Scanning electron microscopic examination of pocket wall epithelium and associated plaque in localized juvenile periodontitis


Abstract. The purpose of this investigation was to describe the morphologic characteristics of epithelial-associated plaque in periodontal pockets of patients with localized juvenile periodontitis (LJP). 25 tissue specimens obtained from 10 adolescent patients were examined by scanning electron microscopy. Specimens were evaluated to determine the following specific features: (1) distribution of microbial colonies on the epithelial surface; (2) topographic features of pocket epithelium associated with microbial colonies; and (3) the predominant microbial morphologic types comprising the colonies. Observations made during this investigation revealed the following. (1) The surface epithelium in the coronal one-third of the pocket wall was essentially healthy in appearance and exhibited no distinct microbial colonies or unusual topographic features. (2) The surface epithelium in the middle one-third area featured randomly-dispersed microbial colonies consisting of 3 major morphotypes: cocci, bacilli and cocccobacilli. Also in this zone, there was morphologic evidence of microbial penetration of the epithelial barrier along intercellular spaces. (4) The apical one-third zone was characterized by lymphocytic infiltration, epithelial cavitation and ulcerations, and singular organisms entrapped in fibrin meshworks. There were no distinct microbial colonies in this zone, although individual spirochetes, fusiforms, filamentous organisms, and short and long rods were observed.

Localized juvenile periodontitis (LJP) is a particularly aggressive and destructive disease process affecting the periodontium of otherwise healthy adolescents (Baer 1971). Characteristically, the disease induces rapid vertical bone loss around first molars and incisors. Originally thought to be a degenerative process (Gottlieb 1923, Thoma & Goldman 1937, Orban & Weismann 1942), a concept since abandoned, the disease is now considered to be infectious in nature (Gottlieb 1923, Thoma & Goldman 1937, Orban & Weismann 1942, Bernier 1949, Ramford 1959).

The concept of inflammatory periodontal disease as a microbial infection implies the possibility of host tissue invasion (Manson & Leenhart 1974). Indeed, considerable evidence exists to establish precedent for this concept (Gillett & Johnson 1982, Saglie et al. 1982).

Bacterial invasion of host periodontal tissues was reported by Beckwith et al. (1927) and since been studied by various investigators using scanning and transmission electron microscopy (Frank & Voegel 1978, Sanz 1987, Wolinsky, Saglie et al. 1987), immunofluorescence (Albini et al. 1987, Zambon et al. 1987) and microbial culture techniques (Christerson, Zambon et al. 1983). Although the majority of such articles are concerned with gingivitis and advanced stage adult periodontitis, a similar invasive character has been described for the bacterial plaque associated with LJP (Gillett & Johnson 1982, Saglie et al. 1982, Carranza et al. 1983). Although not stated, 2 common patterns appear to permeate most descriptions of bacterial invasion and its role in inflammatory periodontal disease: The implication that the soft tissue walls of infected periodontal pockets exhibit a generalized distribution of microorganisms; and that bacterial invasion appears to be a routine observation in advanced stage periodontal disease. Recent studies by Liakoni et al. (1985, 1987) have reported that clinically infected periodontal pockets in LJP patients may, in fact, exhibit a sparse distribution of invading microbes. Thus they conclude it is reasonable to suggest that bacterial metabolic by-products rather than the actual organisms penetrate the tissues and initiate the inflammatory response.

The majority of previous investigations concerned with ultrastructural verification of microbial invasion in LJP or descriptions of the associated plaque are based on limited numbers of specimens and or patient population, i.e., 1 or 2 patients and a corresponding num-
Specimen preparation for SEM

Immediately after surgical dissection of the pocket, soft tissue wall from the involved teeth, the resultant samples were gently rinsed with ice-cold phosphate buffered saline to remove adherent blood. The specimens were then placed in 2% glutaraldehyde in phosphate buffer (pH 7.4) at 4°C for 3–4 h. After fixation, they were rinsed several times in phosphate buffer and subjected to dehydration in a series of graded ethanol solutions (20–100%) and dried in a critical point drying apparatus. All samples were sputter-coated with approximately 200 Angstroms of gold/palladium and subsequently examined with a JEOL JSM-35 scanning electron microscope (SEM).

