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ORIGINAL ARTICLE

Maintenance of the aseptic working field during endodontic treatment

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ABSTRACT

Objective: The aim of this clinical quality study was to determine whether the aseptic working field is maintained during the endodontic procedure.

Materials and methods: Bacterial samples were collected from the rubber dam of 27 patients during endodontic treatment performed by postgraduate students at the Department of Endodontics, University of Oslo. A bacterial sample was first obtained immediately after disinfection of the working field (A), and the second sample was collected just before obturation or dressing with calcium hydroxide cement (B). Aerobic cultivation technique and PCR were used for detection of bacterial growth and species.

Results: All samples were negative on culturing except in one case, which showed positive results with cultivation in both sample A and B. Specie detected with cultivation technique were *Streptococcus mitis*. With PCR technique, 6 samples in 5 patients (11%), showed positive results. Species detected with PCR technique were *Bacteroidales spp. Propionibacterium spp., Bacteroidetes spp., Prevotella nigrescens, Haemophilus parainfluenzae, Neisseria elongata, Alloprevotella tannerae, Capnocytophaga granulosa, Cardiobacterium hominis, Fusobacterium nucleatum* and *Streptococcus mitis.*

Conclusion: The present study showed that an aseptic working field was maintained throughout the endodontic procedure in 81% (22/27) of the cases after disinfection of the rubber dam.

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Introduction

Routine isolation of teeth with rubber dam is recommended in professional treatment quality guidelines for both adults and children [1,2]. An improved prognosis is seen when no cultivable bacteria are found in root canal samples at the time of obturation [3,4]. Asepsis (prevention) and antisepsis (reduction/elimination) are fundamental concepts in endodontics [5]. During the endodontic procedure, it is important that no bacteria gain access to the root canal system. Inappropriate asepsis and antisepsis can lead to contamination and infection of the root canal and subsequent treatment failure. Different medicaments can be applied for the disinfection procedure. The solutions primarily used for disinfection of the operative field in endodontics are hydrogen peroxide, tincture of iodine, chlorhexidine, alcohol and sodium hypochlorite in various concentrations [6].

Disinfection of the working field can also be done by a mixture of chlorhexidine and alcohol [7]. According to the standard of care at the Department of endodontics, Faculty of dentistry, University of Oslo, the working field (rubber dam and tooth) is disinfected for 2 min with 0.5% chlorhexidine in 70% ethanol [8].

Identification of microorganisms can be done with cultivation and/or molecular techniques. Culture-dependent studies have limitations related to low sensitivity and the inability to detect many difficult-to-grow or uncultivable bacteria [9]. Molecular techniques appear to provide accurate results, but have certain limitations due to the chance of false-positive results [10]. Even the presence of bacterial DNA released by breakdown of dead cells may give a positive result [11].

There is little evidence in the literature whether the disinfection of the operative field can be kept aseptic throughout the endodontic procedure. The aim of the present study was to investigate whether postgraduate students in endodontics at the University of Oslo maintained an aseptic working field during the endodontic procedure.

Materials and methods

Clinical material

Bacterial samples were collected from the rubber dam of 29 patients, during endodontic treatment. All patients were referred to the Department of endodontics, Institute of Clinical Dentistry, University of Oslo, Norway, for endodontic treatment. Only upper and lower molars were included in this study. Six postgraduate students in endodontics performed all the treatments. Two patients were excluded because the rubber dam was changed during treatment. The final analysis was based on bacterial samples from 27 patients. Aerobic cultivation technique and PCR were used for detection and identification of bacterial species,

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according to previously described procedures [11,12]. Two teeth were diagnosed with chronic irreversible pulpitis, 19 teeth had chronic apical periodontitis, five teeth had periapical abscess with sinus tract and one tooth had necrosis of the pulp. Thirteen were non-surgical retreatments and 14 teeth were primary root canal treatments (Table 1).

Disinfection of the working field

After rubber dam application, the operative field, including the tooth, clamp and approximately three centimeters in diameter around the tooth, were disinfected with chlorhexidine in alcohol (0.5% chlorhexidine in 70% ethanol). After 2 min, the working field was cleaned with NaOCl 1% to degrade bacterial DNA, in order to establish a zone completely free of bacterial DNA. NaOCl was subsequently neutralized with 5% sodium thiosulfate. In 19 of 27 cases (70%), supplementary efforts were made to improve isolation, that is, Ultradent® LC Block out Resin, Dental floss (Johnson & Johnson, Dentotape®) and Wedjets® Rubber dam stabilizing cord (Table 2 and Table 3).

