A comparative tissue toxicity evaluation of four endodontic materials

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ABSTRACT

The aim of this study was to evaluate the biocompatibility of four commonly used materials in endodontics, namely : zinc-oxide eugenol and epoxy-resin based root-canal sealers, gutta-percha, the principal core material for obturation and calcium hydroxide, used for various endodontic purposes. Six healthy guinea-pigs were chosen for this study. Each material was subcutaneously injected or implanted at two sites in each animal. The tissue was removed after 7, 30 and 60 days and the inflammatory response was assessed. Gutta-percha was found to be the most bio-compatible material. The inflammatory response with calcium hydroxide subsided with time. Amongst the root-canal sealers, zinc oxide-eugenol was highly irritating and the epoxy-resin based sealer was well tolerated.

Key words: Tissue toxicity, gutta-percha, calcium hydroxide, zinc oxide-eugenol, epoxy-resin based sealer.

Introduction

Successful endodontic therapy depends on several factors. A conceptual triad of access cavity design, intracanal preparation and the final distinct step of obturating the canal, unifies the three aspects of sound endodontic therapy.

One of the purposes of obturation is to prevent the penetration of micro-organisms and toxins from the oral cavity via the root-canal into the peri-radicular tissues. According to Seltzer[1], from a histologic point of view, endodontic healing is the closure of the apical foramina adjacent to the root-canal filling material by means of a hard tissue barrier such as cementum with the simultaneous absence of inflammation. The obturating materials should create an environment, which will encourage healing in the damaged peri-apical tissues.

The endodontic obturating materials like sealers and core materials are in contact with the periapical tissues for a prolonged time, thus necessitating an inert and biocompatible nature. Numerous studies have been performed with various materials to evaluate their bio-compatibility.

Studies by Hensten-Petterson and Helgland[2] have shown that zinc-oxide eugenol when applied to cells in culture is decidedly cytotoxic. Catanzaro and Persinoto[3] demonstrated a large influx of macrophages into the lesion with subcutaneous implantation of zinc-oxide eugenol for both short and long periods.

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It has been shown in a tissue culture study that gutta-percha showed little or no cytotoxicity[1]. Wolfsson and Seltzer[4] in an animal study showed that gutta-percha is well tolerated by the tissues.

Studies by Yelsiloy and Feigal[5] indicated that AH26 was mildly cytotoxic to various cell lines. Catanzaro and Percinoto[3] demonstrated that AH26 displayed moderate cytotoxicity to macrophage system when implanted subcutaneously. Contrary to this, it has been also demonstrated that AH26 was devoid of any cytotoxic reaction[1].

It has been shown that calcium hydroxide has an immediate degenerative effect upon cells[1] while implanted calcium hydroxide had a lower toxic effect on rat connective tissues than zinc-oxide eugenol or AH26[3].

Gutta-percha is the most commonly used core material for obturation. Zinc oxide - eugenol and AH-26, an epoxy-resin based cement, as root-canal sealers and calcium hydroxide as a long term intra-canal medication are the other commonly used endodontic materials. Therefore, it was decided to evaluate and compare the tissue response to these materials and compare the results with previously reported studies.

Methods and Materials

Six healthy female guinea pigs weighing between 500-550 gms were chosen to be used in this study. They were obtained from the animal house, GMC Aurangabad. The following materials were tested for biocompatibility.

1. AH26: A resin based root-canal sealer {DENTSPLY}
2. Zinc-oxide eugenol sealer {zinc-oxide powder and pure eugenol for dental use}
3. RC-CAL: A proprietary calcium hydroxide preparation used for apexification {Dentfill Dental Products}
4. Gutta-percha obturating points {Mallifer}

The animals were anesthetized by an intraperitoneal injection of Ketamine hydrochloride (0.1mg/100gm) body weight. This allowed a sustained period of anesthesia during the procedure without respiratiory difficulties to the animals. The animal backs were then shaved after applying antiseptic solution.

AH26 was mixed according to the manufacturer's specifications. Zinc-oxide and eugenol was mixed to the required sealer consistency and RC-CAL was used directly. Sterile gutta- percha points were cut to 5 mm length for implantation.

