

Evaluation of the Biocompatibility of a Newly Prepared Endodontic Biosealer using Subcutaneous Implant on Rabbits

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ABSTRACT

The purpose of this study was to evaluate in vivo the biocompatibility of newly prepared root canal biosealer (ZOGU) after subcutaneous implantation in rabbits

Materials and Methods: Three materials comprised the groups: group I –tg sealer ZOE base root canal sealer served as positive control. Group II–Empty polyethylene tube (without sealer material) as negative control, and group III – newly prepared root canal biosealer (ZOGU). These materials were placed in polyethylene tubes and implanted into dorsal connective tissue of Fifteen Albino healthy male rabbits. Tissue biopsies were collected at 3rd day, 14th day, 28th day after the procedure. The specimens were processed and stained with hematoxylin and eosin (H&E) and examined microscopically. Statistical analysis was performed by Mann-Whitney test, analysis of variance (ANOVA), and Duncan's Multiple Range Test.

Results: The Histopathological analysis considered: severity of the inflammatory reaction, thickness of the fibrous capsule. At 3rd day, all groups induced severe inflammatory reaction, which reduced over time but with different rates. At 14th day, biosealer (ZOGU) showed a moderate inflammatory reaction, while tg sealer ZOE sealer still induced severe inflammatory reactions. At 28th day, mild inflammatory reactions were observed for biosealer (ZOGU) and moderate for tgsealer. tgsealer ZOE sealer induced the thickest fibrous capsule formation in comparison to that of ZOGU sealer for all implantation intervals. Statistical analysis showed no significant difference among all groups to inflammatory tissue response at 3rd day, while there is significant difference between biosealer (ZOGU) and tgsealer ZOE sealer groups at 14th and 28th day. For all implantation intervals there is significant difference in fibrous capsule thickness between biosealer (ZOGU) and tgsealer ZOE sealer groups.

Conclusion: Biosealer (ZOGU) more biocompatible (better tolerated by the tissue) than tgsealer ZOE sealer.

INTRODUCTION

Most root canal filling techniques use core materials associated with endodontic sealers (1). Core obturating materials, such as guttapercha usually occupy space, whereas the endodontic sealers enhance the possible attainment of an impervious seal by serving as a filler for canal irregularities and minor discrepancies between the root canal wall and the core material (2). This three dimensional filling aspect of treatment causes most problems and controversies, due to the wide number of solid core materials and sealers available (3).

According to Grossman (1982), an ideal root canal filling material should not be irritant to periapical tissue (4). Filling materials, sealers and medications that are used in endodontic therapy should be compatible with the periapical connective tissue to rule out the possibility of any harmful response. For many years, root canal sealers most frequently used were those based on zinc oxide-eugenol which have failed to present favorable biological behavior (5). The biocompatibility, sealing ability and favorable clinical applications of both materials to their bioactivity when in contact with tissue fluid (6).

Herbal or natural products have been used in dental and medical practice for thousands of years and have become even more popular today after the constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives in endodontics due to easy availability, cost-effectiveness, increased shelf life, high biocompatibility and lack of microbial resistance (7). Herbal agents have been used in endodontics for reducing inflammation, antiseptics, antioxidants, antimicrobials, and analgesics (8). In general

biosealer is the biocompatible material that applicable to use in endodontic and have the ability to induce an appropriate and advantageous host response during its intended clinical usage (9). New formula of biosealer in this study contain many natural materials in order to improve biological properties, wound healing. The purpose of this study to evaluate the soft tissue response to newly prepared endodontic biosealer (ZOGU) implanted in subcutaneous tissue of the rabbits.

MATERIALS AND METHODS

Preparation of endodontic biosealer

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

The powder consist of:

1. Zinc oxide 62% (PD/Switzerland).
2. Natural rosin 20% (Lebanon).
1. Hydroxyapatite (HAP) 2%, It was synthesized locally from egg shell according to (Taqa and AL-Sandook 2002) (10). 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 H₃PO₄ (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.
2. Bismuth sub-carbonate 15% (BDH/ England).
3. Zinc acetate 1% (BDH/ England).

The powder particle size was standard in size using 25µm sieve, the mixture was mixed using grinder.

The liquid consist of

1. Guaiacol 85% (Sigma /USA).
2. Olive oil 15% (Agrioil/ Italy).

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

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.

