Comparison of Cytotoxicity of New Nanohybrid Composite, Giomer, Glass Ionomer and Silver Reinforced Glass Ionomer using Human Gingival Fibroblast Cell Line

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Aim: *The objective of this study was to investigate the cytotoxic effects of new nanohybrid composite, giomer, conventional and resin modified and silver reinforced glass ionomer cements and compare the biocompatibility of these dental materials in cell culture.* ***Study design***: *Five cylindrical specimens were made of each material, using a mold (2mm. thick and 5 mm in diameter). For HGF, cells were cultured in RPMI-1640 medium. After attaining 80% confluence, cells were treated with different doses of five tested materials for 24h. Then cell cytotoxicity was assessed using MTT assay. The data were analyzed using Kruskal-Wallis and Dunn test.* ***Results***: *The materials evaluated on HGF cells, showed significantly more cytotoxicity in silver reinforced glass ionomer but nanohybrid composite shows mild cytotoxic effect. However, giomer shows no significant cytotoxicity and conventional and resin modified glass ionomer enhance cell proliferation.* ***Conclusions***: *Silver reinforced glass ionomer induced a significant high cytotoxic effect over a wide range of concentration. Therefore, higher attention should be focused on this restorative dental material, which should be chosen for further investigations.*

Key words: *Biocompatibility, HGF, MTT Assay, Restorative Dental Material*

INTRODUCTION

During the last decades the use of amalgam has been critically discussed due to its allergic and toxic potential upon mercury release.[1] Therefore, an increasing variety of dental restorative materials have conquered the market. The decreased number of amalgam restorations is also affected by a high demand for tooth-colored and biocompatible restorations.[1,2] Great strides in dental research have led to a wide range of alternatives to amalgam. Different types of direct restorative materials are used in routine dental practice.[3] The most common, are resin composites and glass ionomer cements. Resin composites are the most aesthetically accepted material with satisfactory physical properties. Filtek Z250 XT is a nanohybrid resin composite that introduced with new technology of nanosciences. They have also an expensive, time consuming and technique sensitive adhesive procedure.[4] Glass ionomer cements may be used in a variety of clinical applications due to the capability to modify their properties by changing the powder/liquid ratio or their formulation.[5] The glass ionomer cements are esthetically more attractive than metallic restorations. In addition, by incorporating fluorine, they show an anticariogenic potential, and they have good chemical adhesion to dental hard tissue.[6-8] Further development of glass ionomer cements led to the development of hybrid versions of these materials known as resin modified glass ionomer cements. It is believed that resin modified glass ionomer cements combine the main advantages of glass ionomer cements such as adhesion to hard dental structure, fluoride release and biocompatibility, with easy handling of light

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polymerized composites.[9] A new fluoride releasing light cured restorative material containing pre-reacted glass ionomer fillers, known as giomer, has been introduced based on the incorporation of pre-reacted glass ionomer fillers.[10] According to the claims of the manufacturer, giomer shows the advantages of a resin composite and a glass ionomer. It has been shown that these hybrid materials can provide almost a perfect seal against bacterial leakage, cause less mechanical and chemical pulpal irritation and inhibited demineralization.[11,12] In a study, it was shown that the two year clinical performance of giomer fillings was similar or slightly better than that of resin ionomers and compomers.[13] Due to the high success rate of giomers in class V restorations, it is considered that these materials can be used in restoring of carious root surfaces.[14] Restorative dental materials are placed in close contact with living tissues of oral cavity. After time, the composition of the restorative materials changes due to chemical and mechanical degradation in the oral cavity. They also have influences on the health of oral tissues in different ways especially by delivering water soluble components into saliva/oral cavity as well as by interacting with adjacent tissues such as epithelia of gingiva and its connective tissue.[15] It seems that, the organic matrix of the dental composite resins, when released into the oral cavity can cause a wide range of adverse biological reactions such as mucosal irritation, epithelial proliferation, oral lichenoid reaction, hypersensitivity and may also cause fibrosis of the adjacent soft tissue.[15,16] Thus the biological and toxic characteristics of the dental materials must be compatible with the oral tissues and even with general health. Therefore, the need for biocompatible dental material implies the necessity of toxicity testing.[17]

Since the knowledge around the cytotoxicity of new dental materials towards human gingival fibroblasts is limited, this study aimed to assess the biocompatibility of giomer and silver reinforced glass ionomer on human gingival fibroblasts and compare it with the biocompatibility of a nano hybrid composite and glass ionomer cements.

