



ISSN Print: 2664-7591
ISSN Online: 2664-7605
Impact Factor: RJIF 5.2
IJAN 2024; 6(1): 128-133
www.pharmaceuticaljournal.in
Received: 09-03-2024
Accepted: 17-04-2024

Naveena Challa
Intern, Kamineni Institute of
Dental Sciences, Narketpally,
Nalgonda (Dist.), Telangana,
India

Varsha Allenki
Intern, Kamineni Institute of
Dental Sciences, Narketpally,
Nalgonda (Dist.), Telangana,
India

Dr. Madanika P
Associate Professor,
Department of Biochemistry,
Kamineni Institute of Medical
Sciences and Research Center,
L B Nagar, K V Ranga Reddy
(Dist.), Telangana, India

Suryakanth Malgikar
Associate Professor,
Department of Periodontics,
Kamineni Institute of Dental
Sciences, Narketpally,
Nalgonda (Dist.), Telangana,
India

Corresponding Author:
Dr. Madanika P
Associate Professor,
Department of Biochemistry,
Kamineni Institute of Medical
Sciences and Research Center,
L B Nagar, K V Ranga Reddy
(Dist.), Telangana, India

Guided biofilm therapy: Current insights

Naveena Challa, Varsha Allenki, Madanika P and Suryakanth Malgikar

DOI: <https://doi.org/10.33545/26647591.2024.v6.i1b.89>

Abstract

Oral cavity harbors most diverse microorganisms in the human body, providing distinct microbial habitats such as teeth and gingival sulcus. The resident microflora contains dynamic bacteria that grow and adhere to enamel pellicle. Periodontitis is most frequent infections of oral cavity and is often recognized as the leading cause of tooth loss in adults. Periodontitis is caused by an imbalance between the host and bacterial interaction leading to clinical attachment loss and bone loss. Although scaling and root planning is considered as a gold standard for removal of the biofilm but it also has its own disadvantages. Recently, a new novel approach has been emerged known as Guided Biofilm Therapy which is helpful in effective removal of dental biofilm.

Keywords: Guided biofilm therapy, scaling and root planing, periodontitis

Introduction

Oral cavity is an ideal place where microbes, oral fluids, nutrients, soft and hard tissues are interacting and produce unique ecosystems ^[1, 2]. Therefore oral cavity has similarities with that of “incubator” in which microbes can be grown based on their temperature, p^H, nutrient and moisture preferences. This is because the warm, moist and nutritious oral environment provides an ideal breeding place for microbial growth and proliferation ^[3]. The presence or accumulation of a microbial biofilm, without any disruption, will lead to loss of symbiosis between the host’s immune-inflammatory responses. Consequently, this may progress to gingivitis and in susceptible individuals, progress to periodontitis ^[4].

Dental Biofilm

A mature Dental biofilm may consist of up to 150 or more different microbial species ^[5]. Dental biofilm is composed primarily of microorganisms encased within extracellular matrix. One gram of plaque contains approximately 10¹¹ bacteria. Biofilm is dominated by bacteria but it also contains virus, yeasts protozoa. Biofilm bacteria is most common cause for most of the dental diseases ^[6]. The structure of the biofilm restricts the penetration of the antimicrobial agents ^[7].

Characteristics of dental biofilm

Composition of Dental Biofilm

Biofilms are group of microorganisms in which the microbes produce extracellular polymeric substances [EPS] such as proteins including enzymes (<1-2%), DNA (<1%), polysaccharides (1-2%), and RNA (<1%) and in addition to these components, water (97%) is the major part of biofilm which is responsible for the flow of nutrients inside the biofilm matrix (Table 1).⁸ EPS is responsible for structural and functional integrity of biofilms. EPS forms scaffold that holds biofilm together and thus helps in cell-to-cell communication and provides adhesive and cohesive forces which are required for biofilm formation. EPS also helps in nutrient cycling, maintaining availability of Deoxyribose Nucleic Acid [DNA] for horizontal gene transfer and acts as protective barrier against biocides, antibiotics, UV radiations, desiccations and host immune defense system ^[8].

