

ORIGINAL RESEARCH

Comparative Histopathological Evaluation of the Biocompatibility of AH Plus, Epiphany, RoekoSeal: An *In Vivo* Study in Subcutaneous Tissue of Rats

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ABSTRACT

Aims and Objectives: The present study is aimed to evaluate the biocompatibility of two new resin-based root canal sealer materials, i.e., Epiphany sealer and RoekoSeal and to compare their biocompatibility with a commonly used resin based AH plus sealer. After implantation in a subcutaneous tissue of rats and to observe local tissue reaction, at different time periods of 10 days and 90 days.

Materials and Methods: Twenty Wistar rats were divided into 2 groups of 10 each for observation after completion of 10 and 90 days following implantation, respectively. Polyethylene tubes loaded with experimental sealer and empty tube (control) were implanted subcutaneously. The subcutaneous tissues from sacrificed rats were analyzed histologically for inflammatory response and were graded with score 0–4. Results were analyzed statistically with Kruskal–Wallis tests.

Results: In this study, there are significant differences in the severity of local tissue response to AH plus, Epiphany, and RoekoSeal sealers 10 days group. Epiphany caused severe local tissue response, AH plus and RoekoSeal showed moderate to severe tissue response. After 90 days, the order of toxicity from highest toxic to lowest toxic for this group is Epiphany Sealer > AH Plus > RoekoSeal. The differences between these materials are statistically significant.

Conclusion: The biocompatibility of a material depends on its composition, location, and interaction with local tissues. The biologic response of the material depends on whether those components are toxic, immunogenic, or mutagenic. The dental personal should be aware of the biologic response of the material, its advantages and disadvantages as well as precautions to be taken while using the material for the safety and health of the patient. From the current study, it is concluded that RoekoSeal, is relatively a biocompatible sealer as compared to AH Plus and epiphany sealers.

Keywords: Histopathological evaluation, Biocompatibility, AH Plus, Epiphany, RoekoSeal, Subcutaneous tissue.

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INTRODUCTION

Endodontic treatment comprises three main procedures: Cleaning and shaping, disinfection, and three-dimensional obturation of the root canal space.^[1] Three-dimensional obturation of the root canal space is an important step in the success of any root canal therapy. The use of a sealer during root canal obturation enhances the possible attainment of a fluid impervious seal and serves as filler for canal irregularities and minor discrepancies between the root canal wall the core filling material.^[2] A number of sealers have been used to obtain fluid impervious seal and a stable filling of the root canal system, generally in association with gutta percha cones, which forms the solid part of the filling, where as the sealers help to fill in all the remaining empty spaces thus providing a three dimensional filling. Whereas sealers help to fill in all the remaining empty spaces between the cones, thus providing a three-dimensional filling.^[2] The sealers vary widely in composition, and since they may contact vital tissues at the tooth apex, their biological properties are considered to be very paramount. In the past, the antibacterial properties of these materials were given much accentuation, and as a result, many of them contain toxic antibacterial substances, antibodies or steroids,^[3] but recently there has been a trend toward the utilization of more biocompatible materials.^[4]

The design and development of a new or novel endodontic material involve extensive materials property testing as well as the evaluation of its biocompatibility. The mechanical properties to be tested include the strength, porosity, setting reactions, working time, and its sealing ability.^[5] For the evaluation of the biocompatibility, any newly invented material to be used in the human body has to undergo several levels of tests subjected according to the internationally accepted

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standards.^[6]

In general, the biocompatibility of a root canal sealer is assessed by a three-step approach. The first step is a series of *in vitro* cytotoxicity assays (primary tests). If the test is favorable material is subjected to animal studies in rats, rabbits, etc. (secondary tests). Finally, the *in vivo* reaction of the target tissues with the material must be evaluated in higher animals or human beings (usage tests).^[7] Implantation studies are a type of assays unique to medical devices. They have been specifically devised for those simulations where an exogenous (usually man-made) construct or material is enclosed in the body or partially entered into it by a breached surface. They are intended to assess the effects of devices/materials (usually polymers or elastomers) which are in direct contact with living tissue. The effects of concern are most commonly for a relatively short-term exposure and are limited to various indicators of local tissue tolerance.^[8] The rummage around for a biocompatible root canal sealer is incessant. Here, we selected three AH Plus Epiphany™ Soft Resin Endodontic Obturation System and RoekoSeal sealer. The rationale of this study is to assess the biological tissue response of the sealers and to draw attention to the role of animal studies in dentistry by evaluate the biocompatibility of sealers using subcutaneous implantation method in rats.

