Research Article

Evaluation of Antibacterial Properties of Metal Brackets Coated with Graphene Oxide Nanoparticles and Its Synergistic Effect with Other Metal Oxides – An In-Vitro Study

Murali Krishna Malineni, Chandrasekhar Gandikota, Swaroopa Ponnada, Hanish Anand, Akshara Gandikota

[1]Post Graduate Student, [2]Professor and Head, [4]Reader
Panineeya Mahavidyalaya Institute of Dental Sciences and Research Centre Hyderabad, Telangana, India

[3]Research Scholar at Siksha O Anusandhan University &
Professor, Panineeya Mahavidyalaya Institute of Dental Sciences and Research Centre Hyderabad, Telangana, India

[5]Private Practitioner, Hyderabad, Telangana, India

Corresponding author: Swaroopa Ponnada; Email: drswaroopaponnada.1611@gmail.com

ABSTRACT

Introduction: The intricate design of the orthodontic brackets creates a favorable environment for plaque formation, resulting in an increased risk of decalcification and white spot lesions. Many preventative and adjunctive procedures are required to mitigate the negative effects of orthodontic therapy. With the progress of nanotechnology, orthodontics has benefitted immensely. The main objective of the study is to evaluate the antibacterial property of metal brackets coated with various concentrations of Graphene Oxide nanoparticles and its synergistic activity with other metal oxides.

Materials and Method: In this study, 120 metal pre-adjusted premolar brackets are equally divided in to 6 Groups based on various concentrations of graphene oxide nanoparticles and their synergistic activity with other metal oxides. The antibacterial properties are evaluated for each group.

Result: Brackets coated with GO 1wt% have shown statistically significant antibacterial property among the subgroups. The synergistic antibacterial action of GO+AgTiO2 have shown statistically significant antibacterial action compared to other metal oxides. The Antibacterial property of the groups are as follows: GO+AgTiO2 > GO 1wt% > GO+ZnO > GO 0.5wt% > 0.25wt% > Control.

Conclusion: The metal orthodontic brackets coated with Graphene oxide and its synergistic activity with other metal oxides have shown statistically significant antibacterial properties with no cytotoxicity affects to Human gingival fibroblasts.

KEYWORDS: Antibacterial properties, Graphene oxide nanoparticles, Silver doped titanium dioxide, Streptococcus mutans, White spot lesions

INTRODUCTION

Multiple plaque-retentive areas are a hallmark of malocclusions because of the irregularities in teeth positioning. Even though the labial surfaces of the teeth are less prone to demineralization, the presence of orthodontic appliances makes intraoral oral hygiene maintenance difficult. The presence of fixed appliances also significantly reduces saliva's self-cleaning ability. Following all the oral prophylactic measures daily, even multiple times reduces the formation of it but doesn't actually eliminate it.[1]

Their intricate design of orthodontic bracket creates a one-of-a-kind environment that makes proper hygiene maintenance difficult. In the field of orthodontics, stainless steel is one of the most used alloys for the fabrication of arch wires, brackets, bands, ligatures, and tubes. Stainless steel demonstrated the highest critical surface tension, surface energy, and increased adhesion, all these properties are responsible for the potential attraction of the microorganisms such as Streptococcus mutans and Lactobacillus acidophilus

colonization more easily. Eliades et al, stated that stainless steel brackets have a higher potential to attract the microorganisms due to their highest critical surface tension property.[2]

As Streptococcus mutans and lactobacilli are often associated with the development of caries. These microorganisms aids in lowering the PH of the plaque by producing acid by products from the fermentable carbohydrates. Freitas et al, stated that the fixed orthodontic appliances have a significant evidence in the quantity and quality of oral microbiota.[3] Similarly, Luchese et al, also reported that orthodontic appliances have shown significant increase in the colonization of streptococcus mutans and lactobacillus.[4]

The low pH of plaque around the orthodontic brackets prevents remineralization and results in enamel decalcification and white spot lesions. The White spot lesion has been defined as "subsurface enamel porosity from carious demineralization that presence itself as a milky white opacity when located on smooth surfaces"[5] According to Fejerskov and Kidd white spot lesions are considered as the first sign of an enamel carious lesion which can be easily noticed by naked eye.[6]

White spot lesion (WSL) is considered as the common sequelae in patients undergoing orthodontic treatment. Incidence and severity of white spot lesions has been proven to increase more in orthodontic patients than non-orthodontic patients and they cause esthetic problems years after treatment.[7-9] The prevalence of white spot lesions ranges from 2 to 96 percent in orthodontic patients.[10] The prevalence of WSL has been reported to be 38% in 6 months, 46% in 12 months after the initiation of the treatment, and 50% at the end of the fixed orthodontic treatment. The incidence was found to be highest in labio-gingival area of the maxillary lateral incisors and lowest in the maxillary posterior segment.[11]

Many oral hygiene regimens like fluoridated toothpaste, fluoride containing mouthwashes, gel and varnish have been used day to day in order to prevent WSL.[7] Although substantial attempts have been made to teach patients about good oral hygiene, the relationship of WSL with fixed orthodontic equipment continues to be a serious clinical problem. Unfortunately, less than 15% of orthodontic patients abide by instructions.[12]