Table 1: Biofilm chemical composition (Adapted from Lu and Collins^[9])

| Sl. No. | Composition | Percentage of matrix (%) |
|---------|-----------------|--------------------------|
| 1. | Microbial cells | 2-5 |
| 2. | DNA and RNA | <1-2 |
| 3. | Polysaccharides | 1-2 |
| 4. | Proteins | <1-2 including enzymes |
| 5. | Water | 97 |

Formation of dental biofilm

Biofilm formation is a dynamic process. The phases distinguished are only arbitrary. The attachment, growth, removal and reattachment are continuous process and the plaque will undergo a continuous reorganization.

Acquired pellicle formation

A bacterium present in the oral cavity does not come in direct contact with the enamel. Salivary proteins and glycoproteins are adsorbed on to the tooth surface forming an acquired enamel pellicle. Pellicle formation starts seconds after the exposure of tooth surface to the oral environment. When salivary molecules bind to the surface, they will undergo a conformational change which leads to exposure of new receptors for bacterial attachment.

Transport of microorganisms and reversible attachment

Generally, microorganisms are transported passively to the surface of tooth with the help of the salivary flow. As the cell approaches, physiochemical forces are generated which are weak but long in range.

Pioneer microbial colonizers and irreversible attachment

The weak physiochemical interactions may become irreversible due to the presence of Adhesins on the microbial surface with complementary receptors in the acquired pellicle. Initial colonizers constitute a highly selected part of microflora. Within minutes coccal bacteria appear on the surface, mainly the *streptococci*. These microorganisms produce IgA1 protease which help them to survive host defence during initial stages of plaque formation.

Actinomyces species are also commonly isolated after 2hrs, as are *Haemophilus* species and *Neisseria* species which obligate anaerobic species are detected only rare in this stage. Once attached, these pioneer population starts to divide and form microcolonies. The irreversible attachment of cells to the tooth involves specific, short, stereochemical interactions between components on the microbial cell surface (Adhesins) and complementary receptors in the acquired pellicle.

Coaggregation / Coadhesion and microbial succession

Overtime, the plaque microflora becomes more diverse there will be a shift of streptococci with increasing proportions of *Actinomyces* and other Gram-positive *bacilli*. Some organisms that are unable to colonize the pellicle coated tooth surface are able to attach to already adherent pioneer species by further adhesion-receptor interactions (Coaggregation / Coadhesion). In addition to this, the metabolism of the pioneer species alters the local

environment and makes conditions more suited for the growth and other bacteria. Gradually, condition becomes more favorable for growth of obligately anaerobic bacteria. Similarly, the metabolism of pioneer species produces nutrients and fermentation products that can be used by other organisms as primary nutrient sources.

Mature biofilm formation

The microbial diversity of the plaque increases over the time due to the subsequent growth. The growth rate of individual bacteria within the plaque slows as the Biofilm matures. Some of the adherent bacteria synthesize extracellular polymers which will make a major contribution to the plaque matrix. A matrix is a common feature of all biofilms and is more than a chemical scaffold to maintain shape of the biofilm. It makes a significant contribution to the structural integrity and general tolerance of biofilms to environmental factors and antimicrobial agents^[10].

Non-surgical management of dental biofilm

Dental biofilm resides in close vicinity of the gingival epithelium. If proper oral hygiene measures are not taken care of, the supragingival biofilm will accumulate along the gingival epithelium and become potential source for the inflammation^[10, 11]. Initially, based on Nonspecific plaque hypothesis, the removal of the dental plaque was aimed at removing bulk of the bacteria^[12]. Later, the focus was shifted to specific bacterial removal based on specific plaque hypothesis^[13]. Oral hygiene measures plays a central role in the non-surgical treatment of periodontitis and aims to control biofilm through frequent mechanical removal by the patient^[14]. Despite of meticulous cleaning, some amount of dental biofilm is remained in undetected areas. Dental anatomical structures such as furcation's, cervical enamel projections, deep grooves and concavities can be a potential ecological niche for bacteria^[15]. Scaling and root planing significantly reduce the subgingival microbial burden by removing dental biofilm, calculus, and bacterial endotoxins^[16]. Although scaling and root planning is considered as a gold standard, it has several disadvantages such as being time-consuming procedure, technically demanding and occasionally discomfort to the patients.^[17] After Scaling and Root Planing the lingual surfaces and furcation's of teeth are more prone to residual calculus.^[18,19] Considering these drawbacks, various technologies and machines have been introduced to remove the dental biofilm to overcome these, vector scaling systems, lasers and air polishing agents have been introduced.

