DECLARATION OF PLAGIARISM CHECK

From,

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To,

The Head of the Department,

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SUB: Declaration of plagiarism check of my dissertation to be submitted to the "Sathyabama Institute of Science and Technology"- 2021.

I hereby declare that I have checked my dissertation for plagiarism using Smart Seo Tools - plagiarism checker software on 02.03.2022. The unique content was 98.62% and the plagiarism content was 1.38%. The plagiarism content corresponds to definitions and terminologies that have to be quoted.

Yours sincerely,

Dr. D.Hemamalini

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Ref: 188/IRB-IBSEC/SIST Dated 30th September 2021

To,

Dr. Hemamalini, D
Post graduate,
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Chennai -119

This is to inform you that the Institutional Bio-Safety & Ethical Committee has approved your study proposal entitled **"Evaluation of shear bond strength and antimicrobial activity Orthodontic adhesive containing silver, Titanium di-oxide and zinc oxide Nano particles",** based on the presentation of the proposal made and recommendations of the Institutional Bio-Safety & Ethical Committee meeting held on 30th September 2021.

The validity of the Ethical clearance is for one year and you are expected to submit six-month progress and the final report of the study to IHBSEC/IRB

Dr. A. Wilson Aruni, PhD, FAMPV
Pro Vice chancellor,
Chair Person, Institutional Biosafety & Ethical Committee (IBEC), SIST

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Beautiful smiles has been an impetus for attractiveness, social appeal and alignment of teeth plays a cardinal role in attaining a perfect smile. Irregularity in the teeth placement has been a major problem globally affecting various age groups

A recent systematic review reported the prevalence of malocclusion among Indian children and adolescents ranged from 28.4% to 66.7%, apart from functional problems it also poses significant impact on social esteem and emotional wellbeing[1,2.] To ameliorate this condition, newer treatment modalities have been recommended since time immemorial, of which fixed orthodontic treatment has been in convention since then. As a coin have two sides, all advantages are accompanied by disadvantages.

Demineralization is an unavoidable side effect related to fixed orthodontic treatment associated with inadequate oral hygiene. Fixed orthodontic appliances create several retentive areas for the accumulation of bacterial plaque. The acidic by-products of these bacteria are responsible for the subsequent enamel demineralization and formation of white spot lesions (WSL) [3,5,6] Despite intensive efforts to educate patients about effective oral hygiene procedures, WSL associated with fixed orthodontic appliances remains a significant clinical problem. The formation of WSL after completion of orthodontic therapy is discouraging to a speciality whose goal is to improve. Oral hygiene regime must include topical fluoride agents such as fluoridated toothpaste, fluoride containing mouth rinse, gel and varnish to prevent or minimize the formation of WSL during orthodontic treatment.[4,7] According to the literature the prevalence of WSLs after orthodontic treatment is about 50% and its prevention is the purpose of every orthodontist [8.]

Reports from clinical in-vivo and laboratory based in-vitro studies state that resin filler based dental composite induces increased plaque accumulation including Streptococcus mutans as compared to adhesion by other dental materials on the enamel

structure.[9] Eid HA et al has stated that there is presence of cariogenic plaque around the resin or the orthodontic treatment adjunct among normal healthy individuals within three weeks of placement of the bands or the brackets[10]

The most common microorganism isolated from the oral cavity is S.mutans that is usually present in the cariogenic plaque usually located on the dental hard tissues. Below the pH of 5.5, microorganisms have shown to have anaerobic activities thereby producing organic acids. Another microorganism commonly located was Lactobacillus acidophilus which also induced demineralization of the dentinal tissues. Subsequently, the microorganisms present in the non-cariogenic plaque (Ph = 5.5) was Streptococcus sanguis which was an indicative of minimal cariogenic plaque activity.[11, 12, 13]

Active research has been done in these aspects and efforts have been made to inhibit the growth of biofilms that contributes to dental caries. A chemical adjunct including Chlorhexidine was proven to inhibit microbial growth, but the duration of activity was for a shorter duration majorly due to an increased solubility of the agents. Other metal substitutes including various metal oxides like, zinc, silver and calcium was documented to have antimicrobial characters. The efficiency of these compounds has been at an increase when used in nano scales.[14]

Nanotechnology has found a major market towards its application in modern medicine. It is a molecular-level technology where several metal oxides have been used towards benefitting mankind from several diseases. Research has shown that the nanoparticles improve the efficiency towards a better anti-microbial property [14] Nanoparticles are incorporated into orthodontic adhesives/cements or acrylic resins and can be coated onto the surfaces of orthodontic appliances to prevent microbial adhesion or enamel demineralization in orthodontic therapy [15]

Studies in the literature established that nanofillers can minimize enamel demineralization with no arbitration of physical properties of the composite.[16] Researchers confirmed that experimental composites composed of silver nanoparticles (nAg) catered an admirable antibacterial properties without negotiating the shear bond strength.[17] Copper and zinc-based nanoparticles are reported to induce relentless noxious effects in animal studies *in vitro*.[18]

In the recent past, there has been considerable scrutiny over regarding the photo catalytic action of titanium dioxide (TiO_2) nanoparticles in medical and dental literature. Studies in the literature have reported that resins embodying TiO_2 nanoparticles display compelling antimicrobial properties which may be applied for preventing recurrent caries and demineralization of the enamel[19] Incorporation of TiO_2 nanoparticles to dental composites also augmented mechanical properties, such as modulus of elasticity, microhardness, flexural strength, and also provided bond strength values that similar or even higher levels than that of the controls not containing the nanoparticles[20]

It is important to mention that the adhesive system toxicity itself is already significant. Adhesive systems components [e.g. Bis-GMA (bis-phenol A diglyceryl methacrylate), HEMA (2-hydroxyethyl methacrylate), TEGDMA (tri-ethylene glycol methacrylate), and UDMA (urethane methacrylate), camphor quinone] are cytotoxic and may induce cell death by necrosis or apoptosis depending on their concentration also causing cell stress through the formation of reactive oxygen species [21].

Cytotoxicity of different nano-particles has been demonstrated in several studies in dose-dependent and time-dependent manners[22,24,25] Generally, Nano-particles are assumed to cause greater toxicity than fine-size particles due to their greater surface

area-to-volume ratio.[22,23] Cytotoxicity of Nano-particles is also determined by other physico-chemical factors including size, concentration, chemical composition, and crystalline structure.[22,23,26,27] Recently, Lanone et al [26]compared the toxicity of 24 Nano-particles in the same experimental set-up on two pulmonary cell lines (A549 and THP-1) and found that copper and zinc-based Nano-particles were highly toxic, whereas titanium showed moderate degree of toxicity. Warheit et al [27] reported severe pulmonary inflammatory/toxicity effects in rats after intratracheal instillation of ultrafine TiO_2 nano-particles which were all chemically derived.

Previous studies reviewed about various Nano incorporated orthodontic adhesive for antimicrobial property, shear bond strength and cytotoxicity nevertheless all of these studies assembled nanoparticles through top-down approach which adds up to the toxic by-products of the chemicals used[28,29,30,31,32,]. Hence resorting to bottom up approach which is considered to be more environmentally friendly, offers superior antimicrobial property and economical alternative was need of the hour[33]. Green pathways are in huge demand with a vision of minimising energy consumption and use of toxic chemicals benefitting environment.[34,35] Therefore, there is a need to develop an eco-friendly, bio-safe processes for the synthesis of Nanoparticles. To our best knowledge literature is in scarce with regard to the cytotoxicity, antimicrobial property and shear bond strength of Nano adhesive prepared through green pathways.

Hence the aim of our study is to wield into green synthesis of three different metal oxide nanoparticles, subsume them into orthodontic adhesive resin and evaluate their cytotoxicity, antimicrobial activity and shear bond strength. It's a first of its kind study wherein a novel Nano adhesive was developed to improve the oral health related quality of life of individuals.

I. **Cytoxicity**

II. **FTIR**

III. **SEM/EDS**

IV. **Nano adhesive antimicrobial activity**

V. **Shear bond strength**

I. CYTOTOXICITY

1. **Allakar et a**l [36] **(2010)** reported the possible uses of nano particles as constituents of prosthetic device coatings, as topically applied agents, and within dental materials are being explored. The latest insights into the application of nanoparticles in the control of oral infections, including their use in photodynamic therapy, will be discussed in this review. In particular, the use of nano-particulate silver, copper, zinc, silicon, and their oxides will be considered in relation to their effects on bacterial populations.
2. **De lima et al** [37] **(2012)** reviewed literation and reported that in recent year's interest in silver nanoparticles and their applications has increased mainly because of the important antimicrobial activities of these nanomaterials, allowing their use in several industrial sectors. This present work concludes that biogenic silver nanoparticles are generally less cyto/genotoxic *in vivo* compared with chemically synthesized nanoparticles. Furthermore, human cells were found to have a greater resistance to the toxic effects of silver nanoparticles in comparison with other organisms.
3. **Nasim et al** [38] **(2020)** studied the cytotoxicity and anti-microbial analysis of silver and graphene oxide nanoparticles. The plant extracts from Andrographis paniculata and Ocimum sanctum Linn were used as reducing agent. The nanoparticles were characterized using UV-visible spectroscopy, FT-IR, XRD and TEM. The antimicrobial activity was completed for oral pathogens. Brine Shrimp Lethality assay

was conducted for cytotoxicity. He concluded silver and graphene oxide bio-based nanoparticles have antimicrobial activity with minimum cytotoxic effects.

4. **Sorasitthiyanukarn et al** [39] **(2021)** conducted a study and reported that curcumin diethyl disuccinate (CDD) is an ester prodrug of curcumin that has better chemical stability in phosphate buffer (pH 7.4) and anticancer activities against MDA-MB-231 human breast cancer cells and Caco-2 cells than curcumin. The synthesis of biogenic nanoparticles, their properties and applications are explored, and their surface modification and implications towards improved properties are emphasized.
5. **Mudi et al 40 (2021)** conducted a study and stated that the crude ethanol extract from the stem bark of Acacia senegal was macerated with 60% aqueous methanol and partitioned into n-hexane, chloroform, ethylacetate and methanol soluble fractions. The fractions were tested for antibacterial activity using disc agar diffusion technique. All the fractions showed good activity against some respiratory tract pathogenic bacteria particularly in n-hexane soluble fraction at 1000 µg/ml, 3000 µg/ml and 5000 µg/ml concentrations. The Brine shrimp test showed highest toxicity in n-hexane soluble fraction with LC50 value of 6.7674 µg/ml. Phytochemical analysis of the fractions revealed the presence of alkaloids, steroids, cardiac glycosides, Tannins, reducing sugars and flavonosides.
6. **Simorangkir et al** [41] **(2021)** conducted a study on Sarangbanua traditional medicinal plant is found in Simalungunand NorthTapanuli Regencies, Sumatera, Indonesia. The Brine Shrimp Lethality Test (BSLT) method was used to determine the toxicity of the extracts. The mortality data were then analyzed by Probit SAS to obtain LC_{50} values. The results showed that the three types of *C. fragrans* leaf extracts had LC_{50} values below 1000 µg/mL, so they were classified as toxic and potentially bioactive. The

$LC5_0$ values of each of the ethanol, ethyl acetate and ethanol extracts were 26.25; 37.50 and 41.97 µg/mL

II. FTIR

1. **Ayona et al**[42] The present study reports the biosynthesis of copper nanoparticle using the leaf extract of Ocimum sanctum. The color change in the Ocimum leaf extract when copper sulphate solution is added indicates the presence of copper nanoparticle. The effect of temperature and time of incubation on the biosynthesis of Cu NP were noted. The characterization of the biosynthesized copper nanoparticle was done by UV Vis spectrophotometer, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD)
2. **Mehrbakhsh et a**l[43] **(2021)** conducted a study to obtain a suitable polymer for this application as well as troubleshoot the main drawbacks such as stress relaxation and water absorption; thermoplastic polyurethane (TPU) elastomers with various compositions were synthesized and characterized by Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) analyses. In vitro oral environment study showed crystability of samples has recovered great portion of relaxed force. Stress relaxation study indicated samples are applicable in oral temperature range.
3. **A. Murei et al**[44] **(2021)** Study aimed to synthesize, characterize and evaluate *Pyrenacantha grandiflora* Baill extracts and gold nanoparticle conjugates against pathogenic bacteria. Gold nanoparticles was synthesized by chemical and biological methods. The nanoparticles were characterized by the use of UV-visible spectrophotometry, followed by transmission electron microscopy (TEM) and energy-

dispersive X-ray analysis (EDX). Gold nanoparticles were conjugated to plant extracts and analyzed with a Fourier-transform infrared spectroscope (FTIR)

4. **Nozha et al** [45] (2022) The study aimed to modify orthodontic adhesive using graphene sheets decorated with silver nanoparticles (Ag-GS) in order to examine their mechanical and antibacterial properties after bonding with orthodontic brackets. Ag-GS was added at 0.35 and 0.55 wt.% into Transbond XT and studied for its antibacterial and mechanical properties.characterisation was done by FTIR

III. SEM EDX studies relavant to nanoparticle characterization

1. **Kashin et al[46] (2011)** conducted a study and mentioned that the Magnetron sputtering was successfully used to obtain nanosized metal films and metal nanoparticles, which can be useful for development of novel methods in organic chemistry. The morphology of the nanosized (5–100 nm) objects obtained was examined by SEM. The pattern of dependence of the morphology and size of the nanosized objects on the nature of the support surface, the metal, and the sputtering conditions is described.

2. **Syed et al** [47] **(2015)** conducted a study with the coating procedure was verified by comparing the surface topography of nanocoated archwires with the commercially available archwires in an environmental scanning electron microscope (ESEM). The ESEM images prove that the surface topography of the coated wires was found to be smoother with less surface deteriorations as compared to the commercially available wires. Commercially available orthodontic wires can be successfully coated using a novel method of sol-gel thin film dip coating method.

3. **Venugopal et al** [48] **(2016)** conducted a study and inferred that SEM revealed that only a meager amount of AgNPs was sparsely deposited on the Ti-AgNP surface with the first method, while a layer of AgNP-coated biopolymer extended along the Ti-BP-

AgNP surface in the second method. After 24 hours of incubation, clear zones of inhibition were seen around the Ti-BP-AgNP microimplants in all three test bacterial culture plates, whereas no antibacterial effect was observed with the Ti-AgNP microimplants.

IV. NANO ADHESIVE ANTIMICROBIAL ACTIVITY

1. **Kasreini et al [49] (2014)** conducted a study to evaluate the antibacterial properties of composite resins containing 1% silver and zinc-oxide nanoparticles on *Streptococcus mutans* and *Lactobacillus*. There were no significant differences in the antibacterial activity against *Lactobacillus* between composites containing silver nanoparticles and those containing zinc-oxide nanoparticles. Composite resins containing silver or zinc-oxide nanoparticles exhibited antibacterial activity against *Streptococcus mutans* and *Lactobacillus*.
2. **Hernandez et al [50] (2017)** conducted the present study to synthesize silver nanoparticles (AgNPs) in situ on orthodontic elastomeric modules (OEM) using silver nitrate salts as metal-ion precursors and extract of the plant *Hetheroteca inuloides* (*H inuloides*) as bioreductant via a simple and eco-friendly method. The results suggest the potential of the material to combat dental biofilm and in turn decrease the incidence of demineralization in dental enamel, ensuring their performance in patients with orthodontic treatment.
3. **Sodagar et al [51] (2017)** conducted a study with the aim to evaluate the antimicrobial and mechanical properties of composite resins modified by the addition of TiO_2 NPs. All concentration of TiO_2 NPs had a significant effect on creation and extension of inhibition zone. The highest mean shear bond strength belonged to the control group, while the lowest value was seen in 10% NPs composite. Incorporating

TiO_2 nanoparticles into composite resins confer antibacterial properties to adhesives, while the mean shear bond of composite containing 1% and 5% NPs still in an acceptable range.

4. **Toodehzaeim et al[52] (2018)** conducted a study to determine the effects of incorporating copper oxide (CuO) nanoparticles on antimicrobial properties and bond strength of orthodontic adhesive. Nano-composites in all three concentrations showed significant antimicrobial effect compared to the control group. With increasing concentration of nanoparticles, antimicrobial effect showed an upward trend, although statistically was not significant. There was no significant difference between the shear bond strength of nano-composites compared to control group (p= 0.695). Incorporating CuO nanoparticles into adhesive in all three studied concentrations added antimicrobial effects to the adhesive with no adverse effects on shear bond strength.
5. **Kambalyal et al [53] (2018)** conducted a study to determine the antimicrobial efficacy of silver, titanium dioxide and zinc oxide nanoparticles against Streptococcus mutans. A significant difference in the colony forming units among all three concentrations of silver (Ag), titanium dioxide (TiO2) and zinc (ZnO) nanoparticles was noted and the antimicrobial effect of nanoparticles was concentration dependent.. Silver, Zinc oxide and Titanium dioxide showed significant antimicrobial effects and the antimicrobial effect of nanoparticles was concentration dependent.
6. **Pourhajibagher et al[54] (2019)** conducted a study to evaluate the antimicrobial properties of an orthodontic adhesive incorporating cationic curcumin doped zinc oxide nanoparticles (cCur/ZnONPs), which can have the highest concentration of cCur/ZnONPs and shear bond strength (SBS) value simultaneously, against cariogenic bacteria including *Streptococcus mutans*, *Streptococcus sobrinus,* and *Lactobacillus*

acidophilus. The present study findings highlight the photo-activated 7.5% wt. cCur/ZnONPs can serve as an orthodontic adhesive additive to control the cariogenic multispecies biofilm, and also to reduce their metabolic activity.

7. **Eslamian et al[55] (2020)** conducted a study to evaluate the effect of incorporating silver nanoparticles (AgNPs) into conventional orthodontic adhesive on its antibacterial activity and the shear bond strength (SBS) to stainless steel orthodontic brackets. The findings suggest that SBS decreased after incorporation of AgNPs [0.3% (*w/w*)], but was still above the recommended SBS of 5.9–7.8 MPa. The nano-adhesive showed significant antibacterial activity which did not change much after 30 days.
8. **Sreenivasagan et al[56] (2020)** conducted a study and reported that Mini-implants have become a major device in orthodontic treatment in this era, and practitioners intend to use for different clinical situations. Cytotoxicity was assessed by testing on shrimp culture. Titanium mini-implants when coated with silver nanoparticles has excellent antimicrobial properties and, hence can be used as a biomaterial in orthodontics but further tests are needed to evaluate the coating during and after placement.
9. **Pourhajibagher et al [57](2020)** conducted a systematic review and meta-analysis to find out whether the association of nanoparticles with the orthodontic adhesives compromises its properties and whether there are exceptional nanoparticles exhibiting excellent antimicrobial potential against cariogenic bacteria along with remarkable mechanical properties. Adding ≤ 5 wt% antimicrobial nanoparticles to an orthodontic adhesive is less conducive to microbial growth than unmodified adhesive and does not influence bracket-enamel bond strength.
10. **Ahmadi et al [58](2020)** conducted a study to evaluate the anti-biofilm activity of an orthodontic adhesive (OA) incorporating curcumin (Cur) doped Poly lactic-co-glycolic acid nanoparticles (Cur-PLGA-NPs), which can have the highest

concentration of Cur-PLGA-NPs and shear bond strength (SBS) value simultaneously, against cariogenic bacteria *i.e., Streptococcus mutans*. No statistically significant difference in ARI scores was observed between the MOA and control (Transbond XT without the Cur-PLGA-NPs).