MATERIAL AND METHOD

The composition of the materials used in this study is shown in table 1. For each test group, 5 cylindrical specimens were prepared by placing the material into stainless steel mold 2 mm thick and 5 mm in diameter. The composite, giomer and resin modified glass ionomer disks were well cured (VIP Junior, Bisco, Schaumburg, IL) at 600 mW/cm^2. Silver reinforced glass ionomer and conventional glass ionomer cement were also prepared. All specimens were made following the manufacturer's instruction. After sterilization at 170ºC for one hour, the disks were immersed in 2.4 ml RPMI-1640 medium supplemented from the disks. Sterilization was performed to avoid contamination of RPMI-1640 medium. Cytotoxicity was tested using the original extract solution along with a series of dilutions. Original extract solution is cell culture medium that specimens were immersed in. The original extract was diluted with fresh medium at dilutions of 1-fold or 100%W/W (1 part of original extract), 1.5-fold or 75%W/W (3 part of original extract + 1 part of fresh medium), 2-fold or 50%W/W (1 part of original extract + 1 part of fresh medium) and 4-fold or 25%W/W (1 part of original extract + 3 part of fresh medium), which were then used for the MTT assay.[18-20]

Cell line, cell culture and restorative dental materials treatment

HGF, human gingival fibroblast cell line, was obtained from the National Cell Bank of Iran (NCBI), Pasteur Institute of Iran. HGF cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mM glutamine, penicillin (100 IU/ml) and streptomycin (100 mg/ml) (All from Gibco, Scotland) at 37ºC in an incubator containing 5% CO2. Harvested cells with trypsin (0.25%) (Sigma, USA) were calculated by neobar slide with trypan blue and then were seeded into 96-well plates (1 × 104 cells/well). The cells were incubated with different concentrations of restorative dental materials at 24 h. Each concentration was examined on six wells of the 96-well plates containing 1 × 10^4 HGF cells. In each experiment, six HGF cultured wells with no restorative dental materials incubation were used as negative controls. [21]

Determination of cell viability

The cell viability was characterized by methyl thiazolyl tetrazolium bromide (MTT, Sigma, USA) assay. The MTT dye was solved in PBS (phosphate buffer saline) at a concentration of 5 mg/ml. Then 10 ml of MTT solution was added to each well containing 100 ml cultured medium. Dissolved yellowish MTT is converted to an insoluble purple formazan by mitochondrial dehydrogenase enzymes during 5 h incubation. The produced insoluble formazan was dissolved in solution containing 100 ml isopropanol (Merck, Germany) and its optical density (OD) was read with an ELISA reader (Organon Teknika, Netherlands) at a wavelength of 540 nm. Six HGF cultured wells were incubated with 100 ml DDW (deionized distilled water) for 10 min and used as positive control and six HGF cultured wells with no sample as negative controls. The percentage of cytotoxicity was calculated according to following formulas:[22]

$$\%\text{Cytotoxicity} = \frac{\text{mean absorbance of negative control}}{1 - \text{mean absorbance of toxicant treated cells}} \times 100$$

$$\%\text{Viability} = 100 - \%\text{Cytotoxicity}$$

Statistical analysis

Results were expressed as mean ±SD. Statistical evaluation of cytotoxicity of five restorative dental materials were assessed by Kruskal-Wallis followed by Dunn's test using by SPSS 20.0 software (SPSS Inc. USA). $P < 0.05$ was considered for statistical significance.

