Evaluation of Antibacterial Properties, Cytotoxic Effects And Wettability of Ceramic Brackets Coated With a Combination of Zinc-Oxide-Tin Oxide Nanoparticles by Two Different Techniques: an In-Vitro Study

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**Abstract:** Orthodontic advancements aim for patient comfort and aesthetics, with ceramic brackets favoured for their cosmetic appeal despite increased plaque buildup. Zinc oxide (ZnO) coatings on brackets have shown a strong antibacterial effect against pathogens like Streptococcus mutans, with ZnO nanoparticles (Nps) exhibiting biocompatibility, making them promising for orthodontic use. Additionally, studies on SnO2 Nps demonstrate their inhibition of bacterial biofilm formation and non-toxic nature, underscoring their potential benefits in orthodontic applications. The objective of this study was to assess the antimicrobial efficacy, cytotoxicity, and wettability of ZnO-SnO2 Nps-coated ceramic brackets. In this in vitro investigation, three equal groups of fifteen polycrystalline ceramic brackets were used. Using the uncoated brackets (group A), dip coating method (group B), and sol-gel method (group C), ZnO-SnO2 Nps were coated on the brackets. The coated brackets were subjected to scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDAX), and Fourier transform infrared spectroscopy (FT-IR). The antibacterial activity was assessed by evaluating the colony-forming units and cell viability by MTT assay. Descriptive statistics, Tukey's posthoc test and One-way ANOVA test were performed. The sol-gel coating on ceramic brackets resulted in an irregular agglomerate structure while dip-coating produced a flaky appearance. Elemental analysis confirmed successful coating, with signal peaks suggesting ZnO-SnO2 presence. Dip-coated brackets showed the highest cell viability and antibacterial activity (94 ± 2 and 23 ± 2) compared to sol-gel-coated (85.2 ± 3.05 and 29 ± 1.41) and uncoated brackets (60.8 ± 5.30 and 40.2 ± 2.31) (p=0.00). Dip-coated brackets exhibited the lowest contact angle (27 ± 2°), enhancing wettability and cell attachment followed by sol-gel-coated brackets (38 ± 2°) and uncoated brackets (49±2°). The efficient application of Zn-SnO2 Nps onto ceramic brackets was achieved via sol-gel and dip coating methods, confirmed through characterisation. Dip-coated brackets demonstrated superior antibacterial properties and cell viability compared to sol-gel-coated ones, with uncoated brackets exhibiting the lowest values.

**Keywords:** Cell viability, antibacterial activity, zinc oxide- tin oxide, nanoparticles

# Introduction

Orthodontic treatments have evolved significantly over time, aiming to optimise patient comfort, aesthetic appeal, and oral health maintenance[(Burden, 2007)](https://paperpile.com/c/SzPSei/ytudi) Ceramic brackets are frequently chosen for their cosmetic appeal, offering an alternative to traditional metal brackets [(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/SzPSei/iI0GO) [(K.-H. Park et al., 2010)](https://paperpile.com/c/SzPSei/2baz); However, they come with the drawback of elevated surface roughness, resulting in greater plaque buildup[(Deepika et al., 2022)](https://paperpile.com/c/SzPSei/QWUs). This heightened accumulation of plaque poses a risk of periodontal conditions and dental caries One such advancement lies in developing novel materials and surface modifications, including incorporating nanoparticles (Nps) onto orthodontic bracket surfaces.[(Zakrzewski et al., 2021)](https://paperpile.com/c/SzPSei/xYlFR) These Nps have a large surface area relative to volume, which raises the surface energy, and they can vary their physical and chemical characteristics, which increases the variability of reactivity [(Harsha & Subramanian, 2022)](https://paperpile.com/c/SzPSei/QTJyT) [(Solanki et al., 2022)](https://paperpile.com/c/SzPSei/osiZp).

The most popular techniques for synthesising Nps are sol-gel, gas condensation, chemical vapour deposition and condensation, vacuum deposition and evaporation, mechanical attrition, chemical precipitation, and electrodeposition [(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/SzPSei/cybI)[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/SzPSei/73aZv)[(Ajay, Suma, et al., 2022)](https://paperpile.com/c/SzPSei/T7Zaz)