V. SHEAR BOND STRENGTH

1. **Poosti et al [20] (2012)** conducted a study with the aim to evaluate shear bond strength (SBS) and antibacterial effects of an orthodontic composite after adding titanium oxide (TiO_2) nanoparticles. No significant difference was found between SBS of conventional and nanocomposites, 24 hours after curing (P = 0.58). Colony count revealed no significant difference in bacterial growth immediately and 30 days after curing in nanocomposite group. Adding TiO_2 nanoparticles to orthodontic composite enhances its antibacterial effects without compromising the SBS.
2. **Heravi et al [59] (2013)** conducted a study to investigate cytotoxicity of Transbond XT adhesive containing 1 wt% titanium dioxide (TiO_2) nano-particles. No significant differences were found in cell viability percentages between the two groups on the other days ($P>0.05$). There was a significant reduction in cell toxicity with increasing pre-incubation time ($P<0.001$). L929 cells showed similar toxicity trends, but lower sensitivity to detect cytotoxicity of dental composites.
3. **Blocher et al. [60] (2015)** conducted a study to determine whether the addition of microsilver or nanosilver particles to an orthodontic primer affects shear bond strength (SBS) and bracket/adhesive failure. No significant differences in SBS and ARI scores were found between the control group and any experimental group. Only experimental groups with nanosilver particles revealed statistically more silver spots on the remaining adhesive.

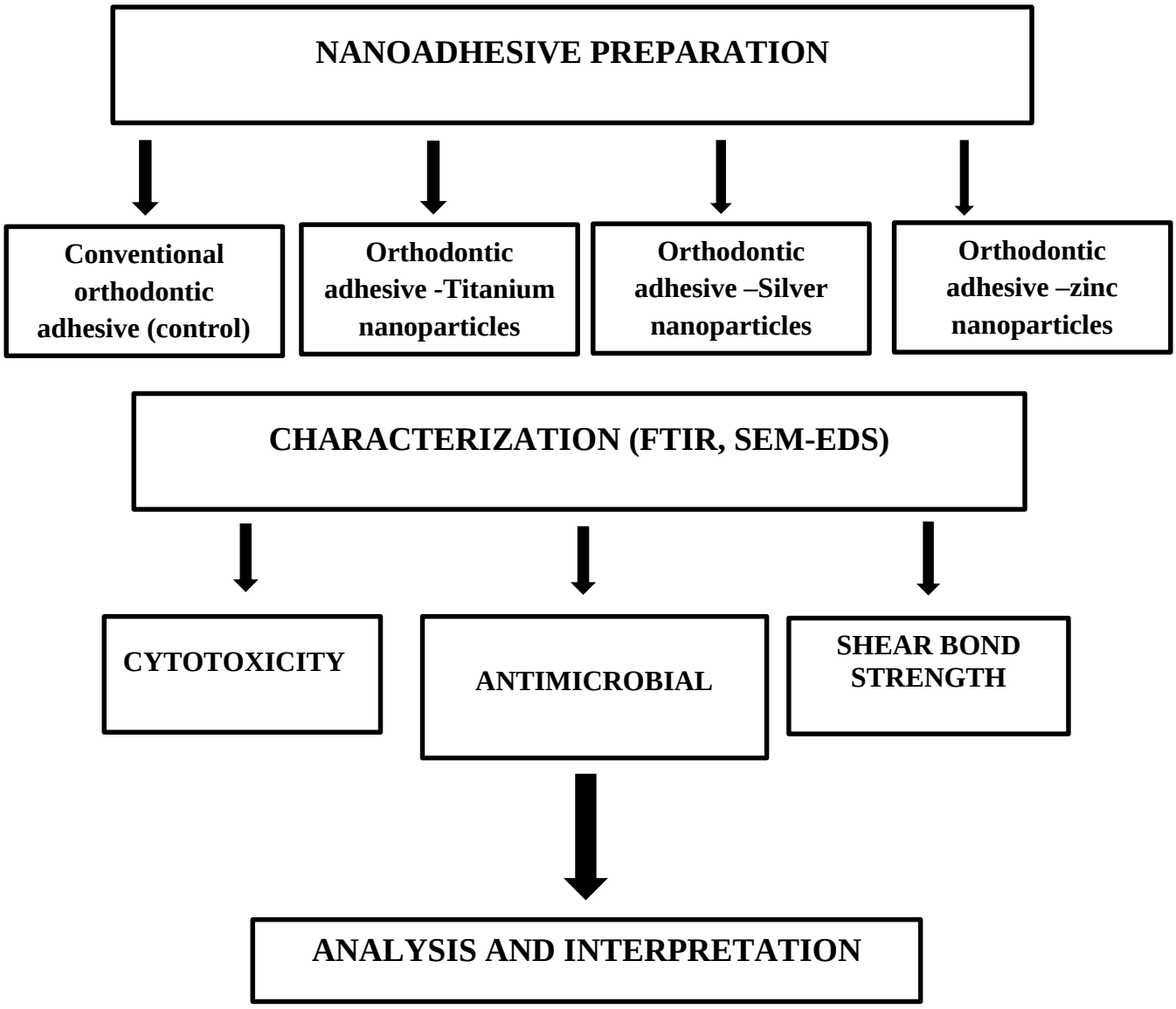
4. **Reddy et al [61] (2016)** conducted a study to investigate the influence of silver (Ag), zinc oxide (ZnO), and titanium dioxide (TiO_2) nanoparticles on shear bond strength (SBS). Group 1 (control): brackets (American Orthodontics) were bonded with Transbond XT primer. Groups 2, 3, and 4: brackets (American Orthodontics) were bonded with adhesives incorporated with Ag, ZnO, and TiO_2 nanoparticles in the concentration of 1.0% nanoparticles of Ag, 1.0% TiO_2, and 1.0% ZnO weight/weight, respectively. Incorporation of various nanoparticles into adhesive materials in minimal amounts may decrease SBS and may lead to the failure of bracket or adhesive.
5. **Hasan et al[62] (2020)** conducted a study to examine the effects of calcium hydroxyapatite nanoparticle incorporation on polymerization as well as the shear bond strength for Heliosit adhesive. A significant difference was found among all the study groups ($p \leq 0.05$) in terms of the degree of conversion and shear bond strength. The 2% wt nanoparticle group showed the highest values for both variables. The lowest value was recorded within the 4% wt nanoparticle group in comparison to the control group. Calcium hydroxyapatite nanoparticle incorporation with a conventional Heliosit adhesive resin to a limited concentration has improved the mechanical properties of orthodontic adhesive.
6. **Hailan et al[63] (2018)** conducted a study to evaluate the shear bond strength (SBS) and adhesive remnant index (ARI) after incorporation (1%) of silver, zinc oxide, or titanium dioxide nanoparticles to an orthodontic bonding agent. There were no significant differences between the groups. The incorporation of silver, zinc oxide, or titanium dioxide nanoparticles into orthodontic bonding agent at concentration of 1% had no effect on shear bond strength, on the other hand, in regard to ARI, a considerably more adhesive remains on the enamel surface following bracket removal

in the test groups than the control group, and this will reduce the enamel damage after debonding.

7. **Behnaz et al[64] (2018)** conducted a study to examine that addition of nano particles could affect the shear bond strength (SBS) below clinically acceptable levels. The SBS was not significantly different at one day, one month or three months (p>0.05) but composites without TiO_2 had a significantly higher mean SBS than composites containing TiO_2 (p<0.001). Addition of TiO_2 nanoparticles to Transbond XT decreased its SBS to the level of SBS of Resilience without TiO_2; thus, TiO_2 nanoparticles may be added to Transbond XT composite for use in the clinical setting.
8. **Gilani et al[65] (2020)** conducted a study to understand the effect of adding different concentrations of nano-silver / hydroxyapatite to orthodontic primer on shear bond strength of bracket-enamel and evaluate the location of bonding failure. Adding 10 % nano-silver / hydroxyapatite to the orthodontic bonding primer would not affect bond strength adversely and can replace conventional bonding to reduce the risk of decay. In all groups, less than half of the adhesive remained on the enamel.
9. **Yaseen et al [66](2020)** conducted a study to investigate the effect of orthodontic resin modified by incorporating Nano Cinnamon powder on the shear bond strength of orthodontic brackets. No significant difference was recorded in the adhesive remnant index scores between the control and the modified resin groups. Findings of this study revealed that the incorporation of 3% Cinnamon Nano particles in orthodontic resin produced an antibacterial effect against *Streptococcus mutans* without compromising the shear bond strength. Incorporation of Cinnamon Nano particles in orthodontic resin may reduce caries formation around brackets during treatment course.

10. **Mirhashemi et al.[67] (2021)** conducted a study to evaluate the effect of the combination of zinc oxide nanoparticles (NPs) and chitosan NPs on the shear bond strength (SBS) of composites used for orthodontic bonding. The adhesive remnant index did not differ significantly among the groups ($P = 0.823$). Incorporation of 1% and 5% zinc oxide and chitosan NPs had no effect on the SBS of composite, and the obtained SBS values were similar to that of the control group.

11. **Farzanegan et al [68] (2021)** conducted an in-vitro study to evaluate the effect of adding different concentrations of chitosan nanoparticles (NPs) and TiO_2 NPs on the shear bond strength (SBS) of an orthodontic adhesive. The results showed no statistically significant difference between groups 1, 2, and 3, but SBS decreased significantly in group 4. No significant differences were found between the groups in terms of ARI scores. It is concluded that the orthodontic composite containing 1% chitosan NPs and 1% TiO_2 NPs has adequate SBS for use in the clinical setting.



FLOWCHART REPRESENTING THE WORK DONE

I. Study Design

This is an in-vitro Nano-laboratory based study to assess the shear bond strength, anti-microbial activity and cytotoxicity of orthodontic adhesive infused with nanoparticles. IRB approval numb: 188/IRB-IBSEC/SIST

II. Sampling Method& Sample Size Calculation

Convenient sampling method was used for the selection of the samples. Using the Prevalence formula we derived at a sample size of 13 per group considering attrition we decided to level off to 15 in each group

Type: Empirical Data Used. Comparisons of the Means between two different group parameters have been taken into consideration,

- Level of significance = 5%,
- Power = 80%,
- Type of test = two-TAILED
- Formula of calculating sample size is

$$n = [(Z_{\alpha/2} + Z_{\beta})^2 \times \{2(ó)^2\}]/ (\mu1 - \mu2)^2$$

Where, n = sample size required in each group, μ1 = mean Value in Group A = 40.31 ,μ2 = mean Value in Group B = 29.17

μ1-μ2 = clinically significant difference = 11.14 ,ó = standard deviation = 8.85$Z_{\alpha/2}$: This depends on level of significance, for 5%

This is 1.96 and Z_{β}: This depends on power, for 80% this is 0.84

- Based on above formula the minimum sample size required per group is 10.
- Considering attrition the minimum sample in each group = 13
- Total sample size to be considered in four groups = 13x4 = 52

III. Sample Distribution

A total of 60 samples were included for the study(fig1). The entire sample was divided into four different groups. (fig2)

Group 1 -Conventional orthodontic adhesive (15 Sample)

Group 2- Orthodontic adhesive with Titanium di oxide (15 Sample)

Group 3- Orthodontic adhesive with Silver Nitrate (15 Sample)

Group 4- Orthodontic adhesive with Zinc oxide (15 Sample)

IV. Nano-particle synthesis

a. Protocol for Green synthesis of Nanoparticles using plant extract

1.5 grams of Neem flower (fig3a) was dissolved in 150 ml of water. The mixture was boiled using heating mantle at 60 to 70 degree Centigrade for 10 to 15 minutes (fig 3b). The mixture was filtered using WATTMAN No. 1 Filter paper and the filtered extract was used for the nano-particle synthesis. (fig 3c)

G**reen synthesis Titanium dioxide-Group-2**

Dilution protocol: 50 mlMol (0.395gms) (50 ml of titanium dioxide nano particles and 50 ml of plant were dispensed in a beaker. Fig4a

Green Synthesis of Silver Nitrate nanoparticle-Group-3

Dilution protocol: 1 mlMol (0.0169 gms) of silver nitrate with the neem flower extract 90 ml of silver nano particles and 10 ml of plant extract were dispensed in a beaker. Fig 4b

Green synthesis Zinc oxide nanoparticle-Group-4

Dilution protocol: -20 mlMol (0.574 gms) 60 ml of zinc-nano particles and 40 ml of plant extract were dispensed in a beaker.(fig 4C)

Centrifugation protocol: The diluted aqueous solution of nanoparticles were centrifuged at 8000 rpm for 10 minutes.(fig5)

Green synthesised nanoparticle: For the settlement of particles, the supernatant material was transferred to a beaker and frequent centrifugation process was carried out to clean $Tio_{2,}$ silver and zinc nanoparticles. The obtained nanoparticle pellet was dry in an oven and stored for further study. (fig 6)

V. Nano-Adhesive Preparation

Nanoadhesive preparation- Group -2

Light cure orthodontic adhesive (Transbond XT) was blended with Titanium (nanospheres 50 nm avg. part. size, 1 % w/w Sigma-Aldrich Biotechnology, St Louis, MO, USA) Using Vortex and IKA ® T25 digital ULTRA-TURRAX ® machine at 3400 rpm for 2 min in a dark room (Rotor stator mechanism).(fig-7)

Nanoadhesive preparation- Group -3

Light cure orthodontic adhesive (Transbond XT) was blended with Silver nanospheres 50 nm avg. part. size, 0.3% w/w Sigma-Aldrich Biotechnology, St Louis, MO, USA) Using Vortex and IKA ® T25 digital ULTRA-TURRAX ® machine at 3400 rpm for 2 min in a dark room (Rotor stator mechanism).

Nanoadhesive preparation- Group -4

Light cure orthodontic adhesive (Transbond XT) was blended with Zinc nanoparticles (nanospheres 50 nm avg. part. size, 1% w/w Sigma-Aldrich Biotechnology, St Louis, MO, USA) Using Vortex and IKA ® T25 digital ULTRA-TURRAX ® machine at 3400 rpm for 2 min in a dark room (Rotor stator mechanism).the prepared nanoadhesives(fig8)

VI. Characterization of the Nano-Adhesives

Fourier Transform Infrared Spectroscopy Analysis (FTIR) (fig9)

FTIR is a very versatile tool for surface characterization of nanoparticles. Under specific conditions, the NPs surface chemical composition can be determined, and additionally, the reactive surface sites responsible for the surface reactivity can be identified**.**

FTIR analysis measures a sample's absorbance of infrared light at various wavelengths to determine the material's molecular composition and structure.

The X-axis Wavenumber axis: -Represents the infrared spectrum, which plots the intensity of infrared spectra. The peaks, which are also called absorbance bands, correspond with the various vibrations of the sample’s atoms when it’s exposed to the infrared region of the electromagnetic spectrum. For mid-range IR, the wave number on the infrared spectrum is plotted between 4,000 to 400 cm-1.

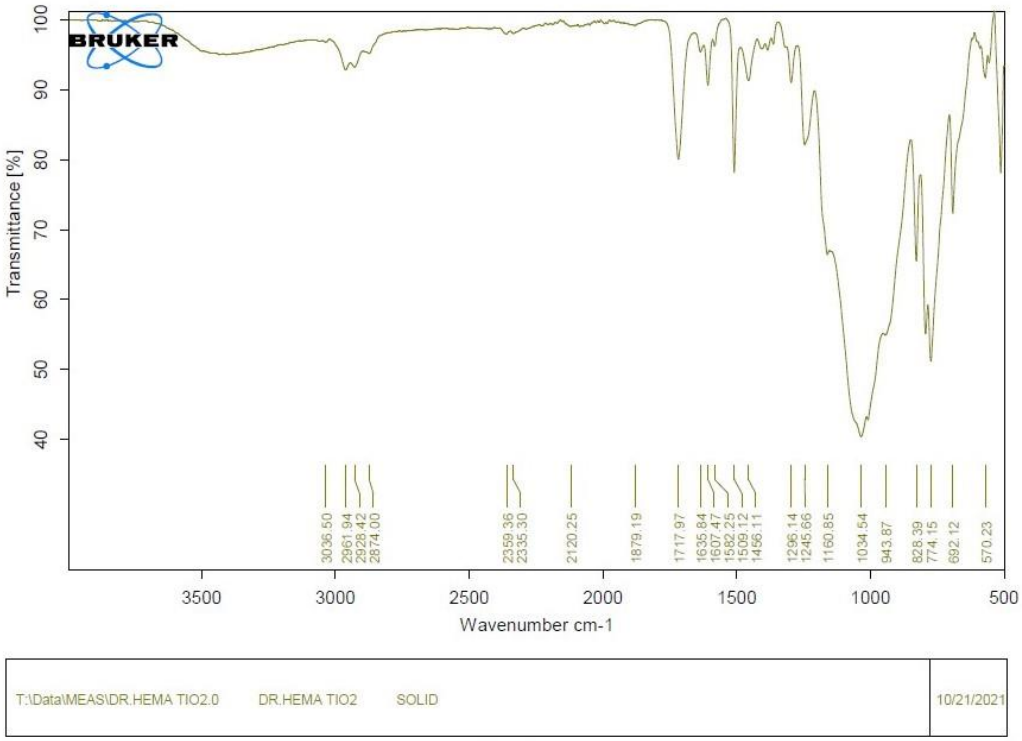
The Y-Axis: Transmittance percentage Axis: The y-axis—or vertical axis—represents the amount of infrared light absorbed or transmitted by the material being analyzed.

The Absorbance Bands: Typically, absorbance bands are grouped within two types: Group frequencies and fingerprint frequencies.

Group frequencies These types of bands are typically seen above 1,500cm-1 in the infrared spectrum and they're usually unique to a specific functional group, making them a reliable means of identifying functional groups in a molecule.

Fingerprint frequencies. The region of infrared spectrum from 1200 to 700 cm-1

The reports from the FTIR analysis have been highlighted in Graph 1, 2 and 3.



