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Electron Beam Irradiation of Resin Luting Agents - a Cytotoxic Evaluation on Dental Pulp Cells

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Authors' contributions

This work was carried out in collaboration between all authors. Author MNH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SS, NDH, SK, and SSS managed the analyses of the study and managed the literature searches. Author GS carried the irradiation of the materials. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of this study was to evaluate the cytotoxicity of three resin luting agents Rely X luting cement, Rely X luting 2 cement and Clearfil SA luting cement before and after electron beam irradiation.
Materials and Methods: Growth and maintenance of cell cultures of human pulp cells was done in Dulbecco's modified Eagle's Medium (DMEM). The test samples were divided into two Categories: Irradiated Category and Non-radiated Category. Samples in Irradiated category were exposed to electron beam radiation at 200Gy. Three subgroups of radiated category and non radiated category were made. All the samples were subjected to MTT assay and spectrophotometric analysis and their cytotoxicity was

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assessed. Statistical analysis was done using t test.
Results: Evaluation of Rely X luting Cement showed that radiated samples of powder, liquid and set cements showed decreased cell viability than non radiated samples. In Case of Rely X luting 2 cement, radiated samples showed increased cell viability for Paste A and Paste B samples. But in set material, irradiated samples showed decreased cell viability as compared to non radiated samples. For Clearfil SA luting Cement, Paste B showed increased cell viability for radiated samples. Paste A and Set cement of radiated samples showed decreased cell viability than non radiated samples.
Conclusion: In the present study , the increased cytotoxicity of irradiated samples may be due to increase in the release of unbound monomers which may be due to chain breakage after irradiation and a reduction in the cytotoxicity which may be due to the cross linking of unbound monomers during irradiation.

Keywords: Electron beam irradiation; resin luting agents; cytotoxicity; pulp cells.

1. INTRODUCTION

A dental luting agent link the fixed prosthesis to the tooth structure. Conventional glass ionomer luting agents attained a high demand due to their properties such as fluoride release, Coefficient of thermal expansion and modulus of elasticity similar to dentin, bonding to tooth and biocompatibility [1-6]. Further research also showed that glass ionomer luting agents had some disadvantages such as high susceptibility for dehydration, and poor physical properties such as slow setting rate and high solubility [7,8]. Due to these limitations, further research leads to the development of resin modified glass ionomer cement. In this new material, basic composition of glass ionomer luting agents are maintained but modified by the presence of resin [2]. In 'resin modified glass ionomer cement', hydrophilic monomers and photoinitiaters were added to improve the physical and mechanical properties of cement [9]. Due to the poor adhesive properties of resin modified glass ionomer cements, further research has occurred in the field of resin modified glass ionomer luting agents that have resulted in the introduction of adhesive resin cements [10]. Although, resin modified glass ionomer cements and adhesive resin luting cements have their own advantages, it is also been confirmed in several literatures that these cements possess cytotoxicity due to the release of unbound free monomers during and after polymerisation, presence of HEMA, and due to the release of ions[11-14] Some literatures have been shown that electron beam irradiation of dental materials can be used as a tool able to increase the properties of dental materials [15-19].

Radiation is commonly used in the biomaterials science for sterilization, surface modification, and to improve bulk properties of materials. The energy sources which is commonly used in the irradiation of biomaterials are high-energy electrons, gamma radiation, ultraviolet (UV) and visible light [20].

Microtron is a high–energy accelerator for protons or electrons which are capable of producing very high currents. A microtron is regarded as a cyclotron in which the kinetic energy of electrons is increased by a constant amount per field change [21].

Electron beam irradiation is described as a method to change the mechanical properties as well as physical properties of polymers. Investigations of Charlesby and Ross showed that electron beam irradiation can be used for improving the properties of polymers [22]. In

general, electron beam irradiation of polymers can give rise to two type of reactions: cross linking, chain breakage [23,24].

Although studies have been done on various dental materials using electron beam radiation to evaluate the changes in their physical and mechanical properties, till date no study has been done to assess biological properties of resin based dental luting agents. If resin based luting agents can reduce the cytotoxicity of material after electron beam irradiation, it can be used as a tool to modify the present day dental luting agent properties [17-19]. Hence, the present study investigated the effect of electron beam radiation on the cytotoxicity of three resin luting agents.

2. MATERIALS AND METHOD

The electron beam irradiation of resin luting cements were conducted with the materials listed in Table 1.

