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Effect of different cleaning regimes on biofilm formation of acrylic based removable orthodontic appliance: A randomized clinical trial

A Protocol Submitted to

the Council of the College of Dentistry, University of Baghdad in Partial Fulfillment of Requirements for the Degree of Master of Science in Orthodontic

By

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Certification of the Supervisor

This is to certify that the preparation and organization of this protocol entitled "**Effect of different cleaning regimes on biofilm formation of acrylic based removable orthodontic appliance: A randomized clinical trial**" had been made by the master student **Safa Imad Khawwam** under my supervision, at the Department of Orthodontics/College of Dentistry/University of Baghdad in partial fulfillment for the Degree of Master of Science in Orthodontics.

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Introduction

Orthodontic treatment is adopted by wide section of society not only for the corrections of malocclusion but also improves mastication, speech and appearance, as well as overall health, comfort, and self-esteem **(Westley, 2010)**. There are two types of orthodontic appliances either removable or fixed appliance. Although the orthodontic appliance has many known benefits, these appliances are also associated with a number of damages and disorders of oral cavity **(Pathak and Sharma, 2013)**. Polymethyl methacrylate (PMMA) is the standard material for denture bases because of its biocompatibility, low cost, ease of processing, high flexural resistance and flexural modulus, and low water solubility **(Leão *et al*., 2018)**; however, the PMMA denture base is susceptible to microbial colonization in the oral environment **(Puri *et al*., 2008)** other factors that favor microbial colonization are porosity, surface roughness, poor denture hygiene, and continual and nighttime wearing of dentures **(Gendreau and Loewy, 2011)**. Rougher surfaces have been found to be more difficult to clean and promote re-growth by surviving organisms as opposed to complete surface re-colonization **(Teughels *et al.*, 2006)**.

Physiologically the human's oral microflora consists of a mixture of organisms, which are common also to other anatomical districts. This bacterial charge is extremely complex, being composed of over 700 different

species of bacteria **(Relman, 2015)**. Biofilm is a matrix-enclosed microbial accretion that adhere to biological or non-biological surfaces **(Hall-Stoodley *et al*., 2004)**. Bacterial biofilms are serious global health concern due to their abilities to tolerate antibiotics, host defense systems and other external stresses; therefore it contributes to persistent chronic infections **(De la Fuente-Núñez *et al*., 2013)**. Formation of biofilm is well-regulated multi step events such as (i) adsorption of molecules (macro and micro molecules) to surfaces; (ii) bacterial adhesion to the surface and release of extracellular polymeric substances (EPS); (iii) colony formation and biofilm maturation. **(Flemming *et al*., 2007)**. Patients need to be aware of the implications for their oral health when undergoing recommended orthodontic treatment. On the other hand, when a patient accepts to undergo orthodontic treatment, including those using removable orthodontic devices, he/she should be reminded that it entails a commitment to a higher regimen of attention towards oral hygiene and health in patient's home care **(Al Makhmari *et al*., 2017)**. Recently, **Rodriguez-Renteria et al. (2021)** investigated after 4 weeks the frequency of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida species on removable orthodontic appliances and found that, of the 55 removable orthodontic appliances studies, Staphylococcus aureus was present on 90.9%, Pseudomonas aeruginosa on 67.7% and Candida species on 32.7% of the appliances. Interestingly, these microorganisms were also found in the support oral mucosa of the 55 children (89.09%; 60%; 30.9% respectively). The authors also concluded a direct relationship between removable orthodontic treatment and an increase in the amount of periodontal pathogenic microorganisms. Surface roughness, incomplete polymerization, or wear caused by daily brushing of the appliance seems to directly influence bacterial and fungal biofilm adhesion to removable acrylic appliances.