Biocompatibility test

Fifteen Albino healthy male rabbits of comparable weight (1.5 ±0.25 kilogram) were selected. The animals were housed in an animal house prepared for this purpose in the college of dentistry/ university of Mosul, the room temperature was maintained at 25°C, they are given free access to water and fed the same diet (pellet and clover) and maintained on a 12 hours' light- dark cycle throughout the study time.

The animals were divided randomly into three groups, five rabbits in each group, for three time intervals (3, 14, 28 days), with three implants per each animal. The tested materials were the new biosealer (ZOGU) in comparison with zinc oxide eugenol based sealer (tgsealer, technical and general Ltd, UK) and empty polyethylene tube without sealer material as negative control.

The rabbits were anesthetized by intramuscular injection of a mixture of 50mg/Kg of body weight Ketamine hydrochloride and 10mg/Kg of body weight xylazine base injected into rabbit's thigh muscle. After 15 minutes anesthetic integrity was checked by testing loss of ear pinch reflex (11).

The animal was laid on its ventral side on the surgical board, the hair was shaved by using lotion, hair remover; the area was washed with tap water. The surgical instruments were sterilized by autoclave at 121°C for 15 minutes, 15 pounds/inch². The surgical area after shaving was disinfected using OTC (Ox tetracycline aerosol spray) before performing the wound.

Three incisions (15 mm-long) were made through the back skin by a scalpel, with subcutaneous pocket was prepared by blunt dissection for each incision. Two sterile one end-opened polyethylene tubes (10-mm long and 1.5 mm inner diameter) filled with fresh endodontic sealer materials immediately after mixing using endodontic file and one empty

(control) were carefully placed into the pockets to a depth of 20 mm in order to prevent smearing of the test material on the outer tube areas. After materials implantation, the margins of the wound were joined and closed with simple interrupted suture (Black silk suture gauge 3/0 Ethicon, Scotland).

Post operatively the animals were kept under observation till recovery from anesthesia; sutures on the dorsal surface were removed after seven days (12). At the end of the experimental periods the animals were sacrificed by anesthetic overdose. The tubes were removed together with the surrounding tissues as a block section (20 x 20 mm), preserved in 10% formaldehyde solution and fixed for 24 hours. The tissue samples were processed for paraffin embedding and 4 µm longitudinal serial sections by using soft tissue microtome (Yidi, china), stained with hematoxylin and eosin.

A blinded pathologist evaluated the specimens at different magnifications to evaluate the intensity of inflammatory reactions to the tested materials-connective tissue interface. Reactions in the tissue in contact with the material on the opening of the tube were scored as (13,14): 0, none or few inflammatory cells and no reaction; 1, less than 25 cells and mild reaction; 2, between 25–125 cells and moderate reaction; and 3, 125 and more cells and severe reaction. Fibrous capsules thickness was evaluated using micrometer lens (Fibrous capsules thickness x 40). An average of value for each material was obtained from 10 separate areas. Results were statistically analyzed by Mann-Whitney test for scores of inflammatory reaction and analysis of variance (ANOVA), and Duncan's Multiple Range Test for fibrous capsule thickness as response to each material according to periods of subcutaneous implantation. P-value ≤ 0.05 was considered as significant.

RESULTS

The intensity of the inflammatory reaction (inflammatory cell scores and fibrous capsule thickness) to all experimental groups at periods of subcutaneous implantation were analyzed histopathologically and statistically.

Histopathological analysis

Control (empty tubes) no sealer

A severe reaction was observed on the 3rd day. The tissue was infiltrated with acute inflammatory cells (lymphocyte and neutrophil) mixed with fibrous tissues reaction (thin fibrous capsule consist from fibroblast and collagen fibers with some extravasated blood (figure 1A and 1B). On the 14th day, the intensity of the reaction was moderate and the tissue was characterized by the presence of chronic inflammatory cells and some areas showed many congested blood vessels with slight increase in fibrous capsule thickness (figure 2A and 2B). The intensity of reaction was greatly diminished (No or few inflammatory cells) on the 28th day, with obvious capsule foundation and signs of reparation (figure 3A and 3B).