Table 1. Resin luting cements used for the study and their composition

Material	Composition
Rely X luting Cement(3M ESPE, St. Paul, MN, USA)	Powder: fluoroaluminosilicate, potassium persulfate, ascorbic acid, opacifying agent Liquid: 30-40% copolymer of acrylic and itaconic acids,25-35% 2-hydroxy ethyl methacylate, 25-35% water.
Rely X luting 2 Cement(3M ESPE, St. Paul, MN, USA)	Paste A: Fluoroaluminosilicate glass, Proprietary reducing agent, HEMA, water, opacifying agent Paste B: Methacrylated carboxylic acid, Bis GMA, HEMA, water, potassium persulfate, zirconia silica filler.
Clearfil SA Luting Cement	Paste A: BIS GMA, TEG DMA, Hydrophobic aromatic dimethacrylate, Silanated barium glass filler, Silanated Colloidal Silica, dl- Camphorquinone, Benzoyl Peroxide, Initiator PasteB: BIS GMA, Hydrophobic aliphatic dimethacrylate, Hydrophobic aromatic dimethacrylate, Silanated barium glass filler, Silanated Colloidal Silica, Surface treated sodium fluoride, Accelarator.

The experiment for cell culture cytotoxicity was done in the Cell Culture Laboratory of Central Research lab, Nitte University, Mangalore, India. The procedure of Irradiation of the resin luting cements was done in Microtron Centre, Mangalore University, India.

The study was conducted in the following steps:

1. Growth and maintenance of cell cultures
2. Sample preparation and Irradiation of the samples for elution
3. Addition of elute or extract to the cells
4. MTT assay.

After obtaining Consent form from patient, human pulp cells were collected from healthy tooth extracting for orthodontic purposes. The tooth root was removed by horizontal section below CEJ with a no. 330 bur in high speed with water spray. The pulp tissue was removed aseptically, rinsed with Dulbecco's modified Eagle's medium (DMEM). Pulp tissue was

placed with a no.15 blade into small fragments and explants were cultivated in 25 cm3 tissue flasks (Orange Scientific, Belgium) containing Dulbecco's modified Eagle's Medium (DMEM, Hi Media Labs, India). It was supplemented with 5% fetal bovine serum (FBS, Hi Media Labs, India), 100UmL-1 penicillin, 100µLmL-1 streptomycin and 2mmolL-1Lglutamine, at 370C in a humidified atmosphere of 95% air and 5% CO2(Nuaire,CO2 Incubator, USA). Sub cultivation was performed with cell cultures treated with 0.25% Trypsin for 4hrs at 4°C. After trypsinization, cells were counted by using a Tryphan Blue Dye on a Nuebaer's Chamber and then seeded at a density of 3.6×104 cells per well in the 96 well Tissue culture plates (Laxbro, India) and incubated for 24 hours in 5% CO^2, 95% air at 37°C, to get sub confluent monolayers of cells.

2.1 Sample Preparation and Irradiation of the Samples for Elution

Electron beam irradiation of resin luting cements were conducted with 200Gy that was previously standardised in various studies [17,18]. The test samples (108 samples)were divided into two categories based on radiation exposure: Radiated Category and non radiated category. In radiated category, all the three resin luting cements were exposed to electron beam radiation at 200Gy. (Microtron, Electron Beam Accelerator, Microtron Centre, Mangalore University). Irradiated category were divided into 3 subgroups. In subgroup 1, all the 3 luting cements powder / paste A were placed in sterile Teflon moulds and were irradiated (18 samples). In subgroup II, all the 3 luting cements liquid / paste B were placed in sterile Teflon moulds and were irradiated. (18 samples) In subgroup III, all the 3 luting cements were mixed and placed on a polytetrafluroethylene moulds with the dimension of 25×2×2 mm and the set cements were placed in sterile moulds and exposed to electron beam irradiation (18 Samples).

Non radiated category also divided into three sub groups. In subgroup I, all 3 luting cements powder/ Paste A were placed in microvials and kept in a humid chamber at 37°C. (18 samples) In subgroup II, all 3 luting cements liquid / paste B were placed in microvials and kept in a humid chamber at 37°C (18 Samples). In subgroup III, all 3 luting cements were mixed and placed on a polytetrafluroethylene moulds with the dimension of 25×2×2 mm and the set cement were placed in microvials and kept in a humid chamber at 37°C (18 samples).

The luting cement extraction of one hundred and eight samples was done following which were subjected to cytotoxicity assay. The test solutions were sterile filtered using a Sterile Filter Unit (0.2µm pore size) (Sartorius Stedim, Biotech, Germany) before being exposed to culture. DMEM culture medium was used as control.

