In Vitro Examination of The Cytotoxicity of Alkasite Restorative Material on Dental Pulp Stem Cells

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Research Article

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Abstract

Background: The clinical suitability of restorative dental materials is also determined by their biocompatibility, in addition to their physical and chemical properties. The present study aimed to examine the cytotoxic effects of three dental restorative materials on human dental pulp stem cells (DPSCs).

Methods: This study examined the cytotoxic properties of a composite (Gradia Direct,GC Europe), a high-viscosity glass ionomer cement (HVGIC; Equia Forte,GC Europe), and an alkasite restorative material (Cention N,Ivoclar Vivadent). In total, 12 samples of each material were prepared for cytotoxicity assays; the cytotoxic effects were determined by taking biomaterial releases into consideration. Cell viability and proliferation were observed and analyzed at intervals of 24- and 72-hours using both the methyl-thiazole-diphenyl-tetrazolium (MTT,Sigma Aldrich) and xCELLigence (RTCA-DP,ACE) cytotoxicity assays. Data were calculated using the RTCA-DP integrated software of the xCELLigence system and the GraphPad Prism 9.1.1 (GraphPad Software, Inc) program. Data from the proliferation experiments were statistically evaluated using the two-way ANOVA test.

Results: Alkasite exhibited the highest cytotoxicity, whereas HVGIC and composite did not exhibit any significant difference compared with the control. No difference in the cytotoxicity of the alkasite and composite was noted between the two time points in the MTT assay; however, the cytotoxicity of HVGIC was higher at 72-hours than that at 24-hours. Similar results were obtained with both assays.

Conclusions: Although alkasite exhibited higher cytotoxicity compared to composite and HVGIC, all materials exerted slightly cytotoxic effects (60%–90% cell viability) on DPSCs. Considering its cytotoxic, aesthetic and mechanical properties, alkasite can be clinically preferred instead other materials in cavities that are not close to the dental pulp.

Introduction

Restorative materials should exhibit good mechanical properties, biocompatibility, and fluoride-releasing ability [1].Amalgam, composites, compomers, and glass ionomer cementsrepresent some common dental materials used for the restoration of primary and permanent dentition [2]. Of these, amalgam has been used most commonly for many years as it exhibits good, long-lasting biomechanical properties;however, the presence of mercury in its composition and itsnonaesthetic properties have made it less popular among dental patients lately [3].In contrast, composite resins, first developed in 1962,meet the increasing aesthetic and masticatory needs of patients while also exhibiting good retention [4].However, they often release residual monomers that may exhibit cytotoxic effects on oral tissues.

The popularity of fluoride-releasing dental materials has increased recentlybecause of their role in caries prevention [5].In this regard, glass ionomer cements are considered particularly advantageous because they are biocompatible, can release fluoride, and can chemically bond to enamel and dentin [6]. However, they also have certain disadvantages such as sensitivity to moisture and poor mechanical

strength.Although resin-modified and HVGICscan minimize thesedisadvantages to some extent,further improvement is necessary [7].

Cention N (IvoclarVivadent, Liechtenstein) is a newly developed dental material and is classified as an “alkasite,” which has been defined as a subgroup of composite materials. Cention N is an aesthetic and highly resistant material, which is particularly suitable for the posterior region of oral cavity. The presence of alkaline fillersin its composition enables the release of fluoride, calcium, and hydroxyl ions that can contribute to the prevention of demineralization and enhancement of remineralization. Therefore, the manufacturer of Cention N states that it combines the best properties of amalgam and glass ionomer cement [8,9].Cention N is a urethane dimethacrylate (UDMA) based self-curing restorative material with optional additional light-curing [9]. Its additional advantages include cost-effectiveness and ease of use without the requirement ofany special equipment or additional skills [8].

In addition to their physical and chemical propertiesof restorative materials, their clinical suitability is determined by theirbiocompatibility—the ability of a material to create an appropriate biological response in the area of application [6,10].It is crucial that restorative materials used in clinical practice do not cause systemic or local cytotoxicityin the oral mucosa, gingiva, and pulpal tissues adjacent to the material [11].Previous studies have reported that cellular and molecular cytotoxicitycan influence pulp vitality and are most commonly mediated by substances that leach out during and after the polymerization of restorative dental materials [12].

Although several studies have examined the cytotoxicity of composites and glass ionomer cements [13-15], evidence regarding the cytotoxic effects of alkasite restorative materials on human dental pulp stem cells (DPSC) remain limited. Therefore, this study aimed to evaluate the cytotoxicity of a newly developed alkasite restorative material on DPSCusing methyl-thiazole-diphenyl-tetrazolium (MTT) and xCELLigence assays and compare thiswith a composite and a high-viscosity glass ionomer cement (HVGIC) routinely used in the clinic.Toourknowledge, this is the first study to examine the cytotoxic effect of alkasite material on dental pulp stem cells.Our two hypotheses were that the cytotoxicity of alkasiteishigher than that of HVGIC and similar to that of composite.

