Endodontic infections and apical periodontitis. An analysis of microbial factors prior, during and after endodontic treatment

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Abstract. - The aim of this study was to develop a test model to quantify the penetration of bacteria into dentinal tubules. The model consisted of two compartments separated by a bovine dentine specimen with a thickness of 1.5-3.1mm. The root cementum was removed from the root surface and the specimens were oriented in the model with the pulpal side facing the inoculated chamber of the test model. One compartment contained the test organism and the other was filled with sterile broth that was evaluated for growth of the test organism. The depth of bacterial penetration was measured in the dentine with or without a smear layer using both a histological and a quantitative recovering grinding technique after 6 weeks of exposure to the microorganisms.

_E. faecalis_ penetrated the dentine significantly deeper than _A. israelii_ \((p < 0.001)\). After removal of the smear layer with EDTA _E. faecalis_ penetrated significantly deeper than in the dentine pretreated with saline only \((p < 0.01)\) or with a combination of saline and sodium hypochlorite \((p < 0.01)\). Microorganisms were found in 89% of the cultured specimens and in 80% of the specimens that were evaluated with light microscopy. Total penetration through the dentine specimen and infection of the broth in the test compartment of the model occurred in only 2 out of 72 specimens. Collection and immediate culturing of infected dentine dust and counting colony forming units (CFU) allowed an overview of the number of bacteria per sample and was more sensitive than microscopy. Removal of the smear layer enhanced bacterial penetration.

The main objective in the treatment of infected root canals is the elimination of micro-organisms from the root canal system because they are the main factor in the development of periapical inflammation. Recently, concern has been expressed about the fate and influence of bacteria left in dentinal tubules after root canal preparation (Abbott 1990, Chong & Pitt Ford 1992, Oguntebi 1994, Peters et al. 1995). It may be questioned whether micro-organisms in the dentinal tubules of roots will survive after instrumentation and obturation, and whether they can multiply to sufficient numbers and produce enough toxins to maintain or develop periapical inflammation (Peters et al. 1995). Because of this potential risk, various disinfection regimens have been tested in vitro to determine the extent to which they kill micro-organisms in the dentinal tubules of roots (Ørstavik & Haapasalo 1990, Siqueira & de Uzeda 1996, Gomes et al. 1996, Heling & Chandler 1996).

In order to study the effect of several treatment modalities on the fate of bacteria in root dentinal tubules, it is necessary to know more about infection of tubules in vitro including the depth of penetration, and the number of bacteria present. In vitro studies have shown that bacteria do penetrate into the tubules (Akpata & Blechman 1982) and remain viable there for periods of up to 10 days (Haapasalo & Ørstavik 1987). It appears that several factors influence the depth of penetration of micro-organisms. For example, the presence of a smear layer prevents complete penetration, whilst etching of the dentine before exposure to the bacteria results in deeper penetration (Safavi et al. 1989).

According to Akpata & Blechman (1982), the more bacteria and the higher their multiplication rate, the deeper the penetration will be. They found that increased exposure times of dentine to bacteria led to an increase in the number of infected tubules and in penetration depth; Ørstavik & Haapasalo (1990) confirmed these observations. In their in vitro study, Enterococcus faecalis and Streptococcus sanguis penetrated the dentinal tubules to a depth of 300-400 μm within 2-3 weeks. Pseudomonas aeruginosa was rarely found in the scanning electron microscopic sections, even after 4 weeks of incubation, while they were found frequently in bur samples. They explained the discrepancy between microscopic observations and culturing by emphasising the susceptibility of Ps. aeruginosa to transportation during the cutting sequence or by the fact that only few cells of Ps. aeruginosa were required for a positive cultural identification; the relatively small numbers passing unnoticed in scanning electron microscopic views. Perez et al. (1993a, 1993b) found a mean penetration depth of 479 μm for Streptococcus sanguis after 28 days of incubation with a maximum value.
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of 792 µm. Love (1996) found that Streptococcus gordonii could penetrate in the dentinal tubules up to 200µm in the coronal and middle portion of the root and to 60µm at the apical extent of the root canal. Siqueira et al. (1996) found that every anaerobic bacteria they tested was able to penetrate into dentinal tubules, all to a different extent.

