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SCIENTIFIC ARTICLES

Participation of Bacterial Biofilms in Refractory and Chronic Periapical Periodontitis

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The aim of this study was to examine morphologically the participation of extraradicular biofilm in refractory periapical periodontitis. Six teeth and five extruded root filling gutta-percha points associated with refractory periapical periodontitis were investigated by scanning electron microscope.

In nine of 11 samples examined, bacterial biofilms were seen at the extraradicular area. The gutta-percha surface was covered with glycocalyx-like structures, and filaments, long rods, and spirochete-shaped bacteria were predominant in the extraradicular sites. Planktonic cells, which were filaments and spirochete-shaped bacteria, emigrated from the glycocalyx structures in some spots. In the extracted teeth, biofilm consisting of both bacteria and glycocalyx-like structures were observed on the periapical root surfaces. Next to the residual periodontal ligament, a few filaments, rods, and fusiforms were attached on the healthy cementum surface.

The present findings suggested that bacterial biofilms formed in the extraradicular areas were related to refractory periapical periodontitis.

Recently, much evidence has been reported that the biofilm environment is often advantageous for bacteria and is important to the characteristics of the bacteria living there (1–3). Costerton et al. (1) reported that biofilm infections share clinical characteristics, and biofilms develop preferentially on inert surfaces or on dead tissue and occur commonly on medical devices and fragments of dead tissue, such as sequestra of dead bone. Moreover, they suggested that biofilms grow slowly and that biofilm infections are often slow to produce obvious clinical symptoms (1). Antibiotic therapy generally ameliorates the acute clinical symptoms, which lead to planktonic cells being released from the biofilm, but cannot kill the bacteria completely within the biofilm (2). Disengagement of planktonic bacterial cells from the biofilm is a natural pattern of the genetic program (3). Dental caries and marginal periodontitis are caused by plaque biofilm, and biofilms are involved in osteomyelitis, cystic fibrosis, necrotizing fasciitis, and bacterial prostatitis (1).

In general, the main etiologic factors of refractory and chronic periapical pathosis are bacteria, their metabolic products, and debris of infected pulp tissue remaining in lateral canals, dentinal tubules, or gaps between the root canal wall and root filling materials. If bacteria in infected root canals can invade the extraradicular area via the apical foramen and the dental tubules and can form bacterial biofilm, they and their components may play etiologic roles in refractory and chronic periodontitis. The presence of bacteria within the endodontic lesions has been investigated previously (4–6). Tronstad et al. (4–6) demonstrated that bacteria might live and maintain endodontic infections within periapical lesions. However, in a study by Walton and Ardjmand (7), no bacterial colonies were seen periapically in monkeys. They concluded that a superficial bacterial mass could contaminate periapical tissues during surgery or extraction and give a false-positive reading upon microbiological sampling in humans. Gutta-percha points from over-extended root fillings in endodontic failure cases, however, have been not examined histologically or morphologically in many studies.

The concept of infected biofilm is relatively new in endodontics, and it has been supposed that no vital microorganisms are present within periapical lesions in chronic periapical infections (8). The purpose of this study was to examine electron microscopically the surface of extracted teeth, root tips, and gutta-percha points removed during surgical or endodontic treatment for the presence of biofilm formation in periapical lesions.

MATERIALS AND METHODS

Subjects and Samples

Five, apically extruded, gutta-percha points removed during endodontic retreatment for chronic periapical pathosis and six teeth extracted for refractory periapical pathosis were used in this study (Table 1). Informed consent was obtained from all patients. In all cases, endodontic treatment had previously been performed by

TABLE1. Characteristics of the specimens

Patient	Gender	Age	Sample No.	Tooth*	Material	Antibiotics
a	F	26	1	14	GP	+
b	F	59	2	5	Tooth	+
c	F	63	3	24	GP	−
c	F	63	4	23	GP	−
d	M	45	5	3	Tooth	−
e	F	58	6	12	Tooth	+
f	M	16	7	31	Tooth	+
g	M	25	8	8	GP	−
h	M	35	9	15	Tooth	+
i	F	42	10	10	GP	−
j	M	50	11	20	Tooth	+

* Numbers denote tooth indices in the ADA system.
GP = gutta-percha point.

