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ORIGINAL RESEARCH

Comparative Evaluation of Tissue Response of MTA and Portland Cement with Three Radiopacifying Agents: An Animal Study

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ABSTRACT

Aim: This study compared the tissue reaction of 80 wt% of White Portland cement (WPC) mixed with 20 wt% of three radiopacifying agents: Bismuth oxide/Iodoform/Zirconium oxide with MTA in rat subcutaneous connective tissue.

Materials and methods: The study was performed in 18 albino rats by implanting the WPC mixed with radiopacifying agents loaded in a polyethylene tube. Empty tubes were used as a control. At the end of 7, 30 and 60 days excisional biopsy of the implant along with surrounding tissues was done and sent for histological examination.

Results: In the 7 days experimental period there was no significant difference between groups in terms of the tissue response. In 30 and 60 days period significant difference was seen between the control (empty tube) and the other groups. But there was no significant difference between WPC mixed with radiopacifiers BiO/Iodoform/ZrO_2 and MTA.

Conclusion: The tissue reaction of the tested materials, White Portland cement (WPC) + Bismuth oxide, WPC + Iodoform, and WPC + Zirconium dioxide were similar to MTA (Pro Root MTA) in all experimental periods 7 days, 30 days and 60 days.

Keywords: Animal study, Iodoform, MTA, Radiopacifying agents, Tissue response, Wistar albino rats, Zirconium Oxide.

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INTRODUCTION

The aim of endodontic treatment is to clean, disinfect and seal the root canal system. Treatment failure due to complex root canal anatomy or iatrogenic errors could be treated by endodontic surgery.

MTA exhibits acceptable *in vivo* biologic performance when used for root-end fillings, perforation repairs, pulp capping, pulpotomy, and apexification treatment.[1] The properties of Portland cement and MTA were found to be comparable with WPC exhibiting similar inflammatory reaction in histological evaluation studies, hard tissue formation, biocompatibility, and antimicrobial property.[2,3]

Type I portland cement is the main component of mineral trioxide Aggregate (MTA) with the addition of bismuth oxide in 4:1 ratio to provide radiopacity. The possible interference of the radiopacifiers with the biocompatibility of Portland cement should be investigated.[4] The implantation of materials in the connective tissue of small animals is considered a suitable secondary test (local toxicity) for the evaluation of the biocompatibility of endodontic materials with more detailed information about the material-tissue reaction at the cellular level.

AIM

The study aims to compare the tissue reaction of WPC (80 wt%) mixed with (20 wt%) radiopacifying agents: Bismuth oxide/Iodoform/zirconium dioxide against MTA (Pro Root MTA) in the subcutaneous connective

tissue of albino rats evaluated histologically using light microscopy.

MATERIALS AND METHODS

Animal Ethical Committee approval was obtained (5/243/CPCSEA). Eighteen male Wistar albino rats, 5 to 6 months old each weighing 200 ± 25 gms were used in this study. Eighteen animals were divided into 3 sets of 6 each for the respective experimental period- 7 days, 30 days and 60 days.

A total of 90 polyethylene tubes of the desired dimension 1.2 mm diameter and 5 mm length were made from sterile B.D. Venflon intravenous apparatus.

An empty polyethylene tube (group I) implanted in each animal was used as the control.

Group II MTA (Pro Root, Dentsply) was mixed according to the manufacturer's instructions with distilled water in the powder-liquid ratio of 3:1.

WPC (Birla White, Grasim Ind Ltd) was mixed with the radiopacifying agents in the ratio of 4:1.

In group III, 80 wt% WPC was mixed with 20 wt% Bismuth oxide (Chen chemicals, India).

In group IV, 80 wt% WPC was mixed with 20 wt% Iodoform (Vikash Pharma, India).

In group V, 80 wt% WPC was mixed with 20 wt% Zirconium dioxide (Lobal Ltd, India).

These three groups were also mixed with sterile saline in the powder-liquid ratio of 3:1.