RESULTS

In this study, we determined the cytotoxicity of five dental restorative materials by treating the HGF cells with various concentrations of these materials (0–100W/W) for 24h followed by MTT assay. Compared to the controls, the lower dose of nano hybrid composite, giomer, conventional glass ionomer, resin modified glass ionomer, and silver reinforced glass ionomer (25W/W)–88.02% ($P < 0.01$), 99.04% ($P > 0.05$), 100.80% ($P > 0.05$), 98.45% ($P > 0.05$) and 98.46% ($P > 0.05$)–and its higher dose (100W/W)–85.93% ($P < 0.001$), 97.75% ($P > 0.05$), 134.86% ($P < 0.05$), 112.29% ($P < 0.01$) and 45.1% ($P < 0.01$)–decreased in total cell number, respectively (Fig. 1). The percentage viability of HGF cells and pairwise comparison for restorative materials at the same concentration are presented in table 2 and table 3, respectively.

Table 1. Test materials name, types, manufacturers, lot numbers and components

Name	Manufacture	Composition
Nano hybrid composite (Filtek Z250 XT)	3M ESPE (USA)	Organic: Bis-GMA, UDMA, Bis-EMA, PEGDMA, TEGDMA, Water Inorganic: 82% (w/w) Zirconia/silica
Giomer	SHOFU (Japan)	Organic: Bis-GMA, TEGDMA Inorganic: 83% (w/w) Multi-functional glass and S-PRG filler based on fluoroboraluminosilicate glass
Conventional glass ionomer (Fuji II)	GC (Japan)	Powder: Flouroaluminosilicate glass Liquid: Polyacrylic acid, 2-Hydroxyl ethyl methacrylate, Urethane dimethacrylate, Camphorquinone, Water
Resin modified glass ionomer (Fuji II LC)	GC (Japan)	Powder: Flouroaluminosilicate glass Liquid: Polyacrylic acid, Itaconic acid, Tartaric acid, Maleic acid, Water
Silver reinforced glass ionomer (HI- DENSE XP)	SHOFU (Japan)	Material type: silver cermet cement

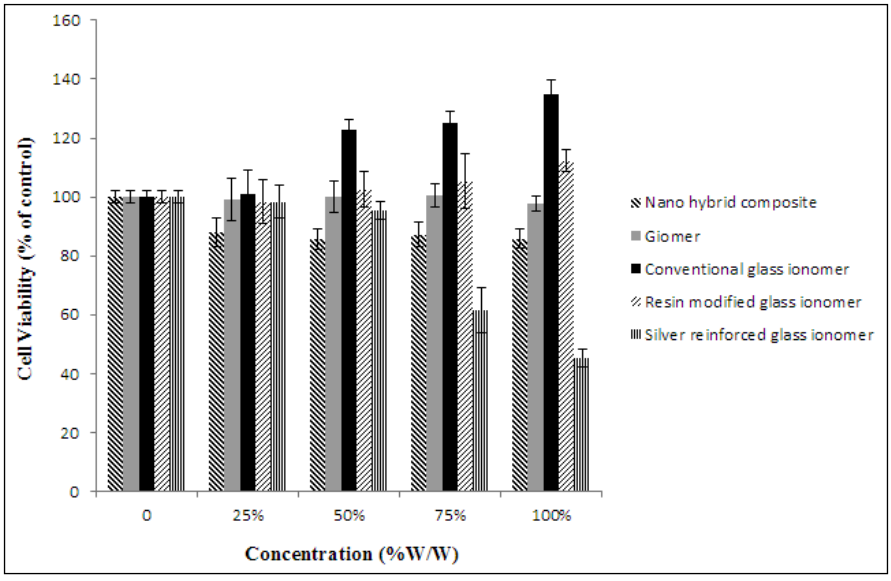
Table 2. The percentage viability of HGF cells exposed to the tested materials (n=6) at 24h

Concentration	Nano hybrid composite	Giomer	Conventional glass ionomer	Resin modified glass ionomer	Silver reinforced glass ionomer
25%	88.02%±5.05 (*P*=0.002)	99.04%±7.16 (*P* =0.893)	100.80%±8.17 (*P* =0.948)	98.45%±7.24 (*P* =0.883)	98.46%±5.72 (*P* =0.431)
50%	85.67%±3.61 (*P*=0.000)	99.94%±5.38 (*P* =0.922)	122.64%±3.76 (*P* =0.036)	102.50%±6.16 (*P* =0.491)	95.46%±3.17 (*P* =0.168)
75%	87.29%±4.12 (*P*=0.001)	100.55%±3.88 (*P* =0.831)	125.15%±3.92 (*P* =0.011)	105.41%±9.16 (*P* =0.272)	61.40%±7.65 (*P* =0.002)
100%	85.93%±3.36 (*P* =0.000)	97.75%±2.65 (*P* =0.781)	134.86%±4.65 (*P* =0.000)	112.29%±3.85 (*P* =0.003)	45.30%±3.12 (*P* =0.000)

