Topography of periodontally involved human root surfaces after different chemical treatment modalities: An *in vitro* scanning electron microscopic study

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**Abstract:** The significance of chemical and conservative treatments of cemental tissue proximal to periodontal pockets has been pointed out in recent years. This *in vitro* scanning electron microscopy (SEM) study aimed to investigate the surface effects of topical applications of 0.1 % cetylpyridinium chloride (CPC) and 2 % sodium lauryl sulfate (SLS) and polishing on the periodontally involved root surfaces of human teeth. Ten single-rooted teeth from 8 patients with advanced adult periodontitis were included. Following extraction, any calculus was removed with extreme care to preserve as much cementum as possible. Eighty root specimens were prepared. Fresh solutions of CPC and SLS were applied for 1, 3 and 5 minutes each to 10 segments of root cementum. A total of 20 segments formed the polished (P) and control (C) groups, respectively. The results showed that the surfaces treated with CPC or SLS differed considerably from polished and control specimens. Depending on time, the surface coating was partly or wholly removed, leaving a nodular cementum structure, uncovering a fibrillar collagen substrate and the openings of dentinal tubules. Scarcely debris was present on both control and polished surfaces, whereas bacteria were observed only on the control specimens. In view of these results, further definitive *in vitro* and *in vivo* research must be done to determine the advantages of chemical treatment and its effect on periodontal regeneration. (*J. Oral Sci. 42, 139-146, 2000*)

**Key words:** Periodontitis; root surface; CPC; SLS; SEM.

**Introduction**

The goal of periodontal therapy is the predictable regeneration of tissues supporting tooth surfaces that were previously exposed owing to periodontal disease (1,2). One of the major reasons for lack of regeneration appears to be the root surface, which presents structural alterations and becomes cytotoxic when exposed to the oral environment (1-4). Conventionally, scaling and root planing with hand scalers are the widely used methods for eliminating calculus, bacterial deposits, and diseased cementum contaminated by bacteria and their products (5-7). Thorough removal of root surface deposits is a key factor for successful periodontal therapy. However, despite visual appearances, scaling does not successfully render the root surfaces free of plaque and calculus, and also locations such as furcations are barely accessible to instruments (8,9).

Until the present, in an attempt to make the root physiologically acceptable for the regeneration of a new connective tissue attachment, root surfaces have been treated with many substances (1,2,10). Conditioning of the root surface by topical application of acid solutions has been introduced as a regenerative procedure to dissolve the smear layer produced by root instrumentation, to aid in detoxification of the root surface and to expose embedded collagen fibers (11-14).

Some studies have indicated, however, that a denuded dentin surface is unsuitable for the regeneration of a connective tissue attachment (15,16). Nyman et al. (17), in an experimental study, suggested that removal of the diseased root cementum for eliminating endotoxins is not
necessary for periodontal health. In addition, several studies have supported the concept that endotoxins bind only superficially to cementum and fail to penetrate into the subsurface tissue (8,18-22). Also, some experimental studies have shown that it is possible to chemically condition the exposed root surface to encourage fibroblasts and save as much cementum as possible to form a new attachment (23,24). In an experimental study in monkeys, Blomlöf et al. (23) found that chemical cleaning of periodontally involved cementum with two detergents, cetylpyridinium chloride (CPC) and sodium lauryl sulfate (SLS), without root planing resulted in significant new attachment.

These studies led to different treatment modalities, superficial and/or chemical cemental curettage (25-27), and polishing (28,29) avoiding excessive or deep removal of the diseased cementum. Polishing has been suggested as an effective treatment method for eliminating bacterial plaque and endotoxins from the diseased root surfaces (28,29). Schwartz et al. (29) showed that plaque bacteria can be almost completely removed from exposed root surfaces with rubber cups alone.

Therefore, preservation of cementum seems to be preferable to deep scaling and root planing, although it may not be routinely applicable in conventional periodontal therapy (4,8,9). Preparation of root surfaces by chemical detoxification has been suggested in order to achieve a predictable healing response and also to preserve cementum on periodontally involved teeth, but the management of cemental tissue proximal to periodontal pockets has been and continues to be controversial (4,5,30). In addition, it is not well established whether detergents or solvents may be used for root conditioning, and how they affect the topography of root surfaces. Therefore, the goal of the present study was to investigate by scanning electron microscopy (SEM) the in vitro effects of two chemical treatments (CPC and SLS) and polishing on the topography of periodontally involved cementum surfaces.

