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ELECTRON BEAM IRRADIATION OF GLASS IONOMER CEMENT TYPE IX ON HUMAN ERYTHROCYTES- A CYTOTOXIC EVALUATION

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Abstract-

Aim: The aim of this study was to evaluate the cytotoxic potential of glass ionomer cement type IX available before and after electron beam irradiation.

Materials and Methods: After blood collection, erythrocyte was separated and kept for cytotoxic study.

A total of 20 samples of GC Fugi IX were prepared on rectangular bar shaped specimens of 25-× 2-× 2- mm and samples were divided into two categories: Non radiated and Radiated. 10 samples of GC fugi IX were irradiated at 10 KGy (Microtrone Centre, Mangalore University, India). The cement extraction was done for a duration of 24 hours, 7 days and 14 days. The test solutions were sterile filtered using a Sterile Filter Unit (0.2 µm pore size) (Sartorius Stedim, Biotech, Germany) and was subjected to the cytotoxicity assay.

Results: Non radiated samples of Fugi type IX on human erythrocytes showed that the percentage of hemolysis was more in non radiated samples than radiated samples after 24 hours and 14 days. The percentage of hemolysis was reduced in radiated samples after 7 days and 14 days.

Conclusions: The reduction in hemolysis after electron beam irradiation of samples may be due to cross linking of unbound acids and increase in hemolysis of non radiated samples may be due to the presence of unbound acids.

Keywords- glass ionomer, blood collection, cytotoxic study

Introduction

The primary objective of a tooth restoration is to remove the carious tooth structure and to fill cavity space with an adequate stable biocompatible material [1]. Conventional glass ionomer cements undergoes setting reaction through an acid-base reaction between an ion leachable glass and aqueous polyacid which are capable of providing properties similar to dentin, bonding to tooth and biocompatibility [2-7]. Most of these materials have to contact or interact with body tissue and fluids, so material's selection must take into consideration not only mechanical and physical properties but also biological compatibility [1]. After operative procedures, the release of components from various dental materials may diffuse through dentinal tubules and potentially attack the target tissues, causing an adverse pulpal reaction [8]. Generally, the biocompatibility of conventional GICs is considered to be good, with minimal release of organic components [9]. Various studies shows that glass ionomer cements release toxic ingrediants such as fluoride, alumina, silica and leaching of polyacrylic acid which are claimed to be cytotoxic [10-12]. Literatures have shown that electron beam irradiation of dental materials can be used as a tool able to increase the properties of dental materials [13-17].

Radiation is used commonly in the field of biomaterials science for sterilization, surface modification and to improve bulk properties of materials. The energy sources commonly used for the irradiation of biomaterials are high-energy electrons, visible light, gamma radiation and ultraviolet (UV) [18].

Microtron is an high - energy accelerator for protons or electrons which found is to be capable of producing very high currents. A microtron is a cyclotron in which the kinetic energy of electrons is increased by a constant amount per field change [19].

Electron beam irradiation is a method to change the mechanical as well as physical properties of polymers. Investigations of Charlesby and Ross showed that electron beam irradiation can be used a tool for improving the properties of polymers [20]. In general, electron beam irradiation of polymers can give rise to two type of reactions: cross linking, chain breakage [21,22].

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 glassionomer restorative material [15-17]. Hence, this study investigated the effect of electron beam radiation on the cytotoxicity of GC Fugi IX glass ionomer cement (GC Corp, Tokyo, Japan).

Materials and Methods

The electron beam irradiation of glass ionomer cements were conducted with the material listed in [Table-1]. The material were used in accordance to manufacturers instructions.

Table 1- GC IX cement used for the study and their composition

MATERIAL	COMPOSITION
95% flouro-alumino silicate glass	40% polyacrylic acid
5% polyacrylic acid powder	10% polybasic carboxylic acid

The present study was conducted in the following steps:

Erythrocyte Seperation

Blood was collected (Central research lab, Nitte University, India) and the whole blood was drawn by antecubital venipuncture into heparinised vacutainers. 1:1 ratio of histopaque was added and centrifuged at 3000 rpm at 10 min. Erythrocytes were collected from the peripheral blood and then washed three times with 0.85% Nacl saline solution. After washing, cells were centrifuged 150g for 5 min and supernatant were discarded. Finally 2% erythrocyte suspension were prepared by using prechilled distilled water and was used for the study.

