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Comparison of Antimicrobial Activity and Mechanical Properties of Elastomeric Ligatures Coated with Chlorhexidine Hexametaphosphate with Acetone and Ethanol Conditioning - An in Vitro Study

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Abstract

Introduction: One of the most critical side effects of fixed orthodontic treatment is white spot lesions formation. Adding antimicrobial coatings to elastomeric modules might be an effective method to prevent white spot lesions.

Aim: The study aims to compare the antimicrobial and mechanical properties of elastomeric ligatures coated with chlorhexidine hexametaphosphate after acetone and ethanol conditioning and to assess surface coating of ligatures through scanning electron microscope.

Material and Method: Orthodontic elastomeric modules were categorized into 3groups - unconditioned or deionised water, ethanol conditioned and acetone conditioned. All three groups were coated with chlorhexidine hexametaphosphate. The antibacterial activity was tested against Streptococcus Mutans, ultimate tensile strength was measured using a universal testing machine. The surface coatings were examined using scanning electron microscopic analysis.

Results: Mean and multiple intra-group comparisons showed a statistically significant difference in antibacterial activity. Ethanol conditioning improved the antibacterial activity without affecting mechanical properties. The antibacterial activity was not enhanced by acetone conditioning and chlorhexidine coating, and mechanical qualities were degraded.

Conclusion: Ethanol-conditioned and Chlorhexidine hexametaphosphate coatings on elastomeric modules showed significant and better antimicrobial activity without affecting mechanical properties. Conditioned and coated elastomeric modules can be used in the prevention of white spot lesion formation.

Keywords: Chlorhexidine Hexametaphosphate, Antibacterial, Elastomeric Modules

Introduction

Fixed appliances are most commonly used and most appropriate for treating dental malocclusions [1]. But, orthodontic treatment with fixed appliances naturally leads to various plaque retention sites due to bands, arch wires, and brackets[2,3], thereby increasing caries and inflammatory reaction in the gingival tissue and subsequent increase of oral bacteria during orthodontic treatment[4].

The principal causative agent of dental caries has been identified as Streptococcus Mutans. It is a gram-positive and anaerobic bacteria. A crucial aspect of S. Mutans virulence is its capacity to produce biofilms resembling dental plaque. S. Mutans plays a significant role in tooth decay by metabolizing sucrose to lactic acid. The mouth becomes acidic as a result of this process, making the highly mineralized tooth enamel susceptible to decay.

The most common side effect of orthodontic treatment is enamel surface decalcification near fixed orthodontic appliances. The most common complication is the formation of WSLs [5].This demineralization is because of an increased number of Streptococcus Mutans, other microbes, low pH, and poor oral hygiene [6]. In some patients, these appear as small lines, and in some, they appear as large decalcified areas [7]. These are characterized by opacity and loss of minerals, which increases the porosity of the tooth's enamel surface and gives it a chalky white appearance.

Self-cleaning becomes difficult because of uneven surfaces of brackets, bands, archwires, and other orthodontic attachments thus, leading to plaque accumulation.

Many approaches have been described to detect white spot lesions, like fibre optic trans-illumination, ultraviolet light application, fluorescent dye uptake, and laser fluorescence. But, photographic images are used routinely[8].

White spot lesions require comprehensive management. Oral hygiene control remains the first preventive measure to control WSLs. Proper tooth brushing twice daily with a power toothbrush or daily irrigation might decrease the accumulation of plaque.

Other methods include fluoride products, CPP-ACP, probiotics, and polyols[9]. Fluoride products include fluoride mouthwashes, gels, toothpaste, fluoride varnishes, fluoride in bonding agents, and elastomers[10]. Fluoride toothpaste with high fluoride concentration is more effective, but it can't be used in patients under 16 years [11]. A systematic review was carried out by **Benson et al.**, which recommended 0.05% sodium fluoride mouthwash daily during orthodontic treatment to prevent enamel demineralization[12]. Fluoride varnishes are proven to be a safe fluoride application method and help decrease caries incidence and enamel demineralization[13].