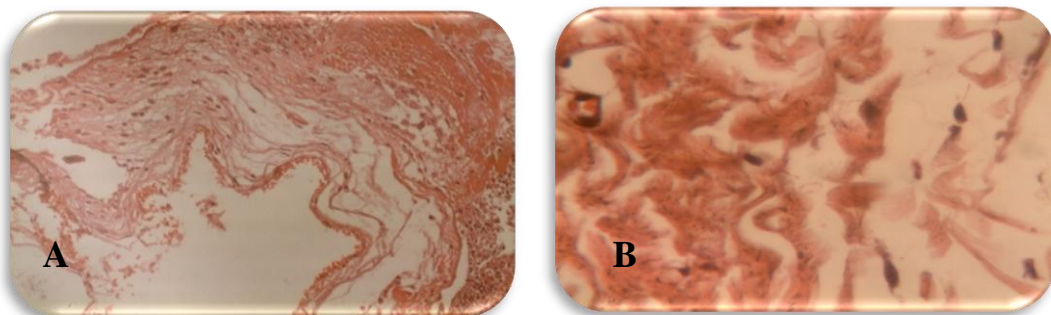


Figure (1): Control group: 3rd day. Severe inflammatory reaction of subcutaneous tissue with acute inflammatory cell infiltration (H&E; A- 10x and B- 40x).

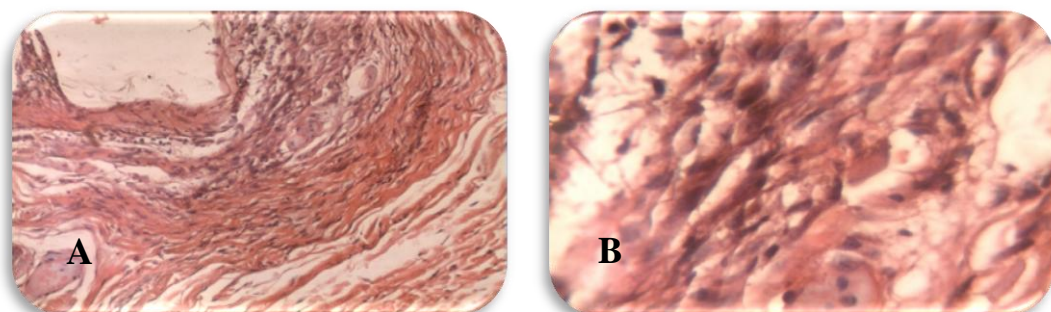


Figure (2): Control group: 14th day. Moderate inflammatory reaction of subcutaneous tissue (H&E; A- 10x and B- 40x).

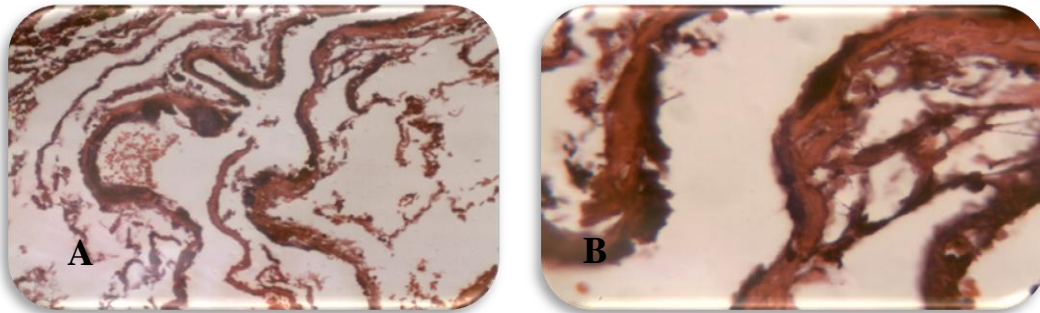


Figure (3): Control group: 28th day. Minimal reaction of subcutaneous tissue with fibrous capsule foundation (H&E; A- 10x and B- 40x).

Experimental biosealer ZOGU

A severe inflammatory reaction was observed on the 3rd day. The tissue was infiltrated with neutrophils and few lymphocytes in area of fibrous capsule (figure 4A and 4B). The intensity of the inflammatory reaction was moderate on the 14th day and the tissue was more organized fibrous capsule. The tissue was infiltrated with macrophages, plasma cells and lymphocytes, with small dilated blood vessels (figure 5A and 5B). On the 28th day, milder inflammatory reaction, infiltration of connective tissue with fibers and fibroblasts was observed. With few dilated blood vessels and a fibrous capsule tissue was present between the tube opening and the tissue (figure 6A and 6B).

