Frontiers in Dentistry

Cytotoxic Effect of Addition of Different Concentrations of Nanohydroxyapatite to Resin Modified and Conventional Glass Ionomer Cements on L929 Murine Fibroblasts

Parisa Golkar[1,2], Ladan Ranjbar Omrani[1,3], Shide Zohourinia[4], Elham Ahmadi[1,3], Faeze Asadian[3,5*]

1. Department of Operative Dentistry, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
2. Dental Materials Research Center, Department of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
3. Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
4. Private Practice, Tehran, Iran
5. Department of Restorative Dentistry, School of Dentistry, Birjand University of Medical Sciences, Birjand, Iran

Article Info

Article type:
Original Article

Article History:
Received: 14 Nov 2020
Accepted: 23 Apr 2021
Published: 17 May 2021

**** Corresponding author:***
Dental Research Center, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Email: faezeasadian@gmail.com

A B S T R A C T

Objectives: In this study we assessed the cytotoxic effect of nanohydroxyapatite (NHA) incorporated into resin modified and conventional glass ionomer cements (RMGICs and CGICs) on L929 murine fibroblasts.

Materials and Methods: In this in vitro study, 0wt%, 1wt%, 2wt%, 5wt%, 7wt% and 10wt% concentrations of NHA were added to Fuji II LC RMGIC and Fuji IX CGIC powders. Eighteen samples (5×3mm) were fabricated from each type of glass ionomer, in six experimental groups (n=3): CG_0, CG_1, CG_2, CG_5, CG_7, CG_{10}, RMG_0, RMG_1, RMG_2, RMG_5, RMG_7, and RMG_{10}. Samples were incubated for 72h. The overlaying solution was removed and added to L929 fibroblasts. The methyl thiazolyl tetrazolium bromide (MTT) assay was performed at 24, 48 and 72h. The wavelength was read by a spectrophotometer. Data were analyzed by ANOVA and Tukey's test.

Results: There was no significant difference in cytotoxicity of the two types of glass ionomers, with and without NHA, except for CG_0 and RMG_0 groups after 72h. RMG_0 group was significantly more cytotoxic than the CG_0 group ($P<0.05$). In CG groups during the first 24h, the cytotoxicity of CG_5 and CG_7 groups was significantly higher than that of CG_1; while, there was no significant difference between the RMG groups. Cytotoxicity significantly decreased in all groups after 24h ($P<0.05$).

Conclusion: Incorporation of NHA into Fuji II LC RMGIC and Fuji IX CGIC did not affect their biocompatibility and therefore its addition to these materials can provide favorable biological properties, especially considering its beneficial effects on the other properties of GICs.

Keywords: Hydroxyapatites; Glass Ionomer Cements; Fibroblasts; Toxicity Tests

➢ **Cite this article as:** Golkar P, Ranjbar Omrani L, Zohourinia Sh, Ahmadi E, Asadian F. Cytotoxic Effect of Addition of Different Concentrations of Nanohydroxyapatite to Resin Modified and Conventional Glass Ionomer Cements on L929 Murine Fibroblasts. *Front Dent.* 2021; 18: 17.

INTRODUCTION

Conventional glass ionomer cements (CGICs) were first introduced in 1970 to replace silicate cements in dentistry. These cements have extensive applications in dentistry due to favorable properties such as fluoride release,

antibacterial effect, chemical bond to tooth structure, having a coefficient of thermal expansion similar to that of tooth structure, modulus of elasticity similar to that of dentin, and biocompatibility. However, these cements have some drawbacks as well such as high technical sensitivity, lower esthetics compared with composite resins, and low mechanical properties, which limit their clinical application. Due to the brittleness and low compressive strength, CGICs cannot be used as restorative materials in occlusal load-bearing areas [1,2]. To overcome the limitations of CGICs, resin-modified glass ionomer cements (RMGICs) were developed. RMGICs have lower moisture sensitivity, enhanced mechanical strength, extended working time, and easy clinical handling; however, the reported results were not in acceptable range. Additionally, they were found to be more cytotoxic than CGICs due to the presence of 2-hydroxyethyl methacrylate monomer in their composition [3].

