

### **Occurrence of invading bacteria in radicular dentin of periodontally diseased teeth: microbiological findings.**

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Bacterial invasion in roots of periodontally diseased teeth, which has been recently documented using cultural and microscopic techniques, may be important in the pathogenesis of periodontal disease. The purpose of this investigation was to determine the occurrence and the species of invading bacteria in radicular dentin of periodontally diseased teeth. 14 healthy teeth were used as controls. Dentin samples were cultured anaerobically. The chosen methodology allowed the determination of the numbers of bacteria present in both deeper and outer part of dentinal tubules, and the bacterial concentration in dentin samples, expressed as colony forming units per mg of tissue (CFU/mg). Invading bacteria was detected in 14 (53.8%) samples from periodontally diseased teeth. The bacterial concentration ranged from 831.84 to 11971.3 CFU/mg (mean $\pm$ standard deviation: 3043.15 $\pm$ 2763.13). Micro-organisms identified included putative periodontal pathogens such as *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Bacteroides forsythus*, *Peptostreptococcus micros* and *Streptococcus intermedius*. These findings suggest that radicular dentin could act as bacterial reservoir from which periodontal pathogens can recolonize treated periodontal pockets, contributing to the failure of therapy and recurrence of disease.

*Endod Dent Traumatol* 1990 Feb;6(1):1-5

### **Similarities in the microfloras of root canals and deep periodontal pockets.**

Kerekes K, Olsen I

Dental Faculty, University of Oslo, Norway.

Although not universally accepted, retrospective histological, roentgenological and microbiological studies have indicated that cross-infection can occur between infected pulps and deep periodontal pockets. This review provides examples of similarities in the microfloras of these adjacent oral sites, supporting the idea that infection spreads from one site to the other. The organisms most often involved are probably bacteroides, fusobacteria, eubacteria, spirochetes, wolinelias, selenomonas, campylobacter, and peptostreptococci. Important qualities of cross-infecting organisms may be the ability to survive in highly reduced environments and motility. Precautions should be taken to prevent in vivo seeding of such micro-organisms, particularly in compromised teeth and hosts.

*Int Endod J* 1990 Mar;23(2):100-106

### **The microbial flora from root canals and periodontal pockets of non-vital teeth associated with advanced periodontitis.**

Kobayashi T, Hayashi A, Yoshikawa R, Okuda K, Hara K

Niigata University School of Dentistry, Japan.

Microflora from root canals and periodontal pockets of periodontally affected teeth were compared in order to elucidate the as yet unknown relationship between pulpal and periodontal disease. Caries-free teeth affected with advanced periodontitis and diagnosed as clinically dead by electric pulp testing were selected. The root canals and periodontal pockets were sampled, and the bacterial flora examined by both culture and interference microscopy. The results indicated that the aerobic/anaerobe ratio in the periodontal pocket was 0.23, while it was 0.0022 in the root canal, the large predominance of obligate anaerobes reflecting the anaerobic environment found in the root canal. Morphological classification obtained from interference microscopy showed similar proportions of morphotypes in the two sites. Results of anaerobic culture demonstrated a significantly higher rate of detection of facultative *Streptococcus* bacteria in the periodontal pocket than in the root canal. The predominant bacterial species common to both regions were *Streptococcus*, *Peptostreptococcus*, *Eubacterium*, *Bacteroides*, and *Fusobacterium* for obligate anaerobes. As for facultative anaerobes, *Actinomyces* and *Streptococcus* were detected predominantly in the periodontal pocket. The occurrence of micro-organisms common to both sites in this study suggests that the periodontal pocket may be a possible source of root canal infections.

*Chung Hua Kou Chiang Hsueh Tsa Chih* 1991 Jan;26(1):28-30

**[Anaerobes in infected canals: a preliminary study].**

[Article in Chinese]

Liang JP

College of Stomatology, Shanghai Second Medical University.

Anaerobes of 17 infected canals with periapical periodontitis were studied. Most of root flora were not only anaerobes but also aerobes. Anaerobes were predominant in chronic periapical periodontitis. Major anaerobes isolated from canals were peptostreptococcus, *B. melaninogenicus* and *B. oralis*. The chi-square results indicated that the peptostreptococcus were significantly related to apical radiolucency and *B. melaninogenicus* were significantly related to percussion or foul smell. Animal experiment results showed that rats inoculated with mixed flora developed many abscesses. But the monoinfected rats had infiltration of PMNs, dilation and hyperemia of vessels. *B. melaninogenicus*, *B. oralis* and mixed flora had liquefied gelatin and indicated these bacteria had collagenase which could dissolve collagen fibers and significantly related to destroy of collagen fibers and bone in periapical tissues.

*J Endod* 1991 Aug;17(8):380-383

**Bacteria in the apical 5 mm of infected root canals.**

Baumgartner JC, Falkler WA Jr

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Ten freshly extracted teeth which had carious pulpal exposures and periapical lesions contiguous with the root apex were placed inside an anaerobic chamber and the apical 5 mm of the root canals cultured. In addition to anaerobic incubation, duplicate cultures were incubated aerobically. Fifty strains of bacteria from the 10 root canals were isolated and identified. The most prominent bacteria cultured from the 10 root canals were *Actinomyces*, *Lactobacillus*, black-pigmented *Bacteroides*, *Peptostreptococcus*, nonpigmented *Bacteroides*, *Veillonella*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, and *Streptococcus mutans*. Of the 50 bacterial isolates, 34 (68%) were strict anaerobes. This study demonstrates the presence of predominantly anaerobic bacteria in the apical 5 mm of infected root canals in teeth with carious pulpal exposures and periapical lesions.

