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Research Protocol

Dentistry Section

Comparative Assessment of Commercially Available and Silver Dioxide Surface Modified Fixed Orthodontic Lingual Retainer for its Anti-adherent and Anti-bacterial Properties: A Research Protocol for an In-vitro study

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ABSTRACT

Introduction: Enamel gets demineralised when they get exposed to acidic environment. Very common issue seen after orthodontic treatment is white spot lesion and also there is a chance of inflammation in periodontal fibres if the fixed lingual retainer is given to the patient. Furthermore, it enhances the plaque and calculus accumulation. So, to minimise these deleterious effects, lingual retainer is modified by surface coating with silver dioxide which shows anti-bacterial and anti-adhesive properties.

Aim: To assess the anti-adherent and anti-bacterial properties of silver dioxide (AgO_2) surface modified Orthodontic lingual retainer against *S.mutans*.

Materials and Methods: This in-vitro cross-sectional study will use 60 orthodontic lingual retainer specimens. The samples will be divided into four groups for testing with 15 specimens in each group. The samples will be divided into four groups: two control groups with commercially available lingual retainer will be used for assessing anti-bacterial and anti-adhesion properties and other two will be of surface treated lingual retainer for testing. Bacterial strains will be taken with sample size 15 in each group then the preparation of photocatalytic silver dioxide-coated orthodontic lingual retainer. Even coating will be ensured by sputtering technique and thickness of coating will be 50-60 nm. Chi-square test will be used to analyse differences in categorical variables.

Expected outcome: The photocatalytic (AgO_2) coated retainer will reduce the bacterial accumulation and adhesion.

Conclusion: Surface modification of lingual retainers with photocatalytic AgO_2 will reduce the bacterial adhesion which can be used to prevent the formation of dental plaque and demineralisation orthodontic treatment, thereby preventing demineralisation of enamel and periodontal breakdown.

Keywords: Demineralisation, Enamel surface, Surface coating, *Streptococcus mutans*, Sputtering technique

INTRODUCTION

Enamel demineralisation, often known as white spot lesions, is a common unintended consequence of orthodontic therapy. Because of their inherent morphologic abnormalities, the oral environment with fixed appliance provides perfect circumstances for microbial colonisation. Patients struggle to keep up with proper dental hygiene, and the appliance gives extra places for bacteria to bind and proliferate. As a result of the increased plaque accumulation and retention areas, the patient is more likely to experience enamel demineralisation around the appliance [1].

As they are attached to the dentition upon orthodontic treatment, lingual retainers, among other fixed orthodontic appliances, may play an important role in enamel demineralisation. This appliance provides a distinct environment that makes cleaning tooth surfaces challenging [2]. Gjermo P et al., in his study showed that on brackets to decrease white spot lesion and to prevent accumulation of plaque [3]. However, orthodontic brackets are in the oral cavity for not more than two years and lingual retainer are given as permanent retention post treatment and they are exposed to oral environment and have higher chances of accumulation of plaque which may further lead to demineralisation.

The tooth wipe is a modality that has been identified as an appropriate adjunct aid to mechanical plaque removal and oral hygiene maintenance in children [4]. Different anti-bacterial solutions in the form of mouthwash were also used to reduce plaque and related disease caused by *Streptococcus mutans* [5]. However, they have drawbacks such as tooth staining, vomiting, or diarrhoea [6]. Specifically, chlorhexidine mouthwash is effective in controlling gingival inflammation when used in conjunction with tooth brushing, but if used for an extended period of time, it may cause staining and a temporary change in taste [7]. Some corrosion of brackets and wires can occur due to mouthwashes being used. The physical properties of the wire deteriorate, and nickel ions are released, which are toxic and allergic to some patients [8].

A study has been demonstrated by Mhaske AR et al., where in orthodontic wires were coated with silver to evaluate anti-adherent and anti-bacterial properties against *lactobacillus acidophilus*. Result showed that surface modification of orthodontic wire can prevent accumulation of bacteria as compared to uncoated one [9].

