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Evaluation of Antimicrobial Effect for Chlorhexidine incorporated Gutta Perchausing FTIR Spectroscopy

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ABSTRACT

Aims: To evaluate the antimicrobial effects for chlorhexidine incorporated gutta-percha after using FTIR Spectroscopy.

Materials and Methods: In this study 80 conventional Gutta-percha (GP) cones the same size (80) were used, first 10cones disinfected by immersion cones with 2%chlorhexidine solution (CHX) for10minutes after that storage in sterile distilled water solution for 24hrs. The chemical incorporation of CHX and GP cones evaluated by using FTIR Spectroscopy. The antimicrobial effect of CHX incorporated gutta percha cones evaluated using the antimicrobial sensitivity test.

The results: There is a Chemical incorporation for a 2% Chlorhexidine solution with GP cones were evaluated using FTIR Spectroscopy, antimicrobial effect of CHX incorporated GP (medicated Gutta- percha) indicated that there are inhibition of growth for all tested microorganisms specially for *Streptococcus Mutans* after 10 minutes immersion of GP cones in 2% CHX solution.

Conclusion: Chlorhexidine incorporated gutta- percha offer additional antimicrobial advantages over conventional gutta-percha increasing the wettability and not the effect on mechanical properties of GP cones.

Key words: Gutta- Percha, Chlorhexidine, FTIR Spectroscopy.

INTRODUCTION

The microorganisms are the major etiological agents in pulpal and periapical disease. Two of the main goals of endodontic therapy are the elimination micro-organisms and their products from the root canal system and the prevention of subsequent reinfection. Persisting bacteria or reinfection bacteria may induce or sustain apical periodontitis [(1,2)].

Guttapercha has been used to fill root canals for a century of years and remain the material of choice in the obturation root canal system. The GPcones taken directly from the manufacture sealed package harbored. Microorganisms were quite low at the time of the opening of the package; clinical use of the packages increased the number of microorganisms contaminating GP cones [(3)].

Evidence for the antimicrobial activity for guttaperchacones is important, Zinc oxide, which is the major component of gutta percha and responsible for some of the antibacterial properties of cone [(4)]. The core filling materials should be free from microorganismsin order to avoid contamination. Therefore the endodontic filling materials should have an antimicrobial effect to avoid microorganism growth [(2)].

Current rootcanal obturation technique involves the use of several chemicals inhibiting the microbial growth and new form of gutta percha cones has an antimicrobial effect without effect on the final outcome of arbitration [(5)].

Brenda *et al.* concluded from their study, chemically disinfected substance like chlor hexidine (CHX) has ability to kill vegetative forms of bacteria within short periods of times [(6)]. Chlorhexidine don't change gutta percha cone properties after exposure for up to30 minutes when used as disinfectant for obturation materials (GP and reselling cones) [(6)].

Therefore, this substance (CHX) is less harmful to the structure of guttapercha and 2%CHX don't produce any changes on gutta percha and resilon surface [(6,7,8)].

The aims of this in vitro study to evaluate the antimicrobial effect of chlorhexidine incorporated gutta percha after using FTIR Spectroscopy.

MATERIALS AND METHODS

A total of 80GP cone size 80 (DIA-Dent, Netherlands, Korea) were used in this in vitro study. Cones disinfected with a 2%chlorhexidine solution (Zeneca Limite, U.K). The first 10 cones were remained fresh without disinfection (control group)and second 10cones (disinfected group) submerging in disposable sterile screw capped tubes contain 20 ml of 2% CHX tested solution as shown in Fig (1), kept for 10 minutes and then cones transferred individually and rinsed insterile distilled water and keptfor 24hours in disposable sterile screw capped tube contain sterile distilled water [6,9]. Then allowed to dry in sterile petri dishes containing sterile filter paper pads [9].