*Bull Tokyo Dent Coll* 1991 Aug;32(3):95-98

**Isolation and classification of anaerobic bacteria from pulp cavities of nonvital teeth in man.**

Hirai K, Tagami A, Okuda K

Tokyo Dental College.

The anaerobic microflora of infected pulp cavities and chronic periapical abscesses was studied. A total of 19 infected nonvital teeth were subjected to this study. The coronal surface was swabbed with 70% ethanol to remove debris and to disinfect. Material in root canal chamber was obtained by sterilized paper points and suspended in reduced transport fluid. The samples were dispersed, diluted, and inoculated on blood agar plates. Isolates were identified by colony characteristics and cellular morphology, fermentation, indole production, nitrate reduction, gelatin digestion, urease production, ability to grow aerobically, API 20A System, and API ZYM System. Anaerobic bacteria were found in 14 pulp cavities. Anaerobic gram-negative rods, *Actinomyces* species, and *Propionibacterium* species were predominant in the root canals. Mixed infection with anaerobes and facultative anaerobes were demonstrated in most of the pulpal cavities of nonvital teeth.

## Dentine tubule infection and endodontic therapy implications.

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A critical review of the literature suggests that the microenvironment of dentinal tubules appears to favour the selection of relatively few bacterial types irrespective of the aetiology of the infection process; coronal dental caries or pulpar necrosis. These bacteria may constitute an important reservoir from which root canal infection and reinfection may occur following pulp necrosis or during and after endodontic treatment. Previous studies of this microflora have utilized microbiological culture techniques which need to be supplemented by those that allow in situ demonstration as well as identification of the bacteria. Newer treatment strategies that are designed to eliminate this microflora must include agents that can penetrate the dentinal tubules and destroy these microorganisms, since they are located in an area beyond the host defence mechanisms where they cannot be reached by systemically administered antimicrobial agents.

*Kokubyo Gakkai Zasshi* 1993 Sep;60(3):408-415

## [Cytotoxicity of anaerobic bacteria isolated from infected root canal].

[Article in Japanese]

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Bacteria in the infected root canal play an important role in the progression of the periapical lesion. The purpose of the present study was to investigate the cytotoxic effects of bacteria isolated from an infected root canal on the periapical lesion. Four obligately anaerobic bacteria were isolated and their sonic extracts (SE) were prepared. The cytotoxic assay of each SE for MC3T3-E1 cell was used with respect to the morphological cell changes and [3H]-thymidine uptake. The following results were obtained: 1. Four obligately anaerobic bacteria were identified as follows: *Porphyromonas asaccharolytica* (P. a.), *Fusobacterium nucleatum* (F. n.), *Eubacterium lentum* (E. l.), and *Peptostreptococcus micros* (P. m.). 2. The protein concentrations which induced the morphological cell changes differed in each SE. The SE from P. m. caused cell abnormalities at 10 micrograms protein/ml, F. n. and E. l., at 30 micrograms protein/ml, and P. a., at 100 micrograms protein/ml. 3. The inhibition of the incorporation of [3H]-thymidine into DNA was concentration-dependent. The SE of P. a. showed less cytotoxicity than the others at lower concentrations; 3, 10, 30 micrograms protein/ml. The SE from F. n. had more cytotoxicity at higher concentrations; 100, 300 micrograms protein/ml. These results suggest that these bacterial sonic extracts have a potential to cause morphological cell changes and inhibit the cell proliferation in different concentrations.

*Infect Immun* 1986 Jul;53(1):149-153

## Black-pigmented *Bacteroides* spp. in human apical periodontitis.

Haapasalo M, Ranta H, Ranta K, Shah H

The incidence of black-pigmented (BP) *Bacteroides* spp. in 62 human dental root canal infections (35 acute and 27 clinically asymptomatic cases of apical periodontitis) in 57 adults was studied. Altogether 37 strains of BP *Bacteroides* were found in 31 infections, always in mixed anaerobic infections. Two different BP *Bacteroides* species were present in six infections. *B. intermedius* was most frequently isolated (15 of 62 canals; 24%) followed by *B. denticola* which was present in 12 cases. Asaccharolytic BP *Bacteroides* species, *B. gingivalis* and *B. endodontalis*, were found in eight cases. BP *Bacteroides* species were found both from symptomatic and asymptomatic infections, but there were also several symptomatic cases from which BP *Bacteroides* species were not isolated. *B. gingivalis* and *B. endodontalis* were present only in acute infections, *B. intermedius* was found both in symptomatic and asymptomatic infections, and *B. denticola* occurred mostly in asymptomatic infections. BP *Bacteroides* species were isolated initially from 9 of the 11 teeth with symptoms at 1 week, but only from 22 of the 51 teeth that were symptomless at 1 week. Two strains of *B. denticola* were resistant to penicillin G at a concentration of 2.4 micrograms/ml, but the MIC of penicillin G for all other strains was 0.6 micrograms/ml or lower. Forty-two randomly selected patients received penicillin V (oral administration, 650 mg, three times daily) during the first week of endodontic therapy. Penicillin had no effect on the occurrence of symptoms after 1 week compared with the control group (20 patients).