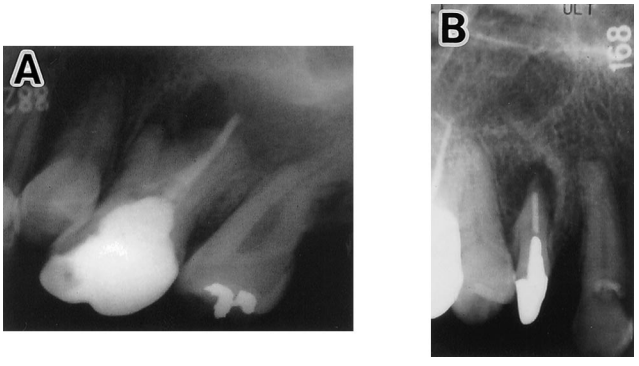

FIG. 1. Radiograph of two specimens at initial examination. (*A*) Filling material (gutta-percha point) is observed outside the palatal root canal (sample 1). (*B*) A large periapical lucency is present (sample 2).

general practitioners, and periapical radiolucency existed. Figure 1 shows a radiograph of two specimens (samples 1 and 2) examined before endodontic retreatment. At the time of sampling, fistulas were seen in five cases (samples 1, 3, 5, 9, and 10). The periodontal pocket depth was less than 3 mm in all cases, and all periapical lesions were isolated from the gingival sulcus. Antibiotics and anti-inflammatory drugs were systemically administered in six cases (samples 1, 2,6,7, 9, and 11) during the endodontic treatment.

Preparation of Scanning Electron Microscopic Observation

The 11 samples were extracted carefully to avoid contaminating them with surrounding bacteria and were then prepared by the methods of Noiri and Ebisu (9) with slight modification. The specimens were immediately fixed with microwave irradiation (2,450 Hz, 500 W) in 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1-M sodium cacodylate buffer for 15 s and immersed in the same fixative for 1 to 2 h at 4°C. After fixation, the extraradicular part of the gutta-percha points was cut longitudinally and separated into two specimens. All specimens were dehydrated in graded aqueous ethanols, and the specimens were dried by using a t-butyl alcohol freeze dryer (JFD-300, JEOL, Tokyo, Japan), coated with OsO4 of approximately 5-Å thickness with a plasma multicoater (PMC-5000, Meiwa Co., Osaka, Japan), and observed by scanning electron microscope (SEM) (JSM-5300, JEOL) operated at 15 kV.

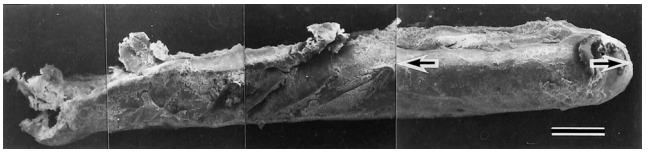

FIG. 2. SEM image of gutta-percha specimen (sample 1). The extruded material (*arrows*) is approximately 2 mm (original magnification ×50; bar = 500 μm).

RESULTS

Extraradicular biofilms were found in nine of 11 samples examined. Figure 2 is a SEM image of a whole gutta-percha point sample (sample 1). In this sample, the length of the extraradicular part was 2 mm, and the whole length of sample was 5 mm at the time of sampling. The gutta-percha surface was almost completely covered with a glycocalyx-like structure (Fig. 3A). In most parts of the extraradicular area, numerous filamentous or spirochete-shaped bacterial cells were aggregated (Fig. 3B). Planktonic bacterial cells were released from the biofilm structure (Fig. 4). A few cocci and short rods were observed on the surface or at the interstice of glycocalyx-like structures [Fig. 5 (A and B)].

Figure 6 shows the whole periapical part of an extracted tooth (sample 2). External resorption was seen in two of six samples examined. The periodontal ligament disappeared within the body of the periapical lesion (Fig. 6). Most periapical root surfaces, which had been in the lesion, were covered with biofilm. At the internal root canal wall of the periapical foramen, fusiform and filamentous bacteria were observed (Fig. 7). Long rods and filaments predominately formed the bacterial biofilms on the extraradicular periapical root surface [Fig. 8 (A and B)]. In some locations, filamentous bacteria jutted out from the biofilm structure (Fig. 8A). Cementum domes, which were found on the healthy cementum surface, were observed next to the residual periodontal ligament, and rods, filaments, and fusiform microorganisms formed small colonies (Fig. 9).

