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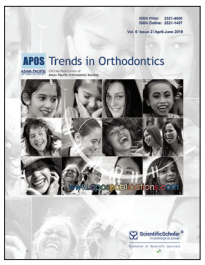

Original Article

Evaluation of antimicrobial property of nanochitosan coated orthodontic brackets against *Streptococcus mutans* and *Lactobacillus acidophilus* – an *in vitro* study

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ABSTRACT

Objectives: The objectives of the study were to evaluate and compare the antimicrobial property of nanochitosan coated and uncoated stainless steel (SS) brackets against *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and Methods: 22 SS orthodontic brackets coated with nanochitosan and 22 uncoated brackets were bonded to the crowns of extracted premolar teeth and prepared for the antimicrobial assay against *S. mutans* and *L. acidophilus*. The bacterial suspensions were incubated in Mueller Hinton broth and cultivated in Petri dish plates. The bacterial colonies were counted after 1, 6, 12, 24, and 72 h intervals using a digital colony counter. Inter and intragroup comparisons were done using independent sample *t*-test and repeated measures ANOVA ($P \leq 0.05$).

Results: The mean colony-forming units (CFU) of *S. mutans* and *L. acidophilus* showed a significant reduction in the coated brackets from 1 h to 72 h ($P = 0.000$). The mean CFU of *S. mutans* and *L. acidophilus* showed a significant increase in the uncoated brackets from 1 h to 24 h and decreased thereafter at 72 h ($P = 0.000$) except for *S. mutans* between 12 h and 72 h where the difference was not statistically significant ($P = 0.837$). The Mean CFU of *S. mutans* and *L. acidophilus* was significantly lesser in coated brackets compared to uncoated brackets at all-time intervals ($P = 0.000$).

Conclusion: Nanochitosan coated orthodontic brackets showed significant antimicrobial properties against *S. mutans* and *L. acidophilus* in short-term up to 72 h compared to uncoated SS brackets.

Keywords: Nanochitosan, Nanocoating, Orthodontic brackets, White spot lesions, Antimicrobial property

INTRODUCTION

The formation of white spot lesions (WSL) on the labial surface of the teeth is a common iatrogenic problem seen during and after fixed orthodontic treatment.[1] Due to the irregular surfaces of brackets and other attachments, there is an increased accumulation of plaque on the labial surface of the teeth. In patients with poor oral hygiene maintenance, this leads to demineralization of enamel and the formation of WSL. It is the first sign of enamel demineralization that is visible to the eye which appears as a chalky white opaque lesion resulting from subsurface enamel porosity.[1] A recent meta-analysis evaluating 14 studies concluded that the incidence and prevalence of WSL in patients undergoing orthodontic treatment were 45.8% and 68.4%, respectively.[2,3]

Researchers have evaluated an array of methods for preventing the formation of WSL during fixed orthodontic treatment. They include patient education on oral hygiene maintenance, antibacterial mouth rinses, fluoride mouth rinses, fluoride dentifrices, or probiotic toothpaste which are dependent on patient compliance and need the active participation of the patient.[4] To overcome this disadvantage non-compliant methods such as the application of fluoride varnish, using fluoride-containing adhesives, and fluoride-releasing modules were introduced.[4]

Various *in vitro* studies were conducted to evaluate the effectiveness of nanoparticles with antibacterial properties in reducing bacterial count when added to orthodontic adhesives or coated to orthodontic brackets and archwires. Nanocoating of brackets, arch wires, and mini-implants reduced biofilm formation and *S. mutans* and *L. acidophilus* count in experimental setups.[5-7]

Nanomaterials are materials with particle size in the range of 1–100 nm and due to their ultra-small sizes, large surface area to mass ratio and increased chemical reactivity, nanomaterials have superior physicochemical properties compared to their non-nanoscale counterparts.[5,6] The large surface area and high charge density of nanoparticles permit them to interact with the surface of the bacterial cells that are negatively charged resulting in enhanced antimicrobial activity.[5-7]

Nanoparticles of various metals and their oxides such as silver, copper, zinc, gold, and titanium are known for their antimicrobial properties. Nanoparticles with antibacterial properties can be added to adhesives or coated on orthodontic attachments as a method to reduce the incidence of WSL during fixed orthodontic treatment.[6,7]

Chitosan is a natural polysaccharide that consists of multiple chains of N-acetyl-D-glucosamine obtained by the alkaline deacetylation of chitin. Chitin is the second most abundant natural polysaccharide after cellulose and is present in shells of insects, marine crustaceans, fungal cell walls, and planktons.[8-11]