*Indian J Med Res* 1997 Jun;105:262-265

### **Anaerobic flora in endodontic infections.**

Chaudhry R, Kalra N, Talwar V, Thakur R

Department of Microbiology, University College of Medical Sciences, Delhi.

Microbiological and clinical data from 56 patients with endodontic infections were evaluated. Samples were collected using autoclaved paper points. Specimens were processed for isolation of aerobic and anaerobic bacteria. Antimicrobial sensitivity and resistance profiles of the recovered isolates was also performed. Forty nine positive cultures (87.5%) were obtained from the 56 consecutive necrotic root canal systems which were sampled. A total of 69 aerobic bacteria and 21 anaerobic bacteria were recovered. Aerobic bacteria were isolated from 35 patients (72%), anaerobic bacteria from 3 (6%) and mixed aerobic and anaerobic bacteria from 11 patients (22%). The most common aerobic isolate was *Klebsiella pneumoniae*. The predominant anaerobic isolate was *Bacteroides* species. One isolate was recovered from 25 patients (51%) whereas in the remaining 24 patients (49%) more than 1 isolate were recovered. These data illustrate the polymicrobial nature of endodontic infections in half the patients studied and the role of anaerobic bacteria in a quarter of them.

*Scand J Dent Res* 1988 Feb;96(1):50-55

### **Ultrastructure of a novel anaerobic gram-positive nonsporing rod from dental root canal.**

Kerosuo E, Haapasalo M, Lounatmaa K, Ranta H, Ranta K

Department of Cariology, University of Helsinki, Finland.

A novel anaerobic Gram-positive rod, strain ES4C, was isolated from a dental root canal infection. The isolate did not produce acids from carbohydrates and showed no glycosidase activity. Most biochemical reactions were identical to *Clostridium malenominatum* with the exception of the production of three aminopeptidases. In addition, no spores were detected. A tetragonally arranged surface layer was consistently found by electron microscopy. The ultrastructure of closely related *Eubacterium* spp. was also studied, but no crystalline surface structures were found. The physiologic and ultrastructural characteristics of ES4C did not allow identification as any known species. The periapical lesion responded to routine root canal therapy, but after 18 months observation the radiologic signs indicated partial healing only.

*Microbios* 1992;71(288-289):225-234

### **The endodontic microflora revisited.**

Drucker DB, Lilley JD, Tucker D, Gibbs AC

Department of Cell and Structural Biology, Turner Dental School, University of Manchester, Great Britain.

The microbial flora of 35 dental root canals were examined, taking care to maintain the viability of obligate anaerobes which accounted for 45% of total isolations, while streptococcal species accounted for 24% of the total species isolated. Individual root canals yielded a maximum of eight bacterial species. A total of 40 different species was isolated of which the most prevalent were the facultative anaerobe *Streptococcus sanguis* and the obligate anaerobe, *Peptostreptococcus micros* (both in 23% of root canals), followed by *Eubacterium aerofaciens* and the 'Streptococcus milleri group' (both 17%) then *Prevotella melaninogenica* (formerly *Bacteroides melaninogenicus*), *Enterococcus faecalis* and *Prevotella oralis* (formerly *Bacteroides oralis*), which were each isolated from 14% of root canals. Highly significant associations were discovered between four pairs of species, viz *P. melaninogenica* with *P. micros*, *P. melaninogenica* with *P. oralis*, *Prevotella corporis* with *Streptococcus morbillorum* and *Actinomyces odontolyticus* with *E. faecalis*.

*Endod Dent Traumatol* 1991 Oct;7(5):226-229

### Scanning electron microscopy of bacteria in the apical part of root canals in permanent teeth with periapical lesions.

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The most apical 2 mm of the root canals of periapically diseased roots were examined for microorganisms by scanning electron microscopy (SEM). Bacteria in this area were observed in 10 out of 12 (83.3%) cases. The two remaining cases exhibited bacteria more coronally, with tissue remnants between the bacterial front and the apical foramen. Rod-shaped bacteria dominated, but filaments, spirochetes and cocci were also seen. Cocci and rods sometimes formed micro-colonies. Occasionally, cocci were seen attached to filaments forming "corn-cob"-like structures. Deposits resembling bacterial plaque were also found inside the root canal. SEM is useful for studying microbial topography of the apical root canal.

*J Endod* 1990 Nov;16(11):534-538

### Localization and identification of root canal bacteria in clinically asymptomatic periapical pathosis.

Fukushima H, Yamamoto K, Hirohata K, Sagawa H, Leung KP, Walker CB

College of Dentistry, University of Florida, Gainesville.

Twenty-one teeth with clinically asymptomatic periapical pathosis (class 3) were extracted and the isolation, identification, and localization of bacteria in the root apex were examined. Mixtures involving several bacteria were isolated from more than 60% of the cases. Scanning electron microscopy revealed bacterial masses to be associated with the apical part of the root canal, but not with the area of apical foramen or on the surface of root apex. Our results indicate that the bacteria in class 3 cases may be derived from organisms which colonized before or during endodontic treatment, but not from anachoresis. The bacteria-positive cases of asymptomatic periapical pathosis have the potential to progress to symptomatic periapical pathosis.