Advantages of fluoride varnish over other topical fluoride methods include protecting enamel without patient compliance, and the fluoride release is continuous for a more extended period. CPP-ACP affects demineralization & remineralisation process by absorption through the enamel. It has anti-cariogenic activity by incorporating nano complexes of calcium and phosphate into dental plaque and tooth surface[14].

Apart from using these external agents, researchers are continually trying to modify the orthodontic biomaterials that would prevent microbial building upon them and are easy to clean. One such attempt is the introduction of self-ligating brackets or ligature-less bracket systems, but they are more expensive than the tie wing brackets, and their clips are prone to breakage.

Recent studies have concentrated on the incorporation of antimicrobial agents in orthodontic adhesives and coated brackets[15,16,17]. These materials sustain in the oral cavity for the entire duration of the treatment, and they are likely to lose their antimicrobial property with time. On the contrary, orthodontic ligatures are located near the tooth surface, and they are replaced regularly during orthodontic treatment. Elastomeric modules may be used as potentially vectors for localized antimicrobial delivery in prevention of white spot lesions formation in orthodontic patients.

The most commonly used agent to prevent microbial build up is chlorhexidine. Chlorhexidine belongs to the cationic bis biguanide class of drugs, and it is effective against both gram-negative(-) and gram-positive (+) bacteria as well as yeasts [18,19]. Chlorhexidine acts by increasing cell wall permeability and causes cell death. Chlorhexidine is used in various products like mouthwashes, gels, and varnishes to prevent dental diseases in orthodontic patients [20]. The main advantage of chlorhexidine is its sustained substantivity over long periods [21]. This property of chlorhexidine can help orthodontic patients as they have monthly appointments.

Ethanol is an organic compound and simple alcohol. It is a volatile, flammable liquid that is colourless and has a distinctive wine-like smell and pungent taste. Many types of mouthwash use ethanol as a dispersant and transporter for the active components [22]. The artificial media chosen for ageing experiments in vitro may include ethanol/water solutions for the majority of polymeric dental materials [23]. By solvent conditioning with ethanol, the functionalization of orthodontic ligatures with CHX-HMP was enhanced in a study [24].

Acetone is an organic compound which is the simplest and smallest of ketones. It is highly flammable and volatile liquid with a characteristic pungent odour. In dentistry, it serves as a solvent in dental adhesives. RM Santana used acetone as an organic solvent for removing metal brackets [25]. When coating chlorhexidine hexametaphosphate on elastomeric modules, Yasmin Kamarudin employed acetone as a conditioning agent. He discovered that acetone conditioning decreased mechanical characteristics and did not improve CHX-HMP absorption or subsequent CHX release[24].

Recently nanotechnology has gained popularity in dentistry. Nanoparticles usually refer to particles with 1-100nm[26]. Nanoparticles can interact efficiently with microbial membranes and provides greater surface area for antimicrobial activity. These nano particles can be used as device coatings, and topical agents, and incorporated within dental materials.

Irania et al in their study used silver nanoparticles on orthodontic brackets as an alternative in preventing WSL formation [28]. Barbour et al. reported on the synthesis of new antibacterial NPs based on the hexametaphosphate salt of chlorhexidine [29].Subramani K *et al.* found that CHX HMP coating orthodontic elastomeric chains have an antibacterial effect that would reduce plaque formation and prevent WSLs [30]. Another study by Kamarudin Y *et al*. observed that elastomeric ligatures, when coated with CHX HMP after ethanol conditioning had a sustained release of CHX and might prevent WSLs in orthodontic patients[24]. The antimicrobial effect of chlorhexidine hexametaphosphate on commercially available elastomeric ligature has not been evaluated. Thus, the purpose of this study is to evaluate and compare the antimicrobial property and mechanical characteristics of chlorhexidine-coated elastomeric ligatures after acetone and ethanol conditioning, and to study the surface coatings of ligatures through scanning electron microscopy.