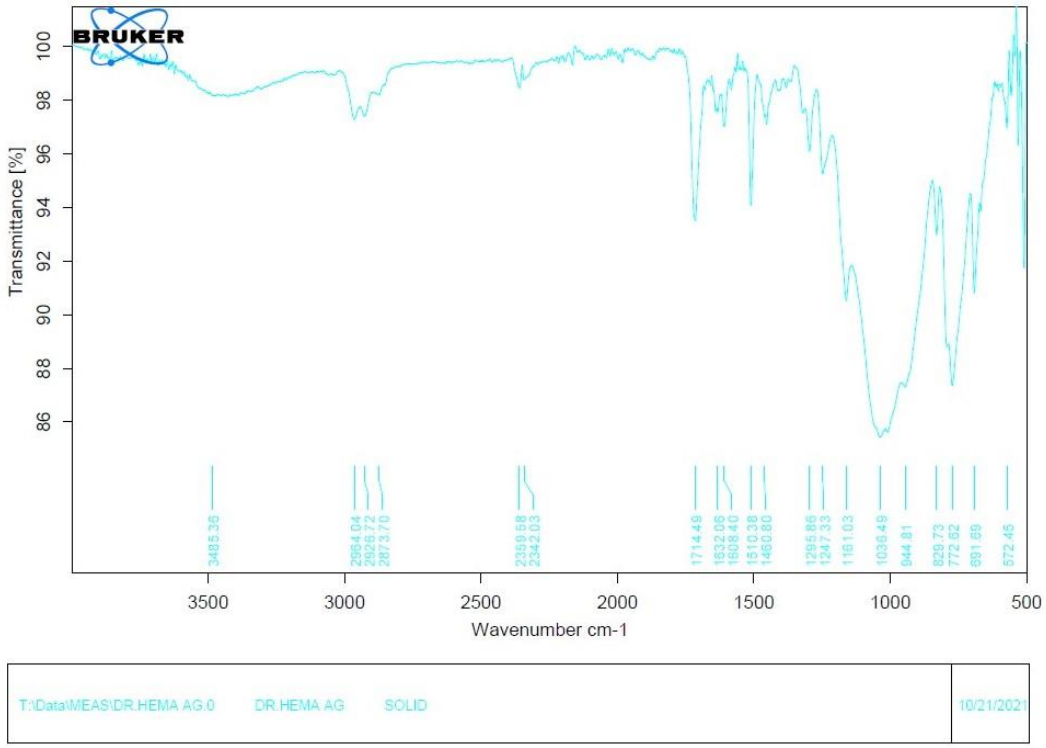
Graph 1: FTIR Analysis for Titanium dioxide (Group -2)

Group 2 Absorpbance bands

- Broad peak at 3036 cm-1 indicative of Amide A N-H stretching ,
- 2,961 cm−1 (methane compound, H-C-H Asymmetric and symmetric stretch). stretching vibrations of alkanes group was formed

- narrow band at 1,717 cm−1 indicates the presence carboxylic group (Formic acid; Hydrogen bonded O-H stretch).
- 1,582 cm−1 corresponding to nitro group (N-H Bend).
- A narrow band of 1607 cm−1 indicates the presence of amide group (C=O Stretch). The broad spike at 1,030 cm−1 is because of the presence of C-N stretching vibrations of aliphatic amines of proteins. As represented in Graph-1.

Inference -Presence of functional groups Amide linkage proteins along with carboxylic indicates the amide linkage protein possibly involved in reduction and stabilization of titanium nanoparticles.

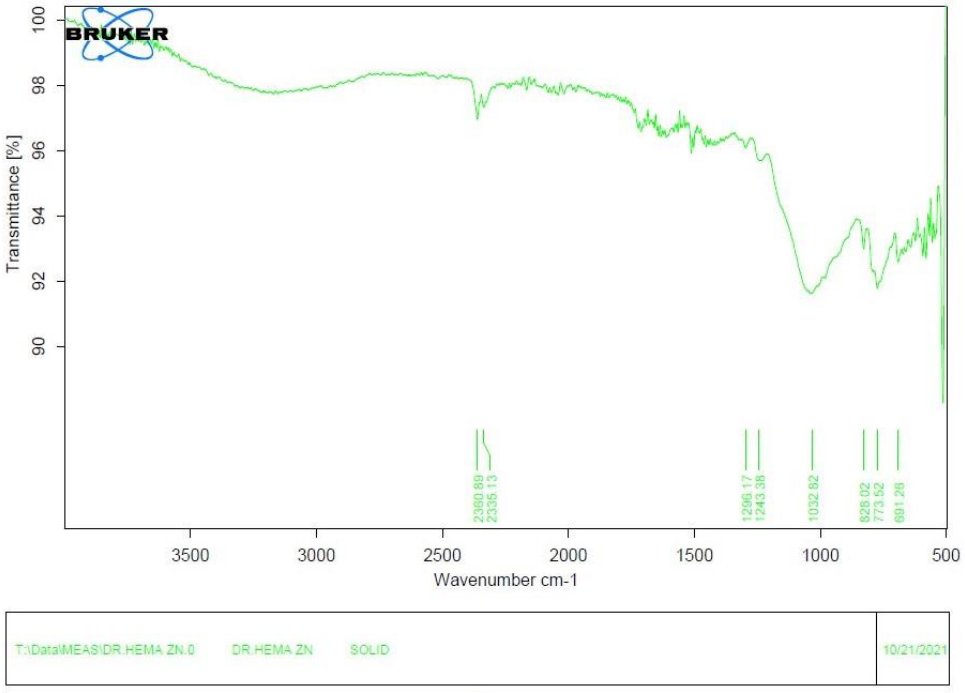


Graph 2: FTIR Analysis for Silver (Group-3)

Group 3-Absorbance bands

- A broad peak was noted ,3414 cm-1 indicated the presence of polyphenolic O-H group and primary amine O-H band
- 2964 cm-1 due to the presence of carboxylic acid (H-C-H Asymmetric and symmetric stretch).).
- A narrow peak was noted at 2357 cm-1 due to the presence of nitrile group (Hydrogen-bonded O-H Stretch).
- A broad peak was at 1714 cm-1 due to the presence of ester compound (C-O Stretch).
- The fifth peak was at 1510 cm-1 having secondary amide group (N-H Bend). As represented in Graph-2.

Inference -Presence of functional groups carboxylic,amine ,phosphate and hydroxyl functional groups involved in reduction and stabilization of silver nanoparticles



Graph 3: FTIR Analysis for Zinc Oxide (Group -4)

Group 4 Absorbance band

- The initial widening was noted at 2360 cm-1 for the nitrile group, with presence of methanenitrile group.
- The next narrow peak for the presence of carboxylic group was seen at 2335 cm-1 (Hydrogen-bonded O-H Stretch) with the presence of methanenitrile group.
- There was a narrow peak attained at 1296 cm-1 (C-O Stretch). Ether group was present.
- An irregular peak was obtained at 1243 cm-1, with the ester group (C-O Stretch).
- The fifth peak was 1032 cm-1 with esthers group with (C-O stretch)
- Intense bands at 546 cm-1 indicating presence of phenols and flavonoids compounds. As represented in Graph-3.

Inference -Presence of functional groups carboxylic, Esthers and phenols and flavonoids indicates presence of zinc oxide nanoparticles

Chemical Compound	**Control**	**Titanium**	**Silver**	**Zinc**
Carboxylic group	Present	Present	Present	Present
Nitrile	Present	-	Present	Present
Ester	Present	-	Present	Present
Amines secondary	Present	-	Present	-
Alkanes	-	Present	-	-
Amides	-	Present	-	-
Ethers	-	-	-	Present
Nitro	-	Present	-	-
Phenolic group				Present
Amine group			Present	

Table I: FTIR Interpretations Based on the Compounds

Characterization of Nanoparticles using Scanning Electron Microscopic and Elemental Dispersive Analysis of Transbond Incorporated with Incorporated with Nanoparticles

Scanning Electron microscopy

The distribution and the elemental analysis of Nanoadhesives were done using scanning electron microscopy. Each sample was placed on carbon-coated conductive tape and observed under an SEM microscope (fig10) (JEOL, JSM-6510LV at 20 kV, Tokyo, Japan) with secondary electrons at 100×, 500× and 2500× magnification, operating at 20 kV.and the images were recorded,

Energy dispersive X-ray spectroscopy

Energy dispersive X-ray spectroscopy (EDX) is an analytical method for analytical or chemical characterization of materials. EDX systems are generally attached to an electron microscopy instrument such as transmission electron microscopy (TEM) or scanning electron microscopy (SEM). EDX is based on the emission of a specimen characteristic X-rays. A beam of high energy charged particles (electrons or protons) are focused into the investigated sample. An electron from a higher binding energy electron level falls into the core hole and an X-ray with the energy of the difference of the electron level binding energies is emitted. EDX analysis gives a spectrum that displays the peaks correlated to the elemental composition of the investigated sample. In addition, the elemental mapping of a sample can be created with this characterization method.

Energy dispersive X-ray spectroscopy (EDS) is a standard method for identifying and quantifying elemental compositions in a very small sample of material (even a few cubic micrometers). In a properly equipped SEM, the atoms on the surface are excited by the electron beam, emitting specific wavelengths of X-rays that are characteristic

of the atomic structure of the elements. An energy dispersive detector (a solid-state device that discriminates among X-ray energies) can analyze these X-ray emissions. Appropriate elements are assigned, yielding the composition of the atoms on the specimen surface. This procedure is called energy dispersive X-ray spectroscopy (EDS) and is useful for analyzing the composition of the surface of a specimen

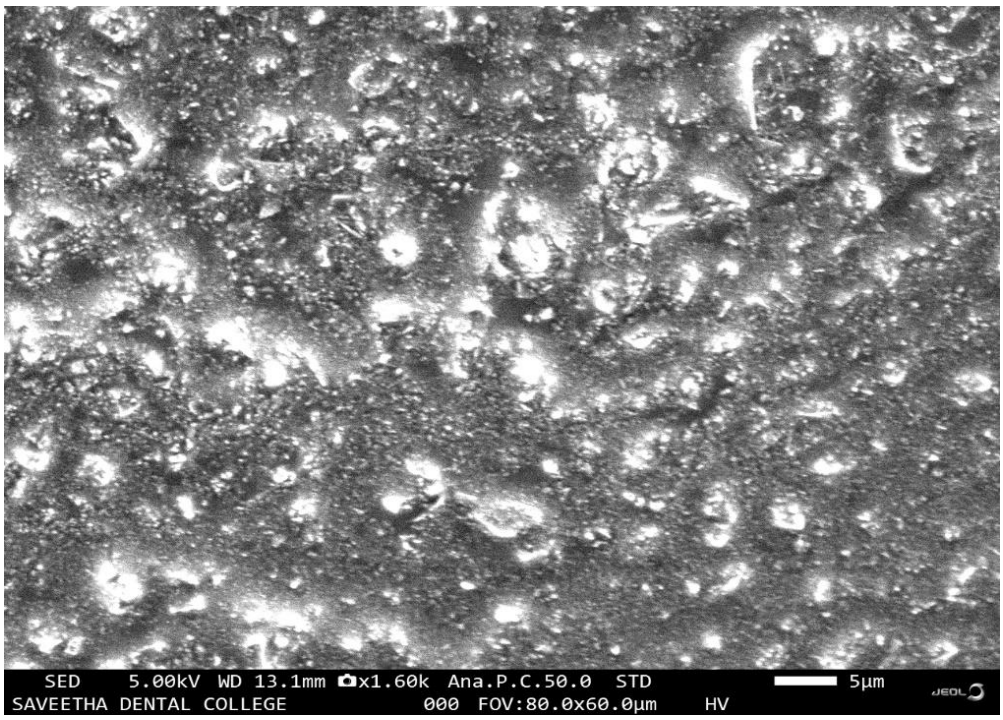
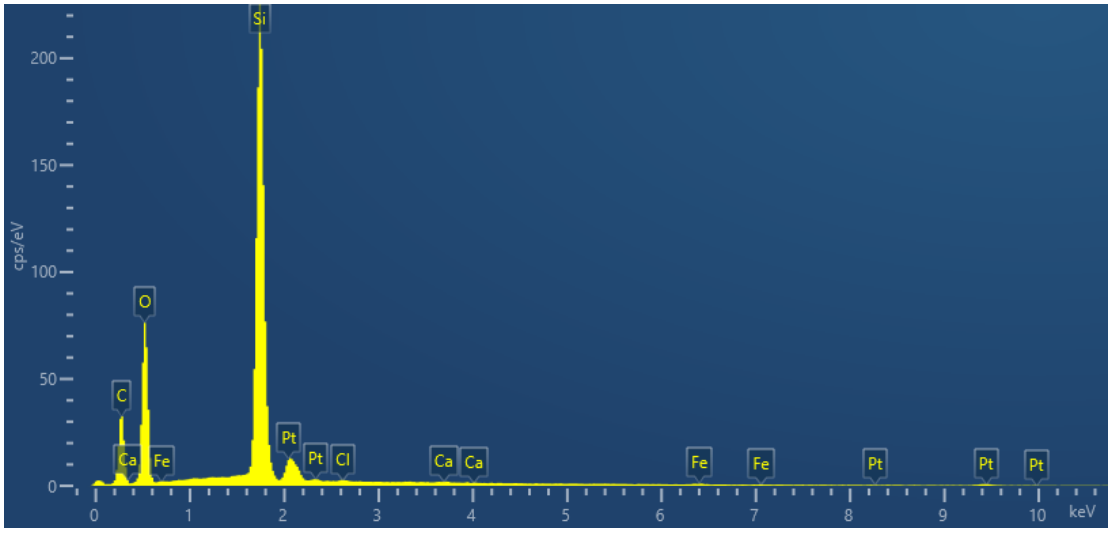


Figure 11: Group 1 Analysis of SEM

The material composition of the Group 1 was determined using SEM and EDX technique (Figure 6a). No nanoparticle was detected in the present Group. The abscissa of the EDX spectrum indicates the ionization energy and ordinate indicates the counts. Higher the counts of a particular element, higher will be its presence at that point or area of interest. The amount of each element is usually presented in number of counts or in weight percentage. The EDX analysis stated the presence of carbon, calcium, oxygen, iron, silicon, platinum and chlorine.

Compounds	Atomic Weight (cps/eV)
Carbon	20
Calcium	<10
Oxygen	80
Iron	<10
Silicon	220
Platinum	20
Chlorine	<10

Table II: EDX Interpretations for Group 1



Graph 4: Group 1 Analysis of EDX

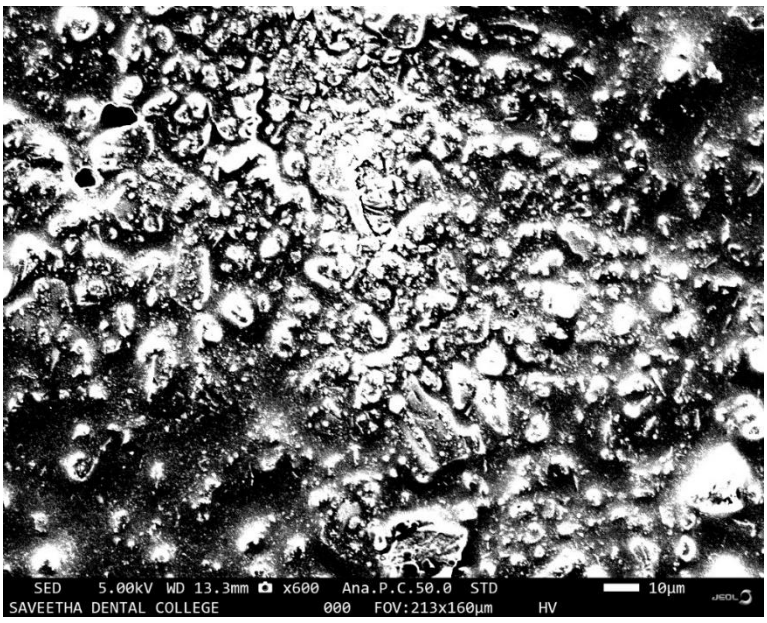
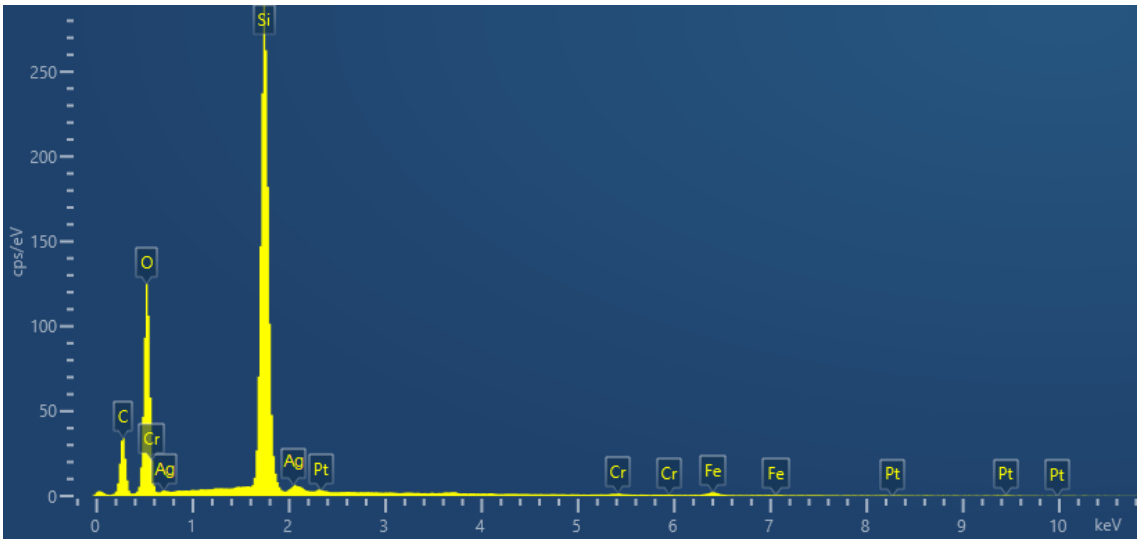


Figure 12: Group 3 Analysis of Transbond with SEM

The analysis was performed using SEM and EDX technique and the presence of silver nanoparticle on the orthodontic adhesive was confirmed. The EDX analysis stated the presence of carbon, chromium, oxygen, iron, silicon, platinum and silver.

