Periapical bacterial plaque in teeth refractory to endodontic treatment


Abstract — It has recently been found that bacteria are able to survive and maintain an infectious disease process in periapical lesions of nonvital teeth. The purpose of this study was to examine the surfaces of root tips removed during surgical-endodontic treatment for the presence of microorganisms. A full thickness flap was reflected under strict surgical asepsis and the periapical lesions were enucleated and removed. About 2–3 mm of the root was cut off, rinsed in sterile saline and placed in 10% neutral-buffered formalin. Upon fixation, the root tips were dehydrated, air-dried and given an electrically conducting coat of gold in a vacuum evaporator. The root tips were then studied in a Jeol, JSM-U3 scanning electron microscope, usually operated at 20 kV. The root surfaces were covered with soft tissue, except at the apex of the roots, where a continuous, smooth and structureless coating was seen, apparently adjacent to the apical foramen. At higher magnification a variety of bacterial forms were recognized in the smooth coating. A bacterial plaque was observed in irregularities of the surfaces between fiber bundles and cells and in crypts and holes. The bacteria were held together by an extracellular material and the plaque was dominated by cocci and rods. Fibrillar forms were recognized as well, often with cocci attached to their surfaces.

In recent studies it has been shown that bacteria are able to survive and maintain an infectious disease process in periapical tissues (1, 2). Periapical lesions refractory to conventional endodontic therapy exclusively yield anaerobic bacteria or are heavily dominated by anaerobes. Black-pigmented and non-pigmenting Bacteroides species as well as anaerobic gram-positive rods and cocci are isolated, as are facultative Staphylococcus and Streptococcus species. The flora of longstanding lesions with fistulae often is dominated by enteric bacteria or Pseudomonas aeruginosa. Yeast, mostly Candida albicans, is isolated as well. The purpose of the present study was to examine microscopically the surface of root tips removed during surgical-endodontic treatment of teeth for the presence of microorganisms.

Material and methods

The patients participating in this study had all been referred to the Postdoctoral Clinic of the Department of Endodontics, University of Pennsylvania School of Dental Medicine for diagnosis and treatment of endodontic diseases. The findings in 10 patients are reported here.

Each patient had a tooth with a periapical lesion that had not responded to previous endodontic treatment. Fistulae were present in conjunction with 5 of the teeth. Periodontal probing was within normal limits. Four patients were retreated conservatively without success. In 6 patients this was not feasible because of large posts in the root canals and extensive prosthetic restorations.

All patients were therefore treated surgically. Following a sulcular incision, a full thickness flap was reflected and the periapical lesion was exposed under aseptic conditions. An incision was made into the lesion and bacterial sampling of the lesion was carried out using sterile endodontic paper points, which were inserted into the lesion towards the location of the root tip. Upon removal, the paper points (at least 3) were placed in vials with 3 ml of

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Moller’s VMGA III transport medium (3). The samples were processed using well-established aerobic and anaerobic techniques (4). The periapical lesion was then carefully enucleated and removed. An effort was made not to touch the root surfaces with curettes or other instruments. About 2–3 mm of the root was then cut off using a sterile fissure bur in a high speed handpiece under irrigation with sterile saline. The root tips were removed by means of sterile pliers, rinsed off in sterile saline and placed in 10% neutral-buffered formalin. Retrograde fillings were then placed in the roots (5) and the flaps were repositioned and sutured in place. All patients received penicillin (or erythromycin in case of penicillin allergy) for 7 days and the immediate postoperative healing was uneventful in all instances.

After 1 month, 1 patient came back with a fistula from the treated tooth. Based upon the results of the microbiological culturing, metronidazole (750 mg × 4 for 10 days) was prescribed and the fistula closed. Complete bony healing was evident radiographically in all patients after 6 months.

Upon fixation, the root tips were dehydrated, air-dried and given an electrically conducting coat of gold in a vacuum evaporator. The surfaces of the root tips were then studied in a Jeol, JSM-U3, scanning electron microscope, usually operated at 20 kV.

Results

Microorganisms were recovered from all periapical samples. The flora was heavily dominated by anaerobic bacteria as described in 2 previous studies (1, 2).

To the naked eye, the surgically removed root tips looked denuded. When examined in the microscope the root cementum was mostly not visible, but was covered with soft tissue or an extraneous smooth and structureless coating (Fig. 1). The smooth coating was seen as a continuous layer at the apex of the root, seemingly adjacent to the apical foramen, which was not visible in any of the specimens. In particular, the presence of a coating layer was obvious when occasional cracks or tears had led to a loosening of the layer from the underlying tooth structure (Fig. 2). In 9 of 10 specimens the distribution of the structureless coating was as seen in Fig. 1. In the 10th specimen only small discontinuous patches of the structureless material were seen in the apex area of the tooth. At higher

![Fig. 1. Scanning electron micrograph of root tip of human tooth removed during endodontic surgery. The root surfaces are covered with soft tissue except at the apex (A) where the root has a smooth structureless appearance. Note that the apical foramen is not visible. × 35.](image1)

![Fig. 2. Higher magnification of apical area (A) in Fig. 1. The smooth appearance of the root in this area is due to the presence of an extraneous, structureless coating layer visualized where cracks and tears have led to a loosening of the layer from the underlying tooth structure (arrow). × 110.](image2)

![Fig. 3. Higher magnification of apical smooth coating demonstrated in Figs. 1 and 2. Colonies of bacteria are seen in the structureless material. × 550.](image3)
Periapical bacterial plaque

Magnification a variety of bacterial forms were recognized in the smooth coating (Fig. 3). The bacteria had formed colonies and were completely covered by the structureless material. In other areas of the smooth coating there was no evidence of bacteria.