Recently, application of nano-sized bio-material particles was proposed to improve the mechanical properties of glass ionomers, such as addition of nano-TiO2, nano-hydroxyapatite (NHA), and silver nano-particles [4-6].

Hydroxyapatite is a naturally occurring mineral, and is the major component of bone and tooth structure [7]. The NHA particles are smaller in size than hydroxyapatite particles and have higher surface charge, which enhance their strength and facilitate their application. In some previous studies, NHA was added to CGICs and RMGICs in 1% to 10% concentrations as a filler with favorable properties and resulted in an increase in flexural strength, compressive strength, wear resistance, antibacterial activity, and bond strength to dentin [5, 8-10].

As a result of high success rate of glass ionomers for root surface restorations, they are the material of choice for these lesions. Since they are in close contact with the gingival tissue, they must be biocompatible [11]. They should not contain toxic or leachable elements since they may be released into the oral environment and result in inflammatory response and tissue damage [12]. To minimize the risk of adverse local and systemic complications, biocompatibility of dental materials must be assessed in vitro and in vivo prior to their use in the clinical setting in order to ensure their safety [13]. L929 murine fibroblasts are routinely used for cytotoxicity testing of dental materials due to their optimal reproducible growth rates and biological response close to that of human cells [14].

No studies have been conducted on cytotoxic effects of adding NHP to CGICs. Few studies have been conducted on the cytotoxicity of adding NHA to RMGIC. Genaro et al. [3] showed that adding 2%, 5%, and 10% NHA to RMGI decreased its toxic effect on odontoblasts; this reduction increased by increasing the percentage of NHA [3]. Motskin et al. [15] revealed that cytotoxicity of NHA is related to its physicochemical properties. While NHA seems to be a safe material, it may be speculated that the reaction of incorporation of NHA into CGICs and RMGIs may result in formation and release of by-products or components that are cytotoxic [16].

It is crucial to carry out studied that evaluate the biological properties of adding nano-particles to RMGICs and CGICs aside from other properties. Thus, this study sought to assess the effect of addition of different concentrations of NHA on the cytotoxicity of RMGICs and CGICs. The null hypothesis was that addition of different concentrations of NHA to RMGICs and CGICs would have no significant effect on their cytotoxicity against L929 murine fibroblasts.

MATERIALS AND METHODS

Preparation of samples:

This in vitro, experimental study was conducted on 18 Fuji II LC (GC Corporation, Tokyo, Japan) and 18 Fuji II (GC Corporation, Tokyo, Japan) samples. The samples were divided into six groups of three with 0, 1, 2, 5, 7, and 10wt% NHA.

According to a pilot study, the amount of powder required to produce three RMGI samples with 1wt% NHA was found to be 2 g. The amount of NHA required to obtain 2 g powder was calculated to be 0.02 g. Thus, to

obtain samples with 1wt% NHA, 0.02 g of NHA powder and 1.98 g of RMGIC powder were mixed. The values in other groups were also calculated as such. The amount of powder required to produce three CGIC samples with 1wt% NHA was found to be 1.7 g. The amount of NHA required to obtain 1.7 g powder was calculated to be 0. 017 g. The values in other groups were calculated as such. The obtained powders for each concentration of NHA of each glass ionomer were mixed in a mortar and pestle for 2 min to obtain a homogenous mixture of particles. The powders were then kept in dark bottles until the experiment.

To fabricate RMGIC samples, the powder and liquid were mixed in 3.2/1 weight ratio. The mixing time was less than 25 s as recommended by the manufacturer. The powder to liquid ratio was 2.7/1 for the Fuji II CGIC. The pastes were transferred to a stainless-steel mold measuring 5×3 mm. The paste was condensed from one side to prevent void formation. The setting time was 180 s for the CGIC. For the RMGIC, a glass slab was placed over the mold and the cement was light-cured with a curing unit (Blue Phase; Ivoclar Vivadent, Schaan, Liechtenstein) with a light intensity of 600 mW/cm^2 for 40 s from both sides (20 s from each side). For each percentage of NHA, three samples were fabricated as such. The group definitions and abbreviations are mentioned in Table 1.

