Efficacy of Chemomechanical Preparation of Root Canal System in Retreatment Cases

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The main goal of this study was to investigate the efficacy of chemomechanical preparation of root canal system in retreatment cases. The root canals of 40 single-rooted teeth with apical periodontitis which had been treated 8–10 years ago (different kinds of cements, pastes and silver cones were used as root filling materials) were thoroughly instrumented. EDTA and 2.5% sodium hypochlorite solutions were used during instrumentation as chemical adjuncts. Using advanced anaerobic bacteriological techniques, pre- and post-instrumentation samples were taken during the first appointment. Before instrumentation bacteria were isolated from 33 of the 40 (82.5%) teeth examined.

The microbiological profile of all the 40 teeth was determined with a special focus on the presence of yeasts, gram-negative facultative enteric rods and *Enterococcus* species. These microorganisms as indicated by several *in vivo* and *in vitro* studies resist chemomechanical cleaning procedures much better than most other microorganisms.

The predominant microorganism in the samples was *E. faecalis*. It was isolated from 21 of 33 (64%) culture-positive teeth. In 19 (57.6%) cases this microorganism was the dominant or the only isolate from the retreated culture-positive root canals. Another microorganism, *C. albicans*, was found in six (18%) of the culture-positive root canals, always together with other bacterial species.

After the completion of the chemomechanical instrumentation bacteriological sampling revealed bacteria in ten of the 33 (30%) culture-positive teeth. *E. faecalis* was revealed in seven out of 10 (70%) culture-positive root canals after completion of instrumentation, five of them in pure culture. Facultative and anaerobic gram-positive bacteria were isolated in three other cases. After completion of chemomechanical preparation, no yeasts or gram-negative facultative enteric rods were found in the second samples. The number of bacterial cells isolated in 10 postpreparation samples was always below 1% of the original counts in the first sample.

Key words: chemomechanical preparation, retreatment, *Enterococcus faecalis*

INTRODUCTION

Bacteria in dental root canals play a decisive role in the development of apical periodontitis (1, 2). Thus, elimination of bacteria from the root canals is the ultimate aim of endodontic treatment. Usually the elimination of bacteria is achieved by a combination of measures such as mechanical cleansing, irrigation with various antibacterial solutions and the deposition of an antibacterial in the canals after preparation. Nevertheless, after a technically successful completion of the instrumentation the treatment may fail, even in cases where bacteriological sampling revealed no bacteria in the root canals (3).

The differences in the microflora of the root canal system of primary and retreatment cases are evident and well-documented (4–6). These differences may be a consequence of the ecological changes in the root canal system, such as shift of the redox potential, changes in the supply and composition of the nutrients because of chemomechanical preparation and medicaments used during primary root canal treatment. *Enterococcus faecalis,* gram-negative enteric rods and gram-positive facultatives such as *Actinomyces* spp. are often found in persistent endodontic infections (7–9). These bacteria can be detected in root canals as monoinfections or as part of a polymicrobial infection. Some of these bacteria, for example, *E. faecalis,* are able to survive for long periods in a root canal without nutrients even in an

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alkaline environment (5). This species can also enter the root canal during the root canal treatment because of inadequate isolation of the operative field, leaking temporary or permanent fillings or because the root canals were left opened for drainage.

The aim of the present study was to evaluate the efficacy of chemomechanical preparation in retreatment of teeth with apical periodontitis.

MATERIALS AND METHODS

Teeth

The material initially consisted of 40 non-vital teeth with apical periodontitis. The examined material was selected from referrals made to the Clinic of Stomatology of Vilnius University. The teeth included in this study had undergone primary root-filling procedures eight and more years before. The examined teeth showed no clinical symptoms. Fourty teeth meeting these criteria and demonstrating radiographic and clinical signs of chronic apical periodontitis were examined using microbiological sampling techniques before and after chemomechanical preparation. The old root fillings were evaluated using radiographs, and the length from the root filling end to the radiological apex was measured. The size of the periapical lesions was also measured from the radiographs as the longest diameter of each lesion.