[(Joudeh et al., 2022)](https://paperpile.com/c/SzPSei/S1VR). Nps coatings on orthodontic brackets have shown promising prospects in enhancing antimicrobial capabilities and biocompatibility. [(Wang et al., 2023)](https://paperpile.com/c/SzPSei/hZUWW) These include silver, copper oxide, zinc oxide, titanium dioxide, and chitosan-based Nps which have significantly enhanced the antibacterial effects against pathogens such as Streptococcus mutans and Lactobacillus acidophilus [(Tiwari & Jain, 2023)](https://paperpile.com/c/SzPSei/HjIfu). In vivo studies have shown a great decrease in the quantity of plaque accumulation hence can potentially reduce the complications associated with prolonged orthodontic therapy. [(P et al., 2022; Siva et al., 2022; Syed et al., 2015; Zeidan et al., 2022)](https://paperpile.com/c/SzPSei/KAD2N+e8jf6+SLXbq+h97Du). These Nps have also been shown to have biocompatibility comparable to standard brackets.[(Metin-Gürsoy et al., 2016; P et al., 2022)](https://paperpile.com/c/SzPSei/KAD2N+vZFrY)

Zinc oxide (ZnO) coated brackets have been extensively studied for their potential antibacterial properties and cytotoxicity [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/SzPSei/hgUet) . ZnO has been shown to have a strong antibacterial effect against a range of bacteria, including Streptococcus mutans and Lactobacillus acidophilus, which are commonly associated with enamel demineralisation [(Zeidan et al., 2022)](https://paperpile.com/c/SzPSei/SLXbq). The antibacterial mechanism of ZnO is attributed to the release of zinc ions, which can disrupt bacterial cell membranes and inhibit bacterial growth [(Jain et al., 2020)](https://paperpile.com/c/SzPSei/VKu6). ZnO Nps have been reported to exhibit biocompatibility and minimum cytotoxicity, making them suitable for potential application in orthodontic materials [(Yun et al., 2022)](https://paperpile.com/c/SzPSei/bhdy). Park et al evaluated the antibacterial effect and cytotoxicity of SnO2 Nps against polymicrobial biofilms of E. *coli* and S. *aureus*. It was noted that the SnO2 Nps significantly inhibited the biofilm formation and the cytotoxicity assays confirmed the non-toxic nature of the Nps [(I. Park et al., 2023; “Recent Advances in Synthesis, Modification, and Potential Application of Tin Oxide Nanoparticles,” 2022)](https://paperpile.com/c/SzPSei/IuFE+VOAN). The objective of the current investigation was to assess the antibacterial, cytotoxic and wettability of ceramic brackets covered with a ZnO-SnO2 Nps mixture[(Graf et al., 2023)](https://paperpile.com/c/SzPSei/L7Z0C).

# Materials and methods

## Brackets

A total of fifteen maxillary polycrystalline ceramic brackets with 0.022-inch slots (Ormco Symetri, California, USA) were utilized. Zinc oxide-tin oxide nanoparticles were synthesized and applied to the brackets using the sol-gel and dip coating techniques. The brackets were categorized into three groups: group A (uncoated), group B (dip coated), group C (sol-gel coated), each containing five brackets.

## Synthesis of ZnO- SnO2 nanoparticles

Tin chloride pentahydrate (SnCl2.5H2O) at a concentration of 0.4 M was dissolved in 40 mL of distilled water. Once a uniform solution was achieved, the ZnO precursor zinc sulfate heptahydrate (ZnSO4.5H2O) was added in specific ratios of 0.25 M and 1 M. Once the homogeneous mixture was obtained, an 8 M aq. solution of sodium hydroxide (NaOH) was gradually added at a uniform rate. After stirring the mixture for 20 minutes it was placed in a microwave oven and exposed to 320 W of microwave irradiation for 10 minutes. The resulting product underwent centrifugation and was rinsed alternately with deionized water and ethanol to remove impurities. Finally, the white ZnO-SnO2 powder was obtained after drying and annealing at 500°C for 2 hours.

## Nanoparticle coating

### Dip coating

To coat ZnO-SnO2 Nps to orthodontic brackets, five brackets were initially placed in an ultrasonic bath with an ethanol solution for 40 minutes at 25°C. Then, 0.1 g of the prepared ZnO-SnO2 Nps (at concentrations of 0.25 M and 1 M) was added to an experimental tube containing the ethanol solution. This mixture was then transferred to a water bath set at 80°C. The nanoparticles were evenly dispersed in the ethanol solution, and the brackets were individually immersed. Various time intervals (10, 15, 20, 30, 40, 50, and 60 minutes) were tested for coating the orthodontic brackets with ZnO-SnO2 nanoparticles. Visual inspection revealed that a 20-minute interval was optimal for achieving the best nanoparticle coating on the orthodontic brackets.

