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Antibacterial Effect of Orthodontic Adhesive Containing Titanium Dioxide Nanoparticles: an Experimental Animal Study

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ABSTRACT:

Introduction: *The most important goal of orthodontic treatment is to improve esthetics in the dentofacial region. Demineralization around orthodontic brackets is a discouraging sequela during and after orthodontic treatment. In order to overcome this phenomena, TiO_2-NPs was added to orthodontic adhesives to assess its antibacterial effect.* ***Materials and Methods:*** *Twenty healthy albino rats were randomly divided into two groups. A small increment of commercial unmodified composite was placed on the lower central incisor of rats for study group. A small increment of composite containing TiO_2-NPs was placed on the lower central incisor of rats for study group. For antibacterial test, a swab was taken every week to for counting S. mutans and total bacterial count in both groups.* ***Results:*** *Results showed significant reduction in bacterial counting for S. mutans and total bacterial count in study group more than that of control group.* ***Conclusion:*** *TiO2-NPs is a promising antibacterial agent and may play a major role in preventing WSLs which developed during orthodontic treatment.*

INTRODUCTION

Orthodontic appliances can affect the self-cleaning ability of teeth, alter the oral micro flora and increase the levels of acidogenic bacteria. These bacterial byproducts causes demineralization and formation of WSLs which formed around fixed orthodontic attachments. These can cause caries thereby leading to poor esthetics and patient dissatisfaction.

Due to antibacterial capabilities of certain NPs, these have been incorporated in orthodontic adhesives to control the oral biofilm and reduce the demineralization around the brackets.

Since certain NPs have antibacterial activity directly on bacteria and indirect by providing smooth surface both inhibit bacterial growth and plaque accumulation, so in the current study we are going to evaluate the antibacterial effect of the composite containing TiO2-NPs.

Review:

The effect of orthodontic treatment on prevalence of S. mutans in plaque and saliva was evaluated on 14patients, with low to moderate caries index. Three patients were treated with 2-4 orthodontic attachments (limited banding), 9 patients treated with 8-22 attachments (extensive banding) and 2 patients with limited and later extensive banding. Samples were collected prior and after banding between 1 and 14 months, plaque levels of S. mutans were assessed. Microbiological results showed that a significant difference existed between subjects treated with extensive banding compared with those treated with limited banding. It was concluded that the

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presence of S. mutans and Lactobacillus and new sites of plaque appearance on the enamel surrounding the orthodontic attachments is common in patients undergoing fixed appliance therapy. This may be further influenced by the duration of the orthodontic treatment and the number of orthodontic attachments [(1)].

Nanocomposite containing calcium phosphate and quaternary ammonium was tested for its antibacterial effect. A composite containing NACP, QADM and two commercial composites were tested as control groups. Six composite discs of each group were inoculated with Streptococcus mutants for 180-days, NACP+QADM nanocomposite and commercial controls. NACP+QADM nanocomposite reduced the biofilm CFU compared with commercial composites. It was concluded that NACP+QADM nanocomposite greatly decreased biofilm metabolic activity, CFU, and lactic acid [(2)].

In a review article, it was stated that inorganic NPs and their nano-composites are considered as good antibacterial agents. The metallic NPs are the most promising as they show good antibacterial properties due to their large surface area to volume ratios, which draw growing interest from researchers due to increasing microbial resistance against metal ions, antibiotics and the development of resistant strains. Metallic NPs could be used as effective growth inhibitors in various microorganisms and thereby are applicable to diverse medical devices [(3)].

The antibacterial effect of chitosan and ZnO-NPs were assessed against S. mutans, S. sanguis and L. acidophilus in four groups. Frist group unmodified composite and considered as control group. Second group 1% chitosan and ZnO-NPs blended to composite. Third group 5% chitosan and ZnO-NPs blended to composite. Fourth group 10% chitosan and ZnO-NPs blended to composite. A total of 162 discs were prepared, sterilized and disc agar diffusion test was performed to assess the antimicrobial effects of NPs. Results showed that the antibacterial effect was observed in 10% group. It concluded that a mixture of ZnO-NPs and chitosan NPs has induced an antibacterial activity in resin composite especially 10% concentrations [(4)].