The soft tissue, which was seen on the root surface outside the apical area with the smooth coating, appeared to be in various stages of degradation (Fig. 4). Fibrillar structures were recognized and in some areas large fiber bundles were seen lying on the root surfaces (Figs. 5, 6). Individual cells could be recognized as well (Fig. 6) and small areas with an apparent intact cementoblast layer were seen (Fig. 7). In some specimens erythrocytes covered parts of the root in spite of a thorough rinsing prior to fixation. Only occasionally was the root surface visible between the soft tissue components (Fig. 8).

A bacterial plaque was observed in scattered areas of the root surface. The plaque was located in irregularities in the surface between fiber bundles and cells (Figs. 5, 6) and in crypts and holes (Figs. 4, 9). The bacteria were embedded in or held together by various amounts of extracellular material. This made it difficult to distinguish individual cells, although the plaque clearly was dominated by cocci and rods (Figs. 5, 6, 9).

However, in areas with minimal amounts of extracellular material a large variation of bacterial forms were recognized (Fig. 8). Due to the fibrillar nature of much of the soft tissue covering the root, filamentous organisms may have been overlooked. Still filamentous or fibrillar forms were recognized, often with cocci attached to their surfaces (Figs. 5, 6, 8, 9).

Fig. 4. Scanning electron micrograph from lateral aspect of root tip. Soft tissue with crypts and holes possibly representing vascular canals is covering the root surface.

Fig. 6. Scanning electron micrograph from lateral aspect of root tip. A bacterial plaque comprising mainly cocci and rods is seen between periodontal cells and fibers. ×6500.

Fig. 5. Scanning electron micrograph from lateral aspect of root tip. Fibrous structures of various sizes are seen. A bacterial plaque is present on and between the fiber bundles. Fibrillar forms with cocci attached to their surfaces are recognized. ×6500.

Fig. 7. Scanning electron micrograph from lateral aspect of root tip. An apparent intact layer of cementoblasts is covering the root surface. ×650.
Discussion

Previous findings (1, 2) that microorganisms may be recovered from periapical lesions refractory to endodontic treatment were confirmed in the present study. Microscopically, this study had clear limitations in that the periapical lesions were removed from the root tips during the surgical operation so that only remnants of granulomatous tissue left on the root surfaces could be examined. However, in spite of this, interesting findings were made that further elucidated the phenomenon of extraradicular endodontic infections.

Microorganisms were observed on the surfaces of all specimens studied. Scattered bacteria of different morphological types were seen, but mostly the organisms were present in aggregates or colonies in crevices and crypts of the tissue left on the root surfaces. An extracellular material was present between the individual bacterial cells, often obscuring their morphology. Still most colonies were dominated by cocci and short rods. Filamentous organisms appeared to be relatively scarce. However, the fibrillar character of much of the soft tissue covering the root may have made it difficult to distinguish both spirochete clusters and filamentous forms. Still filaments with cocci attached to their surfaces were seen (6) although fully developed “corn cobs” were not recognized (7–9). On the whole, the periapical bacterial plaque as seen in the scanning electron microscope appeared similar to periodontal plaque (7, 9, 10).

An interesting finding in this study was the conspicuous presence of a smooth and structureless material outside the main apical foramen. The presence of bacteria embedded in this material might indicate that it represented an extracellular material produced by the bacteria, in all likelihood extracellular polysaccharide (11–13). Extracellular polysaccharide provides a reserve of substrate (14) and may therefore be important for the survival and continuous growth of the bacteria outside the canal. It is also widely held that extracellular polysaccharide acts as an effective diffusion barrier (15, 16). It is conceivable, therefore, that its presence could be one reason that it has proven difficult to eliminate extraradicular bacteria with systemic antibiotic treatment, even after accurate microbiologic diagnosis (17).

However, the structureless material may also be the result of a host response to the infecting organisms. In this regard it should be alluded to the apparent similarities between the material observed in this study and the structureless material observed on tooth surfaces adjacent to periodontal pockets (8, 9). Originally, this material was considered to be a secretory product of the adjacent epithelial cells (18, 19), whereas the present evidence suggests that it may form by adsorption of components of gingival exudate seeping out between the junctional epithelium and the tooth surface incident to inflammation in the marginal periodontium (20). Since the structureless material in this study always was observed outside the apical foramen, it is tempting to suggest an analogous reaction in the marginal and the apical periodontium.

However, the similarities in the microbial flora of the periodontal pocket and the root canal should
be considered in this regard (21). Clearly, the structureless material both apically and marginally in the periodontium may be a combination of bacteria-produced extracellular products as well as components reflecting the local inflammatory reaction.

The question then remains where the extraradicular bacteria came from. It seems logical that the organisms observed embedded in the structureless material outside the apical foramen had come from the root canal. Even on the lateral aspects of the root tip, the bacterial colonies were mostly found in or near crypts or holes in the soft tissue coating. It is not clear what was the origin of these holes, but they often had a regular form and originally might have been nerve-vascular channels leading to accessory foramina and root canals. Thus, the microorganisms in these areas as well may have come from the root canal. However, in our microbiological studies of refractory periapical lesions, bacteria which are not considered common oral bacteria are frequently found (1, 2). An example is the recovery of Bacteroides fragilis from periapical lesions (2). It appears as a distinct possibility, therefore, that hematogenous spreading of bacteria to longstanding periapical lesions may occur.

References

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