Guided biofilm therapy

Guided Biofilm Therapy is a new regimen where there is a sequential removal of plaque and calculus by initially detecting it with a disclosing agent followed by usages of air abrasive powder for removal of plaque and stains. Finally, the subgingival plaque and calculus are removed with a specialized nozzle and eventually scaling with a specialized tip is performed. (Figure 1) explains about the procedure in Guided Biofilm Therapy.

The sequential steps of Guided biofilm Therapy is given as follows

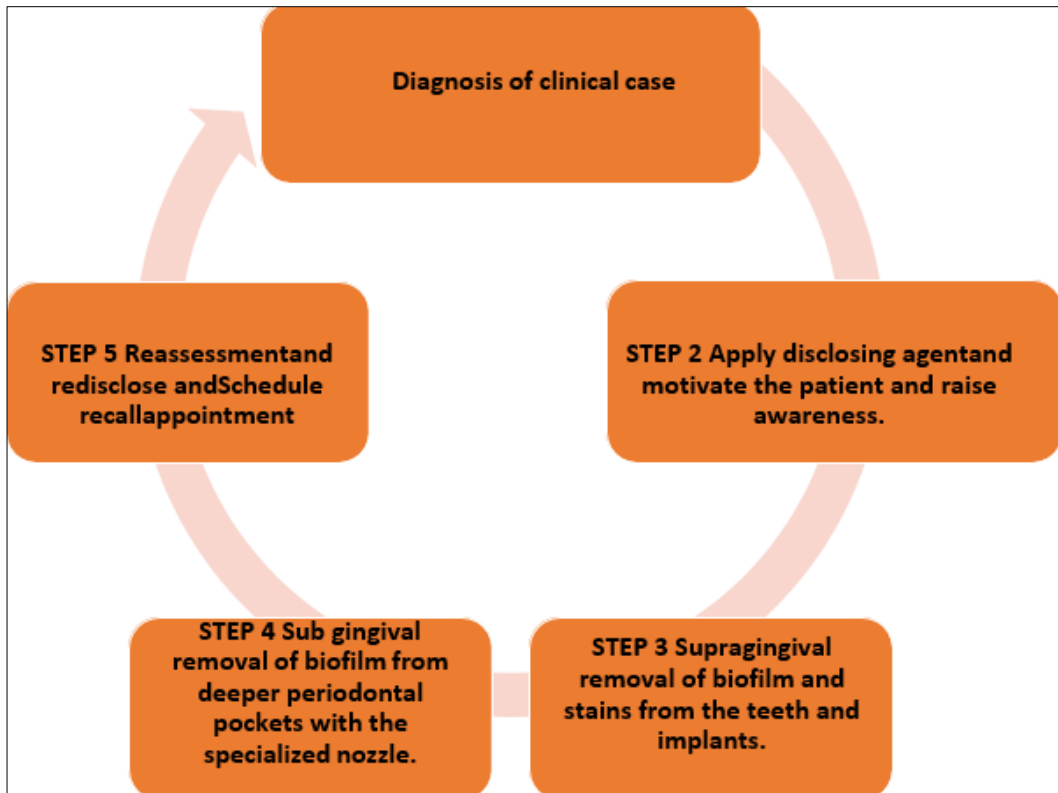


Fig 1: Steps in Guided Biofilm Therapy

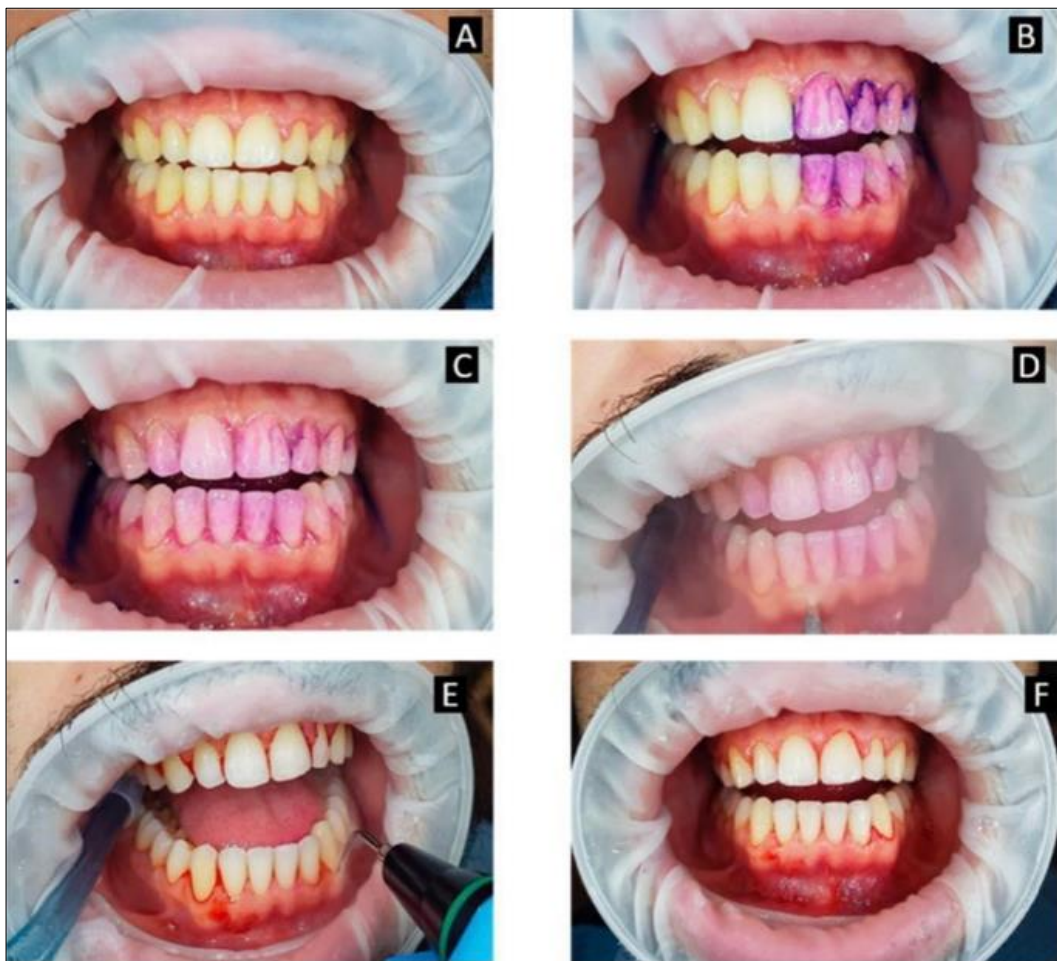


Fig 2: (A-F): Guided biofilm treatment (GBT) procedure in a patient with generalized bleeding on probing with plaque accumulation and localized calculus. (A) Pre-operative view; (B) application of disclosing agent on left half of the patient's mouth; (C) application of disclosing agent over the patient's entire mouth; (D) removal of supragingival biofilm and stains with air-polishing device (AIRFLOW® Handy); (E) removal of the calculus removal from natural teeth with PIEZON® PS allows thorough cleaning; (F) immediate post-operative view of the patient's mouth after implementing the GBT protocol (Adopted from Srivastava *et al.* [36])

Role of disclosing agents

Disclosing agents are the solutions or wafers capable of staining bacterial deposits on the surface of teeth, tongue or gingiva. These are very effective tools in patient education and motivation, helping them to perform adequate plaque control [20].

