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REVIEW ARTICLE

Establishment and maintenance of asepsis in endodontics – a review of the literature

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ABSTRACT

Objectives: Successful endodontic treatment depends on effective measures to eliminate and prevent infection of root canals. Initially treatment should start with isolation and disinfection of the operating field. This review makes an inventory of the available knowledge regarding its establishment and maintenance.

Materials and methods: A literature search was conducted in the PubMed database in order to identify clinical trials examining disinfection or unintentional contamination of the endodontic operative field. A list of 115 articles was obtained and screened. Five relevant articles were identified. These articles were read in full text. The reference lists from these articles were checked manually for additional studies and three studies were obtained. A total of eight articles met the inclusion criteria.

Results: There was a great variety in terms of aim, method, and material of the included studies. None could prove a totally reliable aseptic operative field and not one chemical, or combination of chemicals, were found in more than one study.

Conclusions: No study documented complete asepsis following initial disinfection, and no study could document predictable maintenance of an established bacteria-free surface. Critical appraisal and standardization of the disinfection and aseptic procedures in endodontics are needed.

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Introduction

Asepsis is defined as freedom from infection and the prevention of contact with microorganisms.[1] In surgical procedures it includes the process of removing pathogenic organisms and protecting the surgical field from contamination. In endodontics specific procedures and standards have been developed to attain an aseptic field of operation. This review identifies the studies describing controlled procedures for establishing, and maintaining, an aseptic operating field in endodontics by applying a systematic approach to the PubMed database.

The infectious nature of endodontic disease processes

Miller observed in 1890 that microorganisms can be found in pulp tissue and he described the pulp as a centre for infection, which can form dentoalveolar abscesses.[2] The etiologic role of infection is today well established and confirmed in animal experiments [3] and clinical studies.[4–6]

The oral cavity has a rich and varied microbial flora. More than 700 bacterial species have today been detected.[7] Living conditions in the root canal are drastically different from that offered in other parts of the oral cavity. Results of both culture and molecular studies indicate that only a limited number of the species have the ability to establish themselves in root canals.[8] As the methods for identifying endodontic pathogens have evolved in recent years by the introduction of molecular methods, the list of potentially involved species has grown substantially.[9] The relative pathogenicity and virulence of these organisms will vary, but clinical practice must focus on eliminating and preventing all from entering the root canal system.

Methods for asepsis

Cross sectional analysis of endodontic treatments carried out in the general practice of dentistry [10–16] suggests that dentists in general have a problem to eliminate the bacterial organisms in infected root canals. It is also obvious from these field studies that many of the large number of failures depend on improper aseptic technique.

The rubber dam constitutes a physical barrier that minimizes contamination of the root canal system from bacteria in the oral environment. It further protects the oral cavity and respiratory tract from foreign objects and chemicals. Unfortunately, scientific studies with high levels of evidence for the efficacy of the use of rubber dam are lacking and that

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the outcome of endodontic treatment is significantly improved by its usage. However, a negative impact of not using rubber dam during endodontic treatment can be indirectly inferred. Abbott [17] evaluated 100 referred patients with persisting symptoms after endodontic treatment and noted that in 87% of the cases the root canal treatment had been performed without rubber dam. van Nieuwenhuysen [18] evaluated the outcome of 612 re-treatments and observed that the outcome was significantly better when the teeth were isolated with a rubber dam compared with only cotton rolls. Lin et al. [19] evaluated the tooth survival of initial root canal treatment of 517,234 teeth under a mean observed time of 3.43 years. Tooth survival probability of initial root canal treatment using rubber dams was significantly greater than without the use of rubber dams.

Creating and maintaining an aseptic field for endodontic therapy is not merely accomplished by the use of rubber dam. Antiseptic agents are indispensable for infection control within the entire health care system, and have also been important to attain an aseptic field of operation in endodontics. To determine which active ingredient should be used in a given treatment requires knowledge of the types of microorganisms involved, the tissue/area to be treated, possible adverse effects, and what effect the biocide has under different conditions. 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.