2.2 Addition of Elute or Extract to the Cells in Culture

Cells were diluted in fresh medium and seeded into 96 well plates (3.6×104 cells per well). After incubation for 24 hours, the medium was aspirated from all wells and replaced with 100µL per well of test solution or control medium. The test solutions were added to wells and one column of wells was filled with only culture medium as control group. The plates were incubated for another 24 hours, in 5% CO^2, 95% air mixture at 37°C in a CO^2 incubator (Nuaire, USA) before cytotoxicity was addressed.

2.3 MTT Assay

MTT solution was prepared as 1 mg/ml in phosphate buffer saline just before use. 100µL MTT dye was added to each well containing cells treated with extracts of the sealer and also to the control wells. Plates were incubated in a CO^2 incubator for 4hours.

Optical density was determined by dissolving the MTT-formazan inter cellular reaction product with dimethyl sulfoxide [6.25% v/v 0.1 N NaOH in dimethyl sulphoxide (DMSO)] and the spectrophotometric absorbance was measured at 630 nm using a ELISA microplate reader (Lisa Plus, Aspen Diagnostics, India). Three readings were taken per well and the mean was considered.

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 percentage of cell viability for each resin luting cements was recorded and the results were tabulated and subjected to statistical analysis by using student t test.

3. RESULT

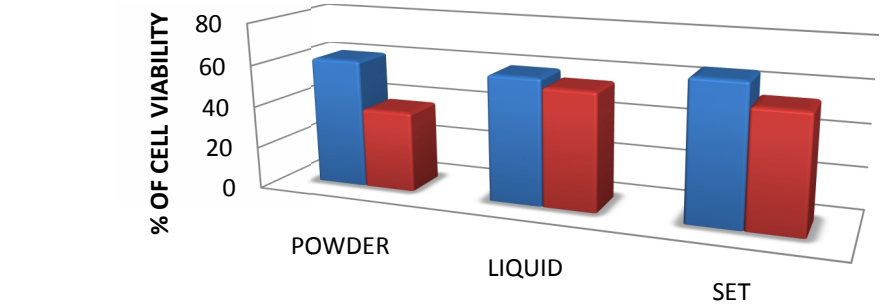
The result of evaluation of cytotoxicity on electron beam irradiation of resin luting cements are depicted in Diagrams 1, 2, 3 and Table 2.

In non radiated group of Rely X luting cement powder, liquid and set samples showed slightly cytotoxicity whereas in Rely X luting cement radiated group, powder, liquid and set cement showed moderate cytotoxicity. It can also be interpreted from the Diagram 1 that radiated group of Rely X luting cement powder, liquid and set cement was reported to have a reduced cell viability than non radiated group.

Non radiated and radiated group of Rely X luting 2 cement of paste A and set cement showed moderate cytotoxicity. But in the mean it was showed that there is a slight reduction in the cytotoxicity of radiated Paste A group. In radiated group of Paste B , it showed only slightly cytotoxic whereas non radiated group of Paste B showed moderate cytotoxicity. It can also be interpreted from the Diagram 2 that Paste A and Paste B was reported to have an increased cell viability for radiated group.

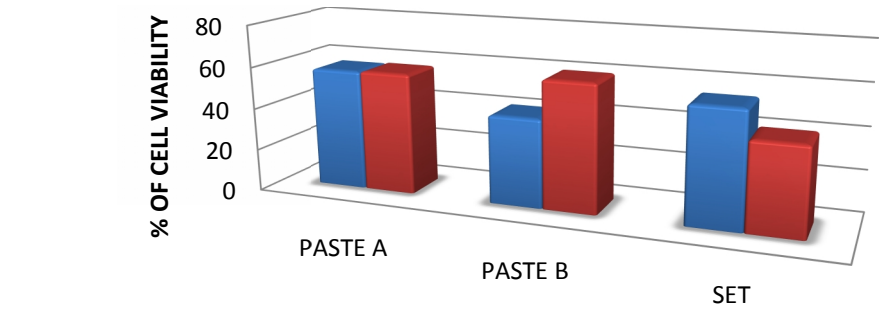
Table 2. Cytotoxicity was rated based on cell viability relative to control as (Dahl et al. 2006) [28]

Interpretation	% Cell Viability
Non-cytotoxic	>90% cell viability
Slightly cytotoxic	0–90% cell viability
Moderately cytotoxic	0–59% cell viability
Strongly cytotoxic	<30% cell viability



