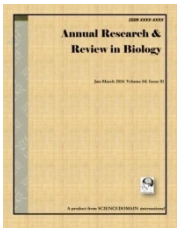

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Effect of Electron Beam Irradiation on Resin– Based Root Canal Sealer –AH Plus: A Cytotoxic Evaluation

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Authors' contributions

Authors may use the following wordings for this section: Author MNH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AP and SSS managed the analyses of the study. Authors NDH and SK managed the literature searches, author GS conducted the irradiation of the samples. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate and compare the cytotoxicity of a resin-based root canal sealer, AH Plus, before and after electron beam irradiation and to assess the effect of three different doses of electron beam radiation (1 KGy, 3 KGy and 5 KGy) on cytotoxicity of the sealer.
Place and Duration of Study: Department of Conservative Dentistry and Endodontics, A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore, India and Microtron Centre; Department of Physics Mangalore University; Mangalore, India between July 2012 and November 2012.
Methodology: Gingival fibroblasts were cultured in Dulbecco's Modified Eagle's Medium

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(DMEM) and sub confluent monolayers of cells were obtained. The study included two categories- Irradiated and Non-irradiated. Both of them were further divided into four groups of paste A, paste B, Mixed and Set sealer of AH plus. Samples in irradiated category were further divided into 3 subgroups based on electron beam irradiation dose-1, 3 and 5 KGy. Sample elutes were prepared using standard extraction procedure. The elutes of all sealers from both the categories were added to the human gingival fibroblast cultures and their cytotoxicity was assessed by MTT assay. The results were tabulated and subjected to statistical analysis using One–way ANOVA (Analysis of variance) test and Tukey's HSD Test.

Results: Cell viability was minimum in Paste M irradiated with 5 KGy irradiation dose and maximum in non-irradiated SET AH Plus sealer. The results of the present study showed positive effect of irradiation in the epoxide paste of AH Plus sealer. The positive effect here is mainly based on experimental data of percentage cell viability and not on statistical significance. Multiple comparisons of different doses of electron beam irradiation on percentage of cell viability in all the samples showed a highly statistically significant difference at 3 KGy of electron beam radiation (*P* value = 0.008).

Conclusion: Further research should to be performed with higher doses of electron beam radiation on epoxide paste in order to notice further improvement in physical as well as biological properties in order to generate an upgraded version of the present dental materials.

Keywords: Electron beam irradiation; resin based sealers; root canal sealers; cytotoxicity; gingival fibroblasts.

1. INTRODUCTION

The primary objective of pulp space therapy is to remove diseased pulpal tissue and to adequately seal the pulp space with a stable biocompatible material [1]. The use of a root canal sealer in conjunction with a core material is considered imperative to produce the highest quality obturations [2]. These sealers are intended to be contained within the root canal [3-6] but they sometimes inadvertently extrude through the apical constriction during placement [7,9]. Even without extrusion, eluents derived from these materials may come into contact with periradicular tissues which causes potential irritation resulting in delayed wound healing [10-15]. Thus, the biocompatibility of root canal sealers is critical to the clinical success of endodontic therapy [16-18]. Although several classes of root canal sealers are currently used in clinical practice including epoxy resin, calcium hydroxide, zinc-oxide eugenol and silicone; all of these have substantial limitations. The popularity of resin-based sealers like AH26 (DENTSPLY, DEtrey, Konstanz, Germany) and AH Plus (DENTSPLY, DEtrey, Konstanz, Germany) is increasing, despite their well-documented toxicity and mutagenicity.[19] Epoxy resin-based sealers have certain favourable characteristics, such as adhesion to tooth structure, long working time, ease of mixing and good sealing ability [20]. AH-Plus root canal sealer is a two paste system and is known to exhibit very low shrinkage during setting and long-term dimensional stability [21]. It has been used in research because of its well-studied and good physicochemical properties.

One method of choice for toxicity screening is cell culture assay [22]. Gingival fibroblasts and periodontal membrane fibroblasts have a common connective tissue origin. The predictability and behavioural similarity of these two types of cells in tissue culture have already been demonstrated [23,24].

Radiation is widely used in the biomaterials science for surface modification, sterilization and to improve bulk properties. Electron beam irradiation is described as a method to change the mechanical properties of polymers [25,26].

