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Commercial ZnO NPs coating on orthodontic bands and its In-Vivo evaluation

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ABSTRACT

Aim: The aim of this study is to compare the antimicrobial activity of Zinc Oxide coated stainless steel orthodontic bands to non-coated orthodontic band.

Materials and Method: A total number of 12 Wistar rats were randomly assigned either to the control group (Non-Coated Orthodontic Bands) or to the experimental group (Coated Orthodontic Bands) to check the antimicrobial activity of the ZnO nanoparticle. Swabs were taken at random intervals and cultured, at the end of the 30th day blood samples were collected and they were euthanized at the end of the experimental period. The antimicrobial activity of the nanoparticle and the biocompatibility of the material were analysed.

Results: Paired T-test and Independent T-test were conducted to test the equality of the antimicrobial property of non-coated stainless-steel bands and Zinc-Oxide coated stainless steel bands. Independent T-test used to compare between groups and Paired T-test was used to compare within the same group.

Conclusion: In our study we found that ZnO nanoparticles had increased antimicrobial activity. Invitro showed no signs of toxicity whereas in-vivo showed mild toxicity. Therefore, further studies are required to check their property on varied concentration of the nanoparticles.

Keywords: antimicrobial activity, ZnO nanoparticle, biocompatibility, cytotoxicity

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INTRODUCTION

Occlusion is known as the inter-relation between maxillary and mandibular teeth upon contact. Any change from its normal relation is known as malocclusion. Malocclusion is considered to be un-aesthetic by the people. It is found that people with malocclusion lack in self-confidence therefore they seek for treatment. There are various methods of treatment, one among them is fixed orthodontic treatment. Fixed orthodontic treatment is the primary treatment option and the most common method for the treatment of malocclusion. It is known that during fixed orthodontic treatment the oral cavity is more prone for colonization of microorganisms [1, 2]. The colonization of micro-organisms results in the formation of inherent morphologic irregularities. Reasons attributed for the bacterial colonisation in people seeking orthodontic treatment is that, they face difficulty in maintaining their oral hygiene and the appliance provides additional sites for microorganisms to bind and colonize. The resultant increase in oral microbial count places the patient at higher risk for enamel demineralization and periodontal disease.

It is known that the incidence of enamel demineralization after fixed orthodontic treatment can involve up to 50% of patients. The incidence of such white spot lesions around orthodontic brackets can be demonstrated within 1 month of treatment [3]. Studies have proved that the bacterial accumulation has been detected at the 10 mm gaps at the adhesive-enamel junction [4]. On comparing various types of fixed orthodontic appliances that is available, it has been found that the brackets play a major role in plaque accumulation as they are attached to the teeth throughout the orthodontic treatment period. The major reason is due to their complex design which provides a unique environment that impedes proper access to tooth surfaces for cleaning. Some studies have stated that stainless steel has the highest critical surface tension and energy, hence it can be expected to have highest plaque retaining capacity [5].

Among several pathogenic organisms it is found that certain organisms such as Streptococcus mutans and lactobacillus play a major part in initiation of white spot lesion [6, 7]. When there is low pH, the number of lactobacilli increases and the number of Streptococcus mutans decreases [8, 9]. This contributes to demineralization of the teeth once the lesions are established. Preventing these lesions is an important concern, for the orthodontist, because they are unesthetic, unhealthy and potentially irreversible.

Accumulation of dental plaque also leads to gingival inflammation [6, 7]. However, the effect of orthodontic treatment on the periodontal tissues in the long term is questionable. Several studies have stated that the gram-negative anaerobe pathogen Porphyromonas-gingivalisis found as a putative periodontal pathogen in subgingival dental plaque has an important role in the onset and progression of periodontal disease and it is implicated as an indicator of periodontal disease [10-12].

The advancements in the field of science have led to the concept of nanotechnology. The term "Nano" is a Greek word which refers to the term dwarf and it denotes the factor 10^{-9} [1]. Nanotechnology deals with working with very small objects. It was found that the nanoparticles possess antimicrobial property, which attracts the attention of medical and dental fields [5]. The reason for incorporating the antibacterial property of nanoparticles in medical and dental fields is that they consist of a particle size smaller than 100 nm. Therefore, they can interact more closely with the microbial membrane, thereby providing larger surface area for antimicrobial activity. It was also found that the bacterial strains are less likely to develop resistance against metal nanoparticles [8]. Nanoparticles can be used either combining with dental materials or by coating the surface which aims to reduce the microbial adhesion.

With the innovation of Nano-technology it was found that they can be incorporated in various fields in-order to improve their property. Studies have stated that the antimicrobial property of silver nanoparticles incorporated nanocomposites and found that they resulted in less microbial adhesion [13, 14]. In their studies used calcium Nano phosphate as an enamel remineralizing agent found better results, have demonstrated the reduction in friction between nanoparticle coated arch wire and self-ligating brackets thereby bringing faster tooth movement [15, 16].

Various studies have demonstrated the effect of silver, zinc oxide, titanium-oxide nanoparticles on multiple organisms [17, 18]. In their study coated NITI orthodontic wire with Zinc–Oxide (ZnO) nanoparticles and compared it with non-coated wire and checked the antibacterial activity of the nanoparticles against S [19]. Mutans and concluded that the coated wires had better antibacterial property than non-coated wires. In their study compared ZnO, CuO and CuO-ZnO nanoparticles coated orthodontic brackets to non-coated orthodontic brackets and concluded that the nanoparticle coated brackets had decreased or no bacterial colonies compared to non-coated brackets.

