Effect of Curcumin on Bacterial Biofilm Adhesion and Surface Roughness of Orthodontic Archwires- In Vivo Study

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ABSTRACT
Background: To evaluate the effect of curcumin on bacterial biofilm adhesion and surface roughness of orthodontic archwires.

Materials & Methods: A randomized trial was conducted with a sample size of 20 individuals having OHIS score between 0-1.2 i.e. good. Effect of curcumin mouthrinse on biofilm adhesion and surface roughness of orthodontic archwires after intraoral exposure of 8 weeks with and without use of curcumin mouthrinse was evaluated and compared.

Results: The mean biofilm attachment and surface roughness after intraoral exposure of 8 weeks without use of curcumin mouthrinse was 5.3489 CFU and 0.2855 µm respectively and mean biofilm attachment of another wire after intraoral exposure with the use of curcumin mouthrinse was 2.3308 CFU and 0.1014 µm which shows significant reduction in biofilm attachment and surface roughness after use of curcumin mouthwash.

Conclusion: Curcumin mouthwash has antimicrobial efficacy and it was biocompatible and acceptable by patients without any adverse effects. The use of curcumin mouthrinse. It also shows indirect effect on reducing surface roughness of orthodontic archwires which is important from clinical point of view as rougher archwires induce greater friction and retard tooth movement, therefore it can be used as an adjunct to orthodontic treatment.

Keywords: Curcumin, orthodontic archwires, biofilm attachment, surface roughness.

INTRODUCTION
During orthodontic therapy, orthodontic attachments like bands, brackets, archwires and the different orthodontic elements (elastics, power chains, sleeves, springs) are used. These elements accelerate the accumulation of bacterial plaque as they create new retention areas that are suitable for bacterial colonization and lead to an increase in the number of microorganisms.

This dental plaque is a highly complex organization in a biofilm form.
Bacterial biofilm is the paramount factor in initiation and progression of gingival and periodontal diseases. Fixed orthodontic appliances significantly enhance the colonization of Streptococcus mutans and Lactobacilli, as per given in several studies. Periodontal pathogens such as Actinobacillus actinomycetemcomitans and Tannerella forsythia have also been significantly associated with gingival inflammation during orthodontic therapy.23

The incidence of enamel demineralization can occur in up to 50% of orthodontic patients and if they are left untreated, they may produce carious. Thus prevention of these lesions has been an important concern for orthodontists.

Biofilm adhesion to orthodontic bands, brackets and archwires also affect surface characteristics of these materials. Increased surface roughness can increase frictional forces as it enhances the contact area between the bracket and the wire. This can, in turn, reduce the orthodontic force by 50% or more, thereby lowering the quality of orthodontic treatment.45

Thus considering consequences of bacterial biofilm, maintenance of oral hygiene is crucial in orthodontic patients. Maintenance of oral hygiene is done by using different plaque control methods which include mechanical and chemical methods. But difficulties to achieve ideal mechanical plaque control due to lack of motivation and of manual dexterity have led many researchers to use other plaque control methods such as chemical agents.

Several clinical trials have been conducted in order to test the efficacy and safety of these products. Among all, chlorhexidine gluconate (CHX) is regarded as gold standard. However, it shows certain side effects such as brown discoloration of the teeth, oral mucosal erosion, and bitter taste on long term use.6 Hence, a need was felt of an alternative medicine that could provide a product already enmeshed within the traditional Indian set up and is also safe and economical.

Various formulations of curcumin in the form of powder, paste, gel, and poultice have been extensively used proving its various pleiotropic effects. It is known for its formidable effect as an anti-inflammatory, antioxidant, and antimicrobial properties.7 Due to these versatile properties, curcumin mouthrinse is used in this study.

Adverse effects of bacterial biofilm adhesion on gingival and periodontal heath, formation of white spot lesion and increase in surface roughness, affects overall orthodontic treatment. However limited literature is available regarding in vivo assessment of biofilm adhesion and surface roughness on orthodontic archwires and effect of curcumin on these parameters. Thus present study was conducted with an aim to evaluate effect of curcumin on bacterial biofilm adhesion and surface roughness of orthodontic archwires after intraoral exposure for 8 weeks without use of curcumin mouthrinse and with use of curcumin mouthrinse.

