

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
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سورة البقرة {32}

**Antibacterial and Antifungal Efficacy of a
New Chlorhexidine Containing Gutta-
Percha Points Used as a Temporary Root
Canal Filling Material**

(A Comparative In Vivo and In Vitro study)

Thesis

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Cairo University,

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(Endodontics)

By

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فاعلية أقمع الكلور هكسدين جاتا بركة الحديثة
المستخدمة كمادة حشو مؤقت للقناة العصبية
كمضاد للبكتيريا والفطريات
(دراسة مقارنة إكلينيكية ومعملية)

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Dedication

*To my Supporting Father, Loving and Caring Mother
for their Continuous Help, Love, Support and for
being there Whenever I Needed them*

*To my beloved Wife and My Newly Born Son for their
Love, Support and her ongoing backup*

*To my Loving and Supporting
Grandmother and Brother*

*Special Dedication for My Father in law (God bless
his soul) and my Mother in law
for their Support & Love*

INTRODUCTION

The vast majority of diseases of the pulp and radicular tissues are associated with micro-organisms. After microbial invasion of these tissues, the host responds with both, non specific inflammatory responses and specific immunologic responses. In 1890 W.D. Miller, the father of oral microbiology, was the first investigator to associate the presence of bacteria with pulpal diseases. Before 1970 only a few strains of anaerobic bacteria were isolated because of inadequate culturing methods. Currently the vast majority of bacteria isolated from an endodontic infection are anaerobic.

In addition to microbial infection, fungi have been associated with cases of secondary or persistent root canal infections. Yeast-like microorganisms also have been found in root canals of obturated teeth in which the treatment has failed.

The goal of clinical treatment is to completely disrupt and destroy bacteria and fungi involved in endodontic infection. Root canal debridement includes the removal of micro-organisms and their substrates required for growth. Chemo-mechanical cleaning and shaping of the root canal system remove a great deal of irritants, but total debridement is impeded because of the complex root canal system with accessory canals, fins and communications between canals. The last decade has seen the development and use of several innovative methods and materials to aid root canal debridement.

If root canal is not completed in a single appointment, then it is recommended to use an intracanal antiseptics to prevent the growth of microorganisms between appointments.

It has been shown that calcium hydroxide does not only exert an antimicrobial effect, but it is also able to neutralize bacterial endotoxin. To facilitate its application, Calcium Hydroxide containing gutta-percha points have been developed recently.

Due to reports that cases refractory to endodontic treatment were associated with calcium hydroxide resistant bacterial strains, e.g. *Enterococcus faecalis* or *Candida* species, research has been focused on alternative substances. Chlorhexidine is known to bind to dental hard tissues having a certain residual effect. The antimicrobial properties of chlorhexidine suggested that it might be a good inter-appointment dressing. Recently, Chlorhexidine containing gutta-percha points have been introduced, allowing easy insertion into root canals.

Because of the increasing interest regarding the role of fungi in root canal infections, particularly as members of secondary or persistent infections associated with failed endodontic therapy and the recent reports of fungal resistance against commonly used intracanal medications, studies must be directed to show the effect of intracanal medications against root canal fungi.

REVIEW OF LITERATURE

Historical Review of Gutta-Percha

Gutta-percha is a solid core filling material used for canal obturation. It is a natural organic polymer of isoprene which was known in dentistry for more than a century. It was first introduced to the Royal Asiatic society in England in 1843 by Sir Jose d'Almeida and was introduced into dentistry in the late 1800s ⁽¹⁾.

Actually, true gutta-percha may not be the product presently used in dentistry. Manufacturers admitted that they have long used balata which is a dried juice of the Brazilian tree, *Manilkara bidentata*. Also gutta-percha comes from the Malaysian tree, genera *Paysonia* or *Palaquium*. Chemically and physically, balata and gutta-percha appear to be essentially identical ⁽²⁾.

Gutta-percha is the most commonly used root canal filling material independently of the applied technique. This is due to its multiple advantages which was cited in the literatures such as: *Compressibility, Dimensional stability, Inertness, Radiopacity, Becomes plastic when warmed* (When heated to 42°C–49°C, there will be change in physical properties that are useful during endodontic treatment), *Tissue tolerance* ^(3,4).

