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MICROBIAL COLONIZATION IN HUMAN PERIODONTAL DISEASE: AN ILLUSTRATED TUTORIAL ON SELECTED ULTRASTRUCTURAL AND ECOLOGIC CONSIDERATIONS

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Abstract

The oral cavity is populated by a prodigious microbial flora that exhibits a unique successional colonization of enamel and subgingival root surfaces. A wide range of oral sites provide different ecologic conditions and are, therefore, populated by different commensal microbial combinations. The sequence of microbial colonization, regardless of location within the oral cavity, commences with the acquisition of salivary and/or crevicular fluid-derived pellicle.

As the process of successional colonization of the gingival crevice area proceeds uninterrupted, achieving critical mass between 10 and 21 days, gingivitis becomes evident at a clinical level. However, at a histologic level, gingivitis may be evident within 2-3 days of plaque accumulation. The inflammatory response sufficiently alters the ecological conditions so as to allow proliferation of supragingival plaque into subgingival areas. The subgingival plaque becomes progressively more Gram-negative and anaerobic in nature as the periodontal pocket deepens, leading ultimately to a chronic, progressive deterioration of the periodontium--adult periodontitis. Both gingivitis and adult periodontitis are characterized by the successive colonization of cocci, short and long rods, filamentous microbes with "corn cob" and "bristle brush" formations, flagellated microbes, and spirochetes.

Localized juvenile periodontitis (LJP), in contrast to the adult form of periodontitis, features a comparatively sparse microbial flora. The subgingival microbial colonization characteristically features cocci, short rods, coccobacilli, and spirochetes.

Key Words: Adult periodontitis, bacterial colonization, gingivitis, localized juvenile periodontitis, microbial ecology, oral microbiology, ultrastructure of microbial plaque, plaque, subgingival plaque, supragingival plaque

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Introduction

Collectively, the periodontal diseases are characterized by inflammatory lesions affecting the supporting tissues of teeth. Based on clinical and radiographic criteria and the history of disease progression, the inflammatory periodontal diseases may be grouped into one of two general categories, i.e., gingivitis or periodontitis. Gingivitis is often subdivided into clinical entities based on the etiology, i.e., marginal gingivitis associated with dental plaque, herpetic gingivostomatitis, HIV+ related gingivitis, and gingivitis conditioned by systemic influence. Likewise, periodontitis is now subdivided based on clinical symptoms and host response, i.e., prepubertal, localized juvenile, adult, rapidly progressing, and HIV+ related periodontitis (Genco, 1990).

The common periodontal diseases (gingivitis and adult periodontitis) are ubiquitous and represent a major worldwide health problem. For instance, reports on gingival health in children and adolescents routinely note gingivitis in at least 90% of the population examined whereas 50% of the adult population exhibit gingivitis (Ramfjord, 1961; McHugh et al., 1964; Bjorby and Loe, 1969; Kirkegaard et al., 1987). Adult periodontitis, a slowly advancing inflammatory lesion featuring resorption of the alveolar bone and formation of periodontal pockets, has been noted to increase in severity and prevalence with increasing age (Miller et al., 1987). Thus, depending on the age group examined, the prevalence of adult periodontitis has been reported to range from 8-34% (Miller et al., 1987; Brown et al., 1989). Although the prevalence and severity of adult periodontitis appears directly related to increasing age, it is unlikely that age per se is a significant factor (Abdellatif and Burt, 1987). Both gingivitis and adult periodontitis are consistently associated with inadequate oral hygiene, regardless of age (Suomi et al., 1971). Furthermore, after reviewing epidemiologic studies conducted in the 1950's and 1960's, Russell (1967) concluded that the relationship between dental plaque and disease was so strong as to exclude all factors other than age and oral hygiene.

Based on the results of epidemiological studies, at least two previously held concepts concerning periodontal diseases have been revised.

First, the common periodontal diseases were long assumed to be the major cause of tooth loss in adults. However, recent data from several studies have shown that, in fact, periodontal disease accounts for relatively few extractions at all ages (Ainamo et al., 1984; Kay and Blinkhorn, 1986; Bailit et al., 1987). Second, it has long been assumed that practically all individuals were susceptible to a generalized adult periodontitis. Evidence now suggests that when good oral hygiene is practiced on a regular basis, adult periodontitis has a relatively low rate of incidence, although gingivitis is common (Pilot and Schaub, 1985; Halling and Bjorn, 1986; Hoover and Tynan, 1986). The positive correlation between dental plaque and disease establishes an association, but not necessarily a cause-and-effect relationship.

A direct cause-and-effect relationship between dental microbial plaque and gingivitis was demonstrated by Loe et al. (1965) and Theilade et al. (1966). Both studies showed that a 10-21 day accumulation of microbial plaque on clinically healthy teeth resulted in the initiation of gingivitis. Further, when oral hygiene was reinstituted, thereby removing the accumulated plaque, the gingivitis resolved. Together, these studies provide convincing evidence that local ecologic alterations occur as a result of inadequate oral hygiene, leading to successional colonization and growth of commensal oral microbes, and result in gingival inflammation. Present day periodontal therapy is based on the doctrine that supragingival plaque accumulation causes gingivitis; that with time, an ecological shift occurs within the plaque mass from a predominately Gram-positive, aerobic flora to a Gram-negative, anaerobic flora; and that more advanced periodontal breakdown is the result of growth and apical extension of the plaque (Slots, 1977; Newman, 1985; Page, 1986).

Thus, the purpose of this paper will be to selectively review the dental literature and present scanning electron microscopic (SEM) evidence to illustrate the following features inherent to the relationship of microbial plaque and periodontal disease: (1) the role of adherence in colonization of oral microbes on tooth and epithelial surfaces; (2) the process of successional colonization; and (3) the differences between supra- and subgingival dental plaques associated with gingivitis, adult periodontitis, and localized juvenile periodontitis.

Materials and Methods

As the title indicates an illustrated tutorial, the authors have used SEM to reinforce the more salient features. Thus, a brief presentation of the materials and methods is offered for the reader's consideration. It should be noted that none of the SEM photographs represent new findings, as similar observations have been made by numerous investigators. They are simply offered for the purpose of illustration.

Figures 1-12 represent specimens of extracted teeth procured in the following manner. All teeth were obtained from five patients between the ages of 15-21 years that were scheduled for extractions for orthodontic reasons. Those teeth selected for study had to exhibit a gingival index and plaque index of 0 (Loe, 1967). Prior to removal, the designated teeth were cleaned of plaque and acquired enamel pellicle via a rubber cup and pumice prophylaxis and the patient requested to cease all oral hygiene for periods ranging from 0-10 days. Teeth were removed at 2 hours post-prophylaxis and at 1, 3, 5, 7, and 10 days.

Figures 13-21 were obtained from specimens of teeth removed for reasons of advanced stage adult periodontitis. These teeth were selected on the basis of a plaque and gingival index of >2.0, pocket depth of >6 mm, and a positive dental history with radiographic support for the diagnosis of chronic adult periodontitis.

Figures 22-27 were obtained from extracted teeth from patients diagnosed as having localized juvenile periodontitis. These patients ranged in age from 14-20 years and exhibited the following clinical and radiographic symptoms: severe vertical bone loss involving first molars and/or incisors; bilateral symmetry to the bone loss pattern; radiographic or historical evidence of rapid disease progression; pocket depths >8 mm; and hard tissue destruction not commensurate with the amount of local irritants (Baer, 1971).