FTIR spectroscopy charts for 2%CHX solution, sterile GP cone samples(control group) and 2%CHX incorporated GP cone samples(disinfected samples) as shown in Fig (2a,b,c) measured by computerizes Fourier Transmittance Infrared Spectroscopy (Broker optics ATR Diamond, Germany) as shown in Fig (3).GP cones (disinfected samples) are put randomly and then pressed to produce a change in the sample as shown in (Fig 3). The FTIR spectroscopy is a technique which is used to obtain an infrared spectrum for absorption, emission and photo conductivity, scattering of liquid,solid or gas[6,7,8] .

FTIR Spectroscopy simultaneously collected spectral data in a wide spectral range, this confers a significant advantage over a dispersion spectrometer[10,11] .

Antimicrobial sensitivity test were used for CHX incorporated GP cone samples, Kirby – bauer method is applied [1,2,12], using Muller – Hinton agar, the ideal inoculums was prepared by taking 4-5 colonies of the isolated test microorganisms (*Staphylococcus aureus, Enterococcus feacalis, Streptococcusmutans, Lactobacillus acidophilus,* and *candid albicans*) by sterile loop and inoculated a tube of broth culture, the tube was incubated for few hours until it become slightly turbid, and diluted to match the turbidity of a McFarland 0.5 standard, which is equal to 10^8 Cell /ml. The sterile cotton swab was used to evenly incubated the entire surface of agar plate and then allowed to dry [13].

Then 60 conventional gutta percha cones impregnated with 20 ml of 2%CHX solution in sterile plates and divided to 5 groups depend on type of microorganisms tested and within same group divided into 4 sub-groups depend on time ofimmersion (5,10,15,20minutes) then the plates were incubated aerobically for 24 hours at 37^0 C [1,2,14], and the antimicrobial effect was determined by measuring the diameter of the zone of inhibition for each strain experiment were performed in triplicate and average value were determined as shown in Tab(1).

RESULTS

Results of FTIR Spectroscopy charts for 2%CHX solution and for GP cone samples before and after immersion in 2% CHX solution as shown in fig (2a,b,c) indicate that, the chemical incorporation for 2% CHX and GP cone samples was calculated by the two frequency techniques using the net peak transmittance areas of C=C stretching vibration at 1482 cm-1 as analytical frequency (before immersion in 2% CHX solution),disappear referred to chemical incorporation with GP cone samples.

Because analytic of GPcone samples only appear at vibration 1636cm^{-1}as a reference and this away from GP sample before immersion, which mean there are chemical incorporation as shown in Fig (2 a,b,c).

Evaluation of the antimicrobial effect of CHX incorporated GP cone samples include the mean diameter of zones of inhibition for all tested microorganisms over different time periods are given in table (1),regardless of time and pathogen strain, CHX incorporated GP cone samples are more effective than regular GP cone samples in inhibition of growth of microorganisms tested, the largest mean inhibition zone with CHX incorporated GP (medicated GP) occur after 10 minute incubation time for most bacterial strain, especially for *Streptococcus Mutance* (32mm),Staphylococcusaureus(30mm),EnterococcusFeacalis(30mm),Candida albicans (31mm), Lactobacillus acidophilus (17mm) in descending order as shown in the table(1), fig (3).

DISCUSIONS

Theterm Fourier transmittance infrared spectroscopy (FTIR) originates from the fact that a Fourier transform a mathematical process which is required to convert the raw data into the actual spectrum for other uses of this kind of techniques it provide information about the chemical bonding or molecular structure of the material whether organic or inorganic[11], the clinical importance of the CHX realest in endodontic cones might be related to it is an immediate antimicrobial effect inside the root canal and during and late obturation time [15] .

Also CHX increase in the surface free energy (wet ability)of gutta percha cones and resilon, there by interfering positively with the adhesion mechanisms, this change can be due to chemical modifications to the surface of the gutta percha cone caused by the action of these solutions, also CHX is the better disinfected solution compared to others that is due to present high values of surface free energy, the cone disinfected with CHX presented smaller contact angles then another disinfectant favoring the interaction between the solid surface (cone) and the liquid materials, also 2%CHX solution doesn't have any significant effect on mechanical properties of GP cones[16,17].

