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Efficacy of local acting antibiotics as intra canal medication

(An in vivo-in vitro study)

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BY

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Summary and Conclusions

Forty five extracted human single rooted teeth were collected from the outpatient clinic of the oral surgery department. All teeth were examined under magnifying loupes for detection of external cracks or fractures to be excluded.

After radio graphic verification of accurate tooth length, root canals were cleaned and shaped using stainless steel K files up to size # 60 MAF utilizing the step back technique. 10ml of Naocl 2.5% were used as root canal irrigant between different file sizes .Final canal flush was done using 2.5% Naocl followed by 17% EDTA for 3 min to remove the smear layer

Biofilm development: A clinical isolate of *E. faecalis* from the Microbiology laboratory (Central laboratories, Ministry of Health, Egypt) was used for biofilm formation. The bacterial strain was inoculated in Brain Heart Infusion broth (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37^{o}C for 24 hours.

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Introduction

Chronic apical periodontitis is one of the most common forms of biofilm-induced diseases that affect humans. The disease develops after dental pulp necrosis. The environmental conditions in the necrotic root canal are conducive to the establishment of a microbiota conspicuously dominated by anaerobic bacteria. Viable microorganisms remaining in ramifications and dental tubules after root canal preparation and disinfection contribute significantly to failure in endodontic therapy. The use of an intracanal medication is indicated as a dressing to promote bacterial elimination.

Entrococcus faecalis in endodontic infection represents a biofilm type of disease, which explains the bacterial resistance to various antimicrobial compounds and the possible subsequent failure after endodontic treatment. To overcome the abovementioned problems, an alternative protocol is to use antimicrobial agents that exhibit substantively, that is, agents that can have a therapeutic effect for a prolonged period. The use of locally acting antibiotic as an intra canal dressing to promote the

elimination of the remaining bacteria is one of the suggested answer but they are not fully studied.

Review of Literature

Endodontic microflora: Role of Entrococcus faecalis in endodontic infection and biofilm formation. Root canal infection is composed of mixed microbial infection in form of a biofilm. Many studies demonstrated that E.faecalis is frequently found in patient suffering from oral infection like gingivitis, periodontities, and teeth with failed endodontic treatment as well as acidic carious lesion associated with persistently infected root canal. Virulence factor of E. faecalis include adherence to host tissue, invasion and abscess formation, modulation of host response and secretion of active component which enhance biofilm formation.

Peciuliene et al. [(1)] studied the occurrence of Enterococcus faecalis in root canals of previously root filled teeth with apical periodontitis requiring retreatment. Twenty-five asymptomatic teeth were included in the study. Avoiding contamination, microbiological samples were taken from the canals before and after preparation and irrigation with sodium hypochlorite and EDTA. Results showed that, E. faecalis was isolated from 14 out of 20 positive culture teeth, either as a pure culture or as a major

component of the flora. Second samples taken after preparation revealed growth in 7 of the 20 teeth. Five of the seven cases were E. faecalis in pure culture. Isolation of E. faecalis was not related to the use of any particular root filling material in the original root filling.

Distel et al. [2] tested the hypothesis that *Enterococcus faecalis* resists common intracanal medications by forming biofilms. *E. faecalis* colonization of 46 extracted, medicated roots was observed with scanning electron microscopy (SEM) and scanning confocal laser microscopy. SEM detected colonization of root canals medicated with calcium hydroxide points and the positive control within 2 days. It also detected biofilms in canals medicated with calcium hydroxide paste in an average of 77 days. Scanning confocal laser microscopy analysis of two calcium hydroxide paste medicated roots showed viable colonies forming in a root canal infected for 86 days, whereas in a canal infected for 160 days, a mushroom-shape typical of a biofilm was observed .These observations supported potential *E. faecalis* biofilm formation in vivo in medicated root canals.

De Paz et al. [3] determined whether there is a pattern for certain bacteria to remain after chemo-mechanical treatment of root canals in teeth with apical periodontitis. Consecutive root-canal samples of 200 teeth receiving root-canal treatment were studied. All samples were from teeth that either presented with clinical or radiographic evidence of apical periodontitis or both. Bacteriological findings were linked to clinical and radiographic parameters including status of the root canal prior to treatment, namely, vital pulp, necrotic pulp or root filled. Results showed that, a total of 248 strains were isolated from 107 teeth giving bacterial growth. Gram-positives predominated (85%). Lactobacillus spp. (22%), nonmutans streptococci (18%), and Enterococcus spp. (12%) were the most common isolates. Gram-negative anaerobes were relatively sporadic.

Gomes et al. [4] examined the root canal micro biota of primary and secondary root-infected root canals. Microbial samples were taken from 60 root canals, 41 with necrotic pulp tissues (primary infection) and 19 with failed endodontic treatment (secondary infection). Strict anaerobic techniques were used for serial dilution, plating, incubation and identification. The root canal micro

flora of untreated teeth with apical periodontitis was found to be mixed, comprising gram-negative and gram-positive and mostly anaerobic. On the other hand, facultative anaerobic and gram-positive bacteria predominated in canals with failed endodontic treatment.

