ABSTRACT

The search for the etiologic agents of periodontal diseases started in the Golden Era of medical bacteriology, when the etiologic agents of many bacterial infections were isolated and characterized. After the initial enthusiasm in establishing the infectious nature and the true agents of periodontal diseases, this concept was virtually ignored for the next four decades. Until the early 1970s treatment regimens based on the non-specific plaque hypothesis were directed towards a non-specific reduction in plaque amount. Later, the specific plaque hypothesis established the role of some microorganisms such as *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, *T. denticola*, *P. intermedia* and *F. nucleatum* in different forms of periodontal diseases. It was recently suggested that these suspected periodontal pathogens seem to not act alone and interactions between species, especially the balance between pathogenic and beneficial species affect both progression of disease and response of tissues to periodontal therapy. Nowadays it is well established that one of the goals of therapy is to control such periodontal pathogens. Among the most commonly used therapies to treat periodontal infections are scaling and root planing (SRP), supragingival plaque control and periodontal surgeries. Many studies confirmed the reduction of “red complex” species by SRP, and apically repositioned flap can lead to an additional beneficial effect in the subgingival microbiota by decreasing levels of “red” and “orange complexes” species. Furthermore, the level of plaque control maintained by the patients has been considered a crucial step in preventing recurrence of destructive periodontitis.

Uniterms: Bacteria; Periodontitis; Toothbrushing; Dental scaling; Surgical procedures.

RESUMO

A busca pelos agentes etiológicos das doenças periodontais iniciou na Época de Ouro da bacteriologia médica, quando os agentes de diversas infecções foram identificados. Após o entusiasmo inicial em estabelecer a natureza infecciosa da doença periodontal, este conceito foi ignorado por quatro décadas. Até o início dos anos 70, terapias baseadas na hipótese da placa não-específica focavam a redução da quantidade de placa. Posteriormente, a hipótese da placa específica determinou o papel de alguns microorganismos como *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythensis*, *T. denticola*, *P. intermedia* e *F. nucleatum* nas diferentes formas de doença periodontal. Recentemente, foi sugerido que estes patógenos periodontais não atuam isoladamente e interações entre espécies, como o equilíbrio entre bactérias patogênicas e benéficas afetam a progressão da doença e a resposta tecidual à terapia periodontal. Atualmente está bem estabelecido que um dos objetivos da terapia é o controle destes patógenos. Dentre as terapias mais frequentemente utilizadas no tratamento da periodontite estão raspagem e alisamento radicular (RAR), controle da placa supragingival e cirurgias periodontais. Muitos estudos confirmaram a redução de espécies do “complexo vermelho” pela RAR, e mostraram que o retalho reposicionado apicalmente pode levar a um efeito benéfico adicional na microbiota subgingival pela diminuição nos níveis de espécies dos “complexos vermelho” e “laranja”. Além disso, o controle de placa mantido pelos indivíduos é considerado determinante para a prevenção da recorrência de doença periodontal destrutiva.

Unitermos: Bactérias; Periodontite; Escovação dentária; Raspagem dentária; Procedimentos cirúrgicos.
BACTERIAL ETIOLOGY OF PERIODONTAL DISEASES

The Early Years

The search for the etiologic agents of periodontal diseases started in the Golden Era of medical bacteriology (1880 to 1920), when the etiologic agents of many bacterial infections were isolated and characterized. Groups of investigators applied the microbiological techniques available at that time to study the oral microbiota. As a result, numerous papers published during this period suggested specific etiologic agents of periodontal diseases, including spirochetes, fusiforms, and streptococci.

After the initial enthusiasm in establishing the infectious nature and agents of periodontal diseases, this concept was virtually ignored for the next four decades. From the mid-1920s to the early 1960s, a series of different factors were considered to cause or to be related with the nature of periodontal lesions. Among them were the concept of passive eruption developed by Gottlieb, local irritation of the periodontium, constitutional defects, and functional occlusal disturbances. Bacteria were thought to be merely secondary invaders in this process.