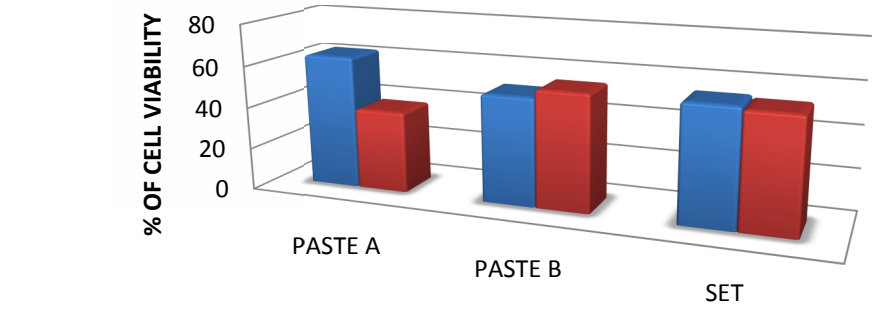
	POWDER	LIQUID	SET
NON RADIATED	61.01	59.5	65.1
RADIATED	38.1	55.5	54.5

Diagram 1. Bar diagram showing the mean % of cell viability of radiated and non radiated groups of rely x luting cement powder, liquid and set cement



	PASTE A	PASTE B	SET
NON RADIATED	57.3	42.03	54.7
RADIATED	57.7	60.5	41.5

Diagram 2. Bar diagram showing the mean % of cell viability of radiated and non radiated groups of rely x luting 2 cement paste a, paste b and set cement



	PASTE A	PASTE B	SET
NON RADIATED	63.1	51.1	54.9
RADIATED	38.6	55.1	53.6

Diagram 3. Bar diagram showing the mean % of cell viability of radiated and non-radiated groups of clearfil sa luting cement paste a , paste b and set cement

In non-radiated and radiated group of Clearfil SA luting Cement, Paste A, Paste B and set cement showed moderate cytotoxicity except for non radiated Paste A. In non radiated group Paste A, it showed only slight cytotoxicity. But in the mean it was showed that there is a slight reduction in the cytotoxicity of paste B radiated group than non radiated group.

When the means of all groups were compared , i.e. Sub group I, II and III of radiated and non radiated samples of Rely X luting Cement, Rely X luting 2 cement and Clearfil SA luting cement did not show any statistical difference in cytotoxicity of radiated and non radiated luting cement components Table 3.

Table 3. Comparison of cell viability of non radiated and radiated luting cement components

		GROUPS	N	Mean	Std. Deviation	t	df	Sig. (2-tailed)
Rely X	Sub Group I powder % cell viability	Non radiated	6	61.01	2.52	1.954	6.063	0.186
		Irradiated	6	38.1	20.11			
	Sub Group II liquid % cell viability	Non radiated	6	59.5	0.80	2.353	10	0.078
		Irradiated	6	55.55	2.81			
	Sub Group III set cement % cell viability	Non radiated	6	65.18	11.69	1.568	6.048	0.255
		Irradiated	6	54.53	1.28			
Rely X Luting 2	Sub Group I paste A % cell viability	Non radiated	6	57.31482	2.69	-0.095	10	0.929
		Irradiated	6	57.68	6.21			
	Sub Group II Paste B % cell viability	Non radiated	6	42.03	24.60	-1.268	6.185	0.323
		Irradiated	6	60.46	5.30			
	Sub Group III set cement % cell viability	Non radiated	6	54.72	0.27	0.998	6.001	0.423
		Irradiated	6	41.48	22.97			
Clearfil SA	Sub Group I paste A % cell viability	Non radiated	6	63.14	7.52	1.627	10	0.179
		Irradiated	6	38.61	25.02			
	Sub Group II paste B % cell viability	Non radiated	6	51.11	2.37	-1.875	10	0.134
		Irradiated	6	55.09	2.80			
	Sub Group III set % cell viability	Non radiated	6	54.90	3.93	0.434	10	0.687
		Irradiated	6	53.61	3.36			

4. DISCUSSION

Resin based dental materials are claimed to cause cytotoxicity to the materials due their presence of HEMA, BIS GMA, TEG DMA. It is also been confirmed that resin modified glass ionomer luting cements release ions such as fluoride, aluminium and strontium which can produce reactive Oxygen Species which are said to be cytotoxic [25].

Electron beam irradiation is a modern method for improving the properties of polymers and composites. It was shown to increase the stiffness of polymers as well as the links between polymer chains [26].

