Biocompatibility of a Conventional Glass Ionomer, Ceramic Reinforced Glass Ionomer, Giomer and Resin Composite to Fibroblasts: *In vitro* Study

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Objective: *This aim of this study was at compare the fibroblast cytotoxicicty of four restorative materials - a conventional glass ionomer cement (GC Fuji Type II GIC), a ceramic reinforced glass ionomer cement (Amalgomer), a giomer (Beautifil II) and a resin composite (Filtek Z350) at three different time periods (24, 48 and 72 hours).* ***Method****: The succinyl dehydrogenase (MTT) assay was employed. Cylindrical specimens of each material (n=15) were prepared and stored in Dulbecco's modified Eagle medium, following which L929 fibroblasts were cultured in 96 well plates. After 24 hours of incubation, the MTT assay was performed to detect the cell viability. The method was repeated after 48 and 72 hours. The impact of materials and exposure times on cytotoxicity of fibroblasts was statistically analyzed using two way ANOVA (P=0.05).* ***Results****: Both time and material had an impact on cell viability, with giomer demonstrating the maximum cell viability at all time periods. The cell viability in the giomer group was significantly different from all other materials at 24 and 72 hours (P<0.05), while at 48 hours giomer was significantly different only with resin composite (P<0.05).* ***Conclusions****: Giomers showed better biocompatibility than conventional and ceramic reinforced glass ionomer cements and, resin composite. Ceramic reinforced glass ionomer demonstrated superior biocompatibility compared to conventional glass ionomer.*

Keywords: *Biocompatibility, fibroblast, glass ionomer cement, giomer, resin composite*

INTRODUCTION

A wide variety of tooth colored materials are available for restoration of carious lesions. The most commonly used materials are composite resin and Glass Ionomer Cement (GIC).[1] GICs are unique in that they chemically adhere to enamel and dentin, release fluoride and thereby exhibit anti cariogenic properties and have a coefficient of thermal expansion similar to tooth structure; however they do have some inherently deficient properties such as low tensile strength, fracture toughness and brittleness.[2] To overcome these disadvantages, several reinforcing materials have been incorporated into GIC.[3] The most recent introduction in this regard is ceramic reinforced GIC (Amalgomer CR, AHL Generic, UK). The manufacturer claims that this material offers the benefits of GIC combined with the strength of amalgam owing to the ceramic reinforcement. There is evidence showed that this material has physicomechanical properties similar to amalgam. The compressive strength and diametral tensile strength of these material have been claimed to be superior to dental amalgam.[4,5]

The knowledge that fluoride release from dental cements exhibits anticariogenicity, lead to the application of pre-reacted glass ionomer (PRG) filler technology in composite resins. These PRG fillers are prepared by the acid-base reaction between a fluoro-alumino silicate glass and polyacrylic acid in the presence of water. Such polymers reinforced by glass fillers are termed giomers.[6]

For any biomaterial, a property of prime importance is its biocompatibility. The cytotoxicity of resin composite and GIC have been extensively evaluated.[7-9] The biological compatibilities of GIC is not conclusive in that, some reports claim that the material is cytotoxic to fibroblast and macrophages, while some claim the contrary.[10-13] The variation could possibly be explained by the nature of different additives in the GIC studied. Against this background, it is important that the biocompatibility of ceramic reinforced GIC, and giomer, be determined. An extensive review of literature showed us that there is no documented evidence on the cytotoxicity of the aforementioned materials to gingival fibroblasts.

The aim of this study was to analyze the cytotoxicity of ceramic reinforced glass ionomer cement, a giomer, a conventional glass ionomer cement, and a resin composite to gingival fibroblasts *in vitro*. The null hypothesis was that there is no significant difference in viability of fibroblasts exposed to these materials.

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Table 1. Materials used in this study

MATERIAL	COMPOSITION	MANUFACTURER, LOT NUMBER AND SHADE
Group I – Ceramic reinforced glass ionomer cement	Powder: Calcium fluoroaluminosilicate glass, polyacrylic acid powder, zirconia crystals, tartaric acid Liquid: Polyacrylic acid (aqueous solution), tartaric acid, water	Amalgomer CR, AHL generic, UK; Lot number: 020906-56
Group II - Giomer	Bisphenol A Diglycidyl ether dimethacrylate (BIS GMA), Triethylene glycol dimethacrylate, alumino fluoro borosilicate glass, alumina, DL-camphoroquinone	Beautifil II, Shofu Inc., Kyoto, Japan; Lot number: 051003, Shade A2
Group III - Glass ionomer cement	Powder: Calcium fluoroaluminosilicate glass, polyacrylic acid powder, iron oxide Liquid: Polyacrylic acid (aqueous solution), tartaric acid, water	Fuji II, GC Fuji Corporation, Japan; Lot number: 1109091; Shade: Yellow brown
Group IV – Resin composite	Silane treated ceramic, silane treated silica, diurethane dimethacrylate (UDMA), Bisphenol A Polyethylene glycol diether dimethacrylate, Bisphenol A Diglycidyl ether dimethacrylate (BIS GMA), silane treated zirconia, polyethylene glycol dimethacrylate, triethylene glycol dimethacrylate (TEGDMA), 2,6-Di - Tert - Butyl - p-cresol (BHT)	Filtek Z350, 3M ESPE, MN, USA; Lot number: 7KF; Shade: A2