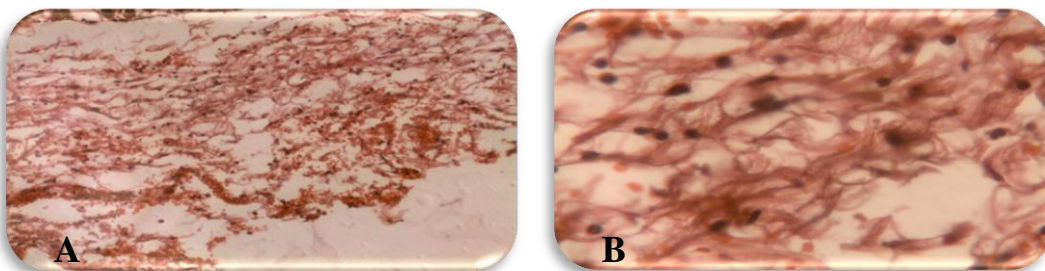


Figure (4): biosealer ZOGU: 3rd day. Severe inflammatory response of subcutaneous tissue (H&E; A- 10x and B- 40x).

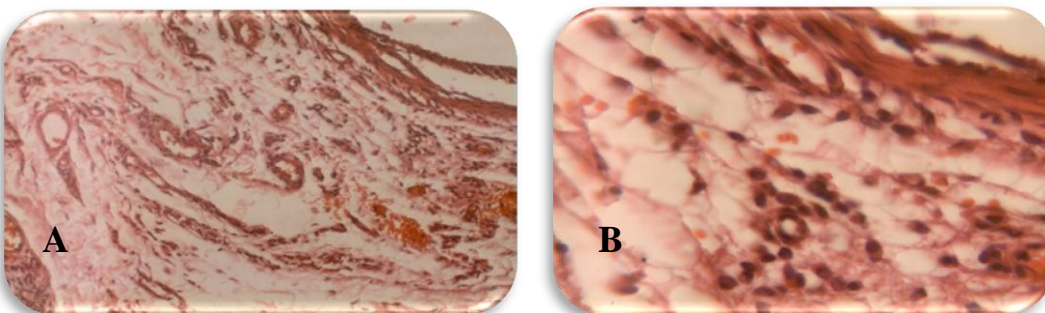


Figure (5): biosealer ZOGU: 14th day. Moderate inflammatory reaction of subcutaneous tissue with fibrous capsule formation (H&E; A- 10x and B- 40x).

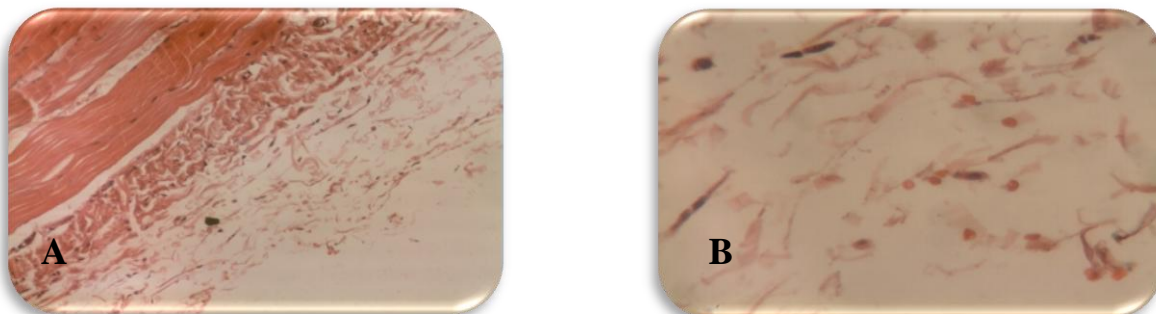


Figure (6): Biosealer ZOGU: 28th day. Mild inflammatory reaction of subcutaneous tissue with few inflammatory cells (H&E; A- 10x and B- 40x).

Tgsealer (ZOE based) sealer

Severe infiltration of large number of mononuclear cells and neutrophils in area of capsule and surrounding tissues on the 3rd day (figure 7A and 7B). The intensity of the inflammatory reaction was remained severe on 14th day, and the tissue was more organized exhibiting the formation of connective fibers. The tissue was infiltrated with many macrophages, plasma cells and lymphocytes in area of capsule and adjacent tissues with many blood vessels (figure 8A and 8B). At 28th day, moderate amount of chronic inflammatory cells was seen with more organization of a fibrous capsule along with granulation tissue with dilated blood vessels and extravasated blood (figure 9A and 9B).

