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Original Article

Nanoparticle Coating Obtained from Agaricus Bisporus On Elastic Ligatures: An *in vitro* Study

Meghana Pasala[1], Sujatha Sanneboina[2], Aparna Kadiveti[1], Gowri Sankar Singaraju[*1], Vivek Reddy Ganugapanta[1], Prasad Mandava[1]

[1]*Department of Orthodontics, Narayana Dental College, Nellore, Andhra Pradesh, India*
[2]*Department of Pharmaceuticals, Narayana Pharmacy College, Nellore, Andhra Pradesh, India*

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The presence of fixed orthodontic appliance favours the plaque accumulation and a compromise in oral hygiene. There are various methods in practice to minimize the white spot lesions (WSL) incidence, but which depend on the patients' compliance. It is prudent to introduce materials and methods which rely less on patient. The purpose of our study is to introduce a new silver nanoparticle (AgNPs) coating onto elastomeric ligatures and to assess its antimicrobial property and durability of the silver nanoparticles coating on the elastomeric ring. Out of a total sample of 69 clear elastomeric ligatures, 44 of them are equally allocated (n=22) to each of the two groups, Group A-test group (AgNPs coated) and Group-B-control (Non coated) for antimicrobial testing and rest for durability testing. The test group elastomeric modules coated with AgNPs were prepared from *Agaricus bisporus* extract and silver nitrate solution and tested for antimicrobial testing against *Streptococcus mutans*. Remaining test sample was utilised for determination of silver ion release(mg/L) when coated elastomeric rings are placed in artificial saliva and analyzed after T1 - 24 hours, T2 – 48 hours, T3 - 2 weeks, T4 – 4 weeks using atomic absorption spectrophotometer (AAS). The results showed a mean inhibition zone of 2.57 + 0.17 mm for antimicrobial activity for test group which is clinically significant compared to the control group which showed no inhibition zone. Friedman test was used to compare the silver release at 4 different time periods T1, T2, T3, and T4 and Wilcoxon rank test for pairwise comparison. The amount of silver ion accumulation into artificial saliva increased continuously as time elapsed and silver ion release is statistically significant between the measured time points ($p = 0.001$). The AgNPs coated elastomeric modules has definite antimicrobial activity compared non coated elastomeric modules. The durability of the coating was shorter.

Introduction

The orthodontic treatment with fixed appliances is challenging for oral hygiene maintenance as it provides increased surface area for plaque adherence. The orthodontic appliances limit the self-cleansing capacity of saliva leading to high risk of incipient caries and white spot lesions (WSL) on dental surfaces [1]. Fejerskov and Kidd defined WSL as the "first sign of a caries lesion on enamel that is detectable with the naked eye"[2]. While the majority of WSL re-mineralize after the removal of appliances, but pre-treatment levels are never regained and can progress to cavitation [3].

The most important component in preventing WSL is good oral hygiene habits, although these depend totally on patient compliance. Therefore, orthodontic biomaterials that are inherently antibacterial or anti-cariogenic, such as adhesives, ligatures, brackets, etc., are of interest and have been tested [4].

The method of ligating archwires is a supplemental factor accounting to dental biofilm retention [5]. Elastomeric ligatures are synthetic elastics made of polyurethane, lie close to enamel and are changed regularly during orthodontic treatment [6,7]. They could serve as a vehicle for the localised distribution of antibiotics, reducing the need on patient cooperation and also improve enamel remineralisation of areas adjacent to the bracket base, that are difficult to clean [8].

One of the major advents in orthodontics is coating these elastomeric surfaces with Nano Particles to prevent microbial adhesion. Silver nanoparticles (AgNPs) are one of the commonly used nanoparticles (NPs) that can potentially combat the dental

* *Coresponding author*
E-mail address: *drgowrisankar@gmail.com* (Dr. Gowri Sankar Singaraju)

biofilm, reducing enamel demineralization during and after the orthodontic treatment. AgNPs demonstrated suppression of bacterial adherence and growth of S. mutans bacteria on orthodontic brackets and wires [9]. Metal coated nanoparticles could give rise to harmful by-products during their production as well as during their usage [10]. Consequently there is an increasing need and demand to create safe, nontoxic, and ecologically friendly (green chemistry) processes for production of these nano particles [11].