Chitosan is active against a broad variety of microorganisms including fungi, algae, and bacteria. The high molecular weight chitosan is specifically more active against Gram-positive bacteria and cationic chitosan interacts with the anionic cell membrane of microbes leading to leakage and disruption of the cell membrane.[10,11]

Bumgardner *et al.* evaluated the tensile bond strength of chitosan coating on titanium and found the bond strength to be adequate and concluded that chitosan can be used as a bioactive and biocompatible coating for orthopedic and craniofacial implant devices.[12]

The current study aims at attempting to standardize a method to coat orthodontic brackets with nanochitosan and evaluating the antimicrobial property of nanochitosan against *S. mutans* and *L. acidophilus* when coated on orthodontic stainless-steel brackets.

MATERIALS AND METHODS

This experimental, *in vitro* study, was undertaken in the Department of Orthodontics, SRM Dental College, Ramapuram. The study design was approved by the Institutional Review Board, SRM Dental College, Ramapuram (SRMDC/IRB/2019/MDS/No.101).

Sample size determination

The sample size was calculated using G Power software with a power of 95%, α error of 5% and $P < 0.05$. The estimated sample size was 44 with 22 in each group.

Materials

Orthodontic brackets

A total number of 48 pre adjusted edgewise stainless-steel maxillary premolar brackets from Gemini series (3M Unitek, Monrovia, Calif.) with MBT prescription and a slot size of 0.022" was used in the study. The brackets were allotted to 2 groups with 26 brackets in Group A constituting the study group and 22 brackets in Group B constituting control group of uncoated orthodontic brackets. The Group A brackets were coated with Chitosan nanoparticles and 4 brackets were randomly selected for scanning electron microscope (SEM) analysis to ensure the uniformity of the coating and the rest of the brackets were used for the microbial assay.

Chitosan particles

Chitosan particles of molecular weight 3800–20,000 Da was procured from HiMedia Laboratories Pvt Limited, Mumbai, India.

Teeth

44 healthy human maxillary premolar teeth extracted for orthodontic reasons without enamel cracks, decalcification spots, surface defects, caries, or restoration were collected and stored in distilled water at room temperature. The roots of the teeth were amputated using a micromotor and carborundum disc, the pulp was extirpated, and the chamber was sealed with flowable composite TE-Econom (Ivoclar Vivadent, Somerset NJ, USA).

Bacterial strain

S. mutans strain Microbial Type Culture Collection and Gene bank (MTCC) 497 and *L. acidophilus* strain MTCC 447 were used and obtained from MTCC, Chandigarh.

Methods

Cleaning of orthodontic brackets before coating

SS brackets were cleaned with deionized water and ethanol at 80°C for 30 min to get rid of the oxidized layer over the surface.

Synthesis of chitosan nanoparticles and coating of orthodontic brackets

The chitosan nanoparticles were synthesized and coated on the stainless-steel orthodontic brackets using the hydrothermal method.[13] A solution of 0.5 g Chitosan in 30 ml distilled water was prepared by continuous stirring for 30 min. Meanwhile 1.5 M solution of acetic acid solution was prepared by mixing 60.05 g of acetic acid in 500 ml distilled water under stirring for the same duration. The acetic acid solution was added dropwise to the chitosan solution under continuous stirring until the pH of the reactants becomes 10.9. This solution mixture was transferred into Teflon lined sealed stainless-steel hydrothermal autoclave reactor (Techinstro hydrothermal autoclave reactor, Indian Institute of Technology, Jammu) along with steel brackets and kept in the hydrothermal oven at a temperature of 90°C for 8 h. The chitosan nanoparticle synthesized by the hydrothermal reaction will precipitate over the stainless-steel orthodontic brackets as a uniform coating. The Teflon-coated SS autoclaves were removed from the furnace after 8 h and allowed to cool at room temperature. The brackets were retrieved then washed with distilled water and kept for drying under ambient temperature. The coated brackets had a dull surface compared to the non-coated brackets [Figure 1].

SEM analysis

After retrieval 4 brackets were selected randomly from the 26 coated brackets and subjected to SEM and field emission scanning electron microscopy (FESEM) analysis to confirm the uniformity of coating. The surface of the nanochitosan coated brackets and noncoated brackets were analyzed with SEM (TESCAN SEM solutions, Alagappa College of Technology, Anna University, Chennai) under × 42, × 70, × 500, × 1000 and × 1500 various magnifications. The SEM images of nanocoated brackets demonstrated a uniformly distributed coating of nanochitosan over the bracket surface compared to the uncoated brackets [Figures 2 and 3].