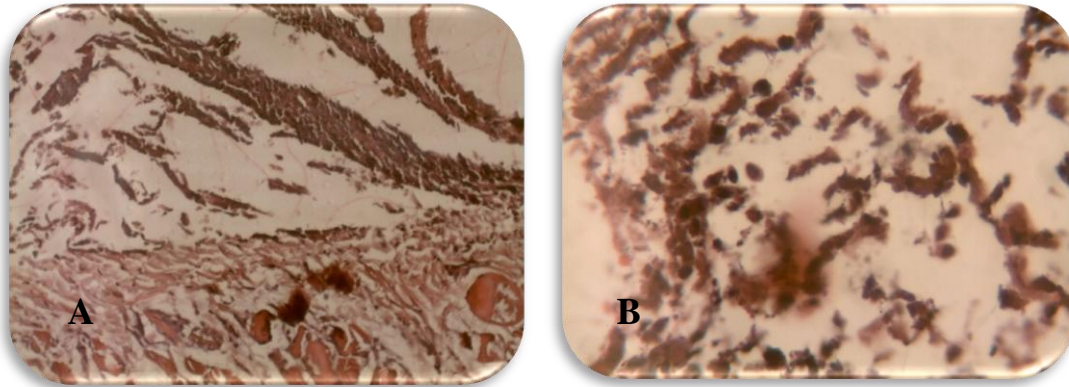


Figure (7): tgsealer ZOE sealer: 3rd day. Severe inflammatory reaction of subcutaneous tissue with acute inflammatory cells infiltration (H&E; A- 10x and B- 40x).

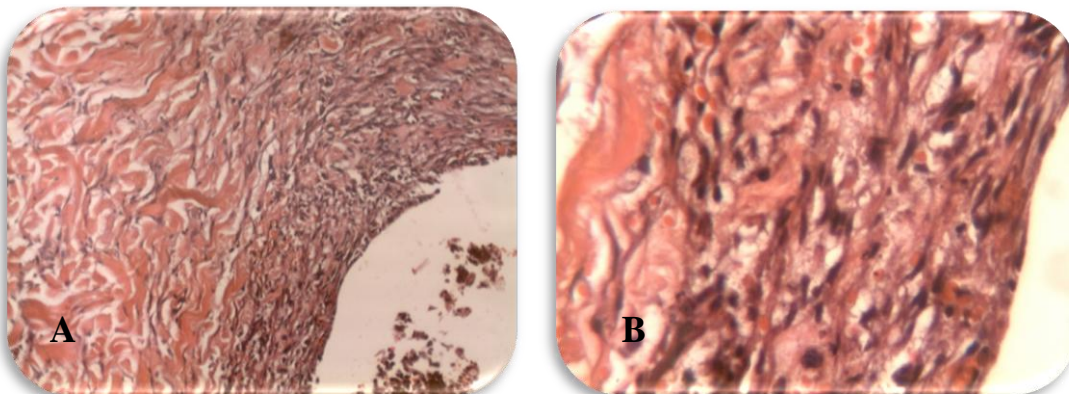


Figure (8): tgsealer ZOE sealer: 14th day. Severe inflammatory reaction of subcutaneous tissue close to the implanted material (H&E; A- 10x and B- 40x).

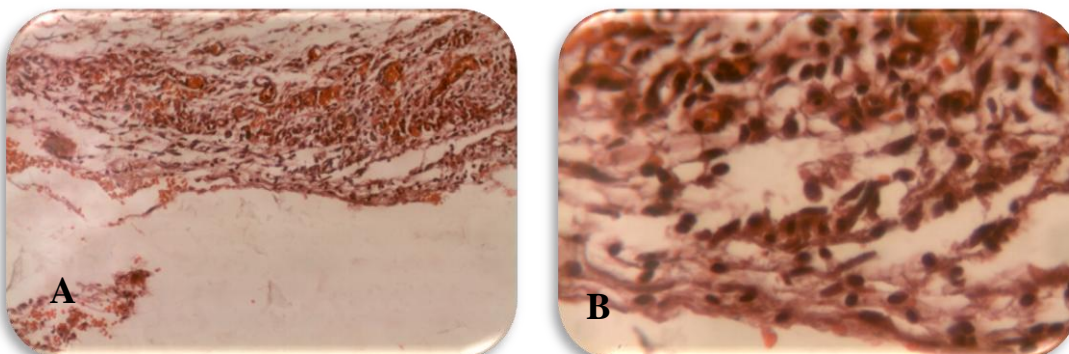


Figure (9): tgsealer ZOE sealer: 28th day. Moderate inflammatory reaction of subcutaneous tissue close to the implanted material (H&E; A- 10x and B- 40x).

