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Histological Evaluation of Bioceramic Sealer Effect in Animal Model after Exposure to Gamma Radiation

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ABSTRACT

Purpose: This study was carried out to evaluate the bone tissue reactions to TotalFill Bioceramic (BC) and Zinc oxide eugenol (ZOE) sealers in gamma-irradiated rats. **Materials and methods:** In this study, round osseous bone defects were produced in the tibia of 48 male Albino rats (weighing120–150g). Group 1: 9 control non-irradiated rats, Group 2: 9 control irradiated rats, and Group 3: 30 irradiated rats in which the surgical cavities were filled with TotalFill sealer in the right tibia and ZOE in the left tibia. The assigned animals were euthanized on 7, 14, and 28 days. For histopathological examination, tissue sections stained with H & E were examined by a light microscope. **Results:** At 7 days, the inflammatory response of all studied groups (1, 2, and 3) showed no significant differences (p-0.092). The inflammatory response significantly decreased from 14 to 28 days in all groups, virtually disappeared in the non-irradiated group whilst intense inflammatory infiltrate was still observed in 40% of ZOE group samples with a significant difference between TotalFill vs. ZOE sealer (p- < 0.009). Bone tissue at 14 days showed severe resorption in many areas in irradiated animals that reversed at 28 days. In group 3 treated with TotalFill sealer, fiber condensation was observed in surgical cavity at 14 days which was replaced by trabeculae of bone tissue at 28 days while this event was focal in ZOE sealer. **Conclusion:** TotalFill sealer proved to be more compatible and has some repair potential than ZOE sealer in irradiated animals.

KEYWORDS

Bioceramic, Sealer, Gamma, Radiation.

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INTRODUCTION

Head and neck cancers are treated by ionizing radiation, either alone or combined with surgery and chemotherapy. The radiation therapy has its damage effect in the tooth structure [(1)]. Bone is influenced by the radiation which leads to a significant change of the bone regeneration capacity. Bone matrix is altered slowly after radiation therapy as a result of injury to the bone remodeling system, leading to a change of the bone regeneration. Dental extractions are contraindicated during one year following radiotherapy [(2)]. Hence, endodontic treatment is ideal.

Endodontic materials utilized for root canal treatment comprise the basic filling material guttapercha and the sealer. Because of the durable direct contact between these materials and periapical tissues, their biocompatibility should be taken into consideration. In this manner, regardless of their chemical composition, endodontic sealers should be associated with adequate biological responses, to ensure the safety of their clinical use. The material is said to be biocompatible when the material coming into contact with the tissue does not cause an unfavorable reaction, as toxicity, irritation, inflammation, allergy, or carcinogenicity [(3)].

A considerable amount of the literature notes that many Bioceramic sealers can promote bone regeneration when the sealer is accidentally extruded through the apical foramen during root canal filling or can also repair the root perforations [(4)]. However, there is not enough data on the effect of Bioceramic sealer histologically, clinically and radiographically after gamma radiation. Therefore, this study aimed to evaluate the biocompatibility of TotalFill sealer and ZOE in filling a bone defect in the irradiated animal model through evaluating histologically the inflammatory response.

MATERIALS AND METHODS

Forty-eight male Albino rats of 120–150g weight were obtained from the animal farm of the Egyptian Holding Company for Biological Product and Vaccines, Egypt. The animals were allowed to acclimatize for 1 week upon arrival before starting the experiment. Animals were kept under standard conditions and were allowed for free access to a standard requirement diet and water ad. Libitum. Animal experimentation was conducted in accordance with the guide for the care and use of laboratory animals. Ethical approval was obtained from REC of the Faculty of Dental Medicine Al-Azhar University EN-F-020-001.

i. Animal Grouping

Group 1: Non-irradiated (control -ve) 9 rats with a surgical cavity in both right and left tibia (without endodontic sealer).

Group 2: Irradiated (control +ve) 9 rats with a surgical cavity in both right and left tibia were exposed to gamma rays (6 Gy) as a single dose (without endodontic sealer).