Pure raw gutta-percha is non toxic, but zinc oxide which is the major component of gutta-percha points is highly toxic and has *known solvents*: the most common solvents are chloroform and xylene. These solvents were used to remove gutta-percha from the canal completely or soften it for compaction.

However, disadvantages of gutta-percha were mentioned such as: *Lack of rigidity*; which bent when subjected to lateral forces, so it can not be easily used in small sizes, *lack of length control*; It permits vertical distortion by stretching unless it is packed against a definite matrix and *become brittle as it ages*; probably through oxidation, also storage under artificial light speeds its deterioration ⁽⁵⁾.

Chemical Structure and Composition

Natural gutta-percha chemical structure mainly has a 1–4 trans-polyisoporene. The crystalline form appears in two phases; the alpha form and the beta form which can be converted to each other. The alpha form comes directly from the tree. Most commercial gutta percha is the beta form.

Gutta-percha is manufactured in the form of cones in both standardized and non standardized sizes. The composition of gutta-percha cones supplied by five manufacturers was found to be 20% gutta-percha, 60 -75 % zinc oxide, with the remaining percentage is combination of various waxes, coloring agents, antioxidants and metallic salts. The particular percentages vary according to **manufacturer** ⁽⁶⁾.

Friedman et al (1975)⁽⁷⁾ performed a quantitative investigation of five brands of gutta-percha points for the respective concentration of gutta-percha, zinc oxide, heavy metal salts and plasticizers and their effect on the mechanical properties in tension. They found that gutta-percha points contain approximately, 20% gutta-percha, 66% zinc oxide, 11% heavy metal sulfates and 3% waxes and / or resins.

Friedman et al (1977)⁽⁸⁾ investigated the relationship between composition and mechanical properties of gutta-percha. Regardless the brand, the mean organic percentage was 23.1%, while it was 76.4% for

non organic part. High percentage of gutta-percha produces stronger, more rigid material. On the contrary, high percentage of zinc oxide increase brittleness and decrease flow.

Marciano and michalesco (1989)⁽⁹⁾ determined the chemical composition and the x-ray diffraction analysis (XRD) of three different sizes of ten gutta-percha cones brands. They eliminated the organic portion by calcitination of gutta-percha cones at 550°C for 4 hours. Qualitative analysis was done to the inorganic part and subtracting the weight of ashes from that of gutta-percha samples to show the amount of organic part. The analysis showed that, there was a great chemical homogeneity among the different used brands and even in different sizes. The organic content ranged from 17.73% to 46.72% (gutta-percha and resin), barium sulfate was 3.28% to 31.23%, while zinc oxide ranged from 36.55% to 74.57% for different brands.

Combe et al (2001)⁽¹⁰⁾ evaluated the presence of alpha- and beta-forms in gutta-percha products used for filling of root canal. Samples of gutta-percha without additives and dental gutta-percha were collected as; group a) pellets of gutta-percha (control material) , group b) Two old gutta-percha (DMS & PD gutta-percha points) , group c) Twelve new current materials (Microflow cartidge, Microflow master cone, Micro seal #3, Micro seal #7, Multiphase I, Multiphase II, Obtura, SuccessFil, Thermafil 70, Ultrafil Regular, Ultrafil Firmest and Ultrafil Endoset, group d) current material that was stored in heater for more than 8 hours at 79 – 83°C (Multiphase II). The measurements were carried by using thermo/gravimetric differential thermal analyzer, which were checked using calcium oxalate standard. The results showed that, only four materials (Thermafil 70, Ultrafil Regular, Ultrafil Firmest and Ultrafil Endoset) contain alpha-form while all other materials contain beta-form.

No weight loss occurs to the materials under the experiment condition. Storage of gutta-percha points for 10 years under ambient temperature and in a heater at 80°C didn't cause any change in the properties of the material.