According to Wilkins, a disclosing agent is a selective dye in solution, tablet, or lozenge form used to visualize and identify dental biofilm on the surfaces of the teeth [21]. According to Raybin, disclosing agent is a solution which when applied on the tooth, makes visible by staining roughness and foreign matter on the tooth. (Foreign matter is meant to include mucinous plaque, calculus and material surfaces) [22]. Disclosing agents work by changing the color of dental plaque so that it contrasts with the white tooth surface. Dental plaque has the ability to retain a large number of dye substances which can be used for disclosing purposes. This property is related to interaction, because of the polarity difference between the components of the plaque and the dyes. The particles are bound to the surface by electrostatic interaction (Proteins) and hydrogen bonds (Polysaccharides) [23].

Application methods for disclosing agents

The disclosing agents in a solution form are applied with a cotton swab and the tablets are chewed and swished. The patient is instructed to rinse and expectorate the disclosing agent. These agents do not stain biofilm-free tooth surfaces unless roughness (i.e., decalcification, pitting) is present (Figure 2). The precautions followed during the application of disclosing agents include:

- To avoid staining the lips, a light coat of non-petroleum or water-based lubricant should be applied.
- Restorations which may permanently be stained by the disclosing agents should be avoided.
- The disclosing agents should not be contaminated by introducing applicators into the storage container bottle. Disposable cups may be used to avoid contamination.
- Erythrosine solutions contain alcohol, which can evaporate over time and alter the concentration of the solution. So, disclosing solution should be used according to the manufacturer's instructions.
- Application of disclosing agent should be avoided before the application of a dental sealant.
- Appropriate protective drapes should be used to protect the clothing from staining.

Limitations of disclosing agents

Disclosing solutions cannot selectively stain bacterial plaque, but stain debris and dental pellicle.

- Disclosing solutions may stain restorations, especially silicate cements and resin restorations.
- These can stain exposed cementum free of plaque.
- The alcohol present in the disclosing agents evaporates slowly, rendering the solution too highly concentrated [24].

Likewise, in GBT the basic principle is to visualize the dental biofilm-the main etiologic agent for periodontal disease and peri-implant disease and subsequently its removal with specialized instruments and equipment. These disclosing agents act as a professional guide to visualize the most inaccessible area of biofilm and thereby achieve

mechanical plaque control by a minimally invasive procedure concept [25].

Air abrasive devices

The usage of air polishing devices was introduced in 1945, wherein aluminium hydroxide [Al(OH)₃] powder was used for cavity preparation. Various technical advances have improvised the devices, and currently, these air polishing devices are used for biofilm removal. The principle of air-polishing device was to deliver the slurry consisting of abrasive air particles (Powder) mixed with water under pressure through a specialized nozzle [26].

The air-polishing device is based on two principles. First, the "venturi" powder chamber principle, where the powder mainly exits from the bottom of the chamber. In this technique the mixture of air and powder is created by the combination of carburetor technique and swirling [27]. In this technique, the amount of air powder discharged through the tube is dependent on the positioning of a sloping deflector at the filler cap [28]. As per the second principle, the air-powder slurry is formed by forcing the pressurized air into the powder chamber and reaching the outlet by swirling action [29]. The amount of powder emission is dependent on the screw setting. It has been observed that the amount of powder emission can be regulated by the first principle, whereas according to the second principle, the setting powder emission is inconsistent. In the second type, a decrease in the powder mass can decrease the powder output [30].

Currently, two systems (Devices) are available for an air-polishing device: a hand-held device and a standalone device. These units are connected with the air turbine coupling of the dental unit. In the hand-held device, the powder chamber is smaller, thus requiring frequent refilling. Furthermore, the coupling unit of the hand-held device is bulkier. Hence, it is not ergonomic to use in inaccessible areas [31].