All teeth, regardless of disease category, at time of extraction were gently rinsed with sodium heparin, 1000 USP units/ml, to remove adherent blood. Specimens were then fixed in cold 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer at pH 7.4 for 4 hours. Following initial fixation, specimens were rinsed in buffer, post-fixed with 1% osmium tetroxide for 4 hours, and then dehydrated in a series of graded ethanol solutions (20-100%) at 15-minute intervals, followed by immersion in hexamethyldisilazane for 45 minutes. Specimens were then stored in a desiccator overnight, attached to aluminum mounts, and sputter-coated with 200 Angstroms of gold/palladium. All specimens were examined using a Phillips 515 SEM at 15 kV and photographs taken at positive angles of 15, 45, and 60 degrees.

Microbial Adhesion

The adhesive nature and structural integrity of microbial plaque have been recognized since the late nineteenth century (Miller, 1890). However, the impact of the adhesion process on dental plaque development and microbial ecology was not fully appreciated until the 1970's. Studies by Gibbons and van Houte (1973 & 1975) were among the first to demonstrate that oral microbes exhibit considerable disparity in their ability to attach to different types of surfaces. The specific nature of microbial adhesion to different tissues, its ecological significance, and its role in bacterial virulence have become areas of intense research interest.

Fundamental to the process of microbial adhesion is the formation of dental pellicle that has been defined as a thin biofilm or cuticle (Scannapieco and Levine, 1990). With respect to microbial adhesion, the salient features of dental pellicle formation include: (1) The ability of pellicle to form on any exposed oral surface such as enamel, root surfaces, epithelia, and biomaterials; (2) various pellicle components are derived from saliva, gingival crevicular fluid, and microbial and cellular products; and (3) selective

deposition of these components onto various oral surfaces results in pellicles of differing composition (Levine et al., 1985).

Numerous studies have demonstrated pellicle formation on cleaned enamel surfaces within 2-3 hours (Hay, 1967; Mayhall, 1970; Sonju and Rolla, 1973). Pellicle adsorbed onto enamel features a diverse composition of salivary glycoproteins including those containing sialic acid, sulfate or phosphate, blood group-reactive substance, immunoglobulins (IgA, IgG, IgM), and various enzymes (Mayhall, 1970; Orstavik and Kraus, 1973 & 1974; Kraus et al., 1973; Sonju and Rolla, 1973; Rolla et al., 1975; Pruitt and Adamson, 1977). Analysis of pellicles from different oral tissues indicates a selective adsorption of the various components. Chromatographic analysis of pellicle amino acid composition indicate glutamic acid, glycine, and serine to be most commonly adsorbed onto enamel surfaces (Oste et al., 1981).

Pellicle formation involves a complex interaction of physical forces, i.e., ionic, hydrophobic, hydrogen bonding, and van der Waals, between oral tissue surfaces and the organic and inorganic components in the surrounding fluids. In the case of enamel and exposed root surfaces, it has been suggested that surface phosphate groups interact with salivary calcium ions to form "bridges" with negatively charged groups (carboxyl, sulfate, and sialic acid) on salivary and crevicular fluid components (Rolla, 1983).

Prior to initial colonization by pioneering microbes, the pellicle, when examined by transmission electron microscopy (TEM) appears to be a thin, amorphous or finely granular, electron-dense layer immediately adjacent to the tissue surface (Meckel, 1965; Leach and Saxton, 1966; Nyvad and Fejerskov, 1987a). The thickness of pellicle can vary from site to site but has been reported to range from 1-4 micrometers (Nyvad and Fejerskov, 1987a). SEM examinations of enamel pellicle reveal a fine granular and/or fibrillar texture and a rough, irregular topography (Fig. 1).

Maturation of the pellicle occurs with time and is characterized by modification of existing and acquisition of additional components from the saliva, gingival crevicular fluid, and oral microbes. Modification of early pellicle may be the result of degradation by enzymes from microbes, desquamated epithelium, and salivary granulocytes (Costello et al., 1979; Rolla, 1983; Kilian et al., 1983). The maturation of pellicle ultimately dictates the composition of the initial microbial colonization by exposure of binding sites that are reactive with specific microbes (Gibbons and van Houte, 1971 & 1973); or conversely, components of mature pellicle, such as albumin and lysosomal enzymes, may inhibit the adherence of specific microbes (Cimasoni et al., 1987). It is apparent that several different mechanisms control the specific interaction between pellicle components and chemical groups on bacterial cell walls. Reciprocative combinations such as enzyme-substrate, carbohydrate-lectin, and antigen-antibody have been suggested as models for microbe-pellicle interactions.

Thread-like extensions emanating from microbial cell walls appear to mediate cell-to-cell and cell-to-pellicle attachments (Newman & Britton, 1974; Tinanoff et al., 1976; Lie, 1977 & 1978). These extensions or intercellular connections are thought to contribute to retention of individual microbes and coherence of the plaque mass (Figs. 2 & 3). Similar structures and a "fuzzy coat" have been described on the surface of bacteria attaching to epithelial cells (Gibbons et al., 1972; McMillan, 1975). Lie (1978) has suggested that some of the thread-like extensions may represent true bacterial organelles similar to polar fibrils while others may be protein or polysaccharide polymers produced by microbes in situ on the pellicle surface. Ultrastructural studies utilizing immunolabeling and ruthenium red staining have demonstrated two classes of cell surface fibrils on Streptococcus sanguis and salivarius. However, the fibrils appear to have species-specific differences as fibrils of Streptococcus sanguis facilitate in vitro adhesion to epithelial cells whereas those of Streptococcus salivarius do not (Handley et al., 1988; Willcox et al., 1989). Attramadal (1977) has proposed that such structures may serve as bridges across barriers or repelling forces until other adhesive forces become effective.

The significance of adhesion for microbial colonization of oral epithelial surfaces is more pronounced than in the case of enamel. Continual desquamation of cells from the epithelial surfaces limits the growth of adherent microbial colonies. Consequently, adhesion becomes the primary factor involved in determining the composition of the microbial flora on oral epithelium. Furthermore, there is evidence suggesting that the rate of epithelial cell turnover is directly related to the extent of microbial colonization (Abrams et al., 1963). Thus, desquamation of epithelial cells would appear to be an important adjunct to host defenses.

In contrast to epithelial surfaces, enamel and root surfaces are not constantly renewed. Consequently, microbial plaque may grow to become tens of microns thick. In such thickened plaque masses, other factors become the primary determinants of composition. For example, microbial metabolism within the plaque mass will produce gradients of factors affecting the growth of various species. Such factor gradients might include the depletion of essential nutrients, accumulation of toxic and inhibitory by-products such as bacteriocins, pH, and redox potentials (Marsh and Keevil, 1986; Takazoe et al., 1984). In general, the distribution of anaerobic microbes in the oral cavity will be related to the redox potential at a particular site. Further, the availability and concentration of nutrients determines the rates at which bacteria grow and replicate. There is evidence for symbiotic relationships between plaque microbes that allow them to avoid direct competition for nutrients (Cowman et al., 1979; Ter Steeg et al., 1988). A specific example is the utilization, by the genus Veillonella, of lactic acid produced as an end product of metabolism by other oral species (Marsh, 1980).

Collectively, the research literature concerning adhesion of oral microbes indicates, among other things, that: (1) attachment of microbes to an oral surface is required for successful colonization; (2) site-specific localization of differing microbes is a result of their affinity for

defined pellicle components and growth conditions that, in turn, are found at specific anatomical sites; (3) microbial adhesion by influencing cellular attachment and accumulation becomes an important virulence factor; and (4) adhesive interactions are mediated through multiple mechanisms ranging from cell-cell to substrate-cell binding (Cisar, 1982; Clark et al., 1984; Gibbons et al., 1988).

