

# NEW PERSPECTIVE ARTICLE

## Endodontic Biofilm: Quo Vadis?

### Biofilm en endodoncia: ¿Quo vadis?

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#### ABSTRACT

The main challenge in root canal treatment is to reduce bacterial load good enough to ensure success and to avoid reinfection. However, the evidence of biofilm along the root canal system and the inability of current instruments and irrigants to eliminate bacterial biofilm have built a barrier toward a higher favorable prognosis of the root canal treatment, which has not change in the past five decades. Regarding this scenery, research in endodontics have been addressed to find new strategies or protocols to fight the war against biofilm. From conventional irrigants solutions to nanotechnology products are been tested, several in vitro and clinical studies are performance every day in this incessant search. So, what is next, where are we going in endodontics biofilm?

#### KEYWORDS

Biofilm; Antibacterial; Nanoparticles; Apical periodontitis.

#### RESUMEN

El mayor reto en el tratamiento de conductos es lograr disminuir la carga bacteriana a un nivel que nos permita tener éxito en el mismo, así como evitar la reinfección del mismo. Sin embargo, dada la presencia de una biopelícula bacteriana altamente resistente dentro del sistema de conductos y a la incapacidad de los instrumentos y soluciones irrigantes empleados durante la desinfección químico-mecánica de eliminar esta biopelícula, no ha sido posible aumentar el porcentaje de éxito en el tratamiento de conductos, el mismo no ha cambiado en los últimos 50 años. Con este escenario de por medio, el campo de investigación en endodoncia se ha enfocado en la búsqueda de nuevas estrategias de desinfección en la lucha contra la biopelícula bacteriana. Están siendo estudiados desde las soluciones irrigantes convencionales hasta productos de la nanotecnología, múltiples estudios tanto clínicos como in vitro se llevan a cabo día con día en esta incesante búsqueda. Cuál será el siguiente paso, hacia dónde vamos?

#### PALABRAS CLAVE

Biopelícula; Antibacteriano; Nanopartículas; Periodontitis apical.

Since 1987 when Nair PNR(1) first described the presence of a “condensed bacterial layer of co-aggregating communities of bacteria on the wall of root canal”, the perspective and understanding of endodontic infections changed forever. Posterior morphological studies supported that the root canal microbiota is usually organized in structured communities resembling typical biofilms (2–4), instead of planktonic cells as was thought in the past based on the findings from classical culture-based studies (5–7).

Biofilm, a concept introduced by Costerton (8) in early eighties, defined as a sessile multicellular microbial community characterized by cells firmly attached to a surface and enmeshed in a self-produced matrix of extracellular polymeric substances (EPS). In bacterial biofilms, independent cells grow and co-aggregate to form microcolonies that are embedded in the EPS matrix, which gives unique characteristics to the biofilm community. This matrix consists of polysaccharides that are responsible for both cohesion and adhesion interactions, proteins serve as an energy and carbon source, and extracellular DNA seems to play an important role in the establishment of the biofilm structure(9). EPS have been called “the dark matter of biofilms” because of the large range of matrix biopolymers and the difficulty in analyzing them (10).

Within a biofilm, bacterial cells are more resistant to harsh environmental conditions than their planktonic counterparts. The matrix protects organisms against desiccation, oxidizing or charged biocides, some antibiotics and metallic cations, ultraviolet radiation, and host immune defences. Therefore this bacteria lifestyle may represent a survival strategy in a nutritionally limited environment (10).

Based on that fact is easy to understand that root canal colonizing bacteria easily develop into a biofilm community trying to protect and survive

from the antibacterial agents and medications used during endodontic therapy in order to achieve the main objectives: to eliminate these biofilms and to prevent reinfection. But, does root canal biofilm form in similar way of others biofilms in nature, i. e. on rocks in rivers and streams or in medical devices such as catheter, contact lenses, etc. For this to happen the pulp would have to become necrotic and liquefied before bacterial invasion. Siqueira *et al.* (11) has proposed a different dynamic for biofilm formation inside the root canal. Caries is a disease caused by biofilms (12). As the caries lesion advances toward the pulp and destroy the last dentin layer, the pulp become exposed to the caries biofilm, the pulp portion beneath the carious lesion becomes severely inflamed, necrotic, and eventually the frontline of infection advances to involve the tissue in the pulp chamber and then moves inside into the pulp in apical direction. All these events occur by compartments, once it reaches the apical portion, it will become necrotic and infected. This hypothesis is supported not only by microscopic findings, but also by microbiological studies of the composition of the microbiota in dental caries and primary endodontic infections (13,14). Where has been observed that several species found in dentinal caries lesions are also present in primary intraradicular infections and participate in the process of pulp inflammation and necrosis as a pioneer colonizers, it is possible that as the infection advances newcomers from saliva act as a “reinforcements”, also latecomers from the fluid phase in the necrotic canal may entry into the biofilm community and replace primary colonizers. Overtime and according to classical ecological theory, process ends when biofilm structure becomes relatively stable and more organized- called climax community (11).