The denture base after processing is finished using acrylic finishing bur and sandpaper and polished using a rag wheel and cone with pumice slurry. The surface roughness of the denture base material has a substantial effect on plaque adherence and microbial colonization of the prostheses. A rough surface creates niches in which microorganisms are protected against mechanical dislodging forces. The bacteria can subsequently spread over the denture surface from these niches. It has been proven that an increase in surface roughness above a threshold of 0.2 μm results in a simultaneous increase in plaque accumulation. This concept is of clinical importance because patients need to have a smooth surface to deter the formation of a biofilm. This is an esthetic concernas well as an overall concern for maintaining good oral hygiene. **Bollen et al. (2017)** found that the surface roughness of acrylic resin can be dependent on the polishing grit. Streptococcus sanguis, Bacteriodes gingivalis, and Candida albicans adhere in very high numbers to roughened acrylic resin versus smooth acrylic resin. **Al Groosh et al. (2015)** investigated the influence of surface roughness and surface dynamics on the attachment of Methicillin-Resistant Staphylococcus aureus (MRSA) onto orthodontic retainer materials and found that the number of bacteria attached to the surface of the tested materials increased over the 48 h time period regardless of the material chemistry and surface topography. There was no significant difference in MRSA biofilm formed on the surface of these materials. However, the scanning electron microscope and the Confocal laser scanning microscopy showed that the distribution of bacterial aggregates increased on the rougher surface of the auto polymerized acrylic samples whereas the pattern of bacterial attachment was more uniformly distributed on the thermoplastic samples.

Some studies have already been conducted in order to investigate the effects of different cleaning protocols for acrylic removable orthodontic appliances and may also help to reduce the risk of oral diseases related to biofilm growth, some different processes have been described such as denture cleaners, enzymatic solutions, chlorhexidine, sodium hypochlorite, or homemade solutions containing vinegar or citric acid **(Eichenauer *et al*., 2011)**. The two major approaches used for denture cleaning are mechanical and chemical methods. Mechanical methods include brushing (using water, soap, toothpaste, or abrasives) and ultrasonic treatment. Mechanical cleaning with brushes is inexpensive and common, but elderly and disabled patients might face difficulties with this method because of poor motor coordination, poor dexterity, and low motivation. There is evidence that mechanical cleaning with toothpastes can result in significant wear of conventional acrylic resins. Chemical method includes immersion of dentures in different cleansing solutions. Immersion in cleansing solution is an inexpensive, easy, and comfortable alternative procedure. Moreover, the cleansing solution can reach undercuts of the denture base that are difficult to clean mechanically, resulting in more efficient cleaning **(Schwindling *et al*.,2014)**.

No study is conducted to evaluate the most effective method used to remove biofilm from removable orthodontic appliance.

Research question:

What is the most effective method of cleaning an acrylic based removable orthodontic appliance?

Research hypothesis

The null hypothesis: There is no difference between the different methods of cleaning acrylic based removable orthodontic appliances.

Aims of the study:

The aims of the current study are:

1. To evaluate the effects of different cleaning regimes i.e. chemical and mechanical on biofilm formation of an acrylic based removable orthodontic appliance.

2. To find out if surface modification i.e. polished acrylic fitting surface, have an impact on cleaning the biofilm formation.

Methodology

Study design:

An in vivo study.

Setting:

It is double-blind, parallel, randomized clinical trial conducted in the orthodontic clinic after obtaining the ethical approval from the ethics committee of the department.

Subjects:

Orthodontic patient will be selected to wear a removable appliance for the study period.

Sample size:

Thirteen orthodontic patients for each group will be selected to wear a removable orthodontic appliance for the study period **(Lima et al., 2006)** and will be instructed for the cleaning protocol according to the manufacturer's instructions.

Inclusion criteria:

• Presence of full upper permanent dentition;

• Well-aligned teeth or mild crowding (less than 3mm);

• No history of sensitivity to any ingredient contained in the cleaning materials;

• Nonsmoker;

• Caries free.

Exclusion criteria:

• Participant taking steroid-based or antibacterial mouthwash or broad-spectrum antibiotics;

• Mouth breather;

• Participant taking medication that reduces salivary flow;

• Pregnant or lactating female **(Albanna *et al*., 2017)**.

All of the study steps were explained to the participants verbally and in writing, written informed consent will be obtained from each participant.

Materials and instruments

Materials used for orthodontic removable appliance fabrication:

• Acrylic powder and monomer (Orthocryl, Dentaurum, Ispringen, Germany).

• 0.7 mm stainless steel wire (Spring hard, Dentarum, Ispringrn, Germany).

• Adam's plier.

• Sticky wax.

• Separating medium.

• Cutter.

• Wax knife.

• Mold material.