Fujun et al assessed the antibacterial effect of orthodontic cement modified with Ag-NPs. A total of 160 extracted upper premolar were randomly divided into 8 groups. Teeth were embedded in dental stone, then orthodontic brackets were bonded to them. The adhesive was consisted of reinforced glass ionomer cement with Ag-NPs, which was added to assess the antibacterial effect of NPs. Five groups of reinforced glass ionomer cement with Ag-NPs with the weight ratio of 1:99, 3:97, 5:95, 10:90 and 15:85 respectively. Another three groups as control consisted of reinforced glass ionomer cement and two composite resin cements. For antibacterial assessment Agar diffusion and direct contact tests. Results showed that NPs provide a good antibacterial effect, which helps in preventing WSLs[(5)].

Mhaske et al evaluated the Ag coated orthodontic wires for antiadherent and antibacterial properties against *L. acidophilus*. Thermal evaporation method was used to treat stainless steel and nickel titanium orthodontic wires with Ag particles. Eight groups were included in this study, consisted of 10 specimens for each group. Four groups for antiadherent test and four groups for antibacterial test. For each test, two controls uncoated, stainless steel and nickel titanium orthodontic wires, and two experimental groups surface modified stainless steel coated with Ag.Results showed that coated wires with superior antiadherent and antibacterial properties in comparison to uncoated wires. It was concluded that Ag coated wires could be used in orthodontic treatment to prevent formation of dental plaque and dental caries[(6)].

An experimental animal study was performed to evaluate the antibacterial effect of Ag-NPs. Mandibular incisor brackets were coated with Ag-NPs. Samples were collected by means of saliva, blood, vestibular smear from the lower lip epithelium, and the plaque sample of the incisor teeth on different periods of time. Caries assessment was performed for

maxillary molars and the sulcus of the mandibular molar teeth on the left side for both smooth and occlusal surfaces of each animal. Results showed that NP release in saliva was not significant and serum samples showed the same results. Caries assessment was significantly decreased in study group for smooth surface caries [(7)].

TiO_2NPs were used surface treatment of orthodontic wires to evaluate its antiadherent and antibacterial properties. Results showed mild difference in weight bacterial adhesion with slight increase in uncoated wires. Coated wires showed a significant antibacterial effect against *S.* mutans in comparison to uncoated wires. For *P. Gingivalis* coated wires showed initial antibacterial effect in first 30 minutes then it was steady. It was concluded that surface treatment of orthodontic wires with TiO_2 NPs could prevent dental plaque, which is developing during orthodontic treatment [(8)].

Dental adhesive was mixed with TiO_2NPs with percentages of 5%, 10%, and 20%. Control samples included a composite without TiO_2. The sample plates were irradiated with the UV light. S. epidermidis was used in the bacterial killing test as the growth of *S.* mutans was not successful. Bacterial suspensions were spread on the samples for each group. UV irradiation was applied while the controls without UV irradiation. Microbiological results showed that a good bacterial killing ability was with TiO_2/adhesive mixtures of 10 and 20 % TiO_2NPs after 2 hours UV irradiation [(9)].

Ceramic brackets coated with TiO_2 were tested for antibacterial effect. Coating was performed using a sol gel dip-coating method. Seven coated and five uncoated brackets were transferred to sterile petri dishes containing bacterial suspensions of S. mutans and C. albicansand illuminated with UV. TiO_2 film coating exerts a significant effect on the microorganisms used under UV. The number of attached cells is reduced and the cells break down after UV[(10)].

Shah et al assessed the anti-adherent and antibacterial properties of surface modified stainless steel orthodontic brackets with TiO_2 against L. acidophilus. A total of 120 stainless steel brackets were divided into four groups. Two control groups which were uncoated, one for anti-adherent, and other for antibacterial test. Another two testing groups coated with a thin film of TiO_2, one for each test. It was concluded that surface modification of orthodontic brackets with TiO_2 could be used to prevent demineralization during orthodontic treatment [(11)].