DISCUSSION

In both the extracted teeth and gutta-percha specimens, similar biofilm structures were seen on the extraradicular structures within the body of the lesion. These structures had been built up over a long time, and it is suggested that these biofilm structures did not result from bacterial contamination during sampling and prepara-

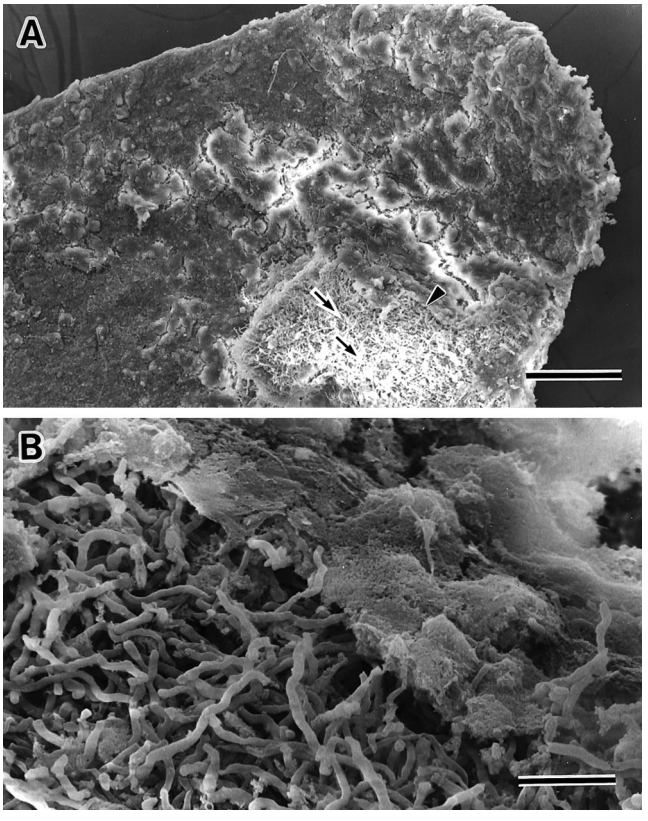

FIG. 3. SEM image of extruded gutta-percha. (*A*) Bacterial cells aggregate without a covering of a glycocalyx-like structure (*arrows*). Other areas are covered with a glycocalyx-like structure (original magnification ×350; bar = 50 μm). (*B*) High magnification of *arrowhead* area in (*A*). Glycocalyx structure is present in the upper right area but not in lower left area. Filamentous or spirochete-shaped bacteria are observed in lower left (original magnification ×3500; bar = 5 μm).

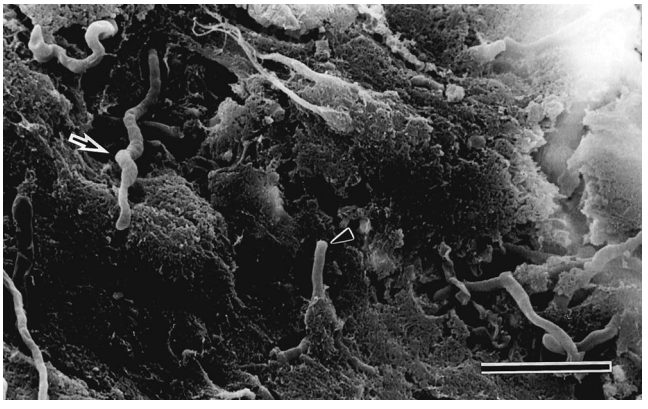

FIG. 4. SEM image of over-filling area from a gutta-percha specimen. Spirochete-shaped bacteria (*arrow*) and filaments (*arrowhead*), which are suggested to be planktonic bacteria, come out from the biofilm (original magnification ×5000; bar = 5 μm).

tion of specimens. Most previous studies showing the presence of bacteria within lesions have been carried out on extracted teeth or surgical biopsies from endodontic failure cases (4–6). However, some histological studies of periapical endodontic surgical specimens did not identify bacteria outside the periapical foramen (10,