### Sol-gel coating

The coating sol-gel solution was prepared by mixing 0.2 gms ZnO and 0.05 gms SnO2 with 20 ml of distilled water in a beaker. A uniform dispersion of the Nps was achived by thoroughly mixing the solution. For 24 hours the CB were immersed in this solution while being agitated with a magnetic stirrer to ensure strong adhesion of the coating. Subsequently, the solution was heated in an electric oven for 5 hours at 80°C.

## Characterisation of the ZnO- SnO2 nanoparticle-coated ceramic brackets

### Scanning electron microscopy analysis

A high-resolution scanning electron microscopy (FE-SEM, JEOL, JSM IT-800), was used to determine the surface morphology of the ZnO-SnO2 coated brackets. The specimens were attached to aluminium stubs using carbon tape and then underwent a gold coating process through sputter coating. The brackets were observed at a resolution of 10 nanometers and 100X magnification. The coated structure was clearly visible at the specified magnification and resolution.

### EDAXs and FT-IR

The brackets coated with ZnO-SnO2 nanoparticles was examined for their chemical composition using field emission scanning electron microscopy (FE-SEM), specifically the JEOL JSM IT-800 model with EDAX analysis (EDAX-OXFORD instrumentation). The EDAX spectrophotometer was used to activate the nanoparticles on the brackets and identify their elemental components. Additionally, FT-IR spectroscopy was used to examine the functional groups and bonding of the Nps. During the analysis, a portion of IR radiation within the wavelength range of approximately 4000-5000 cm-1 was aimed at the sample. The sample absorbed this radiation, transforming it into rotational or vibrational energy, while the remaining energy passed through.

### Contact angle analysis

Contact angle analysis was performed in this study using a Osilla-Goniometer.The contact angle of the orthodontic bracket's surface explains the cell adhesion, cell growth, and cell proliferation. The wettability of coated bracket surfaces generally depend on the average surface roughness, chemical effect, and surface energy. The hydrophilic surfaces improved the biocompatibility of the orthodontic brackets in a physiological solution. Wettability was assessed by contact angles under room temperature and ambient humidity conditions. Approximately 1 μL of water was deposited at four random locations on all 3 groups of samples to reduce the effects of surface irregularities and gravity on the measurements. The average water contact angle was determined by taking three measurements at each point.

### Cytotoxicity Study using MG-63 Cell Line

In Pune, India, the National Centre for Cell Science provided fibroblast-like MG-63 cell lines. These cells were cultured for seven days with CB coated with ZnO-SnO2 Nps using sol-gel and dip coating methods. A negative control group included untreated cell lines. After the seven-day culture period, the cell lines were rinsed with phosphate-buffered saline (PBS) at pH 7.4. The cells were then treated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and incubated for four hours to assess cellular metabolism. Dimethyl sulfoxide (DMSO) was subsequently introduced to dissolve the formazan crystals formed by metabolically active cells. These cells convert MTT to formazan, which has an absorbance near 570 nm. The absorbance at 570 nm was measured to quantify the metabolic activity of the cell lines.

### Antibacterial activity assessment

To evaluate the antibacterial properties, a colony counting method was used for both nanocoated and uncoated brackets. Streptococcus mutans was selected as the bacterial strain, and nutrient agar was used as the culture medium. Nutrient broth containing peptone, beef extract, yeast extract, and NaCl was prepared to support bacterial growth. A suspension containing 1.5 × 10^6 colony-forming units (CFU)/ml of Streptococcus mutans was made. Subsequently, 3 ml of the liquid medium was added to 100 μl of the bacterial suspension in sterile tubes. The tubes were filled with brackets from the experimental groups (sol-gel coated and dip-coated brackets) and the uncoated bracket control group. For the purpose of encouraging bacterial growth and observing any potential antibacterial effects of the nanocoated brackets, the tubes were incubated at 37°C for 60 minutes under visible light. After the incubation period, 10 μl of the suspension was diluted with 10 ml of sterile saline and then plated onto agar plates using 10 μl of this dilution. These plates were incubated at 37°C for 24 hours to allow bacterial colonies to form. The bacterial colonies were then counted to assess the antibacterial properties of the brackets.

