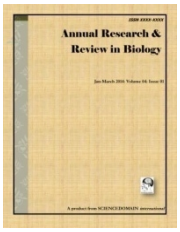

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Penetration of *E. faecalis* into Root Cementum Cause for Reinfection

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Authors' contributions

This work was carried out in collaboration between all authors. Author HR designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author HNM managed the analyses of the study. Author HK managed the literature searches. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Enterococcus faecalis possesses the unique properties such as production of serine protease and specific gene Ace, which aid in binding to extra cellular matrix protein like collagen, aggregation substances that help *E. faecalis* adhesion to recipient bacteria; helps to form biofilms through surface adhesion, which in turn resist alkalinity of calcium hydroxide in the medicated canal. The production of lipoteichoic acid by *E. faecalis* enables binding to PNM cell, erythrocytes, and platelets, and lysis of cell in vitro and in vivo. It can resist the high temperature of 60ºC for 30 minutes and can grow at 10–45ºC in a pH of 9.6, and is the most frequently isolated micro-organism from root-filled teeth. *E. faecalis* can penetrate up to 140 to 160 µm deep into root dentin and root cementum beyond minor constriction in root-filled teeth. The aim of this article is to understand the role of *E. faecalis* beyond minor constriction and its role in causing persisting infection. It was found that the virulent factors and ability of *E. faecalis* to penetrate deep into root dentin and root cementum beyond minor constriction and ability to adapt to environment in adverse conditions are the possible causes for persisting infection in root-filled teeth.

Keywords: Enterococcus faecalis; root filled teeth; persisting infection; root cementum and root dentin.

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1. INTRODUCTION

1.1 *Enterococcus faecalis*

Enterococcus faecalis is predominantly found in the gastrointestinal (GI) tract of humans and most commonly infects the urinary tract, cardiac muscles (endocardium), GI tract wounds, etc. [1] It is the most common microorganism isolated from chronic apical periodontitis in 23–70% of the cases, [2,3] and also most frequently isolated from lesions after root canal-filled teeth. [4,5] *E. faecalis* is most virulent microorganism, [4] all the above factors make *E. faecalis* a dynamic and resistant microorganism. [6] It can withstand high temperatures of 60°C for 30 minutes and can grow at 10–45ºC at a pH of 9.6. When exposed to adverse conditions, the microorganism undergoes transformation and become less sensitive to toxic dosages of heat, sodium dodecyl sulphate, bile salts, acidity and alkalinity, [7,8] and also to sodium hypochlorite, hydrogen peroxide, ethanol and UV irradiation. [9] It can undergo VBNC (viable but non-cultivable) change under stress and can revert to cultivable form in favourable conditions. [10] *E. faecalis* is resistant to calcium hydroxide [11,12] because of proton pump mechanism. [13] Other virulent factors of *E. faecalis* arise due to the production of aggregation substances that help in its bacterial adhesion to recipient bacteria. [1] Its surface adhesion helps to form a biofilm, which in turn resists alkalinity of calcium hydroxide in a medicated canal. [14] The production of lipoteichoic acid by *Enterococcus faecalis* helps in its binding to PNM cell, erythrocytes, platelets etc. and also in the lysis of cell in vitro and in vivo. [1] It has the ability to produce collagen-binding proteins like serine protease and specific gene Ace, whose encoded protein has characteristics of adhesin and therefore implicated for collagen binding present in the root dentin and cementum [15,16].

Enterococcus faecalis can remain viable for 12 months in vivo. It gets entombed after root canal treatment and returns to a viable state under favourable conditions. [17] Its maximum resistance is seen in starvation phase and no resistance is seen during the exponential stage [18].

Literature survey was undertaken from sources such as Central Library in A.B.S.M.I.D.S, Mangalore, HELINET digital library in RGUHS, Bangalore, and through Google search.

