﴿ قالوا سبحانك لا علم لنا الا ما علمتنا

إنك انت العليم الحكيم ﴾

صدق الله العظيم

الآيه(32) سوره البقره

The antimicrobial action of Trypsin on mature Enterococcus Faecalis Biofilm and its effect on Dentin Hardness (in vitro study)

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Dedication

To the soul of my Cousin Noussa

To My Dearest Father

To My Lovely Mother

To My Faithful Husband

To My Sweet Sisters

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List of Contents:

List of Figures

List of Tables:

Since 1943, when marine microbiologist Claude ZoBell [(1)] described the so-called "bottle effect" (referring to the phenomenon that the number of free-living microorganisms in fresh sea water gradually declines when the water is kept in a glass bottle, while the number of attached microorganisms increases) [(1)] we have been aware of the fact that microorganisms are capable of living their life attached to a surface. However, it then took more than 30 years to accept that for microorganisms (both bacteria and fungi) the biofilm mode of life is the rule rather than the exception [(2, 3)].

Biofilms can be defined as cells attached to a surface embedded in an extracellular polysaccharide (EPS) matrix that fills the space between cells [(4)]. This matrix is important in both the formation and structure of the biofilm, and also in the protection of the cells since it may prevent the access of antimicrobials to the cells inside the biofilm and offer protection against environmental stresses such as UV radiation, pH shifts, osmotic shock and desiccation. Biofilms are present in necrotic pulp canal spaces of primary and secondary root canal infections [(5)].

One of the most prevalent bacterial strains found in resistant endodontic infections is *Enterococcus Faecalis* [(6)]. It has been reported that they have the ability to resist mechanical and chemical root canal cleaning procedures and retain viable even in what's called "starvation phase" [(7)]. It was concluded from the previous studies that binding to dentinal walls and biofilm formation are of the most important virulence factors for this bacterial strain [(8)].

Although the eradication of intra-radicular biofilms should be ideally performed by mechanical methods, the instrumentation process cannot directly access a considerable amount of infected tissue owing to the irregularities of the root canal system [(9, 10)]. For this reason, antimicrobial materials are used during instrumentation and as a final rinse before canal filling. Several irrigant solutions have been proposed for use during the final rinse. EDTA and citric acid are used to eliminate the smear layer produced by the action of endodontic instruments on root canal walls [(11, 12)].

The results of clinical studies have shown that at least 50% of root canals can accommodate bacteria

after endodontic procedures with sodium hypochlorite NaOCl–based or chlorhexidine-based protocols [13, 14]. Many antimicrobial agents fail to penetrate the biofilm due to the EPS which acts as a barrier protecting the bacterial cells within. So alternatively, compounds that will degrade the EPS of the biofilm can be used first; rendering the bacteria liable to the effect of antimicrobial agent applied after that.

Enzymes have been proven to be effective for the degrading of the EPS of the biofilms. They remove the biofilm directly by destroying the physical integrity of the EPS through weakening the proteins by a degradation process [15, 16, 17, 18].

Therefore, conducting a study to evaluate the antimicrobial action of Trypsin enzyme when used as an intracanal medication on *Enterococcus faecalis* biofilm model was thought to be of value.

I-Enterococcus Faecalis in Apical Periodontitis:

- ***Prevalence:***

Enterococcus faecalis is a Gram-positive facultative coccus that lives in the human intestinal lumen and under most circumstances causes no harm to its host as well as being a commensal of the oral cavity [19] Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% [20] . In some cases, *E. faecalis* has been found as the only organism present in root-filled teeth with periradicular lesions [21, 22], and in mixed infections it is frequently the most dominant species [23].

The high prevalence of *Enterococcus faecalis* in root canal treated teeth with post-treatment disease, as evidenced by both molecular and traditional culturing methods, suggests that this species may be a key player in endodontic treatment failure [20, 24].

By Using both Real Time PCR and Reverse Transcription PCR methods **Williams et al** [25] found that *E. faecalis* bacterium was up to three times more prevalent in refractory than primary infections at each