Table 3. Dunn test for pairwise comparison for restorative materials at the same concentration

Tested material	Code	Concentration	Significance **P*≤0.05, ***P*≤0.01, *** *P*≤0.001
Nano hybrid composite	A		
Giomer	B	25%	A,B**
Conventional glass ionomer	C	50%	A,D* B,C* A,B** A,C** C,E***
Resin modified glass ionomer	D	75%	B,C* B,E* D,E* A,B*** C,E*** C,D***
Silver reinforced glass ionomer	E	100%	C,D* C,E* A,B** B,C*** B,E*** D,E***

Figure 1. Cytotoxiciy of five restorative dental materials on the HGF cells. Results are expressed as a percentage of viability compared to control and are presented as mean±SD from at least six independent experiments.



DISCUSSION

Non-amalgam filling materials in dentistry include a wide range of components, which may be released in the surrounding tissues and show biologic activity in the organism.[23] However, little data exist on the adverse effects of these compounds.[24] Restorative materials must be biocompatible to minimize the harmful effects on dental tissues enforce by direct contact. We compared the cytotoxic effects of new nanohybrid composite, giomer, conventional and resin modified and silver reinforced glass ionomer cements indicates that probably only giomer demonstrated safe condition between the various materials.

Glass ionomers are favorable restorative materials due to their ease of use and unique biocompatibility among direct restorations. However, brittleness, inferior abrasion resistance and strength, toughness and fatigue performance currently contraindicates the application as permanent filling materials in load bearing areas.[25] Results of MTT cytotoxicity assay of resin modified glass ionomer and conventional glass ionomer, on fibroblast cell line used in this study, showed no cytotoxic effect. Of course, these materials show increase in cell proliferation. It was demonstrated in different studies that resin modified glass ionomer cement showed some degree of cytotoxicity to cultured human gingival fibroblasts by inhibiting cell growth, attachment and proliferation. The differences in the results between those studies and our study might be due to the different methods and materials such as the way to prepare specimens.[26-28] One other reason can be the different method for sterilization the specimens. It was performed to avoid contamination of cell culture medium. Also, some previous studies indicated that some of tooth colored dental restorative materials can cause adverse effects such as cancerogenic, mutagenic and genotoxic changes that might increase cell proliferation. Possible genotoxic effects of these materials can represent a possible step in cell proliferation and tumor initiation. Cytotoxic and tumorigenic effects of xenobiotics may be proved with in vivo studies and after long time intervals. This finding was unexpected, and proves that there are some discrepancies in literature about the amount of cytotoxicity after the placement of different glass ionomer cement and resin modified glass ionomer. Furthermore, materials within the same category may not have behaved in a similar way. Variations in the ion release occur in different products. For example, differences in the pattern and amount of fluoride released were demonstrated among various commercial products.[26]