Each animal was given two rapid subcutaneous injections of 0.1 ml of each test materials using a disposable syringe and an 18 gauge needle. Guttapercha points were placed subcutaneously making incisions and creating subcutaneous pockets. These incisions were later sutured. The eight sites of injection and implantation were separated from each other by 20mm to prevent the interference of one material and its response with the other.

The animals after implantation were maintained on a regular diet. Two animals each were euthanized on the 7th, 30th and the 60th day with an intracardiac injection of sodium pentobarbital. The tissue below each of the eight sites was then surgically removed. Included in each excision was the epithelium, the subcutaneous connective tissue and the underlying muscle tissue . A total of 48 blocks were removed and each placed in 10% formalin solution separately and labelled.

All blocks were processed with the use of standard histologic procedures, serial step sections 5-6 microns thick were prepared and stained with haematoxylin and eosin.

All slides were examined and rated by a pathologist who had no knowledge of the materials used, to eliminate any bias. The

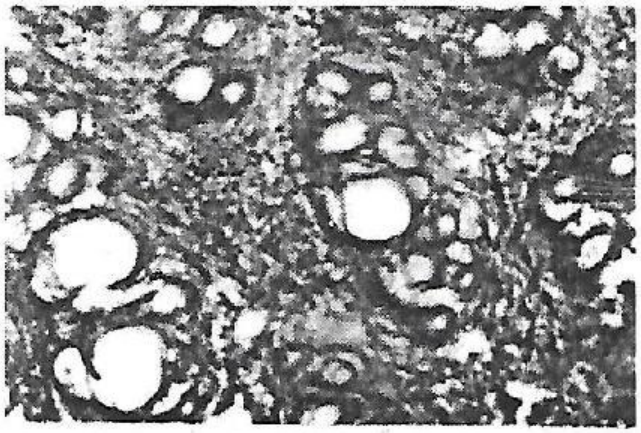

Fig 1. Low power (10x) Gutta-percha showed a near absence of inflammation at 7th day. Similar observations were made throughout the test period

slides were observed under low power {10x} and then under high power {45x}. The state of the surrounding tissue, the occurence and location of fibrous tissue and various types of inflammatory cells were examined. Tissue reactions were graded as mild, moderate or severe according to criteria suggested by Orstavik and Mjor[6].

The scoring criteria used were as follows:

0 **No Inflammation**

1 **Mild Inflammation**
Thickness of reaction zone similar or only slightly wider than normal tissue. Few inflammatory cells

2 **Moderate Inflammation**
Increased reaction zone. Presence of macrophages and / or plasma cells

3 **Severe Inflammation**
Increased reaction zone. Presence of macrophages and plasma cells. Occasional focii of neutrophils, granulocytes and or lymphocytes.

4 **Extreme Inflammation**
Focal areas of necrotic tissue densely infiltrated by inflammatory cells

Results

The frequency and grade of tissue reactions obtained in response to various test materials at various time periods are summarized in Table 1.

Gutta-percha : At 7 days, gutta-percha showed a near absence of inflammation in 75% of the sections while 25% of the sections showed mild inflammation. At 30 days and 60 days observation, all sections showed an absence of inflammation with normal connective tissue pattern of fine interwoven fibres.

Table 1. Tissue reaction to test materials at different time intervals.

Grade of Inflammation		Guttapercha			ZnO-E			RC-CAL			AH 26		
Time period (days)		7	30	60	7	30	60	7	30	60	7	30	60
0	None	3.00	4.00	4.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
1	Mild	1.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	4.00	3.00	4.00	3.00
2	Moderate	0.00	0.00	0.00	1.00	4.00	4.00	2.00	1.00	0.00	1.00	0.00	0.00
3	Severe	0.00	0.00	0.00	2.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00
4	Extreme	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Average Grade of inflammation		0.25	0.00	0.00	3.00	2.00	2.00	2.50	1.25	1.00	1.25	1.00	1.00

Zinc oxide -eugenol : At 7 day observation period, 50% of the sections showed severe inflammation, while 25% each showed moderate and extreme inflammation. The connective tissue showed a dense infiltration of PMN leucocytes and lymphocytes. One section showed the presence of tissue necrosis. At 30 days, all the samples showed a moderate grade of inflammatory response. The inflammatory cells were mainly lymphocytes, macrophages and plasma cells. Fibrous encapsulation was also noted. At 60 days observation, all the samples demonstrated a moderate grade of inflammation with an infiltration of chronic inflammatory cells.