The antimicrobial properties of stainless-steel surfaces can be adjusted before appliance application to prevent enamel demineralization and overcome the difficulties associated with traditional retentive methods. In addition, the advent of an antibiotic-resistant type of bacteria has sparked interest in some metals, notably in the form of nanoparticles.[13]

N. Taniguchi first used the word "nanotechnology" in 1974 at Tokyo Science University. Nanomaterials are substances with parts that are at least one dimension smaller than 100 nm.[14] These elements might take the shape of grains, fibres, clusters, nanoholes, or a mix of these. Sheets are characterised in one dimension; nanowires and nanotubes in two dimensions; and quantum dots in three dimensions.[15] Recently, a lot of interest has been focused on the antibacterial characteristics of semiconductor nanoparticles such titanium dioxide (TiO2), zinc oxide, tungsten oxide, and iron oxide.[16]

In comparison to other metals, silver has long been recognised for its antibacterial properties against gram-positive and gram-negative bacteria, fungi, protozoa, and certain viruses, including those that are resistant to antibiotics. Silver nanoparticles also emit silver ions for 4 months, which will have a long-lasting antibacterial impact. These are biocompatible and do not significantly affect cytotoxicity or mutagenesis.[17] Recent studies have demonstrated that coating orthodontic brackets with titanium compounds can reduce the amount of S. mutans by up to 98%.[18] Zinc oxide nanoparticles (ZnO) was proven to be a good antibacterial agent.[19]

Carbon allotropes have been introduced and used in various fields of medicine and dentistry to date. Graphene has gained prominence among the various Allotropes due to its diverse set of characteristics. Recent research has demonstrated that GO has the strongest antibacterial impact when compared to other graphene-based materials including graphite, graphite oxide, and reduced graphene oxide, especially with regard to dental microorganisms.[20]

Graphene oxide (GO), a form of graphene with more functional groups containing oxygen than graphene itself, has greater active characteristics. According to Dai et al. (2022), the bacteriostatic impact of GO coating was strengthened as graphene oxide concentration increased.[21]

The objective of the present research was to develop a bracket coated with a thin coating of graphene oxide and assess its efficacy against Streptococcus mutans.

This study may help create an innovative approach for effectively preventing enamel demineralization and gingivitis in orthodontic patients.

MATERIALS AND METHOD

This was a prospective in-vitro study done under strict lab setting. The study duration was around 2 months. This study was conducted after obtaining ethical approval from Institutional Ethical Committee (PMVIDS & RC/IEC/ORTHO/DN/365-20)

1. MBT .022" Slot Stainless steel pre adjusted edgewise brackets (Ormco MINI 2000 1st Premolar brackets)
2. Graphene Oxide (GO) nanoparticles.
3. Zinc Oxide (ZnO) nanoparticle
4. Sliver doped Titanium dioxide (AgTiO2) nanoparticles
5. Streptococcus mutans strain (ATCC 25175)
6. Petri Dishes
7. BHI Broth
8. BHI Agar Medium
9. Eppendorf Tubes

This study included 120 MBT 0.022" Slot 1st premolar pre adjusted edgewise brackets (Ormco MINI 2000). These brackets were divided into 4 groups and 3 Sub groups. Uncoated group (Group 1) Consisted of 20 brackets acted as a control. Experimental groups were divided into 3 groups (Group 2,3,4). Group 2 was divided into 3 sub-groups (Sub-Group A, B, C) based on different concentrations of Graphene Oxide (0.25wt%, 0.5wt%, 1wt%) (Fig: 1)

