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Effect of Irradiation on Genotoxicity of Two Resin Modified Glass Ionomer Luting Cements - An in-Vitro Study

KEYWORDS

* Mithra N. Hegde	Shilpa .S.Shetty	Shabin.S
Department of Conservative Dentistry and Endodontics, A.B.Shetty Memorial, Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore, India. * Corresponding author	Nitte University Centre for Science Education and Research, Nitte University, Deralakatte, Mangalore, India.	Department of Conservative Dentistry and Endodontics, A.B.Shetty Memorial, Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore, India.

Nidarsh D.Hegde	Suchetha Kumari	Ganesh Sanjeev
Department of Oral and Maxillofacial Surgery, A.B.Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte, Mangalore, India.	Department of Biochemistry, KSHEMA, Deralakatte, Mangalore, India.	Microtron Centre; Department of Physics Mangalore University; Mangalore, India.

ABSTRACT *The aim of the study was to evaluate the genotoxicity before and after electron beam irradiation of two commercially available resin modified glass ionomer luting cements –Rely X Luting and Rely X Luting plus on human lymphocytes invitro. Samples were prepared according to ISO standards and divided into two groups- Non radiated and radiated groups. The radiated group was exposed to 200Gy of electron beam irradiation. The sealer extraction was done for a duration of 24 hours in DMEM media. Lymphocyte was separated and used for genotoxicity study. Statistical analysis was performed using student't' test. The irradiation of Rely X luting cement with 200Gy dose of electron beam irradiation showed increase in the frequency of DNA damage when compared to that of the non-radiated group. Similar results were also seen in RelyX Luting Plus cement with 200Gy dose. Apoptotic diffusion also showed a significant increase in DNA diffusion in lymphocytes that were incubated with irradiated materials of Rely X Luting and Rely X Luting Plus . Statistical significance was observed in Relyx Luting Plus (p=0.02). Within the limitations of the present study, it is concluded that further research is needed to increase the polymerization of the resin based glass ionomer luting cements and decrease the genotoxicity by pretreatment of the material.*

INTRODUCTION

From the seventies till the present day glass ionomer cements have been used in dentistry.

Along with their positive aspects they have certain negative aspects such as sensitivity to humidity and early, weak mechanical strength. In order to overcome the limitations resin was added to glass ionomer cements which resulted in the development of a new cement in the late eighties called the resin modified glass ionomer cements (RMGIC) [1,2]. RMGICs were formed by the replacement of the polyacid with a modified polyacid grafted with unsaturated groups, and the incorporation of polymerizable hydrophilic resins [3]. HEMA is a hydrophilic resin, added as a co-solvent which polymerizes or copolymerizes with the modified polyacid [4].

Basically RMGIC consist of 80% glass ionomer cement and 20% resin. The composition may slightly vary depending on the brand. [5]

HEMA (Hydroxy Ethyl Methacrylate), of which liquid is polymerized via light methacrylate groups (EGMA, GMA and BIS GMA etc.), tartaric acid, polyacrylic acid and water. Its powder however contains fluoro aluminosilicate glass particles. The qualities of resin modified glass ionomer cement are between Conventional glass ionomer cements and composite resins which means RMGIC is a hybrid materials [1,6].

RelyX Luting cement is a self cure resin modified glass ionomer cement which is composed of powder and liquid. The RelyX Luting cement powder is composed of a radiopaque fluoroaluminosilicate glass (FAS glass). microencapsulated potassium persulfate and ascorbic acid catalyst system. The RelyX Luting cement liquid is an aqueous solution of polycarboxylic acid modified with pendant methacrylate groups. It also contains HEMA, water and small amounts of tartaric acid.

RelyX Luting plus is a self cure radiopaque, fluoride-releasing, resin-modified glass ionomer luting cement consists of a base and catalyst paste. Luting agents for permanent cementation of crown and bridge restorations have to meet many requirements before they can safely be used in humans. An ideal luting agent should be, biocompatible with the tissue that it contacts; able to adhere to tooth substance and restoration; able to prevent leakage by producing a good marginal seal; cariostatic and insoluble in the oral cavity.

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 [7,8]. There is only very little information regarding the biocompatibility effect of electron beam irradiation on genotoxicity of resin luting cements.

So it was the aim of the present study to determine the in vitro genotoxicity of two commercially available resin modified glass ionomer luting cements -Rely X luting and Rely X plus luting cement on human lymphocyte cells before and after electron beam irradiation.

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Materials and method

Dental Material

Resin modified glass ionomer luting cements commercially available as Rely X Luting cement and Rely X Luting plus cement (3M ESPE) were (Table 1&2).

Table 1: Composition of Rely X luting

Powder	Liquid
Fluoroaluminosilicate (FAS) glass	Methacrylated polycarboxylic acid
Potassium persulfate	Water
Ascorbic acid	HEMA
Opacifying agent	Tartaric acid

Table 2: Composition of RelyX Luting Plus

Paste A	Paste B
Fluoroaluminosilicate (FAS) glass	Methacrylated polycarboxylic acid
Proprietary reducing agent	BisGMA
HEMA	HEMA
Water	Water
Opacifying agent	Potassium persulfate
Zirconia silica filler	

Sample preparation

Samples were prepared according to ISO standard -4049 using 25×2×2 mm polytetrafluoroethylene moulds.

