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Original Research Article

Evaluation of biofilm adhesion on various types of orthodontic archwires using Spectrophotometer - An in vivo study

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Abstract

Background: The archwires used in orthodontic practice provide a conducive environment for oral microorganisms and biofilm, enough to cause dental diseases such as caries and periodontal diseases, hence this study is carried out to evaluate the absorbance and concentration of biofilm on 4 different types of retrieved orthodontic archwires used during orthodontic treatment.
Materials and Methods: The study was conducted on 120 orthodontic patients, divided into 4 groups based on type of archwire of same cross section - 0.017*0.025 inch - Group I- Aesthetic Coated Stainless Steel, Group II- Stainless Steel (SS), Group III- Heat Activated Nickel Titanium (HANT) and Group IV- Nickel Titanium(NiTi). After 1 month in the oral cavity, archwires were retrieved, subject to quantitative biofilm measurement using spectrophotometer.
Results -On comparing biofilm absorbance, significant differences among archwires (P=0.0001) were found, except between aesthetic coated SS and SS (P=0.93) and for biofilm concentration, except between aesthetic coated SS and SS (P=0.86) all archwires showed significant difference.
Conclusion: All archwires demonstrate significant biofilm adhesion, with biofilm absorbance and concentration highest affinity for NiTi wires, followed by HANT, SS, and aesthetic coated SS with aesthetic coated SS having the least biofilm retention and microbial colonization.

Keywords: Biofilm adhesion, Orthodontic archwires, Spectrophotometer, Absorbance, Concentration

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1. Introduction

In the course of the fixed orthodontic treatment, archwires play one of the most primary roles in bringing about tooth movement, aiding in improvement of occlusion and aesthetics of the patient.[1] However, during this journey, the placement of bands, brackets and archwires cause formation of newer retentive surfaces on which there is microbial dental biofilm accumulation, leading to iatrogenic decalcification of enamel, sparking the formation of white spot lesions which are the precursors of dental caries.[2] These biofilms are made of highly dynamic and structured communities of microorganisms embedded in a complex self-sustained and produce three dimensional extracellular matrix.[3] This increasing prevalence of bacteria in biofilms acts as the main driver for enamel demineralization and deterioration of periodontal health. The complex nature of biofilms and its intricate three dimensional architecture creates a challenge for measurements regarding the number of viable cells, mass accumulation, biofilm morphology, and other critical properties. One method for biofilm quantification is spectrophotometry. The spectrophotometer measures the amount of biofilm by staining the bacteria in the biofilm as well as the biofilm matrix by crystal violet dye, followed by quantification of the stained dye. The spectrophotometer is one of the most widely used instrument which is highly sensitive for accurate quantification of biofilm even at low concentrations.[4] Its assistance in quantifying proportion of cariogenic bacteria within biofilms surrounding different orthodontic archwires from fixed orthodontic appliances can provide a better insight for choice of orthodontic wires. Orthodontic archwires have evolved from stainless-steel to nickel titanium alloys and recently to aesthetic coated

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archwires with a clear increase in demand for more aesthetic appearing appliances.[5]Archwires, with their varied properties such as surface roughness, surface free energy, and surface topography provide a conducive environment for the adhesion of biofilms. Hence for this study, four archwires of similar cross section, namely Aesthetic Coated Stainless Steel, Stainless Steel (SS), Heat-activated Nickel-Titanium (HANT) and Nickel-Titanium (NiTi) are compared in vivo for total amount of biofilm adhesion by quantifying the biofilm absorbance and concentration with the Spectrophotometer.

2. Materials and Methods

The present study was carried out at the Department of Orthodontics and Dentofacial Orthopaedics, Government Dental College & Hospital, Ahmedabad. It was approved by the Institutional Ethical Committee (IEC). For this study 120 subjects undergoing fixed orthodontic therapy at the Department of Orthodontics are divided into 4 equal groups based on the type of archwire of same cross section.

1. Group I - 30 subjects (0.017" *0.025" Aesthetic Coated SS)
2. Group II - 30 subjects (0.017" *0.025" Stainless SS)
3. Group III - 30 subjects (0.017" *0.025" HANT)
4. Group IV - 30 subjects (0.017" *0.025" NiTi)

After one month of the wires being in the oral cavity, they are retrieved, cut and subject to spectrophotometric analysis for quantification of amount of biofilm adhesion.