Aims and Objectives

This study aims to compare the antimicrobial property of elastomeric modules coated with chlorhexidine hexametaphosphate with acetone and ethanol conditioning. The objective of the study is to assess the antimicrobial property of coated elastomeric modules, mechanical properties of ligatures, and surface coating of ligatures through SEM.

Materials and Methodology

Chlorhexidine Hexametaphosphate Preparation:

Chlorhexidine hexametaphosphate solution was prepared using the **Barbour et al**.[29] method from chlorhexidine digluconate (Yarrow Chem Products, Ghatkopar, Mumbai) and sodium hexametaphosphate (Isochem Laboratories, Angamaly, Kochi). From a 20% solution, 5mM chlorhexidine digluconate was made by mixing 133.7ml of distilled water and 2ml of chlorhexidine digluconate. By combining 0.305gm sodium hexametaphosphate powder with 100ml distilled water, a 5mM solution of sodium hexametaphosphate is created. An aqueous solution of 5mM chlorhexidine hexametaphosphate (CHXHMP)[Fig.1] was made by continuously mixing 100ml of aqueous sodium HMP and 100ml of aqueous CHX digluconate at room temperature.

Preparation of Nanoparticle-Coated Orthodontic Elastomeric Modules

A total of 72 orthodontic elastomeric ligatures (Gray, AlastiK^{TM}; 3M Unitek Orthodontics products, South Peck Road, USA) were divided into following groups

Group 1 (Deionised water or No conditioning)

Group 2 (Ethanol conditioning)

Group 3 (Acetone conditioning)

Before usage, all the groups were cleaned in deionised water and left for an hour to air dry. Following that, for 60 minutes, group 1 modules were submerged in deionised water, group 2 modules were immersed in ethanol, and group 3 modules were submerged in acetone. The modules in all three groups were immediately immersed in freshly made chlorhexidine hexametaphosphate solution for 10 minutes while being stirred. Following a further 30 second submersion in deionised water to remove any remaining unbound material, samples were allowed to air dry for an additional hour.

Antimicrobial Activity of Coated Ligatures:

Disc Agar Diffusion Test (DAD)

This test evaluates an antimicrobial agent's capacity to infiltrate inside agar and create a bacterial inhibition zone. The density of bacterial suspension was adjusted with sterile phosphate buffer saline (PBS) to match the density of Mc Farland standard 0.5. Now a hundred micro liters from bacterial suspensions (~10 to power 8CFU/ml) were spread on the Blood Agar Plate via a sterilized swab, and CHX HMP coated elastomeric modules [Fig.2] were positioned on the surface of the plates 2cm apart from each other. Following incubation for 48 hours, the bacterial growth inhibition zones[Fig.3] were measured using the zone of the inhibition measuring scale.

Ultimate tensile strength

The universal testing apparatus was used to determine the ultimate tensile strength and elastic modulus. U-shaped hooks made of 0.8 mm stainless steel wire that was fastened to the cross-head of a universal testing machine were used to strain elastomeric ligatures on the machine until they snapped [Fig.4].The **Kovatch et al.** guideline was followed, and 5mm/min cross head speed was used for this. The ultimate tensile strength and elastic modulus of the ligatures were calculated from the load extension curve of each elastomeric module. Tensile strength mean and standard deviation values were determined for each group.

Microscopic analysis

The surface of the modules coated with nanoparticles were examined using a scanning electron microscope (SEM). SEM observations of all the groups were made to assess surface changes in coatings (fig.5,6.7).

Observation and Results

Statistics

Mean, median, and standard deviation were calculated using descriptive statistics for each group. The data were expressed in MEAN±SD. IBM Statistical Package for Social Sciences (SPSS 20.0) version was used for statistical analysis. To determine statistical significance between and within the groups, the Kruskal Wallis test and Mann Whitney U test were applied. Statistical significance is defined as a p-value of 0.05 or below ($p \leq 0.05$).