*Endod Dent Traumatol* 1992 Dec;8(6):248-254

### Profiling of *Propionibacterium acnes* recovered from root canal and blood during and after endodontic treatment.

Debelian GJ, Olsen I, Tronstad L

Division of Endodontics, University of Oslo, Norway.

This report describes the first results of an ongoing study of bacteremia after endodontic treatment of teeth with Asymptomatic apical periodontitis. After access cavity preparation, microbiological samples were taken from the root canal under aseptic conditions in 4 single-rooted teeth in 4 patients. In treatment of 2 of the patients, the first 3 reamers (sizes 15-25) were deliberately used to a level 2 mm beyond the apical foramen. In 2 patients the instrumentation ended inside the root canal 1 mm short of the apical foramen. Blood samples were taken from the patients during the endodontic instrumentation and 10 min after the treatment was completed. Using lysis-filtration under anaerobic conditions, the blood was passed through a cellulose membrane filter. The filters as well as the root canal samples were incubated using an anaerobic technique. Anaerobic bacteria were isolated from all root canals. In the 2 patients where overinstrumentation had occurred, *Propionibacterium acnes* was recovered both from the root canals and from the blood samples taken during and after the treatment had been completed. Biochemical profiles, antibiotic susceptibility tests and electrophoresis of soluble proteins revealed that *Propionibacterium acnes* isolated from the root canal and blood samples were identical within patients, but varied between patients. Facultative anaerobic bacteria including *Streptococcus sanguis* were recovered from only one root canal sample and not from the blood samples.

*Endod Dent Traumatol* 1993 Jun;9(3):87-94**Spirochaetes in oral infections.**

Dahle UR, Tronstad L, Olsen I

Division of Endodontics, University of Oslo, Norway.

Oral spirochaetes, which are small-, medium- or large-sized, include species of the genus *Treponema*, many of which have not yet been cultured. They are found in root canal infections, pericoronitis, gingivitis and periodontitis, constituting up to 10% of the flora in endodontic abscesses, 30% in acute necrotizing ulcerative gingivitis, and 56% in advanced marginal periodontitis. The strong proteolytic activity of these organisms probably make them causes of infection rather than consequences. Being able to penetrate tissue, they bring their enzymes, metabolic products, and endotoxins, in direct contact with target cells. This may perturb essential functions of host cells and immunoglobulins. Enzyme activities may also help fulfill the complex growth requirements of spirochaetes in vivo. Reaction between infected periodontal tissue and monoclonal antibodies to *Treponema pallidum* has suggested that uncharacterized pathogen-related oral spirochaetes have surface structures and functions analogue to this well recognized pathogen. This warrants a more intensified search for the role of spirochaetes in oral infections.

*Oral Microbiol Immunol* 1993 Aug;8(4):251-253**Observation of an unusually large spirochete in endodontic infection.**

Dahle UR, Tronstad L, Olsen I

Division of Endodontics and Department of Microbiology, Dental Faculty, University of Oslo, Norway.

Spirochetes in the root canal have been investigated only to a minor extent. Oral spirochetes have been detected particularly in the periodontal pocket, where small, medium-sized and large morphotypes have been reported. In the present darkfield and scanning electron microscopic study we describe a spirochete isolated from the root canal that is 7 times longer (140 microns long) and 5 times thicker (2 microns thick) than usual for *treponemes*.

*Endod Dent Traumatol* 1989 Feb;5(1):1-10**Bacteroides spp. in dental root canal infections.**

Haapasalo M

A summary of a series of bacteriological studies of endodontic infections is presented in this article. The bacteriology of 62 root canal infections was studied with special attention focused on the occurrence, role and taxonomy of *Bacteroides* spp. All infections except one were mixed infections dominated usually by anaerobic bacteria. Four to 6 different species were present in most canals. Species of the genus *Bacteroides* were found more frequently than species of any other genus. Seventy-eight *Bacteroides* strains were isolated from 45 canals. *B. buccae*, *B. intermedius*, *B. denticola*, *B. oris*, *B. oralis*, and *B. gingivalis* were the most common *Bacteroides* spp. At the beginning of the treatment 35 of 62 teeth caused acute symptoms. The results indicated that symptoms were a result of the synergistic action of the mixed anaerobic flora. The presence of *B. gingivalis*, *B. endodontalis*, and *B. buccae* was more often related to acute cases than other *Bacteroides* spp. Black-pigmented *Bacteroides* and a new *Bacteroides*-like organism, *Mitsuokella dentalis*, seemed to increase the probability that acute symptoms would persist one week after the beginning of the treatment. However, the treatment result assessed after 4 weeks and after 1 year was not affected by the composition of the mixed anaerobic flora. Calcium hydroxide was the only canal disinfectant used. Its efficacy was proved by a bacteriological sample at the second appointment in 10 cases. All teeth were asymptomatic at the third appointment. The susceptibility of the isolated *Bacteroides* strains to penicillin G was also studied. Only 2 *B. buccae* strains and 2 *B. denticola* strains were resistant at a concentration of 2.4 micrograms/ml. The patients were randomly divided into 3 groups which received 1) no antibiotics, 2) penicillin V (650 mg x 3) for 7 days, or 3) for 12 weeks. There was no difference between the 3 groups in the healing of the periapical lesion after one year. All patients attended the 1-year control. Fifty cases showed complete healing, partial healing was obtained in 11 cases and in 1 case no healing was observed.