Essentially there are two types of nozzles used for air polishing, namely the supragingival and subgingival nozzle. The supragingival nozzle, otherwise known as the standard nozzle, is basically used to remove the supragingival plaque and stains. On the other hand, subgingival nozzles can be used for treatment of periodontal pockets as well as in peri-implantitis. The supragingival nozzle is available at a 120° or 90° angle for posterior and anterior teeth, respectively [32]. Conversely, the subgingival nozzle is designed to have markings with either two outlets (Acteon) or three outlets (EMS). In the EMS system (PERIOFLOW®), the two outlets are located approximately 2mm above the third nozzle which is situated at the tip. The outlets on the sides allow the exit of the air and powder mixture whereas the third outlet at the tip helps in the emergence of water [32]. It has been reported that a minute change in the size, diameter, length of the tube, and curvature can significantly affect the efficacy of the equipment [33]. It is also of prime importance to keep the nozzle at the correct distance from the tooth structure and the angulation of the slurry with the tooth surface. The incorrect angulation of the handpiece and distance from the tooth structure can have an adverse effect on soft tissues.

Air abrasive powders

The mechanism by which the air abrasive removes the biofilm, calculus, or tooth substance depends largely upon

the particle size, mass hardness, and angularity of the abrasive delivered through the pressurized jet of water. However, the increased pressure and water setting increase the efficacy of the instrument. Furthermore, it is also believed that water enhances the activity of air abrasive powder by removing the embedded particles on the surface. On the contrary, it is also believed that water film on the object will decrease the effect of air abrasives. Additionally, water's kinetic energy will help break the particle and reduce its size, hence adversely affecting its efficiency [34].

Since the 1970s, various air abrasives have been used in clinical practice. Currently, sodium bicarbonate (NaHCO₃), glycine powder, erythritol powder, and bioactive glasses are some of the commercially available air abrasive powders [35]. These air abrasive powders mainly differ in particle size, shape, and consequently in their outcome. The particle size ranges from 1- 250 µm with the glycine powder having a particle size of 45-60 µm and erythritol powder with a particle size of about 14-31 µm. The smallest particle size is found to be of bioactive glass (1-10 µm). Comparing the particle shape of air abrasives, NaHCO₃ has chiseled and sharp edges. The particle shape of glycine is similar to NaHCO₃, but it is less chiseled. Erythritol has extra fine grains whereas bioactive glass has regular shape [35].

Another factor that can influence the treatment outcome is time duration. Instrumentation time is basically user dependent, and it may adversely affect the hard tissue or soft tissue if proper technique is not followed [35]. Additionally, the effectiveness of the technique is also modulated by the amount of powder present in the pressurized chamber. It has been reported that the usage of the slurry in the pressurized chamber decreases the efficacy and efficiency of the device [36].

Guided biofilm therapy in periodontal and periimplant diseases

Many studies have been performed to assess the outcome of GBT on periodontal and peri-implant disease. Few of them reported reduction in Red complex bacteria for individuals who underwent GBT treatment [37] and also there is reduction in pocket depth for patients with periodontitis along with reduction in *Tannerella forsythia* (*T. forsythia*) and *Treponema denticola* (*T. denticola*) with subgingival usage of erythritol as air polishing powder along with reduction in Matrix metalloproteinases (MMP-8) [38]. Furthermore, in another study a significant decrease in the levels of *P. gingivalis* was reported after one month in the group treated with erythritol air-polishing compared to SRP [38]. Contrary to this, there are studies which have observed the same or lesser clinical outcomes of glycine/erythritol air-polishing compared to SRP [39]. Home care oral hygiene alone does not have the ability to completely remove the newly formed bacterial deposits from the residual pockets, which is a well-established fact. Hence, patients are supposed to be put on SPT that needs professional dental biofilm management [40, 41]. GBT has been proven to be as effective as conventional SRP treatment in clinical outcomes. However, GBT was reported to be more comfortable to patients with less pain perception. In another study, the 12 month post-operative count of *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) was less at the test site treated with erythritol with 3% chlorhexidine than the control site receiving SRP. However,

the role of adding chlorhexidine to erythritol cannot be substantiated with the reduction of bacteria [41].