The use of mushrooms in the green synthesis of nanoparticles is a new addition and holds a promising role in large-scale nanoparticle production in less time. *Agaricus bisporus* is one of the safest and efficient mushroom variety that is produced in a controlled, sterilized environment[11]. Previous research suggest that hydroxyl and carbonyl groups of the *Agaricus bisporus* extract are involved in the synthesis of AgNP's[12]. To the best of our knowledge, no research has been conducted to evaluate the antibacterial activity and durability of AgNPs biosynthesized from *Agaricus bisporus* (button mushroom) coated on elastomeric ligatures/modules. The current study introduced elastomeric modules with surfaces coated of AgNPs synthesised from *Agaricus bisporus* (button mushroom). The purpose of this in vitro study was to evaluate the antimicrobial activity and durability of biosynthesized AgNPs coating on elastomeric modules.

Materials and Methods

This *in vitro study* was carried out by the Department of Orthodontics, Narayana Dental College and Hospital, Nellore, Andhra Pradesh(AP), India in collaboration with Narayana pharmacy college, Nellore, and Virtue Metasol lab, Hyderabad. The protocol of this *in vitro* study was reviewed and approved by Institutional Ethical Committee (Reg: D200040814, Ref No. ICE/NDCH/2020/P-24 dated 8-12-2020).

Clear orthodontic elastomeric modules as supplied by the manufacturer (DD plus, USA) of same make of batch and year with shelf life more than 6 months and without any precoating were utilized for the study. Based on a prior study by Bindu SH [13], a minimum sample size of 48 was needed to evaluate the antimicrobial property and silver ion release. A 20% additional sample was taken to account for procedural errors, bringing the sample total to 69.

An initial sample of 44 from the total sample with an equal distribution of n= 22 for each of the test and control groups was designated for the antimicrobial test. The power of the study was set at 0.8 and the significance level at 0.05 with a 95% confidence interval. A second sample of 25 elastomeric ligatures coated with AgNPs was used to measure the release of silver ions at four different time points. (figure 1).