Inclusion Criteria was patients undergoing fixed orthodontic therapy and with good oral hygiene. Exclusion Criteria was patients with any systemic diseases and patients undergoing any antibiotic therapy

Cross section of 0.017" *0.025" wires were inserted in patients undergoing Preadjusted Edgewise 0.022*0.028" MBT fixed mechanotherapy and ligated using stainless steel ligatures. After the archwires were inserted, the participants individually received basic instructions on oral hygiene and care regarding the orthodontic appliance. Patients were recalled after 4 weeks and wires were carefully retrieved so as to not dislodge the adherent biofilm. Posterior segments of 20mm were measured with divider and cut with a distal end cutter. Right posterior segment was subjected to biofilm measurement whereas the left posterior segment was subjected to quantitative microbial measurement

Table 1: Comparison of 'Biofilm Absorbance'on different 0.017*0.025" archwires - Aesthetic coated SS, Stainless Steel, HANT, and NiTi

Aesthetic Coated SS			**0.42**	**0.20**
Stainless Steel			0.45	0.11
Hant			0.65	0.23
Niti			1.00	0.22
One way anova test			0.0001*	
Groups	**Difference**	**95% Confidence Interval**		**P value**
Aesthetic Coated SS vs Stainless steel	0.03	-0.10	0.16	0.93
Aesthetic Coated SS vs HANT	0.23	0.09	0.36	0.0001*
Aesthetic Coated SS vs Niti	0.58	0.44	0.71	0.0001*
Stainless Steel vs HANT	0.20	0.06	0.33	0.0008*
Stainless Steel vs Niti	0.55	0.41	0.68	0.0001*
HANT vs Niti	0.35	0.21	0.48	0.0001*

Table 2: Comparison of 'Concentration 'on different 0.017*0.025" archwires - Aesthetic coated SS, Stainless Steel, HANT, and NiTi

Aesthetic Coated SS		32.08		17.9
Stainless Steel		35.22		10.70
HANT		49.76		11.17
Niti		86.35		20.92
One Way Anova Test		**0.0001***		
Groups	**Difference**	**95% Confidence Interval**		**P value**
Aesthetic Coated SS vs Stainless steel	3.14	-7.48	13.76	0.86
Aesthetic Coated SS vs HANT	17.68	7.05	28.30	0.0002*
Aesthetic Coated SS vs Niti	54.27	43.64	64.89	0.0001*
Stainless Steel vs HANT	14.54	3.91	25.16	0.002*
Stainless Steel vs Niti	51.13	40.50	61.75	0.0001*
HANT vs Niti	36.59	25.96	47.21	0.0001*

Figure 1: Wire is washed in PBS Solution

Figure 2: Wire is stained with 1 percent Crystal Violet for 30 minutes

Figure 3: Stained wire

Figure 4: For better solubilisation of dye in Ethanol, wire is vibrated on the vortex.