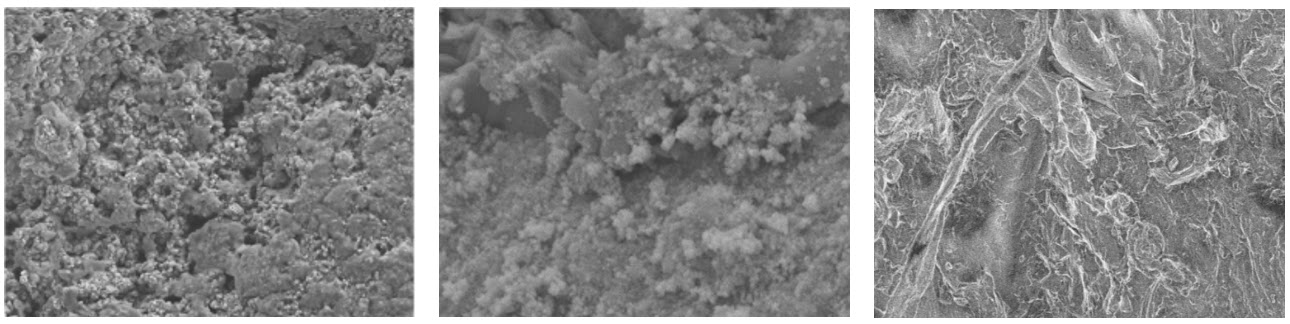
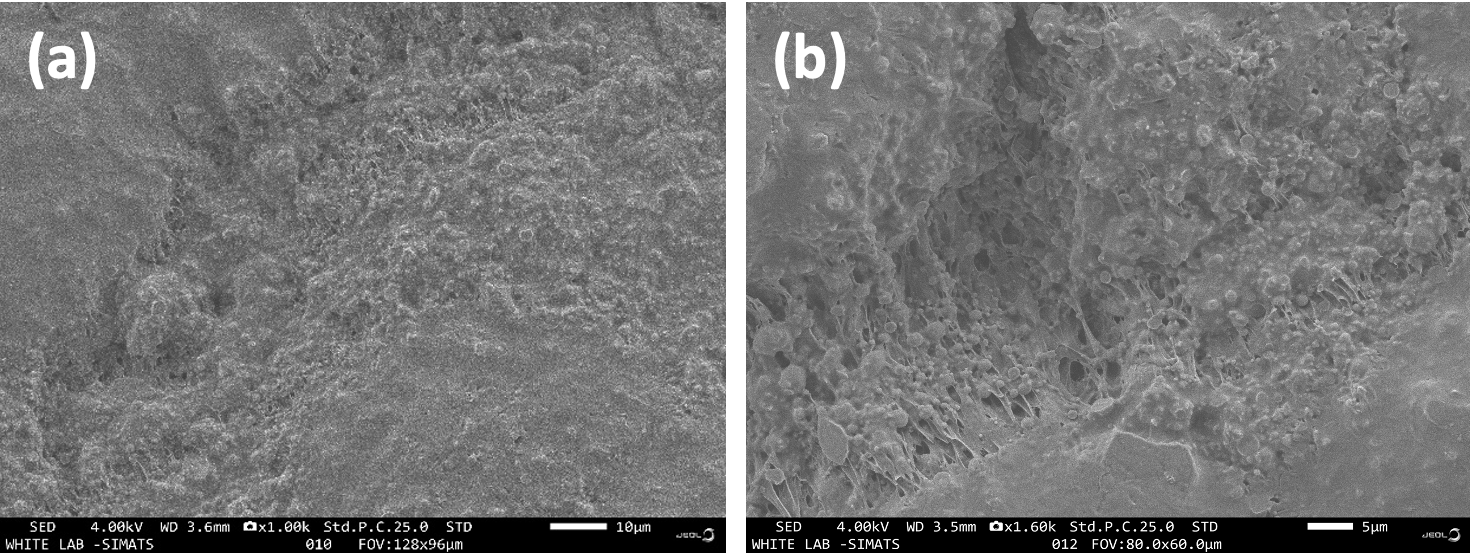
## Statistical analysis

Statistical Analysis was performed using SPSS software package version (version 243.0, IBM, USA). Normality of the data was assessed using the Shapiro Wilk numerical test. The data was found to be normally distributed. Thus, parametric tests such as ANOVA test, followed by post hoc testing using the Tukey test. The p-value <0.05 was significant.

# Results

## SEM analysis

Surface morphological analysis of ZnO-SnO2 nanocoated ceramic brackets by sol-gel and dip-coating methods are shown in Fig. 1. Sol-gel coated brackets displayed a bulkier structure with an agglomerated morphology while dip-coated brackets showed a flaky aggregated morphology.