If bacteria are inoculated from the coronal part of the tooth there are more tubules infected in the cervical area of the dentine than in the middle or apical part (Akpata & Blechman 1982). Bacterial penetration from the pulpal side is deeper when the cementum is removed from the root surface (Haapasalo & Ørstavik 1987, Adriaens et al. 1988). If cementum is not present on the root surface, bacteria are able to colonise the tubules from the periodontal side. The speed of penetration from this side is lower than that from the pulpal side (Haapasalo & Ørstavik 1987, Adriaens et al. 1988). It slows down when bacteria reach the middle portion of the root (in vivo), suggesting that if the source of nutrition (pulp or periodontium) is situated further away, the growth of micro-organisms is reduced (Adriaens et al. 1988). It could be concluded from the work of Adriaens et al. (1988) that one important factor for invasion of dentinal tubules by bacteria may be the availability of a nutrient source. Ingrowth or progress of bacteria can be delayed or prevented by the presence of a smear layer and an intact root cementum and periodontal ligament (Haapasalo & Ørstavik 1987, Safavi et al. 1990).

All these studies have been conducted to either show that bacteria do penetrate the tubules or to develop a model for the evaluation of disinfectants and their action on micro-organisms in the dentinal tubules. Most studies evaluate the penetration depth solely by scanning electron microscopy (SEM) or light microscopy (LM) (Siqueira et al. 1996). In the studies of Akpata & Blechman (1982), Ørstavik & Haapasalo (1990), Perez et al. (1996) and Heling & Chandler (1996) bacteria were cultured after penetration into the tubules. Dentine shavings were collected in broth to test for viability of the bacteria, which are a qualitative measure rather than quantitative. In order to study the effects of several treatment modalities, it is necessary to develop an in vitro model in which it is possible to measure the number of micro-organisms that penetrate and survive in the root dentinal tubules under different circumstances. The purpose of this study was to evaluate the effect of dentine surface treatment on the penetration depth from the pulpal side in root dentine and on the survival of two bacterial species by determining the number of surviving bacteria per milligram of dentin (CFU/mg). More specifically we focused on:

1. The influence of removal of the smear layer on the penetration depth of E. faecalis in root dentinal tubules by determining the colony forming units per milligram of dentine (CFU/mg) in three different layers.
2: The comparison in depth of penetration between *E. faecalis* and *A. israelii*.

3: The comparison between histologic observations of micro-organisms present in root dentine and those made by determining viable bacteria.

*E. faecalis* was chosen as a test organism since it is associated with persistent apical inflammation (Engström 1964, Haapasalo *et al.* 1983, Sundqvist *et al.* 1998, Molander *et al.* 1998) and because *E. faecalis* seems to be the most suitable organism to infect dentinal tubules as it leads to gross infection in most studies where it is used (Akpata & Blechman 1982, Ørstavik & Haapasalo 1990). *A. israelii* was chosen because it is a facultative micro-organism isolated in endodontic infections.

**MATERIALS AND METHODS**

*Test device (figs 1,2)*

Two cylindrical compartments A and B were connected to acrylic washers (C) and rubber-o-rings (D, E) sealing the device to the experimental dentine specimen (F) and controlling the surface area (central opening in C) of dentine to be exposed (1.77 mm²). The device is closed by a screw (G) providing tension on the rubber rings and holding the specimen in place. Metal tubes allowed access to both compartments using slender (27 gauge) needles (Reeder *et al.* 1978).

The dentine specimen between the two chambers was orientated with the pulpal side facing the inoculated chamber (A). Chamber B contains sterile broth to provide a nutrition source on the ‘cemental’ side of the dentine.

**Figure 1** Two cylindrical compartments (A, B) are connected to acrylic washers (C) and rubber o-rings (D, E) sealing the device to the experimental dentine specimen.
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(F). The surface area of dentine to be exposed (1,77mm², center of C) is controlled by the acrylic washers (top of figure). The dentine specimen (F) between the two chambers is orientated with the pulpal side facing the inoculated chamber (A).

Figure 2 The test device is closed by a screw (G) providing tension on the rubber o-rings and holding the specimen in place. Metal tubes allow access to both compartments (A, B) using 27 gauge needles.

