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Mutans Streptococci and Removable Orthodontics

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Abstract

Numerous studies have investigated the influence of orthodontic therapy and appliances on the oral microbial flora. Little is known about the effect of removable orthodontic appliances on oral colonisation by mutans streptococci. The original aim of the present investigation was to assess the mean and the statistical mean difference of colony values of S. Mutans in a removable orthodontic appliance fabricated from two types of acrylic resin for an Iraqi sample. According to certain predetermined sample criteria; the present study consist of eleven consecutive young patients scheduled for orthodontic treatment with removable orthodontic appliances at the department of orthodontics at the college of dentistry/ University of babylon as well as dental student seeking orthodontic treatment. Each subject included in the present study had to wear an upper (hot cured) and lower (cold cured) well adaptive removable orthodontic appliance. A swab was taken from the lingual/palatal gingival and inoculated within the brain heart agar then cultured within Mitis- Salivarius Bacitracin (MSB) agar. The colonies were calculated for each plate, afterwards the colony forming unit (CFU) will be calculated. of the present study showing that all bacterial colony values were higher in the lower than upper arch. However, a statistical non significant difference was registered between the colony mean values of both arches. The present study signify both types of acrylic resins (heat/ cold) can be used as an orthodontic acrylic base in removable appliances within the orthodontic practice with a careful monitoring of patients treated orthodontically for risk of caries development.

Keywords: *mutans streptococci, heat cure acrylic, cold cure acrylic.*

Introduction

Acrylic resins are mostly used as denture and orthodontic base material in dental practice. These are available in different forms according to the polymerization reaction as heat cure acrylic resin, rapid cure auto polymerizing acrylic resin, light cure resin and specialized form resins used for microwave. Usually, heat-cured (polymethyl methacrylate) PMMA is used as the so-called "gum-work" for removable full dentures or removable partial dentures; [1] the cold-cured PMMA is used for denture repair, reline and orthodontic removable appliances involved in thumb deterrent, tipping teeth, block movements, overbite reduction, space maintenance and retention. [2,3]

Nowadays orthodontic treatment is adopted by wide section of society not only for the correction of malocclusion but also improves mastication, speech and appearance, as well as overall health, comfort, and self-esteem. [4] Although the orthodontic appliances has many known benefits, these appliances are also associated with a number of damages and disorders of oral cavity. [5]

Oral cavity is a complex environment supporting a many distinct bacterial species or phylotypes, of which over 50% are yet to be cultivated, residing specifically in diverse niches in the oral cavity and executing different roles. [6,7] Presence of ortho-dontic appliances in oral cavity alters the balanced ecosystem of oral microbiome; as it provides an additional retentive site for food, different physio-chemical environment and surfaces for adhesion and attachments of normal oral microflora. [8] Regarding the long term existence of baseplates of orthodontic appliances (BOA) in mouth and their surface porosities may have a negative impact on oral microbiota, promote the biofilm formation and may contribute to dental caries, gingival inflammation and periodontal disease. [9,10] Streptococcus mutans (SM) is considered one of the main organisms in plaque that contributes to the initiation of caries. Despite being ubiquitous in the oral cavity, SM prevalence often indicates caries susceptibility and poor oral hygiene. [11]

Numerous studies have investigated the influence of orthodontic therapy and appliances on the oral microbial flora. [12,13] Little is known about the effect of removable orthodontic appliances on oral colonisation by mutans streptococci. [14] The original aim of the present investigation was to assess the mean and the statistical mean difference of colony values of S. Mutans in a removable orthodontic appliance fabricated from two types of acrylic resin for an Iraqi young sample.

Materials and Method

Sample enrollment:

According to certain predetermined sample criteria; the present cross sectional study consist of eleven out of seventeen (7 males and 4 females) consecutive young patients scheduled for orthodontic treatment with removable orthodontic appliances at the department of orthodontics with age ranging from 18 to 25 years were enrolled to be included in this study during the period from December 2018 to July 2019. The study sample were reqruited from patients attending the teaching hospital at the college of dentistry/ babylon University as well as dental student seeking orthodontic treatment. As each patient was receiving upper and lower orthodontic appliance; so the study sample were further subdivided into eleven upper as well as lower arch that receiving appliances.

Inclusion sample Criteria:

1) Good oral hygiene 2) Cooperative compliant patient 3) Clinically healthy gingiva 4) Well fabricated upper and lower removable orthodontic appliance 5) Patients' malocclusion is within the scope of removable appliance.

Exclusion sample criteria:

1) History of systemic diseases 2) medicament such as antibiotics, steroids, or nonsteroidal anti-inflammatory drugs at least 3 months prior to appliance insertion 3) Presence of fixed bridges/crowns or partial dentures 4) Smoking habit.