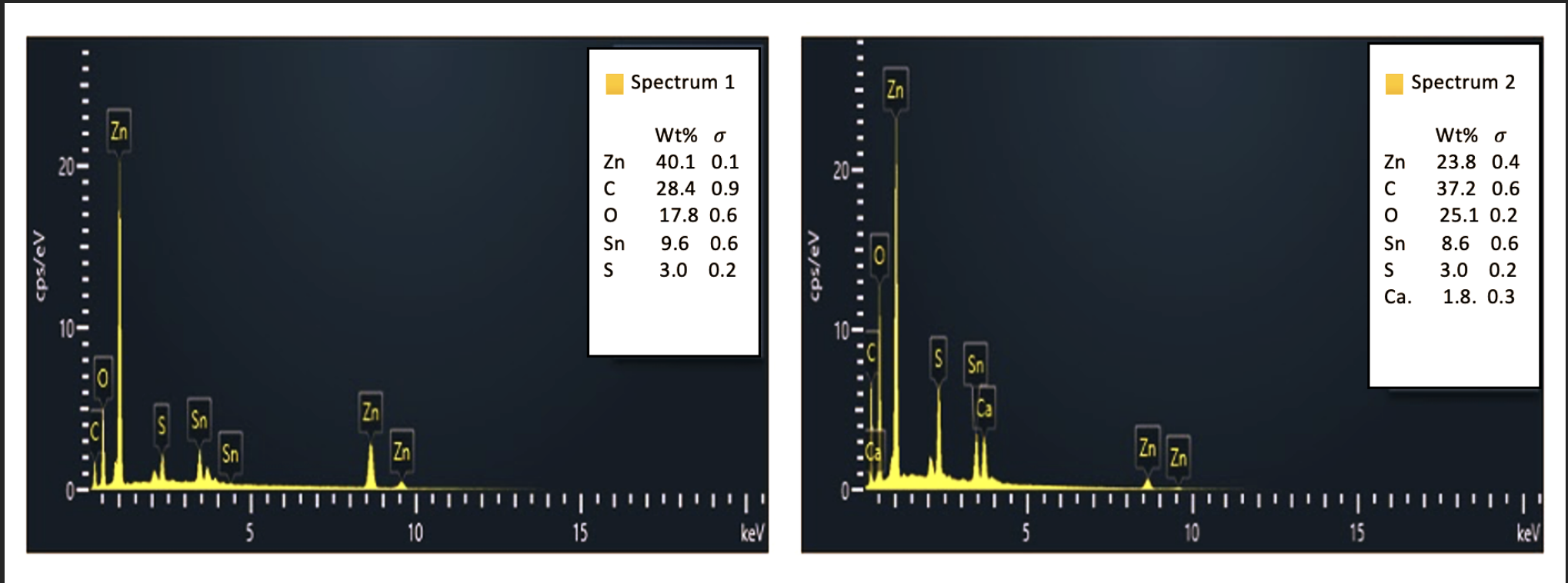


(a) (b) (c)

Figure 1: SEM images of ceramic brackets coated with ZnO-SnO2 Nps (a) by sol-gel method (b) dip coating method (c) uncoated bracket

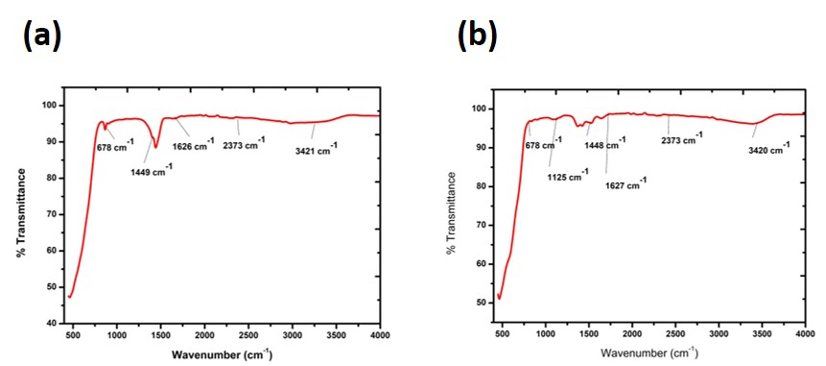
## EDAX Analysis

Elemental composition (Wt%) of ZnO-SnO2 coated brackets is recorded by energy dispersive X-ray spectroscopy (EDAX). The observed results of EDAX for ZnO-SnO2 coating are shown in Fig. 2. The EDAX spectrum clearly shows the presence of Zn, Sn, and O in the elemental distribution. There are noticeable differences in the intensities of the peaks for Zn, Sn, and O. The amount of (wt%) percentage of the elements is shown in the Table (inset). Based on the elements identified in the spectrum, the successful coating of ZnO-SnO2 on the orthodontic bracket is confirmed. The observed results concluded that there are no other impurities were formed during the deposition process.