Table 1. Group definitions and abbreviations

Type of Glass Ionomer	NHP (%)	Group
Fuji II LC	0	RMG_0
	1	RMG_1
	2	RMG_2
	5	RMG_5
	7	RMG_7
	10	RMG_{10}
Fuji II	0	CG_0
	1	CG_1
	2	CG_2
	5	CG_5
	7	CG_7
	10	CG_{10}

NHP: Nanohydroxyapatite

The negative control group contained L929 fibroblasts and RPMI culture medium. The positive control group contained L929 fibroblasts and dimethyl sulfoxide culture medium.

Cell culture and passage:

L929 murine fibroblasts were obtained from the National Genetic Bank of Iran and cultured. The fibroblasts were cultured in flasks at 37˚C and 5% CO2. The culture medium contained 10% fetal bovine serum, penicillin-streptomycin, and L-glutamine. The over-laying medium was discarded and the 4th passage cells were detached from the flasks using 0.2% trypsin. After neutralization of trypsin, the cells were collected by centrifugation, and cell suspension was prepared. Next, 100 µL of the cell suspension containing 5×10^3 cells was added to each well of a 96-well plate, and the cells were incubated for 24 h.

Experiment:

The prepared disc-shaped samples were subjected to UV radiation for 3 h for sterilization. Next, 1 mL of the cell culture medium was added to each sample and incubated at 37°C for 72 h with 95% oxygen and 5% CO_2 (Innova-Co 170; New Brunswick Scientific, Edison, NJ, USA). Afterwards, the overlaying medium was removed and filtered through a membrane with 0.22 µm pore size.

The overlaying medium was discarded, and 100 µL of the extracted medium was added to the cells in a 96-well plate. The methyl thiazolyl tetrazolium (MTT) assay was done at 24, 48, and 72 h after cell incubation. Dimethyl sulfoxide (which is a toxic substance) was added to three wells as the positive control group. In three wells, as the negative control group, only cells and the culture medium were added with no other substance.

MTT assay:

For the MTT assay, 200 µL of the MTT solution (Sigma-Aldrich, St. Louis, MO, USA) and 5 mg/mL concentration of phosphate buffered saline were added to each well and incubated at 37˚C, 95% humidity and 5% CO_2 for 4 h, to convert the soluble MTT yellow salt into insoluble purple formazan crystals by the activity of viable cells. Next, the supernatant was discarded and replaced with 100 µL of dimethyl sulfoxide (DMSO, Gibco BRL, Grand

Island, NY, USA). The plate was agitated for 30 min, and the optical density was read by a spectrophotometer (Pharmacia, Biotech, USA) at 570 nm wavelength. At each time point of 24, 48 and 72 h, the cell viability values were recorded for each cement, and the control groups.

Cytotoxicity was rated based on cell viability relative to controls as: non-cytotoxic >90% cell viability, slightly cytotoxic: 60–90% cell viability, moderately cytotoxic: 30–59% cell viability, and severely cytotoxic: <30% cell viability [17].

Statistical analysis:

One-way ANOVA was applied for the comparison of the groups, which showed a significant difference between the groups ($P<0.05$). Pairwise comparisons of the mean values of the groups were performed using the Tukey's HSD test. Level of significance was set at $P<0.05$.

RESULTS

Table 2 demonstrates the cell viability values obtained for each cement, and control groups at different times. The results showed that there was a significant difference in cytotoxicity of RMG_0 and CG_0 after 72h, and RMG_0 was more cytotoxic ($P=0.042$; 91%±0.01 cell viability of CG_0 in comparison with 86%±0.04 cell viability of RMG_0). RMG_0 was slightly cytotoxic and CG_0 was non-cytotoxic after 72h. Following the addition of NHA, a different trend of cytotoxicity was noted in the two types of cements.