Endodontic treatment was performed under aseptic conditions. During chemomechanical preparation 2.5% sodium hypochlorite and 17% EDTA solutions were used as antibacterial irrigants and calcium hydroxide was used as a temporary root canal dressing material between the appointments after the second microbiological sampling.

Microbiological procedures

The disinfection of the sampling area was carried out according to the procedures described earlier by Moller (10). All teeth were bacteriologically monitored prior and after the chemomechanical root canal preparation. Enteric bacteria and yeasts were chosen as the study bacteria, because they are considered to be difficult to eliminate from infected root canals.

First microbiological samples were taken from the canals of all the 40 teeth immediately following the coronal access preparation and preliminary canal inspection to the full length by a sterile endodontic instrument. Second samples were taken at the end of the first appointment following the chemomechanical preparation. Samples were taken with sterile forceps using sterile paper points inserted into the canal at its apical portion. After sampling the paper points were immediately placed into the transport medium, VMGA III gel (10).

Bacteriological procedures

The transport medium (VMGA III gel) contained glass beads 3 mm in diameter to facilitate the mixing and homogenization of the sample prior to cultivation. All samples were cultivated in the Microbiological Laboratory, Departament of Endodontics, University of Oslo, 2–3 days after sampling. Each bottle with the transport media was thoroughly shaken in a mixer (Vortex, Scientific Industries Inc. Springfield, MA. USA). Serial ten-fold dilutions were made up to 1:10 in sterile peptone water (Bacto peptone, Difco, MI, USA). One aliquot of 0.3 ml of undiluted medium and several aliquots of 0.1 ml of undiluted and two serial dilutions were plated onto several media, using sterile plastic spreaders. The aliquots were distributed:

– on Brucella agar plates (BBL Microbiology Systems, Cockeysville, MD, USA) enriched with 5% defibrinated horse blood, 5 mgl/l of haemin and 10 mg/l of vitamin K_1 for the cultivation of anaerobic and facultative bacteria;

– TSBV agar plates (Tryptic-soy-agar, Difco) enriched with 10% horse serum and supplemented with 75 mg/l of bacitracin (Sigma Chemical Co., ST. Louis, MO) and 5mg/l of vancomycin (Sigma) for yeasts and gram-negative facultative rods (Slots et al. 1988);

– chocolate agar plates (Tryptic-soy-agar base, Difco) with 10% defibrinated horse blood for facultative bacteria.

The plates were incubated at 37 °C in 5% CO_2 and anaerobically. The preliminary characterization of the microbial species was based on aerotolerance, gram staining and production of catalase and indole. Identification to species level was based on the motility test and carbohydrate fermentation patterns. The detection of glycosidase and aminopeptidase enzymes (Roscozym-4-hour Ent., Rosco, Taastrup, Denmark) was used for the identification of facultative gram-negative enteric rods. *Streptococci* isolates growing on bile-esculin agar plates (Difco) were identified to species level by a rapid ID 32 Strep test panel (BioMerieux, Marcy/Etoile, France). Yeasts were identified based on cellular appearance in gram-stained smears, production of glycosidase enzymes (Chromogenic substrate tablets; Rosco) and by comparing silver-stained (Silver Stain Plus, Biorad Laboratories, Richmond, CA, USA) whole cell protein profiles of the isolates in sodium dodecyl sulphate polyacryl amide gel electrophoresis (SDS-PAGE) (reducing sample buffer, 12% gels, Mini Protean II, Biorad) with reference strains (11). One

per cent SDS was used for the extraction of yeast proteins for half an hour at 20 °C with repeated mixing (Vortex, Scientific Industries Inc.). Supernatants obtained after centrifugation (12.000 g, 5 min) were used for the SDS-PAGE.

RESULTS

At the beginning of the retreatment procedure all root canals contained the old root canal filling. The length of the empty space in the root canal varied from 3 to 12 mm taking the radiographic apex as the reference point (SD = 3.9 mm) (Table 1). All teeth had periapical lesions, their initial size varying from 2 to 13 mm with an average diameter of 5.1 mm (SD = 3.3 mm).