The methacrylate based composites are popularly and widely used, efforts are being made to dominate the clinical shortcomings by modern development and refocusing from the filler content to the matrix resin. More recently, the term nanohybrid has been introduced; Companies augment composites with adding nanoparticles to microhybrids to fill the resin gaps between the larger particles. The performance of a composite material is largely dependent upon the fillers it employs. Generally, a composite that has smaller fillers is more polishable and retains its polish better than one containing larger particles. Also, commonly a composite with a higher filler loading provides stronger mechanical properties. However, because they offer better performance, versatility, and a reasonable cost, they are a popular choice for dentists.[29] Earlier, it has been demonstrated that releasing monomer from composite resins is complete in 24 h.[30,31] Therefore, toxic effects from composite resins happen during this time. In this study we showed that significant cytotoxicity was found in all concentrations of nano hybrid composite. These findings can be related with other reports on the induction of oxidative stress caused by TEGDMA and other compounds of resin-based dental restorative materials or common photosensitizes.[32] Concentration of unpolymerized materials and time are the key factors in determining biotoxicity.[33] However, more studies of the effect of residual monomers on apoptosis, oxidative stress, and cellular mechanism, need to be clarifying, so that the cytotoxicity of resinous materials or monomers can be analyzed.[34] Filtek™ Z250 XT nanohybrid universal restorative is a visible light-activated nanohybrid composite designed for both anterior and posterior restorations. Present result is in agreement with the observations of others in previous studies. The cytotoxicity level of resin-based dental composites depends on their chemical composition, leaching medium, and the amount and type of the ingredients that can be extracted from the materials. There is limited evidence from long-term clinical studies of problems resulting in pulp and soft tissue changes using dental composites. However, there are contradictory reports on the cytotoxicity of these dental materials.[15]

In the present study, silver reinforced glass ionomer is the most toxic material.one idea to enhance cement strength and toughness was to merge metallic particles into the glass ionomer cement matrix. Silver powder is premixed for easy homogeneous mixing. The polycarboxylic acid when mixed with the powder produces a plastic paste that gradually hardens with time. Furthermore, it appears that this reaction may also reduce the leaching of glass fillers in the medium.[17] Silver reinforced glass ionomer created for some purposes for example, in non-stress bearing permanent teeth, core build-up and repair of previous impaired restorations. Although we cannot find any paper about the cytotoxicity of this type of silver reinforced glass ionomer, but it seems that silver-tin alloy particles in this specially formulated glass ionomer powder, possibly is the main factor of cytotoxicity in this material. However silver powder that mixes for produce easy homogeneous mixing can cause some degree of cytotoxicity.

In our study, giomer demonstrated acceptable results in cytotoxicity tests. However, there might be some possible explanations such as favorable cellular response in giomer might be attributed to their surface structure, as the surface characteristics of the final restoration can often determine the biocompatibility of the material. The value and nature of leachable compounds in resinous materials can influence their biocompatibility; as a result, lesser degree of toxic substances into the medium by those restorative materials is another possible reason. In resin base materials, monomer-polymer conversion is a very important factor in biocompatibility of resinous materials.[24-26]

It is important to reevaluate chemical, physical, and biological characteristics of these dental materials, since it is obvious that they should meet the requirements either from the standpoint of biosafety, or even from the standpoint of longevity of restorations. In vitro tests are often preferred to quantify biocompatibility aspects in the early steps of the evaluation of a newly presented material, considering time, expense and ethics. While in vitro researches are simpler to conduct, their validity can only be proved by careful and meticulous in vivo studies. The reliability of using cell culture models to test the biocompatibility of dental materials is well established with the help of cultured fibroblast from human oral and dental tissues such

as pulp, gingiva, skin, buccal mucosa, periodontal membrane, oral epithelium and commercially existing cell lines. Human gingival fibroblasts are most frequently indicated in the biological tests of dental materials.[35,36] Like other tissues, normal fibroblast function is critical to obtain the periodontal tissue function for optimal healing. Gingival fibroblasts were selected due to their accessibility and culturing properties.[37] The point that researchers usually do not know the exact composition of materials being tested recommended further studies to evaluate cytotoxicity of the restorative materials for extended period of time and to resemble clinical conditions. In addition, SEM growth assay would seem to be useful in assessing the changes in the morphology of human gingival fibroblasts.

CONCLUSION

Silver reinforced glass ionomer induced a significant high cytotoxic effect over a wide range of concentration. Therefore, higher attention should be focused on this restorative dental material, which should be chosen for further investigations. Nanohybrid composite shows adverse effects in all concentrations and giomer shows no significant cytotoxic effects. The differences in the results about two types of glass ionomers between our study and the previous studies might be due to the different methods and materials such as the way to prepare specimens.