Dentine specimens

From freshly extracted bovine incisors the coronal and the apical third of the root were removed leaving the middle portion to be used for the experiments. The sections were split longitudinally and ground flat at the cemental and pulpal sides (Fig. 1) resulting in a dentine specimen with smear layer. The thickness of the dentine specimens was measured and ranged from 1.5 -3.1mm. A total of 87 dentine specimens were randomly divided in six groups. The dentine specimen between the two chambers was orientated with the pulpal side facing the inoculated chamber (A). Chamber B contained sterile broth to provide a nutrition source on the ‘cemental’ side of the dentine.

Group 1 contained 18 specimens that were exposed to ultrasound (Branson 32, Branson Ultrasonics Corp., Danbury, CT, USA) in saline for 30 minutes. Group 2 (18 specimens) was exposed to ultrasound in 5% NaOCl for 5 minutes followed by saline for 30 minutes. Group 3 (18 specimens), 4 (18 specimens), 5 (10 specimens) and 6 (5 specimens) were treated with ultrasound in 15% EDTA for 5 minutes to remove the smear layer, followed by 5 minutes in 5% NaOCl and 30 minutes in saline. Five specimens of group 5 served as test model controls and were covered with nail varnish (see controls).

Dentine groups 1, 2, 3 and 5 were exposed to E. faecalis (see procedure), group 4 was exposed to A. israelii.

Procedure

All specimens were mounted in the test devices. These contained the dentine specimens and water in chambers A and B before autoclaving at 121°C for 20 minutes. After autoclaving water was removed from the
chambers with sterile 27 gauge needles, replaced with brain heart infusion broth (BHI) (2.5mL) and incubated at 37°C. After one week broth samples were taken from both chambers of all test models using sterile 27 gauge needles. Samples were plated on BHI-agar plates to check for sterility. Thereafter, the chambers facing the pulpal side of the dentine specimens (chamber A, Figure 1) of groups 1, 2, 3 and 5 were inoculated with *E. faecalis* in BHI (10^9 mL^-1). The specimens of group 4 were inoculated with *A. israelii*. Every two weeks half of the infected chamber suspension was aspirated and replaced with fresh broth. The aspirated suspension was checked for purity and vitality of the bacteria. After 6 weeks the chambers on both sides of the dentine specimen were checked for viable microorganisms by plating 4 x 100μl on BHI-agar plates. The dentine specimens were removed from the test model for processing using a grinding technique for quantitative recovery of viable bacteria (group 1, 2, 3, 6).

**Controls (group 5, 6)**

Five dentine specimens of control group 5 were coated with nail varnish before mounting in the test device. This was to check for leakage of the broth adjacent to the specimens that could cause contamination of chamber B with *E. faecalis* without passing the dentinal tubules. The specimens of group 6 were not infected but served as a sterility check for all procedures during the test period.

**Histology**

The other dentine specimens of group 5 were mounted (without nail varnish) and exposed to *E. faecalis* as group 3. After removal from the model after 6 weeks, these five specimens were rinsed in sterile water and processed for histology and light microscopy. They were fixed in modified McDowell fixative (4% para-formaldehyde, 1% glutaraldehyde, 0.1mol/L Na-cacodylate, pH 7.4). Following dehydration through an ethanol series the dentine blocks were embedded in LX-112 epoxy resin (Ladd) and processed for light microscopic examination (Brown & Brenn stain) to check for the presence of bacteria in the dentinal tubules and compare histological penetration depth with the cultured specimens of group 3. Therefore, serial sections 2μm thick were cut, every 100μm, oriented parallel to the dentinal tubules. Deepest penetration of the micro-organisms was scored as presence in the pulpal third, middle third and cemental third segments of the specimens. The infection of the specimen was scored as “heavy” when more than half of the tubules contained bacteria, as “intermediate” when less than half of the tubules were infected and negative if there were no visible bacteria in the tubules.
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Recovery of bacteria by the grinding technique

Dentine samples were taken from the cemental side of the dentine specimen first, since bacterial penetration was initiated from the pulpal side. In this way it was possible to detect the bacteria that penetrated deepest without contamination from the middle and pulpal segments of the dentine specimen.