It is known that the zinc-oxide nanoparticles have antimicrobial activity against Gram-positive and Gram -negative bacteria, fungi, protozoa, and certain viruses, including antibiotic-resistant strains. Because of these properties, zinc-oxide is widely used in medical devices, textile fabric, as a water purifier. Nowadays zinc-oxide nanoparticles are being incorporated in composites, denture base resins etc, for their anti-microbial property. Therefore, the aim of this study is to compare the anti-microbial activity of zinc-oxide nanoparticle coated orthodontic band with the non-coated orthodontic band.

MATERIALS AND METHODS

This study was done on 12 Wistar rats by banding their lower incisors with stainless steel orthodontic band of size 0.125* 0.003. The rats were divided into two groups. Each group consisted of 6 Wistar rats.

Study design

It is an animal study which was designed and samples were randomly selected. This study was approved by the Scientific Review Board (SRB/SDMDS08/17/ORT/25) and the Animal Ethical Committee. Animals for this study were taken from BRULAC (Biomedical Research Unit and Lab Animal Centre), Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, SIMATS, Chennai.

Groups

Study was allocated into 2 groups (experimental study)

- 6 non-coated orthodontic bands (control group).
- 6 ZnO coated orthodontic bands (experimental group).

Inclusion criteria

- Healthy wistar rats with no pathological condition or infections present.
- Rats of 4 months to 6 months old.
- Rats which weighed between 180 gms to 220 gms.

Exclusion criteria

- Rats with any existing infections or pathological conditions were removed from the study.
- Overweighed or under-weight rats.
- Rats which were more than 6 months of age or less than 4 months of age.
- Rats with fractured mandibular incisors, discoloured mandibular incisors or the presence of any intra-oral lesions.

METHODOLOGY

In this study we coated the stainless-steel orthodontic bands with ZnO nanoparticle which was carried out by magnetic sputtering method (Sathyabama University). The substrate and the target were kept at a constant distance of about 7 cm, and sputtering was conducted for a period of about 10 minutes. All orthodontic bands were sputtered at the same time to obtain a thin and a uniform coating of ZnO.

Analysis by Scanning Electron Microscope (SEM)

The surface morphology of the ZnO thin film was investigated (Figure 1) with a scanning electron microscope (nanotechnology department, Sathyabama Dental College and Hospital, Chennai).

Analysis of the cytotoxicity of ZnO nanoparticle

The coated orthodontic bands were subjected to in-vitro cytotoxicity test and they were compared with the non-coated bands as the control group (Figure 2). An in vitro cytotoxicity test using indirect contact method was performed as per the ISO 10993:5. The culture medium from the L929 cell monolayer was replaced with a fresh agar medium. Test samples and the control groups were placed on the cells after incubation at 37°C ± 1°C for 24 hours-26 hours. Monolayer was examined microscopically to determine the cytotoxic effect before and

after removing the test sample from the agar medium. The reactivity was graded as 0, 1, 2, 3, and 4 based on the zone of lysis, vacuolization, and detachment, and membrane disintegration as shown in table 1.

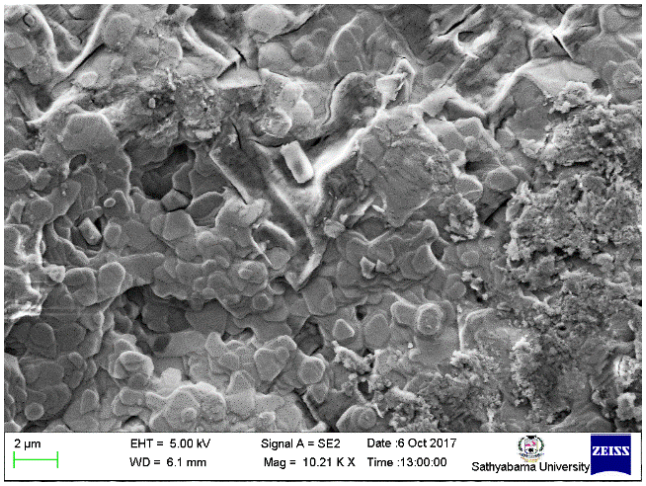

Fig. 1. Shows scanning electron microscopic feature of zinc-oxide coated orthodontic stainless-steel bands

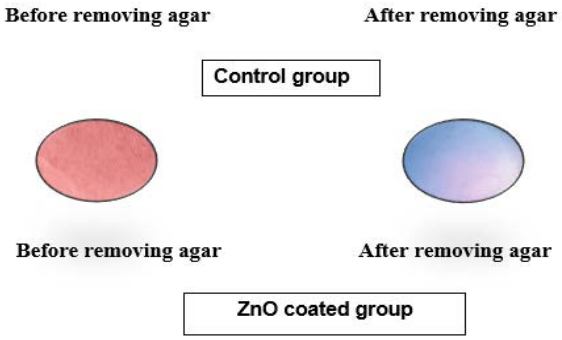

Fig. 2. In-vitro cytotoxicity test

Tab. 1. Grading of reactivity

Grade	Reactivity	Description of Reactivity Zones
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending specimen size up to 1 cm
4	Severe	Zone extending farther than 1cm beyond specimen

Animal model used and animal maintenance

Adult male Wistar Albino rats of about 4 month-6 months of age, weighing 180 g to 220 g were used for the study. Animals were maintained under controlled conditions and in room temperature (23°C ± 2°C), humidity (50% ± 5%) and 14:10 light/dark cycle in the Biomedical Research Unit and Lab Animal Centre (BRULAC), Saveetha Dental College. The animals were fed with standard rat pellet diet and drinking water ad libitum. Experiments were conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC No: SU/CLAR/RD/019/2017). The quarantine procedures and the animal maintenance were according to the recommendations of Canadian Council Guide to the Care and Use of Experimental Animals (1993) and the Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA) Guidelines for laboratory animal facility (2003).