Figure 2: Graph showing the EDAX spectroscopy illustrating the chemical composition of ZnO-SnO2 Nps coated by sol gel and dip coated ceramic brackets

## FTIR Spectra

At 1451 cm-1, Fig. 3 shows a distinct peak characteristic of tetragonal SnO2 nanoparticles. In every sample, vibrational bands between 3420 and 1628 cm-1 were seen as a result of the -OH molecules that were absorbed from the surroundings and deposit into the orthodontic bracket surface. The bands at 2373 and 1400 cm-1 indicate the absorption of CO2 and organic compounds from the atmosphere. A band around 674 cm-1 is present in the SnO2-ZnO ratio, primarily associated with the strong metal-oxygen (Sn-O or Zn-O) vibrations of the ZnO and SnO2 Nps. Additionally, a new peak at approximately 1125 cm-1 corresponds to Zn-O vibration. Furthermore, the vibration at less than 600 cm-1 is linked to Zn-O-Zn.



(a) (b)

Figure 3: (a) (b) Graph showing FT-IR spectroscopy of sol-gel and dip coated ceramic brackets

### Contact angle analysis

The contact angle images are shown in Fig. 4. The contact angle (WCA) value of uncoated bracket was found to be 49±2°, which demonstrates the hydrophilic property of the surface, whereas, the ZnO-SnO2 coated brackets by dip coated method and sol-gel method were 27 ± 2° and 38 ± 2°, respectively showed hydrophilic property which is attributed to the ZnO-SnO2 composite coating.

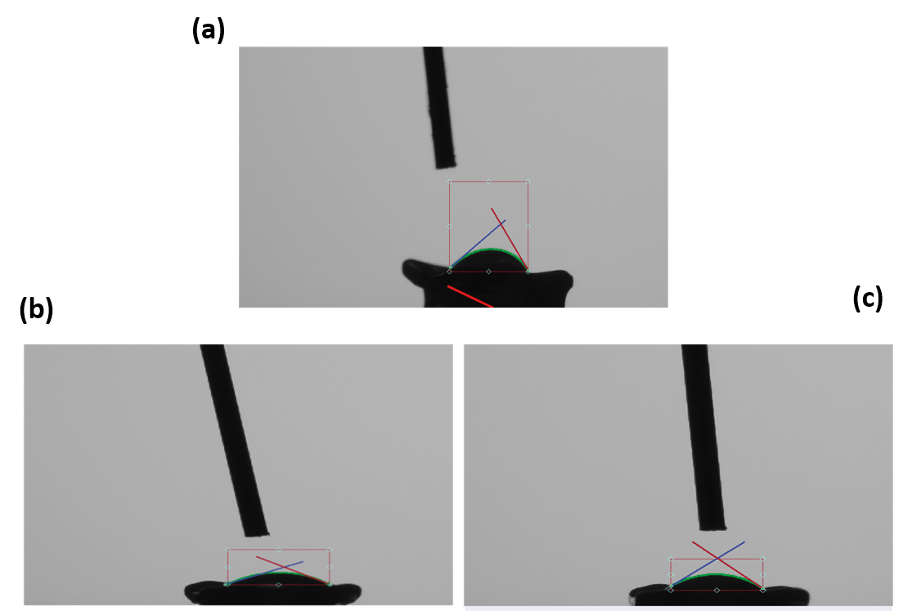
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Figure 4: contact angle analysis of (a) uncoated brackets (b) dip-coated brackets (c) sol-gel coated brackets

### Cell viability

Cytocompatibility results of the fibroblast cell line in dip-coated brackets showed 95.5% compatibility and sol-gel coated brackets exhibited cell growth of 91.7% with the concentration of 100 μg/μL in 24 h incubation. Fig.5 shows the in vitro cytotoxicity test showing live cells on the coated bracket.