Hydrogen peroxide exhibits a broad-spectrum effect against viruses, bacteria, fungi, and spores. Higher concentrations (10–30%) and longer contact time are more efficient.[20] In relation to the disinfection of the operative field, hydrogen peroxide is mainly used in order to eliminate salivary glycoproteins from the surface of the tooth and to reveal any sites of salivary leakage upon application. Chlorine- and iodine-based compounds are microbicidal halogens used for both antiseptic and disinfectant purposes. Sodium hypochlorite is an effective broad-spectrum antimicrobial agent. Iodine has good antibacterial activity against all microorganisms. The ability to penetrate is limited, however, and its effect on biofilms must be preceded by mechanical or chemical dissolution of the biofilm. Alcohols (e.g. ethyl alcohol and isopropyl alcohol) have fast-acting broad-spectrum activity against bacteria, viruses, and fungi in optimal concentrations between 60–90%.[20] Many alcohol-based products contain other biocides such as chlorhexidine, which has a broad-spectrum effect and binds to organic matter.[21]

A properly isolated and disinfected operative field can be seen as a prerequisite to conduct an adequate root canal treatment even though it is a relevant clinical question whether a leakage-free environment really can be obtained. Many different antimicrobial agents have been used over the years, but how effective the disinfection process is and if asepsis is maintained as well as how long it will remain bacteria-free during the endodontic procedure, are relevant issues. This study seeks to see if there is documentation that the operative field can remain bacteria-free following disinfection and during operative procedures.

Table 1. Search strategies for the PubMed-Medline database.

('disinfection/methods'[MeSH Terms] AND 'root canal therapy'[MeSH Terms]) AND 'bacteria/drug effects'[MeSH Terms]) OR ('bacteria/isolation and purification'[MeSH Terms] AND 'root canal therapy'[MeSH Terms]) AND 'rubber'[MeSH Terms]) OR ('Bacteriological Techniques'[MAJR] AND 'Sterilization'[MeSH Terms]) AND 'Anti-Infective Agents, Local'[MeSH Terms]) OR ('bacteria/drug effects'[MeSH Terms] AND 'dental disinfectants/pharmacology'[MeSH Terms]) AND 'root canal therapy/methods'[MeSH Terms]) OR ('disinfection/methods'[MeSH Terms] AND 'root canal therapy'[MeSH Terms]) AND 'bacteria/drug effects'[MeSH Terms]) OR ('rubber dams'[MeSH Terms] AND 'disinfection'[MeSH Terms]) OR ('root canal therapy'[MeSH Terms] AND 'asepsis'[MeSH Terms]) AND 'anti-bacterial agents/therapeutic use'[Mesh Terms]) OR ('Rubber Dams'[MAJR] AND 'Root Canal Therapy/methods'[MAJR])

Material and methods

The literature search was run by one of the reviewers (LM) using the PubMed–Medline database (Table 1). The MeSH-browser was accessed to identify entry terms. Inclusion and exclusion criteria were determined based on the questions at issue and on the aim.

Inclusion criteria: articles published in English between 1950 and 2015; *in vivo* study on human subjects; clinical trials aiming to examine disinfection, or unintentional contamination, of the endodontic operative field; reproducible protocol for disinfection of the operative field; description and analysis of results of the bacteria sampling from the operative field.

Exclusion criteria: studies where checks on disinfection were a subordinate issue.

A list of 115 articles was obtained. Each title and abstract were screened in regard to the inclusion and exclusion criteria. If no abstract was available digitally, the record was obtained in print and the abstract thus screened. A majority of the articles did not meet the inclusion criteria and the screening process identified five relevant articles. These articles were read in full text. The reference lists from each of these articles were then checked manually for additional articles of relevance. Three additional articles were obtained.

Results

Table 2 summarizes the included studies. No study could prove a fully reliable aseptic operative field. Another finding of relevance is that not one chemical or combination of chemicals is found in more than one study.

Discussion

There is a wide range of variation in aims, methods, and materials of the included studies (Table 1). Many of them have small sample sizes or are based on small subgroups.[22,23,26,27] One study [27] reported that all the surfaces that were cleaned with 2.5% sodium hypochlorite showed negative bacteria samples. With the small sample of six subjects, the study provides low strength and too uncertain results. Most studies are carried out without a control group. The population is generally insufficiently described. In the studies, where endodontic treatment was carried out, pulpal diagnosis of the treated teeth is not stated. The description

Table 2. Summary of included studies.