Studies have been done on various dental materials using electron beam irradiation to evaluate the changes in their physical and mechanical properties, but studies to assess their biological properties are very sparse [26,27]. Hence, the aim of the present study is to investigate the effects of electron beam radiation on the cytotoxicity of a root canal sealer using three different doses of 1 kGy, 3 kGy and 5 kGy.

2. MATERIALS AND METHODS

The study included two categories- Irradiated (dose- 1, 3 and 5 kGy) and non-irradiated samples. The sealers were mixed according to the manufacturer's instructions and dispensed into micro vials under aseptic conditions. The two categories were divided into four groups- paste A , paste B, freshly mixed sealer and set sealer discs. All the materials were dispensed into their respective micro vials. The samples to be irradiated were further divided into three subgroups based on the irradiation dose used i.e., 1, 3 and 5 kGy respectively. Institutional ethical clearance was obtained (NU/CEC/clear.2/2012) and patient was explained the procedure of gingival tissue biopsy and the informed consent was duly signed by the patient.

2.1 Growth and Maintenance of Cell Culture

Human gingival fibroblasts were isolated from healthy gingival tissue biopsies (explants) of six patients taken during routine orthodontic extractions. The tissue then was transferred to phosphate buffer saline containing antibiotic solution. The explants were cultivated in 25 cm^3 tissue flasks containing Dulbecco's modified Eagle's Medium (DMEM) (HiMedia Labs,Mumbai, India), supplemented with 5% foetal bovine serum (FBS, (HiMedia Labs,Mumbai, India), 100 U mL^{-1} penicillin, 100 µL mL^{-1} streptomycin and 2 mmol L^{-1} L-glutamine, at 37ºC in a humidified atmosphere of 95% air and 5% CO_2 (Nuaire, CO_2 Incubator, Fernbrook Lane, Plymouth USA). Sub cultivation was done with 0.25% Trypsin (HiMedia Labs,Mumbai, India). After trypsinization, cells were counted using tryphan blue dye and then seeded at a density of 3.6×10^4 cells per well in the 96 - well Tissue culture plates ((HiMedia Labs,Mumbai, India) and incubated to get sub confluent monolayers of cells.

2.2 Chemical Composition of AH Plus

The root canal sealer used for the study was an epoxy–amine resin based sealer AH Plus. The chemical composition of AH plus is given in Table 1.

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Table 1. Chemical Composition of AH Plus

Epoxide Paste A	Amine Paste B
Epoxy resins	Adamantine amine
Calcium tungstate	N,N'-dibenzyl-5-oxa-nonandiamine-1,9
Zirconium oxide	Calcium tungstate
Silica	Zirconium oxide
Iron oxide pigments	Silica
	Aerosil
	Silicone oil

2.3 Sample Preparation

The sealer was mixed aseptically according to manufacturer's instructions and dispensed in microvials (1.5 ml). The material was grouped into 4 categories- pastes A, paste B, Paste M (freshly mixed sealer) and SET (set sealer discs- 3mmX 3 mm). The discs were fabricated in sterile Teflon moulds.The test specimens were allowed to set in a humid chamber at 37ºC for 24 hours.

2.4 Standardization of Dose

The dose of electron beam irradiation to be used was standardized using an 8 MeV Microtron at Microtron Centre, Mangalore University, and Mangalore, India. Doses chosen for the study were 1kGy, 3kGy and 5kGy.

2.5 Groups

The four categories were further divided into two groups-irradiated (n=72) and non-irradiated groups (n=12). The irradiated groups were further divided into three subgroups based on the radiation dose -1kGy, 3kGy and 5kGy (n=24 in each).

2.6 Sealer Extraction

All the materials were now placed in cell culture medium (DMEM) using the ratio 1.25 cm^2 /ml between the surface of the sealer samples and the volume of medium [24]. The sealer extraction was done for a duration of 24 hours. The test solutions were sterile filtered using a Sterile Filter Unit (0.2 µm pore size) (Sartorius Stedim Biotech, Goettingen, Germany) before being exposed to the culture following which the test samples were subjected to the cytotoxicity assay. DMEM culture medium was used as control.