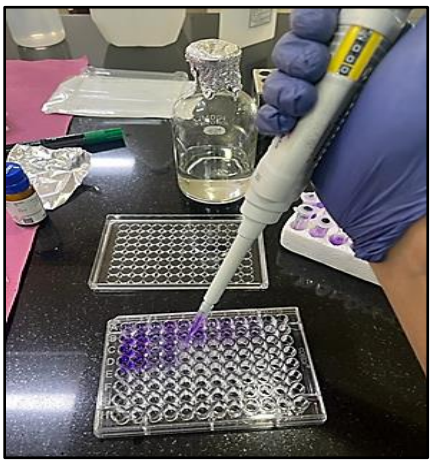

Figure 5: 200 microlitre of each sample is micropipetted into each well

Figure 6: 96 Well Plate is placed into the microplate reader

2.1. Quantitative biofilm measurement

1. Preparation of Phosphate buffered saline (PBS), was prepared by dissolving one tablet of in 100ml of distilled water and stored.
2. The right segment of the wire procured from the patient's mouth was washed in Phosphate Buffered Saline to remove any non-adherent biofilm and debris. It is then stained with 0.5 percent Crystal Violet dye for 30 minutes in an Eppendorf tube.
3. The wire is then washed in distilled water to remove any unabsorbed extra crystal violet and then left to dry for 30 minutes at room temperature and placed in another Eppendorf tube. Patient details, date of insertion, date or retrieval and type of archwire is written on the respective tubes with a marker pen.
4. The crystal violet is solubilised in 1.5ml of 95 percent ethanol for 30 minutes by subjecting the Eppendorf tube to the vortex.
5. After dissolving, 200 microlitre of ethanol is micro pipetted into a 96 well plate and this is then subject to the spectrophotometer at Optical Density of 580nm to calculate absorbance values.
6. The Optical Density is calculated with the help of the scan spectrum curve for Crystal Violet which shows the wavelength at which there is maximum absorbance of the stain.
7. A standard curve for 0.5 percent crystal violet is made at different concentrations (20, 40, 60,80 mM to 200mM) (X-axis) against their respective absorbance values (Y- axis) at optical density of 580nm. From the equation from this standard curve the concentration of crystal violet in the samples are calculated from the respective absorbance values.
8. For this study, spectrophotometer incorporated in the Agilent BioTek Synergy H1 Microplate Reader is used.

3. Results

Data analyzed using SPSS version 26.0: Biofilm absorbance: mean values are Aesthetic Coated SS: 0.42 (±0.20), SS: 0.45 (±0.11), HANT: 0.65 (±0.23), NiTi: 1 (±0.22). - Biofilm concentration: Aesthetic Coated SS: 32.08 (±17.90) mM, SS: 35.22 (±10.70) mM, HANT: 49.76 (±11.17) mM, NiTi: 86.35 (±20.92) mM.

Table: on comparing biofilm absorbance and concentration with 4 orthodontic archwires - Absorbance: significant differences among archwires (P=0.0001), except between aesthetic coated SS and SS (P=0.93). - Concentration: significant differences among archwires (P=0.0001), except between aesthetic coated SS and SS (P=0.86).

4. Discussion

In the oral cavity, the orthodontic appliance creates surfaces and retentive sites with properties such as surface roughness and surface free energy, different to those of the natural oral hard and soft tissue structures. Oral biofilms formed on these surfaces cause demineralization of enamel, leading to white spot lesions which are the precursors of dental caries, gingivitis leading to periodontitis and even bacteremia.40 Bjorn et al showed that white spot lesions are present even 5 years after fixed orthodontic treatment.[6] Today, on the basis of Variable modulus orthodontics that is, use of different materials with different moduli of elasiticity while maintaining the same or similar cross section of archwires, is employed in orthodontic treatment.[7] Based upon this, orthodontic archwires of different materials are used which have different surface characteristics such as surface roughness and surface free energy which influences and increases the amount of plaque formation leading to biofilm adhesion and microbial colonisation over them.[8-9] The microorganisms in this biofilm are responsible for biocorrosion (microbiologically induced corrosion) of orthodontic appliances as they dissipate Fe, Ni, and Cr into the oral cavity, which can be a cause of metallic allergy.[10] Furthermore, biocorrosion from orthodontic archwires also leads to a vicious cycle in which there is increase in biofilm adhesion which inturn causes more corrosion, keeping this cycle intact.[11] Hence, knowledge regarding the growth of cariogenic bacteria in subjects with orthodontic appliances and biofilm adherance to orthodontic archwires can offer a better insight in prevention of white spot lesions and periodontal complications. As archwires of different materials are used according to treatment needs, information regarding affinity to biofilm adhesion and microbiological colonisation will provide the clinician with the better choice in the microbiologic perspective as well, especially in patients with higher risk of dental caries and periodontal diseases. For this study, 120 patients undergoing fixed orthodontic appliance therapy were selected and divided into 4 groups. 4 different orthodontic archwires Aesthetic coated Stainless Steel, Stainless Steel, Heat-Activated Nickel Titanium (HANT) and Nickel Titanium (NiTi) of 0.017*0.025 inch cross- section were placed in each group respectively. The wires are retrieved after 1 month, 2cm of the distal end was cut, stained and subject to the Spectrophotometer for Quantification of biofilm for which biofilm absorbance and biofilm concentration are evaluated.