Microbiological procedures

Microbiological samples were collected with a sterile Omni Swab (Whatman FTA Sigma-Aldrich) with an ejectable head. Samples were harvested from the buccal and lingual tooth surface in the transition area between tooth and rubber dam (Figure 1).

The first sample (sample A) was taken immediately after disinfection of the operative field, and the second sample (sample B) was taken in the same area as sample A, just before final obturation of the root canal or dressing with $Ca(OH)_2$-paste (Figure 2). Due to two different techniques for microbial identification, (cultivation (c) and PCR (p)), two samples A (Ac and Ap) were collected, as well as two samples B (Bc and Bp). Samples Ac and Bc ($n = 54$) were after sampling immediately transferred to a cryotube containing Dental Transport Medium (DMT; Anaerobe systems, Morgan Hill, USA) [13,14]. Samples were placed in room temperature and transferred to Institute for oral biology within 3 days. Microorganisms were cultivated in aerobic conditions. Samples Ap and Bp ($n = 54$) were transferred to a cryotube containing Tris-EDTA buffer (10mmol/L Tris-HCL, 1 mmol/L EDTA, pH = 7,6) (Sigma Aldrich) and immediately placed in a labtop cooler (−20 °C). The samples were transferred to Institute of oral biology within 3 days and placed in a freezer (−75 °C/−76 °C). Bacterial identification was performed by *16S rRNA* gene PCR and Sanger sequencing (Applied Biosystems 3730 DNA Analyzer).

Table 1. Diagnosis (ICD-10) of teeth included.

Diagnosis	Number of teeth
Chronic irreversible pulpitis, K04.03	2
Chronic apical periodontitis, K04.5	19
Periapical abscess with sinus tract, K04.6	5
Necrosis of pulp, K04.01	1
Previously root canal treatment, K04.19	3

Culture and identification

Cryotube with swab and anaerobic dental transport medium was placed in a vortex (Vortex, Scientific Industries Inc., Springfield, MA, USA) for 30 s. The samples were cultured on blood agar plates. The culture plate was incubated for 48 h at 37 °C. After incubation, the plates were inspected for bacterial colony characteristics and simultaneous counting of colonies. Gram staining was performed according to a standard protocol [15]. Identification of the detected colonies on each plate was carried out by Vitek 2 (Biomerieux) compact biochemical analysis [16].

Polymerase chain reaction-based identification

DNA extraction was performed using the MasterPure DNA isolation kit from Epicentre (MCD85201, Epicentre Biotechnologies). Universal primer pairs (forward primer E334 [5′-CCAGACTCCTAC GGGAGGCAGC-3′] and reverse primer E939 [5′-CTTGTGCGGGCCCCCGTCAATTC-3′]) were selected for specific polymerase chain reaction (PCR) amplification of the

Table 2. Results from culture screening, PCR and *16S rRNA* sequencing.

Sample	Supplementary efforts	Number of colonies	Cultivation	*16S rRNA* PCR product	*16S rRNA* sequencing	Diagnosis
3A	–	14 CFU	*S. mitis* (99%)	+	*S. mitis, P. nigrescens, Bacteriodales spp.*	Chronic apikal periodontitis, K04.5 Previolusly RCT, K04.19
3B	-	50–70 CFU	*S. mitis* (96%)	+	*H. parainfluenzae, N. elongata, A. tannerae*	
6B	Ultradent® LC Block-Out Resin			+	*C. granulosa, C. hominis, Propionibacterium spp.*	Chronic irreversible pulpitis, K04.03
7A	Dental floss + Ultradent® LC Block-Out Resin			+	*Bacteroidetes spp., Bacteriodales spp., Fusobacterium nucleatum subsp. Vincentii*	Periapical abscesss with sinus, K04.6
19B	Ultradent® LC Block-Out Resin			+	No obtainable sequencing result	Chronic apikal periodontitis, K04.5
27A	Ultradent® LC Block-Out Resin			+	No obtainable sequencing result	Chronic apikal periodontitis, K04.5