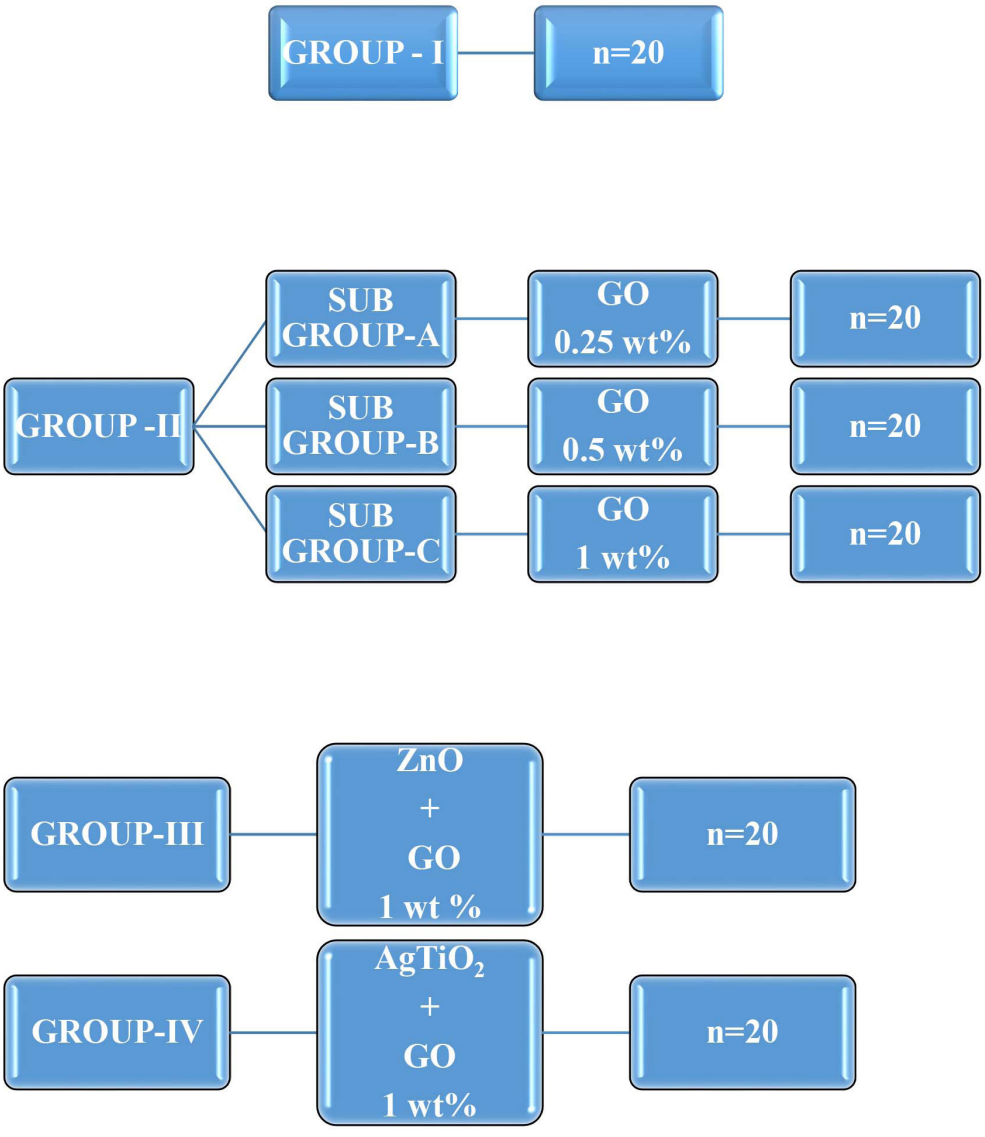

Fig 1: Flowchart representing the sampling of the study

Table 1: Tabular representing the sampling of the study

GROUPS	SAMPLE SIZE	NANO COATINGS
GROUP I	Control Group (n-20)	Uncoated Brackets
GROUP II Graphene Oxide (GO)	Sub Group A (n-20)	Graphene Oxide 0.25wt%
	Sub Group B (n-20)	Graphene Oxide 0.5wt%
	Sub Group C (n-20)	Graphene Oxide 1wt%
GROUP III	Sub Group C + ZnO (n-20)	GO 1wt% + ZnO
GROUP IV	Sub Group C + $AgTiO_2$ (n-20)	GO 1wt% + $AgTiO_2$

The Sub-group showing best antibacterial property was further combined with ZnO nanoparticle, formed Group 3 and with Sliver doped Titanium dioxide nanoparticle formed group 4. (Fig: 2). Graphene Oxide powder was synthesized from purified natural graphite powder provided by Sigma-Aldrich Co., using the Modified-Hummer method.[22]

Control | 0.25 wt% GO | 0.5 wt% GO | 1 wt% GO | GO+ ZnO | GO+ $AgTiO_2$

Fig 2: Clinical variation between the stainless steel brackets coated with different nanoparticles used in the study

The orthodontic brackets were ultra-sonicated in 99% ethanol and 99.5% acetone for 30 mins, in each to remove macroscopic contaminants and then dried in a desiccator. After the brackets are thoroughly dried they are sterilized using an autoclave.[23] Graphene oxide of three different concentrations based on wt% were coated on all the surfaces of Orthodontic stainless steel brackets using electrophoretic deposition.[24] The procedure was carried out at ARCI (International Advanced research Centre for Powder Metallurgy & New Materials, HYDERABAD, INDIA). The assessment of coated brackets was done under Scanned Electron Microscope (Fig: 3, 4 & 5).

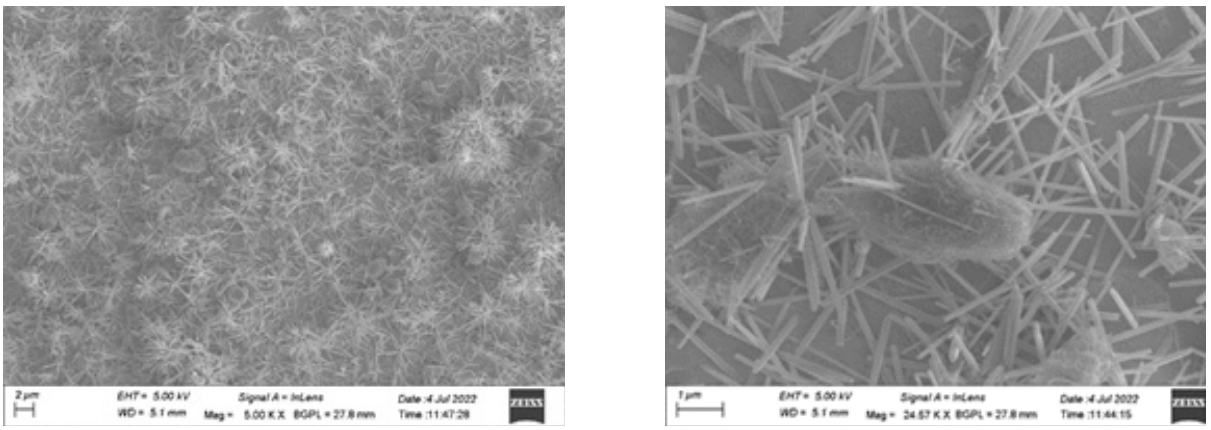

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Fig 3: SEM image of Graphene Oxide nanoparticles on Stainless steel orthodontic brackets