2.7 MTT Assay

Optical density determined by dissolving MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) -formazan intercellular reaction product with dimethyl sulfoxide (DMSO).

MTT solution was prepared as 5 mg/ml in PBS just before use. Cells were diluted in fresh medium and seeded into 96 well plates (2.7×10^4 cells per well). The test solutions were added to 6 wells per group and one row of wells (i.e. 3 wells) was filled with only culture medium as control. After incubation in 5% CO_2, 95% air mixture at 37^0C in an incubator for

24 hours, 20 µL MTT dye (MTT, HiMedia, Mumbai, India) was added. Plates were incubated at 37ºC for 4 hrs. The medium was aspirated from all wells and replaced with 200 µl of DMSO to each well.

Spectrophotometric absorbance was measured at 630 nm using an ELISA micro plate reader (Lisa Plus, Aspen Diagnostics, India).

Percentage of cell viability was calculated from the formula:

$$\% \text{ of Cell viability} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The cytotoxicity was rated based on cell viability relative to control as (Dahl et al, 2006)[28] (Table 2).

Table 2. Cytotoxicity based on cell viability

Interpretation	% of cell viability
Non cytotoxic	>90% cell viability
Slightly cytotoxic	60 – 90% cell viability
Moderately cytotoxic	30 – 59% cell viability
Strongly cytotoxic	<30% cell viability

The percentage of cell viability for each sealer was recorded and the results were tabulated and subjected to statistical analysis.

3. RESULTS AND DISCUSSION

Biocompatibility of materials used in the course of the treatment is considered a prime prerequisite for undisturbed healing.

In this study, the effect of different doses of electron beam irradiation on cytotoxicity of a widely used root canal sealer i.e. AH Plus was evaluated and compared with the non-irradiated materials. Clinically, root canal sealers are inserted into the root canal in a freshly mixed and/or incompletely polymerized stage, but even after setting period, possibility of potentially toxic constituents to be released from the materials by leaching into tissue fluids. Sealer extracts were used rather than direct contact because sealers may not always come in contact with the peri-radicular tissues directly [29].

In the present study, human gingival fibroblasts were cultured. Primary cells, mainly gingival fibroblasts are more sensitive than established cell lines and possess a high degree of differentiation [5]. Evaluation of cytotoxicity of root canal filling materials by using human oral origin fibroblasts is a recognized method because of ease of their isolation, successful growth in culture & method of standardization [30].

Electron beam irradiation (a high-energy dose irradiation) is a modern method to improve the properties of polymers and composites. It is reported to increase the stiffness of polymers as well as the links between polymer chains [31].

Two types of irradiation-initiated reaction can be defined as chain linkage and chain breakage.

During a chemical reaction, radicals, which bring about chain linkage, are initiated from several distinct points. It has been demonstrated that irradiation initiates the radical build-up of all components of a polymer [27]. The entire polymer may simultaneously be newly arranged and cross-linked when irradiated.

In contrast, chain breakage can also occur. This phenomenon happens when using a high-energy dose and specific resins. During chain breakage, the C–C bonds split off and the polymeric structure is broken down [32].

In the present study cytotoxicity of a resin-based root canal sealer, AH Plus is compared before and after electron beam irradiation and the effect of three different doses of electron beam radiation is assessed (1 KGy, 3 KGy and 5 KGy) on the sealer.

From our study we found that Non-irradiated Paste A was reported to have a higher cell viability than the irradiated at 1 kGy electron beam irradiation. It can also be interpreted that higher cell viability was present at a dose of 3kGy and 5kGy as compared to non-irradiated sealer and it was maximum at 5kGy dose of electron beam irradiation and minimum and minimum at 1 KGy; however the difference was not statistically significant (Fig. 1).