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REFRENCES

1. Lohbauer U. Dental glass ionomer cements as permanent filling materials?–Properties, limitations and future trends. *Materials.* 2009;3(1):76-96.
2. Shenoy A. Is it the end of the road for dental amalgam? A critical review. *J Conserv Dent.* 2008;11(3):99-107.
3. Braem M, Lambrechts P, Vanherle G. Clinical relevance of laboratory fatigue studies. *Journal of dentistry.* 1994;22(2):97-102.
4. Taha NA, Ghanim A, Tavangar MS. Comparison of Mechanical Properties of Resin Composites with Resin Modified Glass Ionomers. *Journal of Dental Biomaterials.* 2015;2(2):47-53 %@ 2383-2398X.
5. Nagaraja Upadhya P, Kishore G. Glass ionomer cement: the different generations. *Trends Biomater Artif Organs.* 2005;18(2):158-165.
6. Cho SY, Cheng AC. A review of glass ionomer restorations in the primary dentition. *Journal (Canadian Dental Association).* 1999;65(9):491-495.
7. Dionysopoulos D. The effect of fluoride-releasing restorative materials on inhibition of secondary caries formation. *Fluoride.* 2014;47(3):258-265 %@ 0015-4725.
8. Manhart J, Garcia-Godoy F, Hickel R. Direct posterior restorations: clinical results and new developments. *Dental clinics of North America.* 2002;46(2):303-339.
9. Selimovic-Dragas M, Huseinbegovic A, Kobaslija S, Hatibovic-Kofman S. A comparison of the in vitro cytotoxicity of conventional and resin modified glass ionomer cements. *Bosnian journal of basic medical sciences / Udruzenje basicnih mediciniskih znanosti = Association of Basic Medical Sciences.* 2012;12(4):273-278.
10. Mousavinasab SM, Meyers I. Fluoride release by glass ionomer cements, compomer and giomer. *Dental research journal.* 2009;6(2):75-81.
11. Kimyai S, Mohammadi N, Oskoee PA, et al. Effects of surface treatments of conventional glass-ionomer on shear bond strength to giomer. *Dental research journal.* 2012;9(6):700-705.
12. Morales-Chavez MC, Nualart-Grollmus ZC. Retention of a resin-based sealant and a glass ionomer used as a fissure sealant in children with special needs. *Journal of clinical and experimental dentistry.* 2014;6(5):e551-555.
13. Jyothi K, Annapurna S, Kumar AS, Venugopal P, Jayashankara C. Clinical evaluation of giomer- and resin-modified glass ionomer cement in class V noncarious cervical lesions: An in vivo study. *J Conserv Dent.* 2011;14(4):409-413.
14. Gordan VV, Blaser PK, Watson RE, et al. A clinical evaluation of a giomer restorative system containing surface prereacted glass ionomer filler: results from a 13-year recall examination. *Journal of the American Dental Association (1939).* 2014;145(10):1036-1043.
15. Madhyastha PS, Naik DG, Kotian R, Padma D, Srikant N, Bhat KM. Evaluation of Cytotoxicity of Silorane and Methacrylate based Dental Composites using Human Gingival Fibroblasts. *J Clin Diagn Res.* 2015;9(1):Zc05-08.
16. McParland H, Warnakulasuriya S. Oral lichenoid contact lesions to mercury and dental amalgam—a review. *Journal of biomedicine & biotechnology.* 2012;2012:589569.
17. Mousavinasab SM. Biocompatibility of composite resins. *Dental research journal.* 2011;8(Suppl 1):S21-29.
18. Li F, Weir MD, Chen J, Xu HH. Comparison of quaternary ammonium-containing with nano-silver-containing adhesive in antibacterial properties and cytotoxicity. *Dental materials : official publication of the Academy of Dental Materials.* 2013;29(4):450-461.
19. Salehi S, Gwinner F, Mitchell JC, Pfeifer C, Ferracane JL. Cytotoxicity of resin composites containing bioactive glass fillers. *Dental materials : official publication of the Academy of Dental Materials.* 2015;31(2):195-203.