Materials used for cleaning regimes:

1-Brush (dental or denture brush) with chlorhexidine (CHX) toothpaste.

2-Chemical cleaning i.e. Lacalut cleaning tablet.

3-Combination of brushing with CHX toothpaste and cleaning tablet.

Materials and equipment used form microbial isolation and identification:

1. Calcium alginate swab.
2. Calgon ringer solution.
3. Bijou tube.
4. Glass beads.
5. Phosphate buffered saline (PBS).
6. Anaerobic cabinet.
7. Vortex machine.
8. Culture media.

A. Mitis-Salivarius Agar (MSA): This agar is a commonly used selective medium for isolating and quantifying S. mutans in many cariology clinical and epidemiological studies **(Xiao *et al*., 2016)**.

B. Mannitol salt agar: Is a commonly used selective and differential growth medium in microbiology. It contains a high concentration (about 7.5%-10%) of salt (NaCl), making it selective for Gram-positive Staphylococcus aureus and epidermidis since this level of salt is inhibitory to most other bacteria **(Bachoon *et al*., 2008)**.

C. Blood agar: is enriched medium used to culture those bacteria or microbes that do not grow easily. Such bacteria are called “fastidious” as they demand a special enriched nutritional environment as compared to the routine bacteria **(Sirois, 2020)**.

Other materials:

1- Rubber bowl

2- Type IV stone

3- Gloves

4- Masks

5- Plastic petri dish

Procedure

Construction of acrylic samples with different surface texture:

Acrylic samples i.e. tiles of different surface texture will be made. An acrylic sheet of 1 mm thickness will be made using a mold with technique as shown in figure 3.

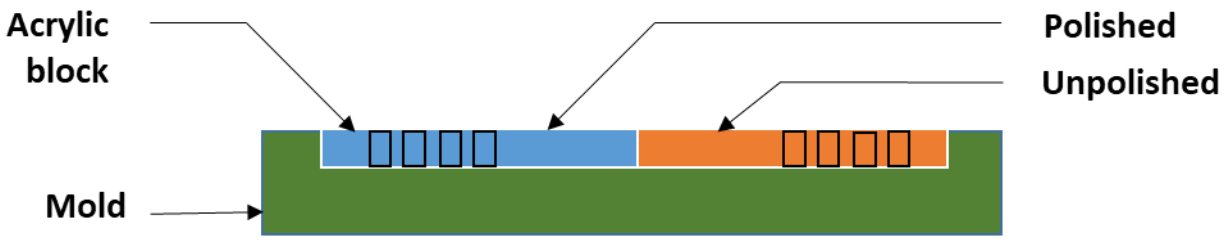

Figure 1: Acrylic samples made from polished and unpolished acrylic sheet.

A half of the sheet will be polished using a conventional polishing technique until a glossy surface appears, whereas, the remaining will be kept without modification. Tiles of 5mm in diameter will be bored using a trephine bur on both sides of the acrylic sheets and each set of tiles i.e. the polished and unpolished samples, will be color coated from its seated surface.

Construction of upper removable orthodontic appliance

An impression will be taken to the maxillary arch using alginate impression material (Hydrogum 5, Zhermack, Badia Polesine, Italy). The negative replica is than poured using type IV stone to make a study model. Four metal discs (5mm in dimeter and 1.2mm thickness) will be placed on both sides of the palate as seen in figure 2; A.

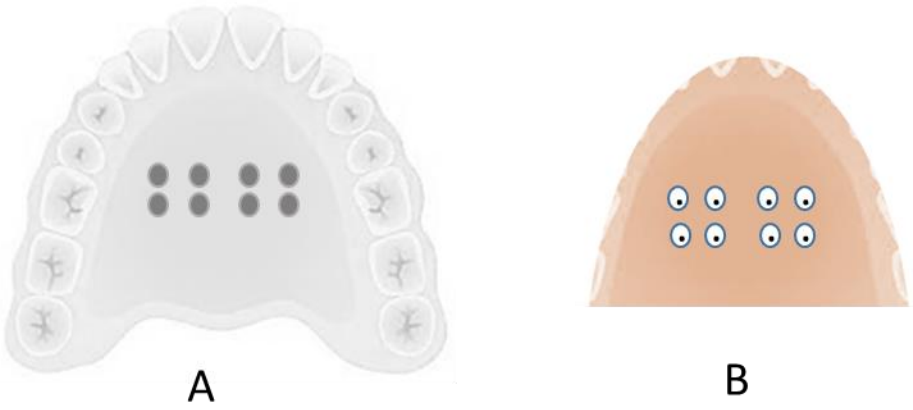

Figure 2: Construction of the acrylic based orthodontic appliance. A, Study model with acrylic tiles; B, Fitting surface of the acrylic removable appliance.