Compounds	**Atomic Weight (cps/eV)**
Carbon	40
Chromium	40
Oxygen	140
Iron	<10
Silicon	280
Platinum	<10
Silver	20

Table III: EDX Interpretations for Group 3



Graph 5: Group 3 Analysis of Transbond with EDX

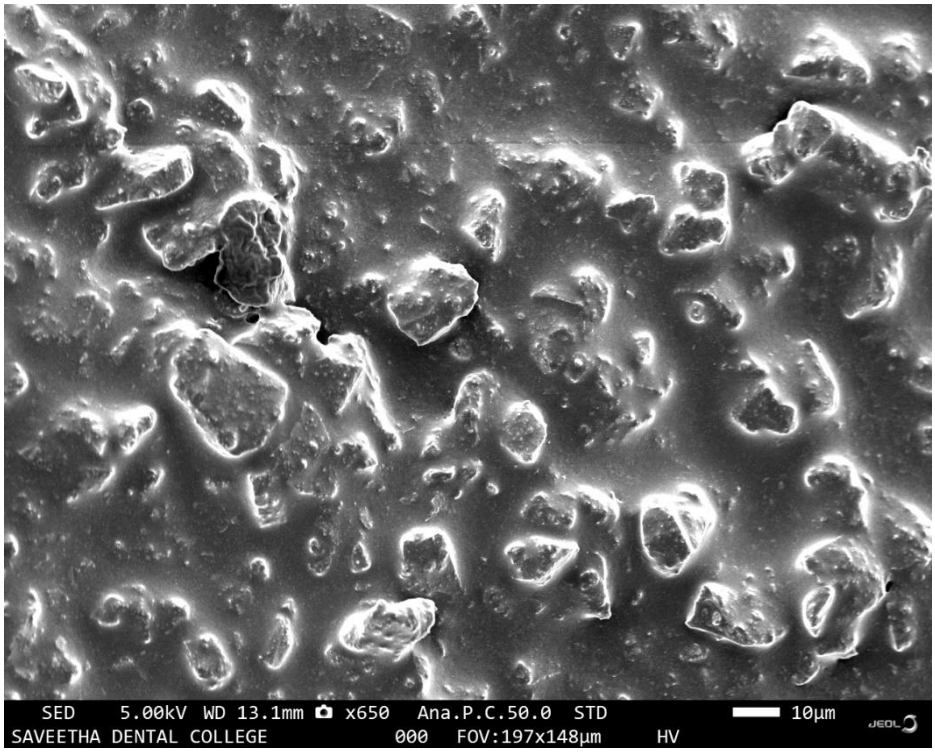
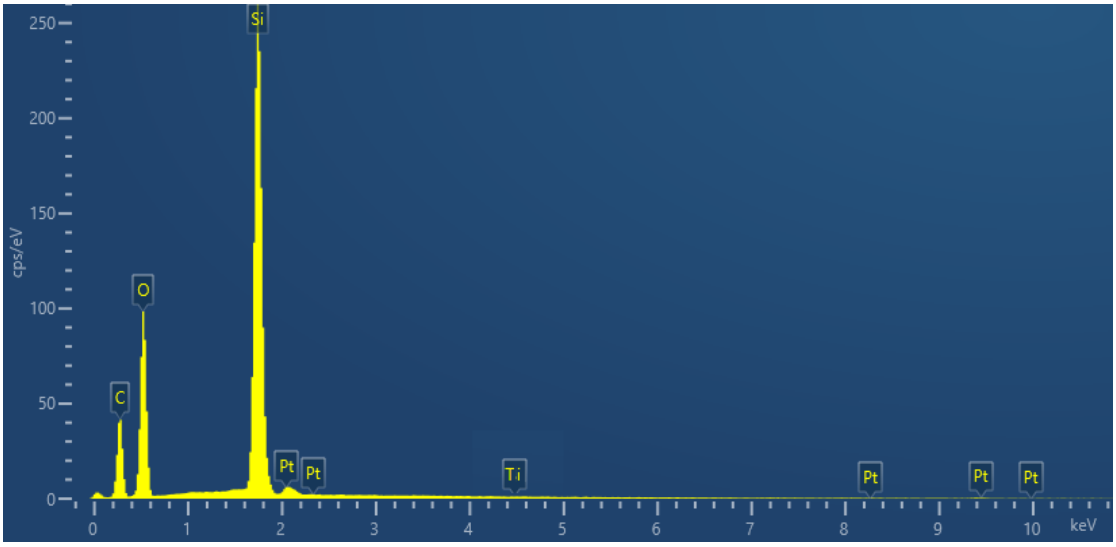


Figure 13: Group 2 Analysis of Transbond with SEM

The analysis was performed using SEM and EDX technique and the presence of titanium nanoparticle on the orthodontic adhesive was confirmed. The EDX analysis stated the presence of carbon, titanium, oxygen, silicon, and platinum.

Compounds	**Atomic Weight (cps/eV)**
Carbon	50
Oxygen	110
Silicon	250
Platinum	<10
Titanium	<10

Table IV: EDX Interpretations for Group 2



Graph 6: Group 2 Analysis of Transbond with EDX

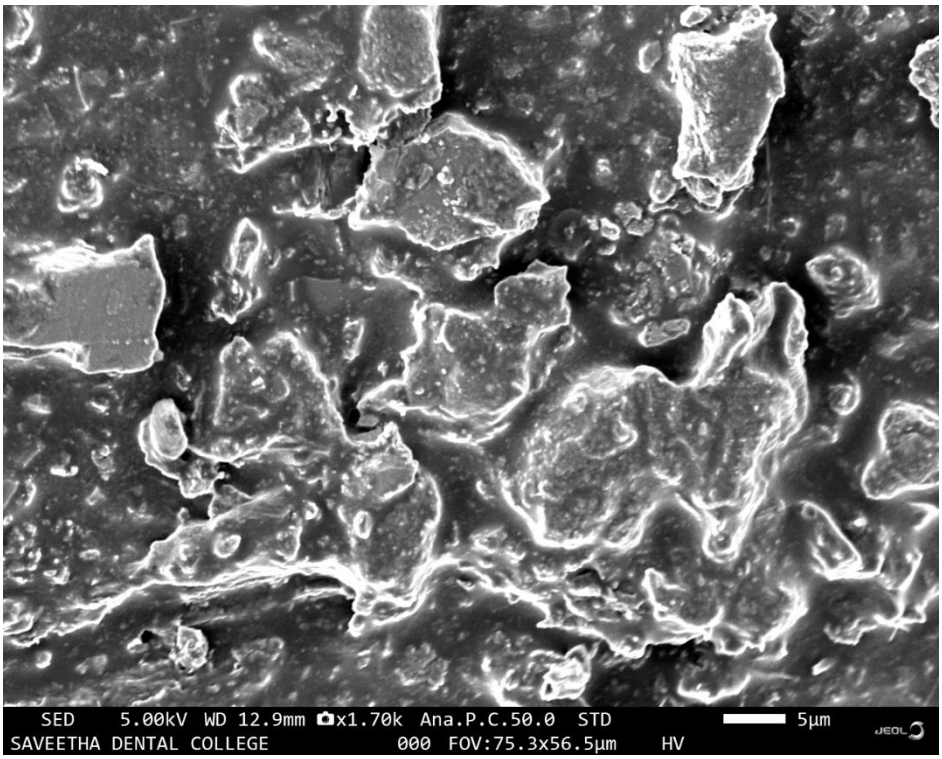
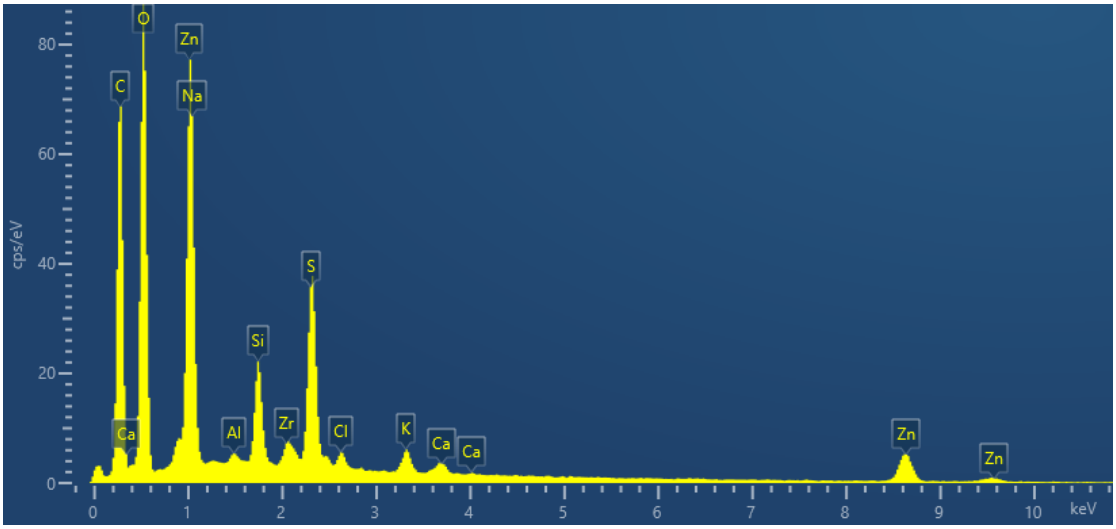


Figure 14: Group 4 Analysis of Transbond with SEM

The analysis was performed using SEM and EDX technique and the presence of zinc nanoparticle on the orthodontic adhesive was confirmed. The EDX analysis stated the presence of carbon, calcium, oxygen, zinc, silicon, sodium, aluminium and potassium.

Compounds	Atomic Weight (cps/eV)
Carbon	70
Calcium	<10
Oxygen	110
Silicon	24
Sodium	80
Zinc	70
Aluminium	30
Potassium	8

Table V: EDX Interpretations for Group 4



Graph 7: Group 4 Analysis of Transbond with EDX

Chemical Compound	Control	Titanium	Silver	Zinc
Carbon	Present	Present	Present	Present
Calcium	Present	-	-	Present
Oxygen	Present	Present	Present	Present
Silicon	Present	Present	Present	Present
Sodium	-	-	-	Present
Zinc	-	-	-	Present
Aluminium	-	-	-	Present
Potassium	-	-	-	Present
Platinum	Present	Present	Present	-

Titanium	-	Present	-	-
Chromium	-	-	Present	-
Silver	-	-	Present	-
Iron	Present	-	-	-
Chlorine	Present	-	-	-

Table VI: Chemical Composition Distribution

Composite disc preparation

For the present study, 60 composite disks (6 mm diameter and 3 mm thickness, nano-adhesive (Transbond XT) disks) were fabricated using plastic molds. Molds were covered by on each side with matrix strips and light cured on each side for 3 s. There was no distance between the LED light tip and the top surface of the composite specimen. Then, specimens were exposed to UV light (15 min) to make sure there is no contamination.

VII. Cytotoxicity

Brine Shrimp Lethality Assay fig15

Salt water preparation:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.

4 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well. Then the nanoparticles discs were placed in each of the wells. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for number of live nauplii's present and calculated by using following formula,

number of dead nauplii/number of dead nauplii+ number of live nauplii×100 were calculated

VIII. Determination of the Antimicrobial Activity of the Nanoparticles

a. Antibacterial Activity (fig 16a)

i. Disk Diffusion method

For this experiment, 60 composite disk (6 mm diameter and 3 mm thickness, 15 silver nano-adhesive,15 titanium naoadhesive, 15 zinc nanoadhesive disc and 15 conventional adhesive (Transbond XT) disks) were fabricated using plastic molds. Molds were covered on each side with matrix strips and light cured on each side for 20s. There was no distance between the LED light tip and the top surface of the composite specimen. Then, specimens were exposed to UV light (15 min) to make sure there is no contamination.

Antibacterial activity of respective nanoparticle discs against the strain Streptococcus mutans, Lactobacillus, Staphylococcus aureus and E.faecalis. Muller Hilton agar (MHA) was utilized for this activity to determine the zone of inhibition. MHA was autoclaved for 45 minutes at 120lbs. Media poured into the sterilized plates and let stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The nanoparticles with different concentration were loaded and the plates were incubated for 24 hours at 37° C. After the incubation time the zone of inhibition were measured.

b. Antifungal activity fig 16b

Candida albicans was used as test pathogen by agar well diffusion assay. Sabouraud's Dextrose Agar is used to prepare the medium. The prepared and sterilized medium was swabbed with test organisms and nanoparticles

with different concentration were added to the wells. The plates were incubated at 28° C for 48-72hours. After the incubation time the zone of inhibition were measured.

IX. Sample Selection

a. Inclusion Criteria

- Maxillary first premolar with intact Buccal and Lingual enamel
- Intact buccal enamel.
- No evidence of caries.
- No developmental defects.
- No cracks
- Teeth without any restorations.

b. Exclusion Criteria

- Premolar devoid of enamel cracks and caries

X. ARMAMENTARIUM:

a. Bonding Materials: fig17a

- 37% Orthophosphoric acid - Scotchbond (3M Unitek, Monrovia, California).
- Transbond XT light cure adhesive primer and paste (3M Unitek, Monrovia, California).
- Micromotor Hand piece with polishing cup.
- Slurry of pumice and mixing well.
- Bracket placement instrument and Explorer and primer applicator tip

- Universal maxillary premolar brackets of 0.022 slot MBT prescription (3M Unitek-Gemini series, Monrovia, California).

b. Light Cure Unit: fig 17b

- High intensity Woodpecker iLED plus light cure unit for 3 seconds

XI. SAMPLE PREPARATION FOR SHEAR BOND TESTING

1. Sixty human maxillary first premolars extracted for orthodontic purpose were collected over 4week period. The teeth were debrided and examined for caries, pre-existing fractures, and restorations. The criteria included intact buccal enamel surface with no cracks caused by pressure of the extraction forceps. The teeth were stored in distilled water for a period of 2 months to prevent dehydration.
2. Sixty premolar teeth were randomly assigned to 4 groups with 15 teeth per group.
3. Each group was given different colour coding
   A. Group 1-Clear
   B. Group 2-Pink
   C. Group 3-Grey
   D. Group 4 -Green
4. Each tooth from the in vitro sample was mounted on colour coded acrylic stubs with roots embedded in a fast set self-cure polymethyl methacrylate resin.
5. The teeth were oriented in such a way labial surface of the tooth was kept perpendicular to the bottom surface of the mould**.**

XII. BONDING PROCEDURE:

1. Teeth were polished with non-fluoridated pumice and prophylactic rubber cup and then rinsed thoroughly with water spray.
2. Enamel surface of the teeth were etched with 37% ortho phosphoric acid (3M Unitek, Monrovia, California) for 30 seconds, and then rinsed with water spray for 30 seconds, then gently dried with oil free air till the etched tooth appeared chalky white.
3. A thin coat of light cure adhesive Transbond XT primer (3M Unitek, Monrovia, California) was applied to acid-etched enamel surface and Transbond XT composite resin (3M Unitek, Monrovia, California) was applied on the bracket base, which was then placed on to the teeth with a bracket placement instrument near the centre of the buccal surfaces with sufficient manual pressure that leads the excess material to flow at the margins of the bracket which was then removed with a exploratory probe before polymerization.

XIII. CURING PROCEDURE:

- Groups are cured with high intensity Woodpecker iLED plus light cure unit for 3 seconds from each of mesial and distal sides of bracket.

Universal testing machine

Universal testing machine (Associated Scientific Engg. Works, New Delhi) with Digital Encoder (Auto Instruments, Kholapur) and FIE make Software (India) was used for measuring mean shear bond strength. Custom made fixture was used for holding blocks.

Instron universal testing machine fig 18a

Shear Bond Strength The shear bond strength was tested using a universal testing machine (Z020; Zwick GmbH, Ulm, Germany).

All the samples are stored in distilled water for 24 hours shear load test in a universal testing machine for shear bond strength. The specimens were placed in a custom made mounting jig in the universal testing machine in such a way that the bracket base was parallel to the shear-peel load. A shear debonding force was applied to the bracket base in an occluso gingival direction at a crosshead speed of 1mm/min. The maximum force necessary to debond or initiate bracket fracture was recorded in Newtons and then converted into Megapascals (MPa) using the formula,

$$\text{SBS (MPa)} = \frac{\text{Peak load at bond failure (N)}}{\text{Bracket surface area (mm}^2\text{)}}$$

(The surface area of the bracket base was 9.08 mm^2 as described by the manufacturer)

XIV. STATISTICAL TESTS USED

The obtained data was analyzed and interpreted using the following statistical tests –

- One-way ANOVA was used to compare the results between the groups.
- Post Hoc Turkey's HSD test was used to do the multiple group comparison.

Figure 1: 60 Extracted Maxillary Premolar Teeth

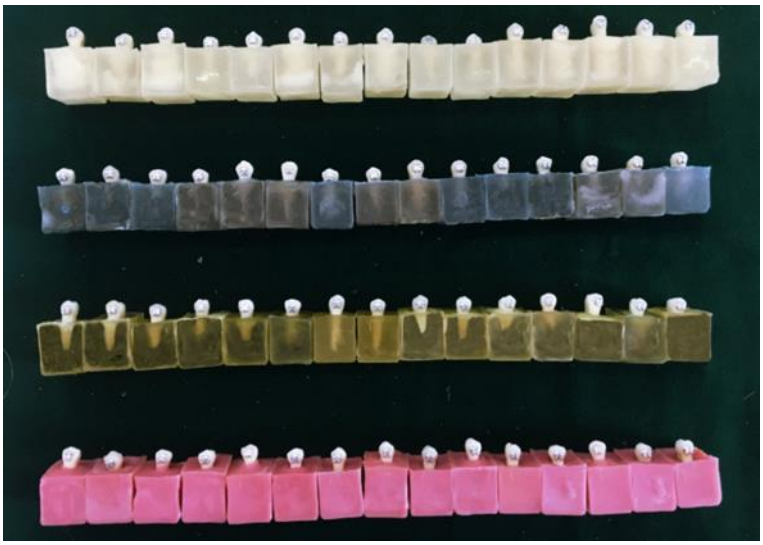


Figure 2: Colour Coding for Various Groups

Figure 3a: Dispensing of Neem Flower Extract

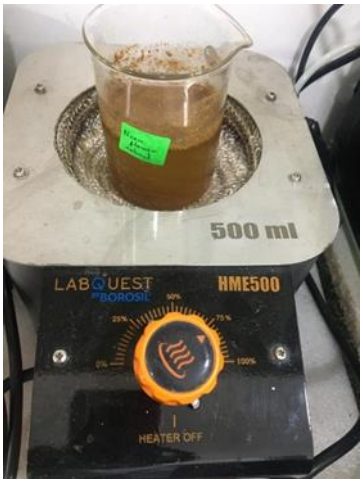


Figure 3b: Boiling of Neem Flower Extract

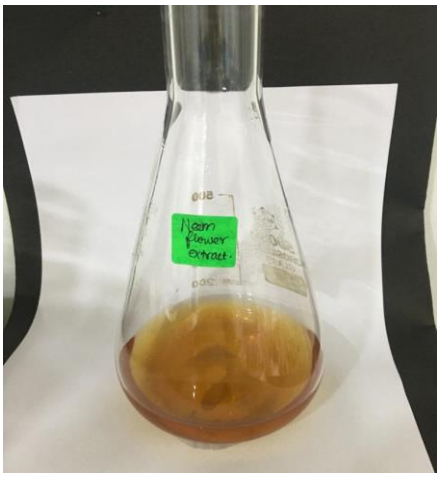
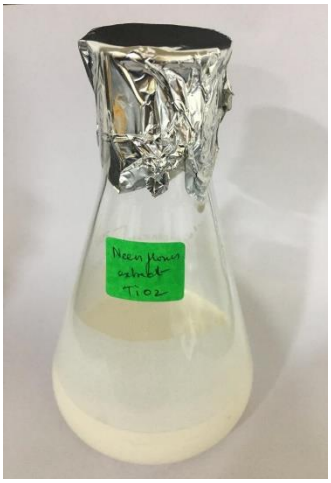
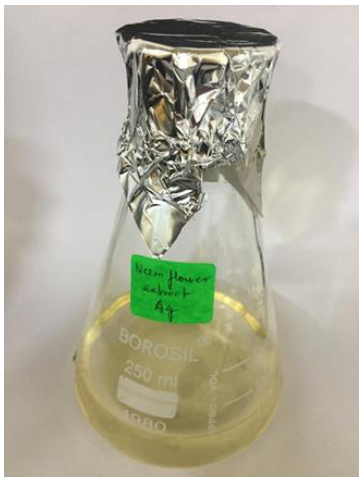
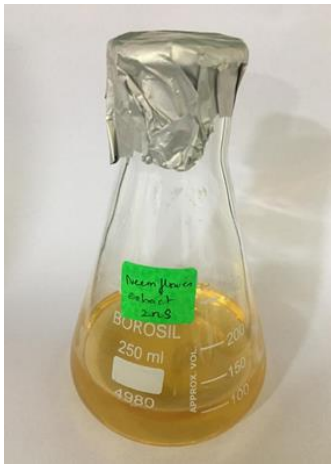