*Atherosclerosis* 1993 Nov;103(2):205-211

### **Dental infections and coronary atherosclerosis.**

**Mattila KJ, Valle MS, Nieminen MS, Vaitonen VV, Hietaniemi KL**

First Department of Medicine, Helsinki University Central Hospital, Finland.

An association between dental and other bacterial infections and coronary heart disease has recently been observed in both cross-sectional and longitudinal studies. To elucidate this topic, the severity of dental infections and coronary atheromatosis was assessed, together with measurements of the conventional coronary risk factors, in 100 individuals (88 men, 12 women, mean age 48, range 28-68 years) referred for diagnostic coronary angiography. Pantomography X-rays and coronary angiograms of the participants were scored blindly by single observers, a dentist and a radiologist respectively. The median pantomography score was 3.0 in male individuals belonging to the highest tertile of coronary atheromatosis score, as compared with 0.0 among the rest of the male participants ( $P = 0.003$ ). The association between dental infections and severe coronary atheromatosis in males remained significant after adjusting for the effect of age, blood lipids, body mass index, hypertension, smoking and social class. No association between dental infections and coronary atheromatosis was observed in the small number of females studied. This observation supports the proposal that bacterial infections play a role in the pathogenesis of coronary atherosclerosis.

*J Periodontol* 1996 Oct;67(10 Suppl):1123-1137

### **Periodontal disease and cardiovascular disease.**

**Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S**

Department of Dental Ecology, University of North Carolina, Chapel Hill, USA.

It is our central hypothesis that periodontal diseases, which are chronic Gram-negative infections, represent a previously unrecognized risk factor for atherosclerosis and thromboembolic events. Previous studies have demonstrated an association between periodontal disease severity and risk of coronary heart disease and stroke. We hypothesize that this association may be due to an underlying inflammatory response trait, which places an individual at high risk for developing both periodontal disease and atherosclerosis. We further suggest that periodontal disease, once established, provides a biological burden of endotoxin (lipopolysaccharide) and inflammatory cytokines (especially  $TxA_2$ , IL-1 beta, PGE<sub>2</sub>, and TNF-alpha) which serve to initiate and exacerbate atherogenesis and thromboembolic events. A cohort study was conducted using combined data from the Normative Aging Study and the Dental Longitudinal Study sponsored by the United States Department of Veterans Affairs. Mean bone loss scores and worst probing pocket depth scores per tooth were measured on 1,147 men during 1968 to 1971. Information gathered during follow-up examinations showed that 207 men developed coronary heart disease (CHD), 59 died of CHD, and 40 had strokes. Incidence odds ratios adjusted for established cardiovascular risk factors were 1.5, 1.9, and 2.8 for bone loss and total CHD, fatal CHD, and stroke, respectively. Levels of bone loss and cumulative incidence of total CHD and fatal CHD indicated a biologic gradient between severity of exposure and occurrence of disease.

*Compendium* 1994 Aug;15(8):976

### **Periodontal disease as a risk factor for heart disease.**

**Loesche WJ**

School of Medicine, University of Michigan, Ann Arbor.

Many individuals with cardiovascular disease appear from epidemiologic studies to have either periodontal disease or to be edentulous. A Finnish group has provided evidence that after conventional risk factors for stroke and heart attacks have been accounted for, there still remains a significant relationship between dental disease and cardiovascular disease. A preliminary analysis of our own investigation of the interrelationship of medical and dental health shows that individuals with a high dental morbidity (ie, edentulous or with many missing teeth) have a high prevalence of coronary heart disease and stroke. A model based on how smoking can predispose to periodontal disease is used to explain how periodontal disease could be a potential risk factor for heart disease.

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Dahle UR, Tronstad L, Olsen I

Division of Endodontics, University of Oslo, Norway.

Oral spirochaetes, which are small-, medium- or large-sized, include species of the genus *Treponema*, many of which have not yet been cultured. They are found in root canal infections, pericoronitis, gingivitis and periodontitis, constituting up to 10% of the flora in endodontic abscesses, 30% in acute necrotizing ulcerative gingivitis, and 56% in advanced marginal periodontitis. The strong proteolytic activity of these organisms probably make them causes of infection rather than consequences. Being able to penetrate tissue, they bring their enzymes, metabolic products, and endotoxins, in direct contact with target cells. This may perturb essential functions of host cells and immunoglobulins. Enzyme activities may also help fulfil the complex growth requirements of spirochaetes in vivo. Reaction between infected periodontal tissue and monoclonal antibodies to *Treponema pallidum* has suggested that uncharacterized pathogen-related oral spirochaetes have surface structures and functions analogue to this well recognized pathogen. This warrants a more intensified search for the role of spirochaetes in oral infections.