Fine dentine shavings were produced with a sterile straight fissure bur mounted in a handpiece and run at low speed (25,000 rpm). Three samples per dentine specimen were taken approximately 0.7mm (range 0.5 - 1.0mm) apart resulting in a cemental, middle and pulpal sample. During the sampling, the specimens were kept in place with sterile forceps. The dentine dust was collected in preweighed sterile petri dishes to establish the weight of the sample in milligrams. It was then transferred to test tubes containing 2mL of sterile saline, vortexed for 10 seconds and plated (100μL) on non-selective (BHI) agar plates. All plates were incubated at 37°C for 7 days and CFU were counted and expressed per milligram of dentine sample.

Statistics

The following variables were tested statistically:
* thickness of dentine within groups and between groups (ANOVA)
* comparisons of CFU per layer (cemental, middle, pulpal) between groups 1, 2, 3
* comparison of the total CFU per dentine specimen (cemental + middle + pulpal) between groups 1, 2, 3
* comparisons of CFU per layer (cemental, middle, pulpal) between E. faecalis (group 3) and A. israelii (group 4) and
* total CFU per dentine specimen (cemental + middle + pulpal) between E. faecalis (group 3) and A. israelii (group 4) all using the Kruskal-Wallis test followed by Mann-Whitney U-test.

The level of significance was set at p < 0.05.
RESULTS

Number of bacteria in the dentine

The numbers of CFU/mg recovered from the dentine samples by the grinding procedure are shown in Tables 1 and 2. In group 1 45% of the dentine samples showed infection (8/18), in groups 2, 3 and 4 this percentage was respectively 45% (8/18), 89% (16/18) and 28% (5/18). In 11% (8/72) no growth was observed from the pulpal or middle grinding

Table 1 Numbers of CFU/mg of groups 1, 2 and 3 (infection with E. faecalis)

<table>
<thead>
<tr>
<th></th>
<th>(Saline, NaOCl) (n=18)</th>
<th>(Saline, NaOCl, EDTA) (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pulp mid cem</td>
<td>pulp mid cem</td>
</tr>
<tr>
<td>(Saline) (n=18) group 1</td>
<td>pulp mid cem</td>
<td>pulp mid cem</td>
</tr>
<tr>
<td>1-10</td>
<td>0 0 0</td>
<td>19-28 0 0 0</td>
</tr>
<tr>
<td>11</td>
<td>2 0 0</td>
<td>29 0 3 7</td>
</tr>
<tr>
<td>12</td>
<td>3 0 0</td>
<td>30 16 20 7</td>
</tr>
<tr>
<td>13</td>
<td>24 0 5</td>
<td>31 80 7 0</td>
</tr>
<tr>
<td>14</td>
<td>29 8 0</td>
<td>32 70 46 1</td>
</tr>
<tr>
<td>15</td>
<td>86 0 0</td>
<td>33 138 2 1</td>
</tr>
<tr>
<td>16</td>
<td>120 86 0</td>
<td>34 141 91 0</td>
</tr>
<tr>
<td>17</td>
<td>168 50 0</td>
<td>35 284 12 0</td>
</tr>
<tr>
<td>18</td>
<td>368 0 0</td>
<td>36 0 516 4</td>
</tr>
</tbody>
</table>

Pulp= Pulpal samples; Mid=Middle samples; Cem=Cemental samples; %pos=percentage of samples that showed infection of the dentine shavings with E. faecalis
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sample while bacteria were detected in deeper layers. In group 2 (Table 1) specimens 29 and 36 did not show bacterial growth from the pulpal sample while growth was observed in the middle and cemental sample. In group 3 (Table 1) no viable bacteria were found in the pulpal and/or middle sample of specimens 40, 42, 49, 53 and 54 although growth was obtained from the deeper segments. In group 1 (Table 1) no viable bacteria were found in the middle segment of specimen 13.

Infection of chambers

Bacteria in the inoculated chambers A were viable during the whole experiment in all groups. Only two specimens of group 3 allowed *E. faecalis* to invade the dentine completely, infecting chamber B. According to cultivation of the dentine dust, the bacterial front reached parts of the outer layer (cemental) in 12 out of 18 specimens (group 3) including the two specimens that showed a positive culture in chamber B. No infection of chamber B was detected in groups 1, 2, 4 and 5. From cultivation of dentine dust, the bacterial front reached the cemental segment of the roots in only 1, 5 and 2 out of 18 specimens in groups 1, 2 and 4 respectively.