Figure 3c: Aqueous Solution of Neem Flower Extract







(a) **(b)** **(c)**

Figure 4: Dilution of (a) TiO2 (b) Silver (c) Nanoparticles

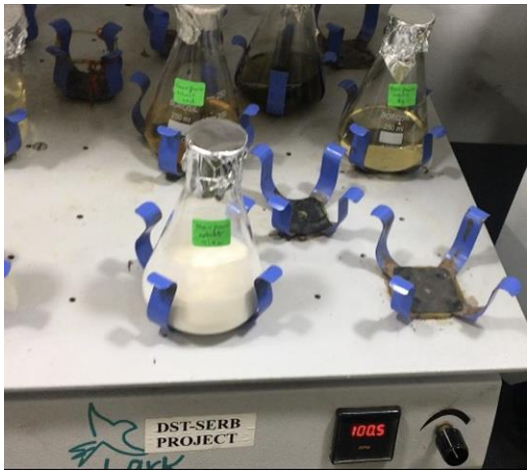


Figure 5: Centrifugation Process

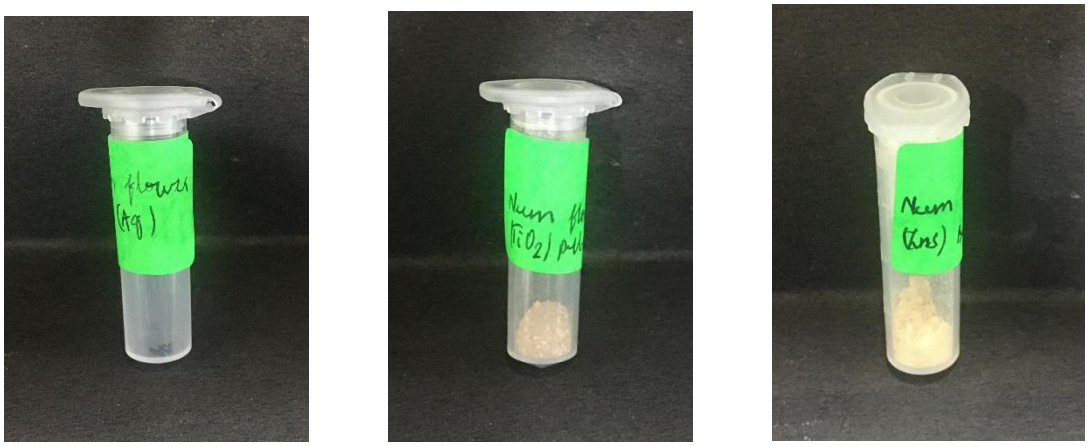


Figure 6: Green Synthesised Nanoparticles

Figure 7: Vortex and Ika ® T25 Digital Ultra-Turrax

Figure 8: Prepared Nano Adhesives

Figure 9: FTIR-Equipment

Figure 10: SEM-EDX Equipment

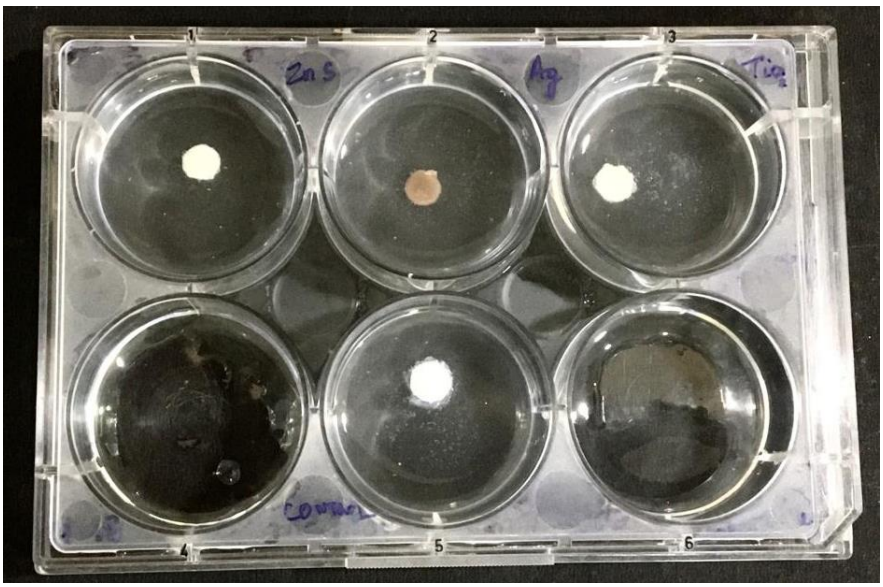


Figure 15: Elisa Plate Containing Nauplii and Nanoadhesive Discs in Each Well to assess the Cytotoxicity

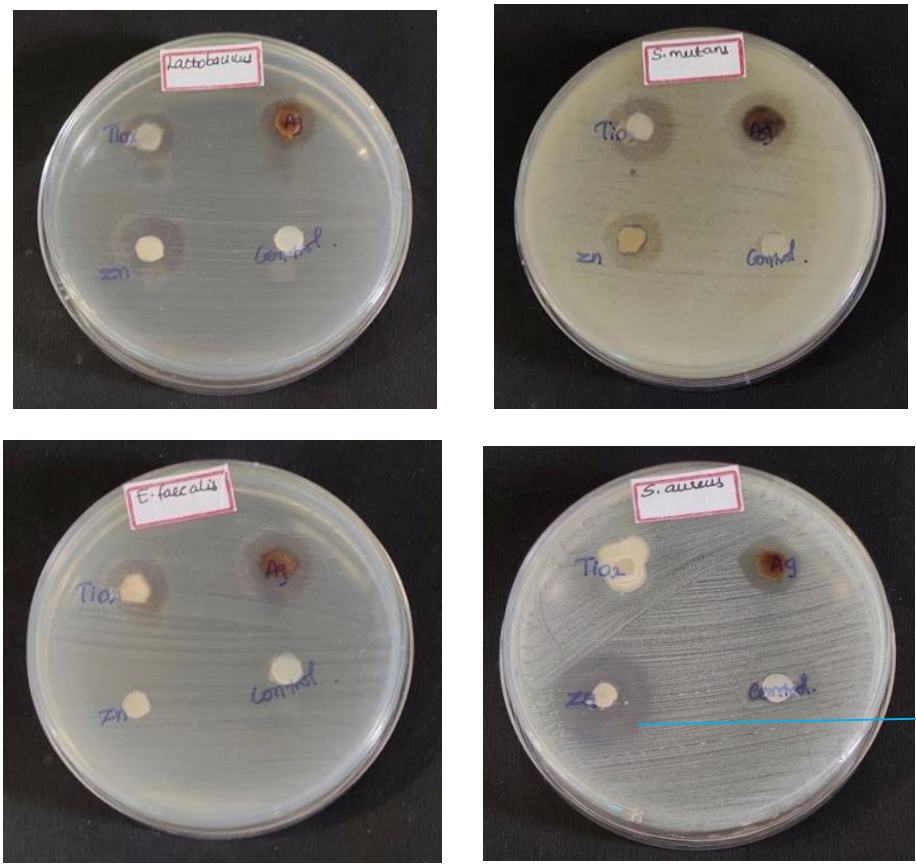


Figure 16a: Petridish Containing Muller Hilton Agar with 3 mm Thickness Nanoparticle Disc Assessed for Anti Bacterial Activity.

Figure 16b: Petridish Containing Sabouraud's Dextrouse Agar with 3 mm Thickness Nanoparticle Disc Assessed for Anti Fungal Activity

Figure 17a: Armamentarium-Bonding

Figure 17b: Light Curing Unit

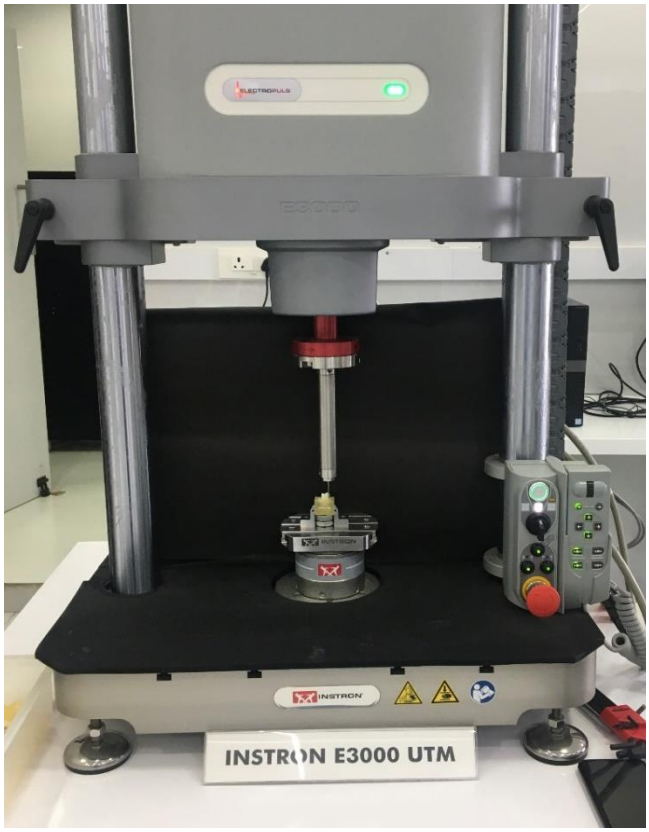


Figure 18a: Universal Testing Machine (INSTRON)

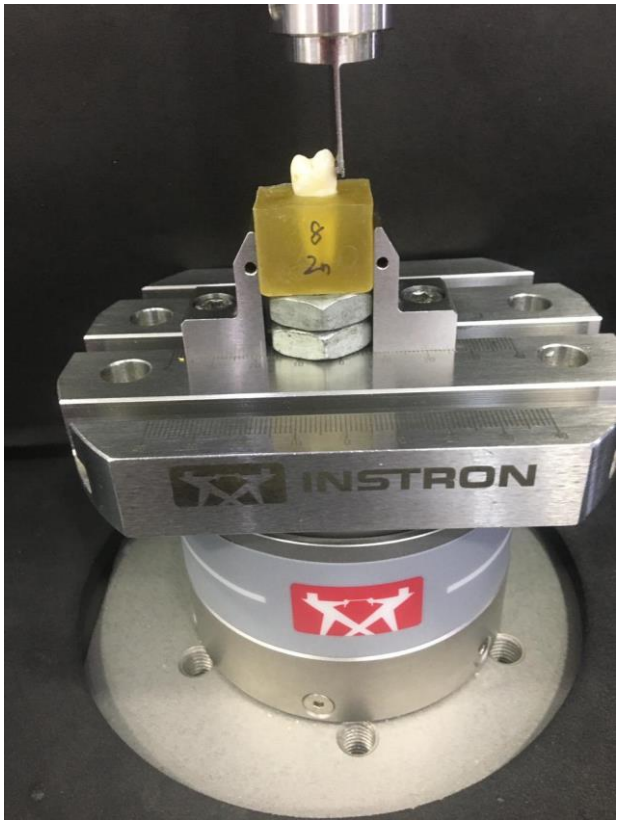


Figure 18b: Shear Bond Testing

RECORDED DATA

I: Zone of inhibition of various microorganisms by the different nano particles

Group	S.Aureus 24h	SMutans_ 24h	E.Faecali_ 24h	LB_24h	C.Albicans_ Transbond
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Control	0	0	0	0	0
Titanium	9	8	7	9	15
Titanium	8	7	8	9	14
Titanium	7	9	9	9	13
Titanium	8	8	9	9	14
Titanium	7	9	10	9	15
Titanium	9	7	9	9	13
Titanium	7	7	8	9	14
Titanium	8	8	7	8	13
Titanium	9	9	9	9	15
Titanium	8	9	10	9	15
Titanium	9	8	8	9	13
Titanium	7	7	9	9	14
Titanium	7	8	7	9	13
Titanium	9	7	8	9	13
Titanium	8	9	9	8	15
Silver	14	12	12	9	15
Silver	13	11	11	10	14
Silver	12	10	13	8	16
Silver	14	12	12	9	15
Silver	13	11	11	10	14
Silver	12	10	13	8	16
Silver	13	12	12	9	14
Silver	12	11	11	10	16
Silver	14	10	13	8	15

Silver	13	12	12	9	14
Silver	12	11	11	10	15
Silver	14	10	13	8	16
Silver	13	12	12	9	14
Silver	12	11	11	10	15
Silver	14	10	13	8	16
Zinc	22	7	0	12	18
Zinc	21	6	0	13	17
Zinc	23	8	0	11	19
Zinc	22	7	0	12	18
Zinc	21	6	0	13	17
Zinc	23	8	0	11	19
Zinc	22	7	0	12	18
Zinc	21	6	0	13	17
Zinc	23	8	0	11	19
Zinc	22	6	0	13	17
Zinc	21	8	0	12	18
Zinc	23	7	0	11	19
Zinc	22	6	0	13	17
Zinc	23	8	0	12	18
Zinc	21	7	0	11	19

II: The Maximum force and maximum load for the different nano particles

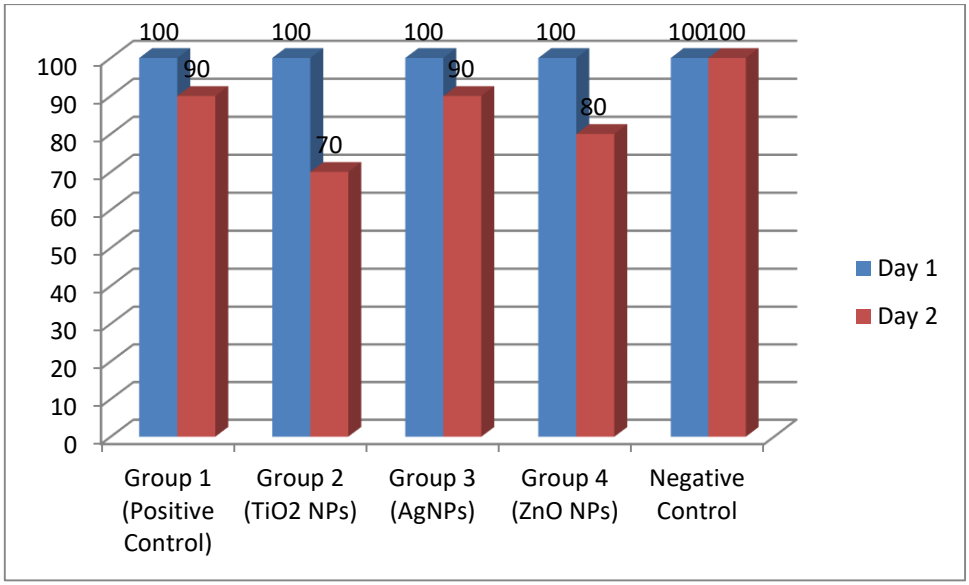
Max Force	Max load	Group
69.79	7.69	**Control**
57.34	6.31	**Control**
73.33	8.08	**Control**
52.72	5.81	**Control**
29.82	3.28	**Control**
103.1	11.35	**Control**
84.44	9.30	**Control**
42.96	4.73	**Control**
76.26	8.40	**Control**
102.82	11.32	**Control**
96.6	10.64	**Control**
68.38	7.53	**Control**
73.28	8.07	**Control**
84.01	9.25	**Control**
91.25	10.05	**Control**
74.61	8.22	**Titanium**
75.76	8.34	**Titanium**
82.63	9.10	**Titanium**

85.13	9.38	**Titanium**
106.43	11.72	**Titanium**
99.49	10.96	**Titanium**
32.8	3.61	**Titanium**
66.57	7.33	**Titanium**
33.84	3.73	**Titanium**
47.36	5.22	**Titanium**
57.53	6.34	**Titanium**
52.49	5.78	**Titanium**
88.32	9.73	**Titanium**
107.22	11.81	**Titanium**
109.02	12.01	**Titanium**
42.79	4.71	**Silver**
67.45	7.43	**Silver**
50.64	5.58	**Silver**
76.93	8.47	**Silver**
58.03	6.39	**Silver**
71.32	7.85	**Silver**
35.41	3.90	**Silver**
62.15	6.84	**Silver**
68.58	7.55	**Silver**
77.55	8.54	**Silver**
44.78	4.93	**Silver**
62.74	6.91	**Silver**
94.7	10.43	**Silver**
79.28	8.73	**Silver**
62.52	6.89	**Silver**
74.76	8.23	**Zinc**
62.59	6.89	**Zinc**
50.3	5.54	**Zinc**
60.45	6.66	**Zinc**
66.02	7.27	**Zinc**
98.3	10.83	**Zinc**
76.27	8.40	**Zinc**
88.32	9.73	**Zinc**
37.86	4.17	**Zinc**
69.04	7.60	**Zinc**
80.69	8.89	**Zinc**
41.98	4.62	**Zinc**
54.08	5.96	**Zinc**
77.89	8.58	**Zinc**
87.66	9.65	**Zinc**

III: The cytotoxic growth for the different nano particles

	Day 1	Day 2
Group 1 (Positive Control)	100	90
Group 2 (TiO2 NPs)	100	70
Group 3 (AgNPs)	100	90
Group 4 (ZnO NPs)	100	80
Negative Control	100	100

CYTOTOXICITY ANALYSIS



Graph 8: Graphical Representation of the Cytotoxicity Values

The cytotoxicity analysis for the Nano material infused adhesive Groups after 24 hours showed: Group 1-control (Transbond XT) had 90% survival of the Nauplii and Group 2-Titanium dioxide Nano adhesive had 70% survival of Nauplii, Group 3-Silver Nano adhesive had 90% survival and Group 4 –Zinc Nano adhesive had 80% survival of the Nauplii. There was also a negative control with no adhesive disc. As represented in Graph 8.

INFERENCE

Among the groups the number of live nauplii was highest in control and silver (90%) followed by zinc (80%) and titanium (70%) however all nanoparticles infused adhesive proved to be biocompatible

ANTIMICROBIAL ACTIVITY

The present study considered four different types of Nano adhesives. The anti-microbial activity of the Nano particles infused adhesive was measured for different

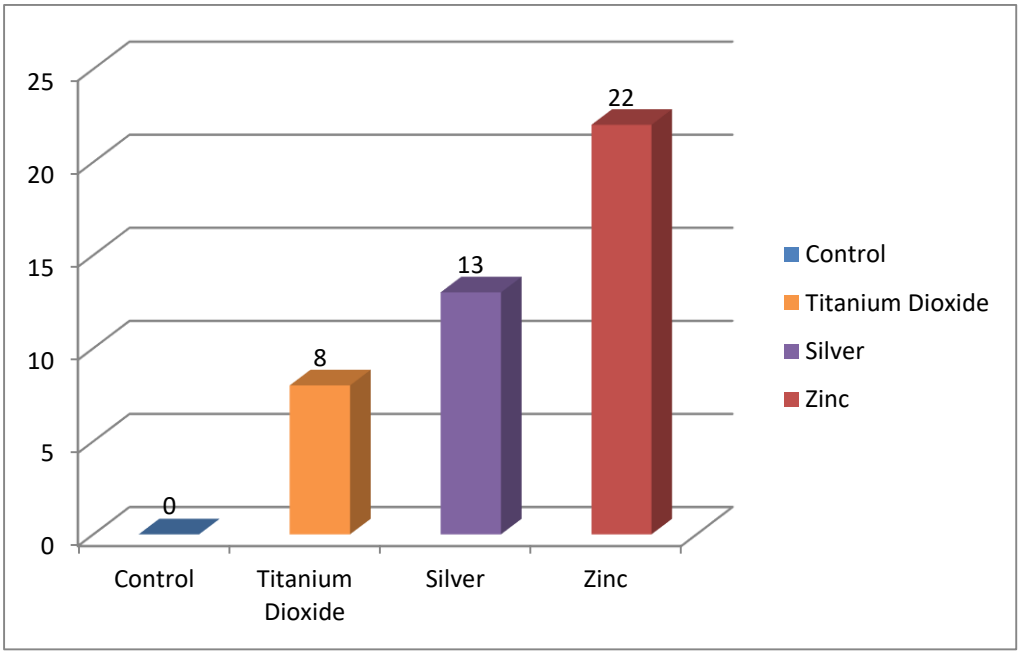
micro-organism at the end of 24 hours. Descriptive statistics were calculated using Mean and Standard Deviation. Inferential statistics were performed to compare between the groups using One way ANOVA. The zone of inhibition among the different nano adhesive as measured after 24 hours.