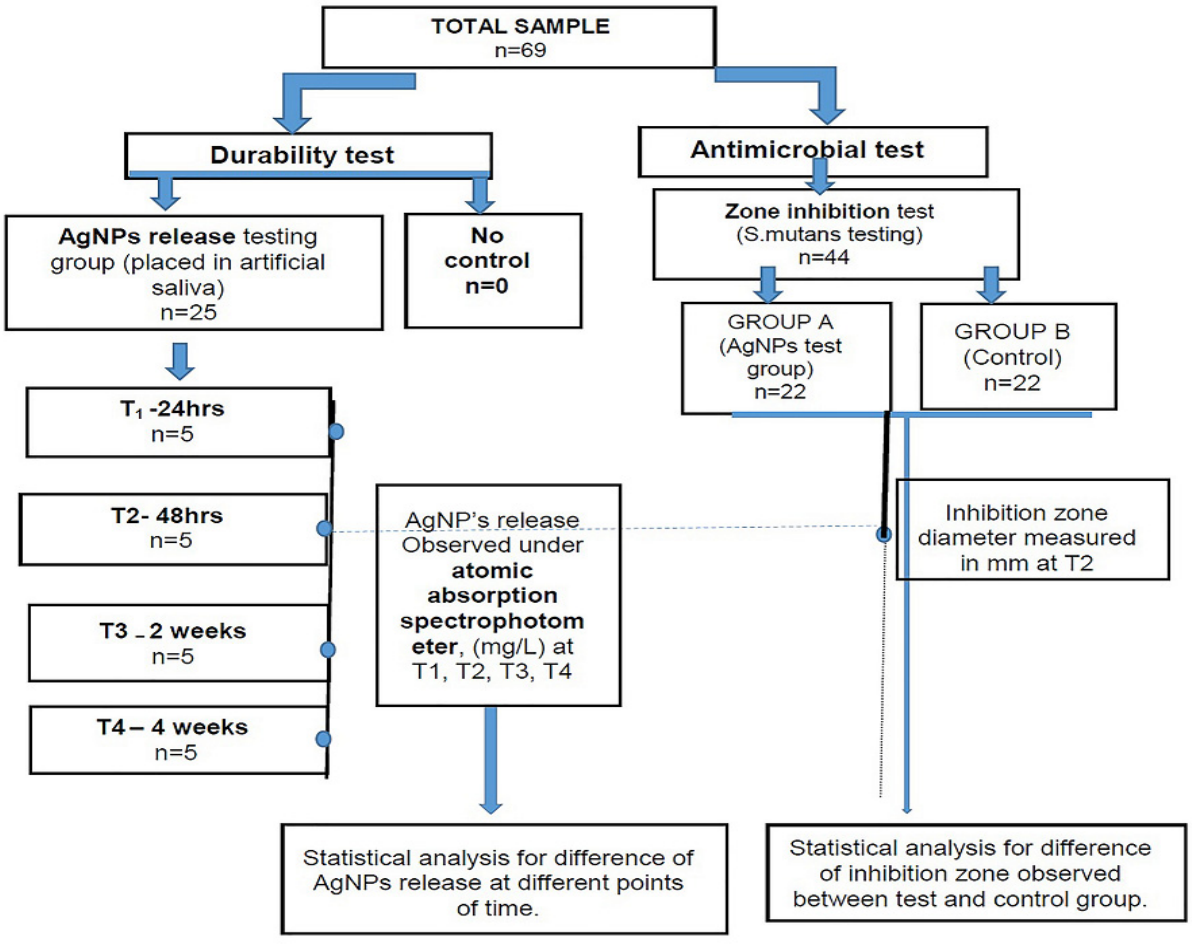


Figure 1: Flow chart showing the methodology of the study

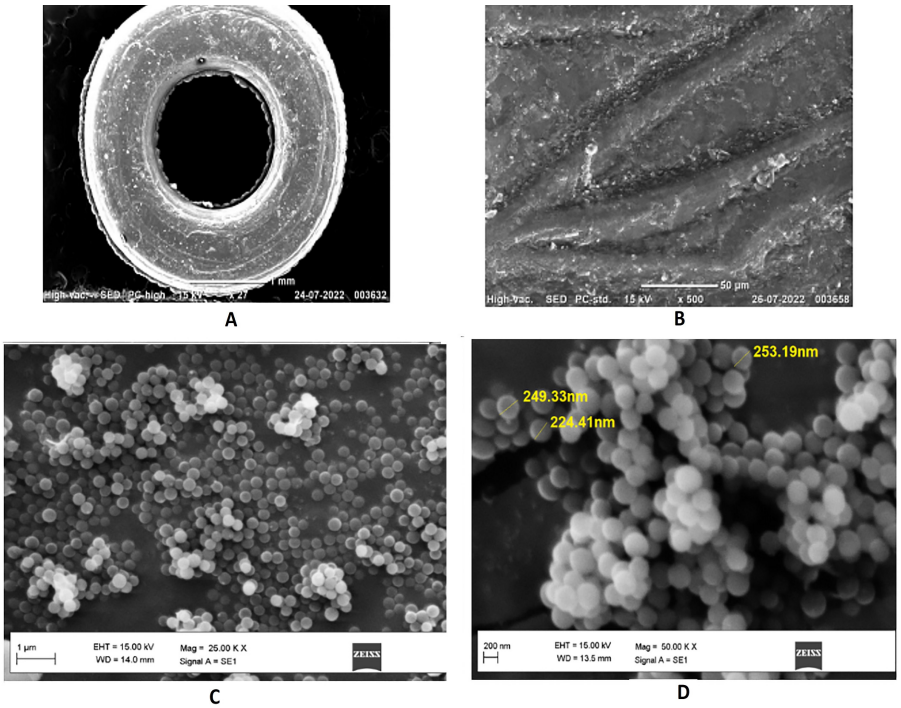


Figure 2: Scanning electron Microscopic examination at various magnifications showing the topographic changes and confirmation of the presence of nano size particles. A- 27X, B- 500X, C- 25KX, D-50KX magnifications

Coating of elastomeric modules with biosynthesized AgNps: The first step involved is the Preparation of *Agaricus bisporus* extract and the methodology was adapted from the earlier study of Manzoor-ul-Haq [14]. Fresh *Agaricus bisporus* (Fresh bowl button mushroom, Fresh bowl horticulture private, Andhra Pradesh, India) were washed thoroughly with vinegar and distilled water to remove any mud adhering to the mushrooms. The washed mushroom was then air-dried at room temperature on bloating paper without overlapping for 3-4 days. The dried mushrooms were cut into small pieces and powdered to attain the finest powdered particles as possible. Five grams of dry powder is weighed using ATOM xs electronic compact scale and added to 350ml of demineralized water (Malayadri 100% Demineralized water) followed by boiling for 10 minutes. The boiled extract solution was then filtered twice through Whatman's filter paper No.1 and stored at 4°C for further experimental procedures.

The next step involved is the preparation of 1mM of silver nitrate solution $AgNO_3$ (99.9%): To obtain 1M $AgNO_3$ solution, 169 mgs of $AgNO_3$ (Silver nitrate LR Particles, Molychem 17980) was dissolved in 1000ml of deionized water. The A.*bisporous* extract was used as reducing agent for 1milli Mol (mM) of AgNO3. For the typical synthesis of AgNPs, the prepared extract of *A.bisporous* was added to 1mMol AgNO3 solution in the ratio of 2:1.

