

COMPARATIVE EVALUATION OF MICROBIAL CONTAMINATION OF LABORATORY FABRICATED HAWLEY'S AND ESSIX RETAINERS, WITH AND WITHOUT DISINFECTION AFTER RECEIVING FROM THE DENTAL LABORATORY- AN IN VITRO STUDY.

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Introduction: Orthodontic treatment plan is incomplete without planning the retention. Usually post treatment, the teeth tend to move to their earlier position. To prevent the relapse, use of retainers after orthodontic treatment becomes mandatory. Removable retentive devices facilitate ease in maintaining good oral hygiene during the treatment¹. The most commonly used removable retainers are the Hawleys and Essix retainers. The retainers permit reorganization of the underlying supportive structures after completion of orthodontic treatment². They are mostly made up of either thermoplastic polymer or polymethylmethacrylate acrylic base³. The selection of retainers from wide range of retainers available, depends on choice of aesthetic of the patient and other parameters. Presently vacuum formed retainers (VFR) are more commonly used. Removable orthodontic devices provide a favourable environment for accumulation of microorganisms and may result in caries, Periodontal diseases and other illnesses⁴⁻⁷. Most common bacteria of oral cavity are Streptococcus mutans and Lactobacillus. Many studies have shown high level of bacterial accumulation on removable orthodontic appliances⁸. The attachment of bacteria to the surface depends on the surface roughness of the material^{9,10}. Higher attachment to the rough surface is due to presence of small pits and grooves^{11,12}. A smooth surface may show less adhesion of

bacteria than the rough surface.¹³

To overcome the bacterial contamination of the orthodontic retainer, proper finishing and disinfection of the appliances should be done before delivering it to the patient. Some studies have suggested that dental laboratories also can be a source of contamination of prosthesis, with potentially pathogenic microbes. Local disinfection can prevent the cross infection from the clinic to laboratory and vice versa.¹⁴ After the appliance is fabricated, it is important to disinfect the appliance before inserting it in patient's mouth. The most common way to disinfect the removable retainer is the use of Hypochlorite's, Aldehydes and Chlorhexidine.¹⁵ Since the removable appliances cannot be sterilized or disinfected by heat treatment, chemical disinfectants are the only choice for the disinfection of the Hawley or Vacuum formed Retainer (VFR).¹⁶⁻¹⁸ It is the duty of the practitioner to prevent or minimize the cross infection to the patient or the team members.¹⁹ Now -a day's patients have become more aware about the disinfection. With increase in the variety of microbial strain and with addition of resistant strains, considerations should be given to disinfection of the dental impression, before sending it to the laboratory. As the Essix and Hawley's retainers are most commonly used for post treatment retention, it is necessary to evaluate the contamination of the retainers before delivering them to the patients. Hence in this study an attempt has been made to analyse the difference in bacterial accumulation

on Essix and Hawley's retainers, with and without disinfection after receiving from the dental laboratory.

Primary Research Question: - Is there any difference in accumulation of bacterial colonies over Hawley's and Essix Retainers, with and without disinfection after receiving from dental laboratory?

Primary Hypothesis: - There is a difference in microbial contamination between laboratory fabricated Hawley's and Essix retainers, with and without disinfection after receiving from the dental laboratory.

Review of Literature:

Bollen et al⁹ in 1997 conducted a study to analyse the effect of surface roughness, on plaque accumulation. In this review, studies on natural teeth; implant abutment; amalgam; gold, composite; acrylic resin; GIC and ceramics were referred. The criteria of threshold surface roughness was employed in the studies referred. The review concluded that that an increase in surface roughness above the threshold roughness, resulted in a simultaneous increase in plaque accumulation, thereby increasing the risk for both caries and periodontal inflammation. J. Sheridan et al²⁰ in 2003 discussed about the Essix appliance and said that Essix appliance is a light, almost invisible removable appliance, that snaps over the teeth and is used mostly in retention. After initial period of full-time wear, the patient is advised to wear retainer only at night during sleep. Two types of plastics, type A+ and C+ are used in fabrication of Essix retainer. Type A is the plastic of choice whenever bite planes, occlusal splints, or various

attachments need to be incorporated in an appliance. It is the material of choice for long-term retention after the orthodontic treatment.