Groups	Mean (in mm)	Std. Dev	Std. Error	95% Confidence Interval for Mean		P Value
				Lower Bound	Upper Bound	
Group 1-(Control)	0.00	0.00	0.00	0.00	0.00	<0.0001*
Group 2-(Titanium Dioxide)	8.00	0.845	0.218	7.53	8.47	
Group 3 (Silver)	13.00	0.845	0.218	12.53	13.47	
Group 4 (Zinc)	22.00	0.845	0.218	21.53	22.47	

*statistically significant

Table VIIa: Staphylococcus Aureus (S. aureus) Zone of Inhibition for Each Nano Adhesive Type

Table VIIa suggests the zone of inhibition of S. Aureus at the end of 24 hours. Group 4 (Zinc) Nano adhesive had the highest inhibition zone (22.00±0.845) mm followed by Group 3 (silver) (13.00±0.845) mm and then titanium (8±0.845) mm. The control group had no zone of inhibition. There was a statistically significant difference observed between the groups ($p<0.0001$). The same have been graphically represented in Graph-9.



Graph 9: Graphical Representation of the S. Aureus Microbial Growth at 24 Hours

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
						Lower Bound	Upper Bound
S. Aureus_24h	GROUP 1 (CONTROL)	Group 2 TiO2	-8.000*	0.267	<0.0001*	-8.71	-7.29
		Group 3 Ag	-13.000*	0.267	<0.0001*	-13.71	-12.29

		Group 4 ZINC	-22.000*	0.267	<0.0001*	-22.71	-21.29
	Group 2 TiO2	Group 3 Ag	-5.000*	0.267	<0.0001*	-5.71	-4.29
		Group 4 ZINC	-14.000*	0.267	<0.0001*	-14.71	-13.29
	Group 3 Ag	Group 2 TiO2	5.000*	0.267	<0.0001*	4.29	5.71
		Group 4 ZINC	-9.000*	0.267	<0.0001*	-9.71	-8.29

Table VIIb: Multiple Group Comparison for the Zone of Inhibition S. Aureus at 24 Hours

Individual group comparison was also made and a statistically significant difference was noted among all the Nano particles. The zinc Nano particles had a significantly high difference from the control for the minimal zone of inhibition. The same have been tabulated in Table VIIb.

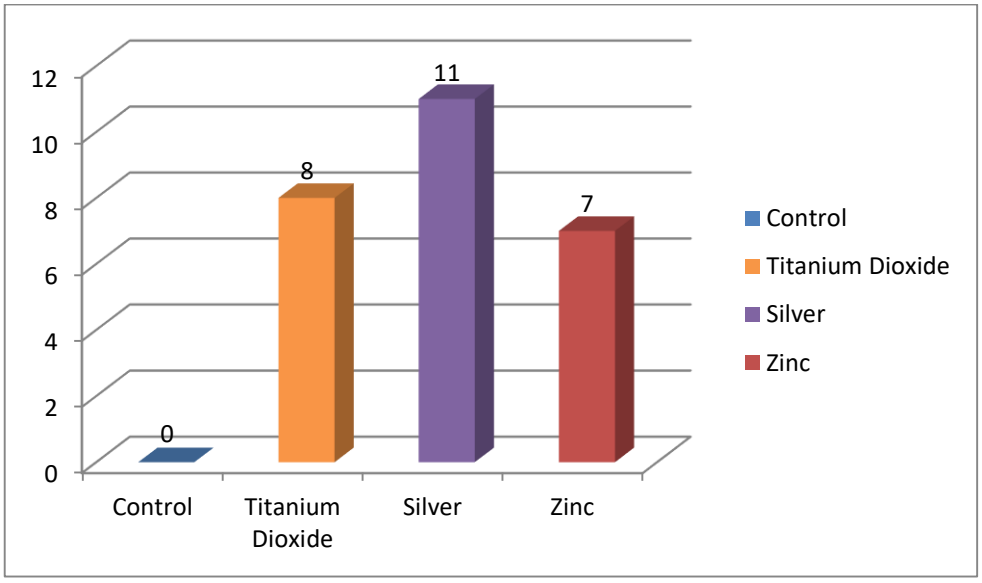
Groups	**Mean (in mm)**	**Std. Dev**	**Std. Error**	**95% Confidence Interval for Mean**		**P Value**
				Lower Bound	**Upper Bound**	
Group 1-(Control)	0.00	0.00	0.00	0.00	0.00	<0.0001*

Group 2-(Titanium Dioxide)	8.00	0.845	0.218	7.53	8.47	
Group 3 (Silver)	11.00	0.845	.218	10.53	11.47	
Group 4 (Zinc)	7.00	0.845	.218	6.53	7.47	

*statistically significant

Table VIIIa: S.Mutans Zone of Inhibition for Each Nano Adhesive Type

Table VIIIa suggests the zone of inhibition of S. Mutans at the end of 24 hours. Silver Nano adhesive (11.00±0.845) mm had highest zone of inhibition followed by titanium dioxide (8.00±0.845) mm and Zinc (7.00±0.845) mm. The control group had no zone of inhibition. There was a statistically significant difference observed between the groups ($p<0.0001$). The same have been graphically represented in Graph-10.



Graph-10: Graphical Representation of the Zone of Inhibition of S. Mutans at 24 Hours

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P Vaue	95% Confidence Interval	
						Lower Bound	Upper Bound
S Mutans _24h	Group 1 (CONTROL)	Group 2 (TiO2)	-8.000*	0.267	<0.0001*	-8.71	-7.29
		Group 3 (Ag)	-11.000*	0.267	<0.0001*	-11.71	-10.29
		Group 4(ZINC)	-7.000*	0.267	<0.0001*	-7.71	-6.29
	Group 2 (TiO2	Group 3 (Ag)	-3.000*	0.267	<0.0001*	-3.71	-2.29
		Group 4(ZINC)	1.000*	0.267	<0.0001*	.29	1.71
	Group 3 (Ag)	Group 2 (TiO2)	4.000*	0.267	<0.0001*	3.29	4.71

*statistically significant

Table VIIIb: Multiple Group Comparison for the Zone of Inhibition S. Mutans at 24 Hours

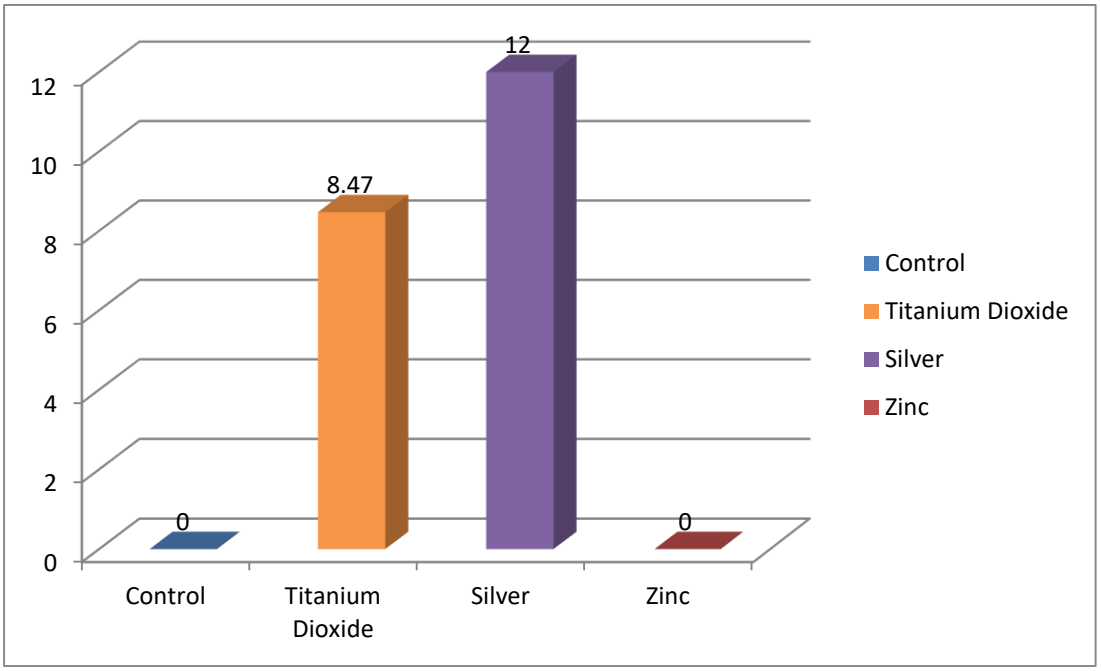
Individual group comparison was also made and a statistically significant difference was noted among all the Nano adhesives. The zinc nano adhesive had a significantly high difference from the control for the minimal zone of inhibition. The same have been tabulated in Table VIIIb.

Groups	Mean (in mm)	Std. Dev	Std. Error	95% Confidence Interval for Mean		P Value
				Lower Bound	Upper Bound	
Group 1-(Control)	0.00	0.00	0.00	0.00	0.00	<0.0001*
Group 2-(Titanium Dioxide)	8.47	0.99	0.256	7.92	9.02	
Group 3 (Silver)	12.00	0.845	0.218	11.53	12.47	
Group 4 (Zinc)	0.00	0.00	0.00	0.00	0.00	

*statistically significant

Table IXa: E.Faecali Zone of Inhibition for Each Nano Particle Type

Table IXa suggests the zone of inhibition of E.Faecali at the end of 24 hours. For the control and zinc there was no zone of inhibition. Silver (12.00±0.845) mm had the highest, followed by Titanium dioxide (8.47±0.845) mm. There was a statistically significant difference observed between the groups ($p<0.0001$). The same have been graphically represented in graph 11.



Graph-11: Graphical Representation of the Zone of Inhibition of E. Faecali at 24 Hours

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
						Lower Bound	Upper Bound
E. Faecali_24h	Group 1 (CONTROL)	Group 2 (TiO2)	-8.467*	.238	<0.0001*	-9.10	-7.84
		Group 3 (Ag)	-12.000*	.238	<0.0001*	-12.63	-11.37

		Group 4(ZINC)	.000	.238	1.000	-.63	.63
	Group 2 (TiO2	**Group 3 (Ag)**	-3.533*	.238	<0.0001*	-4.16	-2.90
		Group 4(ZINC)	8.467*	.238	<0.0001*	7.84	9.10
	Group 3 (Ag)	**Group 2 (TiO2)**	12.000*	.238	<0.0001*	11.37	12.63

*statistically significant

Table IXb: Multiple Group Comparison for the Zone of Inhibition of E.Faecali at 24 Hours

Individual group comparison was also made and a statistically significant difference was noted among all the nano particles. The zinc nano particles had a significantly high difference from the control for the minimal zone of inhibition. The same have been tabulated in Table IXb.

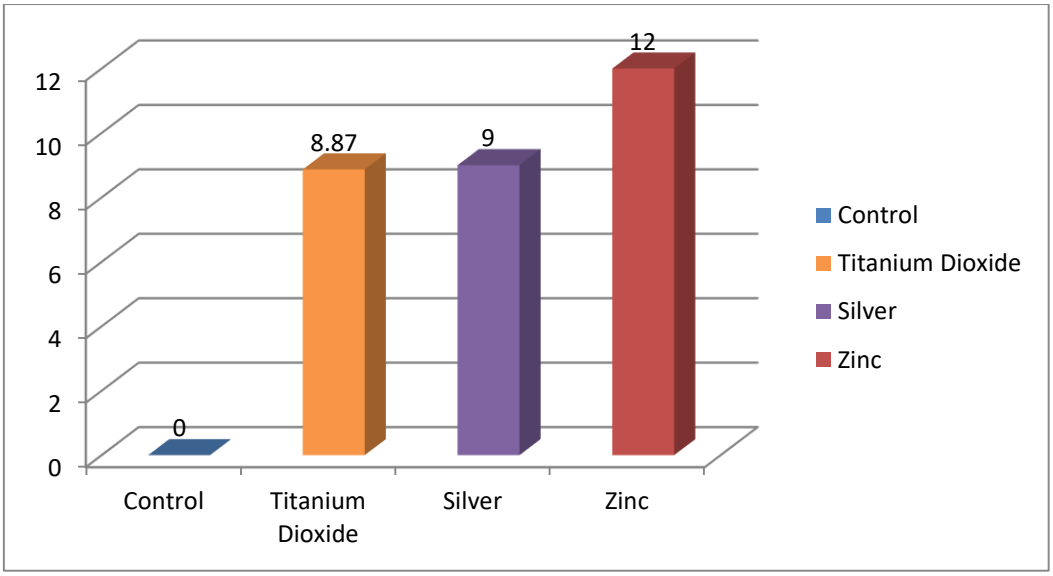
Groups	**Mean (in mm)**	**Std. Dev**	**Std. Error**	**95% Confidence Interval for Mean**		**P Value**
				Lower Bound	**Upper Bound**	
Group 1-(Control)	0.00	0.00	0.00	0.00	0.00	<0.0001*

Group 2-(Titanium Dioxide)	8.87	0.352	0.091	8.67	9.06	
Group 3 (Silver)	9.00	0.845	0.218	8.53	9.47	
Group 4 (Zinc)	12.00	0.845	0.218	11.53	12.47	

*statistically significant

Table Xa: Lacto Bacillus Zone of Inhibition for Each Nano Particle Type

Table Xa suggests the zone of inhibition of Lacto Bacillus at the end of 24 hours. The highest zone of inhibition was seen in Zinc (12.00±0.845) mm nanoparticles, followed by silver (9.00±0.845) mm and titanium dioxide (8.87±0.352) mm. There was no zone of inhibition for the control group. There was a statistically significant difference observed between the groups ($p<0.0001$). The same have been graphically represented in graph-12.



Graph 12: Graphical Representation of the Lacto Bacillus Zone of Inhibition at 24 Hours

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
						Lower Bound	Upper Bound
LB_24h	Group 1 (CONTROL)	Group 2 (TiO2)	-8.867*	0.227	<0.0001*	-9.47	-8.26
		Group 3 (Ag)	-9.000*	0.227	<0.0001*	-9.60	-8.40
		Group 4(ZINC)	-12.000*	0.227	<0.0001*	-12.60	-11.40
	Group 2 (TiO2	Group 3 (Ag)	-.133	0.227	0.936	-.74	.47
		Group 4(ZINC)	-3.133*	0.227	<0.0001*	-3.74	-2.53
	Group 3 (Ag)	Group 2 (TiO2)	-3.000*	0.227	<0.0001*	-3.60	-2.40

*statistically significant

Table Xb: Multiple Group Comparison for the Zone of Inhibition Lacto Bacillus at 24 Hours

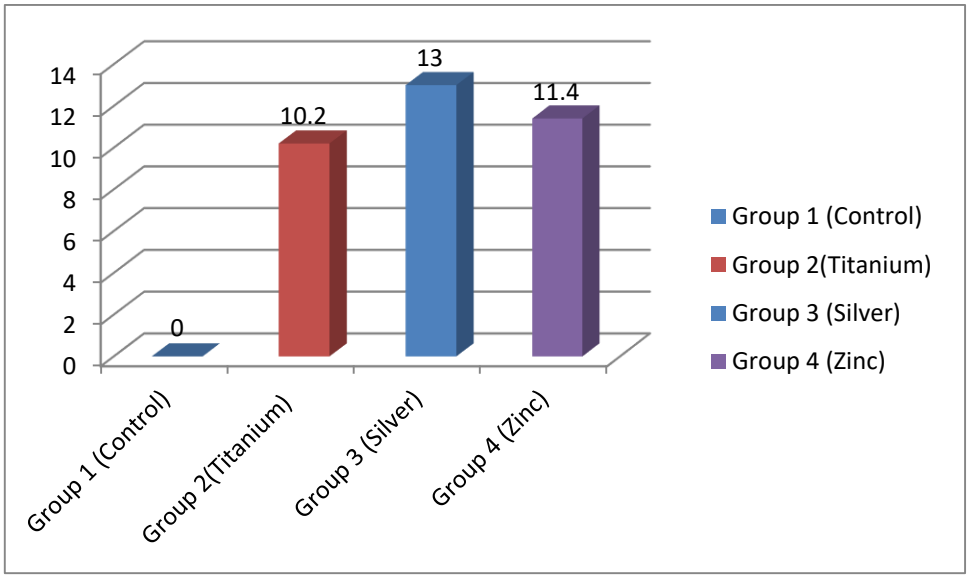
Individual group comparison was also made and a statistically significant difference was noted among all the nano particles. There was statistically significant difference between groups. The same have been tabulated in Table Xb.

	Mean	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Chi Square/ P Value**
				Lower Bound	**Upper Bound**	
Group 1 (Control)	0	0	0	0	0	4.216/ 0.022*
Group 2 (Titanium)	10.20	2.683	1.200	6.87	13.53	
Group 3 (Silver)	13.00	3.317	1.483	8.88	17.12	
Group 4 (Zinc)	11.40	6.269	2.804	3.62	19.18	

*statistically significant

Table XI: Antibacterial Effect of the Nano Particles

The cumulative antibacterial effect of the nanoparticles has been mentioned in Table XI. For Group 1 it was 0±0, for Group 2 it was 10.20±2.683, for Group 3 it was 13.00±3.317 and for Group 4 it was 11.40±6.269. Among the groups silver exhibited better anti-bacterial property followed by zinc and titanium. It has been represented in Graph 13.