Table 1: Comparison of Force at rupture (N) and Extension at rupture (mm) between different groups

Characteristics	Groups	Mean	Median	Standard deviation	P value
Force at rupture(N)	Group 1	24.3500	24.3000	1.28062	0.000*
	Group 2	26.3250	26.4000	.83452	
	Group 3	23.0125	22.8000	.97312	
Extension at rupture (mm)	Group 1	14.2010	14.0040	.58445	0.004*
	Group 2	16.9378	17.4020	1.24103	
	Group 3	14.3099	14.3535	.43623	

* - statistically significant, P-value set as <0.05, Statistical Analysis: Kruskal Wallis test

Inference: There is a statistically significant difference in mean force at rupture and mean extension at rupture for three groups. The mean force at rupture of group 2(26.3250) is highest among the groups and was reduced for group 1(24.3500) and is further reduced in group 3(23.0125). The mean extension at rupture of group 2 (16.9378) is highest and was reduced for group 1(14.2010) and group 3 (14.3099).

Table 2: Pair wise comparison Force at rupture (N) and Extension at rupture (mm)

Characteristics	Pair 1	Pair 2	Mean Difference	P value
Force at rupture(N)	Group 1	Group 2	-1.97500*	.002*
		Group 3	1.33750*	.038*
	Group 2	Group 3	3.31250*	.000*
Extension at rupture (mm)	Group 1	Group 2	-2.73675*	.002*
		Group 3	-.10888	.645
	Group 2	Group 3	2.62787*	.005*

* - statistically significant, P-value set as <0.05, Statistical Analysis: Mann Whitney U test

Inference: There is a significant difference in the mean force at rupture of control group versus all groups. There is no significant difference in the mean extension at rupture of control group and group 3. There is a statistical significant difference in mean extension at rupture of group 2 versus all groups.

Table 3: Comparison of Zones of inhibition (mm) between different groups

Characteristics	Groups	Mean	Median	Standard deviation	P value
Zones of inhibition (mm)	Group 1	8.3750	8.0000	1.18773	0.001*
	Group 2	11.0000	11.0000	.92582	
	Group 3	9.1250	9.5000	.99103	

* - statistically significant, P-value set as <0.05, Statistical Analysis: Kruskal Wallis test

Inference: There is a significant difference in mean antibacterial activity against S. Mutans between the groups. The mean diameter of bacterial inhibition zone in group 2 (11.0000) is having highest mean and it was reduced for group 3(9.1250) and further reduced in control group (8.3750).

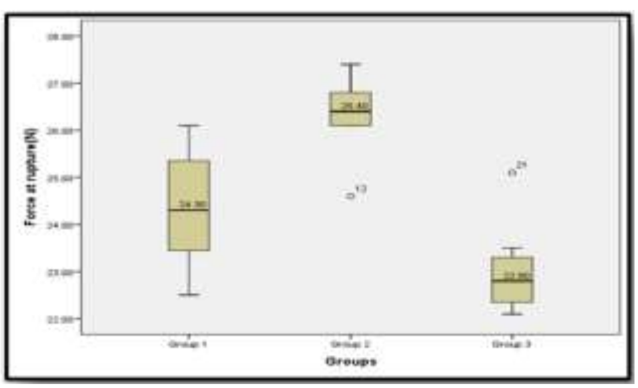
Table 4: Pair wise comparison of Zones of inhibition (mm)

Characteristics	Pair 1	Pair 2	Mean Difference	P value
Zones of inhibition (mm)	Group 1	Group 2	-2.62500*	.001*
		Group 3	-.75000	.234
	Group 2	Group 3	1.87500*	.005*

* - statistically significant, P-value set as <0.05, Statistical Analysis: Mann Whitney U test