Animal grouping

Animals were randomly divided into the following 2 groups with n=6 per group (Table 2).

Tab. 2. Experimental procedure

S. No.	Grouping	Details	No. of Animals
1	Group I	Non-coated orthodontic bands	6
2	Group II	Zinc Oxide coated orthodontic bands	6

Experimental procedure

Experimental procedures were performed under sterile conditions in an animal laboratory surgical room. Rats were anesthetized with ketamine hydrochloride, i.p and xylazine. i.m. at the dosage of 75 mg/kg body weight and 10 mg/kg body weight respectively. After the animals were anaesthetized, the mouth of the rats was kept open with the help of a retractor. After retracting the mouth of the rat, the mandibular central incisor teeth were exposed and a stainless-steel orthodontic band of 3 mm-5 mm height and 6 mm-8 mm length was taken and banded around the mandibular

central right incisor teeth and encircled by applying pressure. Then the measured band was taken out and the two ends of the band were welded with the help of the welding machine (Figure 3 and 4). The same procedure was repeated for all the samples. Then the welded bands were cemented over the teeth by lining the inner walls of the band with "Glass Ionomer Cement". Similar procedures were repeated for all the other subsequent animals for both the control (non- coated bands) and the experimental group. For the experimental group instead of stainless-steel orthodontic band the Zinc Oxide coated orthodontic band were used. Then all the animals were isolated in separate cages as per their groups.

Fig. 3. Formation of bands

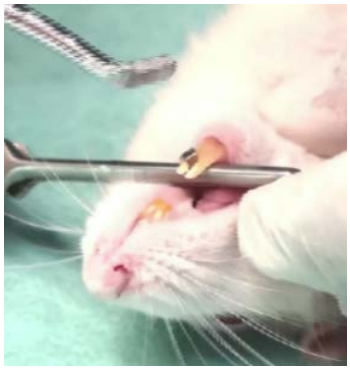

Fig. 4. Banding of the mandibular incisors

Antimicrobial property of Zinc-Oxide coated orthodontic bands

The antimicrobial property of the nanoparticle was assessed by collecting swabs at consecutive intervals of 0 days, 3 days, 6 days, 9 days, 12 days and 30 days by using a sterile cotton swab (Figure 5). From each animal two swabs were collected one from the buccal surface and the other from the lingual surface of the banded mandibular incisors. The collected swabs were inoculated in BHI broth (Brain Heart Infusion Broth) and sabouraud dextrose broth, incubated for a time period of about 6 hours, followed by which 10 microliters of the inoculum were cultured on to BHI agar and Sabouraud dextrose agar (Figure 6). The plates were in-cubated at 37°C for 24 hours. The total no of colonies formed on the agar plates were counted and tabulated. The no. of colonies formed between the experimental group and the control group were compared and statistically analyzed to evaluate the antimi-crobial property of the no. coated orthodontic bands.

Serum biochemistry profile for hepatotoxicity and nephrotoxicity study

At the end of the experimental period of 30 days, blood samples were collected by puncturing retro-orbital venous plexus and the serum biochemistry were analyzed for toxicity (Figure 7). The rats were anaesthetized by ether (Anesthetic Grade) and the blood samples were collected in glass test tubes. The exuded serum was decanted and centrifuged at 2500 rpm for 20 minutes. The clear supernatant serum which was obtained was subjected to liver and renal function tests, such as Bilirubin, Albumin, Total protein, Aspartate Amino S Transferase (AST), Alanine Transaminase (ALT) Alkaline Phosphatase (ALP), Urea and Creatinine.

