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ORIGINAL ARTICLE

Status of bacterial colonization in teeth associated with different types of pulpal and periradicular disease: A scanning electron microscopy analysis

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Abstract *Background/purpose:* The purpose of this study was to use scanning electron microscopy (SEM) to investigate the status of bacterial colonization in differently infected root canals and the damage to radicular dentin.
Materials and methods: Twenty-five freshly extracted teeth were selected for this study (Group A: 8 teeth with pulpitis; Group B: 10 teeth with periapical lesions; and Group C: 7 teeth with failed root canal treatment). After fixation, the teeth were longitudinally split into two halves. The halves were then dehydrated, sputter-coated with gold, and viewed using SEM, descriptively dividing their lengths into apical, middle, and coronal thirds.
Results: In Group A, bacterial infection was mainly located in the coronal third of the root canals and bacteria failed to penetrate into the dentinal tubules. In Group B, bacterial infection was distributed over the entire length of the root canal. The invasion depth of bacteria into the dentinal tubules was approximately 300 μm. In Group C, bacterial infection was mainly focused on the apical third of the root canals. Most of the dentinal tubules had collapsed, and the root canal walls were heavily colonized with dense bacterial biofilm, primarily consisting of cocci. Compared to Group B, the invasion depths were deeper in the apical thirds of root canals ($P < 0.05$).
Conclusion: Bacterial infection was lighter in the root canals with pulpitis than in those with apical periodontitis, which might require special considerations regarding different stages of pulp and periapical pathology in root canal treatment.

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Introduction

Pulpal and periradicular disease is one of the most common infectious diseases threatening human health. Bacteria, usually from dental caries, are the major etiological agents of pulpal and periradicular disease.[1] Related research as previously described has indicated that infected root canals act as habitats for bacteria, which exist in planktonic forms, aggregates, and coaggregates and in the biofilm status in complex communities that are composed of bacterial cells and extracellular matrix.[2] Root canal therapy (RCT) is the most commonly applied, effective method in clinical treatment for pulpal and periradicular disease. The goal of treatment of the disease has been total eradication from the infected root canal systems and prevention of reinfection.[3] According to statistics, RCT has failure rates between 4% and 15%,[4,5] whereas the rate of successful root canal retreatment was approximately 74%.[6,7] The most important factor in endodontic failure is the incomplete eradication of bacteria.[8] With improved bacterial culture and analysis techniques, intracanal bacteria have been extensively studied and exactly classified.[9–11] However, these investigations have not provided sufficient information concerning the bacterial colonization status in the dentinal wall of different types of pulpal and periradicular disease. Knowledge of the status of bacterial colonization could provide us with a better understanding of the disease process. Scanning electron microscopy (SEM) has excellent resolution and can reveal details regarding the bacterial colonization status in natural environments.[10,12–14] Therefore, this study sought to characterize the process of disease and to provide a theoretical basis for the establishment of effective treatment strategies using SEM to examine the bacterial colonization of infected root canals associated with different types of pulpal and periradicular disease. Thus, it can provide us with clinical guidance.

Materials and methods

The study protocol was reviewed and approved by the Ethics Committee of Nanjing University Stomatological Hospital (Nanjing, China). Verbal and written consent was obtained from all of the study participants prior to extraction of teeth.

Patients and specimen collection

The examined material consisted of 25 extracted teeth randomly collected from 25 patients at Nanjing University Stomatological Hospital. Healthy adult volunteers were age 18–70 years. A detailed medical and dental history was obtained from each patient. All patients were in good health and were not taking any medication that would alter bacteria status during the past 3 months. Teeth with periodontitis or fractures were excluded from the study. Teeth were extracted for reasons not related to this study. All samples for the pulpitis group were from the third molars. For primary and secondary apical periodontitis, we collected samples from these cases as the third molars, those that could not meet with the lowest conditions for oral rehabilitation, and the extracted cases for personal reasons.

The extracted teeth were divided into three groups according to the clinical and radiographic examination: Group A (8 teeth): pulpitis; Group B (10 teeth): primary apical periodontitis; and Group C (7 teeth): secondary apical periodontitis.

The inclusion criteria were as follows. Group A: Pulpitis is characterized by a history of provoked and spontaneous dull, heavy, and lingering thermal pain that can be reproduced clinically. Radiographs show the depth of the caries or cavity preparation. The periodontal ligament space and lamina dura are normal.[15] Group B: Presence of clinical signs and symptoms of chronic apical periodontitis (pain, swelling, or sinus tract); no previous endodontic treatment; and diameter of the periapical radiolucent area of at least 3 mm.[16] Group C: Endodontic treatment performed for $>$ 2 years and radiographically visible filling of root canal: (1) the presence of obvious clinical signs and symptoms (pain on palpation, discomfort to percussion, and pain of the sinus tract); and (2) the persistent or emergent periapical radiolucency. The appearance of one of these two items is considered to be a treatment failure.[10]

Sampling procedures

After disinfection of the tooth crown and the adjacent tissues with 2% chlorhexidine digluconate solution, the tooth was carefully extracted. Subsequently, the periodontal ligament and other attachments were removed with a scalpel, and the clinical crown was sectioned at the cementoenamel junction with carborundum disks. Then, the teeth were immediately immersed in 2.5% phosphate-buffered glutaraldehyde solution. The sample teeth were stored at 4°C to provide a total fixation period of 1 week. After fixation, longitudinal grooves (approximately 2 mm) were cut along the entire root length with tapered diamond burs under a water spray. The roots were then split with a chisel into two halves. The root canal length was measured and divided equally into coronal, middle, and apical thirds, which were marked with a scalpel blade.

SEM preparation and observation

Each root half was then gently washed in phosphate buffered saline (pH = 7.2, 4°C), dehydrated in increasing concentrations of ethanol (50%, 60%, 70%, 80%, 90%, and 2 $\times$ 100% for 15 minutes each), critical-point dried using liquid CO_2 replacement, coated with gold, and imaged in a scanning electron microscope (S-3400N, Hitachi, Tokyo, Japan) at a voltage of 20 kV. Thereafter, the entire root half, the radicular dentinal wall, and the dentinal tubules were examined from low to high magnification using SEM. Observation was conducted, and photographic images were obtained.

Statistical analysis

Statistical evaluation of the study results was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) and