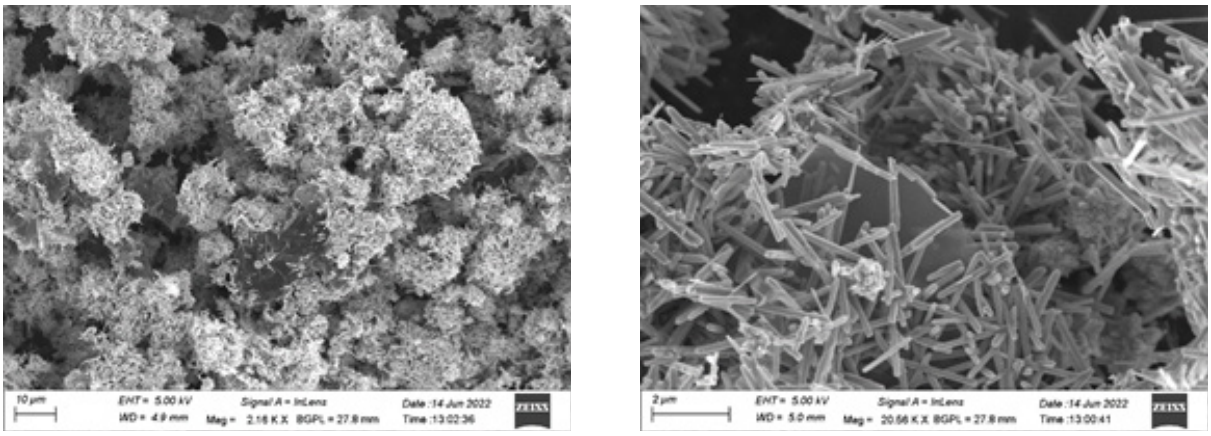

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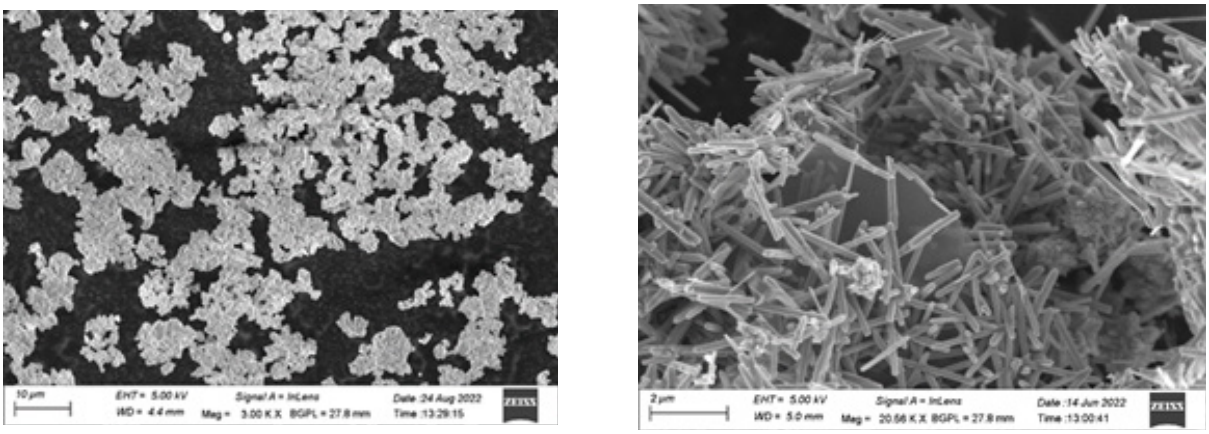
Fig 4: SEM image of GO and its Combination with ZnO nanoparticles on Stainless steel orthodontic brackets



5 K x Magnification 25 K x Magnification

Fig 5: SEM image of GO and its Combination with AgTiO2 nanoparticles on Stainless steel orthodontic brackets

S. mutans (ATCC 25175) in the form of KwikStik (Microbiologics, Minnesota, USA) and Brain Heart Infusion (BHI) agar (HiMedia, Mumbai) (Fig: 6) were chosen to conduct the antimicrobial test.