1.2 *Enterococcus faecalis* in Root Dentin

Studies have shown that *E. faecalis* invades dentinal tubules and strongly gets adhered to type I and type IV collagen, which is abundantly present in root dentin and cementum. [19,20] In the presence of serum and biological cues, *Enterococcus faecalis* has the ability to produce adhesins which act as an activator of adherence to extracellular matrix proteins fibrinogen, fibronectin and collagen [21,*22*]. The virulence factors and collagen-binding properties enable *Enterococcus faecalis* to invade the dentinal tubules [23].

PCR analysis showed that *Enterococcus faecalis* is most commonly isolated in 76% of the cases in root-filled teeth and 82% of the cases in primary infected teeth, [24] because *Enterococcus faecalis* has the ability to colonize deep into dentinal tubules up to 156.2µm, [19] at the same time resisting biomechanical preparation and intra-canal medication. In the view of understanding the viability of *Enterococcus faecalis* deep into root dentin, confocal laser scanning microscope study was done, which showed that the depth of viable *E. faecalis* ranges from 100 to 400µm into dentinal tubules, [25] and in biomechanically prepared teeth, the depth of *Enterococcus faecalis* penetration into the dentin is up to 160

μm. Latest technology such as confocal analysis helps us differentiate between live and dead bacteria [26].

1.3 *Enterococcus faecalis* in Root Cementum

Cementum consists of approximately 60% organic substances, mainly made up of type I collagen [27]. Intact cementum is essential to prevent bacterial penetration into dentin and pulp canals. Bacterial invasion is prominent when cementum layer is damaged. The denudation of the cementum layer occurs in many cases such as in trauma, chronic periodontal disease and in root resorption, paving the way for the microorganism to penetrate deep into root dentin Bacterial retention into prepared tooth leads to reinfection [28].

1.3.1 Most commonly isolated microorganisms from apical 5mm of infected teeth

The most commonly isolated microorganisms from infected root canal at apical 5mm are *Enterococcus*, *Actinomyces, Lactobacillus, Peptostreptococci*, black pigment rods, and *Streptococcus mutans*, which are mainly anaerobic [29]. Anaerobic bacteria were found to be predominant in the culture obtained from intact teeth with apical periodontitis. Of the patients examined, 58 cases required apical surgery. Histological examination and culture techniques showed that 87% were strictly anaerobic, 37% facultative anaerobic and 9% were aerobic bacteria. Therefore, primary infection is dominated by anaerobic bacteria and facultative anaerobic microorganisms are predominant in secondary infection [30]. *Enterococci* are rare in primary endodontic infections, but commonly associated with persistent or reinfection cases. A more likely explanation for the high occurrence of *Enterococci* in filled root canals is that *Enterococci* do not appear to be colonizers of the oral cavity. They are merely transient oral bacteria, unless there is a predilection site such as the unsealed, necrotic or filled root canal. Therefore in root filled teeth, *E. faecalis* gets entombed and changes the micro environment that can create favourable conditions for infection [31].

The incidence of *E. faecalis* was seen in both asymptomatic primary infections and in asymptomatic persistent infections when evaluated by nested PCR but more in secondary infections [32].

1.3.2 Most commonly isolated microorganism from apical 5mm of root filled teeth

The failure of the root-filled teeth is primarily due to the incomplete elimination of microbiota from root canal and bacteria resistant to intracanal medicaments and irrigants, [33] or due to microleakage of post-endodontic restoration and facultative anaerobic bacterium is predominant in the microbiota of root-filled teeth. Biochemical and molecular methods to isolate microorganisms from root-filled teeth with periapical lesions showed that *Enterococcus faecalis* is only enterococcus species isolated from root canal with root-filled teeth [30]. Pulsed field gel electrophoresis (PFGE) technique showed 13 distinct genotypes of *Enterococcus faecalis* that are phenotypically similar [34]. Polymerase chain reaction (PCR) technique showed that *Enterococcus faecalis* is the most frequently isolated microorganism in 77.8% of the root-filled teeth. [35] Thus the above studies show that *Enterococcus faecalis* is the most commonly microorganism isolated from root-filled teeth.