Groups

Two groups- Non radiated and radiated groups.

Irradiation

The set materials in polypropylene vials were exposed to 200Gy of electron beam irradiation at Microtron Centre, Mangalore

Elute preparation

Materials from both the groups were placed in DMEM media using the ratio 1.25 cm^2 /ml between the surface of the samples and the volume of the medium [9]. The sealer extraction was done for a duration of 24 hours. The test solutions were sterile filtered using a Sterile Filter Unit (0.2µm pore size) (Sartorius Stedim, Biotech, Germany).

Blood Sampling:

Lymphocyte Separation:

Whole Blood was drawn by antecubital venipuncture into heparinized vacutainers. 1:1 ratio of histopaque (purchased from Sigma Aldrich) was added and centrifuged at 3000rpm for 10 minutes. Lymphocyte was separated and used for genotoxicity study.

Genotoxicity Studies

Alkaline comet assay:

The alkaline comet assay was performed basically as described by Tice et al. 1991 [10]. The extent of DNA damage was assessed from the DNA migration distance, which was derived by subtracting the diameter of the nucleus from the total length of the comet. Fifty randomly selected cells were examined for each replicate, for each sample or subject. The quantification of the DNA strand breaks was performed using Comet score software by which the percentage of DNA in the tail, tail length and OTM could be obtained directly [10].

DNA diffusion assay

DNA diffusion assay was performed basically as described by Singh et.al. 2004 [11]. It is a simple, sensitive, and rapid method for estimating apoptosis in single cells.

STATISTICAL ANALYSIS

Statistical analysis was performed using student't' test. 'p' value less than 0.05 was statistically significant.

RESULTS

Comet assay

Single cell gel electrophoresis was performed to investigate the effect of electron beam irrradiated and non-radiated material on lymphocyte. The irradiation of Rely X Luting with 200Gy dose of electron beam irradiation showed increase in the frequency of DNA damage when compared to that of the non-radiated group (Table 3). Similar results were also seen in the case RelyX Luting Plus with 200Gy dose (Table 4).

Table 3: Effect of electron beam radiated and non-radiated Rely X Luting on lymphocyte by (comet assay) single cell gel electrophoresis

Rely X Luting	Comet Length	Tail Length	%DNA in tail	Olive Moment
Non-radiated	255.13±91.03	81.93±7.11	19.79±2.32	9.29±1.61
Radiated	345.13±67.10	98.3±5.94	36.79±2.4	11.29±1.55
'P' value	P<0.0001	NS	NS	NS

'P' value <0.05 statistically significant. NS= Not significant

Table 4: Effect of electron beam radiated and non-radiated Rely X Luting plus on lymphocyte by (comet assay) single cell gel electrophoresis

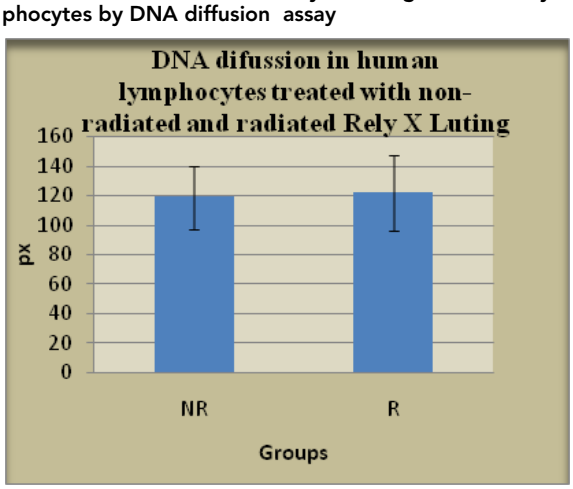
RelyX Luting Plus	Comet Length (px)	Tail Length (px)	%DNA in Tail	Olive Moment
Non-radiated	111.87±20.16	21.18±16.80	54.92±18.95	17.41±8.19
radiated	151.07±53.53	14.76±1.68	51.54±4.54	16.96±1.6
'P' value	P<0.0001	NS	NS	NS

'P' value <0.05 statistically significant. NS= Not significant

DNA diffusion" assay

Non radiated Rely X Luting cement showed an apoptotic diffusion of 118.67±21.35 and radiated samples showed an apoptotic diffusion of 122.02±25.6, but was statistically insignificant (Graph 1,3). Incase of RelyX Luting Plus, it showed an apoptotic diffusion of 153±41.86 and radiated showed an apoptotic diffusion of 174.30±33.2, but was statistically significant with a p value of 0.0216 (Graph 2,3) .