In the CG groups, there was no significant difference in cell viability between CG_0 and other groups ($P>0.05$). However, in the first 24h, increasing the NHA weight percentage from CG_1 to CG_7, significantly decreased the cell viability ($P<0.05$) but further addition of NHA up to 10wt% increased the viability of cells, and the cytotoxicity of CG_5 and CG_7 were significantly higher than that of CG_1 ($P<0.05$). The cytotoxicity decreased from 24h to 48h and 72h. Addition of NHA to RMGIC groups did not affect their cell viability in the first 24h ($P>0.05$). However, after 48h, the cytotoxicity in all groups decreased; while, the cytotoxicity of RMG_0 was significantly higher than that of RMG_1, RMG_2, and RMG_{10} groups. The reduction in cytotoxicity continued for up to 72h; the RMG_2 and RMG_{10} groups were significantly more cytotoxic; however, there was no significant difference between these groups and the RMG_0 group ($P>0.05$).

DISCUSSION

Nano-materials often measure 100nm or smaller in size and have very high surface energy, which significantly affects their physical and chemical properties, compared with mass materials.

Table 2. Mean±standard deviation of percentage of cell viability in different groups

Fuji II LC		**Cell viability**					
Groups		**RMG_0**	**RMG_1**	**RMG_2**	**RMG_5**	**RMG_7**	**RMG_{10}**
Time (h)	**24**	60±0.03	56±0.03	56±0.01	57	55±0.02	63±0.03
	48	77±0.03	90±0.05	88±0.01	79±0.05	83±0.03	89±0.02
	72	86±0.04	91±0.03	81±0.02	81±0.02	92	96±0.02
Fuji ll		**Cell viability**					
Groups		**CG_0**	**CG_1**	**CG_2**	**CG_5**	**CG_7**	**CG_{10}**
Time (h)	**24**	52±0.31	61±0.02	55	51±0.01	50.6±0.01	55±0.02
	48	82±0.22	86±0.07	85±0.02	83±0.03	80±0.04	94±0.03
	72	91±0.1	92±0.32	90±0.01	85±0.03	91±0.47	99.8±0.05

Also, due to high surface energy, nanoparticles form strong bonds to each other and to other materials; this phenomenon is referred to as agglomeration [18]. To improve the esthetic properties of CGICs, nanotechnology has been used for the fabrication of a new RMGIC namely Ketac nano™ (3M ESPE, St. Paul, MN, USA). In this group of RMGICs known as nanoionomers, a combination of fluoro-aluminosilicate glass and unreacted nanoillers confers unique properties to the cements, which are not present in other CGICs [19]. NHA particles, as a nanofiller with optimal biological properties, have also been added to CGICs, and some of the properties of this new cement have been previously investigated [7].

No dental material has all the ideal characteristics [20]. Dental materials are in contact with the tissues and oral fluids. Thus, selection of a material must be based on its physical and mechanical properties as well as its biocompatibility. Therefore, assessment of cytotoxicity and biocompatibility of materials is as important as their physical and mechanical properties [21]. Evaluation of cytotoxicity of materials in vitro is one method to assess their biocompatibility. Assessment of cytotoxicity under controlled and reproducible in vitro conditions can help determine the effect of concentration, type and time of exposure to the material on viability of cells. Many different laboratory tests are available to evaluate the cytotoxic activity. The MTT assay is very sensitive and depends on the potency of mitochondrial dehydrogenase enzyme of vital cells to breakdown the yellow tetrazolium ring of MTT. The blue crystals of formazan are impermeable to cell membrane and accumulate within the vital cells. The level of formazan produced is proportional to the number of vital cells [22].