Fig 6: Streptococcus Mutans Strain (ATCC 25175)

The study availed the use of colony counting approach. The petri dishes were thoroughly cleaned, dried and subjected for sterilization in Hot air oven. The BHI agar was prepared on the sterile petri dishes and placed in incubator. S. mutans was cultured as a suspension in BHI broth at a concentration of 1.5 x 10^6 colony forming units (CFU)/mL. Next, 3 mL of liquid medium in eppendorf tubes were filled with 100 µL of the suspension. Both the experimental group (orthodontic brackets coated with graphene oxide) and the control group (orthodontic brackets without coating) were transferred into the tubes (Fig: 7).

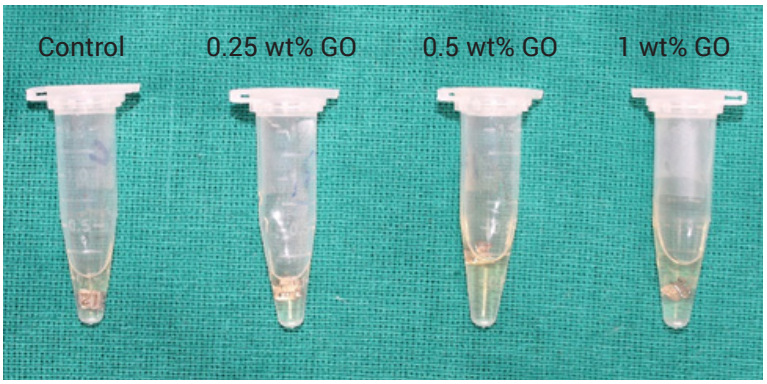


Fig 7: Uncoated and Coated brackets in the Eppendorf Tubes containing Bacterial suspension

The tubes were incubated for 60 min at 37°C under 100 W*2 of visible light. After illumination, 10 mL of sterile saline was added to 10µ L of the suspension, which was then collected using a micropipette from each tube at intervals of 0, 2, 4, 6, and 24 hours and was plated on BHI Agar plates and cultured at 37°C. Antibacterial activity was evaluated by colony counting on each plate after 24 hrs of incubation.[25] (Fig: 8). The concentration of Graphene Oxide which has higher antibacterial property was further used in combination with other metal oxides.

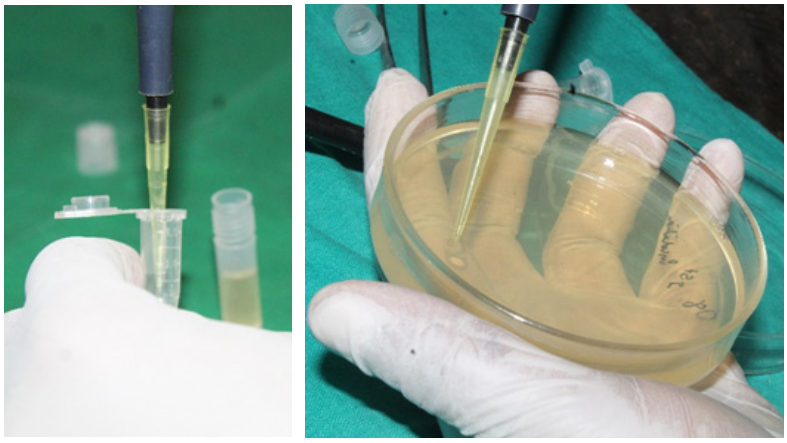


Fig 8: Pipetting and Inoculation of Bacterial Suspension from the Eppendorf tubes

Zinc oxide nanoparticles which were synthesized by reflux digestion method were combined with 1%wt of Graphene oxide nanoparticles[26] and Silver doped Titanium dioxide were combined with Graphene oxide coated on the stainless steel brackets by physical vapor deposition method. After antibacterial assessment of these metal oxides in combination with Graphene oxide, the best coated brackets showing antibacterial property was assessed for cytotoxicity.

Statistical analysis:

The data obtained is entered in excel sheet and subjected for statistical analysis using SPSS software (statistical package for social sciences) software 22.0 (IBM, New York). The normality of the data was derived using Kolmogorov–Smirnov normality test, and the antimicrobial efficacy data did not follow normal distribution, hence non-parametric test was applied. For comparison within the groups at different timelines Friedman Two Way ANOVA was used and for between group comparison Kruskal Wallis test was used. $p<=0.05$ was considered to be statistically significant.

RESULT

In comparison of the antibacterial property of control group and within the subgroups (A, B, C) of experimental group 2, [Table 2,3,4,5] all the subgroups have shown statistically significant antibacterial property after 24 hrs [Table 6]. But in comparison with all the subgroups, 1wt% GO of subgroup C have shown statistically significant antibacterial property after 24hrs of time [Graph 1].

Table 2: Pair wise mean comparison of antibacterial effect within control group between different timelines using Post Hoc analysis – Dunn Bonferroni test.

Comparison between		Mean difference	P value
Baseline	2 hours	-59.6	**0.001***
	4 hours	-189.1	**0.000***
	6 hours	-267.55	**0.000***
	24 hours	-49.7	0.455
2 hours	4 hours	-129.5	0.455
	6 hours	-207.9	**0.000***
	24 hours	9.9	0.455
4 hours	6 hours	-78.45	0.455
	24 hours	139.4	**0.000***
6 hours	24 hours	271.85	**0.000***

Table 3: Pair wise mean comparison of antibacterial effect within subgroup A of experimental Group 2 between different timelines using Post Hoc analysis – Dunn Bonferroni test.