Methodology

This study was approved by the Ethics Committee of Biruni University (2019/34-12) and was conducted in accordance with the World Medical Association Declaration of Helsinki.

Dental materials tested in this study

This study examined the cytotoxic effects of a composite (Gradia Direct, GC Europe, Belgium), a HVGIC; Equia Forte, GC Europe, Belgium), and an alkasite restorative material (Cention N, IvoclarVivadent, Liechtenstein) on DPSC (Table 1).

Preparation of samples

In total, 12 specimens of each material were prepared under sterile conditions in a laminar flow chamber (Heal Force, China) and placed into cylindrical Teflon molds (5.0 mm diameter × 2.0 mm height). Thereafter, the lower and upper surfaces of the materials were covered with transparent matrix tape to prevent the formation of an oxygen inhibition layer; the polymerization phase was initiated by placing the Teflon molds between two glass coverslips to remove excess material and prevent air bubble formation. The materials were cured or set in accordance with the manufacturers' recommendations. An amalgamator device (GC Europe, Belgium) was used to mix materials in capsule form, and a light device (Elipar™ S10; 3M ESPE, St. Paul, MN, USA) was used to polymerize light-cured restorative materials.Thebiomaterials were sterilized using ultraviolet light for 30 minutesbefore thestart of the experiments to prevent bacterial, fungal, or yeast contamination.

Cell culture and experimental design

Human DPSCs (CELPROGEN, 36086-01, USA) were supplied as a cell line and cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma Aldrich, St. Louis, MO, USA), 100 U / mL penicillin / streptomycin (Sigma Aldrich, St. Louis, MO, USA), 100 U / mL L-glutamine (Sigma Aldrich, St. Louis, MO, USA), and 100 U / mL sodium pyruvate at 37°C under 5% CO_2 humidified air. The third passage DPSCs were detached using a 0.05% trypsin–EDTA solution (Sigma Aldrich, St. Louis, MO, USA) and a monolayer was cultured at a concentration of 5×10^5 in 25 cm flask containing DMEM medium.

A total of 12 samples of each material were divided into three subgroups containing four samples each. Freshly prepared samples were placed in 10 ml DMEM and incubated for 24 and 72 hours to obtain eluates.Cytotoxicity was assessed using MTT and xCELLigenceassays as described below, and DMEM was used as control.

Determination of cell viability using MTT assay

Cytotoxic effects of the three tested materials on cell viability and proliferation were evaluated using the MTTassay (Sigma Aldrich Inc., St. Louis, USA). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a stable tetrazolium salt. The production of NAD(P)H in the glycolytic pathways of living cellscan decrease and cause formation offormazan crystals, the concentration of which is directly proportional to the number of living cells at the end of the experiment.

In this study, 3×10^4 cells were grown, placed on 96-well plates, and incubated at 37°C for 24 hours. The next day, 100 µl of different concentrations of the medium in which the biomaterials were stored for 24 and 72 hours were applied to the plates. To allow examination of the effects of these substances on cell viability over time, 10 µl of MTT was added 24 hours after the application of the medium and left to incubate for 4 hours at 37°C in the dark. Thereafter, 100 µl of thesolubilization solution was added to each well andthe plate was kept in the incubator overnight. The absorbance (optical density) of the samples was measured using a spectrophotometer (ELISA reader) at 590 nm, and the number of viable cells in the medium was determined.

Proliferation and cytotoxicity assay of DPSC cell lines usingxCELLigence assay

The xCELLigence system(RocheAppliedScience, and ACEA Biosciences)was used to assess the survival of DPSCs upon exposure to variousdental materials over time. Physiologic changes in the cells were identified and measured by the electronic impedance of the sensor electrodes. This real-time monitoring system provides quantitative information on the biological status of cells, including cell number, viability, and morphology;the relative changes in the electrical impedance are displayed by the cell index system.

Next, 200 µL of the cell suspensions were seeded intoa 16-well E-plate (30,000 cells/well; well volume:250 µL; base diameter of well: 5 mm)in a laminar flow cabinet, placed in the incubator at 37°C and 5% CO_2, and monitored using the RTCA-DP system at 15-minute time intervals for up to 72 hours with or without dental materials. The control samples received only medium and, in accordance with the xCELLigence technical manual, at least three repeats of each experimental condition was performed to facilitate statistical evaluation.

Statistical analysis

Data were calculated using the RTCA-DP integrated software of the xCELLigence system and theGraphPad Prism 9.1.1 (GraphPad Software, Inc) program. Data from the proliferation experiments were statistically evaluated using thetwo-way ANOVAtest, and a*p*-valueof <0.05 was considered statistically significant.

Results

MTTassay results

TheMTT cytotoxicity assay was used to observe and analyze cell survival at intervals of 24 and 72 hours. All restorative materials exhibited varying levels of cytotoxicity against DPSCs, andalkasite sample showed significantly lower cell proliferation after 24 hoursthan the HVGIC sample (p = 0.002).