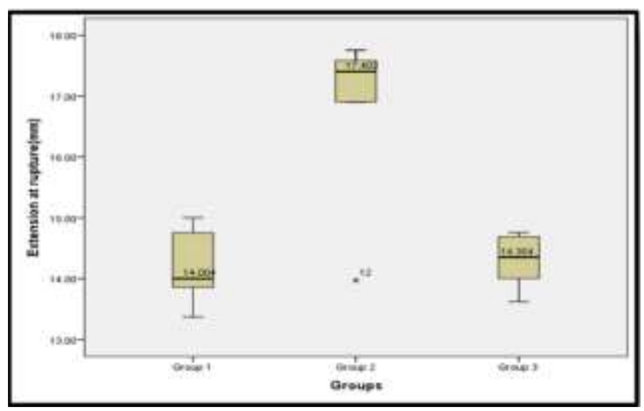
Inference: The antibacterial activity of group 1 and group 3 showed no statistically significant difference. Comparing group 2 to the other groups, there is a statistically significant difference. Zones of inhibition of group 1 compared with group 2 showed a P value of 0.001 which is significant. Group 1 compared with group 2 has no significance with P value 0.234. When comparing group 2 with group 3, P value is 0.005 which shows statistically significant.

Graph 1: Force at rupture (N) in different groups



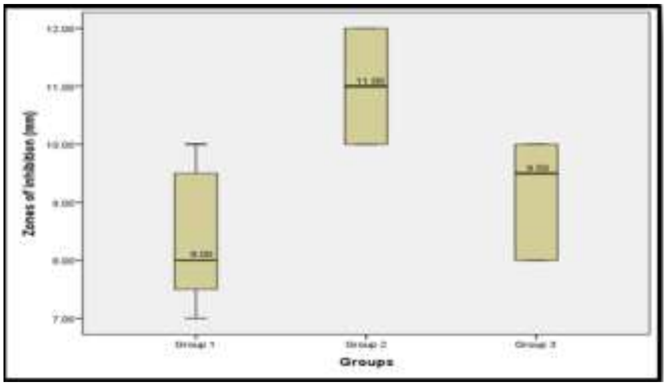
This graph shows the mean force at rupture is highest in group 2 (26.40) followed by group 1(24.30) and least for group 3 (22.80).

Graph 2: Extension at rupture (mm) in different groups



This graph shows mean extension at rupture is highest for group 2 (17.402) and reduced for group 3 (14.354) and group 1 (14.004).

Graph 3: Zones of inhibition (mm) in different groups



This graph shows the mean diameter of bacterial inhibition zone is highest in group 2 (11.00) followed by group 3 (9.50) and group 1 (8.00).

Scanning Electron Microscope Results:

Scanning electron microscopic images revealed uneven coatings of CHX HMP nanoparticles on the surfaces of elastomeric modules in all the three groups.

Discussion

Orthodontics is a branch of dentistry that focuses on correcting skeletal and dental malocclusions along with meeting the aesthetic and functional demands of patients, not only these but it also ensures long-term advantages in terms of oral health of the patients. Fixed orthodontic treatment involves the bonding of brackets to tooth enamel with composite resins and securing the archwire into the bracket slot with elastomeric ligature ties and elastomeric chains. The average duration of treatment is less than 2 years [31].

Oral hygiene is the practise of maintaining a healthy, disease-free mouth. Oral hygiene can directly or indirectly affect the timing, quality, and success of orthodontic therapy. Critical factors in attaining optimal oral hygiene require thorough professional instructions, suitable tools, and patient compliance. Studies have shown that oral hygiene compliance rapidly declines after the initial bonding [32,33,34].

Fixed orthodontic appliances improve aesthetics and have beneficial effects on oral health and the quality of patients [35]. However, it also associates risks and complications of fixed appliance therapy, which leads to plaque accumulation, causing a rapid shift in the microbial flora of plaque resulting in elevated levels of acidogenic bacteria, most notably streptococcus[36].