J. Boersma et al²¹ in 2005 conducted a study in which caries prevalence on the buccal surfaces of teeth in orthodontic patients was determined with QLF and visual examination immediately after removal of fixed appliances. They studied caries prevalence during orthodontic treatment. In the study 97% of all subjects and on average 30% of the buccal surfaces in a person were affected. His study concluded that a positive correlation with caries prevalence was found for the bleeding scores 6 weeks after debonding and lactobacillus counts before debonding.

L. Hichens et al²² in 2007 studied the patient satisfaction of Hawley and Vacuum Formed Retainers (VFR). In all, 196 subjects were randomized to the Hawley group (mean age 14 years 8 months, 63 per cent female, 37 per cent male) and 201 to the VFR group (mean age 15 years, 59 per cent female, 41 per cent male). VFRs were more cost-effective than Hawley retainers from all perspectives. Most subjects showed a preference for VFRs compared with Hawley's retainers. There were also fewer breakages than in the Hawley group.

S. Hilgenberg et al¹² in 2008 evaluated the surface properties of acrylic reins. The purpose of the present study was to evaluate the superficial roughness and contact angle after two types of polishing and the Vickers hardness of three acrylic resins. Five 20 x 3 ± 1 mm diameter discoid specimens were obtained for each group. One side of the specimens was subjected to standard polishing (pumice and whiting slurry), and the opposite side was polished with special tips. The mean roughness and contact angles of the materials were measured. All the groups in the study presented a surface roughness that exceeded this parameter when the specimens were left unpolished, demonstrating that an unpolished provisional fixed prosthesis is unacceptable. After polishing with slurry, the measured roughness of was on lower side.

Jo. Birdsall et al⁴ in 2008 conducted a case study in which patient who developed significant caries and demineralisation due to consumption of large quantities of cariogenic drinks while he was wearing an Essix retainer was analysed. The initial phase of treatment focused on prevention of further disease and included cessation of retainer-wearing, dietary and oral hygiene advice, daily use of a fluoride mouth rinse, plus scaling and prophylaxis. The patient was reviewed two months later, by which time caries progression appeared to have arrested. He concluded that it is particularly important to note that this risk of dental disease continues throughout the retention phase of orthodontic care.

F. Parina et al¹⁷ in 2009 studied the effect of different disinfectants on staphylococcus aureus. A total of 180 impressions of a maxillary model (90 alginate and 90 polyvinylsiloxane impressions) were taken for the purpose of -in vitro study. Half of the impressions were infected with Staphylococcus aureus and the other half were infected with Candida albicans. The results showed that the use of all the four disinfectants reduced Staphylococcus aureus counts. The counts reached to zero in 80% of the cases. There were no statistically significant differences in Staphylococcus aureus count reductions between the four disinfectants evaluated. Micro 10 disinfectant was more effective on alginate; Deconex was more efficient for polyvinylsiloxane and Alprocid had a better efficacy on both impression materials in eliminating Candida albicans.

K.Kitada et al¹⁰ in 2009 conducted a study to detect the opportunistic bacteria and fungi from the oral cavities of orthodontic patients and examine the ability of the organisms to adhere to saliva-coated metallic brackets. Opportunistic bacteria were collected from 58 patients. The orthodontic group was divided into two subgroups: one wore multibracket appliances and the other wore other appliances such as Hawley's type retainer, Begg's type retainer, fixed type retainer, activator, quad helix appliance, lingual arch appliance and maxillary protracting appliance. Seven opportunistic and four streptococcal strains were tested for their ability to adhere to saliva-coated metallic brackets. According to the results more opportunistic bacteria and fungi were detected in the orthodontic group than in the non-orthodontic group. Study concluded that the isolation frequencies of opportunistic bacteria and fungi increase during orthodontic treatment, suggesting the importance of paying special attention to oral hygiene in orthodontic patients so as to prevent periodontal disease and the aggravation of systemic disease in immunocompromised conditions.