This was followed by the in vitro synthesis of AgNPs coating on elastomeric modules as described by Alma E. Hernández et al [7]. Pre-treated elastomeric modules were immersed in 100 ml of silver nitrate (AgNO3) solution for 60 min, and later, 200 ml *of A.bisporous* extract is added to the solution and stirred. The reducing effect of *Agaricus bisporus* on AgNO3 facilitates the coating of silver ions (Ag^+) on elastomeric modules. Further, synthesis of AgNPs was carried out for 48 hours in the darkness to minimize the photo activation of silver nitrate. Orthodontic elastomeric modules were then allowed to dry at room temperature for eight hours. Visual confirmation of the bio reduction of AgNO3 into AgNPs was noticed from a change in the colour of the solution, which went from colourless to yellowish-brown. Characterization of the silver nanoparticles biosynthesized was confirmed by a color change. The elastic modules were very transparent originally; after the process of incorporation of silver nanoparticles on the surface, and change of colour and appeared yellow to brownish.

The coated modules were evaluated and the coating was confirmed by scanning electron microscope (SEM) (JEOL, JCM-6000PLUS at 15 kV, Tokyo, Japan) under 27X, 500X, 25KX and 50 K X magnification. Chemical analysis of the coated elastomers was performed by means of energy dispersion (EDS), with a resolution of 137 eV.

The antimicrobial activity against *Streptococcus mutans (S.mutans)* was tested using the zone inhibition test [13]. The Mutans sanguis agar for *S.mutans* (HiMedia, Mumbai) was prepared as per manufacturer's instructions. The Medium at pH 7.2 to 7.4 was transferred to 9 cm diameter Petri dishes and stored at 2-8° C. The *Streptococcus mutans* (S. mutans, ATCC25175) suspension was prepared in 0.5 McFarland standard concentration (108 bacteria per mL) and transferred to already prepared Mutans sanguis agar culture medium.

The control group and AgNPs coated elastomeric modules were placed in two separate already prepared petri dishes at an interdistance 25mm between any two modules of each for 48 hours at 37°C. Bacterial growth inhibition zone if present was identified after 48 hrs and is measured in millimeters with the help of micrometer (Mitutoyo 0-25mm outside micrometer).

Evaluation of the retentive capacity of AgNPs on elastomeric modules (durability of coating): The AgNP's coated modules (n=25) were randomly distributed into a group of five each and

Table 1: Size of Nano particles and Mass percentage of elemental analysis - Scanning Electron Microscopy and X-Ray Energy-Dispersive Spectroscopy - SEM/EDS

Sample	Ag nanoparticle range (nm)	Mass % by element wise			
		Carbon	Nitrogen	Oxygen	Silver
Sample 1	234.21 - 244.65	46.09	21.81	31.47	0.03
Sample 2	224.41 - 238.45	45.88	20.71	32.52	0.01
Sample 3	240.33 - 250.09	45.33	21.56	33.34	0.01
Sample 4	232.12 - 247.89	46.45	21.02	31.54	0.03
Sample 5	243.65 - 253.19	44.44	20.48	33.99	0.09.
Mean ± S.D		45.63 ± 0.78	21.11 ± 0.56	32.57 ± 1.10	0.02 ± 0.02

immersed into five different test tubes having 5 ml of artificial saliva with gentle shaking. The amount of silver ions leached from the AgNPs coated samples in each of the test tubes was measured (mg/L) using atomic absorption spectrophotometer(AAS) at - after 24 hours, 48hours, 2weeks, 4 weeks, and the average value was calculated for each of different time periods.

The inhibition zone of the bacteria in millimetres for the respective groups and the quantity of silver ion release (mg/L) at time periods mentioned was entered in the data sheet (Microsoft excel 2013) as quantitative continuous data. Friedman test equivalent to repeated measures ANOVA was utilized to compare the amount of silver release from five samples at 4 different time periods T1, T2, T3, and T4. The Wilcoxon rank test was used for pairwise comparison between individual time periods T1 and T2, T3, T4. All the statistical test were carried out using online Social Science Statistics calculator.

Results

Confirmation of AgNPs synthesis:The AgNPs coated elastomeric modules subjected to SEM showed presence of non-uniform, spherical silver particles coating, with size ranging from 224nm to 253nm (figure 2). The SEM/EDS analysis of the impregnated modules with AgNPs exhibited Carbon, as major elements as the module is an elastomeric material and other element identified are Nitrogen, Oxygen and Silver ions (C, N, O, and Ag). The mass percentage of silver and other major ions of coated modules for tested samples was mentioned in the table 1 and micrographs of EDS tested samples is shown in figure 3.