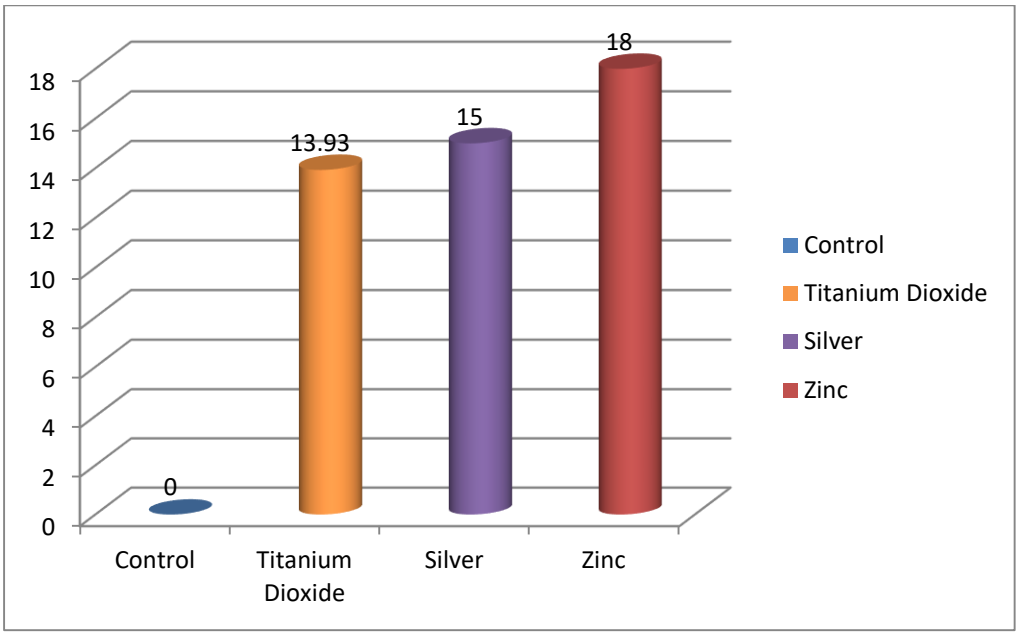
Graph-13: Graphical Representation of the Antibacterial Effect of the Nano Particles

Groups	Mean (in mm)	Std. Dev	Std. Error	95% Confidence Interval for Mean		P Value
				Lower Bound	Upper Bound	
Group 1-(Control)	0.00	0.00	0.00	0.00	0.00	<0.0001*
Group 2-(Titanium Dioxide)	13.93	0.884	0.228	13.44	14.42	
Group 3 (Silver)	15.00	0.845	0.218	14.53	15.47	
Group 4 (Zinc)	18.00	0.845	0.218	17.53	18.47	

*statistically significant

Table XIIa: Candida Albicans Zone of Inhibition for Each Nano Particle Type

Table XIIa suggests the zone of inhibition of Candida albicans at the end of 24 hours. Highest inhibition zone was seen in Zinc (18.00±0.845) mm nanoparticles followed by silver (15.00±0.845) mm and then titanium dioxide (13.93±0.884) mm nanoparticles. There was no zone of inhibition for the control group. There was a statistically significant difference observed between the groups (p<0.0001). The same have been represented in graph-14



Graph-14: Candida Albicans Microbial Presentation for Each Nano Particle Type

Dependent Variable	(I) Group	(J) Group	Mean Diff (I-J)	Std. Error	P Value	95% Confidence Interval	
						Lower Bound	Upper Bound
C.Albicans _Transbond	Group 1 (CONTROL)	Group 2 (TiO2)	-13.933*	0.271	<0.0001*	-14.65	-13.21
		Group 3 (Ag)	-15.000*	0.271	<0.0001*	-15.72	-14.28

		Group 4(ZINC)	-18.000*	0.271	<0.0001*	-18.72	-17.28
	Group 2 (TiO2	**Group 3 (Ag)**	-1.067*	0.271	<0.0001*	-1.79	-.35
		Group 4(ZINC)	-4.067*	0.271	<0.0001*	-4.79	-3.35
	Group 3 (Ag)	**Group 2 (TiO2)**	-3.000*	0.271	<0.0001*	-3.72	-2.28
*. The mean difference is significant at the 0.05 level.							

Table XIIb: Multiple Group Comparison for the Growth of Candida Albicans at 24 Hours

Individual group comparison was also made and a statistically significant difference was noted among all the nano particles. The zinc nano particles had a significantly high difference from the control for the minimal zone of inhibition. The same have been tabulated in Table XIIb.

	CONTROL		**TIO2**		**AG**		**Zn**		**F score/ P Value**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
S. Aureus	0.0	0.0	8.00	.845	13.00	.845	22.00	.845	2377.667 / <0.0001*

S Mutans	0.0	0.0	8.00	.845	11.00	.845	7.00	.845	606.667 <0.0001*
E. Faecali	0.0	0.0	8.47	.990	12.00	.845	0.0	0.0	1309.118 <0.0001*
LB	0.0	0.0	8.87	.352	9.00	.845	12.00	.845	1038.577 <0.0001*
CA	0.0	0.0	13.93	.884	15.00	.845	18.00	.845	1742.034 <0.0001*

*statistically significant

Table XIII : Antimicrobial Effect of the Nanoparticles

The cumulative antimicrobial effect of various Nano adhesive groups is presented in table XIII which showed the zinc nanoparticle had superior antimicrobial effect followed by silver and titanium respectively. The difference was statistically significant.

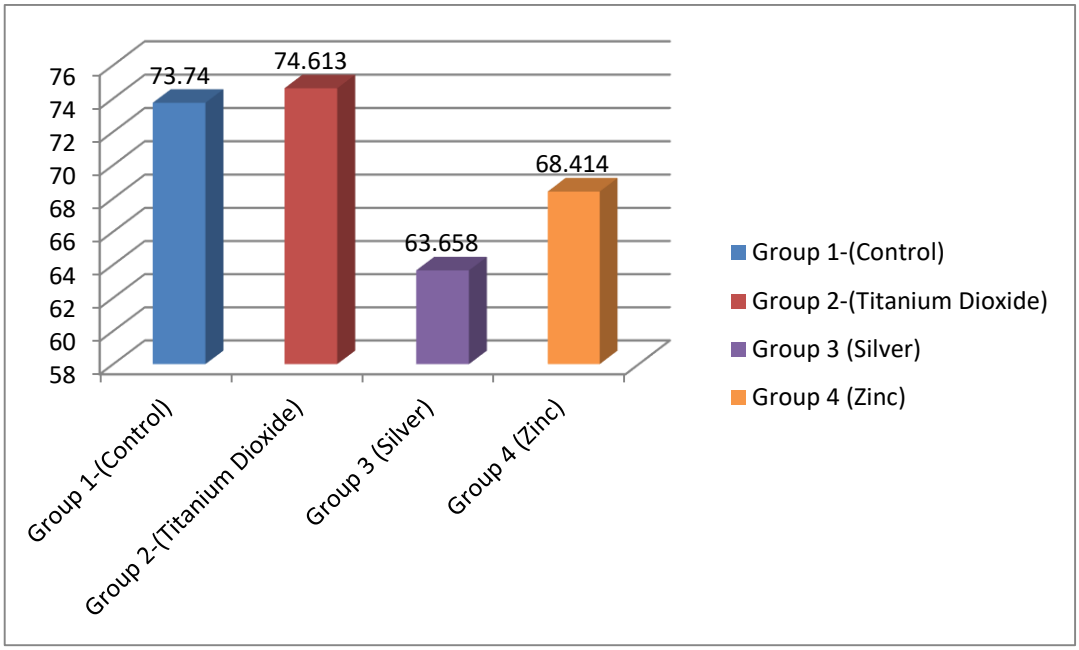
		Mean	**Std. Dev**	**Std. Error**	**95% Confidence Interval for Mean**		**P Value**
					Lower Bound	**Upper Bound**	
Max Force	**Group 1- (Control)**	73.740	21.373	5.518	61.9042	85.5758	0.431

	Group 2- (Titanium Dioxide)	74.613	25.693	6.634	60.3851	88.8416	
	Group 3 (Silver)	63.658	15.742	4.064	54.9402	72.3758	
	Group 4 (Zinc)	68.414	17.467	4.510	58.7407	78.0873	

Table XIVa: Maximum Force Distribution Between the Groups

Table XIVa shows the maximum force required to debond for different Nano adhesive groups. The maximum force to debond for control group was 73.740±21.373N, followed by titanium dioxide with 74.613±25.692N, silver nano particles 63.65±15.742N and zinc nano particles 68.347±17.467N. There was no statistically significant difference between the groups (p=0.431). The same have been graphically represented in Graph 15

Table XIVb states the multiple group comparison for the maximum force required to debond for the different Nano adhesive groups. There was statistically significant difference between groups.



Graph-15: Graphical Representation of the Maximum Force Between the Groups

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Max Force	Group 1 (CONTROL)	Group 2 (TiO2)	-.873	7.46	0.999	-20.63	18.88
		Group 3 (Ag)	10.082	7.46	0.535	-9.67	29.84
		Group 4(ZINC)	5.326	7.461	0.891	-14.43	25.081
	Group 2 (TiO2	Group 3 (Ag)	10.95	7.46	0.463	-8.79	30.7104

		Group 4(ZINC)	6.19	7.46	0.840	-13.55	25.95
	Group 3 (Ag)	**Group 2 (TiO2)**	-4.76	7.46	0.919	-24.51	14.99

*statistically significant

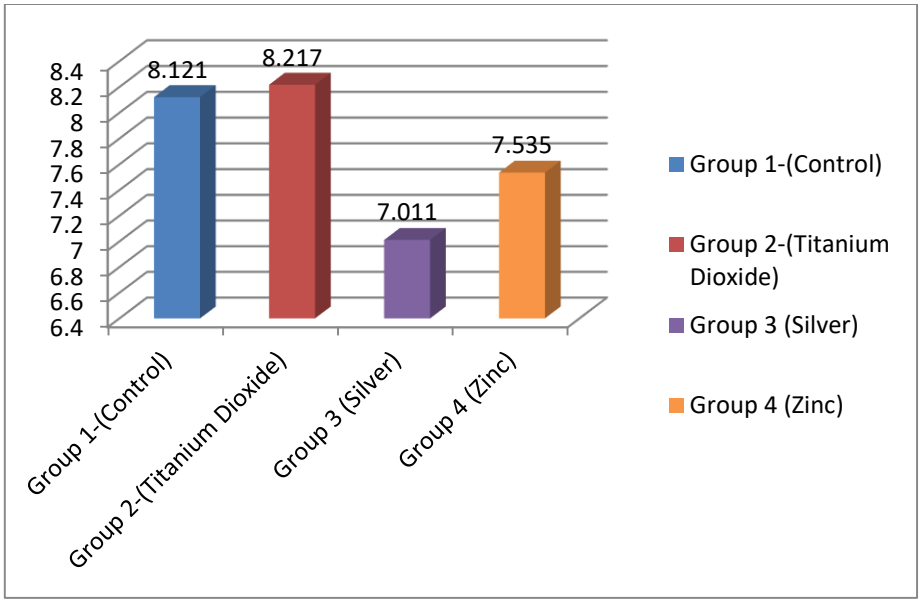
Table XIVb: Multiple Group Comparison for the Maximum Force Distribution Among the Nano Particles

		Mean	**Std. Dev**	**Std. Error**	**95% Confidence Interval for Mean**		**P Value**
					Lower Bound	**Upper Bound**	
Max load	**Group 1- (Control)**	8.121	2.354	.607	6.8176	9.4246	0.431
	Group 2- (Titanium Dioxide)	8.217	2.829	.730	6.6503	9.7843	
	Group 3 (Silver)	7.011	1.733	.447	6.0507	7.9709	
	Group 4 (Zinc)	7.535	1.923	.496	6.4692	8.5999	

Table XVa: Maximum Load Distribution Between the Groups

Table XVa shows the maximum force distribution (shear bond strength) between the groups. The Shear bond strength for the control was 8.121±2.354Mpa, for titanium

dioxide it was 8.217±2.829Mpa, for silver nano particles it was 7.011±1.733Mpa and for the zinc nano particles it was 7.535±1.923. Silver nano particles had presented with least bond strength. There was no statistically significant difference between the groups (p=0.431). The same have been graphically represented in graph 16.



Graph 16: Graphical Representation of the Maximum Load Distribution Between the Groups

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P Value	95% Confidence Interval	
						Lower Bound	Upper Bound
Max load	Group 1 (CONTROL)	Group 2 (TiO2)	-0.0962	.821	.999	-2.272	2.079
		Group 3 (Ag)	1.11	.821	.535	-1.065	3.286

		Group 4(ZINC)	0.586	.821	.891	-1.589	2.762
	Group 2 (TiO2	**Group 3 (Ag)**	1.206	.821	.463	-.969	3.38
		Group 4(ZINC)	0.683	.821	.840	-1.49	2.85
	Group 3 (Ag)	**Group 2 (TiO2)**	0.524	.821	.919	-1.65	2.69
*. The mean difference is significant at the 0.05 level.							

Table XVb: Multiple Group Comparison for the Maximum Load

Table XVb states the multiple group comparison for the maximum force distribution for the different Nano particles. No Statistically significant difference was noted between groups.

A beautiful smile is a gateway to the world. Margaret Wolfe Hungerford once quoted “Beauty is in the mind of the beholder, each mind perceives a different beauty”[69].

Revolutions in the field of science and technology have given promising results in the field of material sciences and one such advancement is nanotechnology. Nanotechnology, which concerns structures at the Nano scale is considered as a vital current technology of the 21st century based on its economic and scientific potential.[69]

The term "Nano" is derived from the Greek word meaning "dwarf". A nanometre is 10^{-9} or one billionth of a meter. The concept and origin of nanotechnology have been attributed to the American physicist and Nobel Laureate Richard Feynman in 1959. Eric Dexler is one of the “founding fathers of nanotechnology” is most known for being the driving force behind the concept of molecular nanotechnology and its potential benefits for humans.[69]

In the field of medicine, nanotechnology has been applied in diagnosis, prevention, and treatment of diseases.[70,71]During the last decade, the use of nanotechnology and nanoparticles has become popular in the design and development of dental biomaterials with improved material characteristics.[72]

The development of Nanotechnology gives better opportunities to both patient and Orthodontist due to its physicochemical, mechanical and antibacterial properties. It can be essentially used in coating orthodontic wires, elastomeric ligatures, and brackets, producing shape memory polymers and orthodontic bonding materials.[73]

The prolonged duration of Orthodontic therapy results in increased accumulation of dental plaque and eventually results in a greater risk of caries. The

demineralization process that progresses to caries is called a white spot lesion (WSL). Enamel demineralization in the form of white spot lesions frequently occurs during and after orthodontic treatment. Katie et al[74] observed, 23.4% of the patients developed at least one WSL during their course of treatment. In the highly cariogenic environment adjacent to orthodontic appliances, this lesion can progress rapidly and may produce carious cavitation. Thus, the prevention of white spot lesions is crucial to maintain the integrity of dentition during orthodontic treatment and scientists are investigating the effect of antibacterial agent incorporation into orthodontic adhesives to prevent white spot formation [7]

Numerous antibacterial agents have been integrated into orthodontic products such as chlorhexidine or fluoride containing mouth rinse, fluoridated toothpaste, fluoride gel and varnish[7]. Borzabad et al,[75] suggested nanoparticles prevent microbial adhesion and enamel demineralization in orthodontic therapy by two strategies either by incorporating them in orthodontic adhesives/cement or acrylic resins. (Nanofillers, silver, TiO2, SiO2, hydroxyapatite, fluorapatite, fluorohydroxyapatite) or coating surfaces of orthodontic appliances with nanoparticles (i.e. coating bracket surfaces with a thin film of nitrogen doped TiO2)[75]

There are various nanoparticles find their use in orthodontics like Silver, Gold, Copper Chitosan, Titanium -Dioxide, Zinc compounds, Silica, Ceria, Alumina, Tin, Copper and Tungsten trioxide[26]. In the present study we chose to use Titanium dioxide, silver and zinc nanoparticles because these are biogenic nanoparticles and exhibit exemplary antimicrobial property.

Titanium nanoparticles Tio_2 resins embodied in resin displays compelling antimicrobial properties which may be applied for preventing recurrent caries and

demineralization of the enamel[20.] Effective catalytic action and properties such as white colour, low toxicity, high stability and low cost have made these nanoparticles an appropriate additive for use in dental materials.[19]

Incorporation of Tio_2 nanoparticles to dental composites also augmented mechanical properties, such as modulus of elasticity, microhardness, flexural strength, and also provided bond strength values that similar or even higher levels than that of the controls not containing the nanoparticles. The activation of N-doped TiO_2 leads to the formation of OH. Free radicals, superoxide ions (O_2), hydrogen peroxide (H_2O_2), and peroxyl radicals (HO_2). These chemicals exert antimicrobial activity, also reacting with lipids, enzymes, and proteins.[20]

Silver nanoparticles have been shown to be materials with excellent anti-microbial properties in a wide variety of microorganisms. Various studies showed incorporating (17 nm) into orthodontic elastomeric modules, orthodontic brackets, wires, and adhesive could potentially combat the microorganism in the dental biofilm decreasing the incidence of dental enamel demineralization during the orthodontic treatments[50] Earlier studies have confirmed silver nanoparticles can release more silver ions, which promotes their antimicrobial effect, while the histological effect of silver nanoparticles generally focuses on inhibition of microbial metabolism, leading to impaired production of extracellular polysaccharides and specific bacterial processes leading to its general dysfunction.[76]

Zinc nanoparticles exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties. Low toxicity and good biocompatibility make it suitable for biomedical usage. Nano Zinc can decrease biofilm formation by inhibition of the active transport and metabolism of sugars as well as disruption of enzyme systems by

displacement of magnesium ions essential for enzymatic activity of the of dental biofilms [77.] It has been observed, that as the concentration of ZnO increases, and the antimicrobial activity also increases, followed by shear bond strength reduction. It is important to underline, that ZnO and CuO coated brackets have been observed with better antimicrobial characteristics on Streptococcus mutans[78]

Orthodontic materials must have specific characteristics such as biological safety, functionality, and acceptable tissue response. They have to pass specific biocompatibility tests to meet regulatory standards. Any material used in the oral cavity might encourage unnecessary disturbance due to its complex and varied environment. Biocompatibility of different types of orthodontic adhesives and their ingredients have been investigated. Most of the studies demonstrated cytotoxic effects of different intensity due to the release of unbound molecules from the structure of cured composites.[21,79,80,81,82,83,84]

Cytotoxicity of different nano-particles has been demonstrated in several studies in dose-dependent and time-dependent manners.[85,23,87] Generally, nano-particles are assumed to cause greater toxicity than fine-size particles due to their greater surface area-to-volume ratio.[27,28] Cytotoxicity of nanoparticles is also determined by other physico chemical factors including size, concentration, chemical composition, and crystalline structure.,[87,89,27] Recently, Lanone et al[26] compared the toxicity of 24 nano-particles in the same experimental set-up on two pulmonary cell lines (A549 and THP-1) and found that copper- and zinc-based nanoparticles were highly toxic, whereas low to moderate degree of toxicity was observed in Titania, Alumina, Ceria, Silver, Nickel and Zirconia-based nanomaterials and no toxicity was observed for Tungsten Carbide.[26]

Hence to curtail the cytotoxicity we adapted a novel way to synthesise nanoparticles from plant extract. The green synthesis of nanoparticles not only renders them biocompatible but also offer outstanding antimicrobial property without affecting the shear strength of the parent material. The aim of our study was to evaluate the cytotoxicity, antimicrobial and shear bond strength of orthodontic adhesive incorporated Titanium -dioxide, Silver and Zinc nanoparticles. The null hypotheses to be tested were that orthodontic composites containing Titanium-Dioxide, Silver and Zinc nanoparticles have (1) No statistically significant difference in Shear bond strength and (2) No statistically significant difference in antibacterial properties compared with conventional composites

In our study Neem flower (Azadirachta indica) extract was used for synthesis of Titanium, Silver and Zinc nanoparticles. The bio-molecules present in the Neem flower extract acts as reducing, capping and stabilizing agent and convert bulk molecules into its nanosized particles. Studies by Shankar et al., (2004) Tripathy et al (2009) found that major chemical constituents in the extract were nimbin and quercetin which has potential antibacterial and antifungal property.Few other authors who indulged in green synthesis were Divya et al., (2013) who synthesized ZnO NPs using leaf extract of Hibiscus rosa-sinensis, Parthiban Sundaramurthy (2015) synthesised ZnO NPs using Pyrus pyrifolia leaf extract, Sajesh Kumar N.K. et al synthesised silver nano particles from neem leaf (azadirachta indica) extract, and B.K.Thakur synthesised titanium dioxide nanoparticles using Azadirachta indica leaf extract. They all felt dispersed aqueous solution of green synthesized nanoparticles showed better stability due to the presence of organic molecules. Hydrophobic nature of organic molecules prevents agglomeration of nanoparticles and causes its effective

dispersion and stabilization in aqueous solution.[91] However, none of the studies incorporated green synthesised nanoparticle into orthodontic adhesive.