When a material is exposed to electron beam irradiation, two types of radiation initiation reaction can occur: Chain linkage and chain breakage.

Whenever a polymer material is exposed to electron beam irradiation, irradiation initiates the radical build up of all components of polymer and the entire polymer may be newly arranged and cross linked [16].

In contrast, electron beam irradiation can leads to chain breakage of the polymer. During reaction, the C-C bonds splits off and the polymer structure is broken down and leads to chain breakage [27].

Some studies have shown that electron beam radiation on resins have resulted in improved fracture toughness properties, improved tensile strength, moduli, and strain to failure properties, high thermal properties, low water absorption values, low resin densities, as well as favourable rheological properties [29]. This may be due to the cross linking of the polymers.

In the present study, the effect of electron beam irradiation of three resin luting agents was evaluated with the non radiated samples of same resin luting agents. Three different components of the luting cements were evaluated in the study, i.e. Powder/ Paste A, Liquid/ Paste B and Set Cement.

In the present study, extracts of luting cements were prepared as per ISO-10993-12 guidelines. Extracts from luting cements microvials were obtained 24 hrs after being placed in cell culture medium (DMEM), following which the radiated and non-radiated samples were subjected to the cytotoxicity assay.

In the present study, Rely X luting cement radiated group shows moderately cytotoxic whereas Rely X luting cement shows only slightly cytotoxic in non radiated group. The increased cytotoxicity of irradiated group may be due to increase in the release of unbound monomers which are present in the Rely X luting cement such as 30-40% copolymer of acrylic and itaconic acids and 25-35% 2-hydroxy ethyl methacylate. This unbound monomer release occurred which may be due to chain breakage after irradiation.

In the present study, radiated group of Rely X luting 2 cement paste A and paste B shows a reduction in the cytotoxicity as compared to the non radiated cement group of paste A and B. This may be due to the cross linking of unbound monomers such as methacrylated carboxylic acid, BIS GMA and HEMA during irradiation. Set Samples of irradiated group of Rely X luting 2 cement shows an increased cytotoxicity as compared to non radiated set

group. This may be due to increase in the release of unbound monomers which may be due to chain breakage after irradiation.

In the present study, Paste A and set samples of radiated group of Clearfil SA luting cement shows an increased cytotoxicity than non radiated group. This may be due to increase in the release of unbound monomers such as BIS GMA, TEG DMA, hydrophobic aromatic dimethacrylate and hydrophobic aliphatic dimethacrylate which may be due to chain breakage after irradiation. Irradiated group of paste B of Clearfil SA luting cement shows a slight reduction in the cytotoxicity than non irradiated group. This may be due to the cross linking of unbound monomers during irradiation and less amount of unreacted toxic particles.

5. CONCLUSION

The present study noted that if the material used for luting agent can provide cross linking rather than chain breakage after irradiation, cytotoxicity can be reduced as well as we will be able to increase the properties of the material. So more investigations should be conducted whether electron beam irradiation can be used as a tool to modify and improve the present day dental materials.

Further studies should be done to evaluate the cell viability of electron beam radiation at different time intervals. Further research should be performed with electron beam radiation on pure resin based materials 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.

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 (ABSM/EC/85/2011).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gao W, Smales RJ. Fluoride release /Uptake of conventional and resin modified glass ionomers, and compomers. J Dent. 2001;29(4);301-6.
2. John F. McCabe-Resin Modified glass-ionomers. Biomaterials. 1998;19:521-527.