Field emission SEM analysis

The brackets were subjected to analysis with FESEM (Jeol Field Emission SEM, Indian Institute of Technology, Jammu) at × 20,000 and × 30,000 magnification to verify the morphology and size of the nanoparticles. The morphological views ensured the uniform distribution of spherical chitosan nanoparticles with an average particle size of ~ 50 nm [Figure 4].

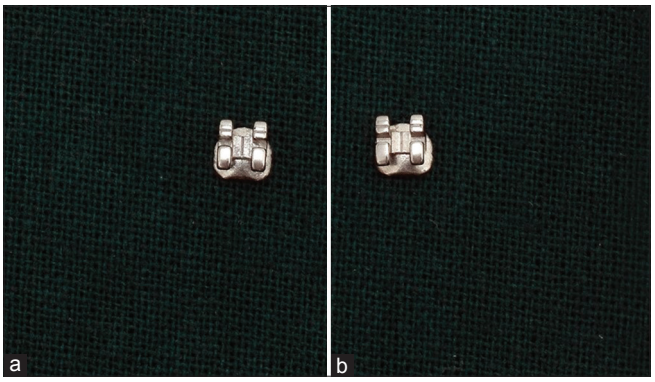

Figure 1: (a) Nanochitosan coated brackets (b) Uncoated brackets.

Bonding

44 maxillary premolar teeth crowns prepared for the study purpose were randomly divided into two groups of 22 each and coated and uncoated brackets were bonded to the teeth using standard bonding protocol by the same operator. The buccal surface of teeth was conditioned with 37% phosphoric acid Eazetech Etchant (Anabond Stedman Pharma Pvt Ltd, Chennai, TN, India) for 30 s, rinsed with water for 20 s and dried with oil-free compressed air for 20 s. The light cure adhesive primer Transbond (3M Unitek, Monrovia, California, USA) was applied on the etched enamel surface and cured for 20 s. The brackets were bonded on the middle third of the enamel parallel to the long axis using Transbond XT Light Cure Adhesive Paste (3M Unitek, Monrovia, California, USA). Excess composite around the margins of the bracket was removed with a dental explorer and photo-polymerized for 20 s. The 22 coated brackets bonded to the human premolar teeth constituted Group A samples and 22 non-coated brackets bonded to premolar teeth constituted Group B samples.

Antimicrobial assay

The teeth and brackets were sterilized using an autoclave at 120°C at a pressure of 15 psi for 15–20 min before the antimicrobial assay. The reference strain of *S. mutans* (MTCC 497) and *L. acidophilus* (MTCC 447) obtained from MTCC and Gene bank were used in this study. The standard sample of *S. mutans* and *L. acidophilus* was cultured and a suspension containing bacteria in a logarithmic phase with a concentration of 1.5×10^5 colony-forming units (CFU)/ml was prepared. Each sample was placed in a separate test tube, 1 ml of the bacterial suspension was added and were incubated at 37°C.

The suspension (10 μL) was taken from each tube in intervals of 1, 6, 12, 24, and 72 h and was cultured on the