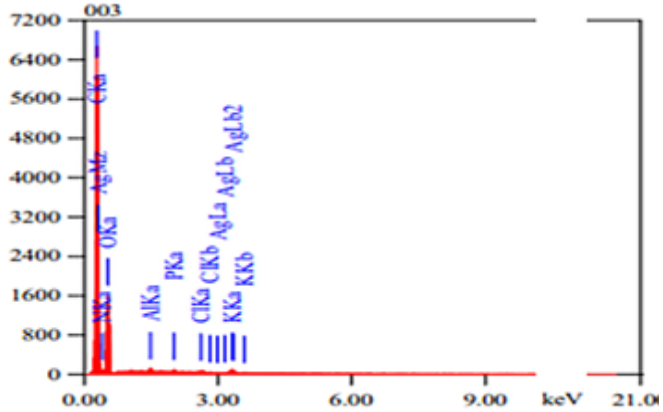


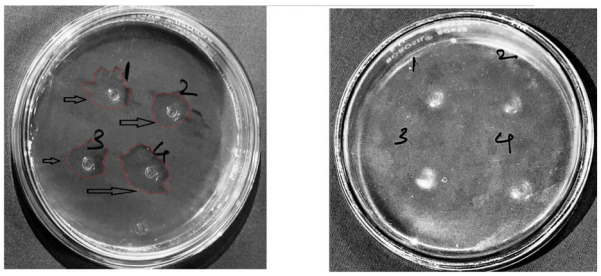
Figure 3: Elemental analysis - micrographs - Scanning Electron Microscopy and X-Ray Energy-Dispersive Spectroscopy - SEM/EDS

2.Anti-microbial activity: The descriptive data of the microbial inhibition zone diameter of the AgNPs coated and control study groups was shown in the (table 2)(figure 4). There was non uniform zone of inhibition, so the minimum and maximum diameters of each sample was noted. The mean inhibition zone of the test samples was 2.57+0.17 mm, which is significant compared to the control group. No inhibition zone was observed for all of the control group samples. As control group showed no inhibition zone, analytical statistical tests could not be performed as it is evident that test sample showed definite antimicrobial activity against *S.mutans* compared to control group.

3.Durability of the coating: The amount of silver ions (ppm) released from AgNPs coated elastomeric modules (n=25) in artificial saliva at 4 different time periods T1=24 hrs, T2=48hrs, T3=2 weeks, T4= 4 weeks was shown in the table 3. The mean ppm measured at T1, T2, T3, T4 are was 2.6+0.51, 6.6+0.67, 14.6+0.67, 18.4+ 0.87. The amount of silver ion accumulation into artificial saliva increased as the time elapsed and was statistically significant between different time points with a p-value of 0.001. Pair wise comparison of silver release between individual different time periods (table 4), showed z value -2.0026, P value of 0.0001. There was statistically significant intrapair differences in the release of silver at T1 compared to that of T2, T3, and T4.

Discussion

Numerous methods can be employed for the synthesis of NPs, but these methods are majorly divided into two main classes i.e. (1) Bottom-up approach and (2) Top-down approach[15]. In the present study, a bottom up procedure was utilized for preparation of green synthesized silver nanoparticles. In orthodontic literature, there was only one study[7] that have evaluated the antimicrobial efficacy of green synthesized silver nanoparticles coated on



A--- Test Group B- Control Group

Figure 4: Test samples showing inhibition zone and control samples showing no inhibition zone

Table 2: Inhibition zone observed in the test and control sample

Sample number	Zone of inhibition - Diameter (mm)			
	Test group (AgNPs coated)			Control group
	Minimum	Maximum	Average	
1	1.50	3.50	2.5	0
2	1.49	3.56	2.52	0
3	1.65	3.83	2.74	0
4	1.51	3.68	2.59	0
5	1.82	3.84	2.83	0
6	1.53	3.91	2.72	0
7	1.31	3.68	2.49	0
8	1.29	3.74	2.51	0
9	1.34	3.64	2.49	0
10	1.63	3.86	2.74	0
11	1.71	4.01	2.86	0
12	1.52	3.63	2.57	0
13	1.04	3.42	2.23	0
14	1.11	3.32	2.21	0
15	1.29	3.53	2.41	0
16	1.31	3.69	2.5	0
17	1.25	3.62	2.43	0
18	1.32	3.73	2.52	0
19	1.44	4.05	2.74	0
20	1.78	3.78	2.78	0
21	1.39	3.79	2.59	0
22	1.43	3.81	2.62	0
Mean ± S.D			2.57 ± 0.17	0

elastomeric ligatures. They have coated elastomeric modules using silver nitrate salts as metal-ion precursors and *Heterotheca inuloides* (H. inuloides) plant extract as bioreductant through a simple and eco-friendly method and is comparable to our study where *A.bisporous* was used as a bioreductant.