Poosti et al evaluated the antibacterial effects of orthodontic composite containing TiO_2NPs. A total of 45 composite discs specimen were prepared. Of the 45 discs, 30 discs were made from nanocomposite and tested for antibacterial properties immediately and 30 days after curing by direct contact test. The antibacterial properties of the remaining 15 discs that were made from the conventional composite were tested immediately after curing as control group. Comparison of antibacterial effects between conventional and nanocomposite demonstrated significant difference between two groups, with nanocomposites having a higher antibacterial activity [(12)].

An in vitro study to determine the antimicrobial efficacy of TiO_2 and ZnO NPs eradication of E. faecalis endodontic biofilms in dentinal tubules. Ninety extracted single-rooted human teeth were instrumented and inoculated with E. Faecalis. After 21 days the positive control group was irrigated with sterile saline. The experimental groups were irrigated with NaOCl/EDTA, TiO_2 NPs, and ZnO NPs. The negative control group was not inoculated, but it was irrigated with sterile saline. Samples were obtained on paper points for culture and Polymerase chain reaction. Results showed that all experimental treatments resulted in a significant increase in dead cells when compared to the controls[(13)].

Stainless steel edgewise brackets were deposited with thin film of TiO_2 using high-vacuum magnetron sputtering equipment. Suspensions of the common oral pathogens S. mutans, L. acidophilus, A. viscous, and C. albicans were prepared at a concentration of 1.5×10^6 CFU/mL each. 100 µL of each suspension was added to 3 mL of liquid

medium in tubes, and the test brackets (coated with TiO_2 thin film) and control brackets (normal edgewise brackets) were placed into these tubes, which were incubated at 25°C and 37°C for 24 h under visible light. TiO_2 coated brackets showed high antimicrobial effect against S.mutans, L. acidophilus, A. viscous, and C. albicans prevented the adherence of S. mutans. In addition, that the bracket coated with the TiO_2 thin film may be effective for the prevention of enamel demineralization and gingivitis during orthodontic treatment[14].

Three Stainless steel orthodontic wires (0.016 × 0.022, 0.016 × 0.022 and 0.014 × 0.018 inch multistranded hammered retainer wire) were used in a study to evaluate the antimicrobial effect of TiO_2 film coated orthodontic arch wires on three oral pathogens. Wires were coated with $TiO_2$by the sol-gel dip coating method. The wires were assessed for their photo catalytic antimicrobial activity against *S. mutans, C. albicans*, and *E. faecalis*. After illumination under UV light, the reduction effiencies of the anatase-coated arch wires were calculated by using CFU. Results showed all anatase coated arch wires with remarkable inhibitor effects against the tested microorganisms under UV light [15].

Thomas et al studied the antibacterial effect of TiO_2 on cariogenic bacteria. For this purpose, 40 samples in the form of swabs from patients having signs and symptoms of dental caries then TiO_2 was added to the collected samples and these samples were checked for the antimicrobial activity by the zone of inhibition obtained. Out of the 40 samples, TiO_2 showed good activity against 18 samples. This study suggested that TiO_2 might have antibacterial activity aganist cariogenic bacteria [16].

Different NPs were tested for their antimicrobial effect as mouthwashes. Four groups of ZnO, CuO, TiO_2 and Ag NPs were tested for antimicrobial effect against S. mutans and S. sangius. The control groups were consisted of 2.0% sodium fluoride and 0.2% chlorhexidine mouthwashes. Results showed that sodium fluoride mouth rinse did not have antibacterial effects against any of the microorganisms. The solution containing TiO_2NPs showed greatest antibacterial activity against S. mutans and S. sangius than any other group [17].

Orthodontic brackets coated with nano Ag/TiO_2 are tested for antibacterial effect against S. mutans, S. sanguis, A. actinomycetemcomitans, F. nucleatum, P. gingivalis, and P. intermedia. Thin film of nano Ag/TiO_2 and nano TiO_2were used for coating on the surface of orthodontic brackets with spin-on deposition. A volume of 0.2mL bacterial culture broth was added on nano Ag/TiO_2 thin film and nano TiO_2 thin film, this procedure was repeated three times for each sample. Results showed that nano Ag/TiO_2 had antibacterial activity to all experimental bacteria. TiO_2 did not show antibacterial activity. Study concluded that nano Ag/TiO_2 coating have antibacterial effect aganist different bacteria and should undergo further clinical orthodontic studies [18].