### Table 2 Numbers of CFU/mg of group 4 (infection with *A. israelii*)

<table>
<thead>
<tr>
<th>EDTA, NaOCl, Saline (n=18)</th>
<th>pulp</th>
<th>mid</th>
<th>cem</th>
</tr>
</thead>
<tbody>
<tr>
<td>group 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55-67</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>68-69</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>70</td>
<td>97</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>71</td>
<td>466</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>72</td>
<td>1480</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

% pos                          | 22   | 17  | 11  |

Pulp= Pulpal samples; Mid=Middle samples; Cem=Cemental samples; %pos=percentage of samples that showed infection of the dentine shavings with *A. israelii*.

Controls

In the five specimens of group 5 (dentine specimens covered with nail
varnish) no leakage occurred from the inoculated chamber A to the sterile chamber B. All grinding samples of group 6 (non-infected) were negative indicating an aseptic technique.

**Histology**

In the 5 specimens of group 5 that were used for histological evaluation (exposed to *E. faecalis*) four out of the five dentine specimens showed intermediate infection (less than half the tubules infected). In three of those the bacteria penetrated only the pulpal segment while the fourth specimen showed penetration up to the middle segment. The fifth specimen did not reveal histological signs of penetration by *E. faecalis* (Table 3).

**Table 3** Histological sections of group 5 (infection with *E. faecalis*)

<table>
<thead>
<tr>
<th>Histology (n=5)</th>
<th>pulp</th>
<th>mid</th>
<th>cem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% pos</td>
<td>80</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

Pulp= Pulpal samples; Mid=Middle samples; Cem=Cemental samples; %pos=percentage of sections that showed infection of the dentine under the light microscope

**Statistics**

The thickness of the dentine specimens within and between groups did not differ significantly.

* Pulpal samples
  Group 3 (EDTA, NaOCl, and saline) showed significantly heavier infection than groups 1 (saline) and 2 (saline and sodium hypochlorite) (p=0.04 and p=0.02). There was no significant difference between groups 1 and 2 (p=0.64).

* Middle samples
  Group 3 showed significantly heavier infection than group 1 (p=0.04). There was no difference between groups 2 and 3 (p=0.76) and groups 2 and 1 (p=0.1).
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* Cemental samples
Group 3 showed significantly heavier infection than groups 1 and 2 (resp. p=0.0003 and p=0.01). There was no difference between groups 1 and 2 (p=0.084).

* Total grinding sample
Group 3 showed significantly heavier infection than groups 1 and 2 (resp. p=0.002 and p=0.009). There was no difference between groups 1 and 2 (p=0.7).

* Comparison between E. faecalis and A. Israelii
There was a significant difference in CFU between group 3 (E. faecalis) and group 4 (A. israelii), in the total dentine sample and at each layer compared separately.
Pulpal sample (p=0.009), middle sample (p=0.01), cemental sample (p=0.001), total grinding sample (p=0.001)

DISCUSSION

The choice was made to use bovine dentine since it is available in large quantities; and all teeth were from the same age group of animals. A pilot study revealed that the autoclaving procedure did not result in cracking of the dentine. During the pilot stages of this study several anaerobic bacterial species were cultured in the model and incubated in an anaerobic chamber. Survival of obligate anaerobes over a period longer than two weeks in a pure culture broth, such as BHI, appeared poor and therefore not suited for the six week evaluation period as anticipated in this study. For this reason the choice was made to compare only a facultative endodontic strain (A. israelii) with E. faecalis.

E. faecalis was chosen as a test organism since it is associated with persistent apical inflammation (Engström 1964, Haapasalo et al. 1983, Sundqvist et al. 1998, Molander et al. 1998) and because it seems to be the best organism for experimental penetration into dentinal tubules, leading to gross infection. Ørstavik & Haapasalo (1990) found that E. faecalis penetrated the entire width of the circumpulpal dentine after only 2 days of incubation. In their model the bacteria had access to both the pulpal and the periodontal side of the dentine. The bacteria were allowed to penetrate the dentine from the pulpal side only in the present study. A longer period of time to penetrate deeper into the root dentine as shown by Akpata & Blechman (1982) is needed. The cementum was removed from all the dentine specimens because Akpata & Blechman (1982), Haapasalo & Ørstavik (1987) and Safavi et al. (1990) found delayed bacterial penetration or no population by microorganisms when the root cementum was left in place.
In the present study penetration of *E. faecalis* was found to be greatly enhanced when the smear layer was removed; the cemental layer was infected in 67% of the specimens in group 3 (12/18) but much less in the specimens of the other test groups (group 1; 6% and group 2; 28%). This is in agreement with the findings of Haapasalo & Ørstavik (1987) and Safavi *et al.* (1989).