The animals were anesthetized with ketamine hydrochloride in all surgical phases as recommended by Miami University, Lab animal anaesthesia.[5] A total of five implants on the dorsal surface of each animal two on the right side and three on the left side was decided to be placed. Five incisions were made on the dorsum of the albino rat, 2 cm from the spine. There should be at least 2 cm distance between the incisions to prevent interaction of the materials. Five surgical pouches were created by blunt dissection (Fig. 1), each for the respective groups. The tubes that were previously loaded with the materials (Fig. 2) were implanted into the surgical cavities (Fig. 3), parallel to the incisions, which could prevent dislodgement or loss of the implant till the experimental periods were over. The position of implant placement was standardized in each group. Incisions were then sutured with a 3-0 silk (Tru Silk Sutures Ind Ltd.). After the surgical procedure, the animals were observed until recuperation of their physical activities and were placed in individual cages under no feeding restrictions.

After the respective experimental periods 7 days, 30 days and 60 days (6 rats in each), the animals were again anesthetized for excisional biopsy of the implant with the surrounding tissues. The animals were exposed to a whole body radiograph (Fig. 4). This will help to locate the implanted tube which was loaded with materials that were radiopaque. Animals were sacrificed by an overdose of anesthetic immediately after removal of the tissue samples (Figs 5A to E) The interface at the opening of the polyethylene tubes between the material and the tissue, was examined and evaluated for the intensity of inflammation.

The inflammatory responses were scored according to the following criteria:[6]

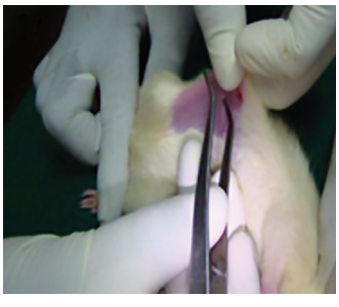


Fig. 1: Preparation of the implantation site

Fig. 2: Preparation of the implantation site

Fig. 3: Implantation of the samples at the preparation site

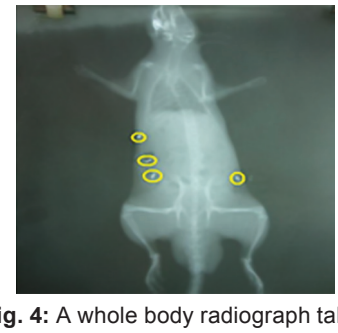
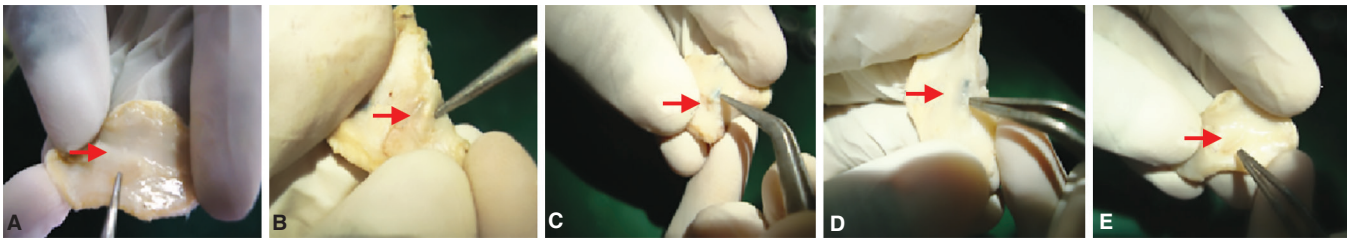


Fig. 4: A whole body radiograph taken to ensure the position of the samples



Figs 5A to E: (A) Tissue sample containing polyethylene tube; (B) MTA; (C) PC+BI2O3; (D) PC+Iodoform; (E) PC+ZrO2

- 0–No reaction (absence of inflammatory cells)
- 1–Mild reaction (presence of mild chronic inflammatory infiltrate)
- 2–Moderate reaction (presence of moderate chronic inflammatory infiltrate, or some eosinophils or giant cells)
- 3–Severe reaction (presence of an intense chronic inflammatory infiltrate, a large number of eosinophils or giant cells)

The values of the scores are given in Table 1. The qualitative data were analyzed using the Pearson Chi-square test in Statistical Package for the Social Sciences (SPSS) version 15. The significance was set at 5% for all analysis. Each group was compared individually with other groups.