Materials and Methods
After obtaining institutional ethical committee approval, 20 individuals having OHIS score between 0-1.2 i.e. good, visiting the Department of Orthodontics and Dentofacial Orthopaedics, at the Swargiya Dadasaheb Smruti Dental College & Hospital, Nagpur were recruited for the study.

Inclusion criteria
i. Individuals of age ranging from 15 to 25 years with satisfactory oral hygiene having Oral hygiene index-simplified score between 0-1.2 i.e. good.
ii. Individuals of permanent dentition with full component of teeth

Exclusion criteria
i. Individuals with gingival and periodontal diseases.
ii. Individuals with systemic disorders.

Study Design
Patients were motivated to follow Modified Bass method in terms of sustaining good oral hygiene. They were instructed to avoid usage of any antibiotics or antibacterial mouthwashes or undergo professional tooth cleaning during the period of the study.

In these selected individuals fixed orthodontic mechanotherapy was initiated with 0.022 inch slot MBT prescription. Initial alignment and levelling was sequentially started from smaller dimension wires like 0.014” NiTi, progressing for higher size wires upto 0.016 x 0.022” SS archwire which was followed by insertion of sterilized 0.017x0.025 inch stainless steel (G&H Manufacturer) archwire. The autoclave
Sterilization was used to sterilize 0.017x0.025 inch stainless steel wire with a protocol of 134°C at 2.1 kg/cm² for 18 mins (12 min sterilization + 6 min drying period).

Archwires were carefully ligated using stainless steel ligatures and were kept for 8 weeks into patient’s oral cavity then they were removed for evaluation of biofilm attachment and surface roughness. These archwire were cut in 2 pieces with the help of sharp cutter. One piece of archwire in molar region was sent for examination of biofilm attachment and remaining piece of archwire was sent for surface roughness evaluation.

**Biofilm attachment of archwires**

The archwires were removed carefully to avoid iatrogenic biofilm dislodgement. Each arch wire was placed into a self-closing plastic container in 0.9% normal saline solution. The name and serial number of the patient were written on the outer surface of each container. It was then sent to Microbiology laboratory within 2 hours of removal of archwire for further testing.

In the Microbiology laboratory cut piece of archwire was rolled on to Blood agar and incubated at 37°C for 48 hours. This is to facilitate growth of microorganisms for identification during colony count test. The amount of bacterial adhesion was quantified using the colony-count method.

**Surface roughness of archwire**

For assessment of surface roughness, cut piece of archwire was placed in the self closing plastic bag.

The name and serial number of the patient was written on its outer aspect and was then sent to the Metallurgical laboratory for surface roughness assessment.

Surface roughness was measured with the help of profilometer (Mitutoyo Surf-test SJ-210). Profilometer tip was positioned on the wire and followed the profile of the surface when the stylus was drawn across the surface of the archwire. For baseline observations, surface roughness of as received 0.017x0.025 inch stainless steel (G&H Manufacturer) archwire was measured.

Another sterilized 0.017x0.025 inch stainless steel archwire was re-placed in patients and it was kept for 8 weeks. The patients were instructed to use curcumin mouthrinse twice daily after brushing. Commercially available curcumin mouthrinse which was marketed by name Turmix mouthwash (standardized to 95% curcumin-tetrahydrocurcumin) was prescribed to every patient, as curcumin in pure form could have discolored composite resin and other elastomerics in orthodontic patients. They were also instructed not to eat or drink for 30 min after use of mouthrinse. After 8 weeks of use of curcumin mouthrinse 0.017x0.025 inch stainless steel was removed and subjected for following the same protocol as mentioned above.

Effect of curcumin mouthrinse on biofilm adhesion and surface roughness of orthodontic archwires after intraoral exposure of 8 weeks with and without use of curcumin mouthrinse was evaluated and compared.
**Results**

Table 1 shows the descriptive statistics for Biofilm attachment of 0.017x0.025 inch SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and biofilm attachment of another wire after intraoral exposure with the use of curcumin mouthrinse.

The mean biofilm attachment after intraoral exposure of 8 weeks without use of curcumin mouthrinse was 5.3489 CFU with a standard deviation of 0.2404 CFU and mean biofilm attachment of another wire after intraoral exposure with the use of curcumin mouthrinse was 2.3308 CFU with a standard deviation of 0.1301 CFU.