Treatment of patients based on the notion of constitutional defects or trauma from occlusion was not effective in controlling periodontal diseases. The absence of a single paradigm for the research into the cause of periodontitis, and the failure of preventive and therapeutic actions to yield predictable results generated profound professional insecurity. Animal studies carried out in the early 1960s brought the microbial etiology of periodontal diseases into focus. Keyes and Jordan demonstrated that periodontal diseases could be transmitted in hamsters from animals with disease to animals without disease by caging them together. Jordan and Keyes also showed that the species now known as Actinomyces viscosus was the only species capable of causing destructive periodontitis in hamsters without disease. Gibbons and Socransky inoculated germ-free mice with organisms from human periodontal pockets and observed an increase in the severity of bone loss in these animals. In addition, strains of streptococci (Streptococcus mutans), as well as Gram-positive rods (particularly Actinomyces naeslundii) isolated from the human oral cavity were shown to induce plaque formation, caries, and alveolar bone loss in gnotobiotic as well as conventional rats.

At the same time, epidemiological and cross-sectional studies demonstrated a close relation between oral hygiene and periodontal pathology and rekindled interest in the importance of bacterial plaque in the etiology of periodontal diseases. A study performed by Schei and co-workers in a Norwegian population showed that individuals with abundant plaque exhibited increased amounts of alveolar bone loss compared to subjects with less dental plaque. The classic study of “experimental gingivitis” by Löe and co-workers demonstrated that plaque led to gingivitis confirming the relationship between plaque accumulation and gingival inflammation. However, at that time, it was thought that periodontal diseases resulted from an overall growth of plaque and that virtually any organism present in the subgingival plaque could contribute to tissue destruction. This concept became known as the “non-specific plaque hypothesis”.

Until the early 1970s, treatment regimens based on the non-specific plaque hypothesis were directed towards a non-specific reduction in plaque amount. It was thought that the composition of plaque was similar from patient to patient and from site to site. However, observations from newer epidemiological surveys indicated that only certain individuals or sites exhibited attachment loss and that some populations in Sri Lanka and Kenya had a large amount of calculus and plaque with minimal or no periodontal attachment loss.

These data contradicted the principles of the non-specific plaque hypothesis. If plaque composition was consistent from individual to individual, why did some subjects who accumulated much plaque fail, even after many years, to develop destruction of the supporting structures? On the other hand, why did some individuals with little detectable plaque develop rapid periodontal destruction? Further, why did some sites in the oral cavity of the same individual lose attachment while others did not?

Re-introduction of Bacterial Specificity

Partial answers to these questions came from more recent studies on the microbiology of periodontal diseases. Listgarten, et al. studied the structure of the microbiota on extracted natural teeth from patients with a diagnosis of health, gingivitis, or periodontitis and observed many differences in terms of plaque composition. The plaque associated with the periodontally healthy tooth was composed of coccoid mainly Gram-positive species. A marked increase in the periodontal mass, as well as a relative increase in the proportion of Gram-negative bacteria, motile rods and filaments characterized the gingivitis-associated microbiota. In samples of teeth from adult periodontitis subjects, an abundant, complex microbiota was observed in the periodontal pockets, presenting a predominantly Gram-negative bacterial population, with a large proportion of spirochetes. Other cultural and microscopic study confirmed the morphological differences in the composition of the subgingival microbiota in subjects with different clinical status.

Besides these morphological studies, the recognition that certain forms of periodontal disease such as necrotizing ulcerative gingivitis (NUG) and localized juvenile periodontitis (LJP) were distinct clinical entities, probably associated with specific microorganisms, led to the re-introduction of specificity in the bacterial etiology of periodontal diseases, the “specific plaque hypothesis”. Listgarten in the 1960s had shown that medium sized spirochetes were present in practically pure culture in the lesions of NUG within the connective tissue and the adjacent
epithelium. The importance of this observation was not realized at that time. The introduction of improved cultural methods, such as continuous anaerobiosis techniques, new culture media, better sampling and dispersion of plaque, facilitated the characterization and identification of new pathogens and later the evaluation of different therapies.