Aims and Objectives

The present study is aimed to evaluate the biocompatibility of two new resin-based root canal sealer materials, i.e., Epiphany sealer and RoekSeal and to compare their biocompatibility with a commonly used resin based AH plus sealer.

The objectives are:

1. To evaluate the biocompatibility of three different root canal sealers: AH Plus sealer (Dentsply), Epiphany Sealer (Pentron, USA), and RoekoSeal (Coltene/Whaledent) after implantation in a subcutaneous tissue of rats and to observe local tissue reaction, at different time periods of 10 days and 90 days.
2. To compare biocompatibility among above-mentioned materials at different time periods after implantation.
3. To observe the histopathological effect of host tissue response, i.e. inflammatory and other tissue-protective mechanisms on the test materials in the *in vivo* environment.

MATERIALS AND METHODS

The materials used for the study were:

- I. AH Plus sealer (Dentsply)
- II. Epiphany Sealer (Pentron, USA)
- III. RoekoSeal sealer (Coltene/Whaledent).

Methodology

The experiment was carried out under the following divisions of the Biomedical Technology Wing, Sree Chitra Institute for Medical Sciences and Technology, Poojappura, Trivandrum.

1. Division of toxicology
2. Histopathology laboratory under implant biology.

The experiment was divided into two parts:

 - I. Animal implantation and autopsy
 - II. Preparation of the slides and histopathological evaluation.

Animal Implantation

Twenty adults Wister rats of either sex, weighting not <20 g were randomly assigned for the study. The animals were included in the study after compulsory clinical examination and only clinically healthy were selected for the study. Care and management of the experimental animals were done according to the committee for the purpose of control and supervision of experiments on animals, and Institutional Animal's Ethics Committee approved guidelines.

The animals for the study were divided into two groups:

- Group A: 10 days period - 10 animals
- Group B: 90 days period - 10 animals

Ethylene trioxide sterilized Teflon tube open at both ends and of dimension 10 mm long with an internal diameter of 2 mm were used as the carrier for the test materials. The test materials were mixed according to manufactures instructions and loaded directly into the Teflon tubes. Empty Teflon tubes were used as negative control.

Dorsal surface of each rat received four different implants consisting of three different test materials and one control. The entire operations were carried out in a sterile environment with all possible precautions of isolation and asepsis of the operating field.

Procedure

Before the test, the fur on either side of the vertebral column was clipped and shaved. Rats were anesthetized using intramuscular injection of ketamine (100 mg/kg) + xylazine (5 mg/kg). Skin of the anesthetized rats was lightly swabbed using 5% iodine in alcohol and air dried. Under aseptic conditions, four separate incisions of approximately 15 mm long were made on the dorsal surface, through the skin into subcutaneous tissue using a scalpel.

Implantation was done after making four separate subcutaneous tunnel pocket on the dorsal surface, on either side of the spinal column by blunt dissection, such that the base of the pocket was 10mm away laterally from the line of incision. The test and control samples were then pushed into the tunnel. The four sites of implantation were separated from each other by at least 25 mm to prevent the interference of one material and its response with the other.

Three test materials were implanted subcutaneously on left upper, lower, and right upper dorsal surface of the animal. Similarly, one control material (empty Teflon tube) was implanted on the right lower dorsal surface of the animal. The incisions were then closed using sterile sutures. This procedure was repeated on 20 rats.

After the procedure, the animals were kept under proper environment and fed with commercial rat feed and water *ad libitum*. Special care was given to the animals during the period following the procedure.

Animal Autopsy

At the end of each observation period, the respective animals were euthanized by an overdose of anesthetic agent (thiopentone). The animals belonging to Group A were euthanized after 10 days, and the animals belonging to Group B were euthanized after 90 days. The test and control implant materials along with the surrounding tissues were surgically removed. The sites of implantations were macroscopically examined for hemorrhage, necrosis, discolorations, and infection. The collected test and control implanted material with surrounding tissues were then fixed in 10% buffered formalin.

Preparation of the Slides

The pieces of skin along with underlying implant were removed and fixed in 10% buffered formalin. All specimens were grossed, and representative cross sections were taken to include all layers of skin and implant site.

