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Review Article

ROLE OF BIOFILM MODELS IN DENTAL RESEARCH: A REVIEW

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ABSTRACT

Oral cavity is an extraordinary environment for several microbial species to attach on tooth surfaces and form dense bacterial biofilms which are prevalent on most wet surfaces representing a common cause of persistent infections. Plaque biofilms are implicated strongly in the causation of dental caries and periodontal diseases. The term biofilm is increasingly replacing 'Dental plaque' in the dental literature, but concepts and existing paradigms are changing much more slowly. The stages of structural organization of biofilm, the composition and activities of the colonizing microorganisms in various environments may be different although the establishment of the micro-community on a surface seems to follow essentially the same series of developmental stages, including deposition of a conditioning film, adhesion and colonization of planktonic microorganisms in a polymeric matrix, co-adhesion of other organisms and detachment of biofilm microorganisms in to the surroundings. The success of any treatment which is targeted against dental caries or periodontal disease is dependant on inactivation of microorganisms present in biofilm and planktonic ambience rather on individual microorganism per se. The complexity of oral environment, financial constraints, ethical problems, poor patient compliance associated with studies of plaque associated oral diseases in humans inevitably direct the attention to development of biofilm models that simulate plaque microorganism. These biofilm models can evaluate microbial interactions in simulated dental plaque and similar biofilms monitoring their physical, chemical, biological and molecular features to a very high degree of accuracy. A range of technologies and microbial systems has been utilized, all with different uses, strengths, and limitations. Hence we review biofilm models used in dentistry which play an important role in developing preventive and therapeutic strategies to combat plaque associated diseases of oral cavity.

Keywords: Biofilm Models, Plaque, Dental Research, Dental caries, Periodontal disease, Candidiasis

INTRODUCTION

Biofilms are referred as structured communities of sessile microbial aggregates, enclosed in a self-polymeric matrix, attached to an inert or living surface¹. Some of the most familiar biofilms are plaque on teeth surfaces, the slippery slime on river stone, and the gel-like film formed on the inside of vase which holds flowers for a week. Biofilm is held together and protected by matrix which in turn protects the cells and show complex intercellular interactions including communication by specific signaling molecules². Some biofilms contain water channels that help distribute nutrient and signalling molecules. Biofilms have tremendous practical importance in the area of industrial, medical and agricultural sciences exhibiting both beneficial and detrimental activities. The resistance of biofilm bacteria against antimicrobials is increasing³. Both the species and numerical composition of

biofilms are dependent on their growth conditions which define the resistance against antimicrobials. These factors seem ultimately crucial for the interaction with the host, resulting in health or disease. P.D. Marsh, a pioneer in oral biofilm experimentation, has described this relation as the ecological plaque hypothesis⁴.

The complexity of the oral environment and ethical problems associated with studies of plaque associated oral diseases in humans inevitably direct the attention to development of biofilm models that simulate the plaque microcosm. Model systems which are more controllable than natural dental plaque are used to explain and predict biofilms behavior⁵. There are considerable difficulties inherent in the modeling of such a heterogeneous and variable biofilms. To explain ecology, pathology, and behavior of plaque biofilm, the model must be realistic, reflecting the very behavior which is under investigation, predicting plaque behavior in vivo in response

to interventions to prevent dental disease. A range of technologies and microbial systems has been utilized, all with different uses, strengths, and limitations. Two complementary microbiological approaches have been considered to generate and study emergent properties in biodiverse model biofilm systems which include the construction of 'synthetic' plaque-like consortia with major plaque species and the evolution of plaque microcosms from the natural oral microflora⁶.

The biofilm models can evaluate microbial interactions in simulated dental plaque and similar biofilms monitoring their physical, chemical, biological and molecular features to a very high degree of accuracy. Laboratory models are best for screening large numbers of agents and in determining their modes of action. They are comparatively cheap to use, non invasive, experimentally controlled, not labor-intensive, could be designed for replicating experiments, and not dependent on patients consent and compliances. There is a limited knowledge about the various biofilm models and their utility in dental research. Hence, the aim of this review is to study various types of biofilm models like endodontic biofilm models, plaque biofilm models, salivary biofilm models, fungal biofilm models and their utility in dental research.