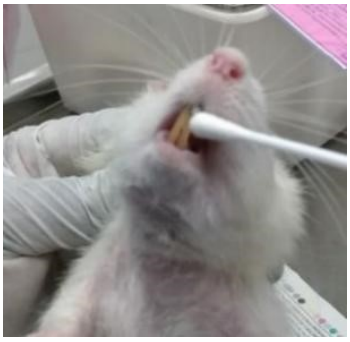

Fig. 5. Swab collection

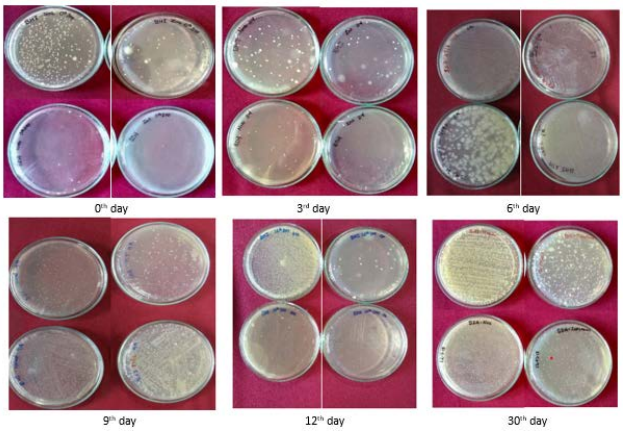

Fig. 6. Culture test on 0th, 3rd, 6th, 9th, 12th and 30th day

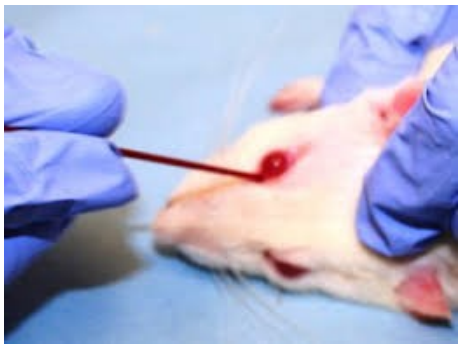

Fig. 7. Blood collection at the end of 30th day

HISTOPATHOLOGICAL STUDY

Euthanasia and tissue harvesting

Animals were euthanized at the end of the intended experimental period (end of 30th day) by administering over dose of anesthesia (Sodium Pentothal-i.p). After the respiration ceases out the animals were transcardially perfused using normal saline and then the tissues were fixed with formal saline. Tissues such as liver spleen and kidney were dissected out and post-fixed in freshly prepared 10% formalin and processed for histopathological investigation. The tissue sections were taken at 5 µm thicknesses and stained with routine Haematoxylin and Eosin staining and permanently mounted in DPX, then analyzed for histopathology.

Light microscopy

Histopathological examinations of Liver, Spleen and Kidney were done using H&E stain for hepatotoxicity and nephrotoxicity (Figure 8).

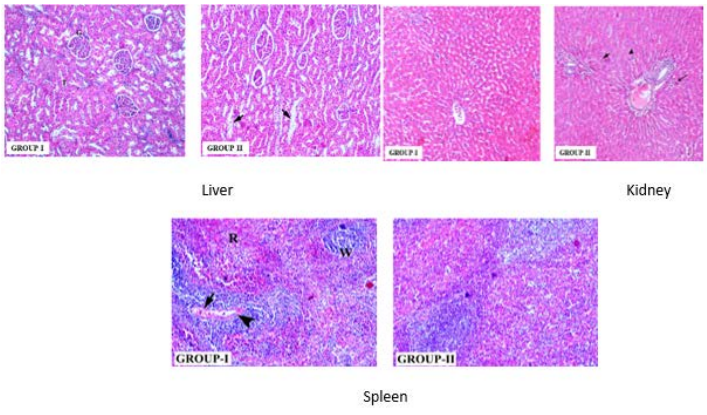

Fig. 8. Histopathological evaluation

Haematoxylin and eosin stain

For light microscopic study and for analyzing the histopathology of liver, spleen and kidney, the fixed tissues were processed for routine paraffin sectioning and stained with Haematoxylin and Eosin [20]. For paraffin sectioning the tissues were hydrated, then dehydrated in graded alcohol series, cleared in chloroform and xylene and then embedded in paraffin wax. For H&E staining the paraffin embedded tissues were sectioned at 5 µm thickness using Rotary microtome (Leica Microsystems, Germany) they were incubated overnight at room temperature, then the sections were deparaffinized and rehydrated through descending alcohol series (100% alcohol, 90% alcohol, 70% alcohol and 50% alcohol) followed by distilled water. Now these sections were stained with Haematoxylin and Eosin and then rapidly carried through ascending alcohol series (50% alcohol, 70% alcohol, 90% alcohol and three changes of 100% alcohol) and then the sections were cleared in three changes of xylene and they were mounted with DPX. Statistical analyses were performed with the statistical package

for social sciences 25 for windows (SPSS Inc, Chicago, Illinios, USA). The results obtained were compiled and tabulated for the 12 samples.

RESULTS

Analysing the cytotoxicity xof ZnO nanoparticle

The results of the Cytotoxicity of ZnO nanoparticles were done by an in-vitro method on a L929 monolayer the results showed that there was no Zone of reactivity which means (grade '0'). Based on the inference in table 1 it shows that the ZnO nanoparticle does not cause any cytotoxic changes which means it is safe to be used as there is no cytotoxicity present.

Antimicrobial activity of Zinc-Oxide coated orthodontic bands

The results obtained from statistical evaluation are given in the following tables. The arithmetic mean and standard deviation were calculated. Paired T-test with a confidence interval of 95% was calculated for both the control group and Zinc-Oxide group. Statistical significance level was established at $p<0.05$. Construction of 95% Confidence Interval (PCI) and a Paired T test and Independent T-test were conducted to test the equality of the antimicrobial property of non-coated stainless-steel bands and Zinc-Oxide coated stainless steel bands.

On comparing between the two groups the Independent T-test showed significant difference, whereas the paired T-test showed that the antimicrobial property of both the groups was significant. On comparing the number of bacterial colonies formed between the control group and the ZnO coated group by independent T-test, it was found that the ZnO group showed less no of bacterial colonies compared to that of the control group in table 3.