Motskin et al. [15] assessed the correlation of properties of NHA particles with their cytotoxicity and bio-stability and showed that particle load was strongly associated with cytotoxicity, and physical and chemical properties of NHA particles. Shi et al. [23] evaluated the effect of size of NHA particles on proliferation and apoptosis of pre-osteoblast cells and reported that particles with 20 nm size had the best effect on cell proliferation and inhibition of apoptosis. In our study, according to the information provided by the manufacturer, the particles were smaller than 10 µm. Size control during the fabrication of NHA particles is difficult, and this may be a possible explanation for the difference in the results of different studies.

The results of this study showed that cell viability in Fuji II cement groups was higher than in Fuji II LC at 72 h; which is in accordance with the results of other studies [24,25]. This result might be due to the release of 2-hydroxyethyl methacrylate resin monomer from Fuji II LC [25].

Assessment of the interaction effect of weight percentage of NHA and type of cement on the cell viability in our study showed that in CG groups, by an increase in weight percentage of NHA from CG_1 to CG_5 and CG_7, the cell viability decreased; however, the cell viability increased again in CG_{10}. There was a significant difference between CG_5 and CG_7 with CG_1 NHA-containing groups. On the other hand, by increasing the weight percentage of NHA particles by up to CG_{10}, a reduction in cytotoxicity was noted. This finding may be due to several reasons. According to a study by Mohammadi Basir et al, [8] in concentrations higher than 5%, nanoparticle agglomerates form within the cement matrix. These agglomerates have the highest frequency in presence of 10wt% NHA. Hydroxyapatite nano-bioceramics are soluble in acidic solutions and after mixing the powder with a poly-acid (glass ionomer cement liquid), calcium ions are released from the surface of NHA particles; consequently, acid-base reactions and cross-linking occur within the cement structure, and subsequently reinforce it [26]. The odds of formation of hydrogen bonds increase as well, which is due to the presence of higher hydroxyl and phosphate ions in the cement matrix [27]. Stronger bonds between the organic and inorganic cement networks increase the mechanical strength of the set cement. Increased chemical reactions may be responsible for higher cytotoxicity of the cement and decreased viability of cells.

On the other hand, it can be speculated that due to ionic interaction of NHA crystals and polyacrylic acids, toxic byproducts or components may be formed and released from the NHA-containing GICs; this statement has been supported by other studies [28,29]. However, according to the results of this study, all groups had moderate cytotoxicity.

There was no significant difference in cytotoxicity between RMGI groups at 24 and 48 h. After 72 h, the cell viability of RMG_2 and RMG_5 was significantly higher than other groups containing NHA; however, their cell viability was not higher than that of RMG_0 group, and all groups had moderate cytotoxicity. Studies showed that adding about the same amount of NHA as in RMG_5 to CGIC and RMGIC groups increased their mechanical properties [6-8, 24]. Mohammadi Basir et al. [8] assessed the mechanical properties of RMGICs containing NHA particles and concluded that flexural strength increased by addition of up to 5wt% NHA; while, further increase in the concentration of NHA decreased the strength. Poorzandpoush et al. [10] assessed the wear resistance of RMGICs containing NHA and concluded that addition of 2wt% and 5wt% NHA increased the wear resistance, and 5wt% yielded the highest wear resistance. They explained that NHA particles are tiny particles containing calcium and phosphorus; when NHA is exposed to polyacrylic acid, the release and cross-linking of ions increase, resulting in higher strength of the cement matrix in 5wt% group. It seems that NHA by up to 5wt% serves as reactive filler and reinforces the cement matrix. The lower cytotoxicity in 2wt% and 5wt% NHA groups after 72 h in this study may also be due to these chemical reactions, and increase in resin crosslinking may reduce the ion release from the RMGI bulk after 72 h [30]. However, long-term storage may show a different trend of cytotoxicity. Genaro et al. [3] stated that adding NHA to RMGI decreased its cytotoxicity, and this reduction increased by increasing the NHA percentage to 10%.