Early Plaque Formation and Gingivitis

Dental plaque formation can be considered as occurring in two phases. The first phase involves microbial adhesion mediated through the dental pellicle. The second phase, plaque maturation, involves growth of the adherent microbes, attachment of additional newcomers, and loss of others due to oral cleansing mechanisms, all of which ultimately result in the process of successional colonization (Gibbons and van Houte, 1980).

Scanning and transmission electron microscopic studies of early plaque formation (2-72 hours) indicate that pioneering microbes adhere initially as randomly dispersed single cells or cell aggregates (Saxton, 1973; Listgarten et al., 1975; Tinanoff et al., 1976; Lie, 1977; Brecx et al., 1981; Nyvad and Fejerskov, 1987a & 1987b). The earliest colonizers are generally coccoid morphotypes followed by rods and filamentous forms (Frank and Brendel, 1966; Ritz, 1967; Schroeder and De Boever, 1970; Newman, 1972). Cultural studies of early supragingival plaque have shown these pioneering microbes to be Gram-positive cocci (Streptococcus sanguis, Streptococcus milleri and Streptococcus mitior), Gram-positive rods (Actinomyces viscosus and Actinomyces naeslundii), and Gram-negative cocci such as Veillonella species (Gibbons and van Houte, 1975; Socransky et al., 1977; Theilade et al., 1982).

With time, the adherent microbes grow to confluence, forming a monolayer of cocci and short rods (Fig. 4). Concomitantly, isolated areas that were initially microbial aggregates may achieve considerable thickness (60-100 micrometers) in a matter of a few hours (Listgarten et al., 1975; Listgarten, 1976). From 8-48 hours, the number of microbes in developing plaque increases 100-1000 fold, indicating a rapid growth phase (Fig. 5). The proliferation of bacteria already present on the teeth accounts for the major part of the microbial mass during early plaque formation (Brecx et al., 1983). The mean generation time has been estimated at 3-4 hours, assuming no cell loss (Socransky et al., 1977). Consequently, it is during the rapid growth phase of plaque formation that successional colonization of microbial morphotypes is most dramatic (Fig. 6).

Once the cocci and short rods have established their domain, they are infiltrated by longer rods and filamentous microorganisms of various diameters and lengths (Fig. 7). TEM and light microscopy studies show that such microbes arrange themselves perpendicular to the tooth surface. Furthermore, as the coccoid organisms replicate they tend to form long columns arranged at right angles to the tooth surface, a phenomenon referred to as palisading (Newman and Britton, 1974; Listgarten, 1976). Thus, the developing plaque quickly takes on the microscopic appearance of a series of parallel rays (of microbes) projecting outward from the tooth surface (Newman, 1972). Palisading is not a feature of any particular species of microbe and may represent nothing more than the plane of division of individual bacteria. Palisading may also facilitate diffusion of nutrients and accumulation of the maximum number of microbes per unit of surface area (Newman, 1980a).

At approximately three days, some of the filamentous forms are covered with cocci and short rods, giving rise to the so-called "corn cob" formations (Listgarten et al., 1973 & 1975). Ultrastructural and microbial characterization studies have determined the central filament of the "corn cob" to consist of either Bacterionema matruchotii or Fusobacterium nucleatum (Takazoe et al., 1978; Lancy et al., 1983) and the "kernels" to consist of Streptococcus sanguis (Mouton et al., 1977 & 1980) (Figs. 8 & 9). At this point, 3-5 days, the microbial mass can reach a thickness of >0.4 mm (Listgarten et al., 1975).

Histopathologic studies of experimental gingivitis have shown the initial lesion of gingivitis to be present at 2-4 days (Payne et al., 1975). Consequently, one may conclude that gingival inflammation can be initiated by a flora dominated by Gram-positive cocci and short rods, with smaller

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Figure 1. Salivary pellicle formation on enamel surface two hours post-polishing and prior to microbial colonization. Original magnification x5,000.

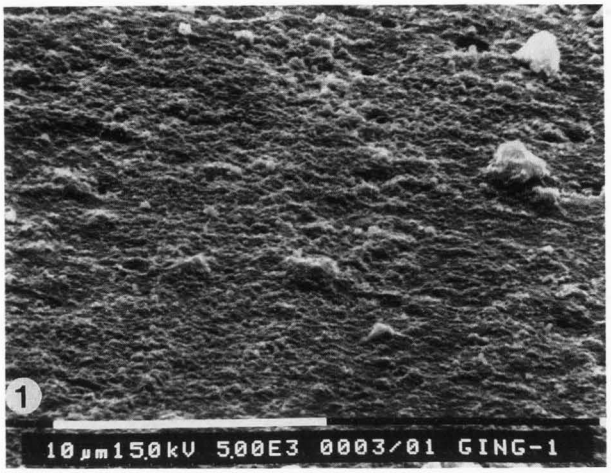
Figure 2. Microbes at periphery of supragingival plaque mass, ten days post polishing, exhibiting thread-like cell-to-cell attachments (small arrows). Microbial morphotypes include cocci, short rods, fusiforms (f-arrow), spirochete (s-arrow), and filamentous organisms (large arrow). Original magnification x6,750.

Figure 3. Supragingival plaque specimen, three days post-polishing, consisting of short rod morphotypes exhibiting cell-to-cell and cell-to-pellicle attachments mediated through thread-like structures. Original magnification x7,400.

Figure 4. Monolayer consisting of coccoid and rod morphotypes attached to enamel pellicle, 1 day post-polishing. Original magnification x5,200.

Figure 5. Supragingival plaque formation representative of the rapid growth phase occurring between 48-72 hours. Majority of microbial morphotypes in this specimen are cocci with a few medium-length rods. Original magnification x2,300.

Figure 6. Supragingival plaque formation representative of 3-5 days of accumulation demonstrating process of successional colonization, i.e., exposed enamel surface (*), layer of cocci and short rods (arrow), with successive layering of longer rods and filamentous organisms (towards top of photograph). Original magnification x156.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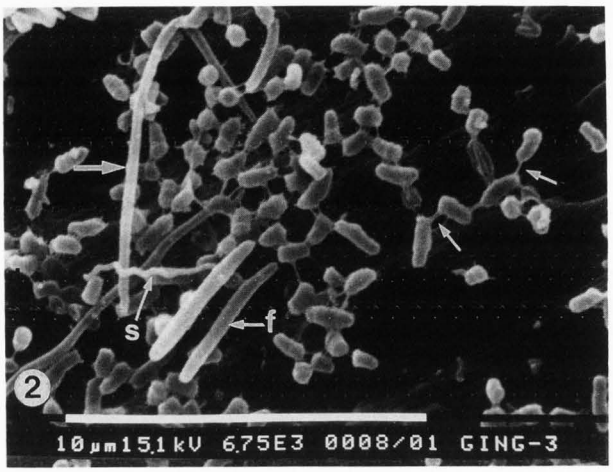
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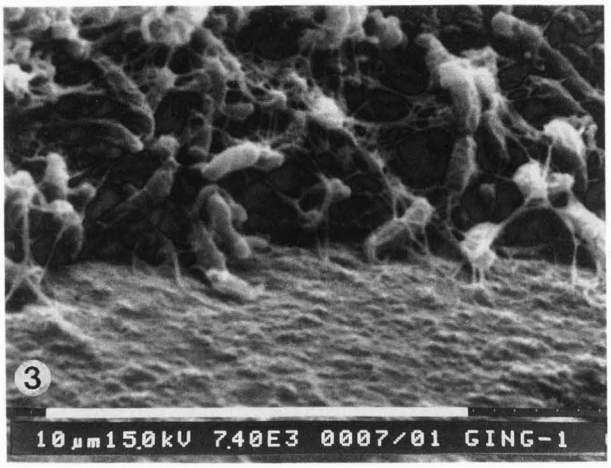
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contributions by Gram-negative cocci. In fact, Sandberg et al. (1988) have shown that phagocytosis and intracellular killing of Actinomyces viscosus is dependent upon neutrophil recognition of type 2 fimbriae. The ensuing phagocytosis results in production of superoxide and release of neutrophil granules, both of which cause host tissue damage. Thus, bacterial surface components that may participate in microbial adhesion (fimbriae) may also initiate gingival inflammation.