Tissues were processed for histological evaluation with automatic tissue processor (LEICA TP1020). The specimens were treated with ascending grades of alcohol for complete dehydration. Chloroform was used as the clearing agent, and the procedure was repeated 3 times to ensure the complete removal of alcohol and then infiltrated with paraffin wax. Then, the tissues were embedded in paraffin wax using an Automatic tissue embedder (LEICA EG 1160).

After paraffin was embedding, the tissues were then sectioned into 5 μ thickness using an automatic rotary microtome (LEICA Germany) and stained by hematoxylin and eosin staining using an automatic stainer

(LEICA, Autostainer XL Germany).

The sections were then examined under transmitted light using a Trinocular microscope (Nikon E600), and the histological features were recorded. Images were captured using a digital camera.

RESULTS AND OBSERVATIONS

Gross Observations of the Specimen

In all samples of skin, an implant was present on the subcutaneous aspect. The implant was a polymer tube 1 cm long and has an internal diameter of 2 mm at both ends. The tube was covered with a well-formed capsule of soft tissue in most cases. The tubes contained a granular material. Cross sections including the epidermis, dermis, and implant with the adjacent tissue were processed for histological examination.

Histopathological Observations

Score	Type of inflammation	Tissue changes
0	No inflammation	Fibrous - capsule formation and absence of inflammatory cells
1	Mild inflammation	Fibrous - capsule formation, few scattered inflammatory cells, predominantly chronic inflammatory cells
2	Moderate inflammation	Fibrous - capsule formation, focal accumulations of inflammatory cells, no tissue necrosis, and disruption of the structural characteristics of the tissues
3	Severe inflammation	Large accumulations of inflammatory cells, foreign-body giant cells, and congested capillaries
4	Abscess formation	

During the 10 days period, the test materials were demonstrable in the histological pictures as numerous foci of black foreign material debris, surrounded by numerous inflammatory cells, the severity of which varied according to material. At the 90 days, these foci of black foreign material were less in number, surrounded by chronic inflammatory cells and macrophages with the ingested material, varying according to material [Figures 1-4].

At first time period, i.e., 10 days of exposure, the mean score of tissue reactions for AH plus, epiphany, and Roekseal sealers along with their median score, standard errors, Kruskal-Wallis H value and *P* value is given in Table 1.

The severity of tissue reactions for all the three sealers differs significantly from each other at 10 days of exposure ($P < 0.001$). Severity of tissue reaction to epiphany sealer is significantly more than AH Plus which is more

Table 1: Scores of severity of tissue reaction for different materials at 10 days of exposure

Material	Mean score	Median score	±SD	Kruskal–Wallis H value	P value
AH Plus	2.67	3.0	0.17	14.769	<0.001
Epiphany	3.00	3.0	0.30		
RoekSeal	2.10	2.0	0.23		
Control	1.30	1.0	0.15		

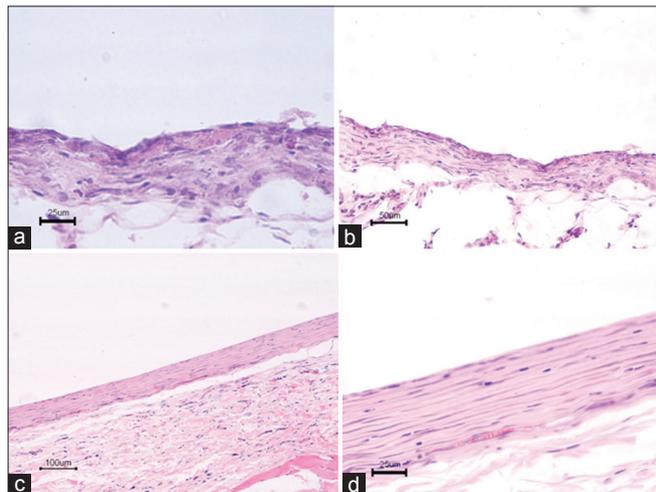


Figure 1: (a) Control group after 10 days. (b) Control group after 10 days magnified view. (c) Control group after 90 days. (d) Control group after 90 days