One of the fundamental property of all Nanoparticles is optical phenomenon, as with AgNPs they have yellowish grey colour, the colour change is due to surface Plasmon resonance (SPR) excitation in the metal nanoparticles[11]. In the current study, similar visible results were obtained regarding colour change, the coated modules turned from colourless to yellowish after 48 hrs confirming the surface coating of Ag particles.

The EDS analysis of AgNPs coated modules in the present study showed a mean mass percent of about 0.043 and atomic percent of 0.02 which is far less compared to the study of Hernandez et al [7], that obtained mass percent of 16.18, and the atomic per cent 2.16. This difference might be due to the variation of bioreductant utilised as extract in the studies. In the current study, the size of

Table 4: Pairwise comparison between different time periods - Wilcoxon rank test

Pairwise comparison		W value	Z value	'p" Value
T1	T2	0	2.0226	0.00001 **
	T3			
	T4			

synthesized nanoparticles was around 220nm which is within the nanoscale range. As per the definition is concerned, the nanoparticles size ranges from 10 nm to 1000nm. However, for nanomedical application the preferential range are within 200nm[16,17]. Various research studies by El-Sonbaty[18], Sujatha [19], Sneha Thomas[20] synthesised AgNPs from *A.bisporous* extract and obtained spherical shaped AgNPs with size ranges of 8–20 nm, 560nm to 710nm, 500nm respectively. The size and shape of AgNPs synthesized in our study is in tune with most these earlier studies.

In the present study the mean inhibition zone (figure-6, table 2) for the test group was 2.57 + 0.17 mm, which is much lesser than the inhibition zones against the other bacterial species tested in the literature. This might be due to the variation in the bacterial species tested (S.mutans) and also the size and concentration of AgNPs that have been coated and also onto the surface coated. However, the mean dimension of inhibition zone obtained in our study (2.5 + 0.1mm) is similar to that of the previous study with 2.0 ± 0.12 mm by Hernandez[7]. In another study conducted by Hima bindu [13], band material coated with AgNPs showed inhibition zone of 4 +0.3mm which is 1.5 times greater than the present study. The AgNPs used in their study was not green synthesized and might have had more antibacterial efficacy. In our study, compared to control group, the minimum inhibition zone exhibited by the modules was 1.04mm and maximum of 4.01mm. A study by PurvaVerma[21] showed a baseline percentage of WSL extension is 0.63 ± 0.04 % mm and increased to 13.87 ± 2.89 % after 1 year of treatment completion. So, when the baseline extension is considered AgNPs coated modules can be effective means for preventing WSL in the initial stages of WSL itself. As the microbial accumulation is more adjacent and around the bracket area, antibacterial effect of the AgNPs coated module of our study may be effective, which should be clinically tested to confirm.

In the present study, the release of silver ions (table-3) into artificial saliva from coated elastic rings at T1, T2, T3, and T4 demonstrated that the mean concentration of silver ion release seems to be doubled at T3 or at the end of second week. The highest concentration of Ag ion release was 18.5 + 0.5 ppm at the end of fourth week. Our data cannot be compared directly with other studies because of difference in the method of coating used, amount of silver nanoparticle deposited on to the testing surface, size and surface characteristics of a sample on which nanoparticle coating was done. A previous study [7]that reported silver nanoparticle coating on elastomeric modules using green synthesis, unfortunately did not

Table 3: Comparison of amount of the silver ion release (ppm) from five different samples at four different time periods - Friedman test

Time period	Sample size (n=5)	Min value	Max Value	Mean	SD	Friedman test value	P value
T1	5	2	3	2.6	0.51	15	0.001*
T2	5	6	7	6.4	0.67		
T3	5	14	15	14.6	0.67		
T4	5	18	19	18.4	0.87		

T1 = 24 hrs; T2 = 48 hrs; T3 = 2weeks; T4 = 4weeks; S.D. - standard deviation; Min-minimum; Max - maximum; (**p < 0.001 - highly significant; *p < 0.05 - significant

evaluate the release of Ag ions. Only few studies have evaluated the release of antimicrobial agents from surface coated elastomeric ligatures and bands. One of the earlier studies[13]evaluated the Silver ion release from Silver nanoparticle coated stainless steel band material. The mean concentration of silver ions released at 24 hours, 48 hours, and 1 week were 0.0236 ± 0.0067 ppm, 0.0221 ± 0.0056 ppm, 0.016 ± 0.0032 ppm respectively with maximum release at 24 hours. The continuous increased release of Ag ions in our study even after 48 hours might be due to improper stabilization of silver coating and the method of coating Silver nanoparticles. In our study the coating procedure is a biological process, whereas in the study mentioned above they used thermal evaporation technique using - vacuum coating unit. The difference might have also arisen due to the number of samples tested and the difference in the surface tested for coatings.