De so et al. proposed that S. Mutans are the primary bacteria responsible for enamel demineralization [37]. S. Mutans is an aerobic bacteria that produces lactic acid as part of its metabolism. They bind to tooth surfaces in sucrose by water-soluble glucan formation, which aids in binding bacteria to the tooth and causes damage to the rigid tooth structure. Unlike the majority of oral microorganisms, S. Mutans is also found on healthy surfaces. Its presence on any surface indicates that the disease may be present or may develop in future. S. Mutans is a strong acid producer hence causing an acidic environment and creating a risk for caries. The most critical virulence property of S. Mutans is the ability to form biofilm known as dental plaque, which offers a fantastic adhesion location for bacterial development and colonisation. This plaque accumulation can be due to self-cleaning difficulty because of uneven surfaces of bands, brackets, wires, and other attachments that lead to the formation of white spot lesions.

On the smooth surfaces of teeth, white spot lesions, also known as white opacity, are caused by subsurface enamel demineralization. Within a month following the insertion of the brackets, WSLs can be detected around the brackets of fixed appliances. These lesions are frequently seen on the buccal surfaces of the teeth, all around brackets, and particularly in the gingival region. Depending on the method of assessment, the extent of white spot lesions ranges from 4.9% to 84% of the tooth surface [38]. Mitechell found that an overall prevalence of 18.5% of the tooth surface was affected.

Elastomeric ligatures are frequently used in orthodontics, and because of their near proximity to the enamel surface, they are convenient and excellent vectors for the delivery of antimicrobials. They reduce the need for patient cooperation in the prevention of WSLs. Although the idea of targeted antibiotic administration is not new, clinical and laboratory research findings have been in consistent. This could be a result of the delivery method or, in rare situations, the experimental layout. Fluoride-releasing ligatures were tested in one study using a randomized crossover clinical trial; however, it was shown that the anti-cariogenic impact was not statistically significant [39], which was attributed to the fluoride release's transient nature [40]. In contrast, a different case-control study found that the use of fluoride-releasing elastomeric ligatures and chains significantly decreased the number of WSLs that were observed [41]. Additionally, elastomeric ligature coatings made of silver nanoparticles have been investigated. Although the duration of the coating and the efficacy were not investigated, a single in-vitro investigation found that silver nanoparticles had local antibacterial efficacy against bacteria like Streptococcus Mutans and Lactobacillus casei [42]. Streptococcus Mutans' capacity to colonise ligatures was examined in a different investigation, but no discernible inhibitory effect was discovered[43].

The introduction of nanoparticles as antimicrobial agents has attracted much attention in the field of dentistry and medicine. The antibacterial properties of these nanoparticles are because of their unique physicochemical properties such as large surface area to mass ratio, small sizes, and increased chemical reactivity. They are a new strategy for treating and preventing dental infections. They cause discrete and distinct types of injuries to microbial cells as a result of oxidative stress, protein dysfunction, or membrane damage [44].

Chlorhexidine has been extensively studied for the possibility of antimicrobial resistance, and results show that when exposed to higher environmental concentrations of CHX, the organism becomes less sensitive to it. However, this change is reversible when the CHX stimulus is removed, indicating that such changes are not true resistance [45]. Because of this, CHX has been considered a potential choice for the creation of antimicrobial materials and products that don't increase the need for antibiotics [46]. The CHX HMP nanoparticles were created by Barbour et al. by mixing CHX with Na HMP at ambient temperature and pressure while swirling rapidly [29]. These nanoparticles could be used in a wide variety of consumer goods and healthcare applications.

Ethanol and acetone are mainly used as solvents in dental adhesives. In a study done by Yamin Kamarudin et al, he employed ethanol and acetone as conditioning agents on elastomeric modules before CHX HMP coating [24]. Acetone conditioning decreased the mechanical characteristics and did not improve CHX-HMP absorption or subsequent CHX release. Conditioning with ethanol did not produce any statistically significant changes to the physical parameters under evaluation. In another study by RM Santana et al, he used acetone as an organic solvent for removing metal brackets [25].