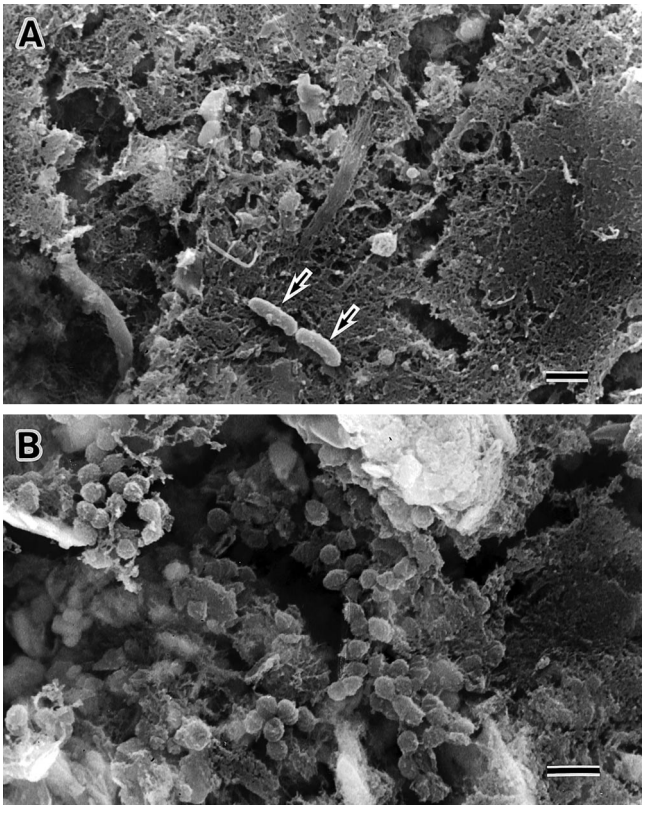

FIG. 5. SEM image of the over-filled area from a gutta-percha specimen. (*A*) A few short rods (*arrows*) are located on the superficial layer of the glycocalyx-like structure (original magnification ×7500; bar = 1 μm). (*B*) Small colony of cocci is observed at the crack in the biofilm structure (original magnification ×10,000; bar = 1 μm).

11). This might occur because of disturbance to the periapical biofilm during the root-end resection. In the excessive root filling cases with periapical lesions, filling materials, for example gutta-percha points, could play a role in the initiation infection. It is considered that the removal of gutta-percha points is relatively easy because of the presence of microgaps between the gutta-percha point and root canal wall, and thus contamination of the sample is less likely compared with both surgical biopsy and tooth extraction cases.

Dahle et al. (12) reported that spirochetes isolated from the root canal were 140 microns long and 2 microns thick. It was suggested these spirochetes were longer and thicker than treponeme. Spirochete-shaped bacteria, which were detected at the extraradicular sites in this study, were large (longer than 20 microns). This study suggested that undetected oral spirochetes were also associated with the extraradicular infections. The bacterial morphotype at the extraradicular sites resembled the findings of Nair et al. (13), who demonstrated the predominance of filaments and the low frequency of cocci and rods in the apical root canals affected with refractory periapical periodontitis. We did not identify the bacterial morphotype in the root canal, but filaments were predominantly observed on the surface of gutta-percha points located in the root canal.

Debelian et al. (14) investigated bacteremia in conjunction with endodontic therapy. They reported that, in 12 of 26 patients examined, 8 bacterial species were recovered from the blood stream and suggested that those bacteria invaded the blood via the peri-

FIG. 6. SEM image of periapical area of an extracted tooth (sample 2). External root resorption (*arrow*) is present surrounding the periapical foramen. PL = residual periodontal ligament (original magnification ×35; bar = 500 μm).

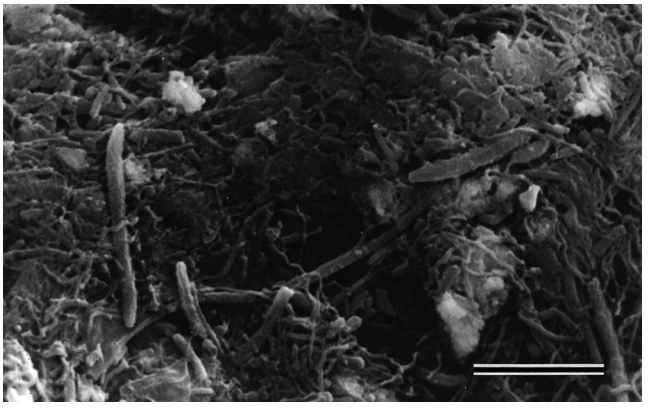

FIG. 7. SEM image of an apex within the body of a lesion of an extracted tooth. Filaments, rods, and fusiform bacteria form a bacterial biofilm at the internal wall of the periapical foramen (original magnification ×5000; bar = 5 μm).

apical foramen from the root canals. The morphotypes of bacteria isolated from blood were predominantly rods and cocci: *Propionibacterium acnes, Prevotella intermedia, Streptococcus sanguis,* and *Streptococcus intermedius*. In this study, filamentous and spirochete-shaped bacteria predominantly formed biofilms at the extraradicular area.

