بسم الله الرحمن الرحيم

(وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ
وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ
فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ)

صدق الله العظيم

أية مئة و خمسة من سورة التوبة

In vitro Assessment of the Antimicrobial Effect of Grapefruit-Seed Extract as an Endodontic Irrigant.

Thesis submitted To Endodontic Department
Faculty Of Dentistry
Ain-Shams University

For partial fulfillment of the requirements of
Master degree in Endodontics

Submitted by

Basant Aly Yehia AbdelRahman
B.D.S., Faculty of Oral and Dental Medicine,
Alexandria University, 2000

(20١2)

Supervisors

Dr. Ahmed Abd El Rahman Hashem
Associate Professor of Endodontics,
Faculty Of Dentistry, Ain-Shams University

Dr. Abeer Abdulhakeem Mahmoud
Lecturer of Endodontics,
Faculty Of Dentistry, Ain-Shams University

Dr. Makram Fahmy Attalah
Lecturer of Medical Microbiology and Immunology,
Faculty of Medicine, Ain-Shams University

Acknowledgement

All praise and thanks be to **Allah**, the most Compassionate, the most Merciful.

I am proud to be supervised by *Doctor Ahmed Abd El Rahman Hashem* Associate Professor of Endodontics, Faculty of Dentistry, Ain Shams University, whom I thank for his inspiration, valuable guidance and for suggesting the main theme of the study.

I would like to express my sincere gratitude to *Doctor Abeer Abdulhakeem Mahmoud*, lecturer of Endodontics, Faculty of Dentistry, Ain Shams University, for her help, co-operation and helpful remarks during this study.

My deep appreciation to *Doctor Makram Fahmy Attalah* lecturer of Medical Microbiology and Immunology, Faculty of Medicine, Ain-Shams University for his effort and support throughout the study.

I dedicate my work

To My Precious Husband

To My Loving Parents

To My Sweet Children

Table of contents

List of Tables:

Table ***Page***

List of Figures

Figure ***Page***

The reduction or eradication of the bacterial population seems to be a justified goal during the course of root canal treatment. Elimination of endodontic infection is quite different from most other sites in the human body. This is mainly because of the special anatomy and physiology of the tooth and of the root canal. Irrigant solutions are used during mechanical instrumentation. The ideal irrigant should be able to kill bacteria, dissolve necrotic tissue, lubricate the canal, remove the smear layer and does not irritate the healthy tissues. Until this time no irrigant possesses all these properties.

Enterococcus faecalis (E.faecalis) is a facultative Gram-positive coccus considered one of the most resistant & virulent strains of the oral cavity. Among it's virulence factors, it can compete with other organisms, invade dentinal tubules & resist nutritional deprivation. This bacterium is often present in persistent endodontic infections & failed endodontic cases. *E.faecalis biofilm* has been used to evaluate the antimicrobial efficacy of irrigants and root canal medications.

Grapefruit-seed extract (GSE®) is a commercially available substance that has received some attention for having antimicrobial properties. The manufacturer claims the

effectiveness of GSE includes successful treatment for dermatologic conditions such as dermatitis, warts, and poison ivy. GSE is made by first converting grapefruit seeds and pulp into an acidic liquid. This liquid is loaded with polyphenolic compounds or *Bioflavanoids.* Grapefruit-seed extract is considered to be effective against more than 800 bacterial and viral strains, 100 strains of fungus, and a large number of single and multi-celled parasites [(1)]. However, the efficiency of this substance as an endodontic irrigant is still unclear.

1- *Enterococcus faecalis* biofilm.

Biofilm is a term, that designates the thin layered condensation of microbes (bacteria, protozoa and fungi) that may occur on various surface structures in nature. Free-floating bacteria existing in an aqueous environment, so-called *planktonic micro-organisms*, are a prerequisite for biofilm formation. Such films may become established on organic as well as inorganic surface substrates where planktonic micro-organisms prevail in water-based solution.

The earliest stage of biofilm formation involves the adsorption of *macromolecules,* from salivary proteins, to the surface, leading to the formation of a conditioning film. The second stage involves *adhesion and co-adhesion* of micro-organisms and strengthening of the attachment through polymer matrix production. The third stage involves multiplication of attached micro-organisms that ultimately will result in a *structurally organized mixed microbial community*. During this stage the inherent characteristics of the micro-organisms and the nature of the micro-environment influence growth and succession of micro-organisms in the biofilm [(2)].

Biofilm formation in root canals, as hypothesized by Svensäter and Bergenholtz [(2)], is probably initiated at some time after the first invasion of the pulp chamber by planktonic oral

organisms after some tissue breakdown. At this point, the inflammatory lesion frontage that moves successively toward the apex will provide the fluid vehicle for the invading planktonic organisms so these can multiply and continue attaching to the root canal walls forming a biofilm.

Enterococcus faecalis, a gram positive, facultative coccus is the most implicated species in post-treatment disease [3]. It lives in the human intestinal lumen and under most circumstances causes no harm to it's host as well as being a commensal of the oral cavity [4]. Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% [3]. In some cases, *E. faecalis* has been found as the only organism (monospecies) present in rootfilled teeth with periradicular lesions [5,6], and in mixed infections it is frequently the most dominant species [7].

Among it's virulence factors, *E.feacalis* can compete with other organisms, invade dentinal tubules & resist nutritional deprivation [5]. *E.faecalis* possesses lytic enzymes, cytosine, aggregation substance and pheromones [5]. It has even proven resistant to inter appointment medications including Calcium hydroxide [8], and to tetracycline irrigation [9].

Nair [10] was probably the first to identify biofilm structures in infected root canals in 1987. Using Transmission Electron Microscopy (TEM) the root canal contents of 33 teeth, to which periapical lesion was attached upon extraction, were examined. It was noted that the major bulk of organisms existed as 'loose collections' of cocci, rods, filaments and spirochetes. While most of these organisms appeared suspended, in what he felt was a moist canal space (*Planktonic phase*), dense aggregates were also observed sticking to the canal walls and forming thin to thick layers of bacterial condensations (*Biofilm*). Amorphous material filled the inter-bacterial spaces and was interpreted as an extra-cellular matrix of bacterial origin. When they occurred, the bacterial condensation showed a palisade structure similar to the one for dental plaque on external tooth surfaces, suggesting similar mechanisms for bacterial attachment as those for dental plaque.

Haapasalo and Orstavik [11] developed a model for in-vitro dentinal tubule infection. Cylindrical dentin specimens, 4 mm high with a diameter of 6 mm and a canal 2.3 mm wide, were prepared from freshly extracted bovine incisors. After removing the cementum the tubules were opened by 4min treatments with 17%EDTA and 5.25%NaOCl. The dentine blocks were autoclaved before being infected with *E.faecalis* for 3 weeks. SEM as well as histological staining and examination under the