MATERIAL AND METHODS

Experimental design

Sample size calculation

The optimum value of the sample size in animal studies could be determined by a value called E value, where E value = Total number of animals – Total number of groups

E value should be ranged from 10 to 20 [19]. In our study, according to E value, the sample size was ranged from 12 to 22 rats.

Using G Power (version 3.1) software for sample size calculation, a statistical results of previous study [7] were used to calculate sample size in the current study, it was found that sample size should be at least 6 rats in each group at significance level 0.05.

Grouping

After acceptance of the ethical committee at Faculty of Dentistry, Suez Canal University, a total of 20Specific Pathogen Free female Albino Rats, were purchased from Faculty of Veterinary Medicine, Suez Canal University. The age of the rats in this study was about five months with erupted lower centrals incisors.

Rats were placed in 10 plastic cages. the rats were randomly divided into two groups; Control group (Group A) which consisted of tenrats (conventional adhesive was used) and study group (Group B) which consisted of tenrats (modified adhesive containing TiO_2-NPs were used).

Preparation of composite-NPs mix:

TiO2-NPs (Nano-powder anatase and rutile phases, particle size of 21±5 nm, P25; Plasma Chem GmbH, Berlin, Germany) were used to modify the orthodontic adhesive to assess their antimicrobial activity. A sensitive balance, was used to measure both TiO2-NPs and the orthodontic adhesive (Transbond XT; 3M Unitek, Monrovia, California, USA) The ratio of NPs to the adhesive was 1% by weight. A sterilized stainless steel spatula and glass slab were used in mixing NPs to the adhesive. The mixing was performed in a semi dark room to a void polymerization of the adhesive. Mixing time was about 5 minutes to ensure proper distribution of the NPs.

Bacterial culture preparation

S. mutants UA 159 were brought on a blood agar from Microbiology Department, Faculty of Medicine, Mansoura University. S. mutans was sub cultured on a blood agar supplemented with 5-10% sheep RBCs. The micro-organismm was propagated by a serial passage on Brain Heart Infusion broth (CM 1135 Brain Heart Infusion, OXOID LTD, Hampshire, England)with incubation at 37 ^{o}C / 16 - 24 hours and cultured on Brain Heart Infusion Agar (CM 1136 Brain Heart Infusion Agar, OXOID LTD, Hampshire, England) supplemented with anti-fungal growth agents (Cyclo-heximide 0.5 ml/ 100 ml agar + Amphotricin - B 1.5 ml / 100 ml agar) were added and mixed thoroughly in a horizontal position[20].Plates were incubated at 37 ^{o}C / 16 - 24 hours, and colonies ranged from 30 – 300 CFU per plated were counted, extremes were rejected. Pure colonies were picked up and resuscitated in physiological saline 0.9%, then counted after subculture on Brain Heart Infusion Agar. A final inoculum size of 12×10^6 was ready to be inoculated into rat's mouth using Pasteur pipette.

Experimental animals preparation

Rats were doused with amoxicillin (25 mg/kg) for two days (on day -3 and -2)to suppress the indigenous flora to enable S. mutans to establish themselves in the oral cavity. Rats were left without antibiotic for 24 hours before bonding. On day -1, rats were infected with 0.50 ml of S.mutans 12×10^6 CFU directly into the oral cavity of each rat, then for 24 hours rats were supplied with water containing 1 ml of a cell suspension (containing the same inoculum size to ensure complete infection) placed in 15ml water in each cage.

Bonding procedures

Bonding brackets on rat's teeth was excluded because bonding failure and subsequent rebonding with orthodontic adhesive containing TiO_2-NPs affects standardization and concentration levels of tested material among rats as observed in a similar study[7]. So it was decided to use orthodontic adhesive directly on tooth surface. A small increment of orthodontic adhesive was placed on the middle-middle third the lower left central incisor for both groups. Modified TiO_2 NPs orthodontic adhesive was used for bonding of the study group while control group was bonded with unmodified conventional orthodontic adhesive. Tooth of choice was dried and etched with 37% phosphoric acid for 20 seconds then rinsed with water, a thin layer of bond was applied on the tooth surface and small increment of orthodontic adhesive was applied by plastic instrument. Then a light cure was applied perpendicularly on the tooth surface for 20 seconds.