*Oral Microbiol Immunol* 1993 Aug;8(4):251-253**Observation of an unusually large spirochete in endodontic infection.**

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Spirochetes in the root canal have been investigated only to a minor extent. Oral spirochetes have been detected particularly in the periodontal pocket, where small, medium-sized and large morphotypes have been reported. In the present darkfield and scanning electron microscopic study we describe a spirochete isolated from the root canal that is 7 times longer (140 microns long) and 5 times thicker (2 microns thick) than usual for treponemes.

*Endod Dent Traumatol* 1989 Feb;5(1):1-10**Bacteroides spp. in dental root canal infections.**

Haapasalo M

A summary of a series of bacteriological studies of endodontic infections is presented in this article. The bacteriology of 62 root canal infections was studied with special attention focused on the occurrence, role and taxonomy of *Bacteroides* spp. All infections except one were mixed infections dominated usually by anaerobic bacteria. Four to 6 different species were present in most canals. Species of the genus *Bacteroides* were found more frequently than species of any other genus. Seventy-eight *Bacteroides* strains were isolated from 45 canals. *B. buccae*, *B. intermedius*, *B. denticola*, *B. oris*, *B. oralis*, and *B. gingivalis* were the most common *Bacteroides* spp. At the beginning of the treatment 35 of 62 teeth caused acute symptoms. The results indicated that symptoms were a result of the synergistic action of the mixed anaerobic flora. The presence of *B. gingivalis*, *B. endodontalis*, and *B. buccae* was more often related to acute cases than other *Bacteroides* spp. Black-pigmented *Bacteroides* and a new *Bacteroides*-like organism, *Mitsuokella dentalis*, seemed to increase the probability that acute symptoms would persist one week after the beginning of the treatment. However, the treatment result assessed after 4 weeks and after 1 year was not affected by the composition of the mixed anaerobic flora. Calcium hydroxide was the only canal disinfectant used. Its efficacy was proved by a bacteriological sample at the second appointment in 10 cases. All teeth were asymptomatic at the third appointment. The susceptibility of the isolated *Bacteroides* strains to penicillin G was also studied. Only 2 *B. buccae* strains and 2 *B. denticola* strains were resistant at a concentration of 2.4 micrograms/ml. The patients were randomly divided into 3 groups which received 1) no antibiotics, 2) penicillin V (650 mg x 3) for 7 days, or 3) for 12 weeks. There was no difference between the 3 groups in the healing of the periapical lesion after one year. All patients attended the 1-year control. Fifty cases showed complete healing, partial healing was obtained in 11 cases and in 1 case no healing was observed.

*Endod Dent Traumatol* 1991 Oct;7(5):226-229

### Scanning electron microscopy of bacteria in the apical part of root canals in permanent teeth with periapical lesions.

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The most apical 2 mm of the root canals of periapically diseased roots were examined for microorganisms by scanning electron microscopy (SEM). Bacteria in this area were observed in 10 out of 12 (83.3%) cases. The two remaining cases exhibited bacteria more coronally, with tissue remnants between the bacterial front and the apical foramen. Rod-shaped bacteria dominated, but filaments, spirochetes and cocci were also seen. Cocci and rods sometimes formed micro-colonies. Occasionally, cocci were seen attached to filaments forming "corn-cob"-like structures. Deposits resembling bacterial plaque were also found inside the root canal. SEM is useful for studying microbial topography of the apical root canal.

*J Endod* 1990 Nov;16(11):534-538

### Localization and identification of root canal bacteria in clinically asymptomatic periapical pathosis.

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Twenty-one teeth with clinically asymptomatic periapical pathosis (class 3) were extracted and the isolation, identification, and localization of bacteria in the root apex were examined. Mixtures involving several bacteria were isolated from more than 60% of the cases. Scanning electron microscopy revealed bacterial masses to be associated with the apical part of the root canal, but not with the area of apical foramen or on the surface of root apex. Our results indicate that the bacteria in class 3 cases may be derived from organisms which colonized before or during endodontic treatment, but not from anachoresis. The bacteria-positive cases of asymptomatic periapical pathosis have the potential to progress to symptomatic periapical pathosis.

*Endod Dent Traumatol* 1992 Dec;8(6):248-254

### Profiling of *Propionibacterium acnes* recovered from root canal and blood during and after endodontic treatment.

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This report describes the first results of an ongoing study of bacteremia after endodontic treatment of teeth with Asymptomatic apical periodontitis. After access cavity preparation, microbiological samples were taken from the root canal under aseptic conditions in 4 single-rooted teeth in 4 patients. In treatment of 2 of the patients, the first 3 reamers (sizes 15-25) were deliberately used to a level 2 mm beyond the apical foramen. In 2 patients the instrumentation ended inside the root canal 1 mm short of the apical foramen. Blood samples were taken from the patients during the endodontic instrumentation and 10 min after the treatment was completed. Using lysis-filtration under anaerobic conditions, the blood was passed through a cellulose membrane filter. The filters as well as the root canal samples were incubated using an anaerobic technique. Anaerobic bacteria were isolated from all root canals. In the 2 patients where overinstrumentation had occurred, *Propionibacterium acnes* was recovered both from the root canals and from the blood samples taken during and after the treatment had been completed. Biochemical profiles, antibiotic susceptibility tests and electrophoresis of soluble proteins revealed that *Propionibacterium acnes* isolated from the root canal and blood samples were identical within patients, but varied between patients. Facultative anaerobic bacteria including *Streptococcus sanguis* were recovered from only one root canal sample and not from the blood samples.