Each specimen examined was initially photographed at a 10× magnification for orientation purposes. Pockets greater than 9 mm in depth required construction of a montage photograph, thereby allowing one to view the entire surface area of the pocket wall. Using topographic landmarks located on the low magnification photographs, the pocket was then visually divided into coronal, middle and apical thirds.

Each one-third of the pocket epithelial surface area was then examined for the following specific features: (1) distribution of epithelial associated colonies; (2) the topographic features of epithelium adjacent to microbial colonies; (3) for the following bacterial morphological types: cocci, short rods, long rods and/or fusiforms, spirochetes, yeast, mycoplasma, and cocccobiacci.

Results

Examination of all 25 tissue samples by scanning electron microscopy failed to reveal any noticeable difference between specimens associated with molars or incisors. In both instances focal microbial colonization of the epithelial surface was randomly dispersed and tended to occur in the middle one-third and the upper aspect of the apical one-third of the pocket soft tissue wall. Epithelial surfaces of the coronal one-third were generally free of obvious microbial colonies although single organisms or small groups were randomly distributed over the surface area.

Every specimen examined exhibited at least one major area of epithelial caviation or micro-ulceration. Generally, these areas were confined to the middle and apical two-thirds of the pocket wall. A uniform feature of all specimens was the presence of a generalized lymphocyte exudation in the apical one-third of the pocket wall. Such areas of lymphocyte infiltration were free of distinct microbial colonies and exhibited only a few isolated micro-organisms.

Spirochetes, fusiforms and long rod shaped organisms were uniformly observed in all specimens but tended to be associated with erythrocyte and fibrin aggregations in and around micro-ulcerations. Definitive microbial colonies, when observed, consisted of cocci, bacilli and cocccobiacci.

One specimen, of the 25 examined, exhibited a localized area of yeast and/or fungus infection manifested by a network of pseudohyphae and blastocandia covering the epithelial surface (Fig. 1). This particular fungus infection of surface epithelium was isolated to the upper aspects of the middle one-third of the pocket wall. None of the 25 specimens examined showed any evidence of mycoplasma infection.

Coronal one-third of the pocket wall

At low magnification (10×) the topographic features of the pocket soft tissue wall of all specimens presented a rather uniform appearance (Fig. 2). The area of marginal gingiva exhibited a smooth regular contour with no evidence of cavitation or crater formation. In most specimens there was a characteristic arrangement of elongated and parallel elevations and depressions best described as striae. This striae appearance may represent a shrinkage artifact resulting from fixation and dehydration.

Higher magnifications (100× - 1000×) of the coronal one-third revealed relatively intact epithelium layer with numerous desquamating cells. However, long narrow surface fissures arranged in parallel fashions were frequently observed. The fissions appeared real and not artificial as adjacent cells exhibited rounded margins as opposed to surface tears and often one could observe migrating lymphocytes within the fissures.

Magnification of 1500× or greater showed the surface epithelium to have tightly adapted intercellular spaces with little evidence of microvilli except in areas of desquamation where one could observe the cell margins (Fig. 3). An occasional "hole" or deep depression was observed to occur between adjacent...
Fig. 1. Yeast infected specimen exhibiting a network of pseudohyphae (arrow) and blastoconidia (insert) ($\times$ 1000 and insert $\times$ 2400; bar = 10 $\mu$m).

Fig. 2. Surface topography of the coronal 8 mm of a 12 mm pocket soft tissue wall. Gingival margin indicated by arrows and boxed area refers to Fig. 4. $\times$ 10 (bar = 1 mm).

Fig. 3. Hole zone between adjacent epithelial cells exhibiting a dispersed population of cocci and short rods (insert) ($\times$ 1200 and insert $\times$ 5400; bar = 10 $\mu$m).

Fig. 4. Rounded plateaus separated by deep surface clefts (enlargement from boxed area in Fig. 2). Distinct microbial colonies were associated with such areas of surface irregularity (arrows). ($\times$ 200; bar = 50 $\mu$m).
Fig. 5. Typical microbial colony of the surface epithelium associated with topographic plateaus and surface clefts in the middle one-third of LJP pockets. Colonies consisted of cocci, short rods and coccobacilli. Note bacteria extending into the cleft between adjacent cells (large arrows) \( (\times 1500; \text{bar}=10 \mu m) \).