Figure (1) : Rats' tooth after bonding procedures

Sampling

Sterile swabs were used to collect samples for bacteriological assessment around orthodontic adhesive for both control and study groups. Rats were neither allowed to eat nor to drink for one hour before sample collection. The samples were collected at different periods as shown in Table (1).

Sample	Time of collection
T0	24 hours after bacterial inoculation before orthodontic adhesive placement.
T1	7 days post bonding
T2	14 days post bonding
T3	21 days post bonding
T4	28 days post bonding
T5	35 days post bonding

Table 1. Time intervals for sample collection used for bacteriological assessment

Bacterial Counts

Swab samples were prepared according to the technique recommended by American Pharmaceutical Association[21]. From the original sample, 1ml was transferred aseptically to a test tube containing 9ml sterile Buffered Peptone Water (BPW) to prepare a dilution of 10^{-1}, from which tenfold decimal serial dilution up to 10^{-6} were prepared to cover the expected range of colonial growth. The dilutions were used to perform bacterial counts as following:

Total Bacterial Count:

Total number of aerobic micro-organisms in swab samples was performed using drop plate Method according to Herigstadet al. [22] using Standard Plate Count Agar (SPCA). Ten micro-litters from each dilution as well as the original samples were aseptically dropped into sterile Petri-dishes onto which 10 ml of Standard Plate Count Agar, previously melted and cooled at 45^{o}C as well as treated with anti-fungal growth agents (Cyclo-heximide 0.5ml/100ml agar + Amphotricin-B 1.5ml/100ml agar) were added and mixed thoroughly in a horizontal position[20].

Plates were inverted and incubated at 37^{o}C for 72 hours. Counting the plates showed 30-300 colonies per plates. The calculation was carried out using the following formula: Log (average CFU/ drop vol.)(Dilution factor)(Vol. scrapped into/ surface area)[23,24].

Total S. mutans count

Total number of S. mutans in swab samples was performed using drop plate Method according to Herigstadet al.[22] using Brain Heart Infusion broth that were incubated after inoculation with 1 ml of samples at 37^{o}C for 72hours. Ten micro-litters from each Brain Heart Infusion broth tubes were aseptically dropped into sterile Petri-dishes onto which 10 ml of Brain Heart Infusion Agar, previously melted and cooled at 45^{o}C as well as treated with anti-fungal growth agents (Cyclo-heximide 0.5ml/100ml agar + Amphotricin-B 1.5ml/100ml agar) were added and mixed thoroughly in a horizontal position[20].

Plates were inverted and incubated at 37^{o}c for 72hours. Counting the plates showed 30-300 colonies per plates. The calculation was carried out using the following formula: Log (average CFU/ drop vol.)(Dilution factor)(Vol. scrapped into/ surface area)[23,24].

Euthanasia of rats

Cervical dislocation is an accepted method for euthanasia of rats [25], at the end of the study rates were sacrificed with cervical dislocation method.

RESULTS

Statistical analysis was performed using the computer program Statistical Package for Social Science (SPSS, Inc., Chicago, IL, USA) version 20.0 was used to analysis the data of this study between the two groups at significance level P value ≤ 0.05.

Paired T-test has shown significant reduction for total bacterial count at 3rd week till the end of study in group B as shown in table (2) and histogram in figure (2).

Total S. mutans count

As noticed in table (3) and histogram in figure (3) S. mutanswas reduced in 1st and 2nd week in group B but it wasn't statistically significant. Meanwhileat 3rd week till the end of study there was statistically significant reduction of S. mutans in the study group.

Table 2: Statistical analysis of total bacterial count of both groups at different periods of time

Time	Total Bacterial Count / CFU		P value
	Group A	Group B	
Zero	6.9684± 0.286	6.9284± 0.250	> 0.05
1st week	6.3853 ± 0.375	5.7884 ± 0.345	> 0.05
2nd week	5.5669± 0.304	5.2032 ± 0.314	> 0.05
3rd week	5.7382 ± 0.107	2.5158 ± 0.653	< 0.05*
4th week	4.1033 ± 0.420	1.5199 ± 0.462	< 0.05*
5th week	5.1711 ± 0.579	1.1892 ± 0.706	< 0.05*
(*); statistically significant using paired T- test at (P value≤ 0.05).			