Statistical analysis

The tables (1, 2, and 3) presented the variation in the mean values and frequency of the inflammatory cells by the type of materials used (Experimental biosealer ZOGU and ZOE tgsealer) in comparison to a control (empty tube only) according to the period since implantation.

Table (1): Descriptive Analysis of inflammatory cell scores of control group.

Periods	N	Min.	Max.	Mean	Scores* (percentage of frequency)				SD
					0	1	2	3	
3 rd day	10	2	3	2.7	—	—	30%	70%	0.48305
14 th day	10	1	2	1.7	—	—	30%	70%	0.48305
28 th day	10	0	1	0.1	90%	10%	—	—	0.31623

*Score of the inflammatory cells.

Table (2): Descriptive Analysis of inflammatory cell scores of Experimental biosealer ZOGU.

Periods	N	Min	Max	Mean	Scores* (percentage of frequency)				SD
					0	1	2	3	
3 rd day	10	2	3	2.9	—	—	10%	90%	0.31623
14 th day	10	1	3	2.0	—	20%	60%	20%	0.66667
28 th day	10	0	1	0.8	20%	80%	—	—	0.42164

*Score of the inflammatory cells.

Table (3): Descriptive Analysis of inflammatory cells scores of tgsealer ZOE sealer.

Periods	N	Min	Max	Mean	Scores* (percentage of frequency)				SD
					0	1	2	3	
3 rd day	10	3	3	3.0	—	—	—	100%	0.0000
14 th day	10	3	3	3.0	—	—	—	100%	0.0000
28 th day	10	2	2	2.0	—	—	100%	—	0.0000

*Score of the inflammatory cells.

Statistical evaluation of tissue (inflammatory cell scores) response to the effect of each sealer according to the periods of subcutaneous implantation showed that there is no significant difference among all groups at 3rd day, (scores: control =2.7, ZOGU=2.9 and tgsealer ZOE=3). At 14th day no significant difference between control group and ZOGU biosealer (scores: control =1.7, ZOGU=2.0) but there is significant difference with tgsealer ZOE group (score: ZOE=3). While at 28th day, there is significant difference among all experimental groups (scores control = 0.1, ZOGU= 0.8 and tgsealer ZOE= 2) (Figure 10).