Median value of biofilm attachment of wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse was 5.4231 CFU and range of 4.4771 CFU to 5.5441 CFU and median value of biofilm attachment of another wire after intraoral exposure with use of curcumin mouthrinse was 2.3222 CFU and range of 2.1461 CFU to 2.5563 CFU.

Table 2 shows the comparison of biofilm attachment of 0.017x0.025 inch SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and biofilm attachment of another wire after intraoral exposure with the use of curcumin mouthrinse by using paired t-test. The difference is statistically highly significant with p-value <0.0001.

Table 3 shows the descriptive statistics for increase in surface roughness of 0.017x0.025 inch stainless steel wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and surface roughness of another wire after intraoral exposure with the use of curcumin mouthrinse.

The mean of increase in surface roughness after 8 weeks of intraoral exposure without use of curcumin mouthrinse was 0.2855 μm with a standard deviation of 0.2969 μm and mean of increase in surface roughness of another wire after intraoral exposure with the use of curcumin mouthrinse was 0.1014 μm with a standard deviation of 0.0789 μm.

Median value of increase in surface roughness of another wire after intraoral exposure with the use of curcumin mouthrinse was 0.0622 and range of 0.0350 μm to 0.2920 μm.

Table 4 shows comparison of increase in surface roughness of 0.017x 0.025 SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and surface roughness of another wire after intraoral exposure with the use of curcumin mouthrinse when compared to surface roughness of as received 0.017x 0.025 SS wire , by using paired t-test. The difference was statistically significant with p-value of 0.002.

**Discussion**

This study evaluated bacterial biofilm adhesion and surface roughness of orthodontic archwires and effect of curcumin mouthrinse on both. Biofilm adhesion and subsequent gingivitis are common in orthodontic patients. Poor oral hygiene can eventually lead to the formation of white spot lesions, decay, and hyperplastic gingival tissue that may require intervention.8

Plaque accumulation also affects surface roughness which may affect the sliding mechanics by influencing the coefficient of friction between the archwire and bracket.

Plaque control by means of regular mechanical removal, with concomitant use of chemical agents aiming to decrease the levels of cariogenic and periodontal bacteria, represents the main prevention methods to the above-cited oral problems.90

Chlorhexidine is a broad-spectrum antimicrobial agent, with activity against streptococci, actinomycetes, gram negative species, yeasts, total aerobes, and anaerobes.11 The high concentrations of chlorhexidine have an immediate bactericidal effect, penetrating the bacterial cell wall and leading to the precipitation of the cytoplasm, whereas lower concentrations are bacteriostatic.12 An unpleasant taste, tooth and restoration staining after long-term use, and the need for frequent applications have stimulated the search for new and more appropriate alternatives with no related side effects.1213

Curcumin has been widely studied throughout literature for its anti-inflammatory1415, anti-oxidant16, antibacterial17 and wound healing properties.18 However; its application in dentistry has been
reported only in the last decade. There is little information in the literature regarding use of curcumin in orthodontic patients. Therefore this randomized controlled study was carried out to evaluate effect of curcumin on bacterial biofilm adhesion and surface roughness of orthodontic archwires.

In this study, effect of curcumin mouthwash on biofilm attachment and surface roughness of orthodontic archwire i.e. .017x0.025 inch stainless steel wire was evaluated. Arch wires represent an ideal model for the study of material alterations occurring in vivo. They can be retrieved and studied during the regular patient treatment visits without any need for the advancement of treatment. Insertion of orthodontic wires creates new surfaces available for biofilm formation. Knowledge regarding the growth and adhesion of cariogenic bacteria to orthodontic materials will offer a better way of prevention of consequences of bacterial biofilm adhesion. The presence of precipitated biofilm found on the orthodontic archwires is in agreement with the findings of other studies of esthetic and metal brackets and orthodontic wires.

In this study, in vivo biofilm detection of 0.017x0.025 inch stainless steel wire was carried out. Table 1 shows the descriptive statistics for biofilm attachment of 0.017x0.025 inch stainless steel wires with 8 weeks of intraoral exposure wherein no curcumin mouthrinse was given and biofilm attachment on another wire with use of curcumin mouthrinse for 8 weeks. Considering baseline bacterial attachment for as-received wires as zero, mean biofilm attachment after 8 weeks of intraoral exposure without use of curcumin mouthrinse was 5.3489 CFU whereas with the use of curcumin mouthrinse biofilm attachment score was less i.e 2.3308 CFU. Table 2 shows the comparison between these two paramenters indicating statistically significant reduction in biofilm attachment with the use of curcumin mouthrinse with p-value <0.0001.