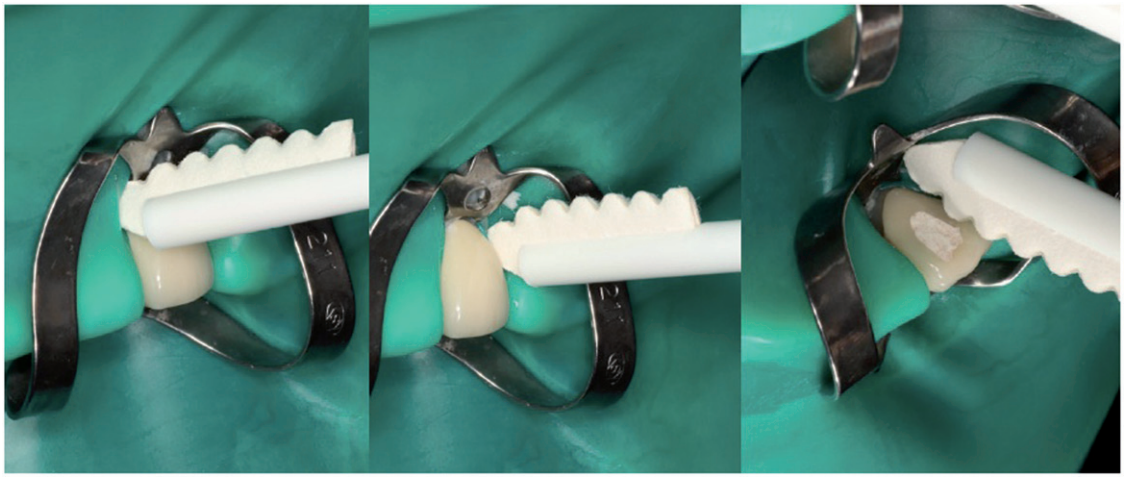

Figure 1. Microbial sampling procedure performed with a sterile Omni Swab.

Table 3. Supplementary efforts for rubber dam placement.

Supplementary efforts for rubber dam placement	Number of teeth
Ultradent® LC Block-Out Resin, Ultradent products, Inc	14
Dental floss, Johnson& Johnson, Dentotape	4
Wedjets® Rubber dam stabilizing cord, Coltene/Whaledent	1

16S rRNA gene. PCR assay was performed in 20μL reaction mixtures, containing 2,6 μL of extracted DNA, 10 μL One taq, 7 μL Milli Q-dH_2O, 0,2 μL forward primer E334, 0,2 μL reverse primer E939. Negative controls consisted of the reaction mix with sterile water instead of the sample. PCR was performed by GeneAmp® PCR System 2700 (Applied BiosystemsTM, Life Technologies Corp., Carlsbad, CA, USA). The PCR temperature profile included an initial denaturation step at 96 °C for 1 min and 8 s, followed by 30 cycles of denaturation step at 96 °C for 30 s, a primer annealing step at 61 °C for 25 s, an extension step at 72 °C for 30 s, and a final step of 72 °C for 4 min, respectively.

Any PCR product from the samples was visualized with gel electrophoresis. The products were ligated into a TOPO cloning vector and transformed into *E.coli* (Topo® TA Cloning® kit K4500-01, Invitrogen). Twenty colonies of each sample were analysed. PCR with M13 primers in front of the insert and the M13 PCR product were sequenced using BigDye® Terminator v1.1 cycle Sequencing Kit and ABI 3730 sequencer (Applied Biosystems). All DNA sequences were analysed, trimmed, and aligned using FinchTV software (version 1.5.0; https://digitalworldbiology.com/FinchTV). The partial gene sequences were identified by a BLAST analysis with *16S rRNA* GeneBank database search on the Human Oral Microbiome database (HOMD) [17].

Results

Cultivation

Of the 54 samples, two samples (3.7%) were positive for growth, both in the same patient (3A and B). Specie identified was *Streptococcus mitis* (Table 2).

PCR and gene sequencing

Of the 54 samples analysed with PCR technique, six samples (11%) yielded a positive PCR product in five different patients. Three of the samples were collected immediately after disinfection (3A, 7A and 27A) and three were collected right before obturation/$Ca(OH)_2$-dressing (3B, 6B and 19B), (Table 2). Two samples (7A and 27A) that showed positive results with PCR right after disinfection showed negative results at sample B. Four samples (6B, 7A, 19B and 27A) that showed positive PCR products, showed no growth with cultivation. Two samples (3A and B) were both positive with culturing and molecular technique. HOMD *16S rRNA* sequence database identified *Bacteroidales spp. Propionibacterium spp., Bacteroidetes spp., Prevotella nigrescens, Haemophilus parainfluenzae, Neisseria elongata, Alloprevotella tannerae, Capnocytophaga granulosa, Cardiobacterium hominis, Fusobacterium nucleatum* and *Streptococcus mitis.*