Although, this concept has been difficult to apply to microbial communities in nature as unexpected disturbances can occur and break the equilibrium, this may well be achieved in the enclosed root canal environment in chronic

cases (11). The “slow-burning” nature of a biofilm infection with starvation and dormancy state may play a significant part in the sustenance of chronic periapical disease (15).

The occurrence of biofilm in infected root canals and beyond it has been widely reported, morphological investigations of teeth with primary or persistent apical periodontitis have pointed out the presence of biofilm not only in the main root canal but also in the anatomical variations, including lateral canals, isthmuses and apical ramifications (16,17). Extraradicular biofilm has also been described attached to the external root surface in cases of endodontic treatment failure (18,19). Different authors based on the observational findings collected through the years have proposed six criteria to determine whether a given infectious disease can be classified as a disease mediated by biofilm communities (20–22). They are as follows (11):

- The infecting bacteria are adhered to or associated with a surface.
- Direct examination of infected tissue shows bacteria forming clusters or microcolonies encased in an extracellular matrix.
- The infection is generally confined to a particular site, and although dissemination may occur, it is a secondary event.
- The infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible microorganisms can be susceptible to killing in the planktonic state.
- Ineffective host clearance. This may be evidenced by the location of microbial colonies in areas usually surrounded by host defense cells. Accumulation of PMNs and macrophages near bacterial aggregates/coaggregates in situ considerably strengthens the point for biofilm involvement with disease causation.
- Elimination or drastic disruption of the biofilm structure and ecology leads to remission of the disease process.

From a clinical point of view, what does it mean we are dealing with an infection mediated by biofilm communities? Should we keep using the same disinfection protocols, or is necessary to look for stronger and efficient biocides? Has the favorable treatment outcomes change lately? What are clinicians doing to succeed in their treatments in spite of biofilm presence?

Certainly, bacterial biofilms in the root canal have become a challenge during the endodontic therapy, the increased antimicrobial resistance of biofilm, their presence in anatomical complexities where uninstrumented portions can be left, and moreover where irrigants are not allowed to penetrate. Clinical studies have shown that even after meticulous chemomechanical preparation and obturation of the root canal system, a significant amount of biofilm still remain intact in inaccessible areas of the canal system where the conventional instruments and irrigating solutions cannot work properly (23). Although, the use of these protocols has shown a satisfactory treatment outcome ranging from 74-92%, the level of favorable outcomes has shown little change for the past 5 decades (24).

It seems that the establishment of new antibiofilm strategies will have to be considered into clinical practice if a rise in the favorable treatment outcome is wanted. At present several new strategies have been developed and are currently being tested in endodontics. They have to be effective but also not cause detrimental physical, mechanical, and/or chemical damages to the root canal dentin (25). So far, the targets of anti-biofilm strategies are especially to prevent the biofilms attachment, disrupt the quorum sensing (QS) and EPS matrix, and reach the dormant cells.

At the adhesion stage of biofilms formation the surface modification of biomaterials is one of the principal strategies. Surface characteristics, such as surface roughness, surface free energy, and

chemistry can influence the type and the feature of the biofilms (26,27). As the QS are actively involved in controlling biofilms formation, studies have focused in a way to inhibit this system, using furanones and RNA III as a QS leading inhibitors. EPS matrix prevent anti-microbial agents from reaching target bacterial cells, disrupting the biofilm matrix via enzyme degradation or by means of ultrasound applications have been some of the utilized antibiofilm methods (28).

Related to the current therapies to remove biofilm from the root canal system, exist different kind of methods that have been used with promissory results. Among them, photodynamic therapy (PDT) which employs a non-toxic dye, and low intensity visible light which, in the presence of oxygen, combine to generate cytotoxic singlet species. There have been several in vitro studies in the literature regarding the use of PDT in root canal disinfection, in conclusion PDT combined with standard chemomechanical preparation of the root canal had the potential to significantly reduce the microbial load. However, the use of PDT is limited by several "tissue specific" factors,

and further research is need it in order to maximize its antibiofilm potential (29).

Another approach is the antibacterial nanoparticles, the potential of nanoparticles to combat infection has increased markedly over the past decade, once they present function as biocide, anti-adhesive, and delivery capabilities. Nanoparticles can be made from zinc oxide, silver, gold, chitosan and functionalized with photosintizers, antibodies, or proteins, providing a better performance as antibiofilm treatment. Currently, several studies are taken place in nanoparticles field in order to establish a new protocol against biofilm, the results so far are provisory, however future research is need it to optimize the potential of nanoparticles (30).

At this point and after this overview regarding endodontic biofilm, the statement Quo vadis? could find the answer in Dr. Kishen words " the newer antimicrobial strategies adapted for endodontic should de direct at disrupting biofilm structure and destroying resident and persister bacteria in a highly irregular environment such as a root canal system"...