MATERIALS AND METHOD

The materials investigated in the study were

Group 1: Ceramic reinforced glass ionomer cement (Amalgomer CR, AHL generic, UK)

Group 2: Giomer (Beautifil II, Shofu Inc, Kyoto Japan)

Group 3: Glass ionomer cement (Fuji II, GC, Tokyo, Japan)
Group 4: Resin composite (Filtek Z350, 3M ESPE, MN, U.S.A).

The composition of the materials used in the study is given in Table 1. For each test group, 15 cylindrical specimens were prepared by placing the material into a stainless steel mold 2 mm thick and 5mm in diameter. A thin Mylar strip (0.8mm, Mylar type D, DuPont, DE, USA) was placed on top of the specimen, followed by a 1mm glass slide on top of the mold to extrude excess material and eliminate air bubbles. The samples were stored in 1 mL of Dulbecco's modified Eagle medium (DMEM, Sigma Aldrich, St.Louis, MO, USA) with 10% fetal calf serum supplemented with 100 IU/mL of penicillin, 100 µg/mL of streptomycin, and 2% L- glutamine. According to ISO standards, the ratio between the surface of the sample and the volume of medium was 0.5 cm^2/ml. L 929 fibroblasts were plated at 30,000 cells cm^{-2} in 96-well plates (Falcon 3072, Becton Dickinson, Oxford, GB). The 96-well dishes were then placed into a humid incubator with an atmosphere of 5% CO2, 95% air for 24 h before use. After this 24-h period, the medium from the 96-well plates was removed and replaced by the test medium. At that time, the 96-well plates were placed in an incubator again for 24 h. The controls consisted of cells incubated with an equivalent amount of the DMEM.

MTT assay

A succinyl dehydrogenase assay (MTT) was performed on the dishes after 24 h of incubation (i.e. 48 h after the beginning of the experiment). The medium was removed and immediately replaced with 100 µL/well of a 0.5% solution of 3-(4,5-dimethylthiazol-2-yl)-2,(-diphenyl tetrazolium bromide) (Sigma Chemical Co., St. Louis, MO). After incubation for 2 h at 37°C, the supernatant was discarded, and the formazan crystals were solubilized with 100 µl/well of dimethylsulfoxide (DMSO, Sigma Chemical Co.). The absorbance of each 96-well dish was determined using an automatic microplate spectrophotometer (E 960, Bioblock, Strasbourg, France) at 550 nm. The absorbance of the wells containing the same medium was averaged as a single measurement and calculated against the control medium. The same experiment was performed at 24, 48 and 72 hours. The medium was renewed after each day.

Data presentation and analysis

The cytotoxicity of the materials was assessed by measuring cell viability that was determined by the MTT assay. The results were calculated relative to the control (100% = no toxicity). The impact of materials and exposure times on cytotoxicty of fibroblasts was statistically analyzed using two wav ANOVA. The alpha type error was set at p=0.05.

RESULTS

The percentage viability of fibroblasts exposed to the test materials at the three exposure times is presented (Table 2). All the materials showed a reduction in cell viability. Two way ANOVA showed that both the variables (material and time) had a significant impact on cell viability. All materials demonstrated significantly higher cell viability in the 24 hour period ($P < 0.05$). Group 2 (Giomer) demonstrated the maximum cell viability at all three periods. The maximum cell viability was demonstrated by giomer at 24 hours (78 ± 1.0%), while the least cell viability was shown by resin composite at 72 hours (28.60±0.54%). The cell viability demonstrated by giomer was significantly different from the other materials in the 24 hour and 72 hour samples ($P < 0.05$). In the 48 hour samples, giomer showed a significant difference only with group 4 - resin composite ($P < 0.05$).