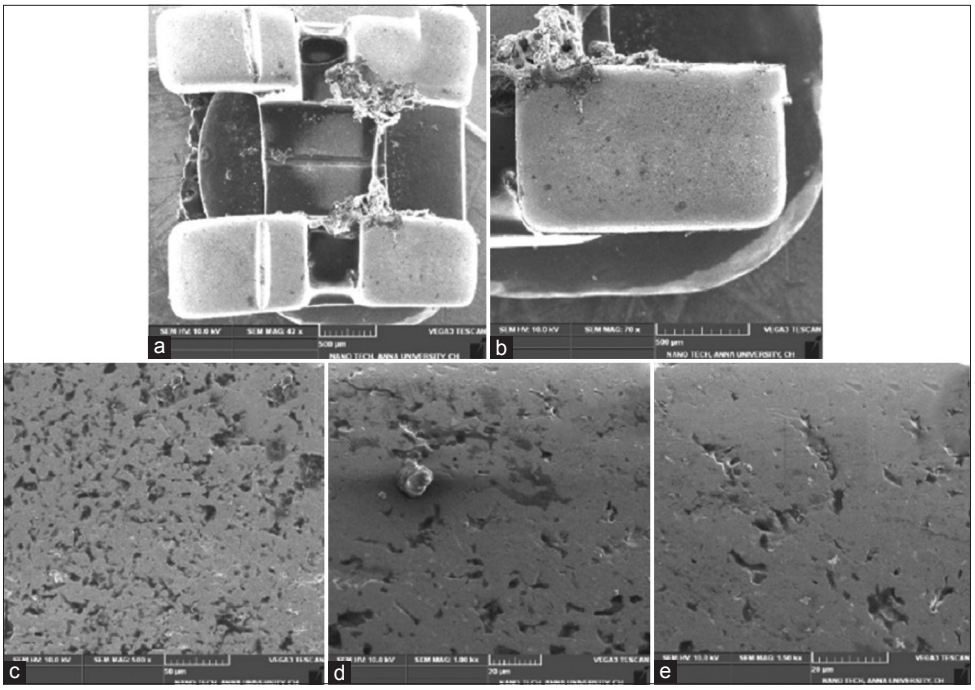

Figure 2: (a-e) Scanning electron microscopy images of nanochitosan coated brackets under various magnifications showing uniform coating of the surface (a- ×42, b- ×70, c- ×500, d- ×1000, e- ×1500 magnification).

plates containing Mueller Hinton broth [Figure 5]. The time intervals of 1, 6, 12, 24, and 72 h were set for investigating the CFU of *S. mutans* and *L. acidophilus* because they are the recommended time intervals used in pharmacology research for *in vitro* evaluation of the antimicrobial activity.[14,15]

The total number of bacterial CFU was counted with a digital colony counter (Deep vision digital colony counter, Praxor Instruments, and scientific Co, India). The Petri dishes were placed on the illuminated pad on the digital colony counter and the colonies were marked with a pen provided to register the count. The number of CFU per ml is calculated by multiplying the registered count by 10^5.

Statistical analysis

Descriptive and Inferential statistics were analyzed using IBM SPSS version 20.0. Descriptive statistics for the CFU, including the mean and standard deviations were calculated for each of the two groups tested. The normality of data was determined using Shapiro–Wilk's test. Inter and intragroup comparisons were done using independent sample *t*-test and repeated measures ANOVA.

RESULTS

The mean CFU of *S. mutans* in orthodontic brackets coated with nanochitosan was 3.12×10^5 CFU/ml at 1 h which subsequently reduced to 1.60×10^5 CFU/ml at 6 h, 1.07×10^5 CFU/ml at 12 h, 0.62×10^5 CFU/ml at 24 h, and 0.43×10^5 CFU/ml at 72 h [Table 1]. The reduction of mean CFU of *L. acidophilus* followed a similar trend from 4.24×10^5 CFU/ml at 1 h to 0.47×10^5 CFU/ml at 72 h [Table 1].

The mean CFU of *S. mutans* in uncoated orthodontic brackets was 2.55×10^5 CFU/ml at 1 h which subsequently increased to 8.17×10^5 CFU/ml at 6 h, 12.16×10^5 CFU/ml at 12 h, 18.73×10^5 CFU/ml at 24 h and decreased to 12.17×10^5 CFU/ml at 72 h [Table 1]. A similar trend was noted for *L. acidophilus* with the mean CFU of 4.71×10^5 CFU/ml at 1 h which gradually increased to 10.15×10^5 CFU/ml at 24 h and decreased to 9.78×10^5 CFU/ml at 72 h [Table 1].

The reduction in the number of CFU with nanochitosan coated group of brackets was significant for both *S. mutans* and *L. acidophilus*. Repeated measures ANOVA showed significant differences between the mean CFU of both *S. mutans* and *L. acidophilus* at different time intervals in the nanochitosan coated group of brackets with a $P = 0.000$ [Table 2].

The increase in the number of CFU of *S. mutans* and *L. acidophilus* in the uncoated brackets group from 1 h to 24 h and the further decrease at 72 h was statistically significant. Repeated measures ANOVA showed significant differences in the mean CFU of both the microbes at different time intervals ($P = 0.000$) except for *S. mutans* between 12 h and 72 h ($P = 0.837$) [Table 3].