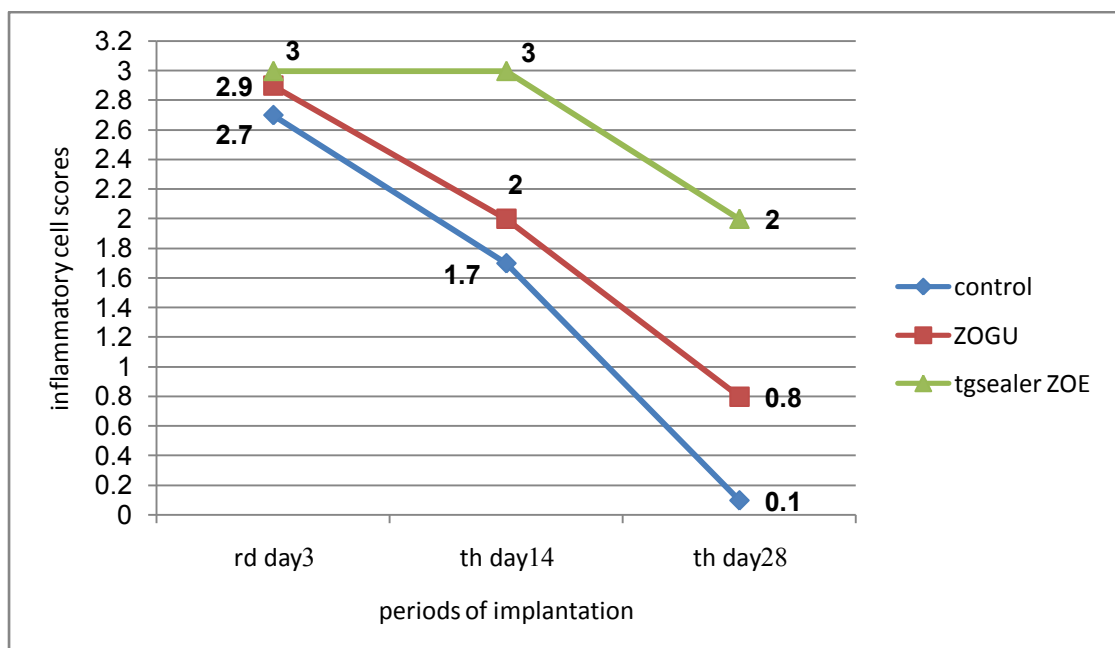


Figure (10): Inflammatory cell scores at 3rd day, 14th day and 28th day periods of implantation.

Generally, the intensity of inflammatory response in all groups of sealers decreased over time, Zinc oxide eugenol sealer had the most severe tissue inflammatory response when compared to biosealer ZOGU and control groups at implantation periods. The result of study revealed formation of fibrous capsule in variable thickness as a response of tissue reaction at side contact with sealer materials and control group.

Analysis of variance (ANOVA) test showed there are highly significant difference among fibrous capsule thickness of groups at 3rd day, 14th day and 28th day periods of subcutaneous implantation (Table 4).

Table (4): Analysis of variance (ANOVA) test for groups

Times		Df	Mean Square	F	Sig.
3 rd day	Between Groups	2	573.333	82.560	.000*
	Within Groups	27	6.944		
	Total	29			
14 th day	Between Groups	2	1235.833	202.227	.000*
	Within Groups	27	6.111		
	Total	29			
28 th days	Between Groups	2	2417.500	104.436	.000*
	Within Groups	27	23.148		
	Total	29			

*Significant at $p \leq 0.05$

Statistical analysis of results revealed that there is no significant difference between fibrous capsule thickness for control and biosealer ZOGU at 3rd and 14th day of subcutaneous implantation. There is significant difference among all groups at 28th day. The ZOE sealer group usually produced the thickest fibrous capsule at all implantation periods (Table 5, 6 and 7).

Table (5): Duncan's Multiple Range Test for capsule thickness of tested sealers and control group at 3rd day's period of implantation

Materials	N	Mean(μm) \pm SD*	Duncan groups**
Control	10	10.5 \pm 2.63523	A
ZOGU	10	12.5 \pm 2.63523	A
tgsealerZOE	10	24.5 \pm 2.63523	B

*Std. deviation**Different letters mean significant different at $p \leq 0.05$

Table (6): Duncan's Multiple Range Test for capsule thickness of tested sealers and control group at 14th days period of implantation

Materials	N	Mean(μm) \pm SD*	Duncan groups**
Control	10	30.0 \pm 2.10819	A
ZOGU	10	30.5 \pm .52705	A
Tgsealer ZOE	10	49.5 \pm 3.68932	B

* Std. deviation ** Different letters mean significant different at $p \leq 0.05$

Table (7): Duncan's Multiple Range Test for capsule thickness of tested sealers and control group at 28th day's period of implantation.

Materials	N	Mean(μm) \pm SD*	Duncan groups**
Control	10	46.0 \pm .00000	A
ZOGU	10	66.5 \pm 4.74342	B
tgsealerZOE	10	76.5 \pm 6.85160	C

Different letters mean significant different at $p \leq 0.05$

* Std. deviation **

DISCUSSION

Endodontic sealers are used in the obturation of root canal systems to achieve a fluid-tight or hermetic seal throughout the canal including the apical foramen and canal irregularities and minor discrepancies between the dentinal wall of the root canal and the core filling material (15). The Biocompatibility of the materials depends on tissue response, histological and pathological findings. The ideal requisite of biosealer should be Noncarcinogenic, Bacteriostatic, Should not discolor tooth, Promote Cementogenesis, Osteogenesis and healing (16).

Such research has been carried out in the field of Endodontics in order to produce a better material. According to the requisites that a sealing material must have, it is possible to establish research parameters for developing new products containing natural ingredients is crucial for successful dental treatment (17).