RESULT

In the 7 days experimental period, there was no significant difference between the various groups. Moderate inflammation was seen in almost all groups. But most of the control (empty tube–group I) showed few inflammatory cells. In the 30 day experimental periods there was a significant difference between the group I (control) with the other groups. But there was no significant difference between group II (MTA) with the other groups (group III WPC + Bi2O3, group IV WPC + CHI3, group V WPC + ZrO_2 and also between group III, group IV and group V (Table 2). In 60 days, mild to moderate inflammation was present in groups II, III, IV, and V. No significant difference was found between these groups. In group I (control empty tube) very mild inflammation with few inflammatory cells was present. In the 60 days experimental period, there was a significant difference between group I with other groups. All the groups in 7 days showed thin fibrous capsule formation. Fibrous capsule increased in thickness in 30 days and it was more organized in 60 days (Figs 6A to E).

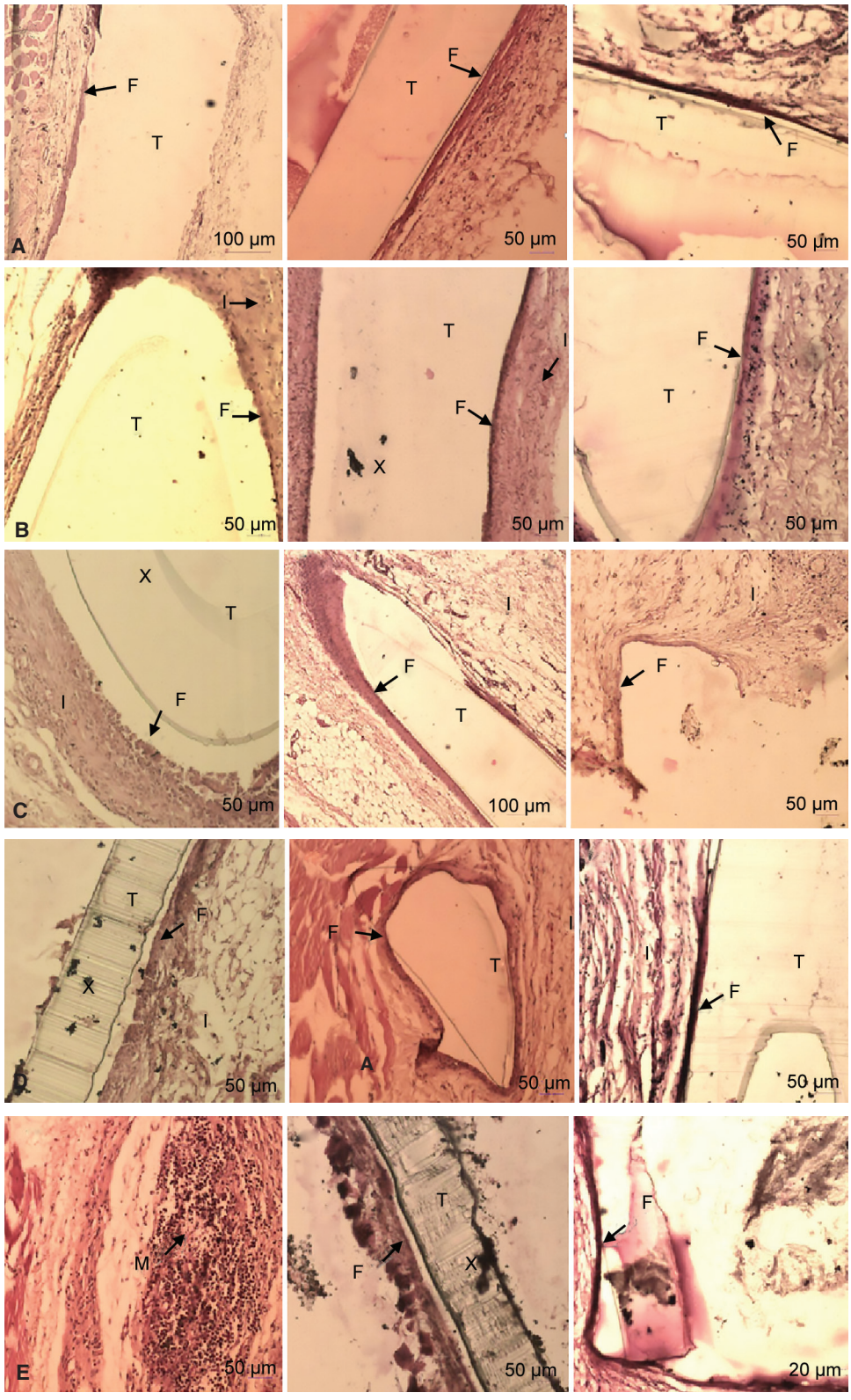
Table 1: The inflammatory scores for groups I to V at the end of 7, 30, 60 days