Tab. 3. Comparing the bacterial colonies between the control group and the ZnO coated orthodontic bands (Independent T-test)

Group Statistics					
	Groups	**N**	**Mean**	**Std. Deviation**	**p-value**
Bacteria-0 days	Control	6	62033.33	3295.856	0
	Zinc Oxide	6	41283.33	3571.788	
Bacteria-3 days	Control	6	66366.67	2557.082	0
	Zinc Oxide	6	21016.67	3205.88	
Bacteria-6 days	Control	6	65800	1306.905	0
	Zinc Oxide	6	33516.67	2877.093	
Bacteria-9 days	Control	6	66266.67	1727.04	0
	Zinc Oxide	6	36150	3430.306	
Bacteria-12 days	Control	6	66200	1680.476	0
	Zinc Oxide	6	20600	2611.513	
Bacteria-30 days	Control	6	67433.33	1561.623	0.003
	Zinc Oxide	6	63283.33	2093.243	

On comparing the number of fungi colonies formed between the control and the ZnO coated group by independent T-test, it was found that the ZnO group showed decreased no of fungi colonies compared to that of the control group in table 4.

Tab. 4. Comparing the no. of fungi colonies formed between the control group and the Zinc-Oxide group (Independent T-test)

Group Statistics					
	Groups	**N**	**Mean**	**Std. Deviation**	**p-value**
fungi-0 days	Control	6	2383.33	348.807	0
	Zinc Oxide	6	633.33	242.212	
fungi-3 days	Control	6	2900	303.315	0
	Zinc Oxide	6	583.33	278.687	
fungi-6 days	Control	6	6133.33	615.359	0
	Zinc Oxide	6	633.33	136.626	
fungi-9 days	Control	6	7766.67	628.225	0
	Zinc Oxide	6	3600	419.524	
fungi-12 days	Control	6	6100	603.324	0
	Zinc Oxide	6	1233.33	393.277	
fungi-30 days	Control	6	9500	857.904	0
	Zinc Oxide	6	4483.33	649.359	

Paired T-test was used to compare the no. of bacterial colonies formed between the consecutive intervals in the control group and it was found that the comparison of 0 day and 3rd days showed significant difference and the other days were not significant which means they were similar. On comparing the 0 day and 3rd days it was found that the 3rd days showed increased colonies than the 0 day which is due to the plaque accumulation in table 5.

Tab. 5. Paired T-test to compare the no. of bacterial colonies formed within the control group between the days

Paired Samples Statistics					
		Mean	N	Std. Deviation	p-value
Pair 1	Bacteria-0 day	62033.33	6	3295.856	0.035
	Bacteria-3 days	66366.67	6	2557.082	
Pair 2	Bacteria-3 days	66366.67	6	2557.082	0.477
	Bacteria-6 days	65800	6	1306.905	
Pair 3	Bacteria-6 days	65800	6	1306.905	0.172
	Bacteria-9 days	66266.67	6	1727.04	
Pair 4	Bacteria-9 days	66266.67	6	1727.04	0.91
	Bacteria-12 days	66200	6	1680.476	
Pair 5	Bacteria-12 days	66200	6	1680.476	0.154
	Bacteria-30 days	67433.33	6	1561.623	

Paired T-test was used to compare the no. of bacterial colonies formed between the consecutive intervals in the ZnO coated group and it was found that all the days showed significant difference except 6th days and 9th days interval. On comparing the no. of colonies formed on each day interval it was found that the 3rd days, 6th days, 9th days and 12th days showed decreased no of colonies formed compared to the 0th day, whereas the 30th days showed more bacterial colonies formed when compared with the other day intervals in table 6.

Tab. 6. Paired t test to compare the no. of bacterial colonies formed within the ZnO coated group between the days

Paired Samples Statistics					
		Mean	N	Std. Deviation	p-value
Pair 1	Bacteria-0 day	41283.33	6	3571.788	0
	Bacteria-3 days	21016.67	6	3205.88	
Pair 2	Bacteria-3 days	21016.67	6	3205.88	0
	Bacteria-6 days	33516.67	6	2877.093	
Pair 3	Bacteria-6 days	33516.67	6	2877.093	0.094
	Bacteria-9 days	36150	6	3430.306	
Pair 4	Bacteria-9 days	36150	6	3430.306	0
	Bacteria-12 days	20600	6	2611.513	
Pair 5	Bacteria-12 days	20600	6	2611.513	0
	Bacteria-30 days	63283.33	6	2093.243	

Paired T-test was used to compare the no. of fungi colonies formed on all the days in the control group and it was found that all the days showed significant differences. On comparing the no. of colonies formed it was found that as the days increased the no. of colonies formed also increased in table 7. Paired T-test was used to compare the no. of fungi colonies formed in the ZnO coated (experimental group) between the consecutive intervals and it was found that the results of the 0th and 3rd days, 3rd days and 6th days were not significant whereas the results of 6th days and 9th days, 9th days and 12th days, 12th days and 30th days showed significant differences. On comparing the no. of colonies formed it was found that as the no. of days increased there were increased no of fungi colonies in table 8, but on comparing the no. of fungi colonies in table 7 and 8 formed on all the days between the control and the ZnO coated group it was found that the no. of colony count of all the days in the control group was increased compared to that of the ZnO group.