*A. israelii* did not grossly infect the dentine although the smear layer was removed (11%, 2/18). An explanation could be that its rough colonial morphology and filaments prevent deeper penetration in dentine.

Of the 20 dentine specimens that had viable bacteria in the cemental segments of the specimens, only two of group 3 (no smear layer) showed complete penetration of the dentine specimen resulting in a positive culture in chamber B of the test device. In these cases, the bacteria had penetrated 2000µm of dentine. This suggests that the viable bacteria in the remaining 18 specimens had not (yet) reached the outer surface, or were not able to infect the culture medium from their topographic position in the dentine specimen. On the other hand, Perez *et al.* (1996) using *S. sanguis*, found that most chambers of their test group were positive after two weeks of incubation and penetration. The difference with our data could be explained by dentine thickness; Perez *et al.* (1996) used dentine 1.3mm thick while our specimens ranged from 1.5 - 3.1mm in thickness. Dentine thickness may be important for nutrient source availability. However, Perez *et al.* (1996) explain the progression of *S. sanguis* through the tubules by an ‘active phenomenon of a regular rate of migration and multiplication’ rather than ‘passive slow culture medium penetration through the tubules’.

Another explanation for the difference in results regarding complete infection of the specimens may be leakage. Although the preliminary seal test showed perfect model sealing, Perez *et al.* (1996) found three receiver compartments of one of their test groups were positive as a result of leakage at the composite-silicone junction. In our study leakage did not occur (group 5).

Although it is important to evaluate if bacteria are able to infect a culture medium after penetration through dentine the present results suggest that complete penetration and infection of chamber B is rare and unpredictable (2/72=3%). Therefore, the grinding of root dentine for evaluation of depth of infection appears a more reliable method. Moreover, grinding can generate quantitative data as is shown in tables 1 and 2, whereas a positive culture medium in chamber B just means that one, several or many micro-organism(s) passed through the dentine and multiplied.

In 11% of the specimens no bacteria were found in the pulpal or middle grinding sample while bacteria were detected in deeper layers. It could be that the amount of collected dentine was insufficient to detect CFU of bacteria or that the viability was too poor for recovery of viable bacteria.
However, 89% of the grinding samples evaluated showed progressive infection from the pulpal to the cemental layer as expected. Light microscopy or SEM show the presence of bacteria in tubules but fail to show viability of the organisms so, the correlation between viable CFU from grinding samples and histological observations is of interest. Ørstavik & Haapasalo (1990) reported overall good correlations between histology and grinding. A grinding sample of dentine dust showing bacterial growth was matched by a histological section showing bacteria. However, they found *Ps. aeruginosa* in bur samples of deeper dentine layers but not in the SEM sections. Although it is possible bacteria were forced deeper in the dentine during the grinding procedure (since in their study it was done from the infected pulpal side to the less-infected cemental side), it raises the question whether histological processing of infected dentine can lead to definite conclusions about penetration depth if these results are not combined with recovery of bacteria from some type of grinding procedure. It is highly probable that bacteria are detected in (deeper layers) of root dentine when using a sensitive grinding technique and go undetected by SEM or LM. In fact, the present results of histology compared with culturing of dentine dust and those of Ørstavik & Haapasalo (1990) with *Ps. aeruginosa* confirm this. The correlation between CFU in the pulpal grinding samples of group 3 (Table 1) and the pulpal aspect of the histological sections (Table 3) is good. The middle and cemental grinding samples contain low numbers of CFU while bacteria are not seen in histological sections. This is not surprising considering the field of vision under the microscope contains only a few micrograms of dentine. Low numbers of CFU/mg remain undetected for that reason. This model provides the possibility to evaluate the action of intracanal disinfectants on micro-organisms in the root dentine, and also shows that the technique of collection and immediate culturing of root dentine dust could be used to gain a better understanding of the infection of root dentine *in-vivo*. 