20. Tamilselvam S, Divyanand MJ, Neelakantan P. Biocompatibility of a conventional glass ionomer, ceramic reinforced glass ionomer, giomer and resin composite to fibroblasts: in vitro study. *The Journal of clinical pediatric dentistry.* 2013;37(4):403-406.
21. Mokhtari MJ, Akbarzadeh A, Hashemi M, et al. Cisplatin Induces Up-Regulation of KAI1, a Metastasis Suppressor Gene, in MCF-7 Breast Cancer Cell Line. *Tropical Journal of Pharmaceutical Research.* 2012;11(4):523-529 %@ 1596-9827.
22. Mokhtari MJ, Motamed N, Shokrgozar MA. Evaluation of silibinin on the viability, migration and adhesion of the human prostate adenocarcinoma (PC-3) cell line. *Cell biology international.* 2008;32(8):888-892.
23. Schwengberg S, Bohlen H, Kleinsasser N, et al. In vitro embryotoxicity assessment with dental restorative materials. *Journal of dentistry.* 2005;33(1):49-55.
24. Gupta SK, Saxena P, Pant VA, Pant AB. Release and toxicity of dental resin composite. *Toxicology international.* 2012;19(3):225-234.
25. Choudhary K, Nandlal B. Comparative evaluation of shear bond strength of nano-hydroxyapatite incorporated glass ionomer cement and conventional glass ionomer cement on dense synthetic hydroxyapatite disk: An in vitro study. *Indian journal of dental research : official publication of Indian Society for Dental Research.* 2015;26(2):170-175.
26. Pourabbas R, Farajnia S, Kimya S, Mohammadnejad L, Johnson A, Nejatian T. In vitro assessment of cytotoxicity of giomer on human gingival fibroblasts. *African Journal of Biotechnology.* 2009;8(20 %@ 1684-5315).
27. Al-Sabek F, Shostad S, Kirkwood KL. Preferential attachment of human gingival fibroblasts to the resin ionomer Geristore. *Journal of endodontics.* 2005;31(3):205-208.
28. Huang FM, Tai KW, Chou MY, Chang YC. Resinous perforation-repair materials inhibit the growth, attachment, and proliferation of human gingival fibroblasts. *Journal of endodontics.* 2002;28(4):291-294.
29. Nayak RS, Valecha S, Pasha A, Mamatha J, Khanna B, Shafiuddin B. A Comparison of Shear Bond Strength of New Nanofilled Composite and Nano-Ionomer Restorative Materials with Traditional Adhesive Material for Orthodontic Bracket Bonding: An In Vitro Study. *Journal of International Oral Health.* 2015;7(10):70 %@ 0976-1799.
30. Cramer NB, Stansbury JW, Bowman CN. Recent advances and developments in composite dental restorative materials. *Journal of dental research.* 2011;90(4):402-416.
31. Archegas LRP, Rached RN, Ignacio SA, Vasconcelos ECd, Ramos DT, Souza EMd. Identification and quantification of monomers released from dental composites using HPLC. *Brazilian Archives of Biology and Technology.* 2009;52(4):855-862 %@ 1516-8913.
32. Gociu M, Patroi D, Prejmerean C, et al. Biology and cytotoxicity of dental materials: an in vitro study. *Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie.* 2013;54(2):261-265.
33. Theodore M, Harald O, Edward J. Sturdevant's art and science of operative dentistry. *Mosby.* 2006;5:807-840.
34. Jiao Y, Ma S, Wang Y, Li J, Shan L, Chen J. Epigallocatechin-3-Gallate Reduces Cytotoxic Effects Caused by Dental Monomers: A Hypothesis. *Medical science monitor : international medical journal of experimental and clinical research.* 2015;21:3197-3202.
35. Samyuktha V, Ravikumar P, Nagesh B, Ranganathan K, Jayaprakash T, Sayesh V. Cytotoxicity evaluation of root repair materials in human-cultured periodontal ligament fibroblasts. *J Conserv Dent.* 2014;17(5):467-470.
36. Moharamzadeh K, Brook IM, Van Noort R. Biocompatibility of resin-based dental materials. *Materials.* 2009;2(2):514-548.
37. McCulloch CA. Origins and functions of cells essential for periodontal repair: the role of fibroblasts in tissue homeostasis. *Oral diseases.* 1995;1(4):271-278.