Choi JY et al., went a step further in this direction by trying to demonstrate that the addition of Silver to Titanium metal appears to result in a synergistic enhancement of photocatalytic anti-bacterial effect against *S mutans*. They concluded that TiO_2-coated metal photocatalyst reactions had antibacterial activity against *S mutans*. Anodic oxidation developed a TiO_2 film with superior anti-bacterial properties to thermal oxidation [10]. There has been no research into the anti-bacterial and anti-adherent properties of a fixed orthodontic lingual retainer coated with silver dioxide (AgO_2). Thus, the proposed study will assess the anti-adherent and anti-bacterial properties of a surface-modified orthodontic lingual retainer coated with Silver di-oxide. The research protocol have been formulated with the aim to assess and compare the anti-adherent and anti-bacterial properties of commercially available and surface modified silver dioxie coated orthodontic lingual retainer against *Streptococcus mutans*.

The protocol is made wth an aim to assess and compare the anti-adherent properties and anti-bacterial properties amongst surface modified silver dioxide coated orthodontic lingual retainer versus uncoated lingual retainer.

MATERIALS AND METHODS

This protocol will be a in-vitro cross-sectional study which will be conducted in Orthodontic and Dentofacial Orthopaedic Department of Sharad Pawar Dental College, Wardha, Maharashtra, India, from April 2023 for an estimated time period of one year, after obtaining the approval of the Institutional Ethical Committee [Ref. no. DMIMS(DU)/IEC/2022/747]. The study will use 60 orthodontic lingual retainer specimens. The samples will be divided into four groups for testing, 15 specimens will be included in each group.

The uncoated lingual retainer groups will serve as the control group for their respective coated lingual retainer experimental group. A sputtering machine with photocatalytic silver di-oxide will be used to modify the surface of the orthodontic lingual retainer. The film thickness of 15-20 mm and even coating will be ensued by sputtering technique [1]. Microbiological assays will be used to evaluate the anti-adherent and antibacterial properties of the photocatalytic AgO_2 coating.

Group 1: Control group - It will consist of 15 uncoated orthodontic lingual retainer that will used for evaluation of bacterial adhesion to the retainer.

Group 2: Experimental group - It will consist of 15 orthodontic lingual retainers coated with photocatalytic AgO_2 thin film which will be used for the evaluation of the bacterial adhesion to the retainer.

Group 3: Control group- It will consist of 15 uncoated orthodontic lingual retainers which will be used for the antibacterial assay.

Group 4: Experimental group - It will consist of 15 surface modified orthodontic lingual retainer coated with photocatalytic AgO_2 thin film which will be used for the antibacterial assay.

Sample size calculation: has been done by Cochrane formula as depicted in Cochrane WG., [11].

n=(Za+Zß)2(d12+d221K)

Δ2

Where Za is the level of significance at 5% i.e 95% confidence interval=1.96

Zß is the power of test=80%=0.84, d1=SD of colony counts in group 3=0.17, d^2=SD of colony counts in group 4=0.27

Δ=difference between two means=4.38-3.92=0.46, K=1, n=$(1.96+0.84)^2$ (0.172+0.272/1)

0.462

=3.77

n=15 samples in each group

Study Procedure

Bacterial strains: *S. mutans* strains will be used for adhesion and viability testing. These will be placed in a 5 mL de Man, Rogosa, and Sharpe (MRS) broth and incubated for 24 hours at 37°C [1]. The adhesion test involves transferring incubate 10% of an overnight cultured broth in 10 mL of MRS broth containing 10% sucrose for 24 hours.

Preparation of photocatalytic silver dioxide coated orthodontic lingual retainer: Sputtering techniques bombard a positive ions from an inert gas (argon) discharge on a solid cathode (target), removing surface atoms or molecular fragments and depositing them on a nearby substrate to produce a thin coating. Substrates will be pushed down to a specified process pressure in a vacuum chamber. Sputtering will be done on an orthodontic lingual retainer (substrate) with silver (Ag) as the target in this investigation. Surface atoms from the titanium target were expelled by a plasma created inside the vacuumed chamber and sputtered onto the lingual retainer (substrate) [1].

Evaluation of bacterial adhesion to lingual retainer: The lingual retainer will be ultrasonicated for 5 minutes into propanol and dried in a desiccator before the adhesion test to remove any potential macroscopic contaminations [1]. The lingual retainer will be pre-weighted with an analytical balance and stored in an airtight container after being cleaned and sterilised in an autoclave. *S. mutans* culture broth will be inoculated at a final concentration of 10% in a sterile beaker containing 10 mL of MRS broth. Lingual retainer extending from canine to canine will then be immersed in suspension and incubated for 24 hours at 37°C under Ultraviolet A (UVA) within the Eppendorf tubes, a black light (Philips Electronics TLD15W/08, F15T8BLB, Blue Bell, Pa, USA) was used. To immobilise the germs, the lingual retainers will be gently taken out and submerged in a 10% formaldehyde solution for 30 minutes [1]. The lingual retainer will be dried in a desiccator for 24 hours after a thorough cleaning with distilled water. An analytical balance will be used to record the weight change of the lingual retainer during the bacterial adhesion test.

