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Genotoxic Effects of Glass Ionomer Type-IX Cements on Human Lymphocytes before and after Irradiation.

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ABSTRACT

The aim of this study was to examine the genotoxic potential of glass ionomer cement type IX available commercially before and after electron beam irradiation. The dental material used in the study was type IX Glass ionomer cement commercially available as GC Type IX (3M ESPE). The study was divided into two groups-Non radiated and radiated groups. The set material was placed in polypropylene vials and exposed to 10KGy of electron beam irradiation. Lymphocyte was separated and used for genotoxicity study. The alkaline comet assay was performed as described by Tice *et al.* 1991. DNA diffusion assay was performed as described by Singh *et.al.* 2004. Statistical analysis was performed using student 't' test. The irradiation of Glass ionomer cement type IX with 10KGy dose of electron beam irradiation showed increase in the frequency of DNA damage when compared to that of the non-radiated group. Statistically significance was observed in olive moment ($p=0.0203$) and tail length ($P<0.0001$) between radiated and non-irradiated groups. Apoptotic DNA diffusion index did not show much difference between non-radiated and radiated groups. However DNA diffusion was higher in radiated group and was statistically significant ($P<0.0001$). From the study it can be concluded that the increase in the frequency of DNA damage after electron beam irradiation may be due to the release of unbound acids because of chain breakage and due to the release of fluoride ions. Further studies are required to study the exact mechanism involved in genotoxicity .

Keywords: Glass ionomer, cytotoxicity, genotoxicity, electron beam irradiation, comet assay, DNA diffusion.

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INTRODUCTION

An important objective in dentistry is the adhesion of restorative materials to tooth structure. Restorative material should resemble the tooth in all respects. It should adhere tenaciously to the surrounding enamel and possess identical properties of the dentin.

A wide range of new dental materials with improved mechanical and physical properties and biological compatibility, for various clinical applications have been developed. However, despite these advances, there is still a need for biomaterials demonstrating high biocompatibility, antimicrobial effects and ideal mechanical properties. Among the recently developed materials, glass ionomer cements (GIC) have gained popularity since their conception in 1972 by Wilson and Kent [1].

During the past 30 years glass ionomer family of restorative materials has evolved into a diverse group of products. Glass ionomers differ from composite resins on several fundamental levels, including composition (water-based vs resin based), setting reaction (acid base reactions vs resin polymerization) and nature of the tooth/restoration interface (chemical adhesion and ion exchange vs micromechanical attachment to acid –dimineralized enamel and dentin). These and other attributes of glass ionomers render them applicable to many restorative situations [2,3].

The biocompatibility of glass ionomer cements is very important because they need to be in direct contact with enamel and dentin if any chemical adhesion is to occur. In an *in vitro* study, freshly mixed conventional glass ionomer cement was found to be cytotoxic, but the set cement had no effect on cell cultures [4].

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 [5,6]. Although studies have been done on various dental materials using electron beam irradiation to evaluate the changes in their physical and mechanical properties [6,7]. Studies to assess their biological properties are very sparse.

Aim

The aim of this study was to examine the genotoxic potential of commercially available glass ionomer cement type IX before and after irradiation.

MATERIALS AND METHOD

Dental Material

The dental material used in the study was type IX glass ionomer cement commercially available as GC Type IX (3M ESPE). The composition of the material is given in Table I.

Table I: Composition of GC Type IX

Powder	Liquid
Silica- 41.9% Alumina-28.6% Aluminium Fluoride -1.6% Calcium Fluoride- 15.7% Sodium Fluoride- 9.3% Aluminium Phosphate- 3.8%	Polyacrylic Acid

Sample preparation

20 Samples were prepared according to ISO standard-4049 using 25×2×2 mm polytetrafluoroethylene moulds [8].

Groups

The study was divided into two groups-Non radiated and radiated groups.

Irradiation

The set materials were placed in polypropylene vials and exposed to 10KGy of electron beam irradiation at Microtron Centre, Mangalore

Elute preparation

All the materials were now placed in DMEM media using the ratio 1.25 cm² /ml between the surface of the samples and the volume of the medium [9]. The cement 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]. After electrophoresis, the slides were neutralized with 0.4 M Tris, pH 7.4, stained with 50μL of ethidium bromide (20μg/mL) and analyzed with a fluorescence microscope (Olympus.40x objective). 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 of the stored images 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]. The DNA diffusion assay described here is a simple, sensitive, and rapid method for estimating apoptosis in single cells. The assay involves mixing cells with agarose and making a microgel on a microscopic slide, then lysing the embedded cells with salt and detergents (to allow the diffusion of small molecular weight DNA in agarose), and finally visualizing the DNA by a sensitive fluorescent [11].

STATISTICAL ANALYSIS

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

RESULTS

Comet assay

To investigate the effect of electron beam irradiated and non-radiated material on lymphocyte, single cell gel electrophoresis was performed. The irradiation of glass ionomer cement type IX with 10KGy dose of electron beam irradiation showed increase in the frequency of DNA damage when compared to that of the non-radiated group. Olive moment and tail length showed statistically significance between radiated and non-radiated groups (Table II).

Table II: Effect of electron beam radiated and non-radiated material on lymphocyte by (comet assay) single cell gel electrophoresis

	Comet Length (px)	Tail Length (px)	%DNA in Tail	Olive Moment
Non-radiated	113.77±46.97	24.70±24.70	51.73±17.61	15.39±4.67
Radiated	120.55±19.9	50.82±20.4	68.27±21.19	26.51±9.92
'P' value	NS	P<0.0001	NS	0.0203

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

DNA diffusion" assay

The "DNA diffusion" assay described as a simple, sensitive, and rapid method for estimating apoptosis in single cells. Non radiated GI type IX showed an apoptotic diffusion of 103.31±14.75 and radiated GI type IX showed an apoptotic diffusion of 125.5±17.94. Apoptotic index did not show much difference between non-radiated and radiated groups. However DNA diffusion was higher in radiated group and was statistically significant (P<0.0001) (Graph I, Fig I).