Historical perspective of Biofilm models

In late 19th century Magitot and Miller, decalcified extracted teeth using pioneer models. Magitot's experiment involved the simple process of placing extracted teeth in bacteriological media⁷. Miller's subsequent experiments in 1890 involved inoculating extracted human teeth with a mixture of bread and saliva, in a conical flask⁸. Dietz circumvented some of the practical difficulties associated with simulating artificial caries by assembling the apparatus under a microscope, which permitted direct observation of developing caries lesions in ground tooth sections, fixed to the microscope stage⁹. In 1952, Pigman developed a model for studying early carious lesions that was an improvement of the pioneer models¹⁰. In 1964, Rowles and colleagues attempted to study the bacteriology of caries and induce caries-like lesions in human enamel under standardized conditions. They designed a system that could be continuously fed with saliva and, intermittent nutrient feeds were used to mimic oral conditions¹¹. In 1975, Bibby constructed an artificial mouth, which he termed the 'Orofax'. In order to preserve the physiochemical properties and prevent mucin precipitation, saliva collected by paraffin stimulation was rapidly frozen to -70 degree Celsius and stored at this temperature within a cold chamber of the Orofax¹². In 1991, Sissons et al reported their advanced multiple artificial mouth system. Here, all the culture units were housed in a single culture chamber, facilitating carefully controlled and identical experimental conditions¹³. Other uses of this system included plaque calcium level measurement using atomic absorption spectrophotometry; fluoride assay by fluoride specific electrodes and, phosphate by molybdate calorimetric method¹⁴. This complex but useful system could be employed for the long-term growth of multiple plaque samples within a standardized, simulated oral environment generated by computer-controlled facilities¹⁵.

Endodontic biofilm models

Root canal is an extraordinary microenvironment for several microbial species to attach on dentin surface and form dense

bacterial biofilms representing a common cause of persistent infections. The success of infected root canal treatment is dependent on inactivation of microorganisms present in root canal biofilm¹⁶. *E.faecalis* has proved a potentially important microorganism to the colonization or overgrowth in endodontic infections, being the dominant microorganism in post-treatment apical periodontitis, and has often been isolated from the root canal in pure culture¹⁷. Model systems were developed by Estrela et al, Ibrahim et al, Gulabivala et al and Berber et al to study antimicrobial strategies in *E.faecalis* endodontic biofilm model using extracted human teeth. Teeth were immersed in sodium hypochlorite solution for disinfection. Access cavity preparation was done for all teeth followed by thorough irrigation and biomechanical preparation. Brain Heart Infusion broth were mixed with 5 mL of the bacterial inoculum containing *E.faecalis* and were inoculated using sterilized syringes of sufficient volume to fill the root canal during a 60 day period. This procedure was repeated every 72 hours, always using 24-hour pure culture prepared. At 60 days, each tooth was removed from its apparatus under aseptic conditions. Sterile paper points were introduced into the root canals and maintained for 3 min for sample collection. Each sample was collected using three paper points, which were individually transported and immersed in a broth. Colony forming units were observed and compared¹⁸⁻²¹. The proposed biofilm models seem to be viable for studies on antimicrobial strategies, and allows for a satisfactory colonization time of selected bacterial species with virulence and adherence properties. The major disadvantage of using such *E.faecalis* biofilms is that it doesn't simulate the entire environment in the root canal and there are several microorganisms present in the root canal which could also affect the prognosis of root canal therapy.

Biofilm models for mechanical plaque removal

In vitro plaque removal studies require biofilm models that resemble in vivo dental plaque. In-vitro single, dual and multispecies biofilm models as well as biofilms grown from human whole saliva using different biofilm models can be generated to study the mechanical plaque removal by various oral hygiene aids. Most studies on dental plaque have focused on single species biofilms, which neglect multi-species interactions as occurring in oral biofilms²²⁻²⁴. But recently a multispecies biofilm model was used by Martinus J et al to study the efficacy of powered tooth brush on an *in-vitro* biofilm model. Different bacterial isolates were sonicated intermittently while cooling on ice for 30-40 seconds. Bacterial adhesion experiments were performed in a parallel plate flow chamber. Human whole saliva from at least 20 healthy volunteers of both genders was collected into ice cooled beakers after stimulation by chewing Parafilm. Fresh human whole saliva from the same volunteers was employed to stimulate biofilm growth of initially adhering bacteria. Saliva was mixed with parallel plate flow chamber containing bacterial adhesions. The flow chamber was mounted on the stage of a phase contrast microscope. Biofilms were grown on and images were taken from the saliva coated bottom plate of the flow chamber. Brushing was carried out on the surface of saliva coated bottom plate for 20 seconds. After brushing the saliva coated plate, it was removed and observed under phase

contrast microscopy. The advantage of this model over previous models is that this model depicts the multispecies interactions accurately and it simulates closely to the natural plaque present *in vivo*²⁵.