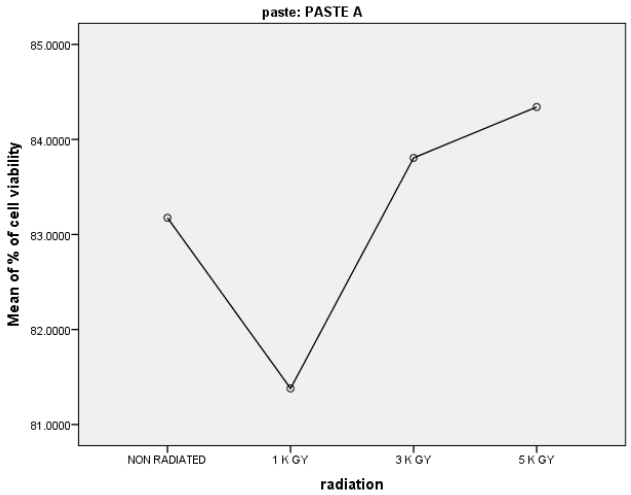

Fig. 1. Effect of different doses of irradiation on cell viability treated with Paste A

Non-irradiated Paste B was observed to have a higher cell viability than the irradiated at 1 kGy and 5 kGy. Paste B irradiated with 3 kGy almost showed similar cell viability as the non-irradiated sealer. The maximum cell viability was observed with non-irradiated sealer and minimum with 5 kGy of electron beam irradiation (Fig. 2).

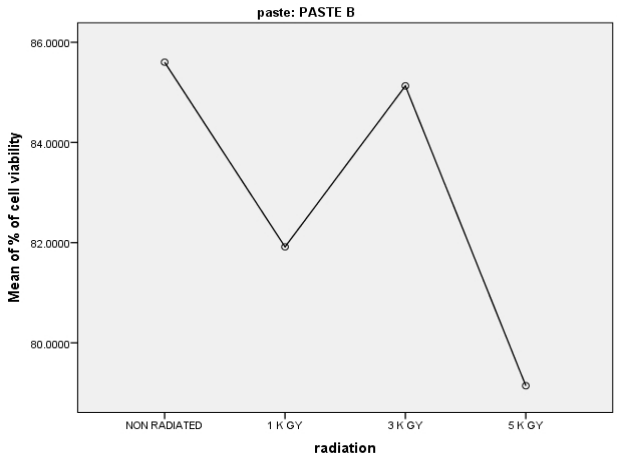

Fig. 2. Effect of Paste B exposed to different doses of irradiation on cell viability

Non-irradiated paste M (Mixed) showed higher cell viability than the irradiated at 1 kGy and 5 kGy and less than the irradiated at 3 kGy of electron beam irradiation. Minimum cell viability was observed at 5 kGy and maximum cell viability was at 3 kGy irradiation (Fig. 3).

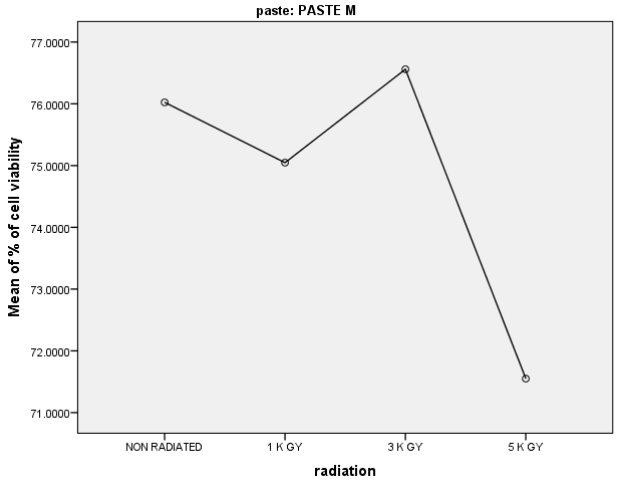

Fig. 3. Effect of Paste M exposed to different doses of irradiation on cell viability

Non-irradiated SET sealer was observed to have higher cell viability than the radiated at all three doses (i.e. I, 3 and 5 kGy). Cell viability reduced at 1 kGy, there was a further reduction seen at 3 kGy after which it increased again at 5kGy dose of electron beam radiation. Cell viability at 1 kGy and 5 kGy were almost equal, maximum was seen with the non-irradiated sealer and minimum was at 3kGy irradiation (Fig. 4).