A study by Hyun sun jeon[22] evaluated the Chlorhexidine release from his study on elastic rings coated with Chlorhexidine. The continuous sustained release of antimicrobial agent was seen from the first hour itself and increased up to 24 hrs but no significant release was seen after 48 hrs. This was similar to our study in the aspect that our study also demonstrated continuous release of silver ions with the passage of time but in contrast to the above study, the release of silver ions continued in our study till the end of 4th week.

The stability of the products obtained is also an essential factor affecting the antibacterial activity with regards to the size and charge of the nanoparticles[23]. Low stability of the generated AgNPs makes them more likely to combine and form larger nanoparticles, which have lower antibacterial activity. This could have been a factor for a lower antibacterial activity observed in our study.

Biocompatibility and stability of AgNPs: Application of AgNPs in elastomeric modules directly contacts with the teeth and surrounding cells and tissues within the oral cavity. Therefore, besides undeniable contribution towards antimicrobial activity of AgNPs, some serious adverse events must be addressed to fulfil its safety requirements [24]. The concentration ranges of AgNPs that can induce toxicity depends on the particle size, temperature, type of medium, Particle crystallinity, surface functionalization, etc. Zhornik[25] demonstrated that the smaller nanoparticles with size less than 20 nm can cause structural modifications, e.g., changes in lymphocyte cell membrane morphology, with no similar effects observed for particles greater than 200 nm in size. It can be assumed until unless proven that the nanoparticle size in our study was greater than > 200nm and might have minor effect on the cell morphology. Further studies should be carried out in order to produce a stabilized nanoparticle using the capping agents so that the release AgNPs into oral cavity can be decreased and further enhancement of antimicrobial activity of the coated elastomeric modules can be expected.

Limitations

One of the main limitations in-vitro simulated study is that the elastic modules were exposed only to artificial saliva and cannot stimulate the multifaceted intraoral environmental milieu. The results obtained should be carefully applied to a clinical situation. However, further in vivo studies conducted in different oral environments are required to validate the findings of the present study. The inhibition zone observed was irregular as the coating was not uniform. We couldn't produce stabilized AgNPs onto elastomeric ligatures as there was increased silver release with time.

Conclusion

The effective size of AgNPs can be synthesized by green synthesis with *Agaricus bisporus* extract. The elastomeric modules coated using green synthesized silver nanoparticles have antimicrobial activity against *S.mutans* compared to non-coated elastomeric modules. The durability of the coating was shorter as there was increased release of silver nanoparticles into artificial saliva with increase in time.