Al – Rifaiy et al¹³ in 2009 studied the effect of mechanical and chemical polishing techniques on the surface roughness of acrylic. The aim of this study was to evaluate the effect of mechanical polishing and chemical polishing on the surface roughness of heat cured and auto cured denture base acrylic resins. Sixty acrylic specimens were used for the study. Thirty heat cured specimens received mechanical conventional lathe polishing using cone with pumice slurry and soft brush with chalk powder. The other thirty heat cured specimens received chemical polishing by immersing in methyl-methacrylate monomer. The sixty self-cured specimens received mechanical and chemical polishing in the same manner. Surface roughness was measured using surface analysing instrument in microns. According to the results, there was no significant difference in surface roughness measured. According to this study a smooth polished surface is important to prevent the bacterial colonization and plaque accumulation.

C. Turkoz et al⁸ in 2011 studied the influence of thermoplastic retainers on streptococcus mutans and Lactobacillus adhesion. The study was conducted to check the thermoplastic retainer influence on the oral flora. Twenty-four orthodontic patients were considered for the study. After debonding, the patients were given thermoplastic retainers (Essix ACE 0.040-in plastic, Dentsply International, York, Pa) for both jaws and instructed to wear them all day. Plaque samples from tooth surfaces and saliva samples were collected from each patient just after debonding (T0), and on day 15 (T1), day 30 (T2), and day 60 (T3) of retention. He concluded that retention with thermoplastic retainers might create oral conditions conducive to Streptococcus mutans and Lactobacillus colonization on dental surfaces. Topaloglu-Ak A⁷ et al in 2011 observed the effect of orthodontic appliance on oral microbiota. This study investigates the effect of fixed and removable orthodontic appliances among children on salivary Streptococcus mutans, Lactobacillus sp. and Candida albicans. Sixty-nine patients were involved in this study. Samples were diluted and plated on MitisSalivarius Agar (MSA), Man Rogosa Sharp Agar (MRS) and Saboroud Dextrose Agar (SDA). The growths on the plates were

examined under a stereomicroscope. The study concluded that long-term utilization of orthodontic appliances may have a negative effect on microbial flora and increase the risk of new carious lesions and periodontal problems.

Groosh et al³ in 2015 conducted a study to check the influence of surface roughness and surface dynamics on the attachment of Methicillin-Resistant *Staphylococcus aureus* onto orthodontic Essix materials. Essix materials have shown favourable surface characteristics for MRSA adhesion and biofilm formation. Clinically simulated samples of auto polymerized acrylic and thermoplastic material were first evaluated using laser non-contact, stylus mechanical profilometries and atomic force microscopy (AFM) followed by contact angle measurement to characterize their surface dynamics.

Finally, an in vitro biofilm assay was carried out using a constant depth film fermentor to assess biofilm attachment. Further work on the effect of surface treatment of auto polymerized acrylic materials on MRSA biofilm will be required to arrive at a definite conclusion.

Kirubakaran et al⁶ in 2017, studied the streptococcus mutans adhesion on nickel titanium and copper titanium arch-wires. Total 16 patients were included in this study. Surface roughness and surface free energy was studied by three-dimensional surface profilometry and dynamic contact angle analysis, respectively. Wires after four weeks of use were tested for the bacterial count. The results suggested that streptococcus mutans adhesion was more common in Cu-NiTi wires. These wires exhibited rougher surface and higher surface energy than compared to NiTi archwires. In a study by Albana et al²³ in 2017, effectiveness of brushing Essix retainer with water only and followed by soaking in one of three commercial cleansing tablets was tested. A split mouth study was conducted with 60 people. A double-blind, parallel, randomized clinical trial with a split-mouth design was completed on 60 volunteers with specific eligibility criteria assigned to all the three groups. Each participant wore an upper Essix retainer (with an electronic microsensor) on a full-time basis for 14 days and used one of the three products on one side of the retainer for 15 min/day. The effectiveness of the cleansing tablets was tested by the scanning electron microscopy, bacterial identification, bacterial quantification, and disk diffusion methods. Using chemical cleansing tablets after mechanical cleansing did not significantly reduce the bacterial count in Essix retainers when compared to use of mechanical cleansing alone. However, the tablets seem to be effective against “cocci” bacterial species.