*In vivo*, the root dentinal tubules of teeth with necrotic pulps will be infected from the pulpal side and micro-organisms are expected to progress through the tubules towards the cemental side. Therefore, an *in-vitro* model should allow bacteria to penetrate from the pulpal side only. Although previous *in-vitro* reports mention that penetration from the cemental side (when cementum is removed) was very infrequent (Safavi et al. 1990) and occurred far less than from the pulpal side (Ørstavik & Haapasalo, 1990) ingress of bacteria from the cemental side (when cementum is removed) could result in false penetration depth readings. Immediate culturing of infected dentine dust and counting CFU allows an overview of the number of bacteria per sample. This could eventually generate quantitative data on the effect of intracanal disinfectants on
bacteria. Haapasalo & Ørstavik (1987) and Heling & Chandler (1996) collected their dentine samples in broth allowing only qualitative type data. Thus, a massive reduction of bacteria during the inclusion of an intracanal disinfectant is overlooked if only a few survivors do grow in such a broth.

The purpose of this study was to evaluate which bacteria penetrated most deeply in root dentine under different circumstances. From the literature it is known that cementum present on the root surface will prevent penetration in-vitro (Haapasalo & Ørstavik 1987, Safavi et al. 1990). For this reason it was decided in the present study to remove the root cementum before exposure to the microorganisms. The danger exists that an artificial situation is created. If extrapolation is possible from the in-vitro situation to the in-vivo situation concern should not be expressed about microorganisms in the dentinal tubules of roots. in-vitro studies show that microorganisms will not or hardly penetrate when cementum is present on the root surface. In cases of endodontic infections, the questions remain whether in-vivo bacteria are able to infect the root dentinal tubules and if there is an acceptable number of remaining bacteria in the root dentine after cleaning and shaping of the root canal system.

CONCLUSIONS

Under the conditions of the study:
1 Removal of the smear layer resulted in deeper bacterial penetration.
2 E. faecalis penetrated the root dentinal tubules significantly deeper than A. Israeliii.
3 Counting colony forming units (CFU) is a more sensitive technique to establish the presence of bacteria in root dentinal tubules than microscopy.
4 Counting colony forming units (CFU) is a more sensitive technique to establish the presence of bacteria in root dentinal tubules because it allows the evaluation of viability of microorganisms.

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Penetration of bacteria in bovine root dentine in vitro

Viable bacteria were detected in tubules of teeth with apical periodontitis.

Abstract:

Samples of teeth with and without periodontitis were collected at different levels from the root to assess the density of bacteria left in the dentine tubules. The dentinal fluid of 36 of these teeth was retrieved from the root canal before and after irrigation with 2% NaOCl. In addition, dentine samples were taken from the root canal walls from the same teeth. Twenty-three percent of the samples contained more than 10^6 colony forming units (CFU) of bacteria, indicating that the teeth were infected. No bacteria were found in the other 77% of samples. A higher level of bacteria was detected in the apical third of the root samples than in the coronal third. No histological signs of bacterial penetration were observed.

In conclusion, it is possible that due to the infected roots, bacteria are present in the root canal system to the teeth. This suggests that anaerobic bacteria in root dentine do not penetrate beyond the apical third of the dentine.
Chapter 3

bacteria. Neapyleae, on the other hand, collected their dentin samples at intervals after only subjective data. Thus, it makes selection of data, during the removal of the intradental bridge, to evaluate the presence of bacteria in such a structure. The purpose of this study was to examine which of the bacteria could penetrate deeply in root dentin under different conditions. Eventually, it was established is known that contamination occurs in the root while the bacteria penetrate into the dentinal tubules by the 3-D visualization of the dentin samples. Bacteria are unable to infect the smear layer, the bacteria in the dentinal tubules of cases, routine clinical practice, may be present in high concentrations in cases of endodontic infections. The general theory that a-vivo bacteria are able to infect the smear layer and if a sufficient number of bacteria are present, then the root canal system in the root due to the smear layer.

Conclusively

Under the conditions of the study,

1. Removal of the smear layer reduced deeper bacterial penetration.

2. E. faecalis penetrated the root dentinal tubules significantly more than A. actinomycetemcomitans.

3. Counting colony forming units (CFU) is a more sensitive technique to establish the presence of bacteria in root dentinal tubules than microscopy.

4. Counting colony forming units (CFU) is a more sensitive technique to establish the presence of bacteria in root dentinal tubules than microscopy.

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