Fig. 6. Surface clefts between adjacent epithelial cells in the middle one-third filled with short rods and cocci (arrows) \( (\times 1200; \text{bar}=10 \mu m) \).

Fig. 7. Epithelial cavitation filled with desquamating epithelial cells (E), erythrocytes (R), and a lymphocyte (L). Note the presence of spirochetes clusters and singular organisms (arrows and insert) \( (\times 1200 \text{ and insert } \times 3300; \text{bar}=10 \mu m) \).

Fig. 8. Spirochetes and a fusiform organism attached to the surface epithelium and possibly penetrating in intracellular cleft \( (\times 3300; \text{bar}=5 \mu m) \).
cells or within a single cell. These areas usually contained a small cluster of cocci and short rods (Fig. 3 and insert). Similar microbial morphotypes were randomly dispersed on the epithelial surfaces but no distinct colonies were noted.

Middle one-third of the pocket wall

In contrast to the topography of the coronal aspects of the pocket wall, that of the middle one-third was characterized by periodic deep surface clefts that delineated rounded plateaus of epithelium (Figs. 2, 4). At low magnification, the pocket wall exhibited gentle crater-like depressions that often contained various types of cellular debris, fibrin, and pooled erythrocytes. Examination of the surface clefts and epithelial plateaus at higher magnifications revealed distinct microbial colonies. In all 25 specimens, these microbial colonies were comprised of relatively pure morphological types, i.e., cocci, bacilli and coccobacilli (Fig. 5). Smaller aggregations of these bacterial morphotypes were often observed under desquamating cells and lying within surface clefts between adjacent cells (Fig. 6).

Other morphological types of bacteria observed in the middle one-third consisted of filamentous organisms, fusiforms, spirochetes, short rods and long rods. These morphotypes were usually observed adjacent to epithelial micro-ulcerations where they were entrapped in fibrin or closely associated with erythrocyte aggregates (Fig. 7). Frequently, fusiforms and spirochetes were observed attached to the epithelial surface or penetrating intercellular spaces (Fig. 8).

Apical one-third of the pocket wall

When examined at magnification of 100 × – 1000 ×, the apical one-third of the pocket soft tissue wall presented two distinct variations in surface character. First, areas of intact surface epithelium uniformly exhibited a pronounced lymphocytic migration towards the pocket space (Fig. 9). There was a conspicuous absence of microbial colonies and only rarely were single bacteria observed in these areas of lymphocyte migration. Also very few cells of the granulocyte series (neutrophils) were identified among the migrating white cell population. Secondly, the apical one-third exhibited a considerable number of micro-ulcerations in the epithelial surface. These ulcerations were identified by the obvious epithelial cavitation filled with erythrocytes, lymphocytes and fibrin (Fig. 10). The ulcerated areas were not associated with microbial colonies although singular spirochetes, fusiforms and short rods were entrapped in the fibrin meshwork.

Discussion

A common dilemma among previous ultrastructural studies of LJP has been the use of an extremely limited sample population and number of specimens. For instance, Allen & Brady (1978), Gillett & Johnson (1982), Saglie et al. (1982), Carranza et al. (1983), Newman et al. (1984) all obtained 4 or less specimens from one patient. In addition, Saglie et al. (1985), Saglie et al. (1986) and Liakoni et al. (1987) based their studies on a sample population of 2 patients. One major study by Gonzalez et al. (1987) used a patient population of twelve from which sixty specimens were procured. However, their report emphasized the presence of yeast rather than the bacterial flora. Due to the limited sample populations, previous studies of a descriptive nature may have empha-
sized anomalous situations as regards the microbial flora associated with LJP. In contrast, this investigation used 25 biopsy specimens taken from 10 different patients.

Studies by, Carranza et al. (1983), Saglie et al. (1985), Newman et al. (1984), and Saglie et al. (1986) have described mycoplasma infected tissues from LJP pocket biopsy specimens. The organisms were attached to epithelial cells, erythrocytes, cementum, and exhibited invasive potential by passing through the epithelial wall into subjacent connective tissues.

The present investigation was unable to confirm these observations as there was no evidence of mycoplasma in any of the 25 biopsy specimens examined. Furthermore, based on examination of published photomicrographs and detailed analysis of written descriptions from the investigations of Allen and Brady (1978), Liljenberg & Lindhe (1980), Gillett & Johnson (1982), Gonzalez et al. (1987) and Liakoni et al. (1987) each failed to reveal the presence of mycoplasma in their specimens. Thus, the presence of mycoplasma in LJP pocket specimens may, indeed, represent the exception rather than the norm.