The wire framework of the removable appliance will be fabricated using a 0.7 mm in diameter stainless steel wire (Spring hard, Dentarum, Ispringen, Germany) to make Adam's clasps on upper first premolars and molars to retain the appliance.

After applying a separating medium, an acrylic base will be fabricated using the orthodontic acrylic powder and liquid (Orthocryle, Dentaurum, Ispringen, German) with a ratio of (2.5 parts powder to 1 part liquid) according to the manufacturer's instruction. Before setting, a sprue or chimney like structure will be created through the acrylic dough to facilitate the metal discs and acrylic sample removal. The appliance then will be removed and the metal tiles will be dislodged using metal pin (Figure 2; B).

The acrylic tiles (5mm in diameter and 1mm thickness) will be placed and fixed into the holes, created by the metal discs, using sticky wax. The acrylic samples will be randomly seated as shown in figure 3.

The appliance will be sterilized using proper sterilization protocol.

Figure 3: The acrylic base containing the acrylic samples.

Method for biofilm detection

The removable appliance will be assigned randomly and blindly and fitted into the oral cavity of the patient for one week. Each individual will be given a pack containing the cleansing pack and an instruction sheet with a web link describing the cleaning regimen for his/ her appliance. The packs are similar in shape and color and will be prepared by a third person who does not know the purpose of the study. The volunteer will be grouped into three groups:

Group 1: patients will be instructed to brush both sides of the removable appliance once a day for 1 minute before bedtime using the toothbrush and the CHX toothpaste provided and water **(Albanna *et al*., 2017)**.

Group 2: patients will be instructed to immerse the appliance in 150 ml cup with tap water and dissolve one cleansing **(Albanna *et al*., 2017)** tablet for 20 minutes according to the manufacture instructions.

Group 3: Combination of the chemical and mechanical methods first submitted to the mechanical method, followed by the chemical method **(Oliveira *et al*., 2009)**.

At the end of the study period, the acrylic samples will be removed carefully and applied into a petri dish containing PBS via a sterile tweezer. The samples will be then immersed twice in a 25ml sterile tubes containing

PBS to remove the planktonic bacteria and inserted into 5ml bijou tube containing 1mm PBS and 5 sterile glass beads. The sample will be vortex-mixed for 1 min to disseminate the biofilm and create a homogenous solution. Ten-fold serial dilutions will be carried out in PBS before the samples are plated onto blood agar (BA), to give the total anaerobic count, mitis salivarius agar (MS), a selective agar for streptococci and Mannitol salt agar (MA) for Staphylococci spp. BA and MS plates will be incubated anaerobically for 2-4 days at 37ºC using anaerobic cabinet whereas the MA will be incubated for 2 days in aerobic condition. All organisms from both selective and nonselective agars will be distinguished by colony morphology and will be characterized by Gram reaction **(Al Groosh *et al.*, 2011)** and other confirmatory tests.

Confirmatory test

1. Catalase testing: Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals) **(Chelikani *et al*., 2004)**. This test demonstrates the presence of catalase, an enzyme that catalysis the release of oxygen from hydrogen peroxide (H2O2). The catalase test is mainly used to differentiate between Gram-positive cocci: members of the genus Staphylococcus are catalase-positive, and members of the genera Streptococcus and Enterococcus are catalase-negative **(Whittenbury, 1964)**.

2. Coagulase testing: Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. In the research laboratory, it is used to differentiate between different types of Staphylococcus isolates. Importantly, S. aureus is generally coagulase-positive **(Ryan and Ray, 2004)**.

Statistical analysis

All the data of the tested samples will be collected and analyzed using SPSS (statistical package of social sciences). In this study the following statistics will be used:

Descriptive statistics

- Mean.
- Standard deviation (SD).
- Minimum and maximum values.
- Statistical tables, figures and charts.