Comparison between		Mean difference	P value
Baseline	2 hours	-262.05	**0.000***
	4 hours	-352	**0.000***
	6 hours	-450.65	**0.000***
	24 hours	-227.35	0.455
2 hours	4 hours	-89.95	0.719
	6 hours	-188.6	**0.001***
	24 hours	34.7	0.357
4 hours	6 hours	-98.65	0.357
	24 hours	124.65	**0.001***
6 hours	24 hours	223.30	**0.000***

Table 4: Pair wise mean comparison of antibacterial effect within subgroup B of experimental Group 2 between different timelines using Post Hoc analysis – Dunn Bonferroni test.

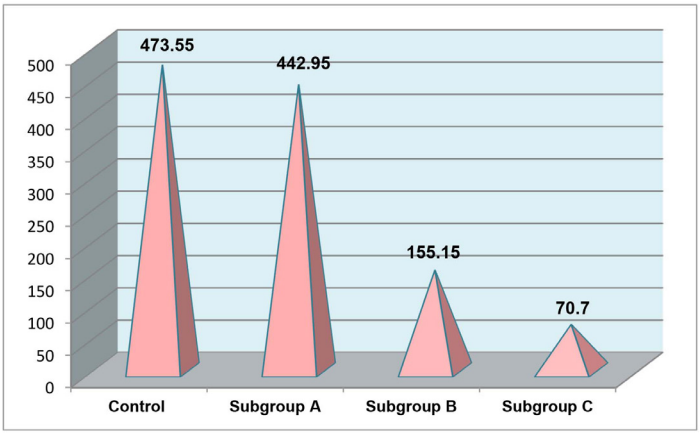
Comparison between		Mean difference	P value
Baseline	2 hours	-50.05	0.455
	4 hours	-168	**0.000***
	6 hours	-371.45	**0.000***
	24 hours	108.55	0.455
2 hours	4 hours	-117.95	0.455
	6 hours	-321.4	**0.000***
	24 hours	158.6	**0.000***
4 hours	6 hours	-203.45	0.455
	24 hours	276.55	**0.000***
6 hours	24 hours	480	**0.000***

Table 5: Pair wise mean comparison of antibacterial effect within subgroup C of experimental Group 2 between different timelines using Post Hoc analysis – Dunn Bonferroni test

Comparison between		Mean difference	P value
Baseline	2 hours	-54.20	0.455
	4 hours	-103.95	**0.001***
	6 hours	-177.5	**0.000***
	24 hours	174.35	0.455
2 hours	4 hours	-49.75	0.455
	6 hours	-123.3	**0.001***
	24 hours	228.55	**0.000***
4 hours	6 hours	-73.55	0.455
	24 hours	278.3	**0.000***
6 hours	24 hours	351.85	**0.000***

Table 6: Mean comparison of antibacterial effect between control and subgroups of experimental group 2 at 24 hours

Groups		Mean	SD	Test statistic	P value
Group 1	Control	473.5500	4.19868	74.101	0.000*
Group 2	Subgroup A	442.9500	6.98476		
	Subgroup B	155.1500	7.70697		
	Subgroup C	70.7000	12.25647		
Pair wise comparison -Post Hoc Analysis					
Comparison between		**Mean difference**		**P value**	
Control	Subgroup A	30.6		0.039*	
	Subgroup B	318.4		0.000*	
	Subgroup C	402.85		0.000*	
Subgroup A	Subgroup B	287.8		0.039*	
	Subgroup C	372.25		0.000*	
Subgroup B	Subgroup C	84.45		0.039*	



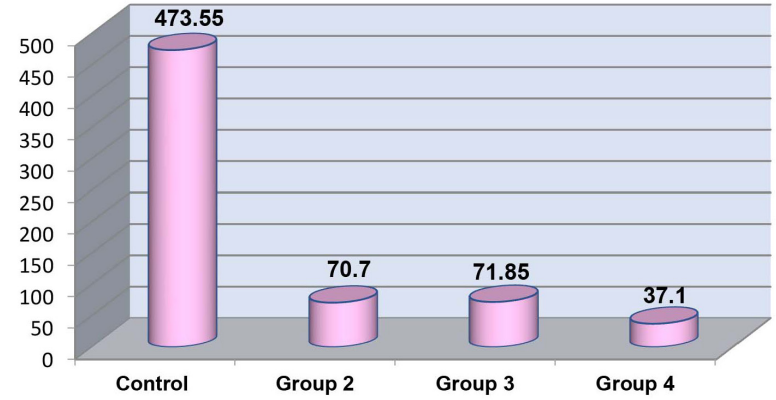
Graph 1: Representing that in comparison with all the subgroups, 1wt% GO of subgroup C have shown statistically significant antibacterial property after 24hrs of time

As 1wt% GO of subgroup C has shown the statistically significant antibacterial property. And it is combined with Zinc Oxide and Silver doped titanium dioxide in order to assess the antibacterial properties.