Calcium hydroxide : At 7 day observation period, 50% of the samples each showed moderate and severe inflammation with the infiltrate consisting mainly of PMNs and lymphocytes. At 30 days period, the inflammatory reaction progressively reduced with 75% of the sections showing a mild reaction and the rest showing a moderate reaction. Macrophages, lymphocytes and occasional plasma cells were noted along with fibroblastic proliferation. The inflammatory reaction subsided to a mild level at 60 days observation. Another important finding was the deposition of calcium salts indicating a probable initiation of calcification in the tissue.

Epoxy-resin based sealer : At 7 day observation period, 75 % of the samples showed a mild inflammatory reaction and the rest demonstrated a moderate inflammatory reaction. The inflammation was in the form of a localised infiltration of PMNs. At 30 days observation, all the samples demonstrated only a mild inflammatory reaction. At 60 days, 75% of the samples showed a mild inflammatory reaction while the remaining specimen showed an absence of any inflammation.

The overall results are summarized in Table 2.

Table 2. Overall tissue response to test materials at different time intervals

	7 days	30 days	60 days
Guttapercha	Mild	Absent	Absent
ZnO-E	Moderate to Extreme	Moderate	Moderate
RC-CAL	Moderate to severe	Mild to moderate	Mild
AH 26	Mild to Moderate	Mild	Mild

Discussion

Long term intra-canal medicaments such as calcium hydroxide and the obturating materials like solid cores and sealers remain in prolonged contact with vital peri-apical tissues. This necessitates that these materials be either inert or bio-compatible, so as to positively effect healing of the peri-radicular tissues. Their bio-compatibility can be evaluated by primary tests such as cytotoxicity tests, usage tests or by secondary tests where subcutaneous and intraosseous implantation methods are used[7].

The subcutaneous implantation and injection technique used in the study possesses several of the qualities desired of a secondary test for the biological evaluation of endodontic materials. The injection technique instead of implantation was preferred, as it eliminated the need for an incision and the consequent surgical trauma. A uniform quantity of sealer was injected each time, to control the area of tissue reaction[8].

Of the materials evaluated in the study, gutta-percha, the most commonly used core material for obturation, appeared to be the least irritating at all the time periods of 7, 30 and 60 days. At the 7th day, of the four specimens, only one showed mild inflammation and the

rest of the specimens showed no inflammation. In the next group of autopsies taken at 30 and 60 days, there was an absence of inflammation. Earlier studies[1,4] have shown that gutta-percha exhibits little or no cytotoxicity and is an extremely inert material. This study also supports their finding.

Zinc-oxide-eugenol ellicited the maximum inflammatory response amongst the test materials. At the 7 day period, of the four specimens evaluated, one specimen showed moderate inflammation, two severe and one specimen showed extreme inflammation. The irritational ability of zinc oxide-eugenol can be primarily attributed to the eugenol content[9]. Zmener et al.[10] and Beagrie et al.[11] have reported that zinc-oxide and eugenol are cytotoxic to several animal and human cell lines and connective tissues. The next group of samples taken at 30 and 60 day time-period, exhibited moderate inflammatory response. This can be attributed to the exponential decrease in the release of eugenol with time due to the progressive hydrolysis of the cement surface as demonstrated by Becker et al.[12]. In order to reduce the cytotoxicity of eugenol, some investigators have suggested replacement of eugenol with glycerin or linoleic acid in the mixture with zinc-oxide[13].

Calcium hydroxide which is used for various endodontic purposes was also evaluated in the study. Of the four specimens taken at the 7 day period, two each showed moderate and severe inflammatory reaction. The initial severe inflammatory reaction can be a result of its high pH (>12). It has been demonstrated that calcium hydroxide has an immediate degenerative effect upon cells and that it disturbs the cell membrane causing degeneration and cell disintegration[1]. With the passage of time, there is a neutralisation of pH and the inflammatory reaction subsides. Three of the four samples taken at 30 day period showed a mild inflammatory response, while one showed a moderate inflammatory response. The reaction subsided still further at the 60 day period showing a mild inflammatory reaction. However, an interesting finding was the presence of nodular calcification in the tissues at 60 days, reconfirming the ability of calcium hydroxide to induce heterotropic ossification, as reported in previous studies[1].