**Materials and Methods**

Ten freshly extracted single-rooted teeth from eight patients, aged 35-45 years, with advanced adult periodontitis were used for this study. All teeth obtained for the study were vital, without a history of acute pain or swelling, free of root caries and cervical restorations, and also showing radiographic evidence of at least 50 % alveolar bone loss, bleeding on gentle probing and attachment loss of 6 mm or more. None of the selected teeth had been subjected in vivo to periodontal instrumentation for at least one year prior to extraction. This study was performed with all ethical considerations, and voluntary consent was obtained from participating patients. All teeth were extracted taking special care not to damage the root surfaces, and then placed individually in sterile capped tubes containing distilled water.

**Preparation of teeth**

Prior to the laboratory phase, the collected teeth were cleaned of blood and saliva with a soft-bristled toothbrush and washed thoroughly with distilled water. The level of connective tissue attachment on each tooth was marked with a diamond marking pencil. All teeth received gentle scaling only for the removal of visible calculus deposits, and no further root planing or instrumentation of the root surface was performed.

The crown of each tooth was removed 1 mm above the cemento-enamel junction with a water-cooled, low-speed circular diamond saw. In order to prepare four surfaces (for CPC, SLS, polishing and control) on the same tooth, each tooth was subdivided longitudinally into four segments. One segment from each tooth was used for the polishing of the cementum surface and one served as a non-treated control. The remaining 2 segments were subdivided horizontally into thirds. All segments were then irrigated with distilled water for 1 minute to remove the grinding debris. The subdivided blocks of one segment were treated with 0.1 % CPC, a cationic detergent, for 1, 3 and 5 minutes, respectively. The subdivided blocks of the other segment were treated with 2 % SLS, an anionic detergent, for 1, 3 and 5 minutes, respectively.

**Treatment of root segments**

Fresh test solutions of 0.1 % CPC and 2 % SLS (99 % purity, Sigma Chemical Co., St. Louis, MO) were prepared immediately prior to use. To allow the agents to wet the surfaces without burnishing, cotton pellets moistened with the CPC and SLS solutions were applied with light pressure onto the root surfaces. During this application, the cotton pellets were changed at 60-s intervals. Specimens for polishing only were polished with a rubber cup and prophylaxis paste (Nupro, 801231, Ash/Dentsply, York, PA) for 10 seconds. No treatment procedure was performed for the control group of surfaces. Following treatments, all the root sections were rinsed with distilled water for 1 minute and prepared for SEM evaluation.

**Preparation for scanning electron microscopy**

Specimens were fixed with 2.5 % glutaraldehyde in phosphate buffer (pH 7.3) for 24 hours at 4 °C. After each specimen had been washed three times for 10 min in phosphate-buffered solution, it was post-fixed by immersion in phosphate-buffered 1.5 % osmium tetroxide (OsO₄) for
2 hours. The specimens were then dehydrated in an ascending graded series of aqueous ethanol solutions. The samples were dried and sputter-coated with a layer of gold, approximately 20 to 30 nm thick, and then examined with a scanning electron microscope (JEOL-JSM-6400).

Photomicrographs at different magnifications were obtained from the root surfaces in order to achieve a representative view of each group.

**Results**

SEM evaluations were made only on the diseased root areas, which were above the reference groove.

**CPC-treated specimens**

Different application times appeared to cause considerable differences in the topographic features of the root surfaces (Fig. 1).

**One-minute application.** Both a smooth layer and, under this layer a mosaic-like structure resembling healthy cementum, were distinguished. This nodular appearance seemed to be gradually masked and attained a more smooth texture, suggesting that this smooth layer may have indeed been a surface coating, similar to that described previously (9,11,18,19,31). In particular, the underlying cementum surface was revealed through the occasional cracks, which supports the view that this was a surface coating. Some remnants of separated coating layers, giving the impression of a brittle structure, were also visible (Fig. 1a). This smooth layer, though not continuous or uniform, varied in distribution but was always observed on the root surfaces of all specimens and tended to mask the details of the root surface.