Plaque and salivary biofilm models

Supragingival plaque as well as subgingival plaque acts as an important factor in the development of gingival or periodontal disease. Several techniques are available today to study multispecies biofilms of oral bacteria, each having its particular advantages and weaknesses. Various multi-species models of dental plaque have been described and applied to problems of clinical relevance, most notably biofilm permeability and chemical control of plaque. These systems usually consist either of flow cells²⁶ or of chemostats modified to allow for insertion and removal of colonizable surfaces²⁷. While these devices have contributed to our understanding of microbial adhesion and biofilm formation, their use has certain drawbacks. They can be cumbersome to construct and/or difficult to maintain over long periods of time. To overcome the drawbacks present in these systems, a Zurich biofilm model was introduced by Guggenheim et al. Microorganisms representative for supragingival plaque were used to generate biofilms. Zurich biofilm model has certain applications with direct or indirect impact on prophylactic dentistry: spatial arrangement and associative behavior of various species in biofilms; multiplex fluorescent *in situ* hybridization analysis of oral bacteria in biofilms; use of the biofilm model to predict *in vivo* efficacy of antimicrobials reliably; mass transport in biofilms, demineralization and remineralization of enamel exposed to biofilms *in vitro*. In this model biofilms were formed either on hydroxyapatite or bovine enamel disks that have been preconditioned in pooled, unstimulated saliva²⁸. Other authors have used different models, such as in-mouth splints or a constant-depth film fermenter using a plaque inoculum for the same purposes^{29,30}.

The study of subgingival biofilms is essential and more valid than supragingival plaque biofilms for the understanding of periodontal disease progression as well as for effective treatment. Shaddox et al developed a subgingival biofilm model using samples of subgingival plaque and saliva which were collected from individuals with generalized chronic periodontitis with a pocket depth >5 mm. Biofilms were established using sterile ceramic calcium hydroxyapatite (HA) disks in a tissue culture plates. Each well was inoculated with sonically dispersed subgingival plaque and incubated in an anaerobic condition at 37 degree C for up to 10 days with change to fresh medium at 48-h intervals. Bacterial isolates were plated onto agar plates and incubated anaerobically at 37 degree Celsius for 5–7 days for total viable counts. Selected target species were detected and monitored by ‘checkerboard’ DNA–DNA hybridization³¹. Another study was conducted by Sanchez et al using bacterial standard reference strains present in subgingival plaque and by using saliva collected from healthy volunteers for development of subgingival plaque. This model studied structure and dynamics of subgingival microbiota using both morphological (confocal laser scanning microscopy; CLSM) and molecular approaches (terminal restriction fragment length polymorphism; T-RFLP)³².

Ledder et al used a biofilm model solely developed using saliva which was collected in a sterile Universal bottle over the course of 30 min by two volunteers (1 male, 1 female) who did not have extant dental disease. In this study Modified drip-flow biofilm reactor (MDFR) delivered a drop-wise, continuous flow of saliva over four HA-coated slides in wells of tissue culture plates. Antimicrobial agents like stannous fluoride, triclosan, zinc lactate and Zinc Lactate with Stannous Fluoride in combination were delivered to each well of hydroxyapatite disk. HA discs were aseptically removed from the tissue culture plate and the removed biofilm or disc was immersed in pre-reduced, half-strength thioglycolate medium. The PCR–denaturing gradient gel electrophoresis was used to quantify plaque after use of antimicrobial agents. It was observed that triclosan was found to be the most potent antibacterial agent, after single and multiple dosage regimens³³.