Group 3: 30 rats were exposed to gamma rays (6Gy), subgroup A: TotalFill BC sealer was injected in rat's right tibia, and subgroup B: Zinc oxide Eugenol sealer was injected in rat's left tibia. Then these subgroups were divided into **a,b,c** division according to follow-up period.

ii. Radiation Exposure

Whole-body gamma-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (137cesium) Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.665 rad /s. Tibial bone defect preparation was done three days after exposure to gamma-radiation in the irradiated groups [(5)].

iii. Tibial bone defect preparation

Animals were generally anesthetized intraperitoneally with ketamine hydrochloride (Ketalar; Pfizer, Sao Paulo, Brazil) of 0.2 mL/100 gm of body weight, associated with dihydrothiazine hydrochloride (Rompum; Bayer, Rio de Janeiro, Brazil), at a dosage of 0.05 mL/100 gm. Then a 20 mm incision was made in both the right and left tibia of each animal after shaving and washing the skin with iodine. The bone defect was produced by a trephine bur #2 with a low-speed drill (Incol; Instrumentos Cirurgicos Oftalmologicos Ltda, Sao Paulo, Brazil). The bur was positioned perpendicularly to the tibia to create a standardized, round osseous defect (5 mm in diameter) in the cortical surface of both the right and left tibia in all groups. In the bone defect rat's right tibia was filled with TotalFill BC sealer (subgroup A) and Zinc oxide eugenol sealer (subgroup B) was injected in rat's left tibia then the wound was sutured. After the experimental procedures, the animals were housed in separate cages. The assigned animals were euthanized on 7, 14, and 28 days [6]. The animals used in the research were disposed of by burial.

iv. Histological analysis

For histopathological examination, the collected tibia was fixed in 10% formalin for 24 h. Fixed specimens were decalcified in 10% formic acid for 7 days. All tissues were set in paraffin blocks after processing the tissues in alcohol and 5 μm thick sections were cut and stained with hematoxylin-eosin then were examined using the light microscope to evaluate any bone change.

Morphometric analysis: Assessment of inflammatory reaction was done on H&E stained slides using the Digital image morphometric software optical micrometer (TSView) at pathology department Girl's Faculty Of Medicine, Al-Azhar University.

Steps for measurements: Quantitative evaluations of inflammatory cells (lymphocytes and polymorphonuclear leukocytes) was done on H&E stained slides at ×400 magnifications using the image analyzer optical micrometer (TSView).

An average value for each material was obtained from the sum of cells counted in 10 high power fields. Inflammatory cells were scored and evaluated[7] as follow:

None or few inflammatory cells (0), Mild inflammatory cells <25 cells (1), Moderate inflammatory cells between 25 and 125 cells (2), Severe inflammatory cells ≥ 125 cells (3).

All measurements for each group were averaged and were subjected to statistical analysis using Chi-square (x2).

RESULTS

Over a period of 7 days, all endodontic sealers and the control groups showed similar moderate and high levels of inflammatory response without significant statistical difference (p-0.092). Over a period of 14, 28 days there was a highly statistically significant difference between groups according to the score of inflammatory cells infiltrate (p-<0.001**). Also, the comparison between TotalFill and ZOE groups showed statistically significant differences (p-0.046*) on 14 days and (p-value 0.009*) on 28 days.

Descriptive Statistical results in different periods for all groups are summarized in (table 1) and (Fig. 1).

Histopathological results of tissue reaction at 7 days:

The bone tissue revealed normal morphology in negative non-irradiated control rats.

In all irradiated animals (+ve Control) and Group 3 injected with Sealer materials, the bone tissue showed slight resorption and decreased number of osteocytes. Inflammatory reaction was moderate to severe in all studied groups with no evidence of repair potential.