Discussion

Rubber dam has been available to the dental profession for over 140 years [18]. The routine isolation of teeth with rubber dam is a prerequisite in root canal treatment. It provides protection against foreign material entering the oro-pharynx, chemical irritation of oral soft tissues, and salivary contamination of the pulp chamber. The literature reports that dental practitioners do not use rubber dam routinely during root canal treatment. Prevalence of rubber dam use by general dental practitioners all over the world is reported as low as 11% to as high as over 90% [18,19]. It has been documented that there is a small but statistically significant improvement in tooth survival when rubber dam was used compared with non-isolated controls [20]. However, the scientific evidence is insufficient regarding how well the operative field is maintained during endodontic treatment. Malmberg et al. found that no study documented complete asepsis following initial disinfection, and no study could document predictable maintenance of an established bacteria-free surface. They concluded that critical appraisal and standardization of the disinfection and aseptic procedures in endodontics are

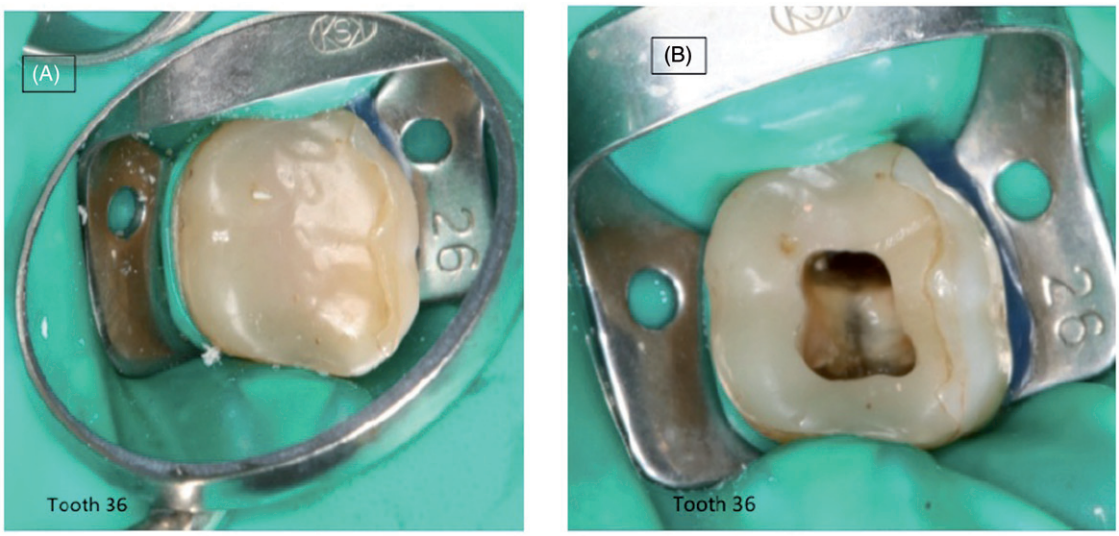

Figure 2. Collection of sample 1A (immediately after disinfection) and sample 1B (before root-filling procedure/$Ca(OH)_2$ – dressing).

needed [6]. Different medicaments can be applied for the disinfection procedure. Möller recommended to clean the operating field with 30% hydrogen peroxide followed by swabbing with 5% or 10% iodine tincture [21]. Ng, et al. evaluated protocols for field decontamination. They compared the method by Möller et al. with a method where 30% hydrogen peroxide was combined with the use of 2.5% sodium hypochlorite. The study showed no significant difference between the two protocols in the ability to disinfect the working field. Neither iodine nor sodium hypochlorite ensured complete disinfection. Significant reduction of contamination was observed after repeated cleansing with sodium hypochlorite [11].

In this study, disinfection was performed with 0.5% chlorhexidine in alcohol, according to the standard of care at the Department of Endodontics, University of Oslo. The primary purpose of the present investigation was to monitor maintenance of asepsis. With the PCR technique residual DNA from bacteria killed by the disinfection procedure would cause false positive samples towards the end of the session. Therefore, an attempt to degrade all bacterial DNA, the rubber dam and tooth were further disinfected with NaOCl, and neutralized with 5% sodium thiosulfate, after the chlorhexidine alcohol wash. NaOCl has been shown not only to kill bacteria, but also to degrade DNA [22]. Even though NaOCl and sodium thiosulfate is not included in the disinfection protocol at Department of Endodontics, the aim of this investigation was to study the maintenance of an aseptic working field. Therefore, an attempt was made to establish a zone completely free of bacterial DNA and live bacteria before treatment. A limitation of the study was that since the original disinfection protocol was altered, the postgraduate students knew that sampling procedure was going to be performed. Random sampling would have been preferred, but then the molecular technique would be incomplete, due to possible false-positive results (the presence of bacterial DNA released by breakdown of dead cells). This may be the reason for the same amount of A and B samples with growth. Expected results would have been more bacterial growth before root-filling procedure (sample B), than immediately after disinfection (sample A) due to contamination during the endodontic treatment. Some postgraduate students disinfected the rubber dam/tooth during the endodontic procedure, for example, after radiograph procedure. This may have attributed to less growth on the B-sample. It can be discussed if disinfection of rubber dam, tooth and oral suction should become a standard routine during an endodontic treatment session to reduce contamination. Another explanation for positive A and, negative B samples might be due to irrigation of sodium hypochlorite during the instrumentation. The excess/surplus of NaOCl may have touched the rubber dam, and thus helped the clinician with disinfection of the operative field during treatment.