Of both, the combination of Graphene oxide with Sliver doped titanium dioxide has shown statistically significant anti-bacterial property compared to the ZnO group and 1wt% GO after 24hrs duration [Table 7], [Graph 2].

Table 7: Mean comparison and pair wise of antibacterial effect between Groups at 24 hours

Groups		Mean	SD	Test statistic	P value
Group 1		473.5500	4.19868	66.730	0.000*
Group 2 (Subgroup C)		70.7000	12.25647		
Group 3		71.8500	11.79775		
Group 4		37.1000	8.70511		
Pair wise comparison -Post Hoc Analysis					
Comparison between		**Mean difference**		**P value**	
Group 1	Group 2	402.85		0.000*	
	Group 3	401.7		0.000*	
	Group 4	436.45		0.000*	
Group 2	Group 3	-1.15		1.000	
	Group 4	33.6		0.000*	
Group 3	Group 4	34.75		0.000*	



Graph 2: In comparison with all the groups, group 4 i.e.; brackets coated with Graphene oxide and Sliver doped titanium dioxide have shown best antibacterial property.

The order of the Antibacterial efficiency of the coated brackets follow the order: GO+AgTiO2 > 1wt% GO > GO+ZnO >0.5wt% GO > 0.25wt% GO.

As, the Group 4 (Brackets coated with the combination of GO+A gTiO2 has shown the statistically significant antibacterial property, this group was further subjected to cytotoxicity tests. The tests signify that 99.6% human gingival fibroblasts are viable with the treatment of the GO+AgTiO2 coated orthodontic brackets.

DISCUSSSION

Aesthetics in dentistry has indeed been prominent among patients, and it is frequently cited as a primary motivation for seeking dental treatment and care. Orthodontic aesthetics includes micro and macro aesthetics, gingival and face aesthetics.[27] Many materials have been introduced into orthodontic practise to improve the aesthetics, clinical efficiency, and patient comfort, among other things. Stainless steel has become an indispensable material in the field of orthodontics. Orthodontic devices have been linked to changes in the oral microbiota, such as increased microbial density, metabolic activity, and pathogenicity.[28] The kind of substrate is known to impact microbial adhesion.[29]

In this present study stainless steel Orthodontic brackets were coated with three different concentartions of Graphene oxide nanoparticles with Electrophoretic deposition method to incorpoarte antibacterial properties to the brackets. The antibacterial properties were evaluated against Streptococcus mutans. In recent studies, Graphene oxide were assessed for the corrosion resistance, Wear resistance, Prevention of decalcification, Osteogenesis, Prevention of De-mineralization, Anti-bacterial property and Cytotoxicity in different areas of Dentistry.[30-33]

The study aimed to evaluate the antibacterial property of the stainless steel brackets coated with graphene oxide nanoparticles. Possessing the antibacterial property is the additional property in orthodontic treatment as it is mainly helpful in overcoming the plaque accumulation around the orthodontic appliances, demineralization and white spot lesions.

The null hypothesis presented, that no difference between the antibacterial effects of the three types of coatings on the orthodontic stainless-steel brackets, was rejected.

In the present study the graphene oxide nanoparticles group was divided into 3 sub-groups based on concentrations. Firstly, intra-group comparison of antibacterial properties between three different concentrations of Graphene oxide (GO) nanoparticles (Sub-group A-0.25wt%, Sub-group B-0.5wt%, Sub-group C-1wt%) against streptococcus mutans were assessed by colony forming units (CFU's). In all the subgroups there is a significant increase in the CFU's from 0 hrs to 6 hrs same as the control group. Whereas after 24hrs of incubation there is a significant decrease in the CFU's in all the subgroups of GO nanoparticles same as the control group [Table 3], [Graph 3]. But within the subgroups we can see there is signification decrease in the CFU's in Subgroup-B (0.5wt%) and Subgroup-C (1wt%), comparatively more in Subgroup C, compared to Subgroup A (0.25wt%).

Graphene oxide coated stainless steel brackets have shown antibacterial property at higher concentration i.e. at GO (1wt%), which was similar to study conducted by Grace ES et al (2016) on Graphene-Oxide Nano sheets towards Gram positive bacteria and Gram-negative bacteria, which have shown that the GO have shown better antibacterial property towards the gram positive compared to gram negative bacteria.[34] Zhao M et al (2020) evaluated the antibacterial effects of Graphene oxide on Streptococcus mutans in both planktonic and biofilm forms and concluded that the GO can be used in dental restorative materials.[35] In supporting to our results, a study conducted by Pourhajibagher M et al (2021) have done doping of N-GO in orthodontic adhesives of different concentrations N-GO (0, 1, 2, 5, and 10 wt%) and concluded that 5 and 10 wt% N-GO significantly reduced S. mutans, based on Disc agar diffusion assay.[36]

As, Subgroup C (1wt%GO) have shown significant antibacterial properties, the GO-1wt% was combined