Materials

1) Transport media 2) Petri dish, test tube, flask, rick 3) Loop and glass Spreader. 4) Micro and electro-pipette. 5) sterile saline and distilled water. 6) Mitis salivarius agar, brain heart agar, agar agar. 7) Bacitracin. 8) Cold and Hot Cure Acrylic. 9) Incubator, Dry oven, Autoclave. 10) Refrigerator. 11) Millipore filter(0.4µm) 12) Gram stain.

Methodology (bacterial isolation):

Each subject included in the present study had to wear an upper and lower well adaptive removable orthodontic appliance. The upper and lower appliances were fabricated from heat and cold cure acrylic resin, respectively following the manufacturer's instructions (Surrey, U.K., England). At the time of 1st appliance activation after 2 weeks of their insertion; a swab was taken from the lingual/palatal gingival area were the acrylic base plates were extended using a selective transport media **(fig. 1)** . After 24 hour of their initial anaerobic incubation at 37º C; the swab was inoculated within the brain heart agar as a broth media that facilitating over growth of bacteria and incubated again for 24 hour. Afterwards and before cultivation, serial dillution was done to be ready for cultivation (0.1 ml of sample is plated) within Mitis- Salivarius Bacitracin (MSB) agar using a sterile spreading glass. After 24 hour of incubated bacterial cultured plates at 37º C, a growth of bacterial colonies (a cluster of clones in bluish distinctive colour) were seen clearly and distributed on the surface of MSB agar **(fig. 2 left).**

The colonies were calculated for each plate using colony counter device, afterwards the colony forming unit (CFU) will be calculated and the data were submitted and stored in an excel program within a computer for satistics.

S. Mutans identification:

The identification of S.mutans is based on (according to the information in Bergeys Manual of Determinative Bacteriology 9th ed.,1994) [15] distinctive colonial morphology on selective and nonselective agar, Gram staining, distinctive cell shape on light microscopy. Morphologically, a gram-stained isolated Mutans Streptococci were viewed under a light microscope at an objective magnification of 40x. The size of the observed colonies ranged from 0.5 to 1 µm. The mutans bacterial cells resembled chains of cocci long as beads in their spatial arrangement. (Fig.2 right).

Figure 1: A transport media with plates

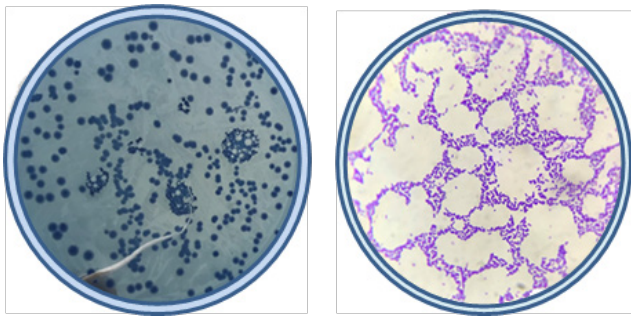

Figure 2: The pictures shows the macro- and micromorphology of Streptococcus Mutans isolated from study sample. (left) S. mutans colonies with a characteristic rounded bluish color grown on the MSB selective agar; (right) The isolated colony of S. mutans is gram stained and viewed under light microscope, the coccus cells arranged in chains.

Colony forming unit

A colony-forming unit (CFU) is a unit used to estimate the number of viable bacteria or fungal cells in a sample. Not all bacterial cells produce colonies; For this reason results are reported as colony forming units (CFU)/ml of bacterial culture. Ideally only plates with 25-250 colonies are used**.** In order to make the calculation of the number of cells/ml in the original samples less formidable, dilutions are designed to be easy to handle mathematically. [16] The number of colonies present in a particular test sample was determined using the formula [17] **CFU/ml= Number of colony × 1/dilution × volume of sample plated.**

CFU/ml= (X) × 10^6 × 10 .

CFU/ml= 0.0X × 10^9 . In present study for a given no. of colonies; a 0.1 ml from a 10^{-6} dilution of the original sample was plated.

Mitis- Salivarius Bacitracin agar (MSB agar):

Following the method of **Geigy [18]; Al-Mudallal et al [19]; Naji [20]** in this agar media preparation:

MSB agar is a selective media were used for cultivation of S. mutans. It was prepared by addition of selective agents: bacitracin antibiotic and sucrose, at the optimal levels determined to the Mitis –Salivarius Agar (MSA).