An unusual observation in the present investigation was the occurrence of an apparent yeast infection in one of the ten patients. Biopsy specimens from this patient were characterized by a network of pseudohyphae and blastoconidia covering the epithelial surfaces of the coronal aspect of the middle one-third of the pocket wall. Gonzalez et al. (1987), observed yeast in 26 of 60 LJP biopsy specimens. However, the yeast infection by their description was in the oral epithelium and underlying connective tissue apical to periodontal pockets. There are 2 major reasons for the apparent disparity between these investigations. First, yeast are known to digest keratin as their sole source of nitrogen (Kapica & Blank 1957). The Gonzalez et al. (1987) study used biopsies taken through keratinized oral epithelium whereas the present investigation was concerned with the nonkeratinized pocket epithelium. Secondly, patients in the Gonzalez study received Spiramycin therapy 15-20 days prior to biopsy. Spiramycin is a macrolide antibiotic that concentrates in the gingiva 40 × greater than in blood serum (Quee 1982). The drug is considered bacteriostatic and as such could possibly suppress competitive micro-organisms and allow for a proliferation of opportunistic yeast. The one yeast positive patient in the present study offered no prior history of antibiotic therapy. However, considering the low incidence of yeast infections in ten biopsied patients (10%) from the present study and the 41% incidence in the Gonzalez study, it would appear that infection by yeast in LJP pockets represents an anomalous situation related to antibiotic therapy.

None of the previous SEM investigations concerning LJP divided their pocket biopsy specimens into coronal, middle or apical thirds. All observations were pooled thereby leaving the impression that the entire pocket wall was uniform in appearance. The present investigation shows each pocket one-third to have rather characteristic features. For example, the coronal one-third exhibited randomly dispersed cocci and short rods with no large aggregations. The epithelium surface was free caviations and/or ulcerations and only an occasional lymphocytic cell was noticed. The apparent lack of tissue degradation in this region is probably related to both a lack of pathogenic microbial flora and host inflammatory cell migration through the epithelial barrier. Clinically, this could account for the relatively normal gingival appearance in many LJP patients.

Epithelial topography of the pocket wall middle one-third featured deep surface clefts and randomly dispersed microbial colonies consisting of cocci, bacilli and cocacobacilli. Other bacterial morphotypes found in this region were usually associated with fibrin meshes and/or aggregates of erythrocytes, i.e., spirochetes, fusiforms, and long rods. Carranza et al. (1983) report similar observations. Those organisms entrapped in fibrin are probably associated with the free floating plaque of the periodontal pocket. Microbial association with erythrocytes is speculated to be related with nutritional requirements. Erythrocytes can furnish hemoglobin, iron, cyanocobalamin (Vitamin B12), and various proteins, lipids, all of which may partially satisfy the nutritional requirements of various gram negative microbes.

Most of the previous SEM studies of the LJP pocket wall have shown extensive colonization of the epithelial surface by cocci, bacilli and cocacobacilli (Allen & Brady 1978, Saglie et al. 1982, Gillett & Johnson 1982, Carranza et al. 1983, and Liakoni et al. 1987). However, none of the investigations discuss the presence of an ulcerated epithelial wall. Further, Gillett & Johnson (1982) specifically state that the junctional epithelium was not ulcerated although they could demonstrate bacterial invasion subjacent to this region. Conversely, the present investigation suggests that epithelial cavitation and/or micro-ulceration with associated erythrocytes extravasation, fibrin formation and a prodigious population of migrating inflammatory cells is characteristic of the apical one-third region of the LJP pocket wall. There was a conspicuous absence of microbial colonies associated with the micro-ulcers suggesting that their origins may result from cytotoxic reactions with the free floating plaque or evolve as a by-product of inflammatory cell degranulation, i.e., lysosomal enzymes. Similar observations are reported by Liakoni et al. (1987) who noted a relatively sparse microbial population and generalized migration of granulocytes through the epithelial wall regardless of the level of clinical inflammation.