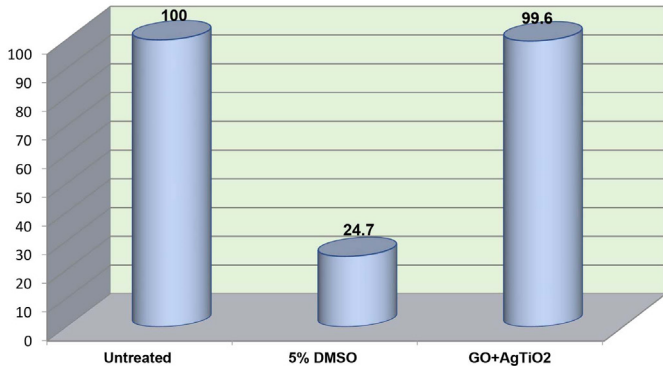
with the Zinc Oxide (ZnO) by a process called Reflux digestion method. During preparation of ZnO by reflux digestion process GO-1wt% and SS orthodontic brackets are added into the flask and subjected to high temperature for better adhesion of GO and ZnO to SS bracket. After the process, coated brackets are evaluated for antibacterial property.

Inter group comparison between the Subgroups of GO and GO+ZnO for antibacterial evaluation against streptococcus mutans showed increase in the CFU's from 0hrs to 6 hrs. But after 24hrs of incubation there is a significant reduction in the CFU's in both GO and GO+ZnO groups. In comparison between these two groups GO group showed high significant reduction in the CFU's after 24 hrs. Similarly, in a study done by Farhangian Z et al (2022) in antibacterial testing on GO, ZnO, GO+ZnO and concluded that the number of colonies in all groups have same range in 24hrs, but little less in GO and GO+ZnO. But the GO have shown better antibacterial properties compared to GO+ZnO. GO have shown significant reduction in the CFU's after 48 hrs.

Whereas, studies conducted by Wang YW et al. (2014)[37], Zhong L et al. (2015)[38] and Zhong L et al (2018)[39] on Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Enterococcus faecalis revealed that synergistic action of GO+ZnO have shown better antibacterial properties compared to GO.

The combination of GO+AgTiO2 material was prepared by Physical Vapour Deposition method. After the process, coated brackets were evaluated for antibacterial property. Inter group comparison between the GO and GO+AgTiO2 for antibacterial evaluation against streptococcus mutans showed increase in the CFU's from 0hrs to 6 hrs. But after 24hrs of incubation there is a significant reduction in the CFU's in both GO and GO+AgTiO2 groups. In comparison between these two groups GO+AgTiO2 group showed high statistically significant reduction in the CFU's after 24 hrs. Studies conducted by De Faria AF et al (2013)[40], Wierzbicki M et al (2019)[41], Cobos M et al (2020)[42] by doping of Go, Ag, GO+Ag in composites and restorative materials towards antibacterial properties concluded that the synergistic action of GO+Ag have shown a significant antibacterial results compared to individual GO, Ag nanoparticles. Based on the statistical analysis, the Group IV i.e. combination of Graphene oxide and silver doped titanium dioxide (GO+Ag-TiO2) have shown better antibacterial properties compared to all other groups evaluated in the study. Therefore, Group IV (GO+AgTiO2) was further assessed for the Cytotoxicity.

The fibroblasts cell viability of test group i.e. Group 4 (GO+AgTiO2) was assessed with untreated fibroblasts and 5% DMSO fibroblasts. The untreated fibroblasts have shown 100% cell viability, 5% DMSO treated fibroblasts have shown 24.7% cell viability, whereas the fibroblasts of the test group have shown 99.6% cell viability, indicating that GO+AgTiO2 coated orthodontic brackets are very less cytotoxic. The observed MTT assay results clearly confirmed that given bracket was non-toxic in nature on Human healthy non-malignant gingival fibroblasts after the incubation period of 24hours. The assay clearly confirmed that the brackets are safe to use for therapeutic purpose or clinical purpose [Graph 3].



Graph 3: The observed MTT assay results clearly confirmed that given bracket was non-toxic in nature on Human healthy non-malignant gingival fibroblasts after the incubation period of 24hours

CONCLUSION

In comparison with the control group, all the subgroups have shown statistically significant antibacterial property at different intervals of time (0, 2, 4, 6, 24 hrs). In comparison with all the subgroups, subgroup C (GO 1wt%) have shown statistically significant antibacterial property at different intervals of time. Inter group comparison shows, the combination of GO+AgTiO2 have shown statistically significant antibacterial property at different intervals of time. The order of the Antibacterial efficiency of the coated brackets follow the order: GO+AgTiO2 > 1wt% GO > GO+ZnO > 0.5wt% GO > 0.25wt% GO. Cytotoxicity assessment of GO+AgTiO2 coated brackets have shown no cytotoxicity affect to Human Gingival Fibroblasts (HGF) as 99.6 % of cell viability have been noticed.

No proper replication of Oral environment as it was an in-vitro study. So further we are continuing a study to replicate the oral environment to evaluate the efficacy in reduction of the white spot lesions in association with GO.

Clinical applications:

The coated graphene oxide brackets have demonstrated a possible decrease in the streptococcus mutans strain, which serves as an indirect preventative measure for the reduction of the white spot lesions. According to the findings, coated graphene oxide brackets exhibit the least cytotoxicity. As a result, they can be applied effectively in orthodontic practice.These coated brackets have also exhibited better biocompatibility with human gingival fibroblast.

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