Laboratory research has demonstrated the acid-base chemical reaction between the acid component of guaiacol with the alkaline constituent of zinc oxide (18). The possibility of a setting reaction between these constituents stimulate the research for biotechnical advances, aiming at developing a new product based on the application of natural materials in endodontics. The new experimental sealer has a powder and a liquid component that are mixed to obtain the final product. The powder is composed of zinc oxide, natural rosin to impart cohesiveness and adhesiveness, natural hydroxyl apatite to enhance the biocompatibility, bismuth subcarbonate to improve radiopacity and zinc acetate to regulate the setting reaction, and the liquid is composed of guaiacol and olive oil to improve smoothness.

Implantation tests are types of secondary tests used to evaluate materials that will contact subcutaneous tissue or bone (19).

In these tests, surgery is performed to make the implantation, the response to tissue injury starts with inflammation and proceeds through the steps of wound healing. Superimposed on this is the "foreign body reaction" due to the presence of material recognized as not belonging to the host. If some form of normal healing sequence is followed the implanted material can be called biocompatible (19, 20).

According to (Williams, 2008) (21), biocompatibility refers to the characteristic of a material that, when in contact with any living tissue, causing an expected, non-toxic reaction, with no damage to the host and with preservation of the function of both the material and the tissue.

The present study was conducted to access the biocompatibility of newly prepared material. The tested materials were placed in polyethylene tubes. Whilst materials to be tested were directly applied subcutaneously in some studies (22), the implantation of the materials in tubes is advocated in others (23, 24). When compared to the direct application of the material, this method helps to provide stabilization of the material in place (25) and to eliminate possible effects caused by different surface structures of individual specimens, thus achieving the standardization of the material-tissue interface (25,26).

In the present study, the reaction observed to the 3rd day specimens may be more likely due to the surgical trauma rather than caused by the materials' toxicity (27). However, it allowed evaluating the behavior of the materials along the experimental time and during natural skin healing process.

A stronger inflammatory response of groups in the beginning and attenuation over time but with different rates. The inflammatory process in experimental biosealer ZOGU specimens had been shown to subside faster than that of tgsealer ZOE. This result related to the composition of material that contains natural components (ingredients) in origin, such as natural HAP. As a one ingredients has a role in improvement biocompatibility, HAP has been shown to be non-toxic and non-mutagenic, encouraging evaluation of this non-resorbable material as a potential root canal sealer (28, 29). It is thought that these properties emerge from the chemical structure of the material. HAP is basically formed by calcium and phosphate ions, also found in neutral bone structure (30).

Guaiacol has good antiseptic and anti-microbial action which improve healing process of tissues (31). It scavenges superoxide radicals and activate the cell proliferation at very low concentration, this result indicates that guaiacol greatly contribute to reconstruction of the injured tissues (32).

All implants were surrounded by a non-inflammatory connective tissue capsule, gradually increasing in thickness, with time indicate the repairing activity, as the result reported by (33, 34). And suggesting the good acceptance of the material by the tissues (35, 36). The fibrous capsule formation is related to a number of factors such as the size, shape and texture of the implant as well as its chemical properties, porosity, deployment location, and the physical and chemical stimuli caused by the implant, being an expected response to the material within the normal range (33,35).

According to (Xavier et al., 2002) (37), it is likely that this capsule prevents the leakage of the biomaterial, therefore reducing the inflammatory response near the site of application and thereby reducing the occurrence of granulomas. tgsealer produce the more intense inflammatory reaction and the thickest fibrous capsule formation in comparison to that of ZOGU sealer for all implantation intervals. Other studies have reported prolonged irritating effect of ZOE

sealers (38,39). Eugenol is the major cytotoxic component in ZOE cements, free-eugenol is still present even after the sealer has been set and released over an extended period, the leach out of zinc-eugenol bases sealers was found cytotoxic to nerve cells as well as to human periodontal ligament (PDL) fibroblasts (40). In addition to possible toxicity of zinc ions (41). Thymol-iodide exhibited a less favorable biological behavior and persisted inflammatory infiltrate, in addition to a discreet formation of collagen fibers in repair procedure (42).

CONCLUSIONS

The outcome of present study showed that the biosealer ZOGU presented good biocompatibility, when the tissue response to implanted material start and proceed through the steps of wound healing, the tissue response diminished faster than that of tgsealer ZOE based type.

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