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Antimicrobial Activity of Newly Prepared Endodontic Biosealer on *Enterococcus Faecalis* - An in vitro study

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Abstract: The aim of the present study was to evaluate the antimicrobial activity of newly prepared root canal biosealer (ZOGU) at various time intervals against *E. faecalis.*

Materials and Methods: Agar diffusion method was used in this study. Tgsealer zinc oxide eugenol base root canal sealer served as positive control. The sealers were freshly mixed and placed in prepared wells in 40 plates of *Enterococcus* agar base media (HiMedia India) inoculated with *E. faecalis*, . Plates were divided randomly, into five test groups according to time interval of incubation, ten plates for each. All plates were incubated at 37˚C. The zone of inhibition of each sealer was measured after 1day, 2days, 3 days and 7 days interval in order to evaluate the antimicrobial efficacy in different time intervals. Statistical analysis was performed by ANOVA, independent sample't' test and Duncan's Multiple Range Test as applicable. P value <0.05 was considered as statistically significant.

Results: Two sealers exhibited antibacterial effect at all incubation periods. There are significant differences between two root canal sealer types at all incubation periods except at 7 days, which indicate no significant differences. The largest inhibition zone formed by (tgsealer) at 1day (13.7mm) followed by (ZOGU) at 1day (13.0 mm),while the smallest inhibition zone formed after 7 days incubation times for two types (tgsealer 11.6 mm and ZOGU 11.5 mm).

Conclusion: There is active antimicrobial activity of ZOGU new root canal biosealer extended for all incubation periods against *E. faecalis.*

INTRODUCTION

Bacteria and their products are considered the primary etiologic agent of pulpal necrosis and periapical lesions [(1)]. Thorough debridement and complete elimination of microbes is of paramount importance before completion of obturation of the root canal. Hence, bacteriological studies were integral part of root canal treatment and two consecutive negative cultures were essential before obturation. To achieve this, the antibacterial effect of irrigants was advantageous [(2,3)]. The other chemical agents that form an inseparable part of root canal treatment are the root canal sealers. Antimicrobial properties of these sealers will ensure elimination of microbes as well as prevent re-infection particularly when bacteriological sampling before obturation is not a routine procedure. Hence the testing of antimicrobial properties of these agents is highly relevant and useful in root canal treatment and worthy of evaluation [(4)].

Antimicrobial activity of root canal sealers that contain substances, such as paraformaldehyde, eugenol, and thymol, help destroy the remaining bacteria [(5)]. On the other hand, severe toxicity of a filling material may be a reason for damage of periapical tissues, thereby abolishing the beneficial effects of the antimicrobial properties of the material [(6)].

To evaluate the antibacterial activities of root canal sealers different methods like agar diffusion test, direct contact test, time kill assay (TKA) have been used[(7-9)]. The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medications[(10,11)]. This method allows direct comparisons of root canal sealers against the test microorganisms and maintain the chemical properties of the tested sealers [(11)].

In the process of sealing root canals, the sealer cement is of paramount importance: it fills spaces unreached by gutta-percha or irrigant agents. This is because cements possess components with anti-microbial characteristics, which persist long after completion of root canal preparation process [(12)].

In recent years, there is an exponential growth in the field of herbal medicine because of their natural origin, easy availability, efficacy, safety and less side effects [(13)]. Medicinal plants constitute a promising source of phytotherapy

drugs and new molecules. The number of studies on this alternative therapeutic system increased in the last decades, as well as their use for several purposes [14].

Guaiacol: is a yellowish aromatic oil is usually derived from guaiacum or wood creosote, present in wood smoke, resulting from the pyrolysis of lignin. This compound contributes to the flavor of many compounds, e.g. roasted coffee [15]. It used clinically as antiseptic, analgesic, sedative drug for the treatment of dental pulp and periodontal tissue, it have cell activating ability with ability to promote cell proliferation in vivo[16].

The aim of the present study was to evaluate the antimicrobial activity of newly prepared endodontic biosealer (ZOGU), at different time intervals (1day, 2days, 3 days and 7 days) in the presence of *E.faecalis*.

MATERIALS AND METHODS

Preparation of endodontic biosealer

The new root canal sealer powder/liquid formula depend on main reaction of zinc oxide and guaiacol as base acid reaction, with adding the other ingredients to improve materials properties, Table (1)

Table (1): Main Components of new root canal sealer.

