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Bacterial association and oral biofilm formation: A bibliometric analysis

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Biofilms are complex aggregations of microorganisms formed in any situation where there is contact with superfaces solids, liquids or gases. This study aims to map the international scientific literature on the formation, bacterial adhesion, prevention and treatment of oral biofilms. There were 921 published articles in 274 different journals indexed in the Web of Science, in the 1991-2015 year period. These articles were written by 2804 authors linked to 695 institutions in 59 countries, notably the United States and the United Kingdom. The 17 articles of greater impact were published in the 1995-2009 year period. The relationship between the most important articles shows that the issue is addressed in a broad and diversified approach, points out to a range of possibilities for prevention and treatment, and uses of experimental methods that list mechanisms and combinations able to enter the structure of the biofilm, elucidate the formation process, to bacterial adhesion and to reduce pathogenic bacterial activity and, in some cases, destroying their structure. The descriptive analysis of the main work has shown potential for the development of the area and contributions to improve the prevention and treatment of oral biofilms.

Key words: Dental plaque, biofilm, bibliometric analysis.

INTRODUCTION

Biofilms are complex aggregations of microorganisms and are formed in situations where there is contact of solids superfaces with liquids or gases. In infectious processes, the bacterials may be present in two ways: as free-living or sessile cells. This second stage of life of the bacteria is seen in microscopic and biological systems with high level of organization called biofilms. These micro-ecosystem, may comprise one or more species of

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bacteria producing films that are deposited and fixate on biotic and abiotic surfaces, forming organized and functional aggregates, allowing a strong adhesion and constitute a defensive mechanism facing hostile environments (Elias and Banin, 2012; Li and Tian, 2012).

The biofilm allows adhesion of bacterial cells to an abiotic surface very quickly, and has been found that an affinity between the organic material and polymeric and hydrophobic surfaces. In the biotic surfaces, adhesion occurs more slowly, mediated by specific processes. The organic matrix, organized in layers produced by these bacteria and gives them greater resistance to anti-microbials, make it difficult to eradicate the source of infection. Several features have been identified as responsible for tolerance to antimicrobials: the protection of proteins and compression of the matrix, low metabolism, resistance genes transfer between species and failure to recognize these structures by the immune system. Another factor considered of impact in this phenomenon is the indiscriminate use of antibiotics, which induce bacterial resistance, increasing the concentrations necessary for combating these micro-organisms and widening the search for new drugs (Preedy et al., 2014; Krasowska and Sigler, 2014; Patel et al., 2015)

The biofilm formation does not occur randomly. The construction of this structure and dispersion of this system are regulated by quorum-sensing, communication between cells dependent on population density. Microorganisms disperse signaling molecules and detect the amount of other microorganisms near them. They are also sensitive to external factors and alter the signaling based on them. Then, there is a communication between species allowing formation of associations and more resistant biofilms (Popat et al., 2012).

Dental plaque or oral biofilm, is a complex biofilm that may be accumulated on the tooth surface or gum and is directly related to periodontal disease and tooth decay (Simoes et al., 2009). For the other hand, the the bibliometric research is a quantitative and statistical technique that concentrates its efforts on visible indicators and academic activity goals, usually of publications and citations (Cronin, 2001). The relevance of bibliometric analysis as a technique for data collection and analysis have been confirmed as one of the argumentative sources in the search for investment resources in research, in academic rankings (Diem and Wolter, 2013; Miguel and Dimitri, 2013). Considering the relevance of bacterial association and formation of oral biofilms and their role in cariogenic and periodontal diseases, this study aimed to map the international scientific literature on the formation, bacterial adhesion, prevention and treatment of oral biofilms.

METHODOLOGY

To carry out a bibliometric study, important selection of the database, keeping compatibility of this choice with the research objectives and the achievement of results was done (Koskinen et al., 2008).

It was specified as database ISI Web of Knowledge/Web of Science for its academic recognition to be considered one of the most comprehensive regular basis covering various areas of scientific knowledge (Santos et al., 2011). The steps for the analysis of selected studies followed the three suggested procedures: the definition of the database and the criteria to be used for the collection; data collection; and the representation and data analysis (Santos et al., 2014).