*Chung Hua Kou Chiang Hsueh Tsa Chih* 1991 Jan;26(1):28-30

### [Anaerobes in infected canals: a preliminary study].

[Article in Chinese]

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Anaerobes of 17 infected canals with periapical periodontitis were studied. Most of root flora were not only anaerobes but also aerobes. Anaerobes were predominant in chronic periapical periodontitis. Major anaerobes isolated from canals were peptostreptococcus, *B. melaninogenicus* and *B. oralis*. The chi-square results indicated that the peptostreptococcus were significantly related to apical radiolucency and *B. melaninogenicus* were significantly related to percussion or foul smell. Animal experiment results showed that rats inoculated with mixed flora developed many abscesses. But the monoinfected rats had infiltration of PMNs, dilation and hyperemia of vessels. *B. melaninogenicus*, *B. oralis* and mixed flora had liquefied gelatin and indicated these bacteria had collagenase which could dissolve collagen fibers and significantly related to destroy of collagen fibers and bone in periapical tissues.

*J Endod* 1991 Aug;17(8):380-383

### Bacteria in the apical 5 mm of infected root canals.

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Ten freshly extracted teeth which had carious pulpal exposures and periapical lesions contiguous with the root apex were placed inside an anaerobic chamber and the apical 5 mm of the root canals cultured. In addition to anaerobic incubation, duplicate cultures were incubated aerobically. Fifty strains of bacteria from the 10 root canals were isolated and identified. The most prominent bacteria cultured from the 10 root canals were *Actinomyces*, *Lactobacillus*, black-pigmented *Bacteroides*, *Peptostreptococcus*, nonpigmented *Bacteroides*, *Veillonella*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, and *Streptococcus mutans*. Of the 50 bacterial isolates, 34 (68%) were strict anaerobes. This study demonstrates the presence of predominantly anaerobic bacteria in the apical 5 mm of infected root canals in teeth with carious pulpal exposures and periapical lesions.

*Bull Tokyo Dent Coll* 1991 Aug;32(3):95-98

### Isolation and classification of anaerobic bacteria from pulp cavities of nonvital teeth in man.

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The anaerobic microflora of infected pulp cavities and chronic periapical abscesses was studied. A total of 19 infected nonvital teeth were subjected to this study. The coronal surface was swabbed with 70% ethanol to remove debris and to disinfect. Material in root canal chamber was obtained by sterilized paper points and suspended in reduced transport fluid. The samples were dispersed, diluted, and inoculated on blood agar plates. Isolates were identified by colony characteristics and cellular morphology, fermentation, indole production, nitrate reduction, gelatin digestion, urease production, ability to grow aerobically, API 20A System, and API ZYM System. Anaerobic bacteria were found in 14 pulp cavities. Anaerobic gram-negative rods, *Actinomyces* species, and *Propionibacterium* species were predominant in the root canals. Mixed infection with anaerobes and facultative anaerobes were demonstrated in most of the pulpal cavities of nonvital teeth.

## Dentine tubule infection and endodontic therapy implications.

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A critical review of the literature suggests that the microenvironment of dentinal tubules appears to favour the selection of relatively few bacterial types irrespective of the aetiology of the infection process; coronal dental caries or pulpar necrosis. These bacteria may constitute an important reservoir from which root canal infection and reinfection may occur following pulp necrosis or during and after endodontic treatment. Previous studies of this microflora have utilized microbiological culture techniques which need to be supplemented by those that allow in situ demonstration as well as identification of the bacteria. Newer treatment strategies that are designed to eliminate this microflora must include agents that can penetrate the dentinal tubules and destroy these microorganisms, since they are located in an area beyond the host defence mechanisms where they cannot be reached by systemically administered antimicrobial agents.

*Kokubyo Gakkai Zasshi* 1993 Sep;60(3):408-415

## [Cytotoxicity of anaerobic bacteria isolated from infected root canal].

[Article in Japanese]

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Bacteria in the infected root canal play an important role in the progression of the periapical lesion. The purpose of the present study was to investigate the cytotoxic effects of bacteria isolated from an infected root canal on the periapical lesion. Four obligately anaerobic bacteria were isolated and their sonic extracts (SE) were prepared. The cytotoxic assay of each SE for MC3T3-E1 cell was used with respect to the morphological cell changes and [3H]-thymidine uptake. The following results were obtained: 1. Four obligately anaerobic bacteria were identified as follows: *Porphyromonas asaccharolytica* (P. a.), *Fusobacterium nucleatum* (F. n.), *Eubacterium lentum* (E. l.), and *Peptostreptococcus micros* (P. m.). 2. The protein concentrations which induced the morphological cell changes differed in each SE. The SE from P. m. caused cell abnormalities at 10 micrograms protein/ml, F. n. and E. l., at 30 micrograms protein/ml, and P. a., at 100 micrograms protein/ml. 3. The inhibition of the incorporation of [3H]-thymidine into DNA was concentration-dependent. The SE of P. a. showed less cytotoxicity than the others at lower concentrations; 3, 10, 30 micrograms protein/ml. The SE from F. n. had more cytotoxicity at higher concentrations; 100, 300 micrograms protein/ml. These results suggest that these bacterial sonic extracts have a potential to cause morphological cell changes and inhibit the cell proliferation in different concentrations.