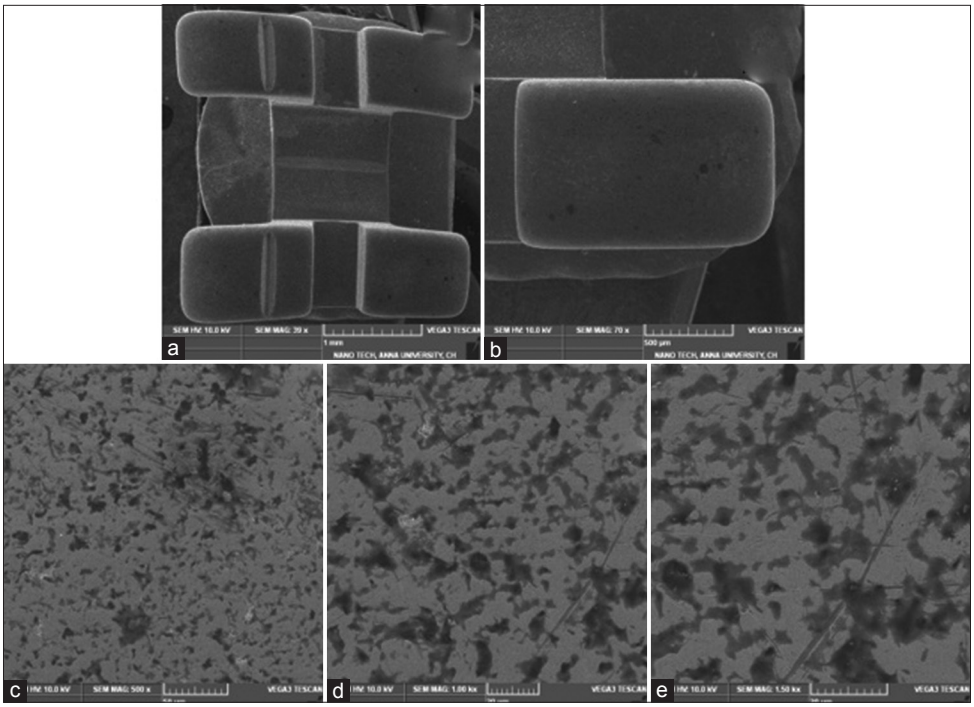

Figure 3: (a-e) Scanning electron microscopy images of uncoated brackets under various magnifications (a- ×42, b- ×70, c- ×500, d- ×1000, e- ×1500 magnification).

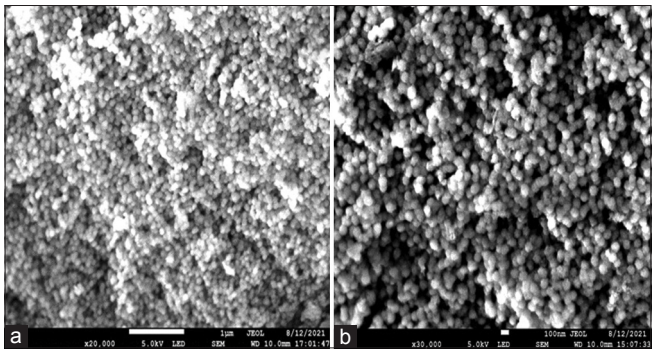

Figure 4: (a and b) Field emission scanning electron microscopy images of chitosan nanoparticles coated over the brackets at ×20,000 and ×30,000 (a- ×20,000; b- ×30,000 magnification) showing uniform distribution of spherical chitosan nanoparticles with an average particle size of ~50 nm.

Intergroup comparison with independent sample *t*-test revealed significantly greater *S. mutans* colonies in the coated group than in the uncoated group at 1 h ($P = 0.000$) [Table 4]. At subsequent time intervals, the numbers gradually increased in the uncoated group and decreased in the coated group. At 6 h, 12 h, 24 h, and 72 h the mean CFU were significantly greater in the uncoated group than in coated group ($P = 0.000$) [Table 4]. The mean CFU of *L. acidophilus* was significantly lesser in the nanocoated group than in uncoated brackets at all-time intervals evaluated with a $P = 0.004$ at 1 h and $P = 0.000$ at other time intervals [Table 4].

DISCUSSION

In the current study, the mean CFU of *S. mutans* and *L. acidophilus* around orthodontic brackets coated with nanochitosan reduced significantly from 1 h to 72 h [Tables 1 and 2]. Nanochitosan coated orthodontic brackets demonstrated antimicrobial activity against both *S. mutans* and *L. acidophilus* when compared to their noncoated counterparts which exhibited no antimicrobial activity. The reduction in the colony count was noted in coated brackets from as early as 6 h and a steady decline was noted throughout the study period whereas a steady and significant increase was noted in the colony counts of both the microorganisms in the uncoated brackets group up to 24 h [Tables 2 and 3].