Orthodontic lingual retainer antibacterial activity assay: *S. mutans* culture broth will be diluted with MRS broth until it attains an optical density of 1.0 at 660 nm. The 10 mL of the diluted bacterial suspension will be transferred to petridishes with uncoated and AgO_2 coated lingual retainers. For 60 minutes, these dishes will be illuminated with a UVA black light with an intensity of 1.0 mW/cm^2 inside the laminar air flow chamber [1]. The 100 mL of the bacterial suspension will be serially diluted and plated onto MRS agar plates after illumination. The survival rate of *S. mutans* by colony forming units (CFUs) will be used to describe antibacterial activity.

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) 22.0 version and graph pad prism 6.0 version will be used. Chi-square test, unpaired t test, student's t test, and Analysis of Variance (ANOVA) will be used for statistical analysis. The $p<0.05$ will be considered as level of significance.

EXPECTED OUTCOME/RESULTS

The photocatalytic AgO_2 coated retainer reduces the prevalence of white spot lesions and enamel demineralisation surrounding the lingual retainer by preventing plaque adherence and accumulation.

DISCUSSION

Surface modification of stainless steel orthodontic brackets with photocatalytic AgO_2 and titanium silver (TiAg) has yielded positive results to reduce the accumulation of plaque and microorganism [1,2].

In an in-vitro investigation by Gilani RA et al., plaque samples from orthodontic patients were studied before and after orthodontic bands and arch wires were placed. The pH, carbohydrate content, and microbial populations of *streptococci* and *lactobacilli* were all measured in the samples. Orthodontic patients had a statistically significant decrease in plaque pH, as well as an increase in carbohydrate content and microbial populations in each milligram of plaque. When compared to groups with uncoated brackets, groups with surface-modified brackets had a statistically significant decrease in *S. mutans* survival expressed as CFU and log of colony count; where log of Colony Forming Unit (CFU) for uncoated was 3.99 compared to coated bracket with log value of 3.48 [1].

The effect of TiO_2 coating on the anti-bacterial and anti-adherent properties of commonly used brackets was investigated in a study by Shah AG et al., They discovered that photocatalyst reactions of TiO_2-coated brackets were anti-bacterial against *S mutans*. As a result, they concluded that photocatalytic TiO_2 surface modification of orthodontic brackets can be employed to inhibit the formation of dental plaque during orthodontic therapy [2].

The study by Mhaske AR et al., was done to assess the antiadherent and antibacterial properties of surface-modified stainless steel and NiTi orthodontic wires with silver against *Lactobacillus acidophilus*. When compared to uncoated wires, orthodontic wires coated with silver had an antiadherent effect against *L. acidophilus*. Uncoated stainless steel and NiTi wires increased in weight by 35.4 and 20.5%, respectively, which was statistically significant (p=0.001), whereas surface-modified wires increased in weight by only 4.08 and 4.4% (statistically insignificant p>0.001). This study concluded that silver surface modification of orthodontic wires can be used to prevent dental plaque accumulation and the development of dental caries during orthodontic treatment [9].

An in-vivo study was conducted to assess carious lesion development related with fixed orthodontic therapy. Premolars that were set to be extracted as part of an orthodontic treatment were fitted with custom-made orthodontic bands. In the absence of fluoride administration, visible white spot lesions appeared within four weeks. Both micro-radiographic and Scanning Electrone Microscopic (SEM) studies revealed weakening of the enamel surface, indicating that the lesions lacked a surface layer. They came to the conclusion that enamel demineralisation associated with fixed orthodontic therapy is a very fast process induced by a high and constant cariogenic challenge in plaque formed around brackets and below ill-fitting bands. Although white spot lesions get remineralise and even disappear, the prime focus should be on preventing carious lesion development during treatment with fixed orthodontic appliances [12].

CONCLUSION(S)

The photocatalytic effects of AgO_2 as a surface coating can benefit orthodontic patients by reducing microbial growth that causes periodontal disease and enamel decalcification.

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