DISCUSSION

The present study compared the biocompatibility of four restorative materials (conventional GIC , ceramic reinforced GIC, giomer and resin composite) to L 929 fibroblasts in vitro, at three different time periods (24, 28 and 72 hours). Cell viability in this study was

Table 2. Percentage viability of fibroblasts (Percentage, means ± standard deviations) exposed to the test materials (n=15) at three exposure times

GROUPS	24 hours	48 hours	72 hours
Group 1 (Ceramic reinforced glass ionomer cement)	78 ± 1.0 [a,A]	66.40 ± 1.51[b, B]	56.50±0.14 [c, C]
Group 2 (Giomer)	81.80 ± 1.10 [b, A]	67.20 ± 1.30 [b, B]	61.40± 0.78 [d, C]
Group 3 (Type II glass ionomer cement)	71.80 ± 1.02 [c, A]	66.40±1.14 [b, B]	53.60±1.19 [e, C]
Group 4 (Resin composite)	42.20 ± 2.16 [d,A]	34.80± 0.45 [c,B]	28.60±0.54 [f,C]

Mean values that share a superscript lower case or upper case letter were not significantly different within the same time point or at different time points respectively (p<0.05)

determined by MTT assay. This assay is based on the reduction of MTT by cells that remain viable after exposure and incubation with a test chemical. This assay is based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble tetrazolium salt MTT into dark blue formazan crystals. The advantages of this method are the simplicity, rapidity, and precision.[14]

Analysis of the data showed that giomer was the most biocompatible material at all three time periods. Also, there was a significant difference in the biocompatibility of the tested materials at all time periods except at 48 hours wherein giomer showed significant difference only with resin composite. The biocompatibility of the materials tested was as follows: Giomer > Ceramic reinforced glass ionomer > Conventional glass ionomer > Composite resin.

The giomer used in this study was Beautifil II (Shofu Inc) which is basically a nanohybrid composite resin incorporating S-PRG fillers and fluoro-boroaluminosilicate glass nanofillers, both of which serve as fluoride sources. The S-PRG in giomer has the glass-ionomer reaction limited to the surface of the glass. The quantity of fluoride ions released from fluoride-releasing resin composites is significantly lower than that from glass-ionomer cements, giomers and compomers.[15,16] We hypothesize that the fluoride release of these resin composites is also lower than those of giomers. In the case of giomers, it is likely that fluoride released from these materials was caused by dissolution of the inorganic fluorides contained within these glasses.[6] The giomers contain little or no glass ionomer hydrogel layer and hence, the fluoro boroaluminosilicate glass and S-PRG fillers may be readily eroded and disintegrated by lactic acid. These materials have shown to release Sr, F, Na, B and Si ions,[6] and, it has been demonstrated that the release of Si ions induces formation of a bone-like apatite layer.[17] However, the exact implications of these ions on the biocompatibility is unknown. This warrants further research.

Release of aluminum and fluoride ions from glass ionomer cements may have stimulatory or inhibitory effect on cells.[18] Although there appears to be no consensus on the biocompatibility of the specific ions, it is generally considered that metal ions are cytotoxic to fibroblasts. An earlier study demonstrated that non-fluoride glasses were least toxic to cells *in vitro*.[19] Conventional glass ionomer cements have been shown to release more fluoride than giomers.[20] In addition to the release of ions, low pH of the cement during setting and maturation can also induce cytotoxicty.[18]

Ceramic reinforced glass ionomers showed better biocompatibility than resin composites and conventional glass ionomers. These materials, commercially available as Amalgomer (AHL generic, UK), contain zirconia as the major reinforcing filler. Although the primary objective of addition of zirconia appears to be towards strengthening and toughening the material owing to its phase transformation from tetragonal to monoclinic during stress, we hypothesize that the addition of zirconia could have also enhanced the biocompatibility of this material. Furthermore, the ceramic filler has been claimed to react partially with the matrix, resulting in an altered polysalt matrix. It appears that this reaction may also reduce the leaching of glass fillers in the medium.[5] In general, quartz and silica based glass fillers are more stable than fillers that contain barium based glasses.[1]

The cytotoxicity of resin composites has been well established in several studies. Triethyleneglycol dimethacrylate (TEGDMA) and 2-hydroxy- ethyl methacrylate (HEMA) in resin monomers have been shown to be cytotoxic and allergenic. These monomers have been established to induce apoptosis *in vitro* and hence suggested to be toxic to the dental pulp and human gingival fibroblasts. The resin composite tested in this study was Filtek Z350. The material does not contain HEMA in its composition. HEMA can be a degradation product from UDMA (Urethane dimethacrylate), which is present in the resin composite.[20] HEMA being a monomer of low molecular weight can easily spread into the buccal fuids in the clinical scenario. The time dependent cytotoxicity of these materials is in accordance with published work.[21]

CONCLUSIONS

Within the limitations of this *in vitro* study, giomers demonstrated the least cytotoxicity to fibroblasts, followed by ceramic reinforced glass ionomer and conventional glass ionomer. The cytotoxicity was time dependent, with the fibroblast survival of all materials differing significantly in the 24 and 72 hour period (P<0.05) while in the 48 hour group, there was significant difference between giomer and resin composite only (P<0.05). Further research is needed to characterize the components of these materials that influence the biocompatibility to fibroblasts. Long term studies and animal studies are needed to evaluate the biocompatibility of these materials to other cell types.

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Disclosure Statement

We affirm that we have no financial affiliation or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed.

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