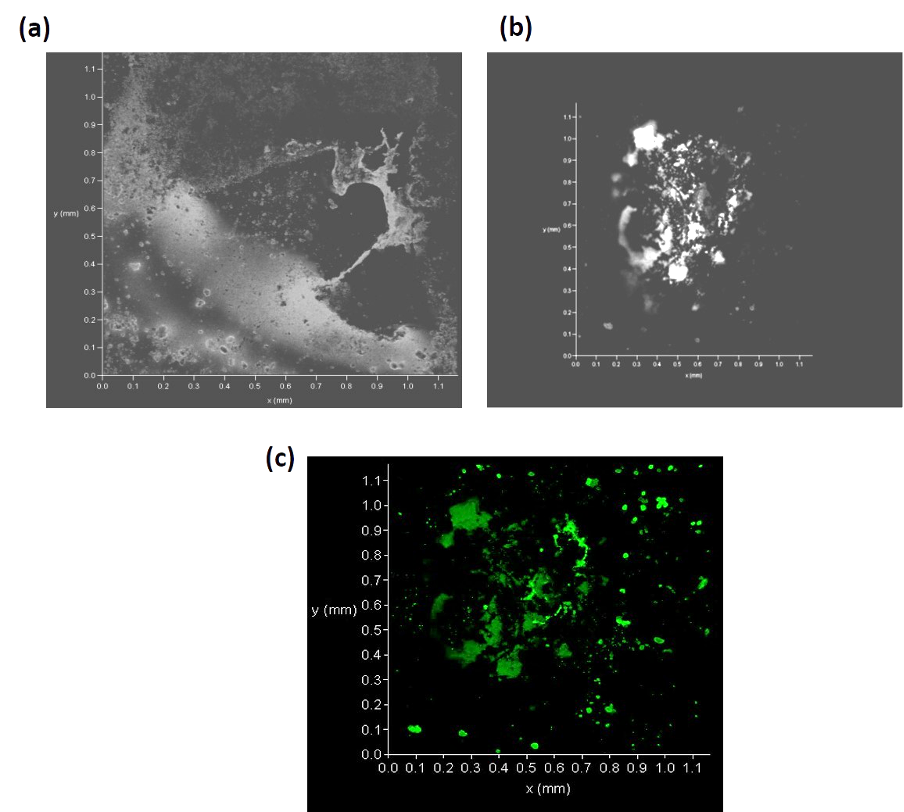


Figure 5: In vitro cytotoxicity test showing live staining on the coated bracket

### Antibacterial activity

The number of colony-forming units was higher in brackets coated by the dip coating method as compared to the sol-gel methd (Table 1). A statistically significant difference was noted between the 3 groups (p=0.00) with mean colony-forming units in groups A, B and C being 40.2 ± 2.31, 23 ± 2 and 29 ± 1.41 respectively.

Table 1: Comparison of mean colony-forming units between the groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Properties | Mean ± SD | | | p value |
|  | Group A | Group B | Group C |  |
| Colony forming units | 40.2 ± 2.31 | 23 ± 2 | 29 ± 1.41 | 0.00 |

# Discussion

This study examined the surface properties, antibacterial efficacy, cytotoxic effects and wettability of CB coated with ZnO-SnO2 Nps using the sol-gel and dip-coating techniques[(Chidambaram et al., 2022)](https://paperpile.com/c/SzPSei/FwUuz)..Sol-gel coated brackets exhibited a more substantial structure with an agglomerated morphology, whereas dip-coated brackets displayed a flaky, aggregated appearance. EDAX spectroscopy confirmed the successful coating of ZnO-SnO2 on the orthodontic bracket was based on the elements identified in the spectrum. The findings indicated the presence of signal peaks for O, Sn, and Zn suggesting the existence of ZnO-SnO2 Nps. In the SnO2-ZnO ratio, a peak at approximately 674 cm-1 was observed, primarily attributed to the bond (Sn-O or Zn-O) of the ZnO and SnO2 Nps using FT-IR. Dip-coated brackets showed the least contact angle. A low contact angle and high wettability of composite coating improved the ion exchange behaviour on the coated surface in physiological solution and enhanced the formation of cell growth and cell attachment[(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/SzPSei/Db5Pp)[(Sushanthi et al., 2021)](https://paperpile.com/c/SzPSei/Wvh4z)[(Harsha et al., 2022)](https://paperpile.com/c/SzPSei/Ol40M). Among the groups, dip-coated brackets had the highest viability, followed by sol-gel coated brackets and the least with uncoated brackets which is due to the difference in the ionic release. Moreover, it is observed that extra ionic release in sol-gel coated sample increases the toxicity levels; which is higher than the dip-coated brackets, which did not release any high-level toxic ions leachable up to 24 h, which exhibits the improved biocompatibility [(Neha et al., 2021)](https://paperpile.com/c/SzPSei/OHSb6)[(Maliael et al., 2021)](https://paperpile.com/c/SzPSei/MZiUB)[(Lakshmi, 2021)](https://paperpile.com/c/SzPSei/SPeYh). Among these two different coated orthodontic materials, dip-coated brackets delivered better compatibility with fibroblast cells. This may be due to the alkaline nature of dip-coated brackets than sol-gel-coated brackets [(Dharman et al., 2021)](https://paperpile.com/c/SzPSei/YQCnG).