Normality test

Data distribution will be tested first using Shapiro-Wilk test to find that whether our collecting data are normally distributed or not.

Inferential statistics

- ANOVA (if the data parametric) or Kruskal Wallis test (if none parametric) to compare the effect of each cleaning agents on biofilm reduction of removable orthodontic appliance and Tukey HSD pot hoc test if there is significant difference between them.
- Independent t-test (if the data parametric) or Mann Whitney test (if none parametric) to compare the effect of each cleaning agents on biofilm reduction on polished and unpolished surface

In the statistical evaluation, the following levels of significance will be used:

Non-significant NS $P > 0.05$

Significant S $0.05 \geq P > 0.01$

Highly significant HS $P \leq 0.01$

Time table:

Duty	Expected time
Patient Identification and allocation	4 months
Intervention period	3 months
Data collection and analysis	2 months
Writing up the thesis	10 months (starting from the beginning of the study)
Total expected time to finish	About 10 to11 months

Budget and Funding: The study is self-funded

Dissemination: Master Thesis.

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Patient Information Sheet

You are invited to participate in a scientific research. Please take your time to read the following information carefully before you decide whether or not you wish to participate. You can ask for clarifications or any more information about the study from the researcher and you can discuss this with outsiders.

Information about the research (to be written by the researcher in a simple language answering the following questions when applicable)

1. Study title: (Effect of different cleaning regimes on biofilm formation of acrylic based removable orthodontic appliance: A randomized clinical trial)

2. What is the purpose of this study? 1. To evaluate the effects of different cleaning regimes such as chemical and mechanical on biofilm formation of an acrylic based removable orthodontic appliance. 2. To find out if surface modification i.e. polished acrylic fitting surface, have an impact on cleaning the biofilm formation.

3. Where will the study be conducted? It will be conducted in the orthodontic clinic

4. What are the procedures to be followed and what will you be asked to do at each visit? Orthodontic patients for each group will be selected to wear a removable orthodontic appliance for the study period and will be instructed for the cleaning protocol according to the manufacturer's instructions.

5. How long will the participation in the study last? One week

6. If you decided to taking part in the study, will the treatment be different from the treatment you would get otherwise? No

7. Who should not enter the study?
   - Participant taking steroid-based or antibacterial mouthwash or broad-spectrum antibiotics 2 months prior to study participation;
   - Smokers;
   - History of sensitivity to any ingredient contained in the cleaning materials;
   - mouth breather;
   - Patients taking any medication that reduces salivary flow;
   - Caries;
   - crowding (more than 3mm);
   - pregnant or lactating female; and
   - absence of some of the upper permanent dentition

8. What will be the benefits of the study?
   a) To the participant? Instruction for the participant about the methods for cleaning and disinfectant their appliances and obtaining good oral hygiene.
   b) To the investigator? (This study is a part of fulfillment of the requirement for the degree of Master of Science in Orthodontic Department).

9. What are the possible risks of taking part? There is no risk

10. If you feel severe discomfort or pain during the study, would you be able to take any relief medication? No

11. Will your participation in the study interfere with your daily activities? No

12. Will you be informed of the results of the study? Yes

If you agree to participate in this study, we will ensure your confidentiality with no one except the study researchers have the right to access your dental (medical) notes.
Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without having to give a reason and without this affecting your future medical care or your relationship with medical staff looking after you.

Thank you for reading this Information Sheet and considering your participation in this study

Consent Form

	Please tick to confirm
I confirm that I have read and understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to withdraw at any time without any medical/dental care affected.	
I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the College of Dentistry – University of Baghdad where it is relevant to my taking part in this research. I give permission to these individuals to have access to my records.	
I agree to take part in the above study.	

Regarding any information and records taken during the research please specify your acceptance to share them as you desire:					
	Personal data	X-rays	Extra-oral photographs	Intra-oral photographs	Others
Confidential					
For consultation					
For teaching					
For conferences					
For publication					

	Name	Signature	Date
Participant			
Parent/guardian (if appropriate)			
Person taking consent			

Person to contact:

Name: Safa Imad Khawwam

Phone No.: 07721417546

Email: safa.khateeb094@gmail.com

1 copy for the participant; 1 copy for the researcher