**Three-minute application.** Root specimens treated with CPC for 3 minutes seemed to lose the smooth surface layer in nearly all areas, leaving an apparent mosaic-like appearance of cementum (Fig. 1b).

**Five-minute application.** This group of specimens displayed a perforated appearance, probably reflecting the effects of the CPC over the root surface. Besides an eroded surface coating, some areas were characterized by exposed dentinal tubules and finger-like collagen projections (Fig. 1c).

**SLS-treated specimens**

Figure 2 is a representation of this group of specimens. The surface morphology of teeth exposed to SLS application showed topographic characteristics similar to those of the CPC group.

**One-minute application.** In this group a smooth appearance of the surface was distinguished in general, whereas some areas were perforated and/or eroded in appearance (Fig. 2a).

**Three-minute application.** The root surface exhibited an irregular architecture, both a smooth and a perforated and/or eroded layered appearance, and the mosaic-like structure of the cementum (Fig. 2b).

**Five-minute application.** In this group, besides the layered surface coating, a collagenous structure and dentin

![Fig. 1](image)

a. Specimen showing 1-minute CPC application which demonstrates the presence of a surface coating (co) by exposing the nodular texture of the underlying cementum (c) surface.

b. 3-minute CPC-treated specimen showing the mosaic-like appearance of cementum, without any covering surface layer.

c. Root surface appearance after application of CPC for 5 minutes. The perforated appearance, with eroded surface coating (co), dentin tubule orifices (arrows) and collagen projections (arrowhead), is shown (original magnification × 850; bar: 10 μm).
tubules were observed. Exposed collagen-like projections protruding from the underlying root surface were visible, similar to the 5-minute CPC specimens, but fibers were in abundance in this group. Fibers that were exposed manifested themselves either as collagen bundles or as a mesh composed of fibers of a much smaller diameter (Fig. 2c).

Polished specimens
All polished specimens showed a smooth surface topography, and patterns due to calculus removal and polishing were visible. Also some debris was detectable on the surface of all specimens (Fig. 3a,b).

Control group
At the sites on the specimens where calculus had been removed, striae and grooves were detected without evidence of distinct debris, unlike the untouched areas (Fig. 4a).

On the other hand, high magnification of the untouched areas showed lobulated structures. Only in this group were the root surfaces not completely free of plaque bacteria, and there was evidence of microorganisms at the surface (Fig. 4b).

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**Fig. 2**

a. Representation of a 1-minute SLS application showing both the smooth and eroded appearance of the surface coating (co).
b. 3-minute SLS application, showing the layered surface coating (co), followed by the nodular appearance of cementum (c).
c. In areas where the nodular cementum has disappeared, exposed collagen-like projections (arrowhead) as bundles or a fiber mesh are visible on the 5-minute specimen of the SLS group. The layered surface coating (co) is also visible (original magnification × 850; bar: 10 µm).

**Fig. 3**

a. Polished cementum specimen displaying a smooth surface with scarce debris (original magnification × 850; bar: 10 µm).
b. Debris and parallel lines possibly caused by a rubber cup or curettes during calculus removal are visible at high magnification (original magnification × 5000; bar: 1 µm).
Discussion

This study was mainly concerned with the changes that occurred on periodontally involved root surfaces following chemical and conservative treatment. We used two surfactants, CPC, a cationic surfactant, and SLS, an anionic surfactant, because of their detergent properties and their inhibitory effects on bacteria associated with periodontal diseases. Solutions of CPC and SLS were applied at a concentration within the range used in toothpastes and mouthwashes, and the time of application varied from 1 to 5 minutes to allow evaluation of time-dependent changes, if any, on root surfaces. In order to allow different intervals of application on the same root specimen, the detergent segments were subdivided horizontally into thirds. Since calculus deposits are virtually always associated with viable microbial deposits and obscure the details of root surfaces, in all groups, calculus was removed from the root surfaces with great care to preserve the underlying cementum layers.

The results of this study confirm previous findings that the smoothness detected at the clinical level does not exist at the SEM level, and the surface possesses a complex structure (11,32).