To collect data, the database of the Web Science™ was used in time range of 1945 to 2015, to allow replication or update this study without the need to carry it again from its beginning. The following search terms were defined: "dental plaque*" and "biofilm*". The quotation marks indicate the exact representation of terms with more than one word and asterisks, the descriptors plural possibilities. These three terms represent the intended association in compliance with the purpose of the study. The data was collected from the search of these terms in the "topic" which is the title of articles, abstracts, keywords, author and keywords created (keywords plus). The results showed the first publication record in 1991.

After the search, there were identified 921 records publications, which were used as set searches for bibliometric analysis proposed in this study. There was no refinement filter to areas of knowledge, countries or languages of the studies, including all records of publications that had the three terms in association.

Later, data collection was performed for the analysis of the material from the export of this data to the bibliometric analysis software package HistCite™ in order to organize information and facilitate analysis. The trajectory of annual evolution of publications; Periodicals with higher records; the authors with the highest number of publications; the number of articles distributed by country of origin of the authors; the more articles cited in Web of Science (global) and those most cited in the set of selected items (local), were analyzed. In addition, to these data generated by the software were identified general aspects of the texts of the 17 articles that compose the two groups: (a) items that received more citations from other papers throughout the database ISI Web of Science™ (GCS) and (B) articles that received more citations of the works in the selection group this bibliometric study (LCS).

RESULTS

921 publications records on formation, bacterial adhesion, prevention and treatment of oral biofilm in the main collection of Web Science™ were identified. These articles were published in 274 different journals indexed to the database and were written by 2804 authors with associations to 695 institutions in 59 countries. To achieve these items, 22,238 references, with an average of approximately 24 references per article were used as demonstrated in Table 1.

The first publication record, dating from 1991 shows experiments that studied factors that influence the capacity for microbial colonization and interactions in biofilms under controlled conditions (Donoghue and Perrons, 1991). The apex of the number of publications was reached in 2015, indicating a growing trend in the interest in this subject, in addition to timeliness and relevance of the subject. The growth percentage of publications in the period 1991-2015 was 8.900%. The

Table 1. Bibliometric analysis-Biofilm / Dental Plaque - publications (1991-2015).

Bibliometric data	Amount
Publications (articles)	921
Indexed journals	274
Authors	2804
Institutions (associations of the authors)	695
Countries	59
Cited References	22.238

Source: Elaborated from the Web of Science data.