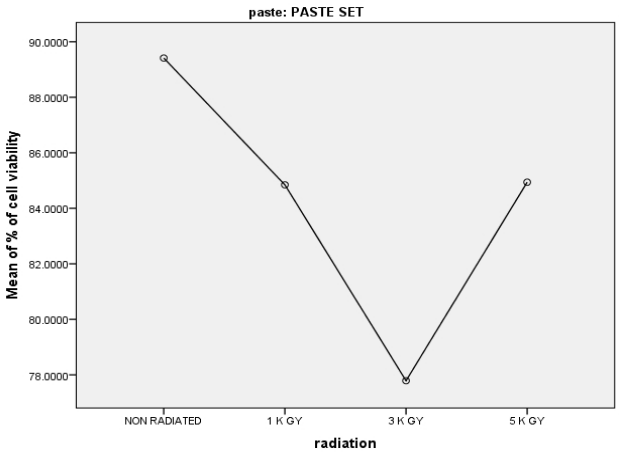

Fig. 4. Effect of Paste Set exposed to different doses of irradiation on cell viability

Statistically significant difference was noticed on comparing non-irradiated and irradiated samples of the set sealer (i.e., *P* value = .008) whereas all the other sealer components i.e. paste A, paste B, freshly mixed paste of AH Plus sealer did not show a statistically significant difference in cytotoxicity (Table 3).

Table 3. Comparison of cell viability of non-radiated and radiated sealer components

Materials		N	Mean	STD. Deviation	Mean Square	F	Sig.
Paste A	NON RADIATED	6	83.1758	5.61026	9.962	0.226	0.877
	1 K GY	6	81.37996	1.702373			
	3 K GY	6	83.80592	6.310139			
	5 K GY	6	84.34153	10.09701			
Paste B	NON RADIATED	6	85.60176	6.428796	54.687	1.709	0.197
	1 K GY	6	81.91556	2.498327			
	3 K GY	6	85.12918	8.679748			
	5 K GY	6	79.14304	2.261068			
Paste M	NON RADIATED	6	76.02395	5.363315	30.434	0.845	0.486
	1 K GY	6	75.04726	1.844433			
	3 K GY	6	76.55955	10.11581			
	5 K GY	6	71.5501	3.105602			
Set	NON RADIATED	6	89.41399	5.937052	138.503	5.217	0.008
	1 K GY	6	84.84562	5.578855			
	3 K GY	6	77.78828	5.168326			
	5 K GY	6	84.94014	3.619762			

In the present study, Paste B (Amine paste), Mixed paste and SET AH Plus sealer showed the maximum cell viability in non -irradiated category.

Only Paste A (epoxide paste) of AH Plus showed maximum cell viability in irradiated category, which was showed a linear rise, higher at 3 kGy and highest at 5 kGy.

It can be interpreted that the considerable effect of electron beam irradiation had only taken place on the epoxy paste of AH Plus sealer. Previous studies have shown the beneficial effect of electron beam irradiation on epoxy resins. During the early 1990's a significant amount of work was conducted on toughening electron beam curable cationic epoxies [32,33].

Studies have shown that electron beam irradiation on these resins have resulted in improved mechanical and thermal properties [32,33]. The results of the present study showed positive effect of irradiation in the epoxide paste of AH Plus sealer. The positive effect here is mainly based on experimental data of % cell viability and not on statistical analysis. The 5 kGy radiated epoxide paste resulted in very minimal cytotoxicity which could be due to increased cross-linking and less amount of unreacted toxic particles.

4. CONCLUSION

Further studies should be done to evaluate the cell viability at different doses of electron beam irradiation at different time intervals. The radiated epoxide paste (paste A) which gave positive result, if mixed with non-radiated amine paste (paste B) on which the effect of irradiation was not beneficial, could result in an improved material.

Only a few studies could be found in general dentistry supporting that electron beam irradiation is able to enhance the mechanical properties. Further research should to be performed with higher doses of electron beam irradiation on epoxide paste in order to notice further improvement in physical as well as biological properties in order to generate an upgraded version of the present dental materials.

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Our thanks and appreciations also go to our colleagues in developing the project and people who have willingly helped us out with their abilities.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

For manuscripts involving human experiments, Authors may use the following wordings for this section: "All authors hereby declare that all experiments have been examined and approved by the institutional ethics committee and institutional ethical clearance was obtained (NU/CEC/CLEAR.2/2012).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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