References

1. Heymann G.C., Grauer D., A Contemporary Review of White Spot Lesions in Orthodontics, J Esthet Restor Dent,25(2),85–95(2013).
2. Ellwood R., Fejerskov O., Cury J., Clarkson B., Kidd E., Dental Caries: The Disease and Its Clinical Management. 2nd ed. Oxford; Ames, Iowa: Blackwell and Munksgaard Oxford (2008).
3. Van der Veen M.H., Attin R., Schwestka-Polly R., Wiechmann D., Caries outcomes after orthodontic treatment with fixed appliances: do lingual brackets make a difference?, Eur J Oral Sci,118(3),298–303(2010).
4. KamarudinY., Skeats M.K., Ireland A.J., Barbour M.E.,Chlorhexidine hexameta phosphate as a coating for elastomeric ligatures with sustained antimicrobial properties: A laboratory study, Am J Orthod Dentofac Orthop,158(5),73–82(2020).
5. Fahning Magno A., Enoki C., Ito I., Matsumoto M., Faria G., Nelson-Filho P., In-vivo evaluation of the contamination of Super Slick elastomeric rings by Streptococcus mutans in orthodontic patients, Am J Orthod Dentofac Orthop, 33, S104-9(2008).
6. Forsberg C.M., Brattström V., Malmberg E., Nord C.E., Ligature wires and elastomeric rings: two methods of ligation, and their association with microbial colonization of Streptococcus mutans and lactobacilli, Eur J Orthod,13(5),416-420(1991).
7. Hernández-Gómora A.E., Lara-Carrillo E., Robles-Navarro J.B., Scougall-Vilchis R.J., Hernández-LópezS., Medina-Solís C.E.,Biosynthesis of Silver Nanoparticles on Orthodontic Elastomeric Modules: Evaluation of Mechanical and Antibacterial Properties. Molecules,22(9),1407(2017).
8. Auxiliary Product [Internet]. TP Orthodontics. [Cited 2022 Feb 17]. Available from: https://www.tportho.com/auxiliary-product
9. Espinosa-Cristóbal L.F., Martínez-Castañón G.A., Martínez-Martínez R.E., Loyola-Rodríguez J.P., Patiño-MarínN., Reyes-Macías J.F, Ruiz F., Antimicrobial sensibility of Streptococcus mutans serotypes to silver nanoparticles, Mater SciEngC, 32(4),896-901(2012).
10. Mansoori GA., Principles of Nanotechnology: molecular based study of condensed matter in small system. World scientific publishing.co.Pte.Ltd, USA,(2005).
11. Narasimha G., Mallikarjuna K., DevaPrasad Raju B., Mushrooms (Agaricus bisporus) mediated biosynthesis of sliver nanoparticles, characterization and their antimicrobial activity, Int J Nano Dimens, 2,29-36(2011)
12. Anthony K.J.P., Murugan M., Jeyaraj M., Rathinam N.K., Sangiliyandi G., Synthesis of silver nanoparticles using pine mushroom extract: A potential antimicrobial agent against E. coli and B. subtilis, J Ind Eng Chem, 20(4),2325–2331(2014).
13. Bindu S.H., Vani S.K., Nirisha G ., Madhuri N ., Deepa B.S., Hemadri S., Evaluation of Antibacterial Effect of Silver Nanoparticle Coated Stainless Steel Band Material–An In vitro Study, Orthod J Nepal,9(2),13-9(2019).
14. Manzoor-ul-Haq V.R., Singh D ., Singh A.K., Ninganagouda S ., Hiremath J., Dried mushroom Agaricus bisporus mediated synthesis of silver nanoparticles from Bandipora District (Jammu and Kashmir) and their efficacy against methicillin resistant Staphylococcus aureus (MRSA) strains, Nano sci Nano technol Int J,5,1–8(2015).
15. Ijaz I., Gilani E., Nazir A., Bukhari A., Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles, Green Chem Lett Rev,13(3),223–245(2020).
16. Biswas A.K., Islam M.R., Choudhury Z.S., Mostafa A., Kadir M.F., Nano-technology based approaches in cancer therapeutics, Adv Nat SciNanosci Nanotechnol,5(4),1-11(2014).
17. Rizvi S.A.A., Saleh A.M., Applications of nanoparticle systems in drug delivery technology, Saudi Pharm J,26(1),64–70(2018).
18. El-Sonbaty S.M., Fungus-mediated synthesis of silver nanoparticles and evaluation of antitumor activity, Cancer Nanotechnol,4(4–5),73–79(2013).
19. Sujatha S., Tamilselvi S., Subha K., Panneerselvam A., Studies on biosynthesis of silver nanoparticles using Mushroom and its antibacterial activities, Int J Curr Microbiol App Sci, 2(12),605-614 (2013).
20. Thomas S., Athar D., Biosynthesis of silver nanoparticles using dried Agaricus bisporous edible mushroom and its antimicrobial activity against human pathogens, Int J Eng Appl Sci Technol,5(1),763–767(2020).
21. Verma P., Ravindra Jain K ., Visual Assessment of Extent of White Spot Lesions in Subjects treated with Fixed Orthodontic Appliances: A Retrospective Study, World JDent,13(3), 246-249(2022).
22. Jeon H.S., Choi C.H., Kang S.M., Kwon H.K., Kim B.I., Chlorhexidine-

releasing orthodontic elastomerics, Dent Mater J,34(3),321-326 (2015).
23. Chen J., Li S., Luo J., Wang R., Ding W., Enhancement of the antibacterial activity of silver nanoparticles against phytopathogenic bacterium Ralstonia solanacearum by stabilization, J. Nanomater,2016,1-15 (2016).
24. Bapat R.A., Chaubal T.V., Joshi C.P., Bapat P.R., Choudhury H., Pandey M., An overview of application of silver nanoparticles for biomaterials in dentistry, Mater Sci Eng C Mater Biol Appl, 91,881-898(2018).
25. Zhornik A., Baranova L., Volotovski I., Chizhik S., Drozd E., Sudas M., Interaction of nanosilver particles with human lymphocyte cells, Adv Nat Sci Nanosci Nanotechnol, 6(2),1-6 (2015).