Graph 1: Graphical representation of genotoxic effects of non-radiated and radiated Rely X Luting on human lymphocytes by DNA diffusion assay



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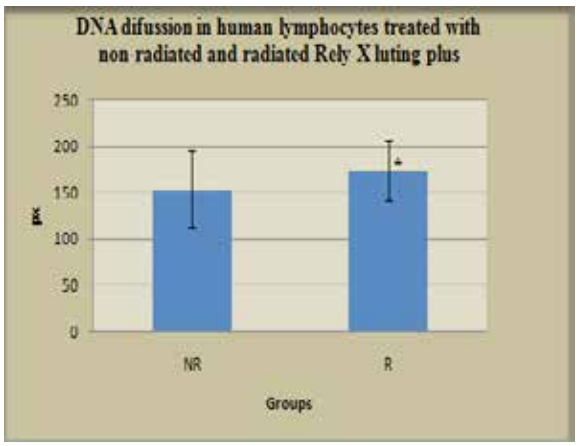
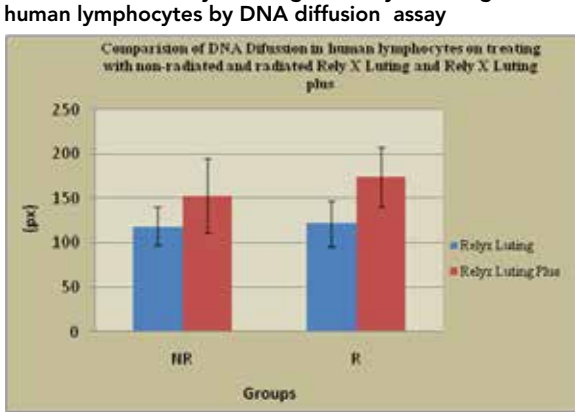
Graph 2: Graphical representation of genotoxic effects of non-radiated and radiated Rely X Luting Plus on human lymphocytes by DNA diffusion assay



Graph 3: Comparision of genotoxic effects of non-radiated and radiated Rely X Luting and Rely X Luting Plus on human lymphocytes by DNA diffusion assay



Discussion

The biological effects of resin-modified glass-ionomer cements as used in clinical dentistry were described recently [12]. It is said that HEMA is released from these materials, which has a variety of damaging biological properties, ranging from pulpal inflammation to allergic contact dermatitis. According to the some of the authors, RMGIs cannot be considered biocompatible to nearly the same extent as conventional glass-ionomers. RMGIs have a better clinical track record than glass ionomer cements [13]. Irradiation-initiated reaction can be of two types- chain linkage and breakage. Radicals, which bring about chain linkage, are initiated from several distinct points during a chemical reaction. It has been demonstrated that irradiation initiates the radical build-up of all components of a polymer [14]. Previously our study showed an increase in flexural strength and modulus of elasticity after radiation of resin modified glass ionomer cements [15]. The entire polymer may simultaneously be newly arranged and cross-linked when irradiated.

The need for evaluating the cytotoxicity and biocompatibility of specific material is as important as the assessment of its physiological or mechanical properties [16]. The present study aimed to determine the in vitro genotoxicity of Rely X luting and Rely X Luting Plus cement on human lymphocyte cells before and after irradiation. Genotoxic assays are important study parameters since it has gained widespread acceptance as an important and useful indicator of biocompatibility and carcinogenicity [17]. Genotoxic analysis was performed using comet assay and apoptotic diffusion assay. Recently it has been shown that single cell gel electrophoresis assay is a suitable tool to investigate genotoxicity of compounds used in dental practice [18,19].Single cell gel electrophoresis or comet assay is increasingly being used in genotoxicity testing as it is a simple and reliable technique [6]. Applicability to various tissues and/or special cell types, its sensitivity for detecting low levels of DNA damage, its requirement for small numbers of cells per sample, general ease of test performance, the short time needed to complete a study and its relatively low cost are some of the advantages of Comet assay.

Apoptosis is a programmed physiological process of cell death which plays a critical role not only in normal development, but also in the pathology of a variety of diseases and the activity of a large number of toxicants.

DNA migration in comet assay slightly increased in case of irradiated Rely X Luting and Rely X Luting Plus when compared to non –radiated group. Statistical significance was observed only in terms of comet length. Apoptotic diffusion also showed a significant increase in DNA diffusion in lymphocytes that were incubated with irradiated materials of Rely X Luting and Rely X Luting Plus . Statistical significance was observed in Relyx Luting Plus .

This toxicity may be attributed to the HEMA present in the Cement and unbound free monomers released by resin during and after polymerisation. Several other mechanisms have also be proposed for mechanism of cytotoxicity of resin Cement. One such mechanism is the Short term release of free monomers during the monomer- polymer conversion. Within the first few hours after initial polymerisation unbound monomers will release due to the defective photopolymerisation, thermal, mechanical or chemical factors, and these free monomers, can exhibit cytotoxic effects. It was expected that at the end of polymerisation, most of the monomers will react to polymer network and the quantity of residual monomers left have been evaluated as no more than 1.5- 5% [20]. However, studies shows that these unbound monomers is enough to contribute cytotoxic effects[21].

Conclusion

Within the limitations of the present study, it is concluded that further research is needed in the field of electron beam irradiation of dental luting agents. The choice of an appropriate irradiation dose which will further increase the polymerization of the material and thereby decrease genotoxicity by preventing the release of monomers is necessary.

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