**Localized Juvenile Periodontitis**

In 1976, Slots24 studied the predominant cultivable microorganisms in juvenile periodontitis and demonstrated that the microbial composition of subgingival plaque taken from diseased sites differed substantially from the samples taken from healthy sites. He found that the microbiota from the control sites consisted primarily of streptococci, Gram-negative and Gram-positive rods. In contrast, the test pockets had a relatively stable microbiota, dominated by Gram-negative anaerobic rods, but facultative cocci and rods were also present; one facultative (capnophilic) Gram-negative rod species was later classified as Actinobacillus actinomycetemcomitans. Other investigations confirmed Slots’ finding that this organism was elevated in active sites when compared with inactive sites in patients with LJP25,26,27. In addition, many other studies showed an increase in the frequency of detection and higher numbers of this organism in lesions of LJP when compared with plaque samples from other clinical conditions including periodontitis, gingivitis and health28,29,30,31. Furthermore LJP patients often demonstrated markedly elevated levels of local and systemic antibodies to A. actinomycetemcomitans27,32,33, 34,35. This organism was also shown to invade human gingival epithelial cells in vitro36. It was also shown that successful treatment of LJP patients was associated with the elimination or reduction of this species, whereas failure was related to persistence of high levels of this organism25,27. Collectively, the data suggested that A. actinomycetemcomitans is a probable pathogen of LJP.

**Chronic Periodontitis**

The recognition that subjects with LJP could be treated successfully with local debridement and systemic antibiotics provided impetus to studies attempting to relate specific microorganisms to the etiology of different periodontal diseases. Another intensively studied probable periodontal pathogen is Porphyromonas gingivalis. Early interest in P. gingivalis and other black-pigmented Bacteroides arose primarily because of their essential role in experimental mixed infections15 and their production of a large number of virulence factors38,39,40. The association of P. gingivalis with disease came from studies that demonstrated that the species was uncommon in health and gingivitis but more frequently detected in destructive forms of disease21,42,43,44. P. gingivalis has been shown to be reduced in successfully treated sites but commonly encountered in samples from sites that exhibited recurrence of post-therapy disease45,46. Studies of ligature-induced periodontitis in animals indicated a very strong relation between P. gingivalis-like organisms and disease25. Like A. actinomycetemcomitans, P. gingivalis has been shown to be able to invade human gingival epithelial cells in vitro48.

**Studies Employing Specific Antisera or DNA Probes**

The development of new techniques using polyclonal or monoclonal antibodies and DNA probes was very important in more precisely discriminating subgingival species, especially those that were difficult to cultivate by traditional cultural methods, such as Bacteroides forsythus and spirochetes.

Initially, B. forsythus (recently reclassified as Tannerella forsythensis by Sakamoto, et al.49, 2002) was thought to be a relatively uncommon subgingival species. However, immunological studies using monoclonal antibodies and fluorescent-labeled polyclonal antisera suggested that this species was more common than previously found in cultural studies and that its levels were strongly related to increased periodontal pocket depth50,51,52,53,54. Haffajee, et al.35, using the checkerboard DNA-DNA hybridization technique, found very high levels of this organism in subgingival plaque samples of 57 patients with adult periodontitis. In addition, the authors observed that T. forsythensis was infrequently detected in epithelial cell samples from healthy subjects. Finally, serum antibody to T. forsythensis has been found to be elevated in many periodontitis patients66.

The spirochetes are another group of organisms implicated in the etiology of periodontal diseases. The difficulty in growing and distinguishing individual species has been the major difficulty in defining species of spirochetes as periodontal pathogens. Clearly, a spirochete has been implicated as the likely etiological agent in NUG by its presence in large numbers in tissue biopsies from affected sites5. However, this species remains uncultivated. In recent years, specific species of spirochetes have been related to periodontal breakdown. Treponema denticola was found to be more common in periodontally diseased than in healthy sites and more frequent in subgingival than in supragingival plaque57,58,59. T. denticola was shown to be decreased in successfully treated periodontal sites but not changed or increased in nonresponding sites60,61. More recently, Haffajee, et al.35 found that almost 35% of the subgingival sites in adult periodontitis patients were colonized with strains of T. denticola. Socransky and coworkers62 observed that together with T. forsythensis and P. gingivalis, T. denticola strikingly related to clinical measures of periodontal disease, particularly increased pocket depth and bleeding on probing63. Additionally, T. denticola-reactive IgA was found to be elevated in aggressive periodontitis patients55. These studies suggested that certain species of spirochetes were important in the pathogenesis of specific forms of periodontitis. However, the studies evaluated only the limited number of spirochetes species that have been successfully cultivated, suggesting the possibility that other spirochetes might be involved59.
Other Possible Pathogens of Periodontitis

Other organisms have been suggested to play a role in disease, including Prevotella intermedia, Campylobacter rectus, Eikenella corrodens, Peptostreptococcus micros, Streptococcus intermedius, and Capnocytophaga spp. However the data on the role of these microorganisms were observed in samples from sites that exhibited less active disease and responded more to the initiation of disease and to the control of post-therapy interactions between species. The presence and levels of these host-compatible or host-beneficial species were critical both to the initiation of disease and to the control of post-therapy disease. The species considered “beneficial” included Veillonella parvula, Actinomyces sp., or the combination of Streptococcus sanguis II (Streptococcus oralis), Streptococcus mitis, V. parvula, and S. intermedius. Clusters of these microorganisms were observed in samples from sites that exhibited less active disease and responded more favorably to therapy.