Author	Aim	Material and method	Results
Ray 1955	Investigate disinfection of the endodontic operative field in situ.	25 volunteers. Intact incisors. 0.5% or 2% bensalkonium chloride in 50% isopropyl alcohol, chloroxylenol and 2% liq. iodii mitis. Bacterial samples cultivated aerobically.	2% benzalkonium rendered sterile surfaces in 94% of the cases. Chloroxylenol assessed to be as effective, while iodine was found to be less effective. No statistical significance stated.
Birch & Melville 1961	Compare the effectiveness of various antiseptics in sterilizing the operative field in situ.	Five test subjects. Intact incisors. 0.1% acriflavine in alcohol solution, iodine tincture, Hibitane (0.5% Chlorhexidine, 70% alcohol) and Savlon (0.05% chlorhexidine, 0.5% cetrimide and 70% alcohol).	Positive samples; Iodine 21/448, Hibitane 30/480, acriflavine 38/480, Savlon 32/320. Statistical analysis showed only significant difference between iodine and Savlon.
Michanovicz 1965	Investigate how effectively Metaphen sterilizes tooth surfaces prior to endodontic treatment.	65 teeth. Metaphen tincture (1:200). Bacterial samples incubated aerobically.	53–61% of the samples were negative. Concludes that tincture of Metaphene does not provide a sterile endodontic operative field.
Möller 1966	Sterilization of the endodontic operative field.	355 teeth. 30% hydrogen peroxide and/or 5% iodine tincture. Positive cultures were analyzed for both aerobic and anaerobic microorganisms.	30% hydrogen peroxide and 5% iodine tincture gave 98% sterile samples from the tooth and 83% from junction between rubber dam and tooth, compared to 40% and 6% respectively when only using 5% iodine tincture. The difference between the methods was statistically significant ($p < 0.001$)
Baumgartner et al. 1975	Compare the effectiveness of povidone-iodine with isopropyl alcohol as disinfectants of teeth isolated by a rubber dam	127 teeth. Disinfection with povidone-iodine or 99% isopropyl alcohol. Bacterial samples incubated aerobically.	No statistically significant difference between povidone-iodine and isopropyl alcohol. Both antimicrobial agents were found to be significantly less effective 15 min after application than after 90 s.
Fors et al. 1985	Examine leakage in the contact area between the tooth and rubber dam during endodontic treatment.	100 teeth. Four groups: no sealing material, wound dressing, silicone adhesive and two-component silicone material. The operative field cleansed with 30% hydrogen peroxide and 0.5% chlorhexidine in 70% alcohol. Bacterial samples incubated aerobically.	Microbiological leakage between rubber dam and tooth was found in 20–53% of the cases. Chi-square analysis showed reduction of bacterial contamination after application of sealing material ($p < 1\%$).
Hermsen & Ludlow 1987	Examine the effectiveness of 2.5% sodium hypochlorite as disinfectant of the endodontic operative field.	Six patients with caries-free molars Disinfection with merthiolate or 2.5% sodium hypochlorite. Bacterial samples were cultured aerobically on blood-agar plates.	Surfaces cleansed with Merthiolate were all positive for bacterial growth. Surfaces cleansed with sodium hypochlorite were all negative.
Ng et al. 2003	Compare the effectiveness of 2.5% sodium hypochlorite and 10% iodine tincture for disinfection of the endodontic operative field.	63 patients. 30% hydrogen peroxide followed by 10% iodine tincture or 2.5% sodium hypochlorite. Bacterial samples were cultured (aerobic and anaerobic) or analyzed by PCR technique.	Neither iodine nor sodium hypochlorite ensured complete disinfection. Significant ($p > 0.016$) reduction of contamination after repeated cleansing with sodium hypochlorite. Sodium hypochlorite more effective at reducing PCR-detected contamination.

of the bacterial sampling, cultivation, and analysis is sometimes flawed and not fully reproducible.[22,23,26,27] Some studies were performed on intact incisors.[22,23] An advantage with this choice is that the group is uniform and provides mutually comparable results, yet findings are not applicable to the clinical reality. One study [26] included teeth with deep coronal damage, which better reflects the daily clinical work. Different antibacterial solutions were tested in the studies. Surprisingly little is discussed about which bacteria were removed, the potential risk of adverse effects on tissues and how the results can be transferable to everyday clinical practice. Hence, there are gaps in knowledge regarding how effective disinfection of the endodontic operative field is in reality and how well an aseptic filed of operation can be maintained during endodontic treatment.

By virtue of the introduction of the PCR technologies irrelevant DNA must also be excluded from the field of operation in endodontics. Ng et al. [28] compared 10% iodine with 2.5% NaOCl after 30% hydrogen peroxide application and noted no difference in disinfection upon culturing, but less frequently detected bacterial DNA after 2.5% NaOCl.

None of the reviewed studies achieved fully reliable aseptic operative fields (Table 2). Of the studies reviewed, the best results were obtained with method A examined by Möller.[4] Möller's method came to have a major impact in Scandinavia and is often quoted as the gold standard. However, after assessing several studies on microbial sampling from the root canal, it can be noted that there exist several interpretations of this 'gold standard'. In some studies [29, 30] disinfection of the sampling area was not more thoroughly described than by referring to the procedure advocated by Möller. Other authors state that they have followed Möller's method, but based on the descriptions it becomes apparent that the interpretation of how this method was carried out varies.