The result of the study coincided with previous studies that had demonstrated the antibacterial property of Chitosan and nano chitosan particles.[16-24] Chitosan exhibited potent antimicrobial properties against cariogenic bacteria when added to dentifrices, mouthwashes, composite resins, orthodontic bonding agents, and impression materials without affecting their mechanical and clinical properties.[16-24] Chitosan acts against bacteria by inhibiting the bacterial enzymes, causing chelation of metal ions and forming polyelectrolyte complexes on the bacterial cell wall.[17,25-30] This specific action of chitosan against *S. mutans* and *L. acidophilus* may be due to the presence of more anionic peptidoglycan and Teichoic acid in the cell wall of

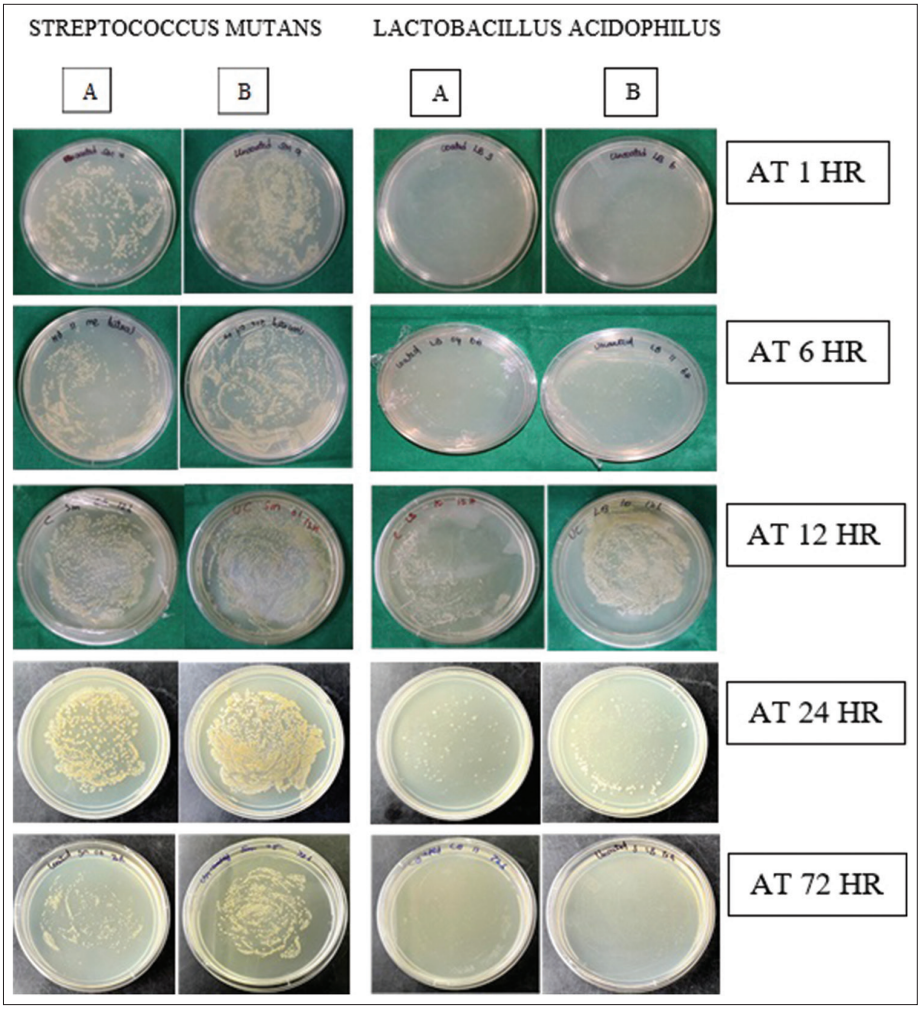

Figure 5: Petri dish plate containing colonies of *Streptococcus mutans* and *Lactobacillus acidophilus* at different intervals throughout the experiment in coated and uncoated brackets group. (A- Nanochitosan coated brackets, B- Uncoated brackets).

Table 1: Descriptive statistics and Wilk's lambda F-value of *Streptococcus mutans* and *Lactobacillus acidophilus* in Group A (nanochitosan coated orthodontic brackets) and Group B (uncoated orthodontic brackets).