Socransky, et al. described 5 major microbial complexes observed in subgingival plaque samples from a wide range of subjects (160 periodontitis and 25 periodontally healthy subjects). The presence and levels of 40 subgingival species were determined in 13,261 subgingival plaque samples using DNA probes. Cluster analysis and community ordination techniques were used to examine the relationships between bacterial species. One complex, called the “red complex”, consisted of 3 tightly related species: T. forsythensis, P. gingivalis, and T. denticola. As mentioned before, this complex strongly related to pocket depth and bleeding on probing. Another complex (“the orange complex”) included F. nucleatum/pontidonticum subspecies, P. intermedia, Prevotella nigrescens, P. micros, C. rectus, Campylobacter gracilis, Campylobacter showae, Eubacterium nodatum and Streptococcus constellatus, and seemed to precede colonization by species of the “red complex”. The so-called “yellow complex” consisted of 6 Streptococcus species: Streptococcus sp., S. sanguis, S. oralis, S. intermedius, Streptococcus gordoni, S. mitis and the “green complex” was comprised of Capnocytophaga ochracea, Capnocytophaga gingivalis, Capnocytophaga sputigena, E. corrodens and A. actinomycetemcomitans serotype a. The fifth, “purple complex”, consisted of V. parvula and Actinomyces odontolyticus. A. actinomycetemcomitans serotype b, Selenomonas noxia and Actinomyces naeslundii genospecies 2 (A. viscosus) did not fall in any cluster or ordination group.

While species within complexes were closely associated, the complexes themselves had specific relationships with one another. The “red” and “orange complexes” showed a significant association, while the “purple”, “yellow” and “green complexes” seemed to be more strongly associated with each other than to either the orange or red complexes. This indicated not only specific relationships between subgingival species, but also suggested a possible pattern or sequence of colonization.

Ximénez-Fyvie, et al. compared the microbial composition of supra and subgingival plaque in 22 periodontally healthy and 23 adult periodontitis subjects. A total of 2,358 samples of supra and subgingival plaque were collected from up to 28 supra and 28 subgingival sites from both the healthy and the periodontitis subjects, and individually analyzed for their content of 40 bacterial taxa using checkerboard DNA-DNA hybridization. T. forsythensis, P. gingivalis, T. denticola, P. intermedia, P. nigrescens and S. noxia were significantly more prevalent in supragingival plaque samples from periodontitis subjects than periodontally healthy subjects. The distributions for P. gingivalis were significantly different between health and disease. The other 2 “red complex” species, T. forsythensis and T. denticola as well as other suspected periodontal pathogens showed similar distributions. Actinomyces species were the dominant taxon in both supra and subgingival plaque from healthy and periodontitis subjects. Both supra and subgingival plaque samples from healthy subjects presented significantly lower total counts than from periodontitis subjects. The main differences found between supra and subgingival plaque, as well as between health and disease, were in the proportions and to some extent levels of Actinomyces, “orange” and “red complexes” species. In another study, Ximénez-Fyvie, et al. suggested that supragingival plaque may play a role as a reservoir of putative periodontal pathogens. They observed that in a population of adult periodontitis subjects, suspected periodontal pathogens were isolated in supragingival plaque samples from sites where subgingival samples were negative for the same species. However, subgingival plaque samples exhibited significantly higher proportions of “red” and “orange complexes” species in comparison with the supragingival plaque samples.

While our knowledge of the bacterial etiology of periodontal diseases has significantly expanded over the years, many questions still remain unanswered; especially how different kinds of periodontal therapies interfere with this highly organized subgingival population.