Elimination of bacteria from the root canal system is essential for long term success of endodontic treatment[17].In this study MGP(CHX incorporated GP) and regular GP cone, were tested against 5 strains microorganisms, it will be important to confirm that the antibacterial effect of MGP cones on various endodontic pathogenic microorganisms help to reduce the overall bacterial population in the infected root canals, rather than merely altering the species composition[18,19].

The antimicrobial activity of root canal obturation material helps to eliminate the remaining residual microorganisms unaffected by either chemo-mechanical preparation or intra canal medication, therefore it has been advocated that the root filling material showed have antibacterial antifungal properties[19].

Factitive anaerobic microorganisms, *E. facalis* is the major pathogen associated with the most root canal complication of periapical periodontitis. Several authors have reported difficulty in elimination *E. facalis ssp*. During root canal treatment [20], calcium hydroxide did not completely eliminate *E .faecalis.* Chlorhexidine was effective against *E. faecalis* [21]. *Candida albicans* was chosen as a test fungal organism in this study because it has been found in infected root canal [22].

CONCLUSIONS

The conclusions of this study indicate that the antimicrobial and antifungal activity for 2%CHX incorporated GP cones offer an advantage over the conventional GP cones, also 2%CHX considering as the safe disinfectant solution for GP cones even after 20 minute disinfection, increasing wet ability of GP and not effect on mechanical properties of cones.

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Fig (1): GP cones submerged in 2%CHX solution in sterile screw caped tube.