3. Bullard RH, Leinfelder KF, Russel CM. Effect of Coefficient of thermal expansion on microleakage. J Am Dent Assoc. 1998;116;871-4.
4. Erickson RL, Glass poole EA. Bonding to tooth structure: A comparison of glass ionomer and composite resin systems. J Esthet Dent. 1994;6:227-44.
5. Costa CAS, Giro EM, Nascimento ABL, Teixeira HM, Hebling J. Shortterm evaluation of pulpo dentin complex response to a resin modified glass ionomer cement and a bonding agent applied in deep cavities. Dent Mater. 2003;19:739-46.
6. Leyhausen G, Abtahi M, Karbakhsch M, Sapotnick A, Geurtsen W. Biocompatibilty of various light curing and one conventional glass ionomer cement. Biomaterials. 1998;19:559-64.
7. Mount GJ. Glass ionomer Cements: Past, present and future. Oper Dent. 1994;19:82-90.
8. Carel Leon Davidson. Advances in Glass ionomer cements. J Appl. Oral Sci. 2006;14;3-9.
9. Cattani–Lorente MA, Godin C, Meyer JM. Mechanical behaviour of glass ionomer cements affected by long term storage in water. Dent Mater. 1994;10:37-44.
10. Rodovic I, Monticells F, Goracci C, Vulicevic ZR, Ferrari M. Self adhesive resin cements: a literature review. J Adhes Dent. 2008;10(4):251-258.
11. Pedro P.C. Souza, Andreza M.F. Aranha, Josimeri Hebling, Elisa M.A. Giro, Carlos A. De Souza Costa. Invitro cytotoxicity and in vivo biocompatibility of contemporary resin-modified glass-ionomer cements. Dent Mater. 2006;22:838-844.
12. Martina Schmid-Schwap, Alexander Franz, Franz Konig, Margit Bristela, TrevarLucas, Eva Piehslinger, et al. Cytotoxicity of four categories of dental cements. Dent Mater. 2009;2:360-368.
13. Adriano Augusto Melo de Mendonca, Pedro Pulo ChavesSouza, Josimeri Hebling, Carlos Alberto de Souza Costa. Cytotoxic effects of hard setting cements applied on the odontoblast cell line MDPC-23. Oral Surg Oral Med Oral Pathol Oral radiol Endod. 2007;104:102-108.
14. Engelmann J, Janke V, Volk J, Leuhausen G, Neuhoff NV, Schlegelberger B, et al. Effects of BISGMA on glutathione metabolism and apoptosis in human gingival fibroblasts *In vitro*. Biomaterials. 2004;25:4573-80.
15. Michael Behr, Martin Rosentritt, Andreas Faltermeier, Gerhard Handel. Electron beam irradiation of dental composites. Dent Mater. 2005;21:804-810.
16. Michael Behr, Martin Rosentritt, Andreas Faltermeier, Gerhard Handel. Electron beam irradiation of denture base materials. J Materials Science: Materials in Medicine 16. 2005;175-181.
17. Mithra N. Hegde, Shabin, Nidarsh D. Hegde, Suchetha Kumari, Ganesh Sanjeev, Shilpa Shetty. Electron beam irradiation effect on flexural strength and modulus of elasticity of resin modified glass ionomer luting cement. Int J Biol Med Res. 2013;4(4):3690-3694.
18. Shabin, Mithra N. Hegde, Nidarsh D. Hegde, Suchetha Kumari, Ganesh Sanjeev, Shilpa Shetty. Electron beam irradiation effect on compressive strength of resin modified glass ionomer luting cement. J Appl Chem. 2013;2(6):1589-1594.
19. Mithra N. Hegde, Aastha Puri, Shilpa Shetty, Nidarsh D. Hegde, Suchetha Kumari, Ganesh Sanjeev. Effect of electron beam irradiation on resin based root canal sealer-AH plus.: A cytotoxic evaluation. Annual Research and Review in Biology. 2013;4(1):163-173.
20. Roberto S. Benson. Use of radiation in biomaterials science. Nucl Instum Meth B. 2002;191:752-757.
21. Arthur Roberts. The microtron as a high-energy, high current particle accelerator. Annals of Physics. 1958;4(2):115-165.

22. Charlesby A, Ross M. Effect of high energy radiation on long chain polymers. Nature. 1953;171:167.
23. Behr M, Rosentritt M, Bettermann K, Handel G. Influence of electron beam irradiation on the alloy-to-resin bond strength. Eur J Oral Sci. 2005;113:429–435.
24. Geer RW, Wilkes GL. Apparent reversal of physical aging by electron beam irradiation- further investigations. Polymer 1998;39:4205-10.
25. Michel Goldberg. *In vitro* and *In vivo* studies on the toxicity of dental resin components: a review. Clin Oral Invest. 2008;12:1-8.
26. Behr M, Rosentritt M, Dümmler F, Handel G. The influence of electron beam irradiation on fibre-reinforced composite specimens. J Oral Rehab. 2006;33:447–451
27. Jenifer Martin. Effect of methacrylate based endodontic 319 sealer Epiphany on rat peritoneal macrophages viability. J Clin Exp Dent. 2011;3(3);216-21.
28. Dahl JE, Frangou-Polyzois MJ, Polyzois GL. *In vitro* biocompatibility of denture relining materials. Gerodont. 2006;23;17-22.
29. Christopher J. Janke, Ronny D. Lomax. Improved epoxy resins cured by electron beam irradiation. 46th Int SAMPE Smposium and Exhibition. 2001;2048-2062.

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