Biological methods are preferred due to its eco-friendly, clean, safe, cost-effective, easy, and effective sources for high productivity and purity. High pressure or temperature is not required for the green synthesis of nanoparticles and the use of toxic and hazardous substances and the addition of external reducing, stabilizing, or capping agents are avoided. The chemical synthesis and stabilization of nanoparticles cause release of toxic by-product which is harmful to the ecosystem. Hence, we resorted to use of plant extract for synthesis of nanoparticles in our study[90]

In the present study extracted nanoparticle were incorporated into the orthodontic adhesive at a concentration of 1% of Titanium di oxide, the rationale behind using this concentration was in concordance with a study done by Poosti et al[20] who concluded TiO_2 nanoparticles of size 21 ± 5 nm can be blended to light cure orthodontic composite paste in 1, 2, and 3%, and all these concentrations have similar antibacterial effects.[20]The reason attributed for use of 1% (w/w) in the present study was lesser concentration may limit the cytotoxic effect of the material used.

Silver nanoparticle concentration used in our study was 0.3 % (w/w). the rationale being previous study by Abbas et al, used 1%, 5% and 10% silver nanoparticles a dose dependent increase in antibacterial property was observed but increased concentration led to more cytotoxicity and reduced shear bond strength. Their small particle size and large surface area could enable them to release more Ag ions at a low filler level, thereby reducing Ag particle concentration necessary for efficacy and curtail their cytotoxicity.[91]

While for Zinc nanoparticles the concentration chosen in the present study was 1% because a study by Spencer et al,[92] inferred that as the concentration of ZnO increases, SBS decreases. Mean bond strengths for the 13% and 23.1% ZnO mixtures were observed to be 5.04 MPa and 4.56 MPa, respectively in his study.[92] The selected concentration of nanoparticles was incorporated into the orthodontic adhesive using Vortex and IKA ® T25 digital ULTRA-TURRAX ® machine at 3400 rpm for 2 min in a dark room (Rotor stator mechanism) [61]

The characterization of Nano adhesive helps us to know the size, shape, functional groups and chemical constituents of the nanoparticles .In the present study the Nano-incorporated adhesives were characterised using Fourier Transform infrared spectroscopy (FTIR), Scanning electron microscopy and Energy Dispersive X ray analysis (SEM-EDX) [93]

FTIR measures the infrared intensity vs. wavelength of light, it is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles. The calculated spectra clearly reflect the well-known dependence of nanoparticle optical properties. In the present study functional groups of phytochemicals (neem flower extract) that induce the nanoparticle synthesis were alkynes and alkanes that are widely seen in secondary metabolites such as carboxylic acids, alkaloids, etc. The green synthesized Titanium Dioxide, silver and Zinc nanoparticle by employing neem flower extract was analysed using FTIR showed characteristic spikes as depicted in Graph 1, 2 and 3. similar reports were obtained by studies done by Sorna et al, Surya et al [94-96]

Furthermore, characterization of the prepared nano adhesive was done using Scanning electron microscope. It is employed to determine the size; shape &

morphologies of formed nanoparticle and it gives high resolution images of the surface of a sample. SEM is capable of magnifying images up to 200.000 times and measures the particle size of conductive and sputter quoted samples[38]

In the present study SEM analysis was carried out to find the surface morphology of Titanium Dioxide Silver and zinc oxide prepared from Neem flower extract concentration using SEM microscope (JSM-6510 LV AT 20KV, Tokyo Japan). The micrographs (Figures 11, 12 and 13) showed that the network formation occurred at Titanium Dioxide, Silver and Zinc oxide nanoparticles. It was clearly indicated that the agglomeration has taken place. From the images it was confirmed that the synthesized Titanium Dioxide, Silver and Zinc oxide nanoparticles were in well agreement with the result obtained from FTIR.

The biosynthesized silver zinc and titanium dioxide nanoparticles were more or less spherical in shape and their crystal size 50 nm, confirming a non-toxic synthesis of nanoparticles through SEM analysis. Chemical analysis was achieved by means of energy dispersive x-ray spectroscopy (EDS; Oxford Abingdon, UK), with a resolution of 137 eV. The chemical mapping allowed seeing the distribution of the different elements that made up of Composite (Graph-4) (Trans bond XT) as well as the Titanium (Graph-4) silver (Graph 5) and zinc (Graph-6) which adhered to the surface confirming the incorporation of nanoparticles in the composite material (Transbond XT) Similar characterisation methodology was followed by Irania et al for characterisation of silver nanoparticles [97]

Once the characterisation of nano-adhesive confirmed the presence of specific nanoparticles. The prepared nano adhesive was subjected to cytotoxicity testing.

Composite discs of 60 nos were prepared (6mm diameter and 3mm thickness) which were used for the purpose cytotoxicity and antimicrobial testing.

Cytotoxicity testing was done using preliminary toxicity screening the brine shrimp lethality assay. Brine shrimp lethality bioassay is a simple, cytotoxicity testing of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (Artemia salina). The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts, etc.[98]

Findings of the present study revealed the highest percentage of viable brine shrimp larvae was observed after exposure to the silver nanoparticle 90%survival which was in par with the control 90%survival which was followed by zinc which exhibited 80% survival followed by Titanium that had 70% surviving nauplii as depicted in Graph-8. The present study highlights Titanium, silver, and zinc nanoparticle incorporated orthodontic adhesive were biocompatible. In concordance with research done by by Zhang, et al, [99](2013), who inferred incorporation of Silver nanoparticles to an adhesive did not render it cytotoxic to human gingival fibroblast.

Farzin Heravi et al,[59] did a Cytotoxicity Assessment of an Orthodontic Composite Containing Titanium-dioxide Nano-particles using human gingival fibroblast which revealed incorporation of 1 wt% TiO2 nano-particles to the composite structure does not result in additional health hazards compared to that occurring with the pure adhesive[41] Thus Biogenic nanoparticles such as Titanium dioxide, Silver nanoparticles, and Zinc Oxide nanoparticles are biocompatible , provide enhanced uptake, easy to produce and are eco-friendly.

Insertion of the fixed orthodontic appliance into the oral cavity creates new stagnation areas that in the presence of carbohydrate and the reduced access by saliva encourages the colonization of S. mutans and L.bacilli. [100-104] It has been found that plaque deposition is greater on resin bonded material than on enamel.[103] Even plaque deposition is greater on gingival side of bracket predominantly colonised by S.aureus Teeth ligated with elastomeric rings exhibited a greater number of cariogenic microorganisms than the teeth ligated with stainless steel ligature wires.[108]

In the present study the anti-bacterial activity of the prepared nano adhesive was assessed using disc diffusion method and antifungal activity was done using Agar well diffusion assay.[20]

The results of present study revealed that for **Staphylococcus aureus,** zinc nanoparticles (Group-4) exhibited a wide zone of inhibition followed by Silver nanoparticles (Group-3) and thereafter by titanium Dioxide (Group-2) whereas the control (Group-1) had no zone of inhibition as shown in Table-VIIa and graph-9 the intergroup comparison revealed statically significant difference between groups as depicted in Table-VIIb. The reason as to why Zinc had maximum zone of inhibition could be attributed to the fact that Zinc nanoparticles can decrease biofilm formation by inhibition of the active transport and metabolism of sugars as well as disruption of enzyme systems by displacement of magnesium ions essential for enzymatic activity of the of dental biofilms.[77]

Thereafter the antibacterial activity of the prepared nano adhesive was tested against **Streptococcus mutans**, strain the zone of inhibition was maximum for Silver Nanoparticles (Group-3) followed titanium dioxide (Group-2) and zinc nanoparticles (Group-4).control (Group-1) did not exhibit any zone of inhibition. As shown in table

VIIIa graph-10. The intergroup comparison of groups revealed statistically significant difference between groups as depicted in table VIIIb. The reason for better antibacterial activity of silver nanoparticles could be because of their catalytic action were it converts the oxygen to active oxygen by light energy in the air or water. The active oxygen produced leads to structural damage in microbes, denaturation of proteins and enzymes of bacteria [16]

Enterococcus.faecalis, was the tested for antibacterial activity of nano adhesives among the groups Silver nanoparticles (Group-3) exhibited maximum zone of inhibition followed by Titanium Dioxide (Group-2),Zinc nanoparticles (Group-4) and control(Group-1) did not exhibit any antibacterial activity as depicted in table IXa and graph-11.The intergroup comparison revealed statistical difference between groups wherein zinc and control were in par. Silver nanoparticles generally focuses on inhibition of microbial metabolism, leading to impaired production of extracellular polysaccharides and specific bacterial processes leading to its general dysfunction.[12,13]

Furthermore, antibacterial activity of Nano adhesive against **Lacto bacillus**, strain was tested among the groups Zinc Nanoparticles (Group-4) exhibited maximum zone of inhibition at the end of 24hrs.followed by Silver nanoparticles (Group-3) followed by Titanium Dioxide nanoparticles (Group-2) and control had nil zone of inhibition as depicted in table Xa and graph-12 The intergroup comparison revealed statistically significant difference between groups as shown in table Xb.

The cumulative antibacterial effect of the Nano adhesives is represented in table XI and graph 13 which denoted maximum antibacterial activity by Silver Nano adhesive (Group-3) followed zinc Nano adhesive (Group-4) and thereafter by

Titanium dioxide Nano adhesive (Group-2) and control exhibited nil antibacterial activity.

Poosti et al,[20] in his study revealed adding TiO2 nanoparticle to composite could have long-term antibacterial effect[11] Similar results were obtained by Elsaka et al,[109] (2011) after incorporating They showed that GI-containing 3% (w/w) TiO2 nanoparticles is a promising restorative material with improved mechanical and antibacterial properties. These nanoparticles are comparatively economical with exemplary mechanical properties and adorable colour.[108]

Ahn SJ et al [16] noted that orthodontic composite adhesives comprising of Nano silver fillers particles prevent enamel demineralization around bracket surfaces without arbitrating their physical properties. Reddy et al found 1% nanosilver content revealed the highest antibacterial effect.[11] Sodagar et al,[51]observed that 5% Ag/HA nanoparticles limit the growth of cariogenic bacteria, and increasing the concentration of Ag/HA nanoparticles did not reveal any compelling curtailment. Even though the exact antimicrobial mechanism of silver nanoparticles has not been established, it has been proposed that the catalytic action of silver converts the oxygen to active oxygen by light energy in the air or water, since silver ions can aid electron deracination from a molecule.The active oxygen thus produced leads to structural damage in microbes[109,110].

Earlier Studies done by various authors proved ZnO nanoparticles possess a broad range of antibacterial spectrum and can kill organisms, which cause caries [113,114, 61] Jones et al, [112] found these nanoparticles have scrupulous toxicity to bacteria, minimally affecting the human cells. It has been proposed by Bates et al,[114] that the antimicrobial action is due to blocking the action of zinc on the electron-transport

chain or by limiting the generation of ATP. Zinc is also known to hinder transport activity by selectively blocking the membrane locations leading to conformational alterations in proteins and enzymes ZnO nanoparticles are also known to act by generating reactive active oxygen species such as H2O2, which are known to hinder the growth of planktonic microbes [114]

In the present study nanoparticles incorporated in orthodontic adhesive were studied for antifungal activity against **Candida albicans** among the groups zinc nanoparticle (Group-4) exhibited showed a greater zone of inhibition followed by silver nanoparticles (Group-3), Titanium Dioxide (Group-2) control did not exhibit any antifungal property as depicted in table XIIa. And Graph-14.The intergroup group comparison showed statistically significant difference between groups the same has been depicted in table XIIb.

The antifungal effect of silver Nanoparticles has received only marginal attention and just a few studies on this topic have been published by Roe et al, [115] and Zeng et al, [116] The only study dealing more specifically with their activity against clinical isolates and ATCC strains of Trichophyton mentagrophytes and Candida spp. was published as late as in 2008 by Kim J et al [118.]

Lili He et.al [119] (2010) study on antifungal activity of zinc nanoparticles reveal that the growth of A.fumigatus and Candida albicans were inhibited at concentrations of 3to12mmll-1ZnONPs which show that ZnONPs show a great enhancement in the antifungal activity due to their unique properties. Exposure to nanoparticles not only altered the growth rate but also affected the onset and length of *Candida albicans* growth phases. The log and the onset of the death phase were shortened and accelerated, respectively. Up to 65% of the Candida albicans were killed after

exposure to 100 μg/mL of titanium dioxide nanoparticles while only 33% were killed with rutile. A higher dosage and incubation time of the nanoparticles increased their toxicity[117,119]

Furthermore, the characterised nano adhesive was subjected to shear bond testing for which sixty maxillary premolars were randomly allocated into four groups- Group -1(control) Group-2(Tiatanium Dioxide) Group-3(Silver) and Group-4 (zinc) The samples were tested using universal testing machine for shear bond strength. The maximum force necessary to debond or initiate bracket fracture was recorded in newtons and then converted into megapascals (Mpa).

In the literature, there are no clear guidelines about shear force limits, but in fact, a good orthodontic biomaterial should allow good adhesion to sustain masticatory forces (with a minimum bond strength of 5–10 MPa). On the other hand, adhesion forces should not be too strong to avoid enamel loss after debonding (40–50 MPa)[57].

In the present study the maximum force required to debond was recorded with control (Transbond XT) (Group-1) requiring maximum force 73.740±21.373N followed by Titanium dioxide Nano adhesive (Group-2) 74.613±25.692N, Zinc Nano adhesive (Group-4) 68.347±17.467N and least force required to debond was for Silver Nano adhesive (Group-3) 63.65±15.742N as depicted in table. XIVa and graph 15 and multiple group comparison revealed no statistical significant difference between groups depicted in XIVb

In the present study Adding AgNPs significantly ($p = 0.009$) reduced the mean (SD) Shear Bond Strength of the nano-adhesive group 7.011± 1.733 MPa compared to Transbond XT 8.121± 2.354 Mpa. Whereas titanium exhibited the bond strength of 8.217 +.2.89Mpa and zinc exhibited 7.535± 1.923Mpa the bond strength which is

depicted in tableXVa graph 16 and the intergroup comparison did not show statistically significant difference as shown in table XVb Hence it can be concluded that bond strength did reduce after addition of nanoparticles in the present study whereas study Ladan Eslamian[55]. Adding AgNPs significantly ($p = 0.009$) reduced the mean SBS so was the iinference of study done by Reddy et al[61] whose concluded ZnO and Tio_2 had redcuced bond strength of 6.50 Mpa and 6.33 Mpa. Respectively compared to the control.[34] The bond strength value of the nano adhesive used in the present study was within the clinically acceptable mean shear bond strength but with a wide range suggesting further clinical investigation. This is in line with recent systematic review finding suggesting mild reduction in SBS of orthodontic adhesive after addition of nanoparticles[57]

Hence Nano adhesive synthesised from plant extract renders it with better antibacterial property without altering the Shear strength of the parent material and proved to be biocompatible.

Few limitations of our study include all the parameters checked were done under in-vitro settings, clinical conditions may differ significantly hence in future, well designed in vivo studies are needed. There is no standard protocol for incorporation of NPs into the orthodontic adhesives, such as optimal w/w concentration of NPs or addition to adhesive or primer; almost all studies are in-vitro studies There is a need for long-term clinical trials to investigate the clinical performance and risk/benefits of incorporating NPs for improving anti-caries properties of orthodontic adhesives. These should identify the right concentration/particle size of NPs and form of preparation (addition to primer vs. adhesive)

The present study is an In-Vitro Nano-laboratory based study, evaluating the cytotoxicity, antimicrobial activity and shear bond strength of Titanium dioxide, silver, zinc Nanoparticles incorporated in Orthodontic adhesive and comparing it with positive controls. The study targeted towards identifying the benefits of Nano particle produced from plant extract and if green synthesis aided in accentuating the antimicrobial property of the Nano adhesive without hindering the shear strength of the parent material.Convenient sampling method was used for the selection of the samples. The following inference was derived.

Cytotoxicity analysis

1. Silver nanoparticle had the highest survival of nauplii (90%) which was in par with control followed by zinc (80%) and titanium (70%). Although all the Nano adhesives proved to be bio-compatible.

Antimicrobial activity

1. For **Staphylococcus aureus,** microorganism, Zinc Nano adhesive (22.00±0.845) mm had the highest inhibition zone followed by Silver (13.00±0.845) mm and then titanium (8±0.845) mm as compared to the control (0.00±0.00).
2. For **Streptococcus mutans,** microorganisms, Silver Nano adhesive (11.00±0.845) mm had highest zone of inhibition followed by Titanium dioxide (8.00±0.845) mm and Zinc (7.00±0.845) mm as compared to the control (0.00±0.00).
3. For **Enterococcus Faecali,** Silver Nano adhesive had the highest zone of inhibition (12.00±0.845) mm, followed by Titanium dioxide (8.47±0.845) as compared to control (0.00±0.00). Zinc Nano adhesive showed no inhibition zone as well.
4. For the **Lacto bacillus,** the highest zone of inhibition was seen in Zinc Nano adhesive (12.00±0.845) mm, followed by silver (9.00±0.845) mm and Titanium dioxide (8.87±0.352) as compared to the control (0.00±0.00).

5. For the **Candida albicans,** group, highest inhibition zone was seen in Zinc Nano adhesive (18.00±0.845)mm followed by silver (15.00±0.845)mm and then Titanium dioxide (13.93±0.884) Nano adhesive
6. Cumulatively, antibacterial property of Silver Nano adhesive was superior to Titanium dioxide and zinc other Nano adhesive groups and as far as antifungal activity is concerned Zinc Nano adhesive stood first.
7. Hence among the Nano adhesive groups Zinc had superior antimicrobial property followed by Silver and Titanium dioxide.

Maximum Force Distribution

1. The maximum force to debond for the control was 73.740±21.373N, for titanium dioxide it was 74.613±25.693N, for silver Nano adhesive it was 63.658±15.742N and for the zinc Nano particles it was 68.414±17.467N. Silver Nano adhesive had presented with the least force.

Maximum Load Distribution (Shear bond strength)

1. The maximum Load for the control was 8.121±2.354Mpa, for Titanium dioxide it was 8.217±2.829Mpa, for Silver Nano particles it was 7.011±1.733Mpa and for the zinc Nano adhesive it was 7.535±1.923Mpa.
2. Silver Nano adhesive had presented with the least bond strength followed by Zinc and Titanium dioxide. However they are within the limit of ideal bond strength required for orthodontics.

Therefore the Null hypothesis is rejected since addition of green synthesized nanoparticles improved the antimicrobial property of the orthodontic adhesive and did not affect the Shear bond strength of the parent material used.

We conclude the study by stating nanoparticles produced by green synthesis render them biocompatible and safe to use in orthodontic adhesives. It stands superior to chemical synthesis by reducing the toxic byproducts, by being more ecofriendly and easy to use. The green synthesized nanoparticles offered exemplary antimicrobial property without compromising the bond strength of the parent material.

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