As plaque development continues, the ecology becomes more complex and suitable for the proliferation of a greater variety of microbial species (Ritz, 1967). Between 10-21 days of plaque accumulation, clinically detectable gingivitis appears and is associated with a complex microflora (Loe et al., 1965; Theilade et al., 1966; Newman, 1980a; Moore et al., 1982a). The microbial flora of gingivitis features, in addition to the previously mentioned morphotypes, flagellated organisms and spirochetes (Fig. 10). These microbes are randomly distributed throughout the plaque mass and are noted to accumulate in the apical portion of the plaque near the gingival crevice (Listgarten, 1976). Proliferation of the plaque microbiota in an apical direction eventually results in development of a subgingival plaque. This directional proliferation also places the plaque microbiota in contact with the oral and crevicular epithelium. The plaque surface layer in contact with gingival epithelium generally contains a high percentage of motile, Gram-negative rods and spirochetes (Listgarten et al., 1975; Theilade, 1977).

The microorganisms that appear later in the microbial succession and in the evolution of the inflammatory lesion probably do so as a result of environmental changes created by the inflammatory proccess and/or the pre-existing microbial flora. Several investigators have shown that a pre-existing gingivitis accelerates plaque formation (Saxton, 1973; Hillam and Hull, 1977; Ronstrom et al., 1977; Brecx et al., 1980). The more favorable environment for plaque formation in the presence of gingivitis may be related to increased crevicular fluid flow containing essential metabolites. Also, the increased numbers of microbes and resultant thickness of plaque offer micro-environments more suitable to the growth and survival of Gram-negative anaerobes.

Plaque microbes are known to synthesize a variety of extracellular polysaccharides and heteropolysaccharide slimes. These extracellular products form an intermicrobial matrix that contributes to the structural integrity of the dental plaque mass (Guggenheim, 1970; Rosan and Hammond, 1974; Van der Hoeven, 1974; Newman, 1980b). Futher, the early pioneering microbes accumulate a variety of cell-bound, host- or bacterial-derived products such as immunoglobulins, salivary glycoproteins, or lysozyme (Brandtzaeg et al., 1968; Pollack et al., 1976). As existing microbes grow and proliferate and salivary-derived microbes become part of the plaque mass, new surface binding sites are presented that result in the addition of more cell-bound materials to the intermicrobial plaque matrix. Matrix components present in saliva are continually adsorbed to the surface of dividing microbes on the plaque periphery (Fig. 11). Such mechanisms probably supplement the in situ synthesis of extracellular materials by plaque microbes and help to create an environment conducive to further colonization by an ever-increasing variety of microbes (Fig. 12).

Subgingival Plaque and Adult Periodontitis

Assuming supragingival plaque is allowed to accumulate at the gingival margin, eliciting gingivitis, the progressive and apically directed microbial proliferation will result in subgingival plaque formation. Gingival inflammation typically is associated with edema and gingival enlargement, increased crevicular fluid flow, and increased cell turnover in the crevicular and junctional epithelia. The environment of the gingival crevice is protected from the normal cleansing mechanisms of the oral cavity. Consequently, the end result is a new ecologic environment which influences the establishment and relative proportions of subgingival microbes. Although subgingival plaque has a profound influence on the progression of gingivitis, adult periodontitis, and localized juvenile periodontitis, the ultrastructural features have not been described to the same degree as these of supragingival deposits. However, extensive cultural studies have defined the predominant microbes associated with both adult periodontitis and localized juvenile periodontitis.

Light microscopic studies of supra- and subgingival plaques indicate a basic similarity (Oshrain et al., 1971). However, ultrastructural studies show subgingival plaque to be distinctly different in microbial composition and distribution

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Figure 7. Periphery of thickened mass of supragingival plaque showing infiltration of long rods and filamentous organisms at ten days post-polishing. Original magnification x1,250.

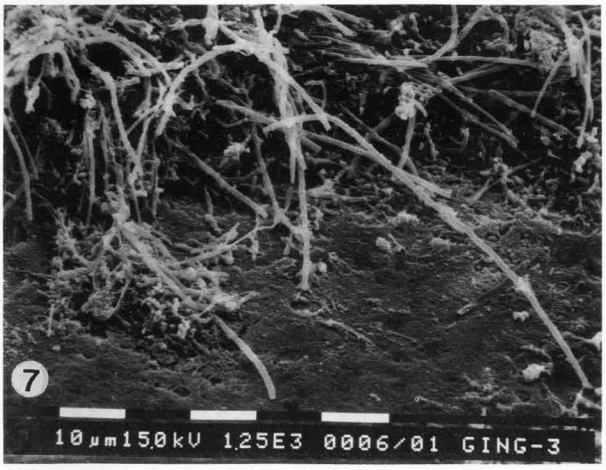
Figure 8. Typical "corn-cob" formation associated with 3-7 day old supragingival plaque accumulation. Original magnification x4,020.

Figure 9. Microbial "corn-cob" in early stages of formation showing central core consisting of a filamentous organisms and "kernels" of cocci. Original magnification x5,200.

Figure 10. Supragingival plaque specimen at the gingival margin, ten days post-polishing, showing the typical Gram-negative morphotypes associated with the appearance of clinical gingivitis, i.e., spirochetes, fusiforms, short rods, and filamentous organisms. Original magnification x2,620.

Figure 11. Early supragingival plaque formation showing microbe (short rods and cocci) embedded in intercellular matrix material, three days post-polishing. Original magnification x4,200.