Fungal biofilm model

Oropharyngeal candidiasis is a frequent problem within immuno-compromised and elderly populations³⁴. It may manifest in different clinical presentations, including pseudomembranous and erythematous forms, causing symptoms such as pain, burning sensation, and altered taste. These can subsequently lead to nutritional compromise³⁵. *Candida albicans* species are implicated in the development of candidiasis. Ramage et al developed a biofilm model using *Candida albicans* reference strains. Efficacy of different antifungal agents and mouthwashes like fluconazole, voriconazole, itraconazole, caspofungin, amphotericin B, nystatin, Listerine, thymol, methyl salicylate, caspofungin, Corsodyl, Oraldene, menthol, ethanol, hexetidine, hydrogen peroxide and chlorhexidine gluconate were checked on the candidal biofilm model. All working stocks of *C. albicans* were maintained at 4°C on Sabouraud agar, and stored indefinitely in Microbank vials at 80°C. Biofilms from *candida albicans* isolates were formed on commercially available presterilized polystyrene flat bottomed 96-well micro titer plates by pipetting standardized cell suspensions into selected wells of the microtiter plate and incubated for 48 hours at 37°C. For sessile testing, mouthwashes were added directly to 10 replicate biofilms for each isolate, including appropriate control samples. Biofilms were treated at room temperature according to the recommended rinse time for each manufacturer (30–60s). A semiquantitative measure of each biofilm was calculated using a XTT [2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H tetrazolium-5-caboxanilide; Sigma]. Plates were then incubated in the dark for up to 2 hours at 37°C. A colorimetric change in the XTT-reduction assay, representing a direct correlation of metabolic activity of the biofilm, was then measured at 492 nm in a microtiter plate reader. Biofilm was more sensitive to Caspofungin, Corsodyl, Listerine, and Oraldene³⁶. The major disadvantage of this model is that *candida albicans* was not isolated directly from patients which possibly doesn’t simulate the disease exactly.

CONCLUSION

There is a very high burden of oral diseases throughout the world which necessitates development of promising strategies

to diagnose, prevent and treat oral diseases. In certain situations *in vivo* studies are difficult to conduct because of the ethical issues involving human subjects, involves high cost and also it is considered to be time consuming. A new era of research in oral biology is now open with promising prospects for preventive dentistry which includes *in vitro* studies involving biofilm models. Biofilm models simulate oral environment artificially which are developed to overcome problems encountered in *in vivo* studies. Biofilm models are best for screening large numbers of agents, to study biological characteristics of plaque, its pathogenicity and formulation of anticaries or anticalculus agents and in determining their modes of action. However, it should be noted that the results obtained from these *in vitro* studies would need confirmation by *in vivo* investigations in human volunteers.

REFERENCES

1. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, LappinScott HM: Microbial biofilms. *Annu Rev Microbiol* 1995;49:711–45
2. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP: The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 1998;280:295–98.
3. Gilbert P, Das J, Foley I: Biofilm susceptibility to antimicrobials. *Adv Dent Res* 1997;11:160–67.
4. Marsh PD, Bradshaw DJ. Physiological approaches to the control of oral biofilms. *Adv Dent Res* 11:176-185. Wimpenny J, Nichols W, Stickler D, Lappin-Scott H, editors. Cardiff, UK: Boline, 1997:13-17.
5. Tatevossian A. Film fermenters in dental research. In: CRC handbook of laboratory model systems for microbial ecosystems. Wimpenny JWT, editor. Boca Raton, FL: CRC Press Inc., 1988:197-227.
6. McKee AS, McDermid AS, Ellwood DC, Marsh PD. The establishment of reproducible, complex communities of oral bacteria in the chemostat using defined inocula. *J Appl Bacteriol* 1985;59:263-75.
7. Magitot E. Treatise on dental caries (Trans by Chandler TH). Boston: Osgood; 1878.
8. Miller WD. The microorganisms of the human mouth. Philadelphia: White Dental MFG; 1890.
9. Dietz VH. *In vitro* production of plaques and caries. *J Dent Res* 1943;22:423–40.
10. Pigman W, Elliott HC, Laffre RO. An artificial mouth for caries research. *J Dent Res* 1952;31:627–33.
11. Rowles SL, Sidaway DA, MacGreggor EA. An apparatus for the production of dental caries *in vitro*. *Arch Oral Biol* 1963;8:311–8.
12. Bibby BG, Huang CT, Zero D, Mundorff SA, Little MF. Protective effect of milk against *in vitro* caries. *J Dent Res* 1980;9:1565–70.
13. Sissons CH, Cutress TW, Hoffman MP, Wakefield JS. A multi-station dental plaque microcosm (artificial mouth) for the study of plaque growth, metabolism, pH, and mineralization. *J Dent Res* 1991;70:1409–16.
14. Sissons CH, Wong L, An YH. Culture and analysis of bacterial biofilms. In: An YH, Friedman RJ, editors. Handbook for studying bacterial adhesion. Totowa: Humana Press; 2000. p. 133.s—69s.
15. Sissons CH, Cutress TW, Pearce EIF. Kinetics and product stoichiometry of ureolysis by human salivary bacteria and artificial mouth plaques. *Arch Oral Biol* 1985;30:781-790.
16. Nair PNR, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after one-visit-endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;99:231-52.
17. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1998;85:86-93.
18. Estrela C, Sydney GB, Figueiredo JA, Estrela CR. A model system to study antimicrobial strategies in endodontic biofilms. *J Appl Oral Sci.* 2009;17(2):87-91.
19. Ibrahim NZ, Abdullah M. Antimicrobial evaluation of sodium hypochlorite and ozonated water on *E.faecalis* biofilm. *Annal Dent Univ Malaya* 2008;15(1):20-26.
20. Gulabivala K, Stock CJ, Lewsey JD, Ghori S, Ng'Y-L, Spratt DA. Effectiveness of electro-chemically activated water as an irrigant in an infected tooth model. *Int Endo J* 2004;37:624-31.
21. Berber VB, Gomez BPFA, Sena NT, Vianna ME, Ferraz CCR, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. *Int Endo J* 2006;39:10-17.
22. Luppens SB, Kara D, Bandounas L, Jonker MJ, Wittink FR, Bruning O et al. Effect of *Veillonella parvula* on the antimicrobial resistance and gene expression of *Streptococcus mutans* grown in a dual-species biofilm. *Oral Microbiol Immunol* 2008;23:183 89
23. Palmer RJ, Kazmerzak K, Hansen MC, Kolenbrander PE. Mutualism versus independence: strategies of mixed-species oral biofilms *in vitro* using saliva as the sole nutrient source. *Infect Immun* 2001;69:5794–5804
24. Adams H , Winston MT, Heersin k J, Buckingham-Meyer K A , Costerton JW, Stoodley P Development of a laboratory model to assess the removal of biofilm from interproximal spaces by powered tooth brushing . *Am J Dent* 15 (Spec) 2002:12 B –17B
25. Verkaik MJ, Busscher HJ, Rustema-Abbing M, Slomp AM, Abbas F, van der Mei HC. Oral biofilm models for mechanical plaque removal. *Clin Oral Investig.* 2010 Aug;14(4):403-9.
26. Larsen T, Fiehn NE: Development of a flow method for susceptibility testing of oral biofilms *in vitro*. *APMIS* 1995;103:339–344.