MECHANICAL THERAPIES

The above section suggested some of the likely pathogenic and beneficial species in subgingival plaque. One of the goals of therapy is to control such organisms. The present section examines the effects of periodontal therapies, not including antibiotics, on clinical and microbiological parameters of periodontal diseases.
Supragingival Plaque Control

Clinical Studies

Since the bacterial etiology of periodontal diseases was established, supragingival plaque control has been considered a crucial step in maintaining periodontal health. The classical longitudinal studies carried out in Sweden by Lindhe and co-workers in the 1970s and 80s established the importance of oral hygiene in the success of different types of periodontal therapies. This group of investigators demonstrated that the level of plaque control maintained by the patients after treatment was more critical in preserving the periodontal status and preventing recurrence of destructive periodontitis than the mode of initial therapy used\cite{80,81,82,83}. It is important to emphasize that in most of these studies the patients received professional cleaning after therapy every 2 weeks. Lindhe, et al.\cite{80} studied the long-term effect of surgical/non-surgical treatment in a group of patients followed for 5 years post-therapy. Patients who during the 5 years of monitoring consistently had a high frequency of plaque-free tooth surfaces showed little evidence of recurrent periodontal disease, while patients who had a low frequency of plaque-free tooth surfaces had a high frequency of sites showing additional loss of attachment. Rosling\cite{82} and Rosling, et al.\cite{83} studied the healing potential of periodontal tissues following different forms of periodontal surgery and confirmed that it was possible to treat periodontal disease successfully, even in advanced stages, in patients who were scrupulously maintained by professional cleaning. Similarly, Nyman and co-workers\cite{81} demonstrated that in plaque-infected dentitions, five different techniques for surgical pocket elimination were equally ineffective in preventing recurrence of destructive periodontitis. Thus, in general, effective supragingival plaque control leads to an improved clinical status for the periodontal patient\cite{84}.

Microbiological Studies

A limited number of studies have examined the effect of supragingival plaque control on the microbial composition of subgingival plaque. While some studies found no effect, others suggested that careful supragingival plaque control decreased the amount of subgingival plaque and/or the levels of specific subgingival species or morphotypes. Studies using darkfield microscopy suggested that it is possible to reduce the number of spirochetes and motile rods in subgingival plaque by implementing professional supragingival plaque control\cite{85,86,87,88}. Similarly, studies of subgingival plaque samples using cultural techniques indicated that professional cleaning decreased the total counts of some specific periodontal pathogens including \textit{A. actinomycetemcomitans}, \textit{P. gingivalis}, \textit{T. denticola} and other black pigmented rods, and spirochetes\cite{85,87,88}. However, other investigations using similar techniques suggested no significant effects of professional supragingival plaque control on the level and/or composition of subgingival plaque\cite{89}. The contradictions among these data may be due to limitations of the microbiological techniques utilized in some of the studies, as well as the limited number of sites and species evaluated. The development of immunological and DNA probes techniques has been extremely helpful in overcoming these problems. Haffajee and co-workers\cite{90} used DNA probes for 40 different species to study the effect of professional plaque control, carried out once a week for 3 months, on the microbial composition of the subgingival plaque sampled from each tooth. A decrease in the levels of the majority of the 40 species, in particular the members of the “red” and “orange complexes” were obtained and maintained for at least 3 months. The species that were most significantly reduced included putative periodontal pathogens such as \textit{A. actinomycetemcomitans}, \textit{P. gingivalis}, \textit{T. forsythensis} and \textit{T. denticola}. However, as seen with other forms of mechanical therapy, supragingival plaque control did not significantly affect the prevalence of any of the presumed pathogenic species supra or subgingivally. It was interesting to observe in this study that at least for 3 months after completion of the professional cleaning phase the microbial profiles of both the supra and subgingival plaque samples in the periodontitis subjects were almost identical to those observed in a second group of 23 periodontally healthy subjects. Ximenez-Fyvie, et al.\cite{91} observed that weekly professional supragingival plaque removal in adult subjects with periodontitis diminished counts of both supra- and subgingival species creating microbial profile comparable to that observed in periodontal health. This microbial profile was maintained for a 9-month period. Similarly, Haffajee, et al.\cite{92} showed clinical and microbiological beneficial effects of supragingival plaque removal in conjunction with different periodontal therapies.