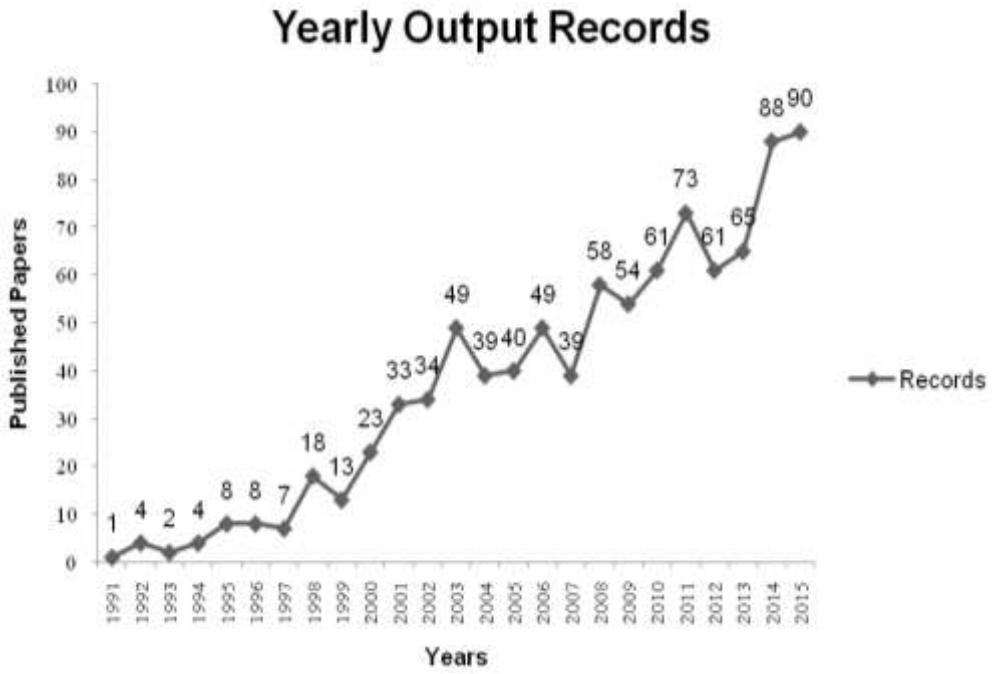

Figure 1. Distribution of publications on dental plaque/biofilm (1991-2015). Source: Elaborated from the Web of Science data.

evolution of the number of publications distributed by years of the records can be seen in Figure 1.

The total quantitative of publications was organized by the corresponding periodic conform in Table 2. The journals with the largest number of published records are the "Archives of oral biology" and "Caries Research", both with 43 articles. However, to identify those journals with the highest impact, was defined an index by dividing the number of citations by the number of published papers, presenting the "Journal of Bacteriology" as the one with the highest rate (79.6). This information become relevant for researchers and research centers to map the academic journals that publish most in the thematic and receive more citations from other studies.

The authors with the highest number of publications were presented in Table 3. Among the 2804 authors identified in the study, Table 3 shows the ten with the highest number of publications. The first is Michael Wilson, a researcher at the post-graduate school in dentistry in Europe, where he also works with Jonathan Pratten. The observation of Table 3 allows the identification of at least three major research centers in the area, "UCL Eastman Dental Institute – United Kingdom," the "University of Otago Wellington – New Zealand" and the "University of Maryland – United States of America", all research centers with a minimum of two researchers and more than forty records of publications. Furthermore, the specific situation in the area of knowledge of dental studies localizes the results in a perspective that corroborates with the initial interest in this study. The quantity of articles by country of origin of the membership of the authors is presented in Table 4.

Table 2. Top journals with more published articles on dental plaque/biofilm (1991-2015).

Journals	Quantity of articles	Citations	Citations/ Quantity
Archives of oral biology	43	1028	23,90
Caries research	43	1289	29,97
Journal of dental research	40	1264	31,6
Oral microbiology and immunology7	35	893	25,51
Applied and environmental microbiology	33	1258	38,12
Journal of clinical periodontology	26	1345	51,73
Journal of dentistry	21	286	13,61
BMC microbiology	20	339	16,95
FEMS microbiology letters	20	753	37,65

Source: Elaborated from the Web of Science data.

Table 3. Authors with more publications on dental plaque/biofilm (1991-2015).

Authors	Quantity of articles	Membership (Institution with Affiliation)	Country
Wilson M	31	UCL Eastman Dental Institute	United Kingdom
Sissons CH	26	University of Otago Wellington	New Zealand
Marsh PD	24	University of Leeds	United Kingdom
Kolenbrander PE	21	University of Mariland	United States of America
Pratten J	21	UCL Eastman Dental Institute	United Kingdom
Xu HHK	20	University of Mariland	United States of America
Wong L	19	University of Otago Wellington	New Zealand
Scannapieco FA	18	University at Buffalo School of Dental Medicine	United States of America
Weir MD	17	University of Mariland	United States of America
Lamont RJ	16	University of Louisville	United States of America

Source: Elaborated from the Web of Science data.

Table 4. Number of articles by origin country and author membership.

Country	Quantity
United States of America	316
United Kingdom	155
Brazil	78
China	73
Japan	71
Netherlands	56
Germany	50
New Zealand	35
Sweden	32

Source: Elaborated from the Web of Science data.

Table 4 indicates the countries of work memberships, with predominance in studies of American origin, followed by the UK and Brazil. It is important to note that the results coincide with the list of authors with most publications only to the first two countries, as Brazil is not in the list shown in Table 3. This may be due to the fact that Brazilian works are more decentralized in authoring or not have specialized centers on the field, despite the amount of individual records.