Epoxy-resin based root-canal sealer, which was also evaluated in this study showed good bio-compatibility. At the 7 day period, three of the four specimens showed a mild inflammatory reaction while one specimen showed a moderate inflammatory reaction. The mild to moderate inflammatory reaction can be attributed to the formaldehyde released from the decomposition of hexamethylene tetra-

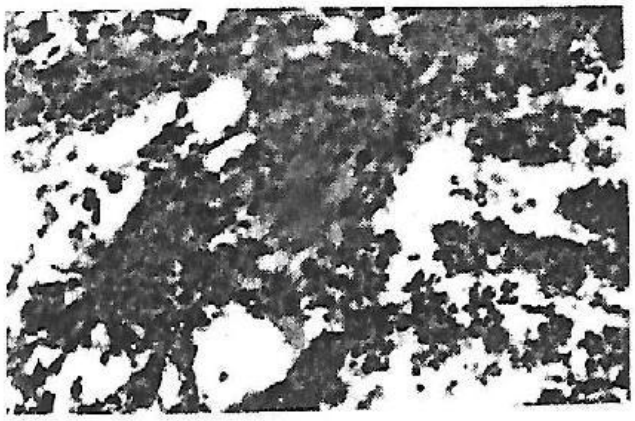

Fig. 2. High power (45x) : Zinc oxide-eugenol at 7 days showing severe inflammatory reaction with dense neutrophilic infiltration and focal haemorrhage. Lymphocytes are also seen in large numbers.

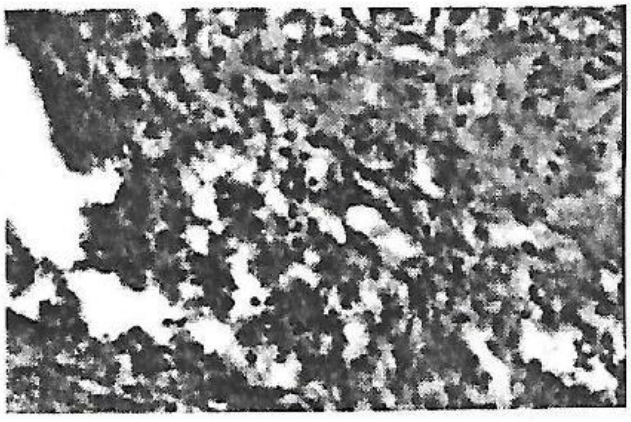

Fig. 3. High power (45x) : Zinc oxide-eugenol at 60 days showing moderate inflammation with chronic inflammatory cell infiltration. Plasma cells and lymphocytes noted.

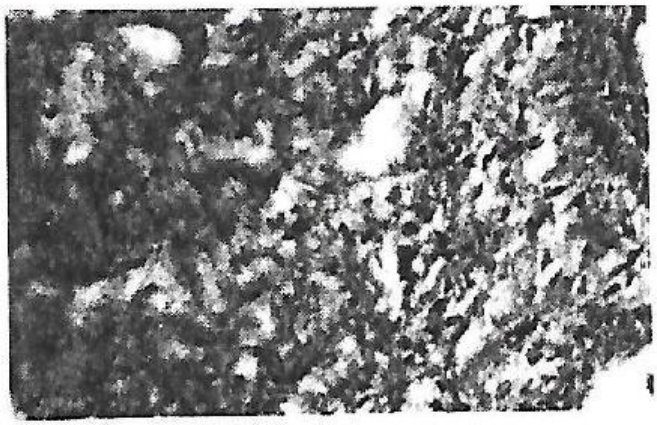

Fig. 4. High power (45x) : Calcium hydroxide at 7 days showing moderate inflammation with lymphocytic and neutrophilic infiltration.