Nakajo et al. [5] isolated and identified alkali-resistant bacteria from the dentin of infected root canals. Bacteria from homogenized dentin powder made up from infected root canal walls were cultured on buffer-enriched Brain Heart Infusion agar supplemented with 4% sheep blood (BHI-blood agar), adjusted to pH 7.0, 9.0 or 10.0. Incubation took place for 7 days at 37°C in an anaerobic condition. Polymerase chain reaction was performed for identification of alkali-resistant isolate. They found that, many bacterial species in infected root canal dentin were alkali-resistant at pH 9.0 and/or pH 10.0, and belonged mainly *to Enterococcus faecium* and *Enterococcus faecalis*

Adib et al. [6] identified cultivable bacterial flora in root filled teeth with persistent per apical lesions and their distribution within the root canal system using an in vitro sampling protocol. Eight freshly extracted root filled teeth with persistent apical periodontiti and evidence of coronal

leakage were used in this study .They were transferred to an anaerobic chamber immediately after careful extraction and sectioned transversely to give a crown and two root segments . The samples were dispersed, serially diluted and cultured on blood agar and fastidious anaerobic agar .The primary growth was subcultured to obtain pure isolates, which were identified by microbiological techniques and commercial enzyme tests. Results of this study showed that, the predominant group of bacteria in root filled teeth with persistent apical periodontitis and coronal leakage was Gram-positive facultative anaerobes of which staphylococci followed by streptococci, and enterococci were the most prevalent.

A collection of Enterococcus faecalis strains from clinical isolates was screened by **Creti et al**.[7] for the presence of virulence factor genes, such as those for collagen-binding protein (ace), endocarditis antigen (efaA), haemolysin activator (cylA), gelatinase (gelE), aggregation substances (asa1 and asa373), a surface protein (esp) and two novel putative surface antigens (EF0591 and EF3314). Data showed that, apart from some genes that were present in all strains (ace, efaA and EF3314), the gelE gene was the most common factor, although its presence did not

correlate with its expression. The genes that encode Esp and CylA were never detected in endocarditis isolates, whereas an association was noted between the esp gene and isolates from urinary tract infection (UTI) and bacteraemia. An aggregation substance gene was always present in commensal strains.

De Marques and Suzart [8] examined the occurrence of known virulence determinants in a group of E. faecalis strains isolated from different clinical sources in Brazil. A total of 95 E. faecalis strains were investigated for the presence of nine virulence genes including aggA, cylA, cylB, cylM, eep, efaA, enlA, esp and gelE by using PCR. The data showed a relatively wide distribution of the virulence genes among the investigated strains. The clinical strains carried at least one and concomitantly up to as many as eight virulence markers, with two or three being the most common pattern. Simultaneous presence of virulence markers was observed among clinical strains regardless of their sources. In this study, the efaA+ esp+ gelE+ profile was the virulence genotype most frequently detected among E. faecalis strains. Finally, there was no significant association between virulence markers and clinical sources.

Rôças et al.[9] applied the PCR-DGGE fingerprinting approach to examine the structure of the bacterial population infecting previously treated root canals of humans associated with persistent periradicular lesions. Samples were taken from 14 filled root canals, DNA was extracted, and part of the 16S rDNA of all bacteria was amplified by PCR and separated by DGGE, generating banding patterns representative of the community structure. Species-specific PCR for the detection of Enterococcus faecalis was also performed. Each sample showed a unique structure of the microbial community. The species-specific PCR assay revealed the presence of E. faecalis in 10 of 14 samples, but DGGE analysis revealed it was not the dominant species. They revealed that the intraradicular bacterial community associated with failed endodontic treatment significantly varied in composition from one case to the other. Persistent intraradicular infections were present in all root-filled teeth.

Takemura et al. [10] investigated the initial biofilm-forming ability of root canal isolates (Enterococcus faecalis, Streptococcus sanguis, Strep. intermedius, Strep. pyogenes, Staphylococcus aureus, Fusobacterium nucleatum, Propionibacterium acnes, Porphyromonas

gingivalis and Prevotella intermedia) on gutta-percha points in vitro. Each bacterial strain was suspended in 100% cell culture medium. The bacterial suspensions were then co-incubated anaerobically with gutta-percha points for 7 days and processed for scanning electron microscopic observation and examined for biofilm presence and thickness. They found that, E. faecalis and Strep. sanguis biofilms were significantly thicker than those of Strep. intermedius, Strep. pyogenes and Staph. aureus. No biofilms were detected on the specimens incubated with F. nucleatum, Prop. acnes, Porph. gingivalis and Prev. intermedia. These findings suggested that Gram-positive facultative anaerobes have the ability to colonize and form extracellular matrices on gutta-percha points.

Lacević et al. [(11)] investigated different microbial morphotypes in the root canal infection associated with chronic diffuse periapical lesion. In forty cases of asymptomatic teeth with radiographically diagnosed diffuse periapical lesion, specimens of infected tissue were taken from the root canals at the beginning of endodontic treatment. Fixation and four different staining methods of the specimens were obtained to provide microscope examination. They found that, all examined root canal

specimens were heavily infected by bacteria. The most commonly identified were cocci mostly grame +ve diplococcic. Bacilli were found in 67%, coccobacilli 37%, fungi 17% while spirochetes were observed in 5% of the cases.

Fosch et al.[12] studied the presence of selected bacteria (Enterococcus faecalis and Treponema denticola) in infected root canals using polymerase chain reaction (PCR) assays. Microbial samples were obtained from 62 teeth in 54 patients with endodontic disease. For each tooth, clinical data including patient symptoms were collected. Teeth were categorized by diagnosis as having acute apical periodontitis (AAP), chronic apical periodontitis (CAP) or exacerbated apical periodontitis (EAP). Results showed that. T. denticola and E. faecalis were each detected in 15 of 62 samples (24%).E. faecalis was found in 60% of teeth with CAP and in 72% of teeth with secondary infection. Statistical analysis demonstrated an association of CAP and secondary endodontic infection with the presence of E. faecalis while EAP was associated with the presence of T. denticola .