Scaling and Root Planing

Clinical Studies

The clinical findings associated with scaling and root planing (SRP) in different forms of periodontal disease have been discussed by the World Workshop of Periodontology\cite{93}. The overall results indicated a clinical benefit from SRP in terms of reducing inflammation and decreasing probing pocket depth and attachment level measurements, especially at deeper sites\cite{93,94,95,96}. Some studies described a loss of clinical attachment at sites with initially shallow pockets\cite{97,98} probably related to mechanical trauma from instrumentation. A number of studies compared the efficacy of SRP with surgical procedures\cite{90,95,96,99,100,101}. In general, surgical therapy produced greater probing depth reduction than non-surgical therapy over the short term. Some of the early differences diminished during long-term follow-up\cite{95,101}. In terms of attachment levels, SRP produced less attachment loss at shallow sites than surgical procedures. Deep periodontal pockets (>6 mm) responded equally favorably to both therapies.
Microbiological Studies

Few studies have documented the microbiological changes associated with SRP. Studies using darkfield and phase contrast microscopy consistently reported significant reductions in the percentage of motile rods and spirochetes after SRP and a concomitant increase in the percentage of cocci and non-motile microorganisms. Other studies, employing cultural techniques, indicated a decrease in groups of organisms such as A. actinomycetemcomitans, P. gingivalis and other black pigmented rods. Several of these studies also noted improvement in clinical parameters such as decreased probing depths, stabilization of attachment levels, and decreased bleeding on probing. These were significantly associated with decreased percentages of spirochetes, motile rods, and P. gingivalis. Other investigations showed minimal long-term effects of SRP on the subgingival microbiota especially for species such as A. actinomycetemcomitans. As mentioned in the previous section, the development of immunological and molecular approaches has been helpful in studying the oral microbiota related to periodontal treatment, especially species that are difficult to cultivate or are even uncultivable. Using these techniques, many studies confirmed the reduction of P. gingivalis by SRP and also suggested an effect on T. denticola as well as T. forsythensis and T. denticola were significantly decreased. However, none of these species or any of the other test species were undetectable post-therapy. Further, the data suggested that A. actinomycetemcomitans was minimally affected by this form of therapy. In some cases, subgingival eradication of P. gingivalis or A. actinomycetemcomitans may require additional periodontal therapy.

Periodontal Surgery

Clinical Studies

Periodontal surgeries are usually performed for 3 different purposes: regeneration, esthetics and infection control. This section will discuss the use of periodontal surgery in controlling periodontal infection.

Since the beginning of the century, a wide variety of surgical procedures have been described for treating periodontal diseases. Once the infectious nature of periodontitis was established, periodontal surgery was used as a means to gain access to the subgingival plaque and calculus. Therefore, since the 1970s the common types of surgical therapies have included the modified Widman flap, described by Ramfjord in 1974 and the apically repositioned flap, described by Friedman in 1962. Most of the clinical results associated with this type of treatment have been discussed in the previous sections of this review. As described before, the early studies carried out by the Swedish group compared different types of surgical procedures. The patients who maintained a high standard of oral hygiene, by receiving professional plaque control every 2 weeks, were able to maintain their periodontal status up to 5 years, irrespective of the type of surgery performed initially. Because SRP was considered the most basic and time-tested form of periodontal therapy, a number of studies compared the surgical procedures to SRP in one way or another. These studies reported no differences in terms of clinical inflammation following non-surgical and surgical therapy, either short or long term. However, Serino et al. compared the clinical effects of modified Widman flap surgery with those of SRP in 64 subjects with advanced periodontal disease and the incidence of recurrent disease during 12 years of maintenance after active therapy. They found that surgical therapy is more effective than SRP in reducing the overall mean probing pocket depth and eliminating deep periodontal pockets. In addition, more SRP-treated subjects exhibited signs of advanced disease progression following active therapy than the surgical-treated subjects.

Microbiological Studies

There has been limited interest in studying the effects of periodontal surgery on the composition of the subgingival microbiota. Reduction in the proportion of spirochetes and motile rods concomitant with an increase in the proportions of cocci has been observed after subjects were treated using apically repositioned flaps. Rawlinson and co-workers using cultural techniques examined the effects of modified Widman flap surgery on the subgingival microbiota of residual periodontal pockets. There was a decrease in the numbers of P. intermedia and an increase in the overall percentage of C. ochracea post-operatively. At 1 year, an increased number of Gram-negative species were detected, including P. intermedia.

Mombelli, et al. using cultural techniques, monitored the levels of subgingival species, for 1 year, in subjects who received apically repositioned flap surgery. They observed a significant decrease in the total anaerobic viable bacterial counts and in the proportion of the Gram-negative anaerobic rods. Furthermore, P. gingivalis, Fusobacterium sp, and C. rectus were detected significantly less often after treatment. Capnocytophaga and A. odontolyticus, on the other hand, were more frequently isolated after therapy.