Conclusion

With the current evidence, it can be concluded that GBT is an effective means of removing biofilm from the tooth or implant vicinity. Compared to SRP, GBT was reported with better patient compliance and less pain perception in non-surgical periodontal therapy or supportive periodontal therapy. Although, in peri-implant diseases, it does help in the reduction of plaque, its usage as monotherapy needs further investigation with long term studies as the clinical outcome is short-lasting.

References

1. Razak FA, Rahim ZHA. Oral microbes and its environment: A review article. Esteem Academic Journal. 2013;9:67-75.
2. Kriebel K, Hieke C, Hilke BM, Nakata M, Kreikemeyer B. Oral biofilms from symbiotic to pathogenic interactions and associated disease-connection of periodontitis and rheumatic arthritis by peptidyl arginine deiminase. Front Microbiol. 2018;9:53.
3. Jiao Y, Tay FR, Niu L, Chen J. Advancing antimicrobial strategies for managing oral biofilm infections. Int. J Oral Sci. 11:28.
4. Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. J Periodontol. 1965;36:177-187.
5. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin. Microbiol. 2005;43:5721-32.
6. Do T, Devine DA, Marsh PD. Oral biofilms: Molecular analysis, challenges, and future prospects in dental diagnostics. Clin. Cosmet. Investig. Dent. 2013;5:11-19.
7. Gilbert P, Maira-Litran T, McBain AJ, Rickard AH, Whyte FW. The physiology and collective recalcitrance of microbial biofilm communities. Adv. Microb. Physiol. 2002;46:203-255.
8. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. Trends Microbiol. 2005 Jan;13(1):34-40.
9. Lu TK, Collins J. Dispersing biofilms with engineered enzymatic bacteriophage. PNAS. 2007;104:11197-11202.
10. Marsh D, Martin V. Oral Microbiology. 5th ed. Churuchhill Livingstone: Elsevier; c2009.
11. Lasserre JF, Brex MC, Toma S. Oral Microbes, Biofilms and Their Role in Periodontal and Peri-Implant Diseases. Materials. 2018;11:1802.
12. Shrivastava D, Srivastava KC, Ganji KK, Alam MK, Zoubi I, Sghaireen MG. Quantitative Assessment of Gingival Inflammation in Patients Undergoing Nonsurgical Periodontal Therapy Using Photometric CIE Lab Analysis. Bio. Med. Res. Int. 2021;30:20-21.
13. Schultz-Hautdt S, Bruce MA, Bibby BG. Bacterial factors in nonspecific gingivitis. J Dent Res. 1954;33:454-458.
14. Loesche WJ. Clinical and microbiological aspects of chemotherapeutic agents used according to the specific plaque hypothesis. Dent Res. 1979;58:2404-2412.
15. Darby I. Non-surgical management of periodontal disease. Aust Dent J. 2009;54:86-95.