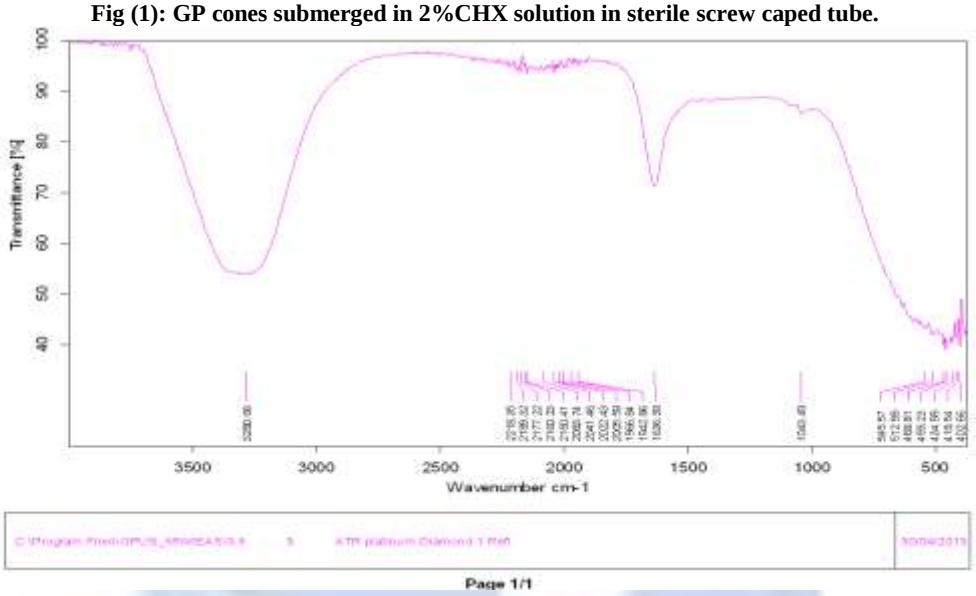


Fig.(2)a:Spectroscopy chart for 2% CHX solution with Aliphatic and Aromatic Peak

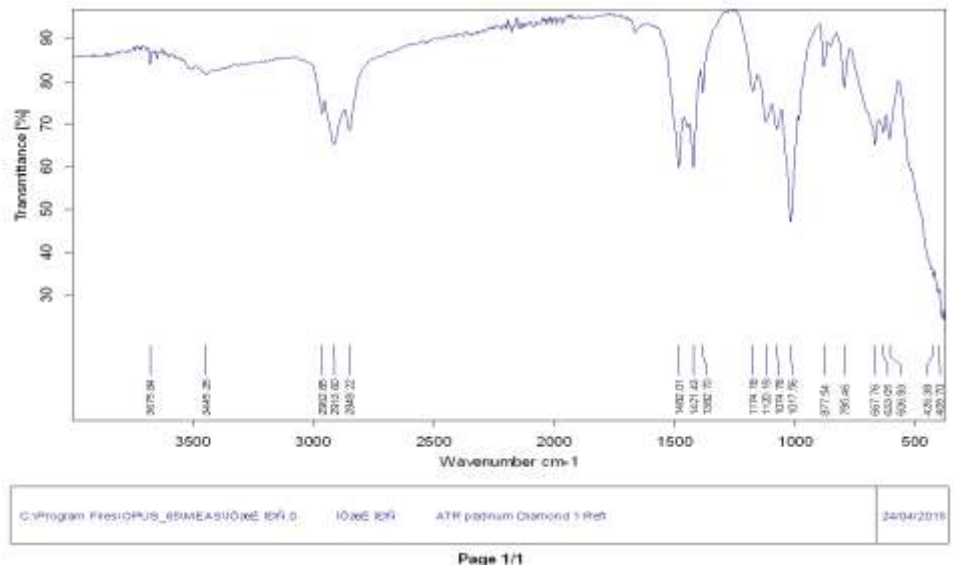
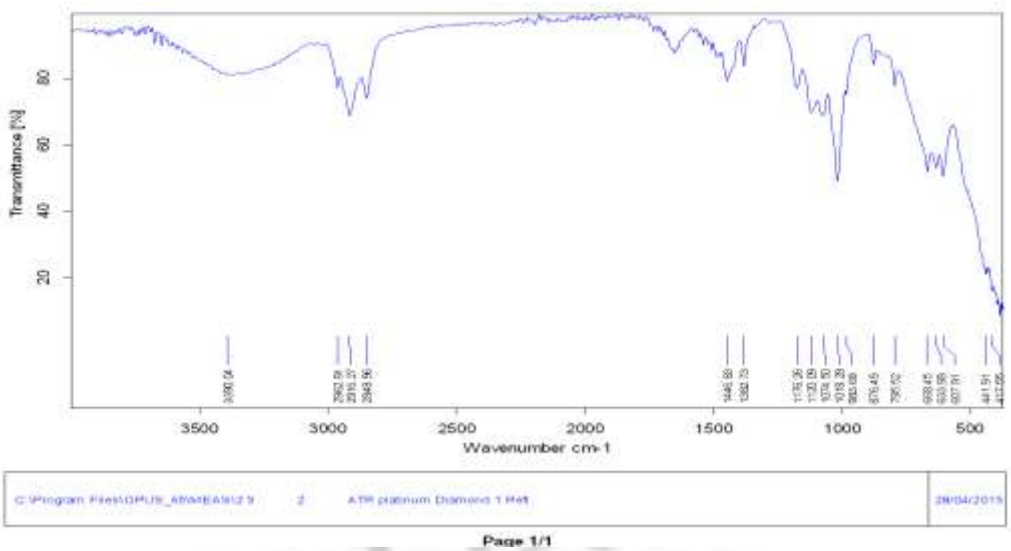
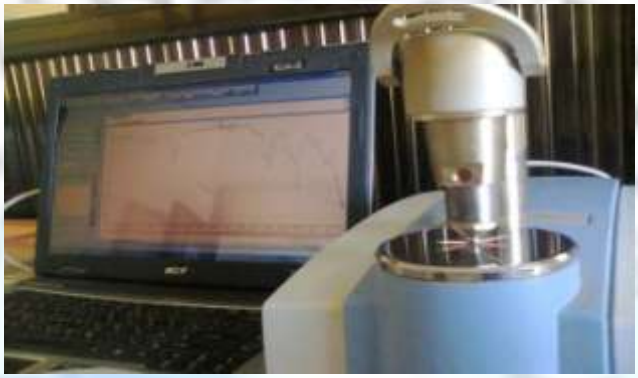


Fig.(2)b: Spectroscopy chart for GP(control) group with Aliphatic and aromatic peak.