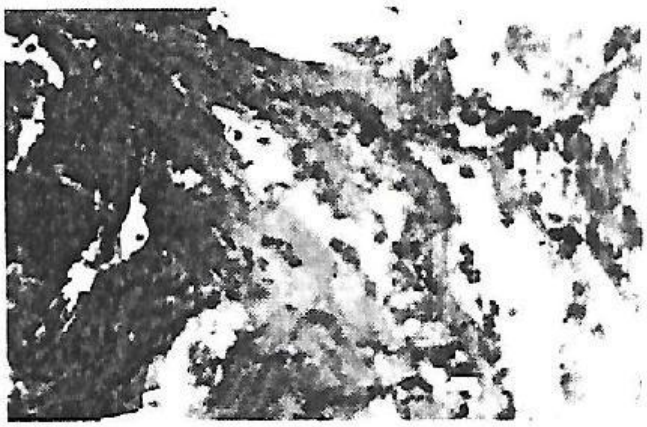

Fig. 5. Low power (10x) : Epoxy-resin based sealer at 60 days. Nil to mild inflammation throughout the test period was noted.

amine component of the sealer[13]. Epoxy-resin based sealer has also been demonstrated to inhibit leucocyte migration[14]. In all the samples taken at the 30 and the 60 day period, only a mild grade of inflammation was noted. This is in confirmation with previous investigations[5].

The above study showed that gutta-percha, the most commonly used core material for obturation, was extremely biocompatible, while amongst the root-canal sealers, epoxy-resin based sealer was more bio-compatible while zinc-oxide eugenol was highly irritating.

Conclusions

1 The subcutaneous implantation and injection technique proved to be suitable for assessing the biocompatibility of endodontic materials

2 Guttapercha proved to the most biocompatible of all the test materials.

3 RC-CAL showed initial severe reaction but later exhibited good biocompatibility with the passage of time.

4 Zin coxide-eugenol showed a severe reaction initially and continued to irritate the tissues for a longer time.

5 AH26 proved to be a very biocompatible material and only showed a mild reaction throughout the study period.

References

1. Seltzer S : Endodontology Biologic considerations in endodontic procedures, 2nd edition, Philadelphia, Lea & Febiger Co .281-325.

2. Henstan-Petterson A, Helgeland K. Evaluation of the biologic effects of dental materials using different cell culture techniques. Scand J. Dent Res.1977; 5:291.

3. Wolfson EM, Seltzer S. Reaction of rat connective tissue to some gutta-percha formulations, J Endodont. 1975 ;1:395.

4. Yelsiloy C, Feigal RJ : Effects of endodontic materials on cell viability across standard pore size filters, J Endodont.1975;1:395.

5. Catanzaro GSA, Percinoto C. Effect of endodontic materials on the influx of macrophages and multinucleated giant cell development in experimental granulomas. J Endodont.1983;9:530.

6. Orstavik D, Mjor IA. Histopathology and X-ray microanalysis of the sucutaneous tissue response to endodontic sealers. J Endodont. 1988 ;14:33-44 .

7. Olsson B, Sliwkowski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endodont 1981;7 : 355-369.

8. Yelsiloy C, Koren LZ, Morse DR , Kobayashi VC. A comparative tissue toxicity evaluation of established and new root canal sealers. Oral Surg Oral Med Oral Pathol. 1988;65 :459-469.

9. Gulati N, Chandra S, Aggarwal PK, Jaiswal JN, Singh M. Cytotoxicity of eugenol in sealer containing zinc oxide. Endod Dent Traumatol. 1991;7 :181-185.

10. Zmener O, Cabrini RL. Adhesion of human blood monocytes and lymphocytes to different endodontic cements, a methodological in-vitro study. J Endodont.1976;12:150.

11. Beagrie GS, Main JHP, Smith DC. Inflammatory reaction evoked by zinc- polycarboxylate and zinc-eugenolate cements: A comparison. Br Dent J., 1972;132: 351

12. Becker RM, Huma WR, Wolinsky. Release of eugenol from mixture of zinc oxide and eugenol in-vitro. J Periodontol. 1983;8: 71.

13. Economides, Kotsaki-Kovatsi VP, Poulopoulos A, Kolokuris, Rozos G, Shore R. Experimental study of the biocompatibility of four root-canal sealers and their influence on the zinc and calcium content of several tissues. J Endodont. 1995; 21:122-127.

14. Doblecki W, Turner DW, Osetek EM, Pelleu GB. Leucocyte migration response to dental materials using Boyden chambers. J Endodont. 1980;6:636.