27. Kinniment SL, Wimpenny JWI, Adams D, Marsh PD: Development of a steady-state oral microbial biofilm community using the constant-depth film fermenter. *Microbiology* 1996;142: 631–638.
28. Guggenheim B, Guggenheim M, Gmür R, Giertsen E, Thurnheer T. Application of the Zürich biofilm model to problems of cariology. *Caries Res.* 2004 May-Jun;38(3):212-22. Review.
29. Wood, S.R., Kirkham, J., Marsh, P.D., Shore, R.C., Nattress, B. and Robinson, C. Architecture of intact natural human plaque biofilms studied by confocal laser scanning microscopy. *J Dent Res* 2000 79:21–27.
30. Dibdin, G. and Wimpenny, J. Steady-state biofilm: practical and theoretical models. *Methods Enzymol* 1999;310:296–322.
31. Shaddox LM, Alfant B, Tobler J, Walker C. Perpetuation of subgingival biofilms in an in vitro model. *Mol Oral Microbiol.* 2010 Feb;25(1):81-7.
32. Sánchez MC, Llama-Palacios A, Blanc V, León R, Herrera D, Sanz M. Structure, viability and bacterial kinetics of an in vitro biofilm model using six bacteria from the subgingival microbiota. *J Periodontal Res.* 2011 Apr;46(2):252-60
33. Ledder RG, Sreenivasan PK, DeVizio W, McBain AJ. Evaluation of the specificity and effectiveness of selected oral hygiene actives in salivary biofilm microcosms. *J Med Microbiol.* 2010 Dec;59(Pt 12):1462-8.
34. Coco BJ, Bagg J, Cross LJ, Jose A, Cross JG, Ramage G. Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. *Oral Microbiol Immunol* 2008;23:377-83.
35. Drage LA, Rogers RS 3rd. Burning mouth syndrome. *Dermatol Clin* 2003;21:135-45.
36. Ramage G, Jose A, Coco B, Rajendran R, Rautemaa R, Murray C, Lappin DF, Bagg J. Commercial mouthwashes are more effective than azole antifungals against *Candida albicans* biofilms in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2011 Apr;111(4):456-60.

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