The highest biofilm absorbance is on NiTi archwires followed by HANT and SS and lowest is for Aesthetic Coated SS. ANOVA showed a statistically highly significant difference between the mean value of biofilm absorbance among all the four archwires(P= 0.0001)Tukey's post hoc analysis found that the mean values of biofilm absorbance

was statistically highly significant(P=0.0001) between all the archwires, except and between Aesthetic Coated SS and Stainless Steel, in which the difference was not statistically significant(P=0.93). In the study by Pritish Polke et al.[12] It was found that there was no statistically significant difference between Aesthetic coated SS and SS archwires for biofilm absorbance (P=0.08). These findings are similar to the present study. Study by Sukriti Raj et al.[13] showed that, uncoated NiTi had the highest bacterial biofilm adhesion whereas coated SS had the least. Seyed Hamid Raji et al.[14] observed that mean bacterial colonization was more on uncoated NiTi wires in comparison to the coated NiTi archwires(P<0.001). Whereas, in the in vitro study by Deise C. Oliveira et al.[15] it was concluded that Aesthetic coated archwires have similar risks of biofilm adhesion incomparison to the uncoated archwires. They compared uncoated NiTi archwires with different coated SS and NiTi archwires in which they found no significant difference. This is because hydroxyl groups of epoxy resins on coated archwires tend to absorb water molecules, consequently, turning the surface hydrophilic. The high hydrophilicity could have been the reason for increase in background staining seen in coated wire rather than staining overall biofilm.

Similar to the mean values of biofilm absorbance, NiTi archwires showed highest biofilm concentration among all the archwires followed by HANT and Stainless Steel whereas Aesthetic Coated SS showed the lowest mean values of concentration of biofilm. Similar findings were found in studies by Pritish Polke et al.[12] in which Aesthetic coated archwires had the least biofilm concentration. ANOVA test showed a statistically highly significant difference between all the archwires.(P=0.0001). Tukey's Post hoc analysis showed that there is statistically highly significant difference between mean values of biofilm concentration of all the archwires except Aesthetic coated SS and Stainless Steel (P=0.86). In the study by Pritish Polke et al.[12] aesthetic coated SS and SS archwires showed no significant difference.(P=0.09). These findings are similar to the present study. Studies by Mahasen Taha et al.[8] and Marwa Ali Tawfik et al.[16] have shown a positive correlation between biofilm adhesion and surface roughness. The increased biofilm concentration on NiTi archwires can be attributed to its increased surface roughness in comparison to Stainless Steel and Aesthetic Coated SS archwires. This was shown in studies By Atia Yousif et al.,[17] Reshma Mohan et al.,[18] and Vincenzo D'Anto et al.[19]

5. Summary and Conclusion

Orthodontic archwires are an integral and active component of the fixed orthodontic appliance but are prone to and act as retention sites for plaque formation leading to biofilm adhesion and microbiological colonisation. This puts the patient at risk for increase in incidence of dental caries and periodontal diseases. The conclusions of the study are:

1. All the archwires show significant amount of biofilm adhesion on them. 6. For biofilm absorbance and concentration, NiTi shows highest adhesion of biofilm, followed by HANT, SS and Aesthetic coated SS. All groups showed significant difference between each other except Aesthetic coated SS and SS. 7. Aesthetic coated SS had the least retention of both biofim adhesion. Hence, it is concluded that, in order to provide a more wholesome orthodontic treatment, keeping the patients' overall prognosis in mind, NiTi and HANT archwires, being the wires with more biofilm adhesion and amount of microbial colonisation should be used for the most optimum and shortest period of time especially in patients with higher risk of dental caries and periodontal complications. Wire progression to wires with lesser biofilm adhesion and microbial colonisation such as to Aesthetic coated SS and SS at the earliest is recommended.

5.1. Limitations of the present study

In the present study, biofilm adhesion and microbiological evaluation was done by placing and retrieving archwires for the period of 1 month. Long-term studies would be more conclusive. On the Aesthetic coating SS archwires,coatings may peel during intervention or in areas where tooth brush can reach easily, can affect the outcome.

For this study, 0.017*0.025 rectangular cross section was taken. Different cross sections can change the outcome. The standardization of eating habits is not possible which may affect the outcome of the study.

6. Source of Funding

None.

7. Conflict of Interest

None.

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