Table (1): *Comparison between groups according to the score of inflammatory cells infiltrate on 7,14 and 28 days.*

Score of inflammatory cells infiltrate	Control Group -ve (N=6)	Control Group +ve (N=6)	Group 3 (a) TotalFill (N=10)	Group 3 (a) ZOE (N=10)	*Chi-square test*	*p-value*
On 7 days						
(0): None /few inflammatory cells	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6.441	0.092
(1): Mild <25 cells	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
(2): Moderate 25-125	4 (66.7%)	2 (33.3%)	2 (20.0%)	1 (10.0%)		
(3): Severe >125	2 (33.3%)	4 (66.7%)	8 (80.0%)	9 (90.0%)		
On 14 days						
(0): none /few inflammatory cells	4 (66.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	32.865 <0.001**	
(1): Mild<25 cells	2 (33.3%)	0 (0.0%)	2 (20.0%)	0 (0.0%)		
(2): Moderate 25-125	0 (0.0%)	4 (66.7%)	6 (60.0%)	3 (30.0%)		
(3): Severe>125	0 (0.0%)	2 (33.3%)	2 (20.0%)	7 (70.0%)		
On 28 days						
(0): none /few inflammatory cells	6 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	53.191 <0.001**	
(1): Mild<25 cells	0 (0.0%)	2 (33.3%)	8 (80.0%)	0 (0.0%)		
(2): Moderate25-125	0 (0.0%)	4 (66.7%)	2 (20.0%)	6 (60.0%)		
(3): Severe>125	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (40.0%)		

*Using: Chi-square test; p-value >0.05 NS; *p-value <0.05 S; **p-value <0.001 HS*

*A comparison between the score of inflammatory cells over different periods in the non-irradiated group showed a highly statistically significant improvement. The level of inflammatory infiltrate decreased after 14 and virtually disappeared on 28 days (p- <0.001**).*

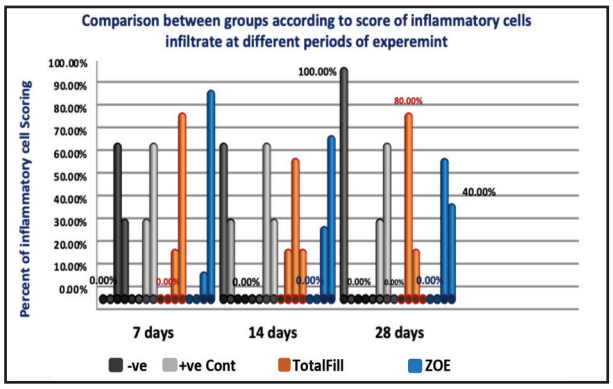

Figure (1): Results of the intragroup histological score analysis of the inflammatory reaction to the materials tested in all periods.

Histopathological results of tissue reaction at 14 days:

Bone of all irradiated animals showed severe resorption in many areas and expansion of the Volkmann's and Haversian canals with an increase in the volume of their connective content. The experimental animals of the non-irradiated group showed a significant decrease in the inflammatory reaction. The inflammatory reaction remained higher in both TotalFill and ZOE than non-irradiated group and near to irradiated group control ranging from mild to severe reaction. The fibrous tissue