Gurgel-Filho et al (2003)⁽¹¹⁾ analyzed the chemical composition of five brands of gutta-percha cones. Non standardized gutta-percha points of brand names *DentsplyTM*, *konneTM*, *TonariTM*, *Obtura SpartanTM* and *Analytic EndodonitcTM* were used. The organic (polymer and wax/resin) and the inorganic (ZnO and BaSO₄) fractions were separated by dissolution in chloroform. Gutta-percha polymer was precipitated by acetone, while ZnO was separated from BaSO₄ by reaction with HCl. Energy-dispersive x-ray microanalysis (EDAX) was used to establish quantitatively the presence of ZnO and BaSO₄ in samples. Results showed that, gutta-percha polymer, wax/resin, metal sulphate and ZnO were found in all samples. Zinc and oxygen were present in all brands as major elements. ZnO and BaSO₄ were identified only in *Obtura SpartanTM* and *Analytic EndodonitcTM*. It was concluded that, the procedure was appropriate for quantifying gutta-percha polymer and wax/resin but not ZnO and BaSO₄. Some brands of gutta-percha didn't contain BaSO₄.

Gutta-Percha Additives

Recontamination of the pulp chamber may occur during restoration of the endodontically treated teeth. Gutta-percha may prevent this contamination if it possesses antimicrobial activity.

Calcium hydroxide has great antimicrobial benefits in endodontics. Recently it was added to gutta-percha to be used as a temporary filling material. However the release of hydroxyl ions from calcium hydroxide was found to have little alkalizing potential as compared to calcium hydroxide preparations.

Chlorhexidine containing gutta-percha points were also introduced in the market as a temporary filling material, which was claimed to have an antibacterial effect on root canal pathogens. Cytotoxicity of standard gutta-percha points with calcium hydroxide, Chlorhexidine containing gutta-percha and MTA was tested. The different gutta-percha points in contact with fibroblasts evoked a time and material dependent reduction in cell growth. Cellular proliferation was only significantly reduced with chlorhexidine containing gutta-percha⁽¹²⁾.

The antimicrobial effect of MGP (10% iodoform MGP) and standard gutta-percha was compared. It was found that neither MGP nor standard gutta-percha demonstrated observable antimicrobial activity against *E.faecalis*. In addition, MGP showed no zone of inhibition on microbes found in oral plaque. It was suggested that, in order to increase the beneficial effects obtained from gutta-percha, the latter should be converted from just being a core material to a material releasing active constituent into periapical tissue and therefore has an active role in many biological operations carried out in human body⁽¹³⁾.

Organic germanium was tried as an additive to gutta-percha. Germanium as an element exists in some plants and it is used in Chinese medicine to treat some diseases. Adding germanium to gutta-percha will result in no alteration in its chemical formula. Organic germanium was found to be released from gutta-percha in vitro and may counteract the toxic or mutagenic properties of root canal sealers used with gutta-percha. In addition, it has a healing power during the release through the periapical tissue, which is an advantage needed after obturation ⁽¹⁴⁾.

Effect of Calcium Hydroxide Containing Gutta-Percha Points on pH of Radicular Dentin

Calcium hydroxide is a popular temporary medication used to disinfect root canals, and has been shown to be effective in a wide variety of situations. The antibacterial effect is due partly to the fact that it produces a pH over 11, preventing the growth and survival of oral bacteria⁽¹⁵⁾. Calcium hydroxide alters the biological properties of bacterial lipopolysaccharides⁽¹⁶⁾, and by inactivating enzymes in bacterial membranes, also upsets transportation mechanisms, resulting in cell toxicity⁽¹⁷⁾. The main advantage of calcium hydroxide is that it has an effect on microorganisms in the absence of direct contact, not only by creating a high pH environment but also by absorbing CO₂ required for bacterial growth⁽¹⁸⁾, also showed that hydroxyl ions can diffuse into dentinal tubules in uninstrumented root canals. The long-term efficacy is the result of two properties: the destruction of bacterial cytoplasmic membranes by the liberation of hydroxyl ions, and the activation of tissue enzymes such as alkaline phosphatases⁽¹⁹⁾.

Schäfer et al (2000)⁽²⁰⁾ compared the alkalization of root dentin after the use of gutta-percha points containing calcium hydroxide and an aqueous calcium hydroxide suspension. Alkalinizing action, on 140