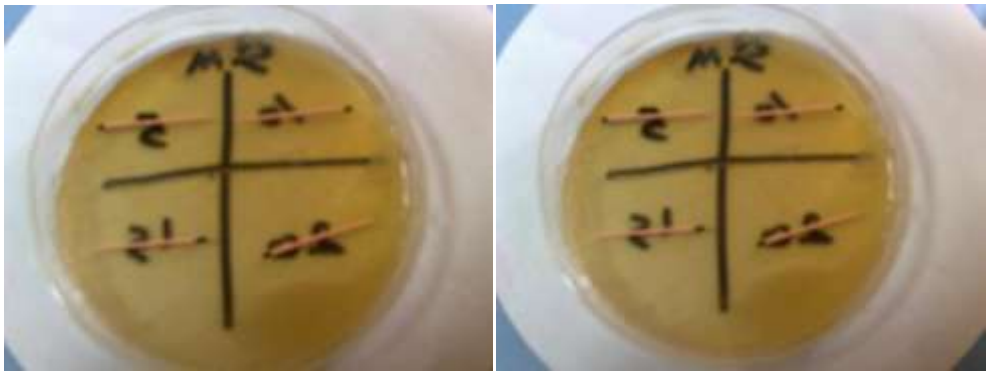
Fig(2)c: Spectroscopy chart of CHX incorporates GP cones withaliphatic and aromatic peak



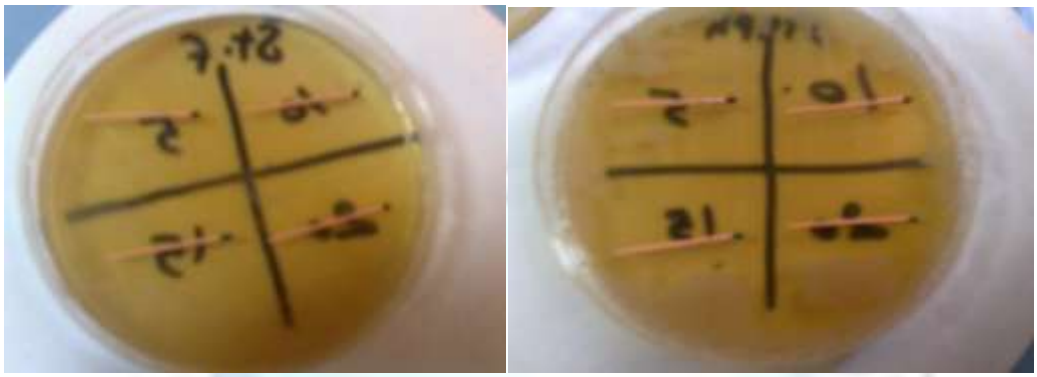
Fig(3): Fourier Transmittance Infrared Spectroscopy(FTIR)

Table (1): Comparison of the antimicrobial effectszone of inhibition (mm) for 2% CHX incorporated GP cone samples after four incubation time (min)

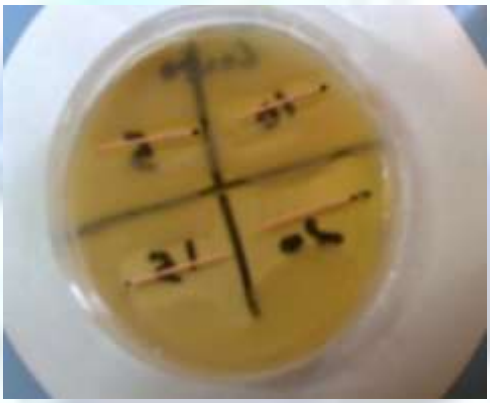
Tested microorganisms	5 min	10 min	15 min	20 min
E. feacalis	13mm	30mm	23mm	**18mm**
L. acido philus	11mm	17mm	20mm	**19mm**
Strep.mutans	25mm	32mm	26mm	**21mm**
Staph. aureus	18mm	30mm	23mm	**20mm**
C. albicans	23mm	31mm	21mm	**17mm**



A:*Strep.mutans* **B:*Staph. aureus***



C: *E. feacalis* **D: *C. albicans***



E : *L. acidophilus*

Fig (3): Comparison the zone of inhibition for 5 different microorganisms tested