To analysis the relationship between the most cited articles, the records were separated into two groups: (a) items that received more citations from other papers throughout the database ISI Web of Science™ (GCS) and (b) articles that received more citations of the works in the selection group in this bibliometric study (LCS). The number of citations and citation relations between these works (represented by lines connecting the circles) indicate the most representative studies on the subject, presenting seminal works and those that later were also very cited (Figure 2).

By observing the relationship between the texts, entitled figures can be identified "authority article "or "base article" (Santos et al., 2014), which are those main references of others who receive large amounts of citations. Are they: Kolenbrander (2000), Loo et al. (2000)

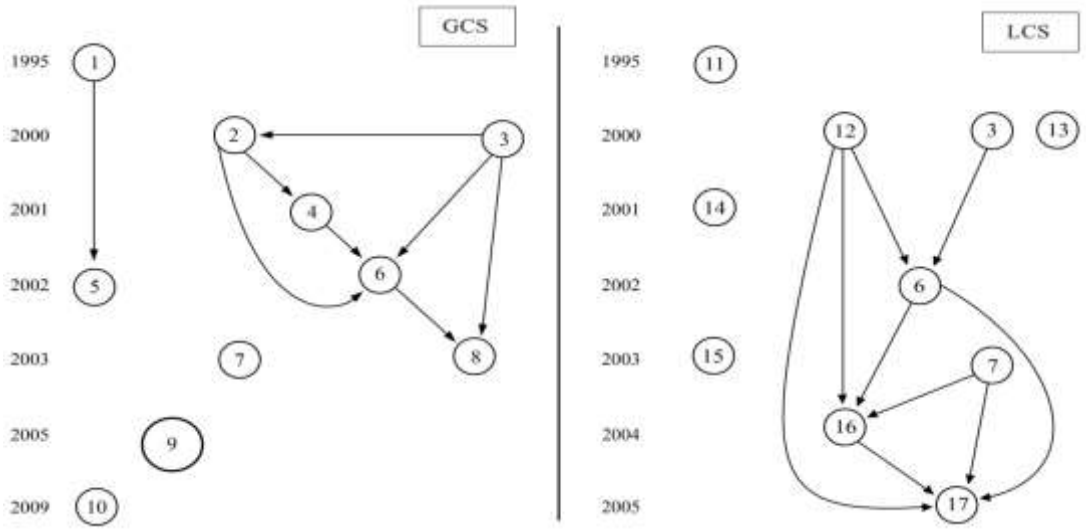

Figure 2. Top 10 most cited articles in Web of Science (Global Citation Score) and most cited by the selected items (Local Citation Score), from the field selection (1991-2015). Source: Elaborated from the Web of Science data. 1– Quirynen and Bollen (1995); 2– Kolenbrander (2000); 3– Loo et al., (2000); 4– Li et al., (2001); 5– Quirynen et al. (2002); 6– Kolenbranderet al (2002); 7– Marsh (2003); 8– Rickard et al. (2003); 9– Pihlstrom et al. (2005); 10 – Hall-Stoodley and Stoodley (2009); 11 – Marsh and Bradshaw (1995); 12 – Wood et al,2000); 13– Rosan and Lamont (2000); 14– Guggenheim et al. (2001); 15– Palmer et al. (2003); 16– Marsh (2004); 17– Marsh (2005).

and Wood et al. (2000). In addition, the "hub articles" or "Connecion articles", which are those that condense important information from previous work by connecting these to other more recent, also receiving large amounts of citations (Santos et al., 2014), They are: Li et al. (2001), Kolenbrander et al. (2002) and Marsh (2004). Older articles of the relationship shown in Figure 1 are: Marsh and Bradshaw (1995) and Quirynen and Bollen (1995).

DISCUSSION

The study is aimed at evaluating the recent advances in microbiology of dental plaque, with particular emphasis on the mechanisms by which the bacteria adhere to the tooth surface and produce a biofilm. It also describe the formation of biofilm on the tooth surface and cites caries and periodontal diseases that arise due to the formation of dental plaque. The study shows that bacteria associated with biofilm formation are more resistant to antibiotics as compared to suspended molecules and suggests that it is necessary to intervene in the formation and development of biofilms. The principal contributions lie in the presentation of the use of some strategies in an attempt to reduce the bacterial activity in the biofilm, such as the use of fluoride, fissure sealant that reduces acid production, combined use of anti-plaque and anti-bacterial that only inhibits the formation of some microorganisms, and vaccination which can raise antibodies against a specific bacterial type (Marsh and Bradshaw, 1995).