Figure 12. View of an isolated microbial colony from a supragingival plaque specimen, five days post-polishing, demonstrating structural integrity of the aggregate and apparent modification of the adjacent pellicle/matrix deposition. Original magnification x2,840.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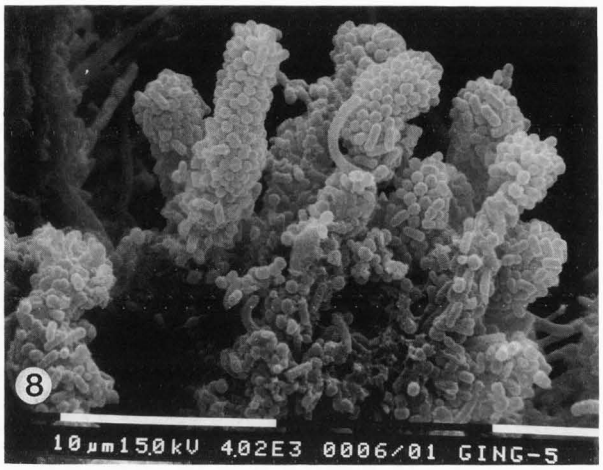
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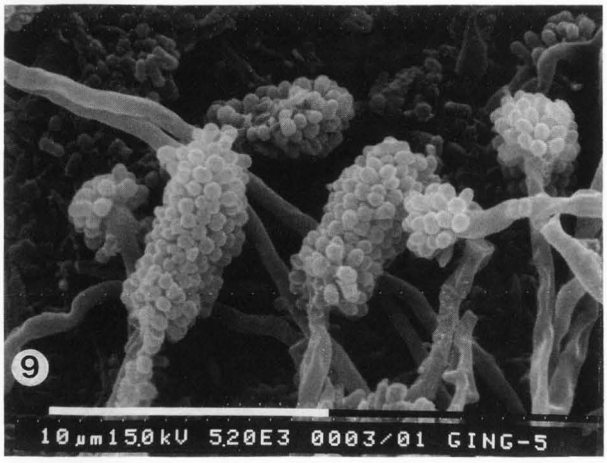
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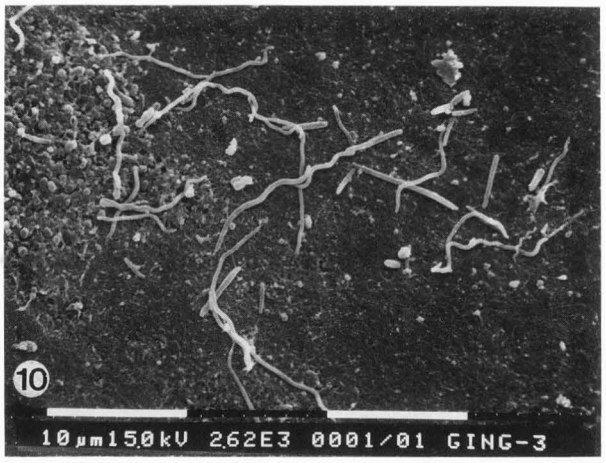
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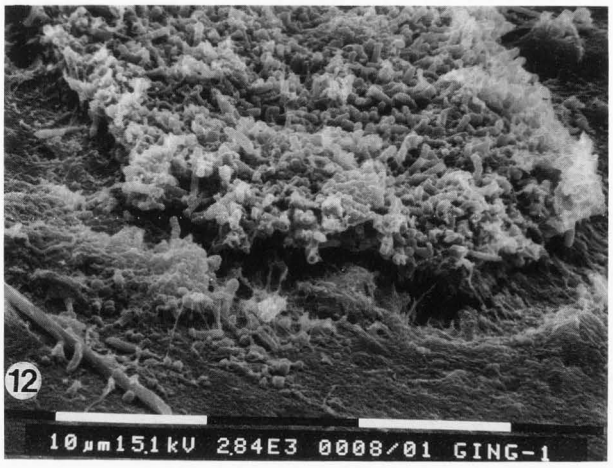
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(Listgarten et al. 1975; Listgarten, 1976; Soames and Davies, 1975; Friskopp and Hammarstrom, 1980; Eide et al., 1983). Successional colonization of subgingival plaque is characterized by a continuous gradient or transition in a coronal to apical direction. Consequently, the more coronal aspects of the periodontal pocket exhibit a prominent population of filamentous microbes with the usual coaggregates of cocci and small rod morphotypes (Figs. 13-15). The deeper pocket areas feature an increasing population of motile-appearing microbes and a decreasing population of cocci, rods, and filamentous organisms. The plaque surface in direct contact with pocket epithelium also differs depending on pocket depth/level. In coronal areas, the surface is characterized by numerous "bristle brush" and "corn-cob" formations (Fig. 16). At deeper levels, the surface plaque features a layer of flagellated microbes, spirochetes, and curved rods (Fig. 17). The deepest portion of the periodontal pocket is known as the plaque-free zone (Fig. 18). Although the term "plaque-free zone" was first used by Brady (1973), observations concerning this area were described by Bass (1946) and Hoffman and Gold (1971). This apically located zone appears devoid of microbial plaque although the cementum is covered with a pellicle (Brady, 1973; Saglie et al., 1975; Eide et al., 1983). Furthermore, there is no evidence of connective tissue attachment to the root surface. Apparently, in vivo, the plaque-free zone is covered by a layer of cells from the most apical aspects of the junctional epithelium.

Epithelial-adherent plaque presents an altogether different picture from that seen on root surfaces. The numbers of microbes are fewer and colony sizes are much smaller and more randomly dispersed. Generally, cocci and short rods are the dominate colonizers of coronally located pocket epithelium (Figs. 19 & 20). In deeper pocket zones, the epithelium may exhibit a variety of adherent fusiforms, filamentous microbes, and spirochetes (Fig. 21). The microbes exhibit a random orientation and are rather loosely adherent to the epithelial surface due to an absence of intermicrobial matrix. Recent morphologic studies have suggested that plaque colonized adjacent to junctional epithelium may represent the apically "advancing front" of the periodontal lesion and be the point of microbial invasion of the lamina propria (Carranza et al., 1983; Sanz et al., 1986). Several TEM studies of advanced-stage periodontitis lesions have noted microbial invasion of the epithelial strata and subjacent connective tissues (Frank, 1980; Saglie et al., 1982; Saglie et al., 1985; Allenspach-Petrzilka and Guggenheim, 1982; Liakoni et al., 1987; Frank, 1988). Microbial invasion of soft tissues has also been noted in acute necrotizing ulcerative gingivitis in both humans and the beagle dog (Listgarten, 1965; Listgarten and Lewis, 1967; Courtois et al., 1983; Maltha et al., 1985) and in localized juvenile periodontitis (Gillett and Johnson, 1982; Carranza et al., 1983; Liakoni et al., 1987). It is important to differentiate between bacterial invasion and translocation resulting from mechanical actions such as oral hygiene, mastication, or manipulation of tissues during biopsy. Bacterial invasion is more likely to result in the presence of selected, proliferating microbes whereas translocation is usually characterized by a heterogeneous mixture of morphotypes (Sanavi et al., 1985).

Cultural studies of the periodontal pocket flora indicate that active disease sites, characterized by continued loss of gingival attachment and persistent bleeding on probing, contain a much higher proportion of Gram-negative anaerobic organisms than do non-disease sites (Slots, 1977; Moore et al., 1982b & 1983; Tanner et al., 1979 & 1984). Genera of anaerobic microbes commonly associated with moderate-advanced periodontitis include Actinobacillus, Bacteroides, Eikenella, Eubacterium, Fusobacterium, Selenomonas, Treponema, and Wolinella (Dzink et al., 1985 & 1988; MacFarlane et al., 1988). The various cultural studies confirm one aspect of selective microbial colonization. Advanced adult periodontitis is characterized by deep pockets resulting from loss of alveolar bone and connective tissues. The microbial environment of deep pockets, in turn, is characterized by low redox potentials, thereby selectively favoring growth and replication of anaerobic microbes (Kenney and Ash, 1969; Mettraux et al., 1984; Savitt and Socransky, 1984). Low oxygen tension in periodontal pockets may be a result of multiple factors such as plaque mass

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Figure 13. Subgingival plaque from the coronal aspect of a periodontal pocket (adult periodontitis) showing a layer of cocci and various rod-shaped morphotypes (*) upon which a thick mat of longer rods and filamentous organisms have colonized. Original magnification x212.

