cause cytotoxic effects was assessed.

Chemical analysis of the coated mini-implants in this study, revealed the formation of ZnO nanoparticles on the min-implant surface (O2= 50.97 atomic%, Zn = 40.30 atomic%, Ti = 6.31 atomic% and AL = 2.42 atomic%). These findings matched with the results reported by Kachoei et al who found in EDAX analysis of ZnO nanoparticles coated wire surface Zn=20% by weight, O=45% by weight, Ti=7.15% by weight and Ni=8.81% by weight. (23) The EDAX analysis of the coated mini-implants confirmed that the mini-implants consisted of

titanium, zinc and oxygen.

On the implant surface, the surface topography of the ZnO nanocoating revealed a uniform layer of spherical nanoparticles with 18 to 80 nm in size. These findings match with the study of Kachoei et al. (23) that showed spherical ZnO nanoparticles which range from 25 to 30 nm in size.

The existence of ZnO nanoparticles with spherical shape on the mini-implant was demonstrated using the SEM method to assess the surface pattern of ZnO nanoparticles deposition. These findings agree with Behroozian et al. (25), who found a consistent coating of spherical ZnO nanoparticles with a restricted size range between 40 and 45 nm on stainless steel wires.

Cytotoxicity assessment of a material can be performed directly by the test sample itself or on an extract from it. Most of previous studies assessing orthodontic materials toxicity have used the eluates collected at variable time intervals. (18-20-21-28) In this study, the mini-implants were put in the cell culture to assess the impact on the cells of the culture. Quantitative methods were more accurate than qualitative ones, so they were employed to measure the dye's reduction and color changes as the optical density of the medium. (26)

Cytotoxicity assays estimate the viable cells' quantity and lay out a measure for cell death through the contact of the material or its eluates.(25) Cytotoxicity causes structural and functional damage to the cell upon exposure to a substance.(26) The material cytotoxicity is considerably affected by the cell line used for the assay ,therefore cells selection remains an important consideration.(27) In dental field, various cell lines such as human gingival fibroblasts, human endothelial cells, keratinocytes, mouse fibroblasts, osteogenic precursor cells from mice, and HeLa cells are utilized to investigate the nanoparticles cytotoxicity. The human lung fibroblast (HLF) is considered a permanent cell line which has better reproducibility and more sensitivity to the toxic effects than other fibroblasts. (28-29) Therefore, in this study, the cytotoxicity of orthodontic brackets coated with zinc oxide nanoparticles was assessed using the human lung fibroblast (HLF) cell line.

Coating the titanium mini-implants with zinc nanoparticles enhances the antimicrobial capabilities and decreases bacterial accumulation, (30) but the possible cytotoxicity is considered an important concern. Several studies reported zinc nanoparticles toxicity; however, they are insufficient to fully comprehend their cytotoxic potential because it depends on a variety of circumstances. One of the micronutrients needed for the body's regular operation is zinc, but if levels are higher than tolerance, they may have harmful effects on the respiratory system, excretory system, neurological system, or digestive system, depending on the type of entrance. (30)

The voltage, specific power on the cathode, magnetic induction, discharge current power, and gas pressure in the working chamber are fundamental elements of the physical vapor sputtering process. The rapidity of layer deposition, repeatability, and the layer composition accuracy are this method's key benefits. The strength of the discharge current and the gas pressure in the working chamber determines how quickly the magnetron sputtering condenses. Thus, by employing radio-frequency (RF) AC and constant current (DC), ZnO layers were developed in the environment of argon using this technique. The target was a disc made of pure zinc (99.99%). (31)

Kim et al. revealed that laser-generated ultra-pure copper nanoparticles cause cytotoxicity to human cells in a cell-dependent manner.(25)They have more toxicity than the microparticles, because they can be absorbed through skin contact, ingestion, and inhalation moreover, high concentrations of smaller particles have highest toxic effects on cell viability. (32) Multiple studies revealed the cytotoxicity of the zinc nanoparticles according to the dose and size of the particles. (33-34) ZnONPs caused oxidative stress and also caused size and dose-dependent cytotoxicity in lung epithelial cells, type II alveolar epithelial cells, hepatic cells, skin fibroblasts, and astrocytes. (33-34) As a result, it was necessary to evaluate nanocoated mini-implants cytotoxicity. In this study, ZnO nanoparticles with an average size (40 nm) were used in coating the mini-implants.

According to Mobeen et al. who compared the release of zinc and copper ions from zinc oxide and copper oxide nanoparticles coated orthodontic brackets in artificial saliva under invitro condition , the zinc oxide coated group exhibited the least cell viability and this may be due to the greater leaching tendency of the zinc ions from the coatings as confirmed by Mobeen et al. who studied ion leach from different nanoparticles coating on orthodontic brackets and noted that the quantity of zinc leached was greater than of copper from the respective coated brackets.(35) They noted that the quantity of zinc and copper ions leached were well below the levels that can elicit systemic toxicity from ingestion in humans until 28th day of immersion. (35) Materials that exhibit cell viability lesser than 70% of the cell viability of the positive control are considered to possess potent cytotoxic activity and cannot be used safely in patients.(21) MTT assay is a sensitive assay with an excellent linearity up to 106 cells per well and even a small change in metabolic activity of the cells can generate a large variation in the findings, allowing one to detect cell stress upon exposure to a toxic agent even in the absence of cell death.