	Components	%	Manufacturer
Powder	Zinc oxide	62	PD/Switzerland
	Natural rosin	20	Lebanon
	hydroxyapatite	2	Locally made from egg shell
	Bismuth sub-carbonate	15	BDH/ England
	Zinc acetate	1	BDH/ England
Liquid	Guaiacol	85	Sigma /USA
	Olive oil	15	Agrioil/ Italy

The final liquid result from mixing of guaiacol (yellowish aromatic oil) and olive oil in small percentage in order to improve smoothness of resulting sealer.

Synthesis of Hydroxyapatite

It is synthesized from chicken egg shell, according to (Taqa and ALSandook 2002) [17] Egg casings were removed for internal crust then wash away the chaff very well to be sure to remove the cover lining. Put them in the oven heat with the temperature 900 °C for a period of one hour to turn material into powder snow-white. Then by the slow addition of 0.6M H_3PO_4 (Phosphoric acid) to the aqueous (molar ratio) suspension of CaO under constant stirring, and formation of Hydroxyapatite .The resultant was filtered and dried at 50°C for 3 hours then sintered in air atmosphere at 1100°C for 2 hours. The powder particle size was standard in size using 25µm sieve, the mixture was mixed using grinder.

The water/powder ratio by volume (2P/1L) was determined after many trials until reach to final formula with desirable setting reaction, consistency and low solubility.

Antimicrobial Properties

Antimicrobial activity test was carried out in the Microbiology Laboratory, Department of Dental Basic Sciences, College of Dentistry, Mosul University.

The antimicrobial efficacy of newly prepared root canal sealer was tested against E. faecalis a common, resistant intracanal pathogen [18], using agar diffusion test (agar-well technique). Zinc oxide eugenol based sealer (tgsealer, technical and general Ltd, UK) served as positive control.

Enterococcus faecalis was sub cultured in a specific *Enterococcus* agar plate (HIMedia India). The plate was incubated at 37°C for an 18 hrs period. A pure single E. *faecalis* colony was isolated from the same cultured plate and inoculated into a sterile screw capped vial containing 5 ml of brain heart infusion broth (BHI). The BHI broth was incubated at 37°C for 24 h, inoculation from a 24-hours growth of the test organisms were adjusted to obtain a turbidity equivalent to the 0.5 McFarland standards scale (approximately 1.5 x 10^8 colony forming units /ml.)[19]. Specific *Enterococcus* agar base media (HiMedia India) was prepared according to manufacturer instruction.

A total of 40 plates of *Enterococcus* agar base media were used: plates divided randomly, into five test groups according to time interval of incubation, (1day, 2days, 3 days and 7 days) with ten plates for each. Bacteria inoculation was done using sterile cotton-tipped application, and filled with the freshly prepared sealers, Figure (1). Two wells of 6 mm diameter wide were punched in each agar plate and the inculated plates with sealers were kept 2 hour at room temperature to allow the diffusion of agents through the agar then the plates incubated at 37°C.

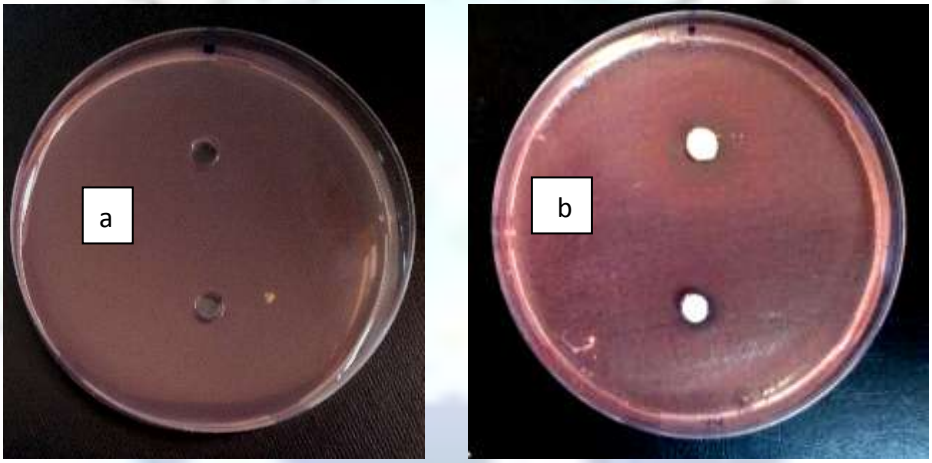

Figure (1): a: agar plate showing two created wells. b: agar plate after application of two types of endodontic sealers.