It is obvious that addition of NHA to powder of CGIC changes the chemical reactions that happen during setting. Due to the difference in the composition and setting mechanism of CGICs and RMGICs, different chemical reactions may occur, and different amounts of materials might release from the cured matrix. As no chemical analysis was done in this study, we could not identify the exact components released. The current study had an in vitro design. Thus, generalization of results to the clinical setting must be done with caution. Future in vivo studies are required to find the most optimal concentration of NHA particles for incorporation into the formulations of cements.

CONCLUSION

Within the limitations of this study, the results showed that addition of NHA to GIC powders by up to 10% did not decrease cell viability.

ACKNOWLEDGMENTS

This study was supported by Dental Research Center, Tehran University of Medical Sciences (grant Number: 93-03-69-26679).

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Sidhu SK, Nicholson JW. A review of glass-ionomer cements for clinical dentistry. J Funct. 2016 Sep;7(3):16.
2. Garoushi S, Vallittu PK, Lassila L. Characterization of fluoride releasing restorative dental materials. Dent Mater. 2018 Jan; 30:2017-161.
3. Genaro LE, Anovazzi G, Hebling J, Zuanon AC. Glass ionomer cement modified by resin with incorporation of nanohydroxyapatite: In vitro evaluation of physical-biological properties. Nanomaterials. 2020 Jul;10(7):1412.
4. Şuhani MF, Băciuţ G, Băciuţ M, Şuhani R, Bran S. Current perspectives regarding the application and incorporation of silver nanoparticles into dental biomaterials. Clujul medical. 2018 Jul;91(3):274.
5. Alatawi RA, Elsayed NH, Mohamed WS. Influence of hydroxyapatite nanoparticles on the properties of glass ionomer cement. J Mater Res Technol. 2019 Jan;8(1):344-9.
6. Munguía-Moreno S, Martínez-Castañón GA, Patiño-Marín N, Cabral-Romero C, Zavala-Alonso NV. Biocompatibility and surface characteristics of resin-modified glass ionomer cements with ammonium quaternary compounds