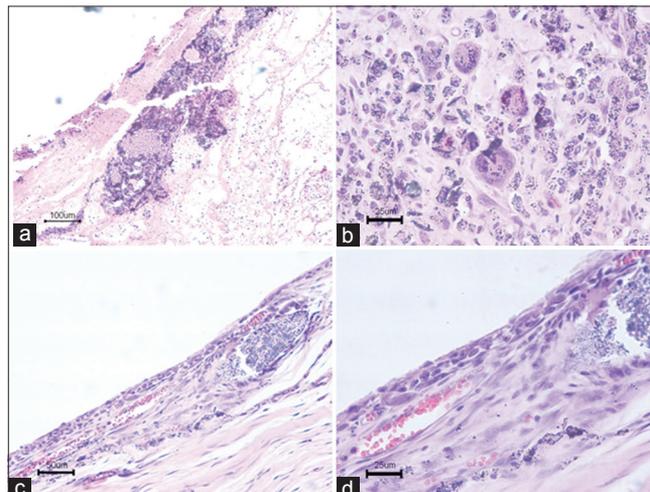


Figure 3: (a) Epiphany group after 10 days. (b) Epiphany group after 10 days magnified view. (c) Epiphany group after 90 days. (d) Epiphany group after 90 days

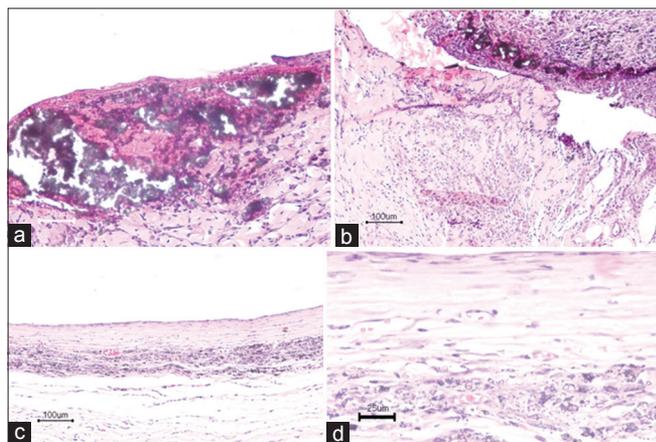


Figure 2: (a) AH plus group after 10 days. (b) AH plus group after 10 days magnified view. (c) AH plus group after 10 days magnified view. (d) AH plus group after 90 days magnified view

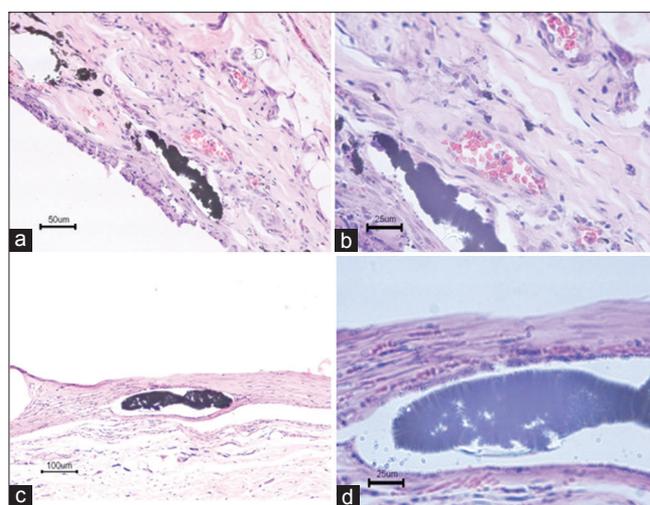


Figure 4: (a) Epiphany group after 90 days. (b) RoekoSeal group after 10 days magnified view. (c) RoekoSeal group after 90 days. (d) RoekoSeal group after 90 days

severe than Roekseal of all the three materials tested, the slides with epiphany caused severe local tissue reaction with a mean score of 3, AH plus, and Roekseal showed moderate-to-severe tissue reaction with a mean score of 2.67 and 2.10, respectively.

At second time period, i.e., 90 days of exposure, the mean score of tissue reactions for AH Plus, epiphany, and Roekseal sealers along with their median score, standard errors, Kruskal–Wallis H value and P value is given in Table 2.

The severity of tissue reactions for all the three sealers differs significantly from each other at 90 days of exposure ($P < 0.001$). Severity of tissue reaction to epiphany sealer is

significantly more than AH Plus which is more severe than Roekseal of all the three materials tested, the slides with Epiphany showed moderate-to-severe local tissue reaction with a mean score of 2.20, AH Plus showed mild-to-moderate tissue reaction with a mean score of 1.75 and Roekseal showed mild tissue reaction with a mean score of 1.00.