The significant reduction in bacterial count is due to the antibacterial property of curcumin which was in accordance to the studies done by Banerjee et al., Niamsa et al., where antimicrobial efficacy of curcumin was proved. This study reconfirms the antimicrobial efficacy of curcumin mouthrinse.

Forsberg et al. evaluated microbial colonization of 12 patients treated by fixed orthodontic appliances and reported that the lateral incisor attached to the archwire with an elastomeric ring exhibited a greater number of microorganisms in the plaque than teeth ligated with steel wire. Therefore in present study archwires were ligated using stainless steel ligatures.

Aging effects on surface archwire roughness, surface topography, fracture, and friction have been examined in only a few investigations on NiTi archwires. There are comparatively less studies showing correlation of debris and stainless steel wires. In the present study choice of rectangular SS (0.017” × 0.025” in) was motivated by the fact that these wires are important during mechanical sliding because of their lower coefficient of friction and lower surface roughness. Also, during retraction, a rectangular SS archwire needs to remain in the oral environment for several months.

In the study done by Joji isac, it was observed that as received stainless steel wires showed surface with striations parallel to long axis. These may be related to mechanical impact during manufacturing. Pitting pattern was also noticed, which may be resulted from chemical interactions during manufacturing whereas in present study surface roughness of “as received” 0.017x0.025 inch stainless steel wire was when tested, it was 0.0363 μm which was taken as baseline measurement.

In the study done by Christoph, quantitative SR analysis performed by Atomic Force Microscopy showed significant differences among “as received” wires from different manufacturers. Therefore in present study surface roughness of one as received 0.017x0.025 inch stainless steel wire of G and H company was tested by profilometer (Mitutoyo SJ-210).

As shown in table 3, the mean of surface roughness after 8 weeks of intraoral exposure without use of curcumin mouthrinse was 0.2855 μm and mean of surface roughness of another wire after intraoral exposure for 8 weeks with use of curcumin mouthrinse was 0.1014 μm. Table 4 shows comparison of surface roughness of 0.017x 0.025 SS wire after 8 weeks of intraoral exposure without curcumin mouthrinse and surface roughness of another wire after 8 weeks of intraoral exposure with use of curcumin mouthrinse. It gives statistically
significant difference with p-value of 0.002. This shows that with use of curcumin mouthrinse surface roughness was statistically reduced.

Hossein Aghili\textsuperscript{33} studied the effect of three mouthwashes on the mechanical properties and surface morphology of orthodontic archwires. In his study 0.05% sodium fluoride (NaF), 0.2% chlorhexidine, and 1 herbal agent i.e Zataria multiflora extract were used wherein fluoride and chlorhexidine seemed to change the mechanical properties and surface quality of the orthodontic wires. However the use of herbal mouthwash used in this study was safer than other mouthwashes due to the lack of chemical components.

Salehi and Momeni Danaie\textsuperscript{34} also studied use of herbal mouthwash in orthodontic patients. He compared antibacterial effects of Persica mouthwash with chlorhexidine on streptococcus mutans. He found significant reduction of streptococcus mutans colonies by persica as well as its lower tooth discoloration effects and unpleasant taste relative to chlorhexidine. In this study the credit of reduction in bacterial biofilm without any sideeffects was given to the use of herbal components.

There are very few studies showing the use of herbal products as mouthrinse in orthodontic patients however there is no study in literature showing the use of curcumin mouthrinse in orthodontic patients and therefore the effect of curcumin mouthrinse in orthodontic patients was evaluated in this study.

Findings of this study suggest that antimicrobial property of curcumin helps in controlling bacterial biofilm attachment in orthodontic patients by significant reduction in bacterial count. Curcumin mouthrinse was also found to have an indirect effect on reducing surface roughness of orthodontic archwires which is important in clinical point of view as rougher archwires induce greater friction and retard tooth movement. To obtain better long standing results in orthodontic patients, long term regime of mouthwash free from side effects is required. Therefore being acceptable for taste, biocompatible to oral tissues as well as free of side effects, curcumin mouthrinse can be successfully used in orthodontic patients.