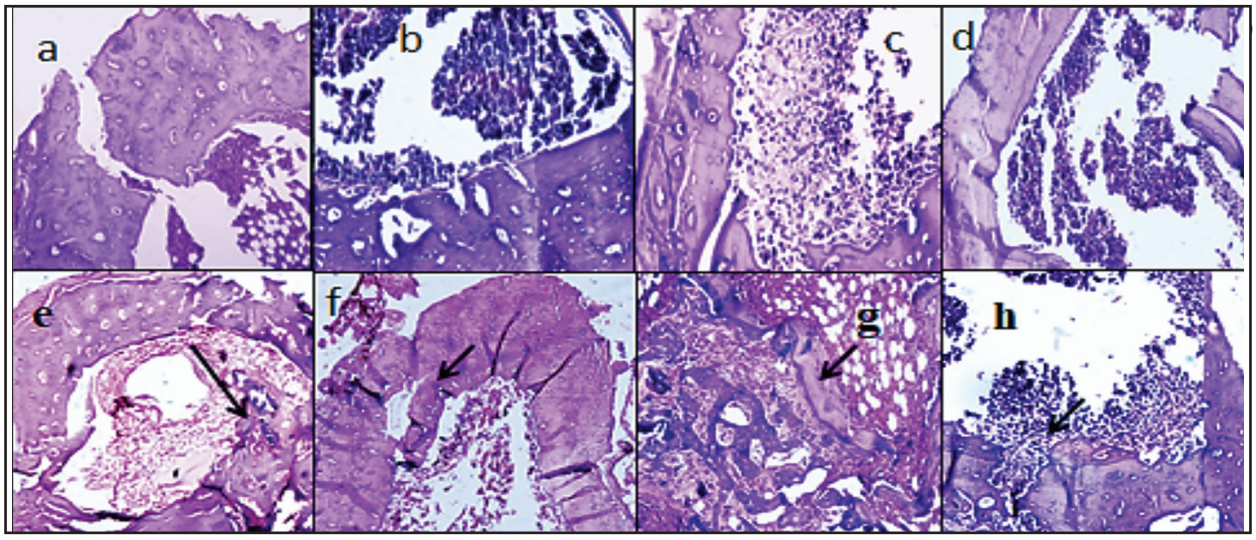

Figure (2): Histological images showing tissue reaction around the bone defects. Day 14 (a) Group 1 showing mild chronic inflammatory cell infiltrate in the surgical cavity (b) Group 2 showing severe bone resorption and severe chronic inflammatory cell infiltrate in the surgical cavity (c) Subgroup A showing moderate chronic inflammatory reaction and scalloping of the inner bone surface with fiber condensation in a large area of a surgical cavity (d) Subgroup B showing severe inflammatory cells and slight bone resorption. Days 28 (e, f) Group 1 and Group 2 showing partially healed wound site (arrow) (g) Subgroup A showing neogenesis of bone trabeculae tissue deposition and mild chronic inflammatory reaction (h) Subgroup B showing fiber condensation in a large area of the surgical cavity and severe chronic inflammatory infiltrate (arrow) (H&E, magnification ×200).

condensation was found in contact with TotalFill sealer with moderate chronic inflammatory reaction Fig.2 (a,b,c,d).

Histopathological results of tissue reaction at 28 days:

Samples from this group no inflammatory response was found in all experimental animals of Group1. The bone tissue restored almost its normal architecture in most cases indicating bone healing by time. The inflammatory reaction remained elevated and intense for the ZOE group than the TotalFill group. Neogenesis of bone trabeculae was observed partially closing the surgical cavity in non-irradiated and irradiated control groups and experimental animals injected with TotalFill sealer whilst fiber tissue condensation was still observed in samples injected with ZOE sealer Fig.2 (e,f,g,h).

DISCUSSION

Ionizing radiation therapy either alone or combined with surgery and chemotherapy.is a common oncologic treatment for head and neck cancers. Despite it is a successful treatment, there are many side effects. The total dose of radiation is pertinent, and important factor to consider for endodontic treatment [8].

Endodontic filling materials remain in direct and intimate contact with vital tissues for a considerably longer time. Almeida et al investigated that bone tissue reaction to endodontic sealers put in direct contact with bone tissue [9].

Bioceramic endodontic materials are used as root repair cements and root canal sealers, which are known as bio-aggregates [10]. They are biocompatible and bioactive, as it is composed of alumina and zirconia particles, calcium silicates, bioactive glass, hydroxyapatite, and resorbable calcium phosphates [11]. Bioceramics are an important class of biomaterials, because of their ceramic origin[12]. A recent systematic review reported that pre-mixed calcium silicate-based sealers had good physicochemical and biological properties in vitro with better results than conventional endodontic sealers [13].

Biocompatibility is considered a key property of root canal sealers, thus demonstrating the significance of the study of the biocompatibility of various endodontic materials [14]. Therefore, to ensure the safety of TotalFill sealer in clinical use in irradiated

head and neck cancer patients, this study aimed to evaluate the bone tissue reactions in a bone defect in the irradiated animal model to evaluate their biocompatibility in filling the root canal by verifying the intensity of the inflammatory infiltrate and tissue repair.

In this study, bone defect in rat's tibia was produced in 48 male Albino rats creating a round, osseous defect (9 non-irradiated –ve control, 9 irradiated +ve control and in 30 rats) the surgical cavities were filled with TotalFill sealer in the right tibia and ZOE in the left tibia. The assigned animals were euthanized on 7, 14, and 28 days. The rats were divided into 3 groups and their tibias were histologically processed and analyzed using a light microscope. The presence of inflammatory cells, and any bone morphological changes were evaluated.