Tab. 7. Paired t test to compare the no. of fungi colonies formed within the control group between the days

Paired Samples Statistics					
		Mean	N	Std. Deviation	p-value
Pair 1	fungi-0 day	2383.33	6	348.807	0.05
	fungi-3 days	2900	6	303.315	
Pair 2	fungi-3 days	2900	6	303.315	0
	fungi-6 days	6133.33	6	615.359	
Pair 3	fungi-6 days	6133.33	6	615.359	0.001
	fungi-9 days	7766.67	6	628.225	
Pair 4	fungi-9 days	7766.67	6	628.225	0.001
	fungi-12 days	6100	6	603.324	
Pair 5	fungi-12 days	6100	6	603.324	0.002
	fungi-30 days	9500	6	857.904	

Tab. 8. Paired t test comparing the t=no of fungi colonies formed in the ZnO group within the experimental days

Paired Samples Statistics					
		Mean	N	Std. Deviation	p-value
Pair 1	fungi-0 day	633.33	6	242.212	o.611
	fungi-3 days	583.33	6	278.687	
Pair 2	fungi-3 days	583.33	6	278.687	0.646
	fungi-6 days	633.33	6	136.626	
Pair 3	fungi-6 days	633.33	6	136.626	0
	fungi-9 days	3600	6	419.524	
Pair 4	fungi-9 days	3600	6	419.524	0
	fungi-12 days	1233.33	6	393.277	
Pair 5	fungi-12 days	1233.33	6	393.277	0
	fungi-30 days	4483.33	6	649.359	

Multivariant analysis was done to compare the no. of bacterial colonies formed between the control and the ZnO group, the results showed that there was significant difference on all the days. On comparing the total no of colony count formed between control and ZnO group on each day interval it was found that the ZnO group showed decreased no of bacterial colony counts formed on all the days interval compared to that of the control group in table 9.

Tab. 9. Multivariant analysis done to compare the no. of bacterial colonies formed between the control and the ZnO group on each day interval

Paired Samples Statistics					
		Mean	N	Std. Deviation	p-value
Pair 1	fungi-0 day	633.33	6	242.212	0.611
	fungi-3 days	583.33	6	278.687	
Pair 2	fungi-3 days	583.33	6	278.687	0.646
	fungi-6 days	633.33	6	136.626	
Pair 3	fungi-6 days	633.33	6	136.626	0
	fungi-9 days	3600	6	419.524	
Pair 4	fungi-9 days	3600	6	419.524	0
	fungi-12 days	1233.33	6	393.277	
Pair 5	fungi-12 days	1233.33	6	393.277	0
	fungi-30 days	4483.33	6	649.359	

Multivariant analysis was done to compare the no. of fungi colonies formed between the control and the ZnO group, the results showed that there was significant difference on all the days. On comparing the total no of colony count formed between control and ZnO group on each day interval it was found that the ZnO group showed decreased no of fungi colony counts formed on all the days compared to that of the control group in table 10 and 11.

Tab. 10. Multivariant analysis done to compare the no. of fungi colonies formed between the control and the ZnO group on each day interval

Descriptive Statistics					
	Groups	Mean	Std. Deviation	N	p-value
fungi-0 day	Control	2383.33	348.807	6	0
	Zinc Oxide	633.33	242.212	6	
	Total	1508.33	957.704	12	
fungi-3 days	Control	2900	303.315	6	0
	Zinc Oxide	583.33	278.687	6	
	Total	1741.67	1241.303	12	
fungi-6 days	Control	6133.33	615.359	6	0
	Zinc Oxide	633.33	136.626	6	
	Total	3383.33	2903.551	12	
fungi-9 days	Control	7766.67	628.225	6	0
	Zinc Oxide	3600	419.524	6	
	Total	5683.33	2234.78	12	

fungi-12 days	Control	6100	603.324	6	0
	Zinc Oxide	1233.33	393.277	6	
	Total	3666.67	2587.499	12	
fungi-30 days	Control	9500	857.904	6	0
	Zinc Oxide	4483.33	649.359	6	

Tab. 11. Liver function test and renal function test (Independent T-test)

Group Statistics					
	Groups	**N**	**Mean**	**Std. Deviation**	**p-value**
Bilirubin	Control	6	0.325	0.01049	0
	Zinc Oxide	6	0.41	0.01414	
Albumin	Control	6	2.6183	0.27007	0.03
	Zinc Oxide	6	2.9117	0.08658	
Total Protein	Control	6	6.715	0.28752	0.512
	Zinc Oxide	6	6.8333	0.31443	
AST	Control	6	63.9167	2.04546	0.298
	Zinc Oxide	6	65.1583	1.8726	
ALT	Control	6	46.7833	2.06632	0
	Zinc Oxide	6	54.5667	2.72886	
ALP	Control	6	170.5883	3.4994	0.023
	Zinc Oxide	6	175.9867	3.43922	
Urea	Control	6	32.7133	1.6501	0.113
	Zinc Oxide	6	34.28	1.47173	
Creatinine	Control	6	0.8183	0.03061	0.004
	Zinc Oxide	6	0.9833	0.10309	

DISCUSSION

Orthodontics is the branch of dentistry which deals with the ar-rangement of the teeth with the help of an additional attachment which is being fixed to the tooth to correct the occlusion. But the major iatrogenic effect of orthodontic therapy is plaque accumula-tion causing enamel decalcification.