## REFERENCES

1. Ramachandran Nair P. N., Harndt E., Winkler K., et al. Light and electron microscopic studies of root canal flora and periapical lesions. *J. Endod.* Elsevier; 1987 Jan; 13 (1): 29–39.
2. Molven O., Olsen I., Kerekes K. Scanning electron microscopy of bacteria in the apical part of root canals in permanent teeth with periapical lesions. *Endod Dent Traumatol.* 1991 Oct; 7 (5): 226–9.
3. Ricucci D., Siqueira J. F., Lopes WSP, Vieira AR, Rôças I. N. Extraradicular infection as the cause of persistent symptoms: a case series. *J Endod.* 2015 Feb; 41 (2): 265–73.
4. Siqueira J. F., Rôças I. N., Lopes H. P. Patterns of microbial colonization in primary root canal infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2002 Feb; 93 (2): 174–8.
5. Sundqvist G. Ecology of the root canal flora. *J. Endod.* 1992 Sep ;18(9): 427–30.
6. Möller A. J. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. *Odontol Tidskr.* 1966 Dec 20 74 (5): Suppl:1-380.
7. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. *Odontol Revy.* 1974. 25 (4): 347–58.
8. Costerton J. W., Cheng K. J., Geesey G. G., Ladd T. I., Nickel J. C., Dasgupta M., et al. Bacterial biofilms in nature and disease. *Annu Rev Microbiol* [Internet]. 1987 41:435–64.
9. Voběrková S., Hermanová S., Hrubanová K., Krzyžánek V. Biofilm formation and extracellular polymeric substances (EPS) production by *Bacillus subtilis* depending on nutritional conditions in the presence of polyester film. *Folia Microbiol (Praha).* 2015.
10. Flemming H-C, Wingender J. The biofilm matrix. *Nat Rev Microbiol.* 8 (9): 623–33. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20676145>
11. Siqueira J. F., Rôças I. N., Ricucci D. Biofilms in endodontic infection. *Endod Top.* 2010 Mar. 22 (1): 33–49.
12. Marsh P. D. Dental plaque as a microbial biofilm. *Caries Res.* 38 (3): 204–11.
13. Lima K. C., Coelho L. T., Pinheiro I.V.A., Rôças I. N., Siqueira J. F. Microbiota of dentinal caries as assessed by reverse-capture checkerboard analysis. *Caries Res.* 2011; 45 (1): 21–30.
14. Munson M. A., Banerjee A., Watson T. F., Wade W. G. Molecular analysis of the microflora associated with dental caries. *J. Clin Microbiol. American Society for Microbiology (ASM);* 2004 Jul; 42 (7): 3023–9.
15. Costerton W., Veeh R., Shirtliff M., Pasmore M., Post C., Ehrlich G. The application of biofilm science to the study and control of chronic bacterial infections. *J. Clin Invest.* 2003 Nov; 112 (10): 1466–77.
16. Nair P. N. R., 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 Feb; 99 (2): 231–52.
17. Ricucci D., Siqueira J. F. Apical actinomycosis as a continuum of intraradicular and extraradicular infection: case report and critical review on its involvement with treatment failure. *J. Endod.* 2008 Sep; 34 (9): 1124–9.
18. Tronstad L., Sunde P. T. The evolving new understanding of endodontic infections. *Endod Top.* Munksgaard International Publishers; 2003 Nov; 6 (1): 57–77.
19. Svensater G., Bergenholtz G. Biofilms in endodontic infections. *Endod Top.* Munksgaard International Publishers; 2004 Nov.

20. Parsek M. R., Singh P. K. Bacterial biofilms: an emerging link to disease pathogenesis. *Annu Rev Microbiol.* 2003. 57: 677–701.
21. Hall-Stoodley L., Stoodley P. Evolving concepts in biofilm infections. *Cell Microbiol.* 2009; 11 (7): 1034–43.
22. Ricucci D., Siqueira J. F. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. *J. Endod.* 2010 Aug 36 (8): 1277–88.
23. Nair P. N. R., 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 Feb; 99 (2): 231–52.
24. Friedman S., Abitbol S., Lawrence H. P. Treatment outcome in endodontics: the Toronto Study. Phase 1: initial treatment. *J. Endod.* 2003 Dec;29 (12): 787–93.
25. Kishen A., Sum C-P, Mathew S., Lim C-T. Influence of irrigation regimens on the adherence of *Enterococcus faecalis* to root canal dentin. *J. Endod.* 2008 Jul 2016 Sep 20]; 34 (7): 850–4.
26. Kostakioti M., Hadjifrangiskou M., Hultgren S. J. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med.* 2013 Apr; 3 (4).
27. Jansen B., Kohnen W. Prevention of biofilm formation by polymer modification. *J. Ind Microbiol.* 1995 Oct 15 (4): 391–6.
28. Balaban N., Giacometti A., Cirioni O., Gov Y., Ghiselli R., Mocchegiani F., et al. Use of the Quorum-Sensing Inhibitor RNAIII- Inhibiting Peptide to Prevent Biofilm Formation In Vivo by Drug-Resistant *Staphylococcus epidermidis*. 2003;187.
29. George S., Kishen A. Photophysical, photochemical, and photobiological characterization of methylene blue formulations for light-activated root canal disinfection. *J. Biomed Opt.* 12 (3): 34029.
30. Shrestha A., Kishen A. Antibacterial Nanoparticles in Endodontics: A Review. *J. Endod.* 2016;42(10):1417–26.

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