EVALUATION OF THE ANTIMICROBIAL EFFECT OF ROOT CANAL SEALERS AMOXICILLIN COMBINATION AGAINST ENTEROCOCCUS FAECALIS. (An In Vitro study)

Thesis

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By

Sherif Al-Hassan Abdo Heidar B.D.S, 2007 (October 6 University)

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SUPERVISORS

Prof. Dr. Alaa Dyab

Professor in Endodontics Department, Faculty of Oral and Dental Medicine, Cairo University

Dr. Heba Abdallah El-Bialy

Associate Professor in Radiation Microbiology Department, Atomic Energy Authority

Dr. Karem Galal Abd-AlQader

Lecturer in Endodontics Department, Faculty of Oral and Dental Medicine, Cairo University

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To my father, Prof.Dr. Hassan Heidar who granted me moral values in my life and during my academic journey,

To my mother who offered unconditional love,

To my kind sister yasmine and helpful brothers

Mostafa and Hossam.

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LIST OF ABBREVIATIONS

ADA American dental association

ADT Agar diffusion Test

ATCC American type culture collection

BHI Brain Heart Infusion broth

CDC Centers of Disease Control

cfu Colony forming unit

DCT Direct contact Test

 E_{max} Maximal effect

FDA Food and Drug Adminstration

I.M. Intramuscular

ISO International Standards Organization

MBC Minimal inhibitory concentration

mg Milligram

MH Müller-Hinton

MIC Minimal inhibitory concentration

mm Millimeter

ml Milliliter

MTA Mineral Trioxide Aggregate

NiTi Nickel Titanium

PBS Phosphate Buffered Saline

PCR Polymerase chain reaction

SEM Scanning electron microscope

St. St. stainless steel

TEM Transmision electron microscope

THA Todd-Hewitt Agar

THB Todd-Hewitt Broth

TSA Tryptone Soy Agar

TSB Tryptone Soy Broth

μl Microliter

μm Micrometer

INTRODUCTION

Ancient Greek scholars Hippocrates and Aristotle wrote about dentistry, including decayed teeth treatment. Literally, endodontics is a Greek term; *endo* "inside" and *odons* "tooth". Endodontics is the dental specialty recognized by the American Dental Association to deal with the tooth pulp and the tissues surrounding the root of a tooth.

In 1904, Frank Billings, directed the attention to the relationship between oral sepsis and bacterial endocarditis. Few years later, E.C. Rosenow developed the theory of "focal infection" in a study of bacterial aspects of root canal therapy; the theory demonstrated that bacteria present in diseased organ could cause infection at distant site by hematogenous spread.

Like all health care clinical disciplines, dentistry is focused on the prevention and elimination of disease. Endodontics targets the prevention and elimination of pulp space infections and associated inflammatory sequelae.

In the past few years, *Enterococcus faecalis* has been the focus of increased interest in medicine and dentistry. A recognized pathogen in post-treatment endodontic infections, probably because the specie is the best to adapt and tolerate the ecologically demanding conditions in the filled root canal and causative organism in endocarditis.

Endodontic literature in regards to the achievement of a hemetic seal, as the ultimate goal of endodontics, contributing to optimize root canal therapy outcomes. In general, there is an agreement that most common cause of endodontic failure, is the incomplete obturation of the root canal system.

The standard root filling is a combination of core material and sealer cement; core acts as a piston on the flowable sealer, causing the sealer to closely adapt to the dentin walls *i.e.* sealer occupies the critical situation, bonding to dentine.

With the increased concern regarding the survival of *Enterococcus faecalis* in obturated root canals and the reports of it's drug resistance, studies must be directed to investigate ways of it's elimination from root canals.

Adding antibiotics to sealers can enhance their antimicrobial effect and provide an important advantage in reducing the critical concentration of microbes necessary for a favorable host response. During endodontic therapy, clinicians may incorporate antibiotic regimen as part of armamentarium for optimum treatment. Root canal infection can't be eliminated neither by the host defense mechanisms nor by systemic antibiotic therapy. This can be explained by the absence of a blood supply in a necrotic pulp which impedes the transport of defense cells as well as systemically administrated antibiotics to the infected site. In addition to systemic administration of antibiotics relies on patient compliance. Accordingly, alternative ways for delivering antibiotics should be considered.

Although few publications available in the literature regarding the root canal sealer antibiotic combination, these publications stated that this combination is a promising strategy that deserves further research.

REVIEW OF LITERATURE

The influence of bacterial persistence in the root canals on treatment outcome is an important issue in endodontics. Bacteria play a major role in persistence or emergence of apical periodontitis lesions after root canal treatment. Several studies revealed that the outcome of the endodontic treatment is significantly influenced by the presence of bacteria in the root canals at the time of filling indicating that persisting bacteria can survive in treated canals and induce or sustain periradicular tissue inflammation. Thus underpinning the concept that the eradication of bacteria from the root canal system should be the ultimate goal of the endodontic treatment, **Siquiera and Roças 2008**⁽⁸⁸⁾.

I. The ecology of root canal system

The ecology is the science of relationship between organisms and their environment also called "Bionomics".

The root canal system represents a unique environment for growth and proliferation of microorganisms, accordingly it has undergone several studies using scanning electron microscope (SEM) and micro computed tomography (μ CT) to asses it's morphological and biochemical characteristics that may alter bacterial defensive mechanisms or inhibit delivery/action of antimicrobials.

There is a general agreement that mechanical root canal preparation using current instrumentation and techniques significantly reduce bacterial population but it fails to completely eradicate bacteria from root

canals. Moreover, there is evidence that mechanical instrumentation leaves significant portions of the canal untouched, **Mannan** *et al.* **2001**⁽⁴³⁾, **Peters** *et al.* **2001**⁽⁵⁹⁾, **Mohammadi and Abbott 2009**⁽⁵¹⁾.

Garberoglio and Brannstrom 1976⁽²³⁾ Studied the human dentinal tubules by scanning electron microscope. The study shown that the dentinal tubules traverse the entire width of dentine with a conical conformation, the largest dentinal tubule diameter located near the pulp (mean, $2.5 \mu m$) and the smallest diameter in the periphery near enamel or cementum (mean $0.9 \mu m$).

Nair *et al.* 2005⁽⁵⁴⁾ observed the microbial status of the root canal system, during various stages of root canal treatment. The study concluded that the anatomical complexity of the root canal system and the organization of the flora as biofilms in inaccessible areas of the root canal system, disable the complete removal of intercanal bacteria by contemporary instruments and irrigation alone in one-visit treatment. A fact necessitating the stringent application of adjunctive chemical measures to disrupt the bacteria and reduce the intraradicular microbial load to the lowest possible level aiming to expect a highly favorable long-term prognosis of the root canal treatment.

Aravind *et al.* 2006⁽¹⁾ analyzed the ecological conditions (nutrients, oxygen tension, and bacterial relationships) in the root canal. This ecological conditions were regarded as favorable for the growth of anaerobic bacteria capable of fermenting amino acids and peptides. Anaerobic bacteria thrive in an oxygen free environment as root canal space even with very limited amount of nutrients due to the low oxygen content of a closed root space. According to this study, anaerobic bacteria are given an ideal atmosphere for growth and survival within root canals.