	Timeline	Mean	SD	Wilk's Lambda F	*P*-value	Mean	SD	Wilk's Lambda F	*P*-value
		$\times 10^5$ CFU/ml				$\times 10^5$ CFU/ml			
		Streptococcus mutans				*Lactobacillus acidophilus*			
Group A	1 h	3.12	0.292	5163.1	0.0001	4.24	0.72	1569.2	0.0001
	6 h	1.60	0.05			2.68	0.345		
	12 h	1.07	0.054			1.07	0.022		
	24 h	0.62	0.04			0.72	0.043		
	72 h	0.43	0.03			0.47	0.037		
Group B	1 h	2.55	0.22	2875.3	0.0001	4.71	0.07	3775.4	0.0001
	6 h	8.17	0.54			5.27	0.17		
	12 h	12.16	0.31			9.56	0.21		
	24 h	18.73	0.65			10.15	0.14		
	72 h	12.17	0.38			9.79	0.08		

SD: Standard deviation, CFU: Colony-forming units

Table 2: Repeated measures ANOVA for intragroup comparison of mean colony-forming units of *Streptococcus mutans* and *Lactobacillus acidophilus* in Group A samples (Orthodontic brackets coated with Nanochitosan).

(I) factor 1	(J) factor 1	Mean Diff (I-J)	SE	*P*-value	Mean diff (I-J)	SE	*P*-value
		Streptococcus mutans			*Lactobacillus acidophilus*		
1 h	6 h	1.52	0.069	0.000*	1.57	0.149	0.000*
1 h	12 h	2.04	0.068	0.000*	3.18	0.153	0.000*
1 h	24 h	2.50	0.058	0.000*	3.5	0.151	0.000*
1 h	72 h	2.69	0.061	0.000*	3.78	0.154	0.000*
6 h	12 h	0.52	0.012	0.000*	1.61	0.074	0.000*
6 h	24 h	0.98	0.019	0.000*	1.95	0.066	0.000*
6 h	72 h	1.16	0.013	0.000*	2.20	0.073	0.000*
12 h	24 h	0.46	0.017	0.000*	0.34	0.011	0.000*
12 h	72 h	0.64	0.015	0.000*	0.60	0.012	0.000*
24 h	72 h	0.18	0.010	0.000*	0.25	0.010	0.000*

*$P<0.05$, SE: Standard error

Table 3: Repeated measures ANOVA for intragroup comparison of mean colony forming units of *Streptococcus mutans* and *Lactobacillus acidophilus* in group B samples (Uncoated orthodontic brackets).

(I) factor1	(J) factor1	Mean diff (I-J)	SE	*P*-value	Mean diff (I-J)	SE	*P*-value
		Streptococcus mutans			*Lactobacillus acidophilus*		
1 h	6 h	−5.62	0.160	0.000*	−0.56	0.038	0.000*
1 h	12 h	−9.61	0.100	0.000*	−4.85	0.041	0.000*
1 h	24 h	−16.18	0.157	0.000*	−5.44	0.041	0.000*
1 h	72 h	−9.63	0.122	0.000*	−5.08	0.024	0.000*
6 h	12 h	−3.99	0.124	0.000*	−4.29	0.036	0.000*
6 h	24 h	−10.56	0.155	0.000*	−4.88	0.046	0.000*
6 h	72 h	−4.00	0.080	0.000*	−4.52	0.039	0.000*
12 h	24 h	−6.57	0.112	0.000*	−0.59	0.063	0.000*
12 h	72 h	−0.014	0.066	0.837*	−0.23	0.054	0.000*
24 h	72 h	6.56	0.101	0.000*	0.36	0.024	0.000*

*$P<0.05$, SE: Standard error

gram-positive bacteria that interact with the cationic amino group of chitosan leading to cell death.[17,28]

Intergroup comparisons revealed a significant difference between the mean colony counts of both the bacteria around the nanochitosan coated and uncoated brackets at all the time intervals tested [Table 4]. Studies have shown that coating orthodontic brackets with nanoparticles of metal and metal oxides with antibacterial properties reduced the number of cariogenic bacteria in an experimental setup.[6,7] Nanoparticles particles due to their smaller size and increased surface area demonstrate enhanced antimicrobial properties than their non-nanoscale counterpart making them potentially useful in the prevention of oral biofilms and the reduction of cariogenic bacteria.[30]

In uncoated brackets, colony count significantly increased from 1 h to 24 h and decreased at 72 h for both *S. mutans* and *L. acidophilus*. The statistically significant difference was

Table 4: Independent sample t test for intergroup comparison between mean colony forming units of Streptococcus mutans and Lactobacillus acidophilus of Group A (coated brackets) and Group B (uncoated brackets) at different time intervals.