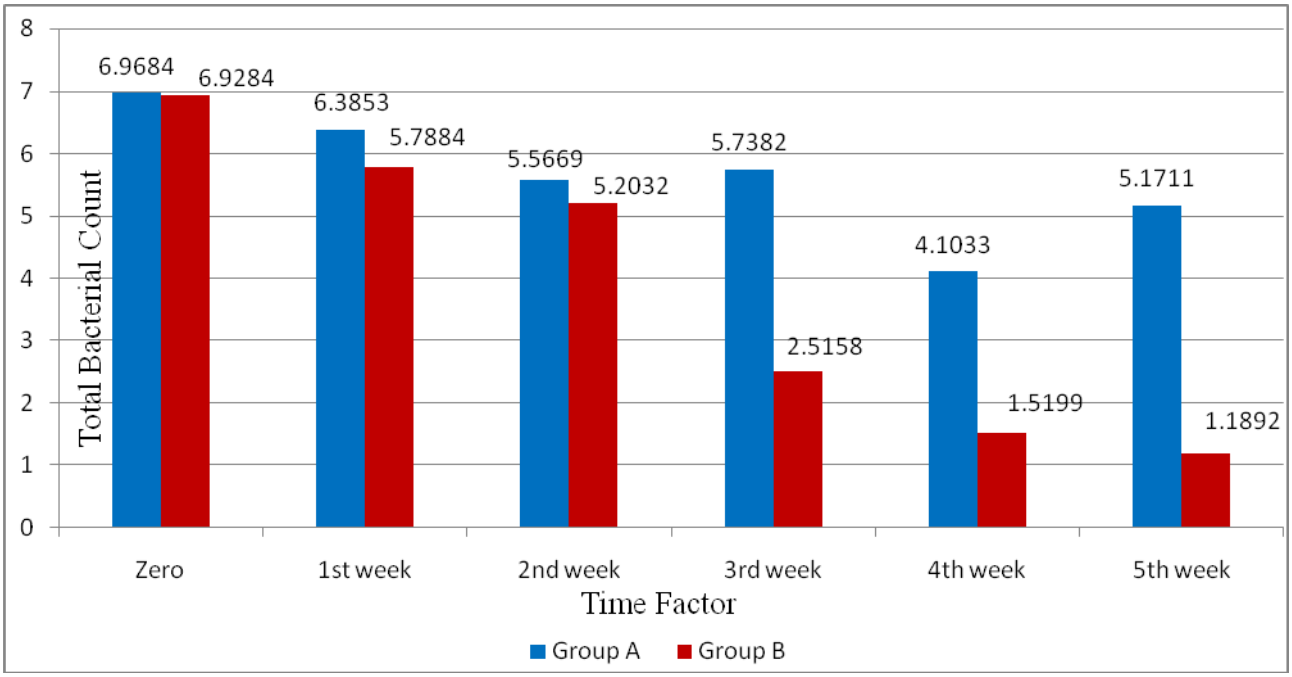

Figure 2: The mean total bacterial count of both groups at each week

Table 3. Statistical analysis of *S. mutans* count at different periods of time.

Time	S.mutans Count / CFU		P value
	Group A	Group B	
Zero	5.4640± 0.415	5.7194 ± 0.241	> 0.05
1st week	3.9449 ± 0.920	3.2255 ± 0.787	> 0.05
2nd week	3.0197 ± 0.692	2.7240 ± 0.620	> 0.05
3rd week	3.0991 ± 0.227	0.7954 ± 0.330	< 0.05*
4th week	2.7491 ± 0.303	0.1778 ± 0.177	< 0.05*
5th week	1.7992 ± 0.630	0.0000 ± 0.000	< 0.05*
(*); statistically significant, using paired T- test at (P value≤ 0.05).			