DISCUSSION

Dental materials share with other fields of biotechnology the problems of biocompatibility that is the

Table 2: Scores of severity of tissue reaction for different materials at 90 days of exposure

Material	Mean score	Median score	±SD	Kruskal–Wallis H value	P value
AH Plus	1.75	2.0	0.37	24.925	<0.001
Epiphany	2.20	2.0	0.29		
RoekSeal	1.00	1.0	0.29		
Control	0.20	0.0	0.13		

interaction of manufactured compounds with body tissue and fluids.

Biocompatibility is important as sealer invariably comes in contact with the periradicular tissues and affects them. When this happens, there may be destruction and inflammation of periradicular tissues, which manifest as pain, tenderness, and sometimes even swelling of the affected area.^[35] Formaldehyde-containing sealers (e.g. N2), the toxicity is so severe when it extrudes into periapical areas it presents as severe inflammation and sometimes permanent damage of nerve and leads paresthesia if its near nerve bundles.^[3] Hence, a root canal sealer and its elutable substances should be critically evaluated for biocompatibility.

The AH plus sealer showed moderate-to-severe local tissue reaction (mean score of 2.67) at the 10 days' time period. Out of the nine slides analyzed, six had severe inflammatory reactions which obtained a score of 3. Three of the slides showed moderate form of inflammatory reactions which obtained scores of 2. At the 90 days' time period, AH plus showed mild-to-moderate tissue reaction with a mean score of 1.75. Toxicity may be related to the epoxy component of AH plus and the various amines in the material.^[22,23] Our results are in agreement with many other authors, who have found AH plus to be cytotoxic^[24,25,26,22] and genotoxic.^[27,28]

The epiphany sealer showed severe local tissue reaction (mean score of 3.00) at the 10 days' time period. Out of the 10 slides analyzed, all of them showed moderate-to-severe tissue reaction with a mean score of 2.20. Out of the nine slides analyzed, five slides showed moderate form of reaction and obtained a score of 2. Three of the slides showed severe inflammatory reactions and obtained a score of 3. The epiphany sealer is a sealer which has resin matrix composed of UDMA, PEGDMA, ethoxylated bisphenol dimethacrylate, and bis GMA resins. The study done by Nielsen *et al.*^[29] showed that the sealer set under Phosphate Buffered Saline, an uncured layer remains on the surface., so there is a very strong probability that it may leach out toxic monomers into tissues. This may result in the severe toxicity for 10 days group that decreases to some degree at 90 days group.

The Roekoseal sealer showed moderate-to-severe local tissue reaction (mean score of 2.10) at the 10 days' time period. Out of the 10 slides analyzed, three had severe inflammatory reactions which obtained a score of 3. Five slides showed a moderate form of inflammatory

reactions; two showed mild local tissue response. They obtained scores of 2 and 1, respectively. At the 90 days' time period, Roekoseal showed mild tissue reaction with a mean score of 1.00. Out of the nine slides analyzed, three of slides showed no inflammation, three slides showed mild form of reaction, and three of the slides showed moderate inflammatory reactions. They obtained scores of 1 and 2, respectively.

Polydimethylsiloxane (PDMS), main component of Roekoseal, is widely used silicon-based organic polymer and is generally considered to be inert, non-toxic and non-flammable. This may be the factor responsible for the better acceptability of Roekoseal by tissues.

PDMS has also been used as a filler fluid in breast implants, in knuckle replacement implants. Activated dimethicone, a mixture of PDMS and silicon dioxide is used in over-the-counter preparations as an anti-foaming agent and carminative. A variant of it is also used as a food additive. These results are in agreement with many other authors who found Roekoseal to be significantly less cytotoxic^[23,30-34] and caused less local tissue reaction^[24] than other commonly used resin-based sealers.

There is a significant difference in the local tissue response of the three sealers tested by this method. The toxicity of the sealer varied from highest to lowest in the order.

Epiphany >AH Plus> Roekoseal.

The differences were statistically significant at different time periods also.

CONCLUSION

The biocompatibility of a material depends on its composition, location, and interaction with local tissues. The biologic response of the dental material depends on whether those components are toxic, immunogenic, or mutagenic at the released concentration. The endodontic therapy does not aim just at the rehabilitation of the particular tooth alone but is, of course, concerned with the overall human body. The dental personal should be aware of the biologic response of the material he/she uses in the patients, its advantages and disadvantages as well as precautions to be taken while using the material for the safety and health of the patient. From the current study, it is concluded that Roekoseal, is relatively a biocompatible sealer as compared to AH Plus and epiphany sealers.

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