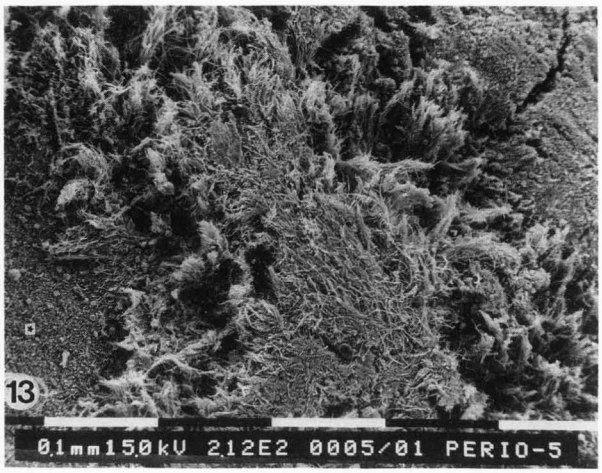
Figure 14. High magnification view of area from Figure 13 (*) showing layer of cocci, short rods, fusiforms, and scattered filamentous organisms. Original magnification x3,380.

Figure 15. High magnification view of area from Figure 13 showing thick colony of long rods (foreground) and longer filamentous organisms (top). Original magnification x2,400.

Figure 16. Specimen of mature subgingival plaque from the coronal aspect of a periodontal pocket (adult periodontitis) featuring "corn-cob" formations. Photograph on right is enlarged from the rectangular area noted in the left photo. Original magnification of left photo x163 and right x1,040.

Figure 17. View of an advancing plaque front from the apical portion of a periodontal pocket (adult periodontitis) composed of cocci and rods embedded in a plaque matrix with spirochete, fusiform, and filamentous morphotypes on the surface. Original magnification x2,400.

Figure 18. Overview of the root surface of a tooth extracted because of advanced staged adult periodontitis showing the plaque-free zone (PFZ) at the most apical extent of the periodontal pocket. Cemento-enamel junction (short arrows) and fibrous connective tissue attachment (long arrows). Original magnification x17.9.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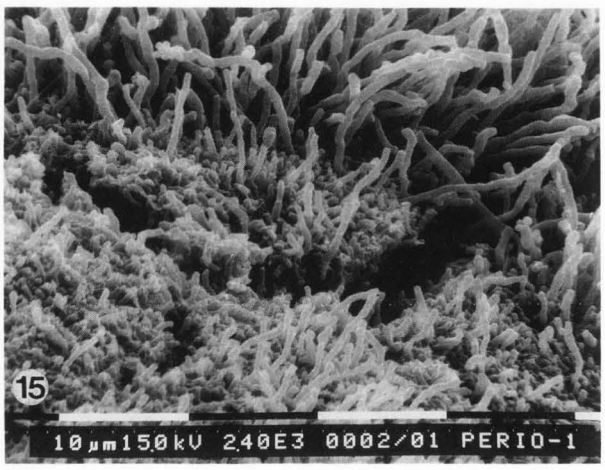
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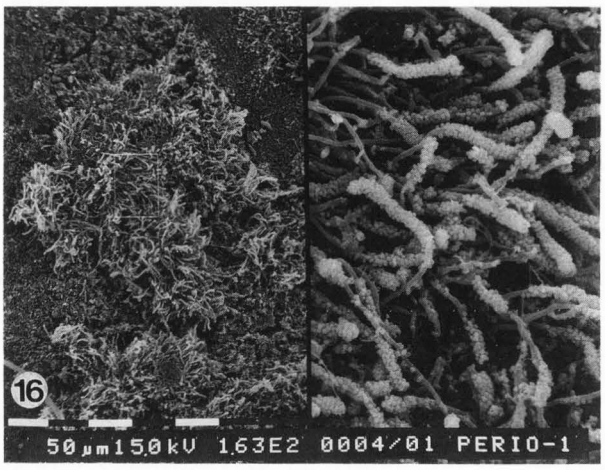
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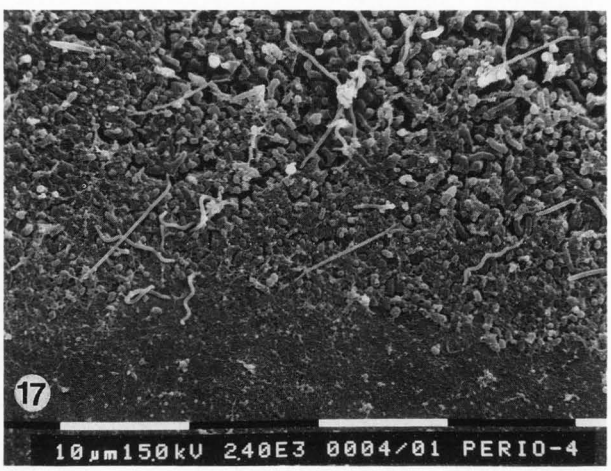
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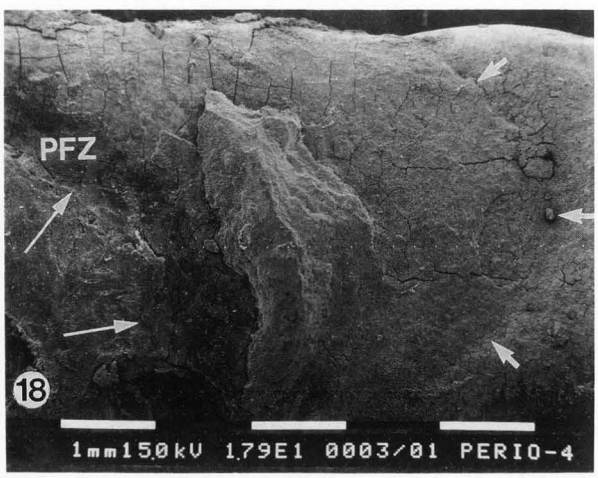
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functioning as a barrier to oxygen diffusion, consumption of oxygen by dense infiltrates of inflammatory cells, and bacterial metabolites that chemically reduce molecular oxygen.

Subgingival Plaque and Juvenile Periodontitis

Localized juvenile periodontitis (LJP) is characterized by rapid loss of the alveolar bone and connective tissue supporting the permanent first molar and incisor teeth in patients between the ages of 11-21 years. Generally, the extensive destruction of the periodontium is not commensurate with the amount of local irritants, e.g., plaque, calculus, or materia alba (Baer, 1971).

The microbial plaque associated with LJP may be characteristic and different from that of other periodontal diseases. The predominant microorganisms are Gram-negative, anaerobic rods, comprising 78% of the cultivable flora (Newman et al., 1976; Slots, 1976; Newman and Socransky, 1977; Liljenberg and Lindhe, 1980). Actinobacillus actinomycetemcomitans, a Gram-negative anaerobic coccobacillus, has been proposed as the primary pathogen as it is found in over 90% of LJP cases, but only 50% of adult periodontitis cases, 36% of normal adults, and 20% of normal juveniles (Slots et al., 1980). Actinobacillus actinomycetemcomitans is quite virulent, producing a leukotoxin, collagenase, and various phosphatases and bone-resorbing factors (Nisengard et al., 1988). Other investigators have expanded the list of microbes associated with LJP to include Bacteroides gingivalis (Savitt and Socransky, 1984), Bacteroides intermedius (Moore et al., 1985), Fusobacterium nucleatum (Asikainen et al., 1987), Eikenella corrodens (Mandell, 1984), and various spirochetes (Asikainen, 1986).