It is reasonable for extraradicular infections to be caused during the acute phase of periapical periodontitis. However, from a biofilm infectious viewpoint, it is possible to infect extraradicular sites with endodontic flora in both the chronic and acute phase because biofilms are resistant to host immune responses and could function as a focus of acute infections (1). In cases of refractory periapical periodontitis, bacteria in the infected root canal might invade extraradicular sites and form a biofilm on the periapical root

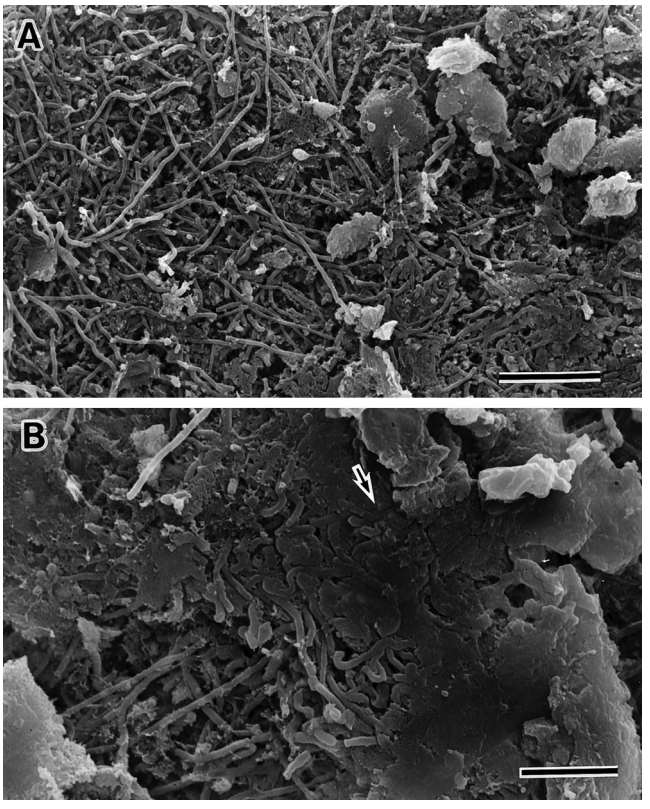

FIG. 8. SEM image of an apex within the body of the lesion of an extracted tooth. (*A*) Filaments or long rods predominantly colonize and constitute a mixed bacterial flora on the periapical root surface (original magnification ×2000; bar = 10 μm) (*B*) Mature glycocalyx-like structure (*arrow*) and filaments form the biofilm (original magnification ×3500; bar = 5 μm).

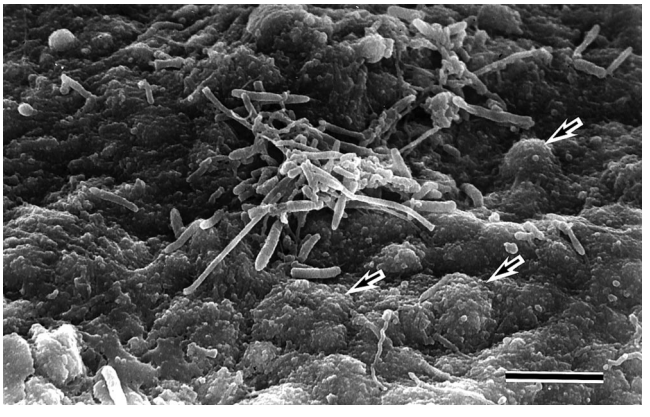

FIG. 9. SEM image of an apex of an extracted tooth. Cement domes (*arrows*) and a microcolony are seen on the root surface next to the residual periodontal ligament (original magnification ×3500; bar = 5 μm).

surface within the lesions. The findings of this study, in which bacterial biofilms were detected at the extraradicular area within the body of lesions, is circumstantial evidence of the presence of bacteria within periapical lesions.

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