Sample Preparation and Irradiation of the Samples for Elution

A total of 20 samples of GC Fugi IX were prepared on rectangular bar shaped specimens of 25-× 2-× 2- mm according to ISO standard -4049 by placing the freshly mixed cements into polytetrafluoroethylene molds held between 2 glass slides.(18) After the material setting, Specimens were removed from mold and kept in 37°c distilled water . The test samples were divided into two categories based on radiation exposure: Radiated Category and non radiated category. A total of 10 samples were exposed to electron beam radiation at 10 KGy. (Microtron, Electron Beam Accelerator, Microtron Centre, Mangalore University). The cement extraction was done for a duration of 24 hours, 7days and 14 days. The test solutions were sterile filtered using a Sterile Filter Unit (0.2µm pore size) (Sartorius Stedim, Biotech, Germany) and was subjected to cytotoxicity assay.

Hemolysis Assay

This assay was performed as per the method described by Black, et al. [19], with slight modification. A 200µl of erythrocyte and 200 µl of sample elute was incubated at 37°C for 1 hour and Centrifuged at 1800rpm for 10min. Optical density was measured at 540nm. The percentage of hemolysis was calculated using the formula,

$$H\% = A_t / A_\alpha \times 100.$$

Statistical analysis was done by using t test.

Results

The result of evaluation of cytotoxicity on electron beam irradiation of GC fugi IX are depicted in [Table-2]. Non radiated samples of Fugi type IX on human erythrocytes showed that the percentage of hemolysis was more in non radiated samples than radiated samples after 24 hours and 14 days. The percentage of hemolysis was reduced in radiated samples after 7 days and 14 days.

Table 2- Hemolytic Effect of GC Fugi IX Before and After Electron Beam Irradiation

GC Fugi IX	24 hrs.	7days	14 days
Non irradiated	78.18±10.13	32.57±12.28	38.56±4.68
Irradiated	58.90±2.28	35.04±1.09	34.26±7.71

Discussion

The need for evaluating the cytotoxicity of restorative material is as important as the assessment of its physiological or mechanical properties. Sufficient biocompatibility of the materials used in the course of the treatment is an essential need as they have to contact or interact with body tissue and fluids.

A glass ionomer cement is a two component system which is commercially available in the form of powder and liquid. Glass ionomer cement forms by a reaction of an acid-decomposable flouroaluminosilicate glass powder with an aqueous solution of acidic polymers such as poly(acrylic acid) or acrylic acid /itaconic acid copolymers. Glass ionomer cement usually achieve a maximum fluoride release 24 h after initial setting and that studies shows that fluoride release has significant potential for toxicity [20-22].

In the present study, the effect of a single standardised dose of electron beam irradiation on cytotoxicity of Fugi GC IX was evaluated with the non-radiated components of the same cement.

Electron beam irradiation is an emerging method for improving the properties of dental materials. It was confirmed that the radiation can increase the stiffness of polymers as well as the links between polymer chains [23].

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

When a 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 [13,14].

In contrast, electron beam irradiation can break the chain of the polymer. The phenomenon occurs when exposed to a high energy dose and specific resins. During reaction, the C-C bonds splits off and the entire polymer structure is broken down and leads to breaking of chains [24].

In the present study, the percentage of hemolysis was more in non radiated samples at 24hr and 14 days of incubation. The reduction in hemolysis after electron beam irradiation of samples may be due to cross linking of unbound acids that is present in Fugi type IX. The percentage of hemolysis was more in non radiated samples may be due to release of unbound acids that was present after the polymerization. It can be also be interpreted that considerable effect of electron beam irradiation had taken place on GC fuji IX GIC which showed minimal cytotoxicity at 10 KGy which could be due to increased cross-linking and less amount of unreacted toxic particles.

Conclusion

The present study noted that if the material used for restorative material can provide cross linking 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 in the field of electron beam irradiation of dental materials so that we may be able to modify and improve the present day dental materials.

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Conflicts of Interest: None declared.

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