Mitis- Salivarius Agar (MSA)

Is the main components of the prepared media that suppress the growth of most microorganisms but allows the growth of Streptococcus *Spp.* This media prepared according to the manufacturer's instruction by dissolving 90 g of MSA in 1000 ml distilled water. Afterwards sterilized by autoclave at 121ºC 1.5 bars per square inch for 15 minutes, and left to cool until 45 ºC. To inhibit bacteria other than Mutans Streptococci; bacitracin and sucrose were added to the Mitis agar medium; since a relative resistance of S. mutans to high concentration of both bacitracin and sucrose had been reported.

Bacitracin antibiotic

Under aseptic condition, bacitracin antibiotic solution was added to the agar media. A bacitracin stock solution was prepared by dissolving 0.2661g of bacitracin powder in 100 ml of de-ionized water. This will supply concentration of 200 IU /L (1 unit of bacitracin =0. 0133 mg). [18] Millipore filter (0.4µm) was used to sterilize bacitracin solution. A new fresh solution was prepared every 2-3 weeks and stored in a refrigerator.

Sucrose

After the sterilization of the Mitis agar medium till cooling, sucrose was added to provide concentration of 200 g/L. Sucrose solution was sterilized by Millipore filter(0.4µm). 200 g/L of sucrose can inhibit the growth of S. sobrinus and S.cricetus and enhance the growth of S.mutans.[19] Moreover, The inclusion of sucrose leads to the formation of glucans and the appearance of colony formation to aid identification.

After the addition of these two components to the Mitis agar, a Mitis -Salivarius Bacitracin (MSB) agar will be form. The latter formed media then poured into

plates while solidify and stored in a refrigerator (after overnight incubation at 37 ºC) till use thereafter.

Results

The results of the present study showing that all bacterial colony values were higher in the lower than upper arch. However, a statistical non significant difference was registered between the colony mean values of both arches.

Table (1): Reveals the mean, maximum, minimum, standard deviation, error values for the upper and lower *Streptococcus Mutans* colonies.

Variable	meanU/L	mean difference	t.test	p.value	df
USC	127	-21.09	-.65	.995	20
LSC	148				

*Each colony value is multiplied by 10^7.

Table (2): Reveals the mean difference between upper and lower *Streptococcal* colonies and independent t-test. A non significant difference was found between both colony values of upper and lower arches.

variable	no.	Mean/*cfu**	Max	Min	SD	SE
USC	(11)	127	263	50	56	23
LSC	(11)	148	285	72	43	22

*P-value ≤ 0.05 was significance

Discussion

Plaque accumulation followed by enamel demineralization and gingivitis is a well-known complication in orthodontic therapy when fixed or removable appliances are used. [21] When use the orthodontic appliances, the acrylic plates are placed in contact with teeth, and thus the resulting plaque accumulation commonly is due to food retentive configuration of the acrylic materials. [22] Regarding the long term existence of base plate of orthodontic appliances in mouth and their surface porosities may have a negative impact on oral microbiota, promote the biofilm formation and may contribute to dental caries, gingival inflammation and periodontal disease. [9,10] Streptococcus mutans (SM) is considered one of the main organisms in plaque that contributes to the initiation of caries. [11]

For this reason it is necessary to investigate the degree of adhesion as well as virulence of S. Mutans using the two types of orthodontic acrylic resins in isolated study by CFU calculation.

Regarding the present study result; the colony forming unit for the lower S.colonies are higher than the upper colonies in all values (mean, minimum, maximum) as the mean lower S. colonies is (148×10^7 cfu/ml) while (127×10^7 cfu/ml). Such higher lower colony units of S. mutans in spite of smaller mandibular surface area suggesting that cold cured acrylic resins of removable orthodontic appliance is more favourable environment for mutans S. colonization than hot cured resins. However, a non statistical mean difference was found between the S. colony units for both acrylic resins (P. value = 0.9) which can be attributed to sample size methodological factors. It was found that the use of removable appliances may lead to the creation of new retentive areas and surfaces, which favour the local adherence and growth of Mutans Streptococci. [23] In cold-cured acrylic resins the problem is even more prominent, because they display more surface porosities than the heat-cured ones. These porosities hamper the complete removal of dental plaques, such that mechanical cleaning often turns out to be inadequate. Surface roughness may contribute to the positively correlated rate of microbial colonization and plaque maturation on

surfaces. [24] Lewis also showed that heat cured PMMA, often the kind used for making a Hawley retainer, showed the most bacterial adherence, specifically by S. mutans. Importantly, S. mutans adhered more, or to the same extent to acrylic, as it did to enamel. A study demonstrated that the subgingival flora did not change, implying that the periodontal condition of the patient was not affected. [25] Moreover; although bacterial levels may increase with orthodontics, patients with removable retainers are similar to healthy nonappliance wearers demonstrating no increased gingivitis or periodontitis. [23]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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