The second study aimed to examine the influence of roughness and surface free energy in the bacterial adhesion process. The authors sought scientific evidence on bacterial adhesion to oral surfaces. As a result, it was Quirynen and Bollen, (1995) that evidenced that the roughness of oral surfaces has a direct impact on bacterial adhesion and biofilm formation and maturation. The increase in surface roughness and free energy directly influenced the bacterial adhesion, plaque formation, thus contributing to the occurrence of future charitable processes and periodontal infections (Quirynen and Bollen, 1995).

Authority article number two, in the shown relationship in Figure 1 (GCS), explores the complexity of the process of biofilm formation and maintenance, as regards the genetic composition, types and multiple species of bacteria groups. This study also emphasizes how future perspectives, the association of new technologies such as electronic confocal laser microscopy to investigate the communication between bacteria and demonstrate more objectively this phenomenon, the formation of biofilms (Kolenbrander, 2000).

One of the authority articles, number three, represented in Figure 1, (GCS), characterized the biofilm formation of *Streptococcus gordonii* in vitro to identify formation of biofilms with genetic defects. In the experiment, the formation of biofilms was altered by changes in pH, osmolality and carbohydrate content. The results suggest that bacterial physiology has a fundamental role in biofilm

formation. The researchers believe that the study of genes can provide information that will assist the understanding of biofilm formation process of oral streptococci (Loo et al., 2000).

The contribution of this study sums up the importance of isolating defective biofilms (mutants) for the understanding of mechanisms for control and prevention of biofilm from the identified defects in the genes. The authors support the idea that understanding the processes involved in the biofilm formation and development through studies of the genes associated with them can provide strategies for the control and prevention of infections mediated by biofilms. This may well trigger the development of agents to combat effectively the bacterial association (Loo et al., 2000).

Study represented in Figure 1 (GCS), with the number four, investigated the biofilm formation by *Streptococcus mutans* and considered the results through genetic analysis. It showed that the *S. mutans* grown in biofilm is more successful. Furthermore, dead cells in the biofilm can serve as donor material resistant to antibiotics. *S. mutans* have higher development capability within the biofilm and is even able to absorb genetic material of dead individuals of the same species, including transferring material resistant to antibiotics which conferring greater resistance to the biofilm (Li et al., 2001). A literature review, represented in Figure 1 (GCS), with the number five, sought risk of infection in cases of oral implant. One of the mentioned risks is the accumulation of bacteria by plaque formation. Analyzed studies indicate that, in studies performed both in animals and in humans, the microflora in the case of implants is similar to that present in periodontitis. However, the authors pointed out that the formation of plaque is not the only risk for infection, but the presence of smoking and poor oral hygiene contributes greatly to the development of infection in oral implants. In the case of implants, it is necessary that the patient undergoes a screening prior to minimize the risk of acquiring infection (Quirynen et al., 2000). This work (Quirynen and Bollen, 1995) mentioned that *in vivo* studies have examined the surface roughness on plaque formation and the relationship with periodontal inflammation.

Article authority, number six, the relationship shown in Figure 1 (GCS) on the communication between the oral bacteria, citing Article Two (Kolenbrander, 2000), shows that oral bacteria interact with their environment, form mixed-species communities and adhere to the surface. Many of the physical interactions that occur between organisms of this community are known. The adhesins are of various molecular sizes and species that support adhesins compete with each other for binding to the polysaccharide receptor. Thus, it is postulated that the co-aggregation and co-adhesion are essential for communication between species and help establish spatio-temporal patterns of development (Kolenbrander et al., 2002) Citing the articles three (Loo et al., 2000) and four (Li et al., 2001), shows that the possibility of oral streptococci in biofilms has been verified and can communicate via a signaling and detection system. A signaling system is essential for genetic competence in *S. mutans* and is involved in biofilm formation (Kolenbrander et al., 2002).