The purpose of this study was to evaluate and compare ultimate tensile strength and antibacterial properties of an elastomeric module coated with CHX HMP after conditioning with acetone and ethanol. The ability of novel salt, CHX HMP, to release CHX over several weeks or months under aqueous conditions has been reported elsewhere [29,47]. However, the antibacterial property of conditioned (ethanol and acetone) and unconditioned elastomeric modules have not been reported.

In the present study disk agar diffusion test showed significant growth inhibition against S. Mutans which were coated with CHX HMP. Elastomeric modules coated with CHX HMP followed by ethanol conditioning showed the highest antibacterial activity among the groups and there was no significant difference in antimicrobial activity in unconditioned and acetone-conditioned modules.

The results of this study are in accordance with the study done by Kamarudin et al. in which he measured the release of CHX from orthodontic elastomeric modules after ethanol and acetone conditioning [24]. He concluded that CHX release was enhanced by conditioning with ethanol. In a study done by Hyun-Sun JEON et al, he used different combinations of polymers and solvents for the prevention of oral disease in orthodontic patients [21]. He concluded that the antibacterial activity of CHX can be adjusted according to combinations of polymers and solvents.

Subramani et al stated that orthodontic elastomeric chains coated with CHX HMP nanoparticles can offer antibacterial activity to help reduce biofilm development and prevent white spot lesions and offers promising clinical applications [30].

In terms of ultimate tensile strength, the results of the present study showed that the force at rupture increased in ethanol-conditioned modules compared to the control group and decreased in acetone-conditioned modules. The mean extension at rupture was highest in ethanol-conditioned modules and decreased in unconditioned and acetone-conditioned modules. The results showed that ethanol conditioning did not significantly affect maximum extension and maximum force delivery.

The present study is in accordance with the study done by Kamarudin et al. in which he tested the mechanical properties of CHX HMP coated modules and concluded that ethanol-conditioned elastomeric modules did not affect force and extension at rupture [24]. In another study done by Subramani et al., he coated elastomeric chains with CHX HMP nanoparticles and concluded that the coatings did not alter the force decay of elastomeric chains [30].

The scanning microscopic analysis images revealed that elastomeric modules were coated with CHX HMP nanoparticles in all three groups but the coatings are not uniform. As a consequence of solvent conditioning, there were no observable differences between the surface CHX HMP coating. Acetone-conditioned modules showed a colour change after conditioning and there was no colour change in other groups as judged by eye.

The findings of the present investigation suggested that coatings on elastomeric modules with ethanol conditioning have the highest antibacterial activity. Acetone-conditioned and CHX HMP coatings altered the mechanical properties of modules.

Conclusion

Plaque accumulation and decalcification are the common drawbacks of fixed orthodontic treatment. Since fixed appliances are recommended to be used for a longer period, the risk of carious lesions may be expected on the bonded tooth surfaces. This study compares the antimicrobial activity of elastomeric modules coated with chlorhexidine hexametaphosphate (CHX HMP) with acetone and ethanol conditioning and this study also assesses the surface coating of ligatures through SEM and mechanical properties.

5mm CHX HMP was prepared by continuously mixing 100ml of aqueous sodium HMP and 100ml of aqueous CHX digluconate at room temperature. A total of 72 modules were divided into three groups of 24 each; Group 1 no conditioning, Group 2 ethanol conditioned, and Group 3 Acetone conditioned. After conditioning, CHX HMP was used to coat the elastomeric modules. The coated modules were tested for ultimate tensile strength using Universal Testing Machine. The force required for the rupture of elastomeric modules was recorded and the extension at rupture was also measured. Antibacterial analysis against Streptococcus mutans was performed using blood agar plates. The antibacterial property was determined by measuring the zones of inhibition around the module. SEM analysis was done to determine changes in the surface of coated modules.

Statistical analysis was performed to know the significance of the difference between the groups. The analysis showed that there was a statistically significant difference in antibacterial activity within the three groups with the highest antibacterial activity in ethanol conditioned group. There was a statistically significant difference in ultimate tensile strength within the three groups. SEM analysis revealed the surface coatings of chlorhexidine in all three groups.