Over a period of 7 days, all endodontic sealers and the control groups showed moderated and high levels of inflammatory response, However, no significant statistical difference was found among the groups. This is consistent with the result of another study [15] who evaluated inflammatory reactions in non-irradiated experimental animals. Tissue morphology at this period of the experiment showed nearly normal morphology in all groups with some decreased number of osteocyte in irradiated animals with no evidence of repair potential. Our results were not in agreement with a new study [10]. They found intense immature hard tissue deposition in contact with EndoSequence sealer and absence of inflammatory infiltrate at 7 days in non-irradiated experimental animals.

At 14 days the inflammatory infiltrate decreased in non-irradiated groups more than other irradiated groups with a highly statistically significant difference between all groups according to the score of inflammatory cells infiltrate and with a statistically significant difference between TotalFill vs ZOE groups. Yet, the inflammatory tissue response by TotalFill and ZOE sealers did not differ statistically from the irradiated control group versus TotalFill and vs ZOE. Understanding these results at this period (14 days) need more researches. Fourteen days post-gamma-irradiation, bone of all irradiated animals showed severe resorption in many areas and expansion of the Volkmann's and Haversian canals with an increase in the volume of their connective content. Fiber condensation was observed in bone tissue implanted with TotalFill while this event was not found in the ZOE sealer group. This is maybe an early response to bone repair. According to other authors [16], inflammatory response, fiber condensation and formation of hard tissue barrier, are related to bone healing and regeneration.

On 28 days the inflammatory infiltrate nearly disappeared in –ve control group and decreased in other groups. There were highly statistically significant differences between all groups according to the score of inflammatory cells infiltrate and statistically significant difference between TotalFill vs ZOE. Neogenesis of bone trabeculae was observed partially closing the surgical cavity in non-irradiated and irradiated control groups. Totalfill group showed bone trabeculae tissue deposition in some samples while fibrous tissue condensation was still observed in samples injected with ZOE sealer. This is contradicting another study that found complete hard tissue barrier formation deposition closing the surgical cavity in the EndoSequence group and focal areas of the surgical cavity in the control group [10]. Formation of trabeculae of hard bone tissue traversing the surgical cavity observed in the TotalFill group indicated the beginning of the process of linear closure of the experimental defect. Its reparative capacity may be explained by the presence of calcium (Ca2+) rendering the sealer the ability to maintain a pH that makes the medium favorable to tissue repair [17]. The mechanism of repair stimulation by deposition of mineralized tissue depends on pH and the ability to release Ca2+. This is also in agreement with other authors who postulated in their study that the presence of

ceramic particles in the modified MTA materials rendering the sealer the ability to maintain a pH around 9.0 [13].

The material is considered biocompatible if the inflammatory reaction reduces to non-significant levels over time [18]. In the current study, there was a highly statistically significant decrease of inflammatory cells infiltrate score in TotalFill Group over the different periods through the experiment so they can be considered biocompatible. Its biocompatibility can be considered more rapid when compared to that of ZOE sealers in irradiated animals. This can be explained by the presence of Calcium hydroxide, which accelerates the tissue repair process and improve the biological properties of endodontic sealers [19].

On the other hand, ZOE was associated with high levels of inflammatory infiltrate during all periods from 7 to 28 days of the experiment. Although there was a statistically significant decrease of inflammatory cells infiltrate score over the different periods through the experiment yet severe score of inflammatory cells infiltrate persisted in 40% of experimental animals in this group. The persistence of intense inflammatory infiltrate in the ZOE group indicate the less favorable biological behavior than TotalFill sealer, this is consistent with what was reported earlier [20].

CONCLUSION

The TotalFill sealer presented the lowest levels of the inflammatory response and provided the re-establishment of the original bone structure. Its biocompatibility can be considered more rapid when compared to that of ZOE sealers in irradiated experimental animals. TotalFill BC sealer can be used safely in irradiated head and neck cancer patients.

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