Timeline	*t*-value	df	*P*–value	Mean difference
Streptococcus mutans				
1 h	7.22	42	0.000*	0.57
6 h	−56.28	42	0.000*	−6.58
12 h	−164.57	42	0.000*	−11.09
24 h	−131.37	42	0.000*	−18.12
72 h	−145.22	42	0.000*	−11.74
Lactobacillus acidophilus				
1 h	−3.06	42	0.004*	−0.47
6 h	−31.70	42	0.000*	−2.59
12 h	−189.05	42	0.000*	−8.49
24 h	−309.28	42	0.000*	−9.42
72 h	−516.08	42	0.000*	−9.32

*$P<0.05$, df: degree of freedom

found at different time intervals except for *S. mutans* between 12 h and 74 h [Tables 2 and 3]. The possible reason for this may be due to the lag phase noted in the bacterial growth curve due to the reduction of nutrients.[8] However, this conclusion can be reached only by further long-term studies.

Nanochitosan coating over metal substrates such as dental implants and NiTi alloy had been successfully tried and evaluated for bond strength and corrosion resistance.[12,24-26] Mareci *et al*. used Nd: YAG pulsed laser deposition technique to coat the NiTi wires with a thin compact layer of chitosan as verified by the scanning electron microscopic images.[24] Laser ablation produced thin layers of polymers on the biomaterial substrates that did not induce an inflammatory response or repulsion by the immune system.[25] Bumgardner *et al*. used deacetylated chitosan to coat the titanium implants using a silanation reaction to create a chemical bond with the implant quality titanium and the bond strength was not affected by the gas sterilization.[12] In our current study, the hydrothermal technique was used to coat the orthodontic brackets with nanochitosan and the uniformity of the coating was verified by scanning electron microscopic images obtained at different magnifications.[13] The bonded brackets were autoclaved before antimicrobial assay as steam sterilization does not modify the structure and degree of acetylation and bonding of chitosan irrespective of its physical form.[30]

S. mutans and *L. acidophilus* count higher than 10^5 CFU/ml of saliva indicates a high risk of developing caries and can be considered as the threshold amount of mean CFU that could cause the development of WSL as mentioned in previous studies.[31-34] The mean CFU of *S. mutans* at 24 h and 72 h in the coated brackets group was $0.62 \pm 0.04 \times 10^5$ CFU/ml and $0.43 \pm 0.03 \times 10^5$ CFU/ml respectively which was well below the threshold concentration for the development of enamel decalcification. A similar trend was noted in the mean CFU of *L. acidophilus* in coated brackets group indicating that the antimicrobial effects of nanochitosan on *S. mutans* and *L. acidophilus* may reduce the bacterial count below threshold levels that will prevent the development of WSL.

The results of this study have shown that nanochitosan coating on brackets has a short-term antimicrobial property against *S. mutans* and *L. acidophilus* for preventing WSL. However, fixed orthodontic treatment usually takes several months to a few years to complete in clinical orthodontics, hence studies evaluating the antimicrobial effect for an extended period that can be correlated to the duration of fixed orthodontic treatment.

Further investigation on surface characteristics, corrosion property, durability of the coating, and clinical investigations are recommended before considering nanochitosan coating as a viable method for reducing WSL around orthodontic brackets. Nanocoating of orthodontic brackets in routine orthodontic practice may be difficult because of the cost, personnel requirement, and application time involved. This method can be reserved for patients with high caries index and an increased risk of enamel decalcification during orthodontic treatment.

CONCLUSION

The following were the conclusions drawn from the study:

1. Nanochitosan coated orthodontic brackets showed significant antimicrobial properties against *S. mutans* and *L. acidophilus* in short term up to 72 h compared to uncoated brackets
2. The reduction in the number of colonies was significant from as early as 6 h in nanochitosan coated brackets for both *S. mutans* and *L. acidophilus*
3. Nanochitosan coating can be considered a viable method to reduce WSL around orthodontic brackets and further long-term studies are recommended to assess the antimicrobial, mechanical, and bio corrosive properties of the coating over stainless steel orthodontic components.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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