Nam Kim et al. found that when latex rubber bands are stretched up to 3 times, the cytotoxic particles may quickly separate from the band surface and release into the air due to the increased length outside of the extracting media. However, when the bands are incubated without stretching, the attached cytotoxic particles, which may contain more harmful materials, can be released more through the latex rubber bands. (36)

Friction of the mini-implants resulted in a decrease in the cytotoxicity, which is more relevant to the clinical

outcome in which it is relatively a less harmful event to the patients due to the detachment of ZnO nanoparticles when using the ZnO nanocoated mini-implants in an orthodontic treatment. (37)

Overall, in this study regression analyses highlight the potential cytotoxic effects of ZnONPs at higher concentrations, as evidenced by the decreasing trends in optical density and cell viability percentage for the "Coated" and "ZnONPs" groups. The significant p-values for these groups suggest that the concentration of ZnONPs has a statistically significant impact on the measured variables.

So, very weak cytotoxic effects human fibroblast cells were detected under
these experimental conditions.

LIMITATIONS

Studies on the durability of ion release from these nanoparticle-coated mini-implants, changes in the physical properties of the incorporated material, retention of the biopolymer coating on clinical application, and, most importantly, the toxicity related to the oral tissues were the limitations of this study that need future researches to support ZnONPs use as a suitable implantable biomaterial.

CONCLUSION

At lower concentrations, ZnONPs did not significantly affect cell viability.but at higher concentrations, ZnONPs exhibited cytotoxic effects, potentially due to their antimicrobial properties, but.

REFERENCES

1. Le Guéhennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007; 23:844-54.
2. Schierholz JM, Beuth J. Implant infections: a haven for opportunistic bacteria. J Hosp Infect 2001; 49:87-93.
3. Zitzmann NU, Berglundh T, Ericsson I, Lindhe J. Spontaneous progression of experimentally induced periimplantitis. J Clin Periodontol 2004; 31:845-9.
4. Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J. Long-standing plaque and gingivitis at implants and teeth in the dog. Clin Oral Implants Res 1992; 3:99-103.
5. Lindhe J, Meyle J. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. J Clin Periodontal 2008;35(8 Suppl): 282-5.
6. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. J Clin Periodontol2008;35(8 Suppl):286-91.
7. Wadström T. Molecular aspects of bacterial adhesion, colonization, and development of infections associated with biomaterials. J Invest Surg 1989; 2:353-60.
8. Liao J, Anchun M, Zhu Z, Quan Y. Antibacterial titanium plate deposited by silver nanoparticles exhibits cell compatibility. Int J Nanomedicine 2010; 5:337-42.
9. Meredith DO, Eschbach L, Riehle MO, Curtis AS, Richards RG. Microtopography of metal surfaces influence fibroblast growth by modifying cell shape, cytoskeleton, and adhesion. J Orthop Res 2007; 25:1523-33.
10. Chang YY, Huang HL, Lai CH, Hsu JT, Shieh TM, Wu AY, et al. Analyses of antibacterial activity and cell compatibility of titanium coated with a Zr-C-N film. PLoS One 2013;8: e56771.
11. Oh EJ, Nguyen TDT, Lee SY, Jeon YM, Bae TS, Kim JG. Enhanced compatibility and initial stability of Ti6Al4V alloy orthodontic miniscrews subjected to anodization, cyclic precalcification, and heat treatment. Korean J Orthod 2014; 44:246-53.
12. Cho YC, Cha JY, Hwang CJ, Park YC, Jung HS, Yu HS. Biologic stability of plasma ion-implanted miniscrews. Korean J Orthod 2013; 43:120-6.
13. Ciobanu CS, Massuyeau F, Constantin LV, Predoi D. Structural and physical properties of antibacterial Ag-doped nano-hydroxyapatite synthesized at 100°C. Nanoscale Res Lett 2011;6:613.
14. Buckley JJ, Lee AF, Olivic L, Wilsonb K. Hydroxyapatite supported antibacterial Ag3PO4 nanoparticles. J Mater Chem 2010; 20:8056-63.
15. Afonso Camargo SE, Mohiuddeen AS, Fares C, Partain JL, Carey PH, 4th, Ren F, et al. Anti-bacterial properties and biocompatibility of novel SiC coating for dental ceramic. J FunctBiomater. 2020; 11:33. doi: 10.3390.
16. Bahrami, R., Pourhajibagher, M., Badiei, A., Masaeli, R., &Tanbakuchi, B. Evaluation of the cell viability and antimicrobial effects of orthodontic bands coated with silver or zinc oxide nanoparticles: An in vitro study. Korean Journal of Orthodontics, 2023, 53(1), 16-25.
17. Kao CT, Ding SJ, Min Y, Hsu TC, Chou MY, Huang TH. The cytotoxicity of orthodontic metal bracket immersion media. Eur J Orthod. 2007 Apr;29(2):198-203.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983 Dec 16;65(1-2):55-63.

19. Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. J Immunol Methods. 1986 Nov 20;94(1-2):57-63.
20. Gruber S, Nickel A. Toxic or not toxic? The specifications of the standard ISO 10993-5 are not explicit enough to yield comparable results in the cytotoxicity assessment of an identical medical device. Front Med Technol. 2023 Jun 14; 5:1195529.
21. Buranaamnuay K. The MTT assay application to measure the viability of spermatozoa: A variety of the assay protocols. Open Vet J. 2021 Apr-Jun;11(2):251-269.
22. Risha, H., Refai, W., & Hashem, A. Coating of stainless steel arch wires with Silicon Carbide & Aluminum Oxide nanoceramic particles: Topographical & mechanical assessment. Egyptian Orthodontic Journal, (2024). 66(1), 63-76.
23. Kachoei M, Nourian A, Divband B, Kachoei Z, Shirazi S. Zinc-oxide nanocoating for improvement of the antibacterial and frictional behavior of nickel-titanium alloy. Nanomedicine (Lond). 2016 Oct;11(19):2511-27.
24. Kim, Yea-Seul, Kim, Kuk Ki, Shin, Seon-Mi, Park, Seung Min, Hah, Sang Soo. Comparative Toxicity Studies of Ultra-Pure Ag, Au, Co, and Cu Nanoparticles Generated by Laser Ablation in Biocompatible Aqueous Solution. Bulletin of the Korean Chemical Society. 2012 Oct 20;33(10):3265–8.
25. Murray PE, Lumley PJ, Ross HF, Smith AJ. Tooth slice organ culture for cytotoxicity assessment of dental materials. Biomaterials. 2000 Aug;21(16):1711-21.
26. Xie Y, Williams NG, Tolic A, Chrisler WB, Teeguarden JG, Maddux BL, Pounds JG, Laskin A, Orr G. Aerosolized ZnO nanoparticles induce toxicity in alveolar type II epithelial cells at the air-liquid interface. Toxicol Sci. 2012 Feb;125(2):450-61.
27. Wataha JC, Hanks CT, Sun Z. Effect of cell line on in vitro metal ion cytotoxicity. Dent Mater. 1994 May;10(3):156-61.
28. Wang J, Deng X, Zhang F, Chen D, Ding W. ZnO nanoparticle-induced oxidative stress triggers apoptosis by activating JNK signaling pathway in cultured primary astrocytes. Nanoscale Res Lett. 2014 Mar 13;9(1):117.
29. Wataha JC, Hanks CT, Sun Z. Effect of cell line on in vitro metal ion cytotoxicity. Dent Mater. 1994 May;10(3):156-61.
30. Ingle AP, Duran N, Rai M. Bioactivity, mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. Appl MicrobiolBiotechnol. 2014 Feb;98(3):1001-9.
31. Rogov, A. & Kapustin, Yuriy & Martynenko, Vitaliy. Factors Determining the Efficiency of Magnetron Sputtering. Optimization Criteria. Technical Physics. Vol. 60. (2015). pp. 283–291.
32. International Organization for Standardization. International standard ISO 10993-5, Biological evaluation of medical devices. 1992. Part 5: Tests for cytotoxicity: in vitro methodsGeneva (SH1992).
33. Kermanizadeh A, Gaiser BK, Hutchison GR, Stone V. An in vitro liver model--assessing oxidative stress and genotoxicity following exposure of hepatocytes to a panel of engineered nanomaterials. Part FibreToxicol. 2012 Jul 19; 9:28.
34. Hsiao IL, Huang YJ. Effects of various physicochemical characteristics on the toxicities of ZnO and TiO nanoparticles toward human lung epithelial cells. Sci Total Environ. 2011 Mar 1;409(7):1219-28.
35. Mobeen, N., Duraisamy, S., & K, R. (2022). Evaluation of the Ion release from nanoparticles coated orthodontic brackets - In vitro Study: Original research. International Journal of Orthodontic Rehabilitation, 13(3), 10–21.
36. Nam Kim K Jung-Hwan L, Eun-Jung L, Jae-Sung K, Chung-Ju H, Cytotoxicity Comparison of the Nanoparticles Deposited on Latex Rubber Bands between the Original and Stretched State. Journal of Nanomaterials 2014; 08:07.
37. Eliades, Theodore &Zinelis, Spiros & Papadopoulos, Moschos & Eliades, George. Characterization of retrieved orthodontic miniscrew implant. Am J Orthod Dentofacial Orthop.2008.06.019.