*Infect Immun* 1986 Jul;53(1):149-153

## Black-pigmented *Bacteroides* spp. in human apical periodontitis.

Haapasalo M, Ranta H, Ranta K, Shah H

The incidence of black-pigmented (BP) *Bacteroides* spp. in 62 human dental root canal infections (35 acute and 27 clinically asymptomatic cases of apical periodontitis) in 57 adults was studied. Altogether 37 strains of BP *Bacteroides* were found in 31 infections, always in mixed anaerobic infections. Two different BP *Bacteroides* species were present in six infections. *B. intermedius* was most frequently isolated (15 of 62 canals; 24%) followed by *B. denticola* which was present in 12 cases. Asaccharolytic BP *Bacteroides* species, *B. gingivalis* and *B. endodontalis*, were found in eight cases. BP *Bacteroides* species were found both from symptomatic and asymptomatic infections, but there were also several symptomatic cases from which BP *Bacteroides* species were not isolated. *B. gingivalis* and *B. endodontalis* were present only in acute infections, *B. intermedius* was found both in symptomatic and asymptomatic infections, and *B. denticola* occurred mostly in asymptomatic infections. BP *Bacteroides* species were isolated initially from 9 of the 11 teeth with symptoms at 1 week, but only from 22 of the 51 teeth that were symptomless at 1 week. Two strains of *B. denticola* were resistant to penicillin G at a concentration of 2.4 micrograms/ml, but the MIC of penicillin G for all other strains was 0.6 micrograms/ml or lower. Forty-two randomly selected patients received penicillin V (oral administration, 650 mg, three times daily) during the first week of endodontic therapy. Penicillin had no effect on the occurrence of symptoms after 1 week compared with the control group (20 patients).

### Occurrence of invading bacteria in radicular dentin of periodontally diseased teeth: microbiological findings.

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Bacterial invasion in roots of periodontally diseased teeth, which has been recently documented using cultural and microscopic techniques, may be important in the pathogenesis of periodontal disease. The purpose of this investigation was to determine the occurrence and the species of invading bacteria in radicular dentin of periodontally diseased teeth. 14 healthy teeth were used as controls. Dentin samples were cultured anaerobically. The chosen methodology allowed the determination of the numbers of bacteria present in both deeper and outer part of dentinal tubules, and the bacterial concentration in dentin samples, expressed as colony forming units per mg of tissue (CFU/mg). Invading bacteria was detected in 14 (53.8%) samples from periodontally diseased teeth. The bacterial concentration ranged from 831.84 to 11971.3 CFU/mg (mean $\pm$  standard deviation: 3043.15 $\pm$ 2763.13). Micro-organisms identified included putative periodontal pathogens such as *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Bacteroides forsythus*, *Peptostreptococcus micros* and *Streptococcus intermedius*. These findings suggest that radicular dentin could act as bacterial reservoir from which periodontal pathogens can recolonize treated periodontal pockets, contributing to the failure of therapy and recurrence of disease.

Endod Dent Traumatol 1990 Feb;6(1):1-5

### Similarities in the microfloras of root canals and deep periodontal pockets.

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Although not universally accepted, retrospective histological, roentgenological and microbiological studies have indicated that cross-infection can occur between infected pulps and deep periodontal pockets. This review provides examples of similarities in the microfloras of these adjacent oral sites, supporting the idea that infection spreads from one site to the other. The organisms most often involved are probably bacteroides, fusobacteria, eubacteria, spirochetes, wolinelias, selenomonas, campylobacter, and peptostreptococci. Important qualities of cross-infecting organisms may be the ability to survive in highly reduced environments and motility. Precautions should be taken to prevent in vivo seeding of such micro-organisms, particularly in compromised teeth and hosts.

Int Endod J 1990 Mar;23(2):100-106

### The microbial flora from root canals and periodontal pockets of non-vital teeth associated with advanced periodontitis.

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Microflora from root canals and periodontal pockets of periodontally affected teeth were compared in order to elucidate the as yet unknown relationship between pulpal and periodontal disease. Caries-free teeth affected with advanced periodontitis and diagnosed as clinically dead by electric pulp testing were selected. The root canals and periodontal pockets were sampled, and the bacterial flora examined by both culture and interference microscopy. The results indicated that the aerobic/anaerobe ratio in the periodontal pocket was 0.23, while it was 0.0022 in the root canal, the large predominance of obligate anaerobes reflecting the anaerobic environment found in the root canal. Morphological classification obtained from interference microscopy showed similar proportions of morphotypes in the two sites. Results of anaerobic culture demonstrated a significantly higher rate of detection of facultative *Streptococcus* bacteria in the periodontal pocket than in the root canal. The predominant bacterial species common to both regions were *Streptococcus*, *Peptostreptococcus*, *Eubacterium*, *Bacteroides*, and *Fusobacterium* for obligate anaerobes. As for facultative anaerobes, *Actinomyces* and *Streptococcus* were detected predominantly in the periodontal pocket. The occurrence of micro-organisms common to both sites in this study suggests that the periodontal pocket may be a possible source of root canal infections.

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