The reason is plaque retention around brackets resulting in poor oral hygiene which in turn results in lowering the pH around the bracket which inhibits the enamel remineralization process which increases enamel decalcification around the brackets [21]. The ini-tial phase is "White Spot Lesion" (WSL) is defined as "subsurface enamel porosity from carious demineralization" that is present as "a milky white opacity when it is located on smooth surfaces" [22]. Studies stated that fixed orthodontic treatments are more prone to plaque accumulation lowering the pH value in orthodontic pa-tients than non-orthodontic patient [23]. Such plaque accumu-lation pre-dispose to increased risk of caries. With the invention of nanoparticles, they are incorporated to orthodontic materials thereby improving the quality of treatment. Studies proved the anti-adherent and antimicrobial property of the nanoparticles but their biocompatibility is questionable [24]. In our study we have coated the orthodontic bands with the ZnO nanoparticle by the magnetic spluttering method in order to check the cytotoxicity and the biocompatibility of the ZnO nanoparticle.

Various authors have incorporated various methods to check the biocompatibility of the material. In his study compared the ZnO NPs and Ag NPs in different concentrations (10 ug/ml, 25 ug/ml, 50 ug/ml, 75 ug/ml, and 100 ug/ml) and the cells were incubated at 12 hours, 24 hours, and 36 hours [25]. The cells were observed with 200-fold magnification by optical microscope. The results showed cell shrinkage at minimum concentration of ZnO NPs and cell apoptosis at higher concentration. He concluded that ZnO NPs exhibited greater toxicity.

In our study we have coated the stainless-steel orthodontic band with ZnO nanoparticle and did an in-vitro cytotoxicity on a L929 monolayer, cells didn't show any zone of reactivity which means there is no cytotoxicity and the nanomaterial is safe to use. It should be noted that in-vitro tests cannot entirely predict the overall biocompatibility of a material and in-vivo use of the mate-rial must be questioned [26].

Conducted an animal study using silver nanoparticles via respira-tory and gastrointestinal tracts as they were considered as the main entry portals of Nano silver into the human body [27]. Nano silver (5000 mg/kg) was given through gavage to mice, for rat's daily intake of oral dosage of Nano silver was given up to 9 mg/kg as they were found to be safe, the histopathological evaluations were done at the end of 14 days. Both the studies showed lowest adverse effects in rats which were given high doses of nano-silver particles for a longer-term (90 days). Therefore, even if the patient swallows the bracket during treatment, it's not possible to reach the above-mentioned daily dose therefore it is safe to coat brackets. Recently an inhalation tests was been done to test the cytotoxicity; results showed no toxic effect in rats [28]. In his study used N-doped TiO_2-xNy [29]. On considering the biocompatibility of the bracket coated with the TiO_2-xNy thin film he coated the orthodontic brackets with N-doped TiO_2-xNy thin film and checked the cytotoxicity of the material, the results showed that the cytotoxicity score was grade 0, which means no cell lysis and cells grew well adherent to the surface to

the bracket. Animal experiments showed that the bracket coated with the TiO_2-xNy thin film didn't cause mucosa irritation, systemic toxic-ity or genetic toxicity and the film exhibits high biocompatibility. In their study coated the orthodontic brackets with different phas-es of TiO_2 and checked their biocompatibility by using an in-vitro cytotoxicity test on a L929 monolayer which was similar to the methodology of our study [30]. The anatase coated brackets were assessed for 6 days, the inference showed that the viability of cells in anatase phase decreased to 89% on first day, 77% on third day and 89% on sixth day. The rutile phase coated brackets showed that the cell viability reduced to 40% on the first day and 21% on the sixth day. There was no statistically significant difference be-tween the anatase coated brackets and the normal brackets. They found that the rutile coated brackets showed significantly greater cytotoxic effects than the control group and the anatase coated brackets. Therefore, they concluded that the rutile had greater an-tibacterial effects than anatase and more cytotoxic. On consider-ing the cytotoxicity effect the anatase phase of TiO_2 showed lesser cytotoxicity and had antibacterial effect.

In our study we coated stainless orthodontic bands with ZnO nanoparticle and subjected it to SEM to check the uniformity of the coating. Then the coated orthodontic bands were cut and pre-formed to the shape of the Wistar rats' mandibular incisors and cemented with GIC cement. Swabs were collected on consecu-tive days of 0, 3, 6, 9, 12 and 30. Culture test were done to check their antimicrobial property the no. of colonies formed in both the groups were compared to the non-coated control group. It was found that the no. of colonies formed in the ZnO coated group were less compared to the non-coated group proved its antimicro-bial activity. At the end of the 30th day blood samples were taken from both the control group and the ZnO coated group to check the hepatotoxicity and nephrotoxicity. The results showed slightly increased valves than the control group which means there is mild cytotoxicity.

Orthodontic treatment is a long-term procedure. Therefore, it is necessary to evaluate the effects of coated brackets over a longer period of time. In this study a single tooth was banded to check the cytotoxicity and their antimicrobial effect, so further more studies are required to assess it over a larger surface area and longer period of time. With relevance to the toxicity, further studies are required to evaluate the cytotoxic effects with reference to particle sizes and their concentrations.