Based on morphologic criteria of identification, the migratory inflammatory cell population noted in the present study was predominately lymphocytic with few granulocytes. Consistent with this observation is the fact that several authors have reported that active periodontal lesions are populated by cells of the B-lymphocyte series (Seymour & Greenspan 1979, Davenport et al. 1982, Seymour 1987). Specific and/or nonspecific antibody production by B-cells may contribute to inhibition of bacterial colonization and thereby account for the absence of such colonies in the apical one-third of the LJP pocket.

Indeed, it should be noted that the requirements of tissue processing in buffer rinses both before and after fixation removed weakly attached migrating host cells and microbes from the epithelial surface. Obviously, this would exclude observation of motile bacteria and those comprising the unattached plaque associated with deeper pocket areas. Thus, the present observations represent those bacteria firmly affixed to the surface epithelium or trapped within intercellular spaces.

Although in direct contrast to the observations of Muller-Glaueter and Schroeder (1982), the lack of a significant population of neutrophilic granulocytes in the middle and apical one-
third areas of the pocket may also be a result of tissue processing. Neutrophils are known to be more active than lymphocytes in terms of migration rate and general mobility and, therefore, may exhibit a less tenacious surface attachment. Although bacterial penetration of host tissues was not within purview of this investigation, the observed epithelial characteristics associated with colonized plaque may offer clues regarding the mechanisms involved. Saglie et al. (1983) have suggested that initial points of entry for bacteria are the undersides of desquamating epithelial cells and along their intercellular spaces. Observations made during the present study would seem to support this concept (Figs. 5, 6, 8) as do those of Carranza et al. (1983) using transmission electron microscopy. Further support of this idea is derived from the suggestions and observations of Takarada et al. (1974), Schroeder (1977), that microbial plaque and large numbers of inflammatory cells cause tissue degeneration and increase epithelial permeability. Examination of the apical one-third region of the soft tissue pocket wall in the present study are supportive of this perception (Figs. 9, 10). Finally, Birkedal-Hansen et al. (1982) demonstrated that Bacteroides gingivalis, Actinobacillus actinomycetemcomitans and Capnocytophaga all produce an epitheliotoxin that may alter the capacity of epithelium to function as a defensive barrier to bacteria. The important clinical implications to the bacterial invasion through sulcular epithelium and into connective tissue is that these bacteria remain in the tissue after scaling, root planing and surgical procedures, therefore possibly playing a role in refractory periodontitis.

Future research is necessary in the examination of the root surfaces of these LJP patients, comparing and contrasting those observations with those of the soft tissue pocket epithelium findings of this study.

Zusammenfassung


References


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Résumé

Microscopie électronique à balayage pour l'examen de l'épithélium de la paroi des poches parasodontales et l'examen de la plaque qui lui est associée dans la parodontite juvénile localisée

Le but de cette étude était de décrire les caractéristiques morphologiques de la plaque associée à l'épithélium dans les poches parasodontales de patients atteints de parodontite juvénile localisée (LJP). Le microscope électronique à balayage a été utilisé pour examiner 25 spécimens provenant de 10 adolescents atteints de LJP. L'examen visait à déterminer les caractéristiques spécifiques suivantes: (1) distribution des colonies microbiennes sur la surface épithéliale; (2) caractéristiques topographiques de l'épithélium de la poche associé à des colonies microbiennes; et (3) types morphologiques prédominants auxquels appartiennent les colonies. Les observations faites au cours de cette étude ont mis en évidence les faits suivants: (1) l'épithélium de la surface du tiers coronaire de la paroi des poches avait un aspect essentiellement sain, on ne pouvait pas y distinguer de colonies microbienne ni de caractères topographiques inhabituels. (2) L'épithélium de la surface du tiers moyen présentait des colonies microbienne dispersées au hasard et consistant en 3 types morphologiques principaux, des coques, des bacilles et des cocci-bacilles. On trouvait aussi dans cette zone des signes morphologiques de la pénétration microbienne à travers la barrière épithéliale le long des espaces intercellulaires. (4) La zone du tiers apical était caractérisée par une infiltration lymphocytaire, une cavitation épithéliale avec ulcérations et quelques organismes pris au piège dans les treillis de fibrine. On ne pouvait pas distinguer de colonies microbienne dans cette zone, mais on pouvait y discerner des éléments isolés: spirochètes, fusiformes, organismes filamentaux, bâtonnets courts et longs.


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