Day	*Reactions*	*Group I*	*Group II*	*Group III*	*Group IV*	*Group V*
7*	Nil	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Mild	2 (33.3%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	0 (0%)
	Moderate	2 (33.3%)	4 (66.7%)	4 (66.7%)	5 (83.3%)	4 (66.7%)
	Severe	0 (0%)	1 (16.7%)	1 (16.7%)	0 (0%)	2 (33.3%)
30+	Nil	3 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Mild	3 (50%)	2 (33.3%)	2 (33.3%)	3 (50%)	1 (16.7%)
	Moderate	0 (0%)	4 (66.7%)	4 (66.7%)	3 (50%)	5 (83.3%)
	Severe	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
60±	Nil	4 (66.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Mild	2 (33.3%)	4 (66.7%)	3 (50%)	4 (66.7%)	2 (33.3%)
	Moderate	0 (0%)	2 (33.3%)	3 (50%)	2 (33.3%)	4 (66.7%)
	Severe	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2: Represents the *p* values obtained with pairwise comparisons of the five groups at 7, 30 and 60 days postoperative period

Pairwise comparison of groups (Pearson's Chi square test)	*p value*		
	7 days	*30 days*	*60 days*
Pair I Group I and II	0.261	0.027*	0.036*
Pair II Group I and III	0.261	0.027*	0.027*
Pair III Group I and IV	0.164	0.048*	0.036*
Pair IV Group I and V	0.083	0.011*	0.018*
Pair V Group II and III	1.000	1.000	0.558
Pair VI Group II and IV	0.574	0.558	1.000
Pair VII Group II and V	0.513	0.50	0.248
Pair VIII Group III and IV	0.574	0.558	0.558
Pair IX Group III and V	0.513	0.505	0.558
Pair X Group IV and V	0.211	0.221	0.248

*Values represent significantly different variables



Figs 6A to E: Histopathological images. Group I 10. 7 days, showing thin fibrous capsule 11. After 30 days (X 100 magnification). 12. After 60 days, Fibrous capsule thick and organize (X100 magnification). Group II 13. 7 days showing inflammatory cell infiltration (X 100 magnification) 14. After 30 days showing increased fibrous capsule thickness & moderate inflammatory infiltration (X 100 magnification)15. Sixty days showing organized and thick fibrous capsule. (X 100 magnification). Group III 16. 7 days showing severe mononuclear inflammatory infiltration. (X 100 magnification) 17. 30 days showing a moderate inflammatory reaction (X 40 magnification) 18. Sixty days showing mild Inflammatory reaction with fibrous capsule formation (X 100 magnification). Group IV19. 7 days showing severe plasma cell infiltration (X 100 magnification). 20. After 30 days showing the increased thickness of the fibrous capsule (X 100 magnification).21 Sixty days showing the increased thickness of fibrous capsule and mild inflammatory reaction. (X 100 magnification). Group V 22. After 7 days showing severe plasma cell infiltration (X 100 magnification). 23. 30 days showing fibrous capsule and tube with the material. (X 100magnification) 24. 60 days showing organized & thick fibrous capsule with few inflammatory cells (X 200 magnification)