Fors et al.,[26] for example described the method as disinfection with 30% hydrogen peroxide for 5 min, followed by application of 5 or 10% iodine tincture. But in the trial the operative field was cleansed with 30% hydrogen peroxide for 5 min followed by swabbing with 0.5% chlorhexidine in 70% alcohol. No reason was given as to why chlorhexidine was chosen instead of iodine tincture.

In many studies where bacterial sampling is made from the root canal, a control of the sterility of the operative field is taken before sampling the root canal. Yet the protocol for disinfection of the operative field is often not reproducibly described. The bacteria sampled from the operative field only served as a control, and were not the main objective of these studies.[29–31] The results were merely mentioned in a brief sentence or in a subordinate clause.

It has been suggested that if plaque were removed from the teeth before disinfection it would increase the likelihood of establishing a bacteria-free operative field. Given our knowledge of biofilms, removing plaque before applying the rubber dam seems justified. Mechanical cleansing reduces the microbial burden, thus increasing the ability of the biocides to penetrate the surface and operate more efficiently. Möller [4] notes that it is important to remove debris and plaque from the tooth surface first, since an aseptic environment cannot be achieved within a reasonable time with antiseptic agents alone. Baumgartner et al. [25] noted no increased disinfection when plaque was removed from the tooth surface before disinfection. The authors speculated that the manner in which the disinfectant was applied (rubbed with cotton pellets against tooth and rubber dam for 15 s) had removed the plaque. Perhaps the result would have been different if they had cleansed the surfaces with 30% hydrogen peroxide before applying iodine/isopropyl alcohol, given that hydrogen peroxide has a protein solving effect.

Michanowicz's [24] study is interesting from the perspective that it attempted to mimic how the disinfection process is performed in everyday clinical work. The disinfection process took 10 + 30 s, after which 30–90 s were required until the root canal treatment was started. In Möller's study [4] the cleansing of the operative field took 3–4 min. In the study by Fors et al. [26] the procedure took over 5 min. In the latter case, the teeth were polished or cleaned before application of the rubber dam. Probably there is a discrepancy between the methods used during controlled clinical conditions in scientific trials and the methods used in the everyday clinical practice. We know very little about how disinfection of the operative field is performed, and how effective it is in the general practice of dentistry.

The scientific evidence is insufficient to assess how well the aseptic operative field is maintained during endodontic treatment. During treatment the rubber dam is exposed to stresses, which may contribute to leakage. It is thus unclear how large the risk of leakage and contamination is. Baumgartner et al. [25] obtained significantly more positive bacterial tests after 15 min, compared to 90 s after cleansing. Fors et al. [26] found 20–53% positive cultures 60 min after disinfection. It is unclear whether these conditions were due to insufficient initial disinfection of the operative field, or if contamination took place during treatment. The study noted, however, less contamination of the operative field when sealing material had been used, which could imply leakage. Ng et al. [28] noted a significant reduction in contamination of the operative field after repeated disinfection with sodium hypochlorite. Perhaps it is necessary to clean the operative field repeatedly during endodontic treatment, in order to remove any bacteria that may have entered from outside the root canal. The question is how practical and applicable this would be in the everyday clinical setting. For an aseptic technique to be used clinically, it is advantageous if it does not require too much effort and contains too many different steps.

Surprisingly few studies have examined how effective the disinfection process is and how well a bacteria-free operative field is maintained during the subsequent endodontic treatment. The included studies show a wide variation in terms of aims, methods, and materials. After reviewing the literature, it can be concluded that there is limited scientific evidence to assess how efficient an aseptic endodontic operative field can be established under ideal and controlled clinical conditions. How well it works under more everyday clinical settings is unclear. Furthermore, there is insufficient scientific evidence to assess how well asepsis is maintained during endodontic treatment.

Our endodontic treatments aim to achieve good asepsis, high quality and at the same time require being cost-effective. This means that one should choose agents and materials that provide the best antimicrobial performance. At the same time the agents and materials must pose a low health risk for both patients and staff, as well as provide a low environmental impact. If we are to use solutions with negative environmental effects, there must be scientific evidence to support their superior efficiency over solutions with a lesser negative environmental impact. In order to make an informed decision reliable scientific evidence are needed.

Conclusion

No study reported complete asepsis after initial disinfection, and no study reported reliable maintenance of an established aseptic field of operation. The most predictable procedures appeared to require extensive efforts in terms of time spent on both mechanical and chemical disinfection procedures. A relevant finding is that not one combination of chemicals used for disinfection is found in more than one study. Critical evaluation and standardization of the disinfection methods and aseptic procedures in endodontics are needed.

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The authors deny any conflicts of interest related to this study.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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