The sampling procedure was performed with sterile Omni swabs. The Omni swabs are quite stiff and may not enter all small areas in the junction between rubber dam and tooth. This may have led to a higher incidence of false negative samples. Other studies have used sterile paper points during sampling procedure [11,21].

Another limitation of the study is the inclusion of teeth with different diagnoses. Four samples with infected pulps and one sample with vital pulp showed all positive PCR product/growth. It would have been optimal to include only teeth with vital pulp to ensure no bacterial contamination from the root canal. Nevertheless, the results of this study indicate that contamination from the root canal does not occur frequently during endodontic procedure performed by postgraduate students.

Culture techniques are widely established for the detection of bacteria, but more recent molecular techniques (PCR) have been used increasingly for studies of the root canal microbiota [23–25]. An advantage with cultivation technique is that detected bacteria are viable, but many bacteria are difficult to grow, or are as-yet-uncultivable, which in turn may lead to false-negative results. Polymerase chain reaction (PCR) provide opportunities to examine if any bacterial DNA

exist in the sample [26]. The PCR technique is relatively rapid and has facilitated the analysis of a far wider range of bacteria than was previously possible. An important benefit of these PCR-based molecular approaches is their potential to detect organisms that are present in low numbers [13], which is the case on a disinfected operative field. In the present study, four samples with positive PCR products showed no growth with cultivation technique.

A bacterium detected in this study is the facultative anaerobic polysaccharide producing *Streptococcus mitis* suggesting leakage of saliva as a possible reason for contamination of the operative field. During treatment, the rubber dam is exposed to stress (radiograph procedure, oral suction), which may contribute to leakage of saliva or gingival fluid between rubber dam and tooth. *Neisseria elongata* and *Cardiobacterium hominis* are Gram-negative bacteria found in the human pharynx and respiratory tract. Thus, detection of these species in the present study may indicate a possible contaminant from the patient or/and the operator. *C hominis* have also been reported in a case with endocarditis [27]. *Propionibacterium spp.* are common inhabitants of the skin. It is possible that the *Propionibacterium spp* detected in the present study were contaminants from the patient or operator [28].

In standard procedures in endodontics, it has been suggested that plaque and calculus should be removed from the teeth before isolation with rubber dam and disinfection [29]. In the present study, no specific attempts at removal of plaque/calculus were recorded. The *16S rRNA* sequencing detected *Capnocytophaga granulosa, Fusobacterium nucleatum* and *Prevotella nigrescens*. *C. granulosa* and *F. nucleatum* are species typically found in subgingival plaque, and *P. nigrescens* and *F. nucleatum* are species involved in the pathogenesis of periodontal disease [30]. These organisms may thus originate from plaque, calculus, or gingival fluid from the patient treated. Some of these species are also found in root canal infections (*F. Nucleatum, P. Nigrescens,*), [9,31] suggesting contaminants from the root canal during treatment.

In this study, a light-cured resin (Ultradent® LC Block-Out Resin, Ultradent products, Inc.) was used on the rubber dam/tooth junction in 14 of 27 cases (52%). Four of the cases with block out resin showed positive PCR product, and only two of the cases without the use of light cured resin showed positive growth. These results are in contradiction with Fors et al. who found that the introduction of an elastic adhesive sealer to the rubber dam tooth/junction reduced microbial leakage. However, this study only used culture technique [12].

Conclusion

The present study showed that an aseptic working field was maintained during the endodontic procedure in 81% (22/27) of the cases after use of chlorhexidine in alcohol and sodium hypochlorite as rubber dam disinfectant. In 24 cases (89%), the working field was free from bacteria right before obturation/dressing probably due to supplementary disinfection throughout the endodontic procedure.

Disclosure statement

No potential conflict of interest was reported by the authors.

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