An in-vitro study by Kumar et al. assessed the antibacterial effectiveness of ZnO-SnO2 Nps by measuring the zone of inhibition. Their findings concluded that after 16-18 hours, there was no discrepancy in the zone of inhibition between the control group and ZnO-SnO2 Nps. However, the current study observed increased antibacterial activity in brackets coated with ZnO-SnO2 Nps by colony-forming unit method [(Kumar & Jain, 2024)](https://paperpile.com/c/SzPSei/WbONI). Zeidan et al. in 2022 conducted an in-vitro investigation comparing the antibacterial effects of Ag, ZnO, and a combination of Ag/ZnO Nps coatings on metal orthodontic brackets [(Zeidan et al., 2022)](https://paperpile.com/c/SzPSei/SLXbq). The study evaluated the antibacterial effectiveness against Streptococcus mutans and Lactobacillus Acidophilus through colony-forming unit (CFU) counts immediately after coating. In the uncoated bracket group, the survival rate of S. mutans bacterial cells was 1403.75 ± 4.20% CFU, whereas in the ZnO-coated group, it measured 1157.50 ± 5.40% CFU, and in the Ag/ZnO-coated group, it recorded 767.50 ± 9.60% CFU [(Katyal et al., 2021)](https://paperpile.com/c/SzPSei/2ymI)[(Jabin et al., 2021)](https://paperpile.com/c/SzPSei/wkQk).

In the present study, uncoated ceramic brackets exhibited 40.2 ± 2.31 CFU/mL, whereas dip-coated brackets showed 23 ± 2 CFU/mL and sol-gel-coated brackets showed 29 ± 1.41 CFU/mL, indicating that a combination of ZnO-SnO2 Nps coated on ceramic brackets exhibited superior antibacterial activity compared to ZnO and Ag-ZnO-coated metal brackets(Chehelgerdi et al., 2023). Duraisamy et al in 2024 conducted an in-vitro study evaluating the antibacterial effect of hybrid nanocoating of metal orthodontic brackets on streptococcus mutans [(Duraisamy et al., 2024)](https://paperpile.com/c/SzPSei/6frd). The study concluded that brackets coated with copper oxide-zinc oxide nanoparticles exhibited superior antibacterial properties compared to those coated with silver-zinc oxide and silver-copper oxide. Uncoated brackets showed the highest count of 249.03 ± 3.91 CFU/mL at 24 hours, followed by 29.95 ± 1.28 CFU/mL in the silver-zinc oxide group and 25.17 ± 3.97 CFU/mL in the copper oxide-zinc oxide group (Saadh et al., 2024). The research assessed the antibacterial activity, noting that brackets coated with ZnO-SnO2 using the dip-coating method displayed fewer CFUs compared to those coated with Ag-ZnO and Cu-ZnO nanoparticles.

Bahrami et al in 2005 performed an in-vitro study evaluating the cell viability of orthodontic bands coated with Ag and ZnO Nps using MTT assay [(Bahrami et al., 2023)](https://paperpile.com/c/SzPSei/HkEr). The relative percentages of viable cells in the control, ZnO, and Ag groups were 98%, 91.8%, and 85.7%, respectively, compared to an untreated control (100%). Brackets coated with ZnO-SnO2 nanoparticles using the dip-coating method exhibited higher cell viability at 95.5% compared to those coated with ZnO alone. Hence the combination of ZnO-SnO2 Nps was noted to be superior. The research was carried out in a bio-laboratory setting, which does not fully replicate the conditions found in the oral cavity. Consequently, the durability of the orthodontic brackets with the coating applied was not assessed in an oral environment. Furthermore, the optimal weight percentage (wt%) of Nps necessary to ensure effective cell viability remained unclear from this study and the potential for concentration-dependent toxicity of ZnO-SnO2 Nps was not addressed in the current investigation.

## Scope of the studies

Further investigation is warranted to validate the observations and elucidate the underlying mechanisms contributing to the differences in bacterial adhesion and antibacterial efficacy between the two bracket types. Furthermore, assessing the durability of the ZnO-SnO2 coating on orthodontic brackets through animal studies under oral medical conditions is imperative. Additionally, research is needed to determine the optimal dosage of ZnO-SnO2 to prevent concentration-dependent toxicity.

# Conclusion

Zn-SnO2 Nps were efficiently applied onto ceramic brackets using both sol-gel and dip coating techniques. The presence of these Nps was confirmed through characterization. Brackets coated via the dip-coating method exhibited superior antibacterial properties and cell viability compared to those coated via the sol-gel method, while the lowest values were observed in uncoated brackets.

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