T–tube; F–fibrous capsule; X–Test material; M–mononuclear infiltration; I–Inflammatory cells

DISCUSSION

The biological properties of new endodontic materials used in furcal perforations, root end filling and as apical barrier must be investigated. The secondary or local toxicity tests were designed to produce evidence of sub-acute toxicity after longer periods in soft or hard tissues, essentially for screening purposes.[7] The implantation of the materials in tubes was advocated in many studies to simulate clinical condition.[2,3,8]

Twenty percent bismuth oxide is the radiopacifier used in MTA. At least 15% bismuth oxide must be added to WPC to give it sufficient radiopacity.[9] Coomarswamy et al. proved that the addition of bismuth oxide decreased the mechanical stability.[10] But Saliba et al. proved that the addition of bismuth oxide did not seem to affect the compressive strength of Portland cement.[11] The concentration of 15% of bismuth oxide resulted in a significant reduction in the inflammatory response in comparison with the other concentrations evaluated.[12] The addition of 20% nano bismuth oxide (50–80 nm) enhanced the physical properties, push-out bond strength and compressive strength of calcium silicate cements without any significant changes in radiopacity than the regular particle size (10 μm) of bismuth oxide.[13] Since there were different schools of thought about the physical and biological properties there was a need to search for an alternative radiopacifying agent.

Recently niobium oxide (NbO) microparticles and nanoparticles were added with portland cement replacing bismuth oxide.[14] Tantalum pentoxide was used in bio-aggregate.[15] Juliana et al. tested the pH and antimicrobial activity of portland cement associated with different (bismuth oxide, calcium tungstate, zirconium oxide) radiopacifying agents.[16]

We used Iodoform and zirconium dioxide as radiopacifying agents in our study.

Group II (MTA) and Group III (WPC + Bi_2O_3) had a similar inflammatory response at all the experimental periods of 7, 30 and 60 days. Many previous studies also concluded that PC + Bi_2O_3 exhibits similar tissue reaction as MTA.[3,8] Mangala et al. evaluated the biocompatibility of the Indian Portland cement Birla white in pellet forms.[17]

Group II (MTA) and Group IV (WPC + Iodoform) showed similar tissue reactions, and there was no significant difference between them in all experimental periods. The previous study proved that PC + iodoform showed a similar inflammatory reaction when compared to MTA.[2] Iodoform has been successfully used in paste form along with $Ca(OH)_2$ in root canal treatment for infected primary teeth.[18] Iodoform had no significant impact on the products and extent of hydration after 7 days.[19] Iodoform can be considered an alternative to bismuth oxide owing to the similarity in radiologic properties, tissue reaction, and anti-inflammatory properties.

Group II (MTA) and Group V (WPC + ZrO_2) exhibits a similar inflammatory reaction which is consistent with the results of other studies.[20] The ZrO2 associated with the Calcium silicate cements provides satisfactory physicochemical properties and better biological response than Bismuth oxide.[21] The zirconium oxide acted as an inert filler and did not participate in the hydration reaction of the Portland cement.[22] ZrO_2 can also be considered as an alternative radiopacifier to bismuth oxide.

CONCLUSION

The tissue reaction of the tested materials, white Portland cement (WPC) + bismuth oxide, WPC + iodoform, and WPC + zirconium dioxide were similar to MTA (Pro Root MTA) in all experimental periods of 7 days, 30 days and 60 days. But all these materials showed more inflammatory response than the control (empty tube) in both 30 and 60 days.

The result from our study supports the idea that Portland cement has the potential to be used in clinical situations similar to those in which MTA is being used.

CLINICAL SIGNIFICANCE

The role of radiopacifying agents in dental materials is well established and analyzing the biological properties of these agents will facilitate in the better clinical application of these materials.

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