1.3.3 Role of biofilm at root apex

Bacterial adaptation determines the pathogenicity of the microorganism. Bacterial adaptation occurs due to biofilm formation, [36] physiological modification, stress response [37] and by subpopulation methods. [38] Biofilm forms on calcified structures like enamel or cementum when planktonic bacteria in liquid form adhere to conditioning polymeric matrix formed on inorganic structures, multiplying bacteria in the process and getting detached to form the biofilm. [39] *Enterococcus faecalis* has the ability to form this kind of a biofilm. [4] *Enterococcus faecalis* present in biofilm are 1000 times more resistant to antibacterial agents, phagocytosis and antibodies than non-biofilm-producing bacteria. [40] The ability of *Enterococcus faecalis* to produce biofilm under stress is one of the causes for persisting periapical infection [41,42].

1.3.4 Penetration of *Enterococcus faecalis* into root cementum

The ecological plaque hypothesis proposed by Marsh [43] states that pathogenic microorganisms are routinely found in dental plaque at normal pH. It is also found that the absence of carbohydrate diet does not lead to a change in the structure of enamel, but in presence of carbohydrate diet, bacteria produce acid, leading to demineralization and dental caries due to low pH. Similarly in intact cementum and in unfavourable conditions *Enterococcus faecalis* adheres to root cementum. [44] *Enterococcus faecalis* penetrates deep up to 140µm into root cementum due to cementum microporosities produced in vitro by exposing the cementum to an acidic pH. [45] Further penetration of *Enterococcus faecalis* up to 160µm into root cementum is found when severity of infection increases, by exposing the cementum to the acidic pH. Apical roughening is also observed, which implies apical resorption to mimic chronic apical periodontitis in vitro. [46] The above in vitro studies show that as severity of the infection increases at the root apex. The *Enterococcus faecalis* penetration is also deep and chances of persisting infection also increases [45,46].

2. DISCUSSION

For any bacteria to be pathogenic and virulent in nature, it should first attach to the host, then multiply and grow, invade the host, survive the host defence mechanism and adapt to stressful conditions. All these properties are exhibited by *Enterococcus faecalis*, which makes it not so easy to eliminate from the root canal system.

Enterococcus faecalis has the ability to produce adhesin, Ace and serine protease, [15] which are collagen-binding proteins. With the help of these proteins *Enterococcus faecalis* adhere to collagen present in root dentin and cementum. *Enterococcus faecalis* resists biomechanical preparation (BMP) and can penetrate deep up to 140µm into root dentin. [26] It cannot be completely eradicated by multiple or single visit RCT, [47] irrigation or intra canal medications [48,49] and *E. faecalis* biofilm formation is seen when root canal is filled with intra-canal medication [12] because it produces certain virulence factors therefore survives in such environments [1].

Studies have shown that one of the causes of persisting infection is due to penetration of *E. faecalis* deep in to root cementum beyond minor constriction in adverse conditions. [45,46] With the help of apex locator, [50] setting the apex locator manually up to root apex and extending the BMP up to the root apex may reduce the percentage of *E. faecalis* getting into the root cementum beyond minor constriction, but studies have shown that extending the working length beyond minor constriction leads to extrusion of material, which, in turn,

provides an opportunity for foreign body reaction and sever inflammatory reaction even in the absence of pain [51].

One of the indications of revascularisation of non-vital teeth is microorganism-free root canal. [52] But revascularisation is not a possible option due to *E. faecalis* penetration deep into root cementum [45,46].

3. CONCLUSION

Enterococcus faecalis is most resistant microorganism causing persisting infection or reinfection, so its eradication from the root canal system completely is the need of the hour. As severity of infection increases, penetration of *E. faecalis also increases*. So early the intervention at initial stages of infection, less are the chances of *E. faecalis* penetration as also are the chances of persisting infection. Intervention at the early stages of infection will increase the success rate of root canal treatment or apicoectomy will be the treatment of last resort for successful root canal treatment in advanced stages of apical infection.

The clinical significance of this study is that adhesion and penetration of *Enterococcus faecalis* deep into root cementum provide long-term source for subsequent infection and is the possible reason for persisting infection after root canal treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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