There are comparatively few studies devoted to the description of dental plaque and microbial morphotypes associated with LJP and their relationship to the root surface and/or pocket epithelium (Listgarten, 1976; Westergaard et al., 1978; Allen and Brady, 1978; Saglie et al., 1982; Carranza et al., 1983; Liakoni et al., 1987; Verderame et al., 1989). Collectively, these papers show the subgingival microbial plaque of LJP to be relatively sparse when compared to that of adult periodontitis. Plaque located near the gingival margin in LJP pockets appears to be similar in morphotypic composition to that of adult periodontitis (Figs. 22 & 23), i.e., cocci, long and short rods, filamentous microbes, fusiforms, and spirochetes (Listgarten, 1976). Deeper pocket areas are inhabited by cocci, coccobacilli, short rods, a few filamentous microbes, flagellated microbes, and various spirochetes (Figs. 24 & 25). The numbers of microbes appear to decrease as pocket depth increases and those at the greatest depths are limited to cocci, short rods, coccobacilli, and spirochetes (Fig. 26). The plaque-free zone in LJP specimens is not bacterial free as it consistently features dispersed populations of short rods and spirochetes (Allen and Brady, 1978; Verderame et al., 1989).

Epithelial-associated plaque in LJP pockets consists of a few randomly dispersed cocci near the gingival margin. At mid-pocket (Fig. 27), the epithelium is populated by dispersed colonies consisting of cocci, bacilli, coccobacilli, and spirochetes (Verderame et al., 1989). Epithelium at the deepest levels of LJP pockets exhibits relatively few microbes, and those observed are usually spirochetes and short rods (Verderame et al., 1989).

Addendum

Microbial plaque-induced gingival inflammation is not a unique host response directed towards a few types of specific bacteria. The inflammatory response is initiated when any bacterium or its metabolic by-products gain access to the subepithelial connective tissues. It is probable that most members of the subgingival microbial flora have the inherent ability to initiate inflammation and thereby contribute to the inflammatory periodontal disease process. Thus, it follows that all bacteria of the subgingival flora could be considered "pathogens".

The host inflammatory response directed against the subgingival microbiota is often self limiting and results in minimal tissue damage, as in gingivitis. However, if the commensal

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Figure 19. Surface of epithelium from the coronal aspect of a periodontal pocket (adult periodontitis) undergoing colonization by cocci and short rods. Original magnification x6,900.

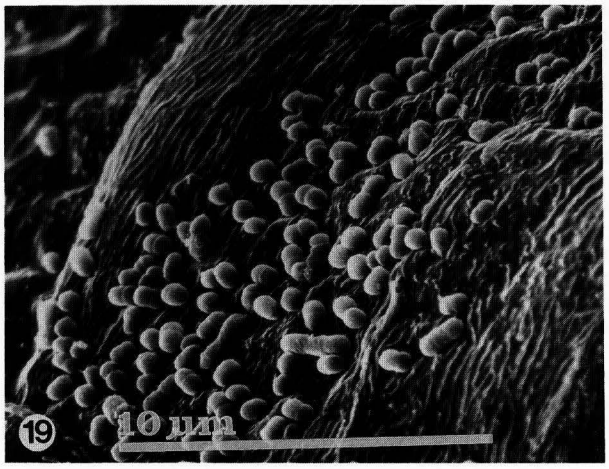
Figure 20. Epithelial surface from the mid-depth region of a 7 mm periodontal pocket (adult periodontitis) featuring several adherent short rods. Note the dense network of cellular micro-ridges and centrally located depression which may represent an endo- or exocytoic vesicle area. Original magnification x7,000.

Figure 21. Epithelial surface from the apical one-third of an 8 mm periodontal pocket (adult periodontitis) exhibiting numerous microbial morphotypes, i.e., fusiforms, long rods, spirochetes, and filamentous organisms of various diameters. Original magnification x3,100.

Figure 22. LJP specimen showing the phenomenon of successional colonization on subgingival root surface near the gingival margin. When compared to Figure 6 (supragingival colonization in gingivitis), the LJP specimen exhibits a comparatively sparse microbial population. Original magnification x1,550.

Figure 23. Subgingival colonization of root surface from the coronal aspect of a periodontal pocket associated with LJP. The microbial morphotypes are similar to those found in adult periodontitis. Original magnification x2,980.

Figure 24. Microbial morphotypes typical of the more apical aspects of periodontal pockets associated with LJP, i.e., short rods, fusiforms, coccobacilli, and flagellated microbes. Original magnification x7,400.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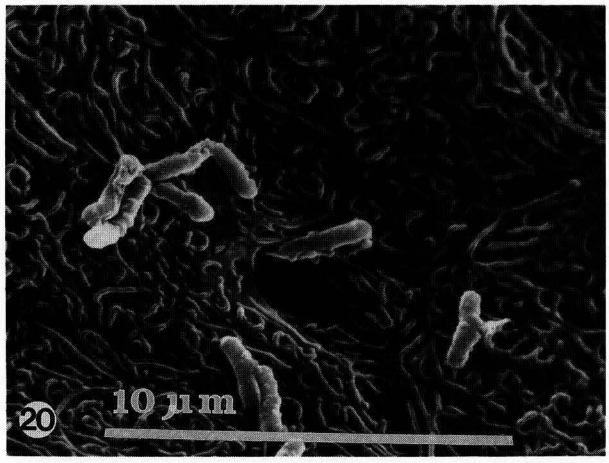
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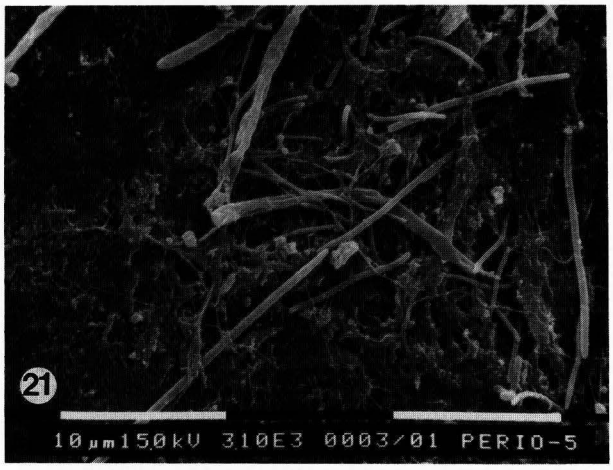
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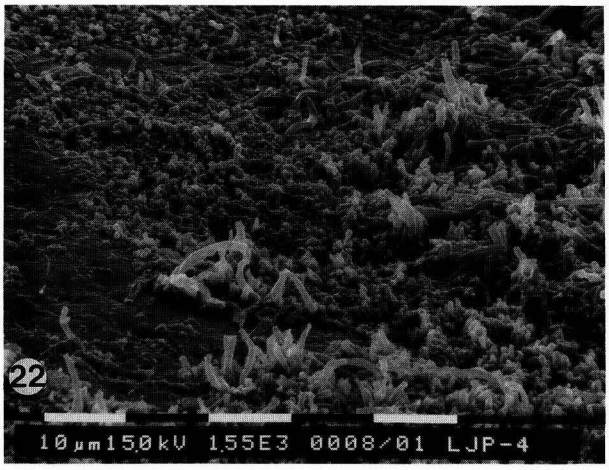
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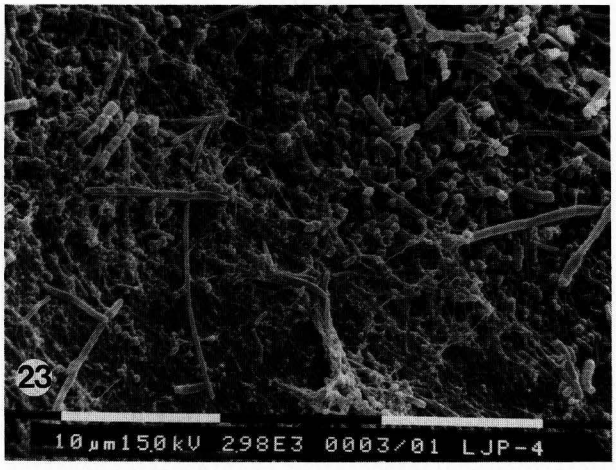
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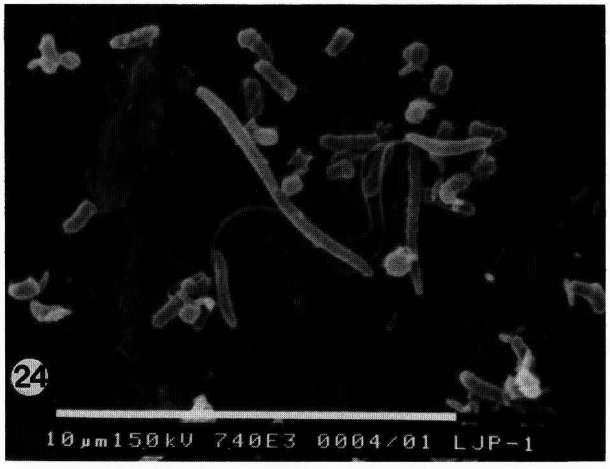
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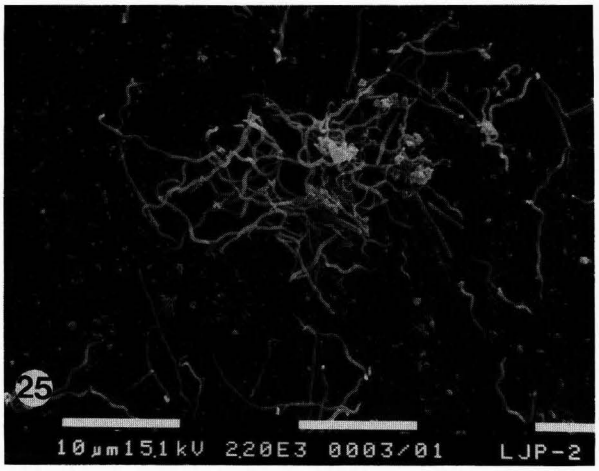