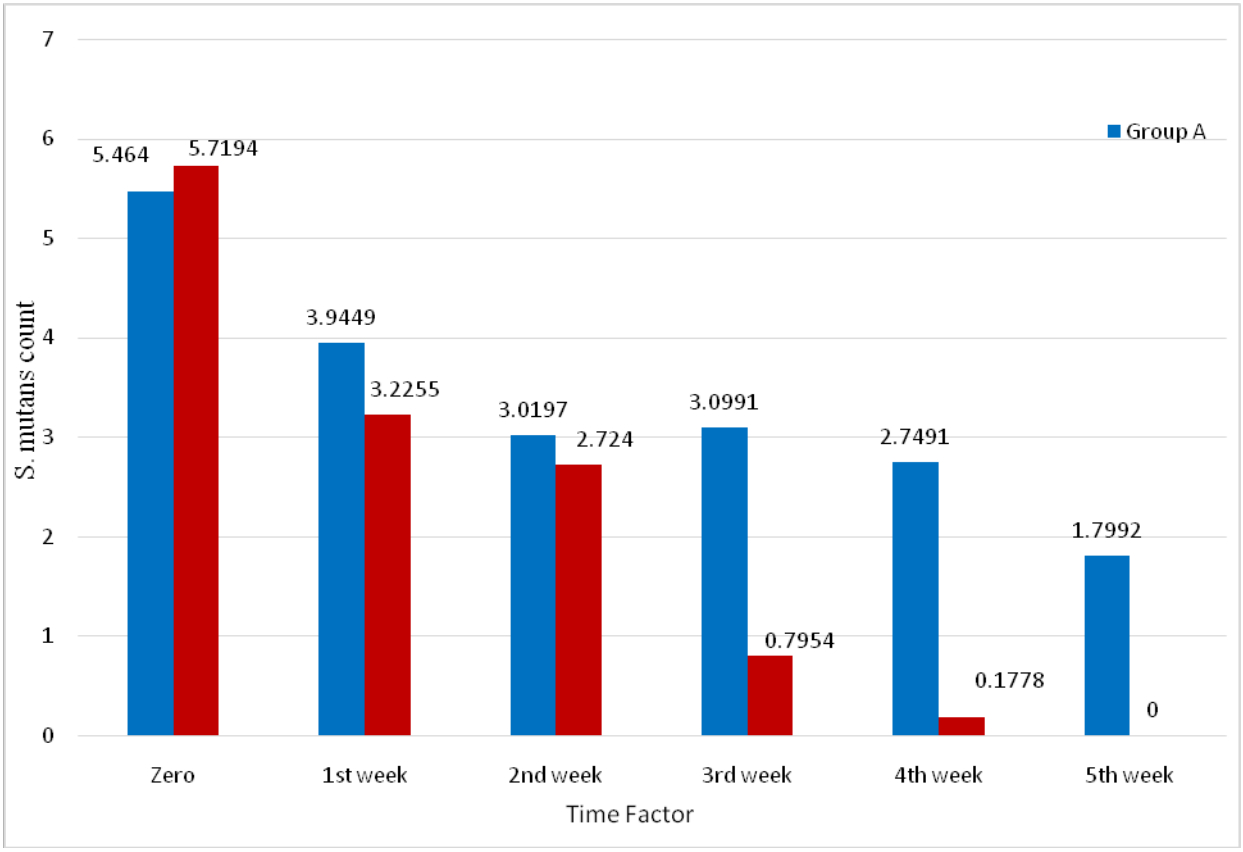

Figure 3: The mean S. mutans of both groups at each week

DISCUSSION

In an attempt to overcome one of the major drawbacks of fixed orthodontic treatment, which is the demineralization and formation of WSLs, researchers investigated a lot of methods. Dental plaque and microbial flora are the major etiological factor in the formation of WSLs. The effect of banding and bonding procedure on the presence of *S.* mutans, which plays a major role in the formation of WSLs was evaluated [(1)].

Because of their unique physical, mechanical and chemical properties, NPs have been utilized for modifications in orthodontic brackets, wires and adhesives. TiO_2-NPs was the material of choice to be added to orthodontic adhesives. Its extreme small size provides a large surface area which interacts with the surface wall of the bacteria increasing cellular permeability leading to cellular death [(3)]. The present study assessed the antibacterial effect and the biocompatibility of composite containing NPs in an experimental animal study.