16. Park EJ, Kwon EY, Kim HJ, Lee JY, Choi J, Joo JY. Clinical and microbiological effects of the supplementary use of an erythritol powder air-polishing device in non-surgical periodontal therapy: A randomized clinical trial. *J Periodontal Implant Sci.* 2018;48:295-304.
17. Cobb CM, Sottosanti JS. A re-evaluation of scaling and root planing. *J Periodontol.* 2021 Oct;92(10):1370-1378.
18. Fleischer HC, Mellonig JT, Brayer WK, Gray JL, Barnett JD. Scaling and root planing efficacy in multirrooted teeth. *J Periodontol.* 1989;60:402-409.
19. Rabbani GM, Ash MM, Caffesse RG. The effectiveness of subgingival scaling and root planing in calculus removal. *J Periodontol.* 1981;52:119-123.
20. Eaton KA, Kieser JB, Davies RM. The removal of hot surface deposits. *J Clin Periodontol.* 1985;12:141-152.
22. Wilkins EM. Clinical practice of the dental hygienist. 12th ed. Philadelphia: Lippincott Williams & Wilkins; c2016.
23. Raybin M. Disclosing agents: their importance and uses. *The Dental Outlook.* 1943;4:159-162.
24. Lang NP, ØStergaard E, Loe H. A fluorescent plaque disclosing agent. *J Periodontal Res.* 1972 Feb;7(1):59-67.
25. Viorica C, Ion IR. Dental plaque - Classification, formation, and identification. *Int. J. Med. Dent.* 2013;3(2):139-143.
26. Mensi M, Scotti E, Sordillo A, Agosti R, Calza S. Plaque disclosing agent as a guide for professional biofilm removal: A randomized controlled clinical trial. *Int. J. Dent. Hyg.* 2020;18:285-294.
27. Petersilka GJ, Schenck U, Flemmig TF. Powder emission rates of four air polishing devices. *J Clin. Periodontol.* 2002;29:694-698.
28. Donnet M, Fournier M, Schmidlin PR, Lussi A. A novel method to measure the powder consumption of dental air-polishing devices. *Appl Sci.* 2021;11:1101.
29. Prof U, Nardi GM. Systeme d'aero-POLISSAGE COMBI Touch. Available from: www.mectron.froumectronfrance@mectron.fr [Accessed 15 March 2024].
30. Momber A, Kovacevic R. Principles of Abrasive Water Jet Machining. 9.6. New York, NY: Springer; c1998.
31. Barnes CM. The management of aerosols with air polishing delivery systems. *J Dent. Hyg.* 1991;65:280-282.
32. Barnes CM. An in-depth look at air polishing. Omaha, NE: University of Nebraska Medical Center; c2010.
33. Petersilka GJ, Bell M, Mehl A, Hickel R, Flemmig TF. Root defects following air polishing. *J Clin. Periodontol.* 2003;30:165-170.
34. Conserva E, Pisciotto A, Bertoni L, Bertani G, Meto A, Colombari B, *et al.* Evaluation of biological response of STRO-1/c-Kit enriched human dental pulp stem cells to titanium surfaces treated with two different cleaning systems. *Int. J. Mol. Sci.* 2019;20:1868.
35. Sultan DA, Hill RG, Gillam DG. Air-polishing in subgingival root debridement: A critical literature review. *J Dent Oral Biol.* 2017;2:1065.
36. Reinhardt B, Klocke A, Neering SH, Selbach S, Peters U, Flemmig TF, *et al.* Microbiological dynamics of red complex bacteria following full-mouth air polishing in periodontally healthy subjects: A randomized clinical pilot study. *Clin. Oral Investig.* 2019;23:3905-3914.
37. Shrivastava D, Natoli V, Srivastava KC, Alzoubi IA, Nagy AO, Johani AI, *et al.* Novel approach to dental biofilm management through guided biofilm therapy (GBT): A review. *Micro-organisms.* 2021;9:1966.
38. Hagi TT, Hofmanner P, Eick S, Donnet M, Salvi GE, Sculean A, *et al.* The effects of erythritol air-polishing powder on microbiologic and clinical outcomes during supportive periodontal therapy: Six-month results of a randomized controlled clinical trial. *Quintessence Int.* 202;46:31-41.
39. Tsang YC, Corbet EF, Jin LJ. Subgingival glycine powder air-polishing as an additional approach to nonsurgical periodontal therapy in subjects with untreated chronic periodontitis. *J Periodontal Res.* 2018;53:440-445.
40. Kargas K, Tsalikis L, Sakellari D, Menexes G, Konstantinidis A. Pilot study on the clinical and microbiological effect of subgingival glycine powder air polishing using a cannula-like jet. *Int. J. Dent. Hyg.* 2015;13:161-169.
41. Flemmig TF, Arushanov D, Daubert D, Rothen M, Mueller G, Leroux BG. Randomized controlled trial assessing efficacy and safety of glycine powder air polishing in moderate- to-deep periodontal pockets. *J Periodontol.* 2012;83:444-452.
42. Wennstrom JL, Dahlen G, Ramberg P. Subgingival debridement of periodontal pockets by air polishing in comparison with ultrasonic instrumentation during maintenance therapy. *J Clin. Periodontol.* 2011;38:820-827.