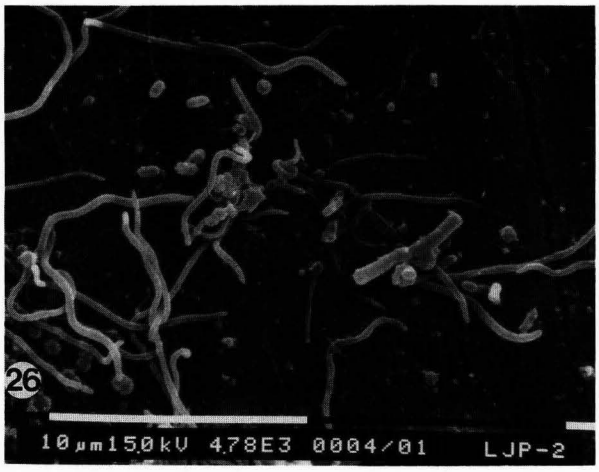

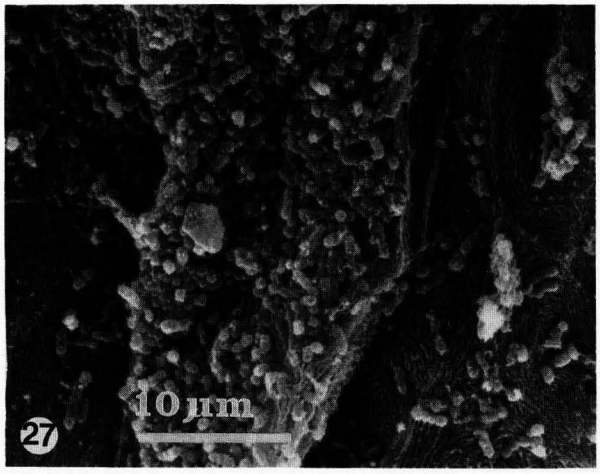

microbiota is allowed to reach critical mass, in terms of numbers, the microbes may simply overwhelm the host's ability to cope, as in adult periodontitis. The microbial flora may also harbor bacteria that specifically alter the host defensive response, as in LJP.

The term "periodontal pathogen" could be restricted to those microbes that exhibit specific mechanisms which alter host defensive responses and thereby cause accelerated destruction of the periodontium. However, it is highly unlikely that such "pathogens" function in isolation. To the contrary, such "pathogens" are members of a complex microbial community and cannot, as such, fulfill the criteria of Koch's postulates. Thus, reference to specific members of the microbial complex as "pathogens" creates obvious conceptual problems, not only from a scientific viewpoint but also when applied to the antimicrobial aspects of periodontal therapy.

Figure 25. Middle one-third root surface of a 10 mm periodontal pocket from an LJP specimen showing a colony of mixed spirochete and fusiform morphotypes. Original magnification x2,200.

Figure 26. Mixture of large spirochetes and coccobacilli on root surface at the most apical extent of a 9 mm periodontal pocket from an LJP specimen. Original magnification x4,780.

Figure 27. Epithelial surface from the mid-region of an LJP periodontal pocket showing colonization by a mixture of cocci, bacilli, and coccobacilli. Original magnification x2,800.

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Discussion With Reviewers

H. N. Newman: What is the significance of stagnation in plaque accumulation, especially with regard to dental disease?

Authors: Stagnation of microbial plaque may result from various factors such as inadequate oral hygiene, reduced salivary flow, dental restorations with overhanging margins, and soft textured diets. All of these factors would appear to favor plaque retention. In protected environments such as interproximal regions and periodontal pockets, plaque can achieve considerable thickness and stagnation may result in lowered oxygen tensions within the plaque mass which favors the proliferation of Gram-negative anaerobic organisms. Further, stagnation of plaque with the associated intermicrobial matrix may allow the concentration of metabolic by-products such as acids, enzymes, endotoxins, and various bacterial cell wall products.

H. N. Newman: Are the structural features of plaque as described indicative of pathogenicity or commensalism?

Authors: With few exceptions, the microbes associated with the common inflammatory periodontal diseases and dental caries must be considered members of the indigenous and/or commensal oral flora. Obviously, microbes may exist in varying states, ranging from mutual symbiosis that is

benign to the host to extreme parasitic symbiosis where the microbes are detrimental to the host and may be described as pathogenic. Further, within the oral ecosystem, individual microbes may be neutral, antagonistic, or synergistic with one another. The structural features of dental plaque noted in this paper probably represent microbial commensal relationships arising from the production of metabolites in the ecosystem. For example, there is evidence that spirochetes and Bacteroides species benefit from a complex dental plaque that provides low oxygen tensions and growth factors. Another often-cited commensal relationship is the utilization by Veillonella of lactic acid excreted by Streptococci and Actinomyces (Loesche, 1968; Savitt and Socransky, 1984). The phenomenon of microbial coaggregation is another example of commensalism. However, with the exception of the "corn cob" formation, there is little in vivo data to demonstrate the significance of coaggregation as an ecological determinant in dental plaque.