Table 1. **Rrelationship between lesion size and old root canal filling quality**

Distance to radiographic apex	Mean size of the periapical lesion	Number of teeth
3–8 mm	4.2 mm (SD = 1.2)	18
9–12 mm	7.1 mm (SD = 2.5)	22

Bacteria were found in 33 out of the 40 (82.5%) root canals. The microbiological profile of the 40 root-filled teeth with chronic apical periodontitis was determined with a special focus on the presence of yeasts, gram-negative facultative enteric rods and *Enterococcus* species in the flora, as these are considered microbial species more resistant to conventional endodontic treatment procedures.

In the first sample microbial growth was detected in 33 out of 40 teeth. There were significant differences between the numbers of bacterial cells found in different root canals. In the first samples the number of bacterial cells per root canal varied from 40 cfu to 7×10^7 cfu (Table 2).

E. faecalis was the most common finding in the enteric bacteria group. It was the predominant species found in the samples prior to instrumentation of the root canal space. *E. faecalis* was isolated from 21 out of 33 culture-positive teeth (64%). In 19 out of 21 canals where this microorganism was detected it was found to be the dominant or the only isolate. Yeasts were found in six teeth (18%), always together with one or more bacterial species.

Table 2. **Initial size of periapical lesions and the number of bacterial cells in initial samples from root canals**

Size of lesions	Number of bacterial cells
2–4 mm	1.4×10^7 cfu
5–7 mm	4.8×10^6 cfu
8–13 mm	2.4×10^7 cfu

The antimicrobial effect of the chemomechanical preparation was examined by microbiological monitoring of the root canal space. The second bacteriological sample was taken immediately after the completion of chemomechanical preparation. Bacteria were revealed in ten out of the 33 (30%) culture-positive root canals. In 30% of the prepared root canals the number of microbial cells was greatly reduced, but not totally eliminated. The number of bacteria in the second samples was always below 1% of the cfu counts of the first microbiological sample. These results show that a proper chemomechanical preparation of the root canal reduces the number of bacteria in the root canals, but cannot totally eliminate them.

E. faecalis was found in 7 samples out of 10 positive second samples, five of which exhibited a pure culture of this microorganism.

Facultative and anaerobic gram-positive bacteria were isolated in three other cases. No yeasts and gram-negative facultative enteric rods were found in the microbiological samples after completion of chemomechanical preparation.

DISCUSSION

All teeth in the present study had infected root canals and periapical lesions. The essential role of bacteria in the initiation, propagation and persistence of apical periodontitis has been established (1, 6). It is generally accepted that endodontic therapy is aimed at the elimination of bacteria from the infected root canal. This is usually accomplished by a thorough chemomechanical cleaning of the root canal, followed by a complete filling of the root canal space. Cleaning, shaping and irrigating greatly reduce the numbers of cultivable bacteria. However, several studies have shown that it is impossible to achieve a sterile root canal space in all cases, even by thorough cleaning, shaping, and irrigating with disinfectants or antiseptics during one visit (3, 12). Therefore, concern exists as to the fate and consequences of microorganisms left in the root canal. It has been shown that they may multiply rapidly in 2–4 days (almost to original numbers) in cases when the canal is not filled or not dressed with a disinfectant between visits (3).

The reasons for the occurrence of enteric bacteria in persistent root canal infections is poorly understood. The observations of the present study indicate that both the high number of visits and the lack of an adequate seal (which was a generally accepted treatment at the time when the treatments had been done earlier) significantly increase the probability of finding enteric bacteria in the root canal

and indicate that these bacteria may enter the root canal during the treatment.