F. Akgun et al¹⁸ in 2019 studied evaluation of the efficacy of different cleaning methods for orthodontic thermoplastic retainers in terms of bacterial colonization. In this prospective cross-over study, a total of 21 volunteers were included. The methods used were peroxide-based cleanser tablets (PBCTs) plus brushing, control (only brushing), and vinegar plus brushing, respectively. Conclusion of the study was that, the application of PBCTs and vinegar to Vacuum formed retainers (VFRs) at sequential time intervals resulted in similar bacteria counts. The higher *Lactobacillus* counts and similarly higher *Streptococcus mutans* counts on the VFR samples indicate that mechanical cleaning only is not adequate to obtain hygiene. Very little attention has been paid towards the contamination of retainers from the orthodontic Laboratory. Contamination and infection risks are unknown. Hence it is important to study the

microbial contamination of Essix and Hawley's retainer, with and without disinfection after receiving them from the dental laboratory.

Primary Objectives: - To assess and compare the microbial contamination of laboratory fabricated Hawley's and Essix retainers, with and without disinfection after receiving from the dental laboratory.

Methodology:

STUDY DESIGN: Experimental in vitro study.

STUDY SETTING: Department of Orthodontics and DentofacialOrthopedics
Department of Microbiology.

STUDY POPULATION: This is an in vitro study.Essix and Hawley's retainers will be used in this study.

SAMPLING TECHNIQUE: Convenience sampling method.

Sample size: In total 40 samples of retainers are required (20 Hawley's retainers and 20 Essix retainers).

SAMPLE SIZE ESTIMATION Level of significance = 5%, Power = 80%,Type of test = two-sided Formula of calculating sample size is: Sample size for clinical trial (outcome variable on ratio scale)

Testing null hypothesis.

$$n = 2 S^2 (Z_1+Z_2)^2 / (M_1-M_2)^2$$

$$n = 2 [0.95 (1.644+0.841)^2]$$

t tests – Means: Difference between two independent means (two groups)

Analysis: A priori: Computer required sample size

Input: Tail(s)= Two

Effect size d = 0.95

α err prob = 0.05 Power (1-β err prob) = 0.80

Allocation ratio N2/N1 = 1

Output: Non-centrality parameter δ = 2.928

Critical t = 2.028

Df = 36

sample size group 1 -19

sample size group 2 - 19

Total sample size = 38

Actual power = 0.8130

A power analysis was established by G*Power, version 3.0.1(Franz Fauluniversitat, Kiel, Germany). Total calculated sample size of 38 rounded to 40 retainers (20-Essix, 20 – Hawley's) would yield 80% power to detect significant differences, with effect size of 0.95 and significance level at 0.05.

METHOD OF SELECTION:

INCLUSION CRITERIA:

- Essix retainer.
- Hawley's retainer.

EXCLUSION CRITERIA:

- Fixed orthodontic retainer.
- Other removable orthodontic retainers.

OPERATIONAL DEFINITIONS:

Disinfection: The process of killing harmful and objectionable bacteria, cysts and other microorganisms except spores by various agents from a surface, medium or an article is called as disinfection. Orthodontic Retainer: Orthodontic retainers are custom-made devices, usually made of wires or clear plastic, that hold teeth in position after realigning the teeth.

STUDY METHOD:

- All the steps in procedure will be conducted in required disinfected conditions.
- 40 samples of retainers will be required for the study.
- Models which are to be used for fabricating the retainers will be retrieved from a disinfected impression.
- All 40 swabs will be coded accordingly
- The samples those are to be disinfected will be disinfected using Korsolex by soaking them for 30 min in disinfecting solution.
- Swab (S1a) will be collected from the fabricated Hawley's retainer after receiving from the dental laboratory.
- Swab (S1b) will be collected from the fabricated Hawley's retainer after disinfection.
- Both the swabs will be placed in test tube containing 1ml of Nutrient broth.
- All the samples will be stored in a sterile test-tube which will be placed in sterile zip bag.
- The S1a, S1b will be then inoculated in sterile Nutrient agar plates and plates are further incubated at 370C for 24 hrs.
- Swab (S2a) will be collected from the fabricated Essix retainer after receiving from the dental Laboratory.
- Swab (S2b) will be collected from the fabricated Essix after disinfection.
- Both the swabs will be placed in test tube containing 1ml of Nutrient broth.
- All the samples will be stored in a sterile test-tube which will be placed in sterile zip bag.
- The S2a, S2b will be then inoculated in sterile Nutrient agar plates and plates are further incubated at 370C for 24 hrs.
- The microbial count will be then assessed and compared.