In view of the findings of the present study, the following conclusions are drawn:

1. Ethanol-conditioned and CHX HMP coated elastomeric modules showed significant and better antimicrobial activity compared with unconditioned and acetone-conditioned modules.
2. There is deterioration in mechanical properties in acetone-conditioned and CHX HMP-coated modules with no significance in ethanol-conditioned elastomeric modules.

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Legend Figures

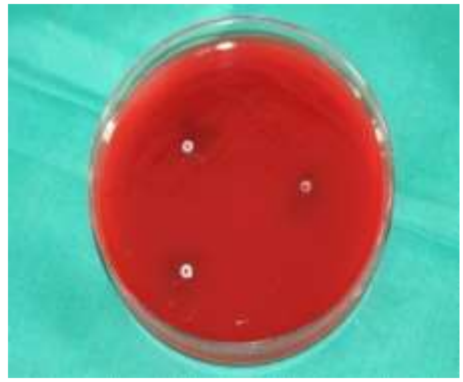

Figure 1: Chlorhexidine Hexametaphosphate solution

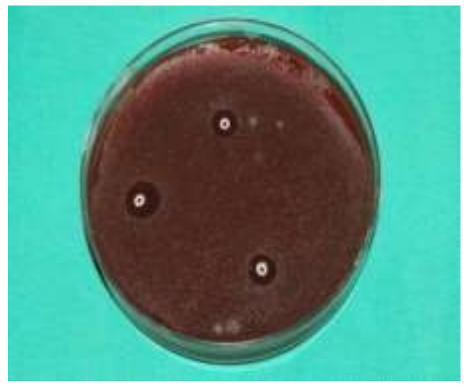

Figure 2: Disc Agar Diffusion Test

Figure 3: Zones of Inhibition

Figure 4: Ultimate Tensile Strength

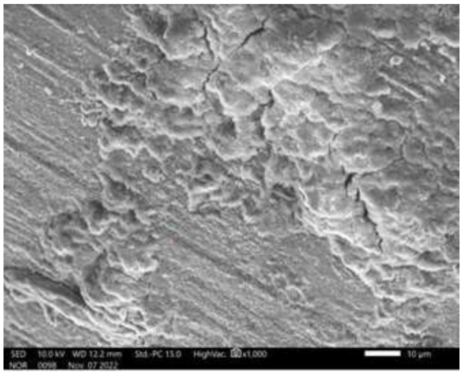

Figure 5: SEM images of elastomeric modules with no conditioning and CHX-HMP coatings

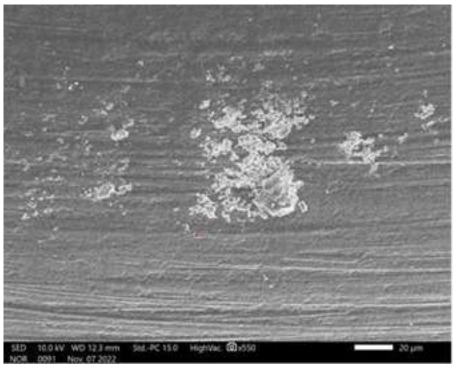

Figure 6: SEM images of elastomeric modules with ethanol conditioning and CHX-HMP coatings

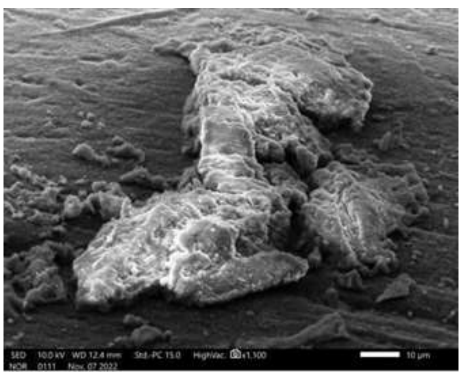

Figure 7: SEM images of elastomeric modules with acetone conditioning and CHX-HMP coatings