Our results confirm and add to the study of Molander et al. who found *E. faecalis* in 54% of the culture-positive cases (5). Calcium hydroxide for the years has been and still is the most commonly used root canal dressing in Scandinavia. In Lithuania calcium hydroxide was started to be used as an intracanal interappointment dressing only 6–8 years ago. *E. faecalis* is the most resistant species to calcium hydroxide (12). Our result is an indication that rather than the previous treatment, it is the present ecological conditions in a incompletely filled root canal that play a key role in exerting a selective ecological pressure on the composition of the infective flora. Obviously this is an indication that the root filling materials (such as resorcin formalin) gradually lose their antibacterial activity in the root canal to an extent that allows survival and even growth of bacteria. These bacteria must be ecologically strong enough to survive in the environment of incompletely filled root canals where the availability of nutrients may often be limited, compared with primary apical periodontitis.

Chemomechanical preparation in the present study was done by a thorough instrumentation of the whole root canal and irrigation with copious amounts of EDTA and 2.5% sodium hypochlorite, which is highly bacteriocidic. Nevertheless, residual bacteria were found in the canals after completion of their preparation and irrigation. The numbers of the isolated bacteria were much lower than in the first samples, as could be expected after removal of the previous root filling material and the necrotic infected pulp tissue. Gomes et al. showed *E. faecalis* to be the best survivor after instrumentation and irrigation by 2.5% sodium hypochlorite (13). However, although data have cumulated in support of an important role of *E. faecalis* in endodontic infections resistant to therapy, direct evidence is still lacking. In addition, despite the lower prognosis in retreatment, the percentage of successful cases seems to be much higher than the number of canals without *E. faecalis*.

Besides, some failures occur even after seemingly perfect root canal treatments. Bacteria are able to colonize the dentine tubules. It can be the reason of some failures, although *in vitro* most bacteria in dentinal tubules died within 24 h after removal of the nutrient medium (14). Concern has been expressed as to their survival after root canal therapy, since *in vivo* conditions may permit nutrient supply to bacteria into dentinal tubules. Therefore, the penetration of various antiseptics into the dentinal tubules has been evaluated. Our results confirmed findings of other studies and showed that even in thoroughly cleaned root canals microorganisms are found in microbiological monitoring. It means that application of interappointment medicament to the root canal system is recommedable, because conventional chemomechanical procedures alone do not eliminate all microorganisms from the root canal system.

We know that after a proper chemomechanical preparation the initial number of microorganisms is reduced in the main root canal, but even if all bacteria initially present in the tubules would remain viable at the end of the clinical session, their numbers are certainly quite small, again compared with the initial number in the root canal system that caused (periapical) pathology (12). *E. faecalis* penetrated the dentinal tubules within 2–3 weeks to a depth of 300–400 μm (14). Obturation of the root canal system also deprives the remaining microorganisms their nutrition and leaves them no space to multiply to sufficient numbers to cause or maintain the disease.

Further research is necessary to answer the question: do bacteria survive in dentinal tubules, and if they survive do they grow to pathologically significant numbers?

CONCLUSIONS

1. Root canals should not be left open during endodontic therapy.

2. Conventional chemomechanical root canal preparation and interappointment dressing must be used routinely.

3. The high level of asepsis throughout the whole endodontic treatment must be maintained.

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References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965; 20: 340–9.
2. Sundqvist G. Ecology of the root canal. J Endod 1992; 18: 427–30.
3. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981; 89: 321–8.
4. Fabricius L, Dahlen G, Ohman AE, Moller AJR. Predominant indogenous oral bacteria isolated from infected root canals after varied times of closure. Scand J Dent Res 1982; 90: 134–44.
5. Molander A, Reit C, Dahlen G, Kvist T. Microbial status of root filled teeth with apical periodontitis. Int Endod J 1998; 31: 1–7.