CONCLUSION

It is known that the White spot lesions and gingivitis are common sequalae of fixed orthodontic appliance therapy. Studies showed that nanoparticles have increased antimicrobial and reduced frictional property. In our study we found that ZnO nanoparticles had increased antimicrobial property. Invitro cytotoxicity test showed no signs of toxicity whereas the in-vivo cytotoxicity test showed mild toxicity based on the statistical values of the blood sample whereas the H&E staining of the organs such as liver, kidney and spleen showed no signs of cytotoxicity. Therefore, further studies are required to check their property on varied concentration of the nanoparticles.

REFERENCES

1. Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. J Dent Res. 1970;49:320-324.
2. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod. 1982;81:93-98.
3. Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. Eur J Orthod. 1986;8:229-234.
4. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. Eur J Orthod. 2001;23:475-484.
5. Eliades T, Eliades G, Brantley WA. Microbial attachment on orthodontic appliances: I. Wettability and early pellicle formation on bracket materials. Am J Orthod Dentofacial Orthop. 1995;108:351-360.
6. Wennström JL. Mucogingival considerations in orthodontic treatment. Semin Orthod. 1996;2:46-54.
7. Bollen AM, Cunha-Cruz J, Bakko DW, Huang GJ, Hujoel PP. The effects of orthodontic therapy on periodontal health: a systematic review of controlled evidence. J Am Dent Assoc. 2008;139:413-422.
8. Menzaghi N, Saletta M, Garattini G, Brambilla E, Strohmenger L. Changes in the yeast oral flora in patients in orthodontic treatment. Prev Assist Dent. 1991;17:26-30.
9. Polson AM, Subtelny JD, Meitner SW, Polson AP, Sommers EW, et al. Long-term periodontal status after orthodontic treatment. Am J Orthod Dentofacial Orthop. 1988;93:51-58.
10. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ. Prevalence of Porphyromonas gingivalis and periodontal health status. J Clin Microbiol. 1998;36:3239-3242.
11. Tanner AC, Maiden MF, Zambon JJ, Thoren GS, Kent RL Jr. Rapid chairside DNA probe assay of Bacteroides forsythus and Porphyromonas gingivalis. J Periodontal Res. 2010;33:105-117.
12. Dahlen G. Microbiological diagnostics in oral diseases. Acta Odontol Scand. 2006;64:164-168.
13. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, et al. In vitro testing of antimicrobial activity of bone cement. Antimicrob Agents Chemother. 2004;48:4084-4088.
14. Ahn SJ, Lee SJ, Kook JK, Lim BS. Experimental antimicrobial orthodontic adhesives using nanofillers and silver nanoparticles. Dent Mater. 2009;25:206-213.
15. Carvalho FG, Brasil VL, Silva Filho TJ, Carlo HL, Santos RL, Lima BA. Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion. Braz Oral Res. 2013;27:463-470.
16. Katz A, Redlich M, Rapport L, Wagner HD, Tenne R. Self-lubricating coatings containing fullerene-like WS2 nanoparticles for orthodontic wires and other possible medical applications. Tribol Lett. 2006;21:135-139.
17. Freitas RA. Nanodentistry. J Am Dent Assoc. 2000;131:1559-1565.
18. Borzabadi-Farahani A, Borzabadi E, Lynch E. Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. Acta Odontol Scand. 2014 ;72:413-417.
19. 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;11:2511-2527.
20. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. Elsevier; 2008.
21. Kim YS, Song MY, Park JD, Song KS, Ryu HR, et al. Subchronic oral toxicity of silver nanoparticles. Part Fibre Toxicol. 2010:6;7:20.
22. Tsabari H. Inorganic Fullerene-Like Nanospheres (IF-WS2) Acute Oral Toxicity, Acute Toxic Class Method in the Rat. Final Report, Israel; Harlan Biotech; 2005.
23. Haist I. Test for sensitization (Local Lymph Node Assay-LLNA) with Inorganic Fullerene-like WS2 Nanospheres, Germany, BSL Disservice; 2005.
24. Zachrisson BU, Zachrisson S. Gingival condition associated with partial orthodontic treatment. Acta Odontol Scand. 1972;30:127-36.
25. Kang T, Guan R, Song Y, Lyu F, Ye X, Jiang H. Cytotoxicity of zinc oxide nanoparticles and silver nanoparticles in human epithelial colorectal adenocarcinoma cells. 2015;60:1143-1148.
26. Ramazanzadeh B, Jahanbin A, Yaghoubi M, Shahtahmassbi N, Ghazvini K, et al. Comparison of antibacterial effects of ZnO and CuO nanoparticles coated brackets against Streptococcus mutans. J Dent (Shiraz). 2015;16:200-205.
27. Metin-Gursoy G, Taner L, Akca G. Nanosilver coated orthodontic brackets: in-vivo antibacterial properties and ion release. Eur J Orthod. 2017;39:9-16.
28. Moore GE. Acute Inhalation Toxicity Study in Rats-Limit Test. Product Safety Laboratories. NJ, USA: Dayton; 2006.
29. Cao B, Wang Y, Li N, Liu B, Zhang Y. Preparation of an orthodontic bracket coated with a nitrogen-doped TiO(2-x)N(y) thin film and examination of its antimicrobial performance. Dent Mater J. 2013;32:311-316.
30. Baby RD, Subramaniam S, Arumugam I, Padmanabhan S. Assessment of antibacterial and cytotoxic effects of orthodontic stainless steel brackets coated with different phases of titanium oxide: An in-vitro study. Am J Orthod Dentofacial Orthop. 2017;151:678-684.