Albino Rats were selected, according to the recommendations by colleagues in the faculty of Veterinary Medicine, due to their high survival rate in similar researches. In addition to ease of manipulation and their tolerance to procedure of such studies.

S. mutans was selected for counting due to its major role in development of dental plaque resulting in the formation of WSLs and demineralization around orthodontic brackets [(1)]. The genetic background of S. mutants may differ from rat to another one which may exhibit different resistant levels against the tested material. It was decided to use S. mutants UA 159 with the same genetic background to ensure same response of S. mutants to TiO_2-NPs. Other bacteria weren't neglected, so that a total bacterial counting including all bacteria around composite was performed.

Before inoculation of S. mutants, rats were dosed with amoxicillin (25 mg/kg) for two days to suppress the indigenous flora to enable S. mutans to establish themselves in the oral cavity [(7)].

The effect of 1% of TiO_2-NPs by weight was chosen to be studded in the present study because Poosti et al [12] found that 1, 2, and 3% shows same antibacterial effect. They used 1% by weight of TiO_2 with composite to form discs and tested for its antibacterial effect against S.mutans.

In the other hand Cai [9] used TiO_2 in different concentrations (5, 10 and 20%) against S. epidermidis (in vitro study). He found that the 10 and 20% concentrations poses antibacterial effect more than 5%. This study was performed for only 2 hours with ultraviolet irradiation, and the study didn't extend in time to assess the antibacterial effect in addition to smaller concentrations wasn't included.

Antibacterial effect of TiO_2-NPs

According to the results of the current study composite containing TiO_2-NPs were shown a significant reduction in bacterial count for both S.mutans and total bacterial count.

The results of the present study were congruent with those of Chuna el al [8]. They coated orthodontic wires with TiO_2 to assess its antibacterial effect against S. mutans and P. Gingivalis. A different experimental setup was performed by Ozyildiz et al [10], they coated ceramic brackets with TiO_2 and tested with S. mutans and C. albicans, with same results. TiO_2-NPs were evaluated, by Shah et al[11], for its antibacterial effect with a different bacteria which was L. acidophilus and showed cytotoxic effect on L. acidophilus as stated in this study.

The antibacterial effect of TiO_2 which was proven in the current study was matched with results of O'Hara [13] who used a different in-vitro study. He tested the antimicrobial efficacy of TiO_2 against E. faecalis of endodontic biofilms in dentinal tubules.

The results of Cao at al [14] came in agreement with the current study. They tested stainless steel edgewise brackets coated with thin film of TiO_2. In addition study of Özyildiz et al [15] showed same results when they used coated wires with TiO_2 against cariogenic bacteria including S. mutans.

Thomas et al [16] reported similar results to those of the present study. They collecting swabs from patient with active dental caries and TiO_2 was added to these samples. In like manner, Ahrari et al et al [17] assessed the antibacterial effect of four NPs including ZnO, CuO, TiO_2 and Ag-NPs against S. mutans and S. sangius. They found TiO_2 was shown highest antibacterial effect.

On the other hand the results of Zhang et al [18] came in contrast to the result of the current study. They evaluated the antibacterial effect of TiO_2 coated on brackets against oral pathogens including S. mutans for only 240 minutes, while in the current study the assessment of TiO_2 was extended to 35 days reflected significant antibacterial effect which started at third week.

Different NPs exhibited similar antibacterial effect which matched with the current study. Chenget al [2] tested composite discs containing NACP and QADM for their antibacterial effect against S. mutants. Mirhashemi et al [4] assessed the effect chitosan and ZnO-NPs by mixing them to composite, they were shown cytotoxic effect against S. mutants.

Ag-NPS were shown antibacterial effect against S. mutants with different orthodontic materials. These results were confirmed by Fujun et al[5] when they mixed Ag-NPsto glass ionmer cement, while Mhaske et al[6] used thermal evaporation method to coat orthodontic wires with Ag-NPs. Also, Metin-gürsoyet al [7]coated the brackets with Ag-NPs using physical vapor deposition.

CONCLUSION

According to the results of the present study TiO2-NPs is a promising antibacterial agent and may play a major role in preventing WSLs which developed during orthodontic treatment.

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