Zones of inhibition of microbial growth around wells containing the sealer materials were measured and recorded after incubation period. Three measurements were made for each material and the average of three values was calculated.

Data were processed and analyzed using SPSS (statistical package for social science) version 19. The analysis of variance (ANOVA), and Duncan's Multiple Range Test and independent sample 't' test were performed to know the effects of each variable and to reveal the statistical significance. P-value <0.05 was considered as significant.

RESULTS

Analysis of variance (ANOVA) test for two root canal sealers (tgsealer and newly prepared ZOGU) of 5% listed in Table (2), each of sealers showed highly significant differences for antimicrobial activity which represented by inhibition zone formation at incubation periods. Independent sample 't' test reveal the effect of incubation times intervals (1day, 2days, 3 days and 7 days) on the antimicrobial activity, Table (3). The result showed there are significant differences between two root canal sealer types at all incubation periods except at 7 days, which indicate no significant differences.

The result of study represented through Duncan's Multiple Range Test, in Table (4) and (5), Figure (6) revealed presence of antimicrobial action of newly prepared biosealer (ZOGU) in relation to that of (tgsealer) overall incubation time intervals. The largest inhibition zone formed by (tgsealer) at 1day (13.7mm) followed by (ZOGU) at 1day (13.0 mm), while the smallest inhibition zone formed after 7 days incubation times for two types (tgsealer 11.6 mm and ZOGU 11.5 mm).

There is descending or decreasing of antibacterial efficacy with time. After 7 days incubation there are approximated antibacterial efficacy of two endodontic sealers with no significant differences.

Table (2): Analysis of variance of tested endodontic sealers.

Material		df	Mean square	F	Sig.
tgsealer	Between groups	3	8.492	35.965	.000
	Within groups	36	.236		
	total	39			
ZOGU	Between groups	3	4.090	34.846	.000
	Within groups	36	.117		
	total	39			

Table (3): The effect of incubation times intervals (1day, 2days, 3 days and 7 days) on the antimicrobial activity of two sealers.

Time	t-test for Equality of Means		
	t	df	Sig.
1day	4.583	18	.000
2day	3.902	18	.001
3day	2.449	18	.025
7day	.429	18	.673

Table (4): Duncan's Multiple Range Test for tgsealer antimicrobial efficacy at incubation times intervals.

Time	Diameter of Inhibition Zones(mm) Mean±SD
1day	13.7±0.483 A
2day	13.2±0.421 B
3day	12.4±0.516 C
7day	11.6±0.516 D

Different letters indicate significant differences.

Table (5): Duncan's Multiple Range Test for antimicrobial activity of new root canal biosealer (ZOGU) throughout incubation time intervals

Time	Diameter of Inhibition Zones(mm) Mean±SD
1day	13.0±0.000 A
2day	12.45±0.437 B
3day	12.0±0.000 C
7day	11.5±0.527 D

Different letters indicate significant differences.

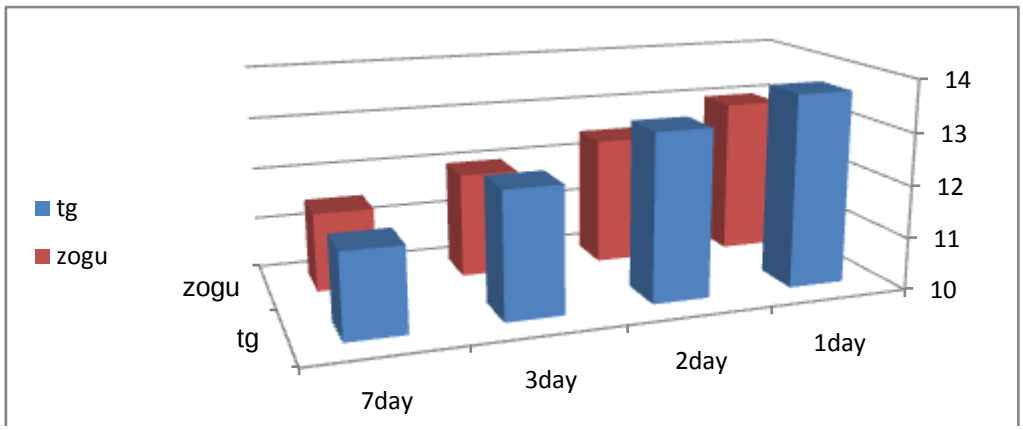

Figure (2): antimicrobial efficacy of root canal sealers at incubation time intervals.