or silver nanoparticles: An in vitro study. J Nanomater. 2018;2018.
7. Bilić-Prcić M, Rajić VB, Ivanišević A, Pilipović A, Gurgan S, Miletić I. Mechanical Properties of Glass Ionomer Cements after Incorporation of Marine Derived Porous Cuttlefish Bone Hydroxyapatite. Materials. 2020 Jan;13(16):3542.
8. Mohammadi Basir M, Ataei M, Rezvani MB, Golkar P. Effect of incorporation of various amounts of nano-sized Hydroxyapatite on the mechanical properties of a resin modified glass ionomer. J Dent Sch. 2013 Mar 15;30(4):216-23.
9. Moshaverinia A, Ansari S, Moshaverinia M, Roohpour N, Darr JA, Rehman I. Effects of incorporation of hydroxyapatite and fluoroapatite nanobioceramics into resin cements (GIC). Acta Biomater. 2008 Mar 31;4(2):432-40.
10. Poorzandpoush K, Omrani LR, Jafarnia SH, Golkar P, Atai M. Effect of addition of Nano hydroxyapatite particles on wear of resin modified glass ionomer by tooth brushing simulation. J Clin Exp Dent. 2017 Mar;9(3):e372.
11. Koohpeima F, Mokhtari MJ, Doozandeh M, Jowkar Z, Yazdanshenas F. Comparison of Cytotoxicity of New Nanohybrid Composite, Giomer, Glass Ionomer and Silver Reinforced Glass Ionomer using Human Gingival Fibroblast Cell Line. J Clin Pediatr Dent. 2017;41(5):368-73.
12. Zarrabian M, Ostad SN, Abbasi MA, Mohseni ME. In vitro evaluation of mutagenicity and cytotoxicity of four root canal sealers. J Dent Med. 2007 Jun 15;20(3):205-11.
13. Wataha JC. Predicting clinical biological responses to dental materials. Dent Mater. 2012 Jan;28(1):23-40.
14. Aydın N, Karaoğlanoğlu S, Oktay EA, Süloğlu AK. Evaluating Cytotoxic Effects of Highly Esthetic Dental Composites. Braz Dent Sci. 2020 Jan;23(1):8.
15. Motskin M, Wright DM, Muller K, Kyle N, Gard TG, Porter AE, et al. Hydroxyapatite nano and microparticles: correlation of particle properties with cytotoxicity and biostability. Biomaterials. 2009 Jul;30(19):3307-17.
16. Coelho CC, Grenho L, Gomes PS, Quadros PA, Fernandes MH. Nano-hydroxyapatite in oral care cosmetics: Characterization and cytotoxicity assessment. Sci Rep. 2019 Jul;9(1):1-0.
17. Dahl JE, Frangou-Polyzois MJ, Polyzois GL. In vitro biocompatibility of denture relining materials. Gerodontology. 2006 Mar;23(1):17-22.
18. Naguib GH, Al-Hazmi FE, Kurakula M, Al-dharrab AA, Hosny KM, Alkhalidi HM, et al. Zein Coated zinc oxide nanoparticles: Fabrication and antimicrobial evaluation as dental aid. Pharmacology. 2018 Jan;14(8):1051-9.
19. Davidson CL. Advances in glass-ionomer cements. J Appl Oral Sci. 2006,14 (SPE):3-9.
20. Sasanaluckit P, Albustany KR, Doherty PJ, Williams DF. Biocompatibility of glass ionomer cements. Biomaterials. 1993 Oct;14(12):906-16.
21. Six N, Lasfargues JJ, Goldberg M. In vivo study of the pulp reaction to Fuji IX, a glass ionomer cement. J Dent. 2000 Aug;28(6):413-22.
22. Hii SC, Luddin N, Kannan TP, Ab Rahman I, Ghani NR. The biological evaluation of conventional and nano-hydroxyapatite-silica glass ionomer cement on dental pulp stem cells: A comparative study. Contemp Clin Dent. 2019 Apr;10(2):324.
23. Shi Z, Huang X, Cai Y, Tang R, Yang D. Size effect of hydroxyapatite nanoparticles on proliferation and apoptosis of osteoblast-like cells. Acta Biomater. 2009 Jan;5(1):338-45.
24. Xie D, Yang Y, Zhao J, Park JG, Zhang JT. A novel comonomer-free light-cured glass-ionomer cement for reduced cytotoxicity and enhanced mechanical strength Dent Mater. 2007 Aug;23(8):994-1003.
25. Noorani TY, Luddin N, Rahman IA, Masudi SM. In vitro cytotoxicity evaluation of novel nano-hydroxyapatite-silica incorporated glass ionomer cement. J Clin Diagn Res. 2017 Apr;11(4):ZC105.
26. Aoki H. Science and medical applications of hydroxyapatite. J Anal At Spectrom.1991;1991:123-34.
27. Moshaverinia A, Ansari S, Movasaghi Z, Billington RW, Darr JA, Rehman IU. Modification of conventional glass-ionomer cements with N-vinylpyrrolidone containing polyacids, nano-hydroxy and fluorapatite to improve mechanical properties. Dent Mater. 2008 Oct;24(10):1381-90.
28. Najeeb S, Khurshid Z, Zafar MS, Khan AS, Zohaib S, Martí JM, Sauro S, Matinlinna JP, Rehman IU. Modifications in glass ionomer cements: Nano-sized fillers and bioactive nanoceramics. Int J Mol Sci 2016 Jul;17(7):1134.
29. Goenka S, Balu R, Kumar TS. Effects of nanocrystalline calcium deficient hydroxyapatite incorporation in glass ionomer cements. J Mech Behav Biomed 2012 Mar;7:69-76.
30. Sharafeddin F, Shoale S, Kowkabi M. Effects of different percentages of microhydroxyapatite on microhardness of resin-modified glass-ionomer and zirconomer. J Clin Exp Dent. 2017 Jun;9(6):e805.