6. Sundqvist G. Bacteriological studies of necrotic dental pulps. Umea: Umea University. Odontological dissertation No. 7, 1976.
7. Bender NA, Selzer S. Combination of antibiotics and fungicides used in treatment of the infected pulpless tooth. J Am Dent Assoc 1952; 45: 293–300.
8. Engstrom B. The significance of enterococci in root canal treatment. Odontologisk revy 1964; 15: 87–106.
9. Haapasalo M, Ranta H, Ranta K. Facultative Gram-negative enteric rods in persistent periapical infections. Acta Odont Scand 1983; 91: 458–63.
10. Moller AJR. Microbiological examination of root canals and periapical tissues of human teeth. Odontol Tidskr 1966; 74: 1–38.
11. Maiden MF, Tanner A. Identification of oral yeasts by polyacrylamide gel electroforesis. Oral Microbiol Immunol 1991; 6: 187–90.
12. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramono chlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985; 1: 70–5.
13. Gomes BP, Lilley JD. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. Int Endod J 1996; 29: 235–41.
14. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990; 6: 142–9.

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CHEMOMECHANINIO ŠAKNŲ KANALŲ PARUOŠIMO EFEKTYVUMAS PERGYDYMŲ ATVEJAIS

S a n t r a u k a

Pagrindinis šio darbo tikslas buvo įvertinti chemomechaninio šaknų kanalų paruošimo efektyvumą pergydymų atvejais. Tyrimui buvo atrinkta 40 dantų šaknų kanalų, kurie buvo gydyti prieš 8–10 metų ir kuriems buvo indikuotinas endodontinis pergydymas. Ankstesnio gydymo metu šių dantų šaknų kanalai buvo užpildyti įvairiomis medžiagomis: pastomis, cementu, sidabriniais kaiščiais.

Naudojant pažangias mikrobiologines technologijas, iš šaknų kanalų prieš ir po chemomechaninio jų paruošimo (naudoti EDTA ir 2,5% natrio hipochlorito tirpalai) buvo paimti mikrobiologiniai pasėliai.

Didžiausias dėmesys tiriant šaknų kanalų pasėlius buvo skiriamas gramneigiamų fakultatyvinių enterinių mikroorganizmų, enterokokų bei grybelių paieškai, nes atlikti tyrimai *in vivo* ir *in vitro* parodė, kad būtent šios mikroorganizmų rūšys yra labiausiai atsparios endodontinio gydymo procedūroms.

Iš 40 tirtų pasėlių 33 (82,5%) buvo rasta mikroorganizmų. Mikrobiologiniuose šaknų kanalų pasėliuose, paimtuose prieš chemomechaninį šaknies kanalo paruošimą, vyravo *E. faecalis:* jo rasta 21 iš 33 teigiamų šaknų kanalų pasėlių. *C. albicans* buvo rastas 18% teigiamų šaknų kanalų pasėlių.

Po chemomechaninio šaknies kanalo paruošimo mikroorganizmų ląstelių pasėliuose buvo rasta 10 iš 33 (30%) teigiamų prieš šaknies kanalo paruošimą pasėlių. Septyniuose iš 10 pasėlių buvo rastas *E. faecalis.* Pasėliuose po chemomechaninio šaknies kanalo paruošimo *C. albicans* nebuvo rasta. Antrajame pasėlyje rastų mikroorganizmų ląstelių skaičius nesiekė 1%, lyginant su pirmuoju pasėliu.

Šio tyrimo rezultatai parodė, kad endodontinio gydymo metu mikroorganizmų ląstelių šaknų kanaluose gerokai sumažėja, tačiau visiškai pašalinti jų dažniausiai nepavyksta. Todėl norint išvengti mikroorganizmų ląstelių ženklaus padidėjimo, būtina naudoti antimikrobinį preparatą kaip laikiną šaknies kanalo užpildą tarp apsilankymų. *E. faecalis* radimas pergydomų dantų šaknų kanaluose įrodo, kad endodontinio gydymo metu būtina laikytis aseptikos reikalavimų ir nepalikti atvirų šaknų kanalų. Laikantis šių taisyklių galima užkirsti kelią šio mikroorganizmo populiacijos plitimui šaknies kanale.

Raktažodžiai: chemomechaninis paruošimas, pergydymas, *Enterococcus faecalis*