DATA COLLECTION CHART:

Sr.no	Swab collected from Hawleys retainer after receiving from laboratory		Swab collected from Essix retainers after receiving from laboratory.	
	Microbial colonies Before disinfection	Microbial colonies After disinfection	Microbial Colonies before disinfection	Microbial Colonies after disinfection.
	(S1a)	(S1b)	(S2a)	(S2b)

STUDY MATERIALS AND EQUIPMENTS: All the materials and equipments in the study will be used under strict disinfected condition.

Materials and equipments:

- Sterile cotton swab.
- Irreversible hydrochloride impression material.
- Impression trays.
- Dental stone.
- Test tube
- Incubator
- Nutrient broth. Sr. No. Swab collected from Hawley’s retainer after receiving from laboratory.
- Nutrient agar (Peptone, meat extracts, NaCl, Agar, Distilled water).
- Disinfectant for disinfection of removable orthodontic device. (Korsolex)(Glutaraldehyde 15.2g, 1,6-dioxahexane-19).
- The experimental study will be conducted in the Department of Orthodontics and DentofacialOrthopedics after obtaining the consent waiver from the institutional ethical committee as it is an in vitro study.
- In total 40 retainers are involved in the study.
- Data will be collected in tabulated format and then statistical data analysis will be carried out using appropriate tools.

STATISTICAL ANALYSIS

- Statistical analysis will be performed using Statistical Package for Social science (SPSS) version 21 for Windows (SPSSInc, Chicago, IL).
- Descriptive quantitative data will be expressed in mean and standard deviation respectively.
- Data normality will be checked by using Shapiro – Wilk test.

- Confidence interval is set at 95% and probability of alpha error (level of significance) set at 5%.

Power of the study set at 80%.

- Intragroup comparison of mean change in microbial colonies between Essix and Hawley's retainers with and without disinfection will be done using paired 't' test.
- Intergroup comparison of mean change in microbial colonies between Essix and Hawley's retainers will be done using unpaired 't' test.

EXPECTED RESULTS: It was decided to observe the result of study without bias and review if the material used in fabrication and the surface morphology of two types of retainers has any effect on plaque accumulation and cross-infection. The efficacy of disinfectant as related to surface morphology of retainer will also be observed.

DISCUSSION

Orthodontic treatment plan is incomplete without planning retention. Presently commonly used retainers are the Hawleys retainer and the Essixretainer. The additional parameters to be considered during selection of retainers are the esthetic perspective of patient and ease of disinfection of these appliance. The risk of contamination of the appliance during its fabrication in laboratory and cross infection from laboratory to clinic and vice-versa should be prevented to patients or team members. Consideration towards disinfecting impressions before sending to lab is important to prevent cross infection. Bollen et al⁹ in their study in 1997 reviewed that increase in surface roughness above the threshold roughness, resulted in simultaneous increase in plaque accumulation and increase in bacterial load.

K. Kitada et al¹⁰ in 2009 conducted a study to detect opportunistic bacteria and fungi from oral cavities of patients undergoing orthodontic treatment and the ability of organism to adhere to surfaces. The study stressed on importance of paying attention to oral hygiene in orthodontic patients to prevent periodontal disease and aggravation of systemic disease in immunocompromised condition. Hence in this study an attempt is made to analyse the difference in bacterial accumulation on Essix and Hawleys retainers, with or without disinfection after receiving from dental lab.

STUDY PERIOD – Approximately 18-20 months.

CONCLUSION:

- Prevention of cross infection from clinical area to laboratory and vice-versa should be considered during fabrication of appliance and its delivery to patients
- Necessary disinfection protocol should be exercised at every stage from transport of impression from clinic to lab and from laboratory back to clinic before delivery of retention appliance to patients.

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Gantt chart: - GANTT CHART: Study period is 18-20 months approximately