Study discusses the properties of dental plaque in order to elucidate its composition and the relationship between the biofilm and the oral microflora. The author considers the hypothesis that biofilms can be combated not only directly with the impediment of its formation, for example, but also through environmental relations between microorganisms. Contribution to the knowledge on the prevention and treatment of the biofilm is the possibility of action for understanding and working knowledge of oral microflora, this why treatments are restricted to combat the symptoms without worrying about the ecological characteristics of the mouth. With the understanding of the peculiarities of oral flora, a holistic care becomes feasible (Marsh, 2003).

In the research on the processes of coaggregation, it was found that genetically distinct bacteria join together by means of specific molecules to form complex aggregates and give origin to complex biofilms. The researchers suggest that there is evidence that these formations are possible in dental plaque in which occurs such junction between different bacteria and biofilm formation. The coaggregation among different species gives greater resistance to the biofilms (Rickard et al., 2003).

Then, the relationship presented in Figure 1, in Article nine (Pihlstrom et al., 2005), discuss the factors that cause periodontal diseases, especially gingivitis caused by biofilms which are related to other factors such as the use of tobacco which contribute to the development of gingivitis. The authors state that, to treat periodontal disease, it is necessary to control the biofilm and other risk factors. Further, the diseases may be treated with systemic antibiotics. But with the use of these, there is a risk of provoking the development of resistant microorganisms. It also point out that the greatest risk factor for periodontal diseases are the biofilms that form when oral hygiene is poor. In this work, the most common ways to prevent and treat biofilms are: oral hygiene, flossing and mouthwash.

The last study presented at the global graph of citations, number ten, investigates attempts to analyze the characteristics of biofilms (phenotype) of different bacterial species. The authors analyze several studies through a review and suggest that there are few investigations that seek to understand how the biofilm interferes with infectious diseases in humans. Understanding the inflammatory response of the host is now been understood. The authors believe that clinical studies of infections caused by biofilms are relevant to understanding of the interaction between bacteria and biofilm in the host's inflammatory response. From new

studies, it will be possible to review the strategies of prevention and treatment of biofilm (Hall Stoodley and Stoodley, 2009).

The first authority article in the relationship shown in Figure 1 (LCS), the local citation graph contains as research problem, the removal of intact plate so that the biofilm can be viewed entirely in the laboratory, or in its natural state and integral to the identification of possible structures or spaces that could be useful in understanding and combating biofilm. The authors conclude that knowing the biofilm structure with their respective spaces is fundamental to the application of therapies to desired targets within the plaque. Circulatory channels have been identified in dental plaque that can function as a path for application of therapeutic agents within the biofilm. The authors also suggest that there are common features in all bacterial biofilm structures, thus this study is very important because it can provide the developing of new biofilm treatments (Wood et al., 2000).

Still in 2000, another study, represented by the number thirteen in Figure 1 (LCS), is a review which discusses the molecular basis of bacterial adhesion to tooth surface. The adhesion process provides stability of bacterial structures and is performed by a multitude of molecules that have critical roles within the biofilm structure. The adhesin, as a adherence protein complex, will define the success or failure of the dental plaque adhesion, to rely on their performance at the time of bacterial adhesion (Rosan and Lamont, 2000).

In the following year, a study represented by the number fourteen in Figure 1 (LCS), describes a supragingival plaque model grown in the laboratory containing the bacteria: *Actinomyces naeslundii*, *Veillonella dispar*, *Fusobacterium nucleatum*, *Streptococcus sobrinus* and *Streptococcus oralis*. In the experiment, researchers developed a biofilm model very useful for preclinical testing of agents to combat biofilms. It was introduced in the experiment chlorhexidine and was observed that there were significant losses on the viability of the biofilm similar to losses *in vivo*. It was still possible to observe the antibacterial effects of chlorhexidine. The search is important for bacterial adhesion studies and coaggregation (Guggenheim et al., 2001).