In vitro application of CPC solution for 1 minute and SLS solution for 3 minutes partially removed a smooth layer from the root surfaces, leading to the exposure of a nodular structure. This smooth layer was similar to a surface coating which has been reported previously, and also seemed to be mineralized, as it tended to flake off from the subjacent cementum (9,11,18,19,31,33). Eide et al. (18,19) have observed, using SEM, a mineralized surface coating on dental cementum which is due to periodontal disease. They concluded that this coating is derived from components of inflammatory exudate within periodontal pockets, that this might be a reservoir of cementum-associated endotoxin, and that it corresponds to the dental cuticle. Furthermore, Friedman et al. (31) showed the presence of a surface coating, which they named the dental cuticle, on the exposed cementum of adult teeth affected by periodontitis, and indicated that this structure may both mediate bacterial adhesion and adsorb components from the periodontal pocket. Although several theories have been proposed with regard to the origin and formation of this coating, its origin still remains unclear (18,31).

It was interesting to note that, in specimens having CPC applied for 3 minutes, the surface coating was almost totally removed, leaving a nodular appearance; on the other hand, in specimens having SLS applied for 3 minutes, besides the nodular appearance, this coating still remained and seemed to have a layered structure. In their transmission electron microscopy (TEM) study, Friedman et al. (31) stated that the surface coating was not homogeneous, but had a layered structure, suggesting an appositional growth pattern.

The present results showed that a 5-minute application of CPC or SLS solution to the root surface partially demineralized the outer smooth root surface, opened the dentinal tubules, and exposed collagen fibres. This finding was similar to the demineralization effect produced by acid solution applications on root surfaces, as previously described (2,11). Specimens were obtained from a root segment extending from the cementoenamel junction to the attachment level, so variations in fibril exposure and differences in the surface appearance may possibly be
explained by (apart from the effects of detergents) the variable anatomical structure of the cementum layer or thickness and/or mineralization of the surface coating, as mentioned before (19,33), or a combination of these variables.

Where CPC or SLS had been applied, the appearance of a distinct surface coating with an underlying nodular structure raised the question of whether the relatively smooth root surfaces observed in the polishing and control groups were, in fact, surfaces that had lost prominences due to the absence of Sharpey fibers or whether the prominences were masked by a surface coating. As we prepared the experimental and control surfaces on the same tooth segments, we propose that the underlying nodular cementum was probably masked with a surface coating in the polishing and control groups. As mentioned before, the smooth surface structure seen in periodontitis-involved root surfaces is caused by a coating on the surface of the normal cementum (11,33,34).

In this study, among the groups, bacteria and a lobulated coating layer were observed only in the control group. This finding is in accordance with that of Friedman et al (31), who reported that, when the surface coating was covered by subgingival plaque, or with scattered bacteria, the most common appearance of the coating was of an irregularly lobulated surface. According to these findings, it can be speculated that the lobulated surface coating may gain a smooth structure following polishing or detergent applications.

It is not well known whether the surface coating interferes with attachment in humans in vivo. In their in vitro tissue culture TEM study, Fukazawa et al. (9) showed that superficially cutted cementum was devoid of any surface coating, and many cells were attached not only to the experimentally scaled cementum but to the diseased cemental surface as well, and although apparently attached, cells proximal to calculus or a surface coating suffered definite cytotoxic damage. As mentioned by Eide et al. (18,19), endotoxins of the microorganisms and the components of the gingival exudate may be incorporated into the surface coating during its formation and mineralization, and therefore this coating may also be a carrier matrix for exogenous cytotoxic substances, previously thought to be located in the cementum. The key question that must be answered appears to be whether any pretreatment of cementum should aim to eliminate this coating, and correspondingly, periodontal regeneration needs to be investigated by in vivo studies.

The results of this study are limited to the physical root surface changes and the potential of the tested solutions. Although chemical treatment of periodontally involved root surfaces aims to preserve the cementum, in this study CPC and SLS treatments resulted not only in the partial and/or total loss of the surface coating, but with application for 5 minutes exposed a fibrillar collagen substrate and dentinal tubules as well. Further studies are needed to establish whether the morphological surface changes produced by the application of CPC and SLS solutions may provide a biologically acceptable environment favorable for connective tissue cell repopulation of the diseased root surfaces and to investigate the solution concentrations, application times and the appropriate detergent to maximize their benefits as root surface conditioners.

References
previously damaged by periodontitis. J. Periodontol. 65, 168-176