Mombelli, et al. determined the distribution patterns of P. gingivalis, P. nigrescens and A. actinomycetemcomitans in 852 subgingival microbial samples after scaling and root planing and modified Widman flap procedures in sites with persisting pockets greater than 5mm. P. nigrescens showed a high frequency in a subject and site levels. Persisting P. gingivalis was detected in a high percentage of subjects, but in a low proportion of sites. Only 2 subjects were A. actinomycetemcomitans positive after treatment. According to Van der Velden, et al. smokers, the periodontal surgery was less effective on clinical parameters and on the
composition of the subgingival microbiota than in nonsmokers. Although Levy et al.\textsuperscript{118} had similar findings in 7 current smokers, the authors suggested that more subjects should be included in further studies in order to confirm the association between cigarette smoking and clinical and microbiologic surgical therapeutic outcomes in subjects with periodontal disease. Tuan, et al.\textsuperscript{119} observed that when periodontal flap surgery was accompanied by osseous recontouring, this procedure reduced periodontal pocket depths and levels of major periodontal pathogens such as \textit{P. gingivalis} and \textit{A. actinomycetemcomitans}.

Levy and co-workers,\textsuperscript{118,120} using the checkerboard DNA-DNA hybridization, studied the changes in the composition of subgingival plaque following surgical elimination of periodontal pockets by apically repositioned flap in 18 subjects with chronic periodontitis. Members of the “red complex” (Socransky, et al.\textsuperscript{62} 1998), \textit{T. forsythensis}, \textit{P. gingivalis} and \textit{T. denticola}, and certain species of the “orange complex” (Socransky, et al.\textsuperscript{62} 1998) such as \textit{C. rectus} and \textit{C. gracilis}, were reduced after SRP and showed further reductions after the surgical phase. Certain suspected periodontal pathogens, such as \textit{F. nucleatum ss polymorphum} and \textit{P. nigrescens} were less affected by SRP but showed reductions in mean counts after surgery. One of the most interesting aspects of this study was the further improvement of microbiological and clinical parameters at sites that received SRP only after periodontal surgery had been completed at the deeper periodontal pockets. The authors suggested that the reduction in pocket depth by surgical means and the associated decrease in reservoirs of periodontal pathogens may be important in achieving sustained periodontal stability. Thus, periodontal surgery appears to be an important part of the armamentarium to control periodontal infections.

**FINAL THOUGHTS**

It took more than a century of research in order to determine the microbial etiology of periodontal diseases. In regarding the early literature, investigators went through several phases alternating different thoughts about the etiology of these diseases, causing a certain delay in the diagnosis, prevention and treatment of periodontitis. Most of these controversies were due to laboratory techniques limitations, such as the impossibility of identifying some important periodontal pathogens using traditional microbiological tests as microscopic and cultural techniques. The failure to find the right causative agents of disease often led to a therapeutic frustration, changing the course of the studies in periodontology. This is probably the reason why we observe certain phases in the periodontal research history where the concept of microbial etiology of periodontal diseases was completely abandoned. New diagnostic techniques, based on immunology and molecular biology knowledge, developed by the end of the 80’s allowed a great evolution in the periodontal microbiology field and initiated “the modern search” for the etiological agents of destructive periodontal diseases. These studies indicated that certain microorganisms or groups of microorganisms relate to disease initiation and progression, while other species, considered to be beneficial, are associated with periodontal health. This knowledge was very important and marked a new phase in terms of periodontal research, where it was possible to define microbiological endpoints for therapy. Nowadays it is generally accepted that the treatment of periodontal infections is an ecological problem, where it is necessary to suppress or eliminate pathogens without suppressing host-compatible species. The changes in the subgingival microbiota after mechanical periodontal therapies such as scaling and root planing, supra gingival plaque control and periodontal surgery was reviewed in this paper. It is important to emphasize that other forms of periodontal therapies, such as the use of systemically antibiotics as adjunctive to mechanical therapies, are also being systematically studied and will be reviewed in a following paper.

**REFERENCES**

7- Bunting RW. Is pyorrhea a local or constitutional disease? Dent Cosmos 1922;64:731-7.
263

MICROBIOLOGICAL BASIS FOR PERIODONTAL THERAPY


MICROBIOLOGICAL BASIS FOR PERIODONTAL THERAPY