DISCUSSION

Enterococcus faecalis is a facultative anaerobic bacterium, commonly isolated in failed root canals. The ecological changes such as nutrients, oxygen tension and bacterial interrelationship that occur in the root canal during and after treatment favor these facultative anaerobic microorganisms. E. *faecalis* can survive with even scant amounts of substrate and as a single microorganism [(1)], and grow to establish mono-infections that are difficult to eradicate using conventional root canal procedures. E. *faecalis* has been used extensively in studies of root canal disinfection because this bacterium is easy to grow in the culture medium and, rapidly and efficiently colonizes in medium [(20)]. Hence, E. *faecalis* was selected as the test microorganism in this study also. The agar diffusion method has been widely employed to investigate the antimicrobial activity of dental materials, however. This procedure does not depend only on the material toxicity to a given microorganism, but may also be influenced by the diffusion and affinity of the material in the culture medium. Hence the plates were kept for two hours at room temperature (allow the diffusion) as suggested by Gomes et al.2004 [(11)], So a material presenting easier diffusion will produce larger zones of inhibition of bacterial growth [(19)].

In this study, freshly mixed sealers were immediately transferred into agar plates. Because of various transitory or permanent products, material should be tested immediately after mixing and after a period of time when it is assumed that it has reached its final chemical structure. The results of the present study revealed that both sealers showed antibacterial activity substantiated by the formation of growth inhibition zones against E. *faecalis* at all incubation time intervals. Tgsealer (ZOE sealer) produced significantly largest inhibitory zones at 1day, 2days, 3 days against E. *faecalis* which was in accordance to similar inhibitory activity of zinc oxide eugenol based sealers by Kothari A and Langalia (2013)[(1)], Cavalcanti et al.(2010)[(21)] and Saha et al.(2010)[(22)]. presence of eugenol is a potent antibacterial agent, which plays a major role within activity of ZOE based sealers[(23)], Its strong antibacterial activity have frequently been found to induce adverse effects during and after treatment; they were also cytotoxic or even mutagenic[(9,24)]. In addition to that most endodontic sealers have inherent antimicrobial properties; antimicrobial compounds such as Iodoform, thymoliodide, and paraformaldehyde are added to enhance the antibacterial activities. These compounds may be responsible for the antimicrobial effects of the sealers, which would maintain the sterility of root canal system and thus potentiate repair [(25)], tgsealer material contain Thymol–iodide According to the manufacturer's description (technical andgeneral Ltd, UK).

The antimicrobial activity of newly prepared endodontic sealer ((ZOGU) which contain Guaiacol) produced through the main components of the material without adding specific antibacterial agent. It is activity continued over all incubation times' intervals. Guaiacol antibacterial action is due to disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents. Extensive loss of cell contents or the exit of critical molecules and ions will lead to cell death [(26)].

Phenolic dental medicaments including phenol, p-chlorophenol, p-eugenol, isoeugenol, and guaiacol have been used also for disinfection and sedative treatment for pulpitis in dental practice [(27)].

The antibacterial activity of materials was decreased with time compared to the freshly-mixed ones, this result confirm this of Bodrumlu and Semiz.(2006)[(28)] . This can be attributed to decrease in the releasing of antibacterial components from the tested materials. None of the sealers totally inhibited the microbial growth, and the antimicrobial activity of each sealer decreased with time and depended on the microbial susceptibility to them. Thus, endodontic treatment must be carried out under aseptic conditions, using a powerful irrigant solution, an intracanal medicament when necessary, a

sealer with antimicrobial activity and an effective coronal seal to prevent coronal microleakage in order to increase the chances of successful root canal treatment [(11)].

CONCLUSION

Under the conditions of this in vitro study, it was concluded that ZOGU new root canal biosealer inhibited the growth of E. *faecalis* at all incubation times.

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