**Conclusion**

Based on results obtained from this study, following conclusions were drawn

1. Mean biofilm attachment of 0.017x0.025 inch stainless steel wire after intraoral exposure for 8 weeks without use of curcumin mouthrinse was 5.3489 CFU.
2. Mean biofilm attachment of 0.017x0.025 inch stainless steel wire after intraoral exposure for 8 weeks with the use of curcumin mouthrinse was less i.e 2.3308 CFU.
3. The difference of biofilm attachment of 0.017x0.025 inch SS wire after intraoral exposure for 8 weeks without use of curcumin mouthrinse and with use of curcumin mouthrinse was statistically significant.
4. The surface roughness of as received 0.017x0.025 inch stainless steel wire was 0.0363 µm.
5. The mean surface roughness of 0.017x0.025 inch stainless steel wire after 8 weeks of intraoral exposure without use of curcumin mouthrinse has increased to 0.3218 µm.
6. The mean surface roughness of 0.017x0.025 inch stainless steel wire after intraoral exposure with the use of curcumin mouthrinse was 0.1376 µm, which was less as compared to without use of mouth rinse.
7. The difference of surface roughness of 0.017x0.025 inch SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and with use of curcumin mouthrinse was statistically significant.
8. Curcumin mouthrinse was biocompatible and acceptable by patients without any adverse effects. Thus, the results obtained from the present clinical trial, prove the antimicrobial efficacy of curcumin mouthrinse. It also shows indirect effect on reducing surface roughness of orthodontic archwires which is important from clinical point of view as rougher archwires induce greater friction and retard tooth movement, therefore it can be used as an adjunct to orthodontic treatment.

**References**

1. Lundström F, Krasse B. Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine


**Table Legends**

**Table 1**: Descriptive statistics for Biofilm attachment of 0.017x0.025 inch SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and biofilm attachment of another wire after intraoral exposure with the use of curcumin mouthrinse

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Biofilm Attachment (without mouthrinse)</th>
<th>Biofilm Attachment (with mouthrinse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.3489</td>
<td>2.3308</td>
</tr>
<tr>
<td>SD</td>
<td>0.2404</td>
<td>0.1301</td>
</tr>
</tbody>
</table>
Table 2: Comparison of biofilm attachment of 0.017x0.025 inch SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and biofilm attachment of another wire after intraoral exposure with the use of curcumin mouthrinse

<table>
<thead>
<tr>
<th>Biofilm Attachment (Log\textsubscript{10}CFU)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without mouthrinse (n=20) 5.3489±0.2404</td>
<td>&lt; 0.0001 (HS)</td>
</tr>
<tr>
<td>With mouthrinse (n=20) 2.3308±0.1301</td>
<td></td>
</tr>
</tbody>
</table>

*Obtained using paired t-test; HS: Highly Significant

Table 3: Descriptive statistics for increase in surface roughness of 0.017x0.025 inch stainless steel wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and surface roughness of another wire after intraoral exposure with the use of curcumin mouthrinse

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Increase in surface roughness (without mouthrinse)</th>
<th>Increase in Surface roughness (with mouthrinse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.2855</td>
<td>0.1014</td>
</tr>
<tr>
<td>SD</td>
<td>0.2969</td>
<td>0.0789</td>
</tr>
<tr>
<td>Median</td>
<td>0.1467</td>
<td>0.0622</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.0510</td>
<td>0.0350</td>
</tr>
<tr>
<td>Maximum</td>
<td>1.0060</td>
<td>0.2920</td>
</tr>
</tbody>
</table>

Table 4: Comparison of increase in surface roughness of 0.017x 0.025 SS wire after intraoral exposure of 8 weeks without use of curcumin mouthrinse and surface roughness of another wire after intraoral exposure with the use of curcumin mouthrinse.

<table>
<thead>
<tr>
<th>Increase in surface roughness Ra (µm)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without mouthrinse (n=20) 0.2855±0.2969</td>
<td>0.002 (S)</td>
</tr>
<tr>
<td>With mouthrinse (n=20) 0.1014±0.0789</td>
<td></td>
</tr>
</tbody>
</table>

*Obtained using paired t-test; S: Significant