Isolated in the figure, but very cited among the group object of research articles, the article number fifteen Figure 1 (LCS) tests the hypothesis that the adhesion between bacteria influence the development of plaque. The study is the first to demonstrate interactions mediated by coaggregation of bacteria during the initial phase of the plaque. There is a relationship between communities of mixed species and early plaque formation. The study is an example to further investigate, isolate and characterize bacterial interactions during the initial stage of plaque (Palmer et al., 2003).

By means of advanced technology, it was identified in article represented by the number sixteen in Figure 1 (LCS), that the biofilm has void spaces, and channels. The study focuses on understanding the plaque as a biofilm of mixed cultures in order to propose new strategies for controlling biofilms. Such understanding will result in future studies on more effective agents for inhibiting plaque, mechanisms able to interfere with the communication and bacterial degradation of the biofilm structure and identification of pathogenic clones may improve diagnosis and predict disease susceptibility (Marsh, 2004).

Study represented by the number seventeen in Figure 1 (LCS), shows that biofilms are complex structures organized and populated by communities of micro-organisms that communicate through gene transfer or through molecules. Through a review, the author evaluates whether the plaque exhibits properties consistent with those of a typical microbial biofilm communities. The results show that the plaque has a structure with an extracellular matrix of a different composition and communication are performed by means of gene transfer, such organization increases the success of the plaque as it increases it's metabolic efficiency, gives greater strength and increased virulence, that is, the plaque exhibits characteristics that are typical of biofilms and microbial communities in general (Marsh, 2005).

Based on other articles (Marsh, 2003, 2004), dental plaque is defined as a multiple community of microorganisms as a biofilm, this community is embodied by an extracellular matrix of bacterial polymers (Marsh, 2005).

The twelfth study of Figure 1 (LCS) demonstrates that microscopic techniques have been developed to investigate biofilms and show that the plaque behaves like a typical biofilm (Wood et al., 2000). The article represented by the number seventeen of Figure 1 (LCS) corroborates to the results that indicate that the plaque has a structure containing pores or channels that point areas where there is interaction at the interface tooth (Marsh, 2005). When citing the work represented by the number six of Figure 1 (LCS), how the communication between the organisms of the plate was carried out by means of small molecules, was explained.

It is noticed that, as shown in Figure 1, - Citation Score - GCS, the top 10 most cited article in the Web of Science, was number nine of the authors, Pilhlstrom et al. (2005), which sought evidence of bacterial coaggregation and development of multi-species biofilm, with 976 citations throughout the database. Top 10 most cited article in The Local Citation Score - LCS, was the work of Kolenbrander et al. (2002), which sought evidence of communication among the oral bacteria, with 77 citations within the selection carried out in the set of articles published in the period 1991 to 2015 on training, bacterial adhesion, prevention and treatment of oral biofilms. Interestingly, the temporal gap, even in a cut that includes recent articles, those most cited have over 10 years of publication and this may be indicative of a

maturation period of the results and the interstices between its release and subsequent citations it receives. The international literature dealing with the subject in a broadly diversified way, points to a range of possibilities for the prevention and treatment of oral biofilms, as well as experimental methods and possibilities of use in studies for this purpose. The prevention of oral biofilm is a complex process because it involves several species of microorganisms in their composition. The studies analyzed the importance of understanding how formation occurred and observed bacterial adhesion. From this understanding, it is possible to list effective strategies to prevent the development of oral biofilms.

Conclusion

There were 921 published articles in 274 different journals indexed in the Web of Science, in the 1991-2015 year period, on training, bacterial adhesion, prevention and treatment of oral biofilms. These articles were written by 2804 authors with ties to 695 institutions in 59 countries, notably the United States and the United Kingdom. The 17 articles of greater impact were published in the 1995 to 2009 year period.

The substances that penetrate and destroy the structure of the biofilm have been already discussed in articles by means of *in vitro* experimental studies that have been listed for mechanisms and combinations able to penetrate the biofilm structure and reduce pathogenic bacterial activity and, in some cases, destroy bacterial structure.

The scientific evidence on this subject can contribute to the development of new research in order to fill gaps in knowledge on the prevention and treatment of oral biofilm and implement effective public policies of oral health care.

Conflict of Interests

The authors have not declared any conflict of interestS.

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