Graph I: Graphical representation of genotoxic effects of non-radiated and radiated glass ionomer IX on human lymphocytes by DNA diffusion assay

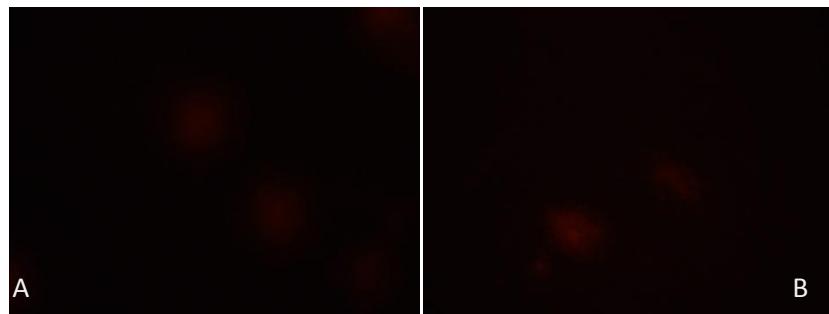
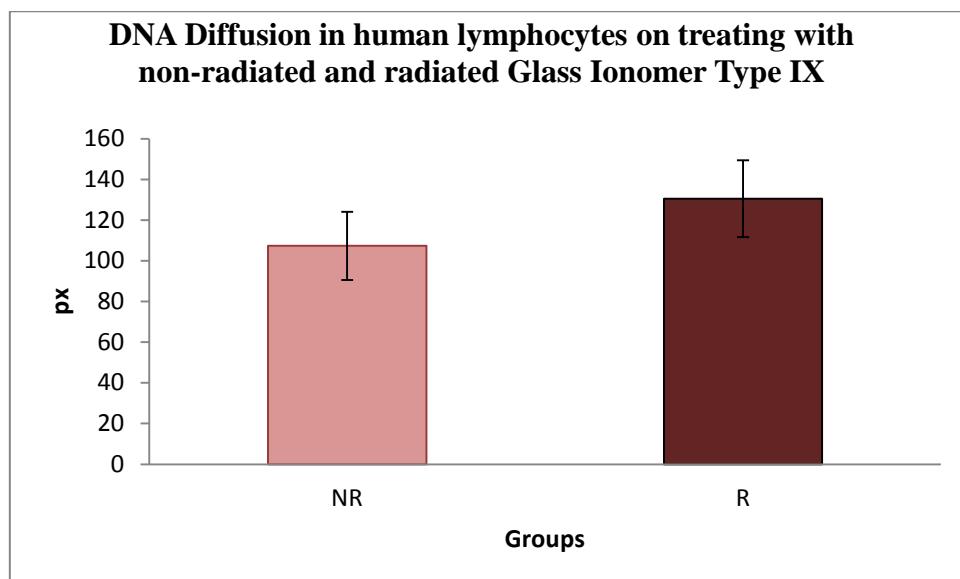


Figure I: Apoptotic Diffusion : A. Non-radiated, B. Radiated

DISCUSSION

Cytotoxicity and genotoxicity testing has gained much importance in the assessment of biocompatibility in dental material research [12-14]. Glass Ionomer cement usually forms by a reaction of fluoroaluminosilicate glass powder with an aqueous solution of acidic copolymers such as polyacrylic acid or acrylic acid/itaconic acid copolymers. Studies showed that Glass Ionomer Cement releases fluoride and acids after setting and this has shown to have a potential for cytotoxicity [15,16]. Studies show that electron beam irradiation of the dental materials can be used as a method to increase the properties and to reduce the cytotoxicity of dental materials. [5-9,16-18]. 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 [19]. 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 [20,21].

In this study we have investigated the genotoxic effects of elutes derived from types of available dental cements, glass ionomer cement type IX on normal cultured human lymphocytes. Genotoxic assays are important study parameters since it has gained widespread acceptance as an important and useful indicator of biocompatibility and carcinogenicity [22].

Genotoxic analysis was performed using comet assay and apoptotic diffusion assay. Single cell gel electrophoresis or comet assay is increasingly being used in genotoxicity testing as it is a simple and reliable technique [23]. The advantages of the alkaline comet assay include its 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.

Recent studies have shown that single cell gel electrophoresis assay is a suitable tool to investigate genotoxicity of compounds used in dental practice [24,25].

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. Radiated materials induced significant enhancement of DNA migration in the Comet assay when compared to that of non-radiated materials as a possible sign for genotoxic effects. Apoptotic diffusion also showed a significant increase in DNA diffusion in lymphocytes that were incubated with irradiated materials.

Literature have shown that increase or decrease in the cytotoxicity of dental material after electron beam irradiation depends on the irradiation-initiation reaction such as chain linkage and chain breakage [19,20,21]. In the present study material was irradiated as an attempt to increase the polymerisation reaction through cross linking but irradiation did not show any favourable changes.

In the present study, irradiation of Glass Ionomer type IX with 10 KGy dose of electron beam irradiation showed increase in the frequency of DNA damage when compared to that of the non radiated group. This may be due to the release of unbound acids because of chain breakage and due to release of fluoride ions.

CONCLUSIONS

From the study it can be concluded that increased polyacrylic acid and fluoride release may be involved in the genotoxicity of non-radiated and radiated glass ionomer type IX. Further studies are required to study the exact mechanism of genotoxicity.

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