Although the alkasite and composite samples exhibited nosignificant changes in DPSC proliferation over time (24–72hours), the HVGIC samples demonstrateda significant decrease(79.7%) in proliferation rates after 72 hours (p =0.01) (Fig.1). No statistically significant difference was observed between the materials and the control after 72 hours; however, all materials in this study exhibited numerical decreases in cell viability. Therefore, they were found to be slightly cytotoxic (Table 2).**XCELLigenceassay results**The effects of the HVGIC, alkasite, and composite samples on DPSC cells were monitored for 72 hours using a real-time cytotoxicity analysis system. Cell index valuesincreased from 0.23 before application of HVGIC and composite samplesto the DPSC cells to 0.51 after 24 hoursfrom the application. In contrast, the cell index valueafter alkasite application was 0.35 (Fig.2). The cell index value increasedin the first hour after the application of the release medium of alkasite (24th hour); however, the cells reached a plateau thereafter (25th hour), and the cell viability decreased compared with the control. Monitoring theviabilityof cells released from the HVGIC and composite samples and the

applied medium for 72 hours using the xCELLigence systemshowed that the cell index values were similar to that of the control, with continuous cell growth and proliferationcessationbeing observed after a while.A significant decrease in DPSC proliferation was observed in the alkasitegroup after 24 and 72 hours compared with the control (p<0.01) (Fig.3), whereas a significant increase was detected in the HVGIC and composite groups after 24 hours compared with thealkasite group. Although no statistically significant difference was noted between the HVGIC and composite groups after 24 hours, the increase in proliferation observed in the HVGIC group compared with the control was statistically significant. Alkasite demonstrated the highest cytotoxicity on cell viability after 72 hours (Table 3). No differenceswere observed in the cytotoxicity of alkasitewhen comparing the two time points,whereas both HVGIC and composite exhibited a significant increase in cytotoxicity at 72 hours compared with that at 24 hours.

Discussion

Dental materials, which have better mechanical and chemical properties, are gradually developed for more aesthetic and long-lasting restorations of decayed teeth. In addition to having good mechanical, chemical, and aesthetic properties, dental materials should also exhibit suitablebiocompatibility as they can directly or indirectly affect the surrounding structuresvia the substances they release during and after setting. These leachable substances can migrate through the dentinal tubules and damage the dental pulp, which highlights the importance of understanding the cytotoxic effects of restorative dental materials used in deep cavities [16].The present study examined and compared the cytotoxicity of a newly developed alkasitematerial, HVGIC, and composite on DPSCs and found that all three materials exhibited slightly cytotoxic effects, although this was statistically significant only in the alkasite group.

The amount of ions and residual monomers released from the material, its composition, and the concentration of filler particles play a role in its cytotoxicity [17,18].The present study found no statistically significant differencesin cytotoxicity among the control, HVGIC, and composite,although the alkasitematerial demonstrated significant cytotoxic effects. This may be attributed to the chemical composition of the material: in contrast to compositesthat releasedonly monomers and HVGICsthat released only ions, alkasiteswere capable of releasing both (particularly, UDMA and fluoride),which potentially increased their cytotoxicity. In addition, previous studies have found that the amount of residual monomer increases with higher filler content in resin-based materials, thus decreasing the cell proliferation [18].The filler content of the alkasite restorative material (78.4%) was higher than that of the composite (73%)examined in this study, which might have resulted in greater cytotoxic effects. To the best of our knowledge, only a single previous study by Awad et al. [19]has investigated theeffects of alkasites on human gingival fibroblast cells and found greater cytotoxicitythan that ofcomposites. The results of the present study were in accordance with this.

Neither of the assay methods used in this study revealed any statistically significant differences between the composite and the control,although a numerical decrease in cell proliferation was observed in the former.Da Silva et al. [20]havesuggested that a material is considered nontoxic or slightly toxic if the cell viability exceeds 90% or ranges between 60%–90%, respectively. In accordance with this, all materials

evaluated in the present study were considered slightly cytotoxic after 72 hours, and these findings were in agreement with those of previous studies [15,21-23].

An increase in cell proliferation was also observed in the HVGIC group after 24 hours, and this was in accordance withthe finding of Ersahan et al. [16]who observedno cytotoxicity with HVGIC in their study.On the contrary, they reported an increase in cell proliferation, suggesting that the material used was biocompatible. This might be attributed tothe small-particle glass-filler technology used, low-setting exothermic reaction, and rapid neutralization [24].

Elution times may play a role in the amount of substances released by dental materials and the subsequent cytotoxicity produced. In the present study, HVGIC samples, which set chemically, exhibited a significant difference between the two timepoints examined, and this was in agreement with the reports of previous studies [15,25].The increased cytotoxicity of HVGIC can be explained by its longer setting reaction, which results inlarger concentrations of ions in the eluates obtained after 72 hours. These findings were in accordance with those reported by Al-qathami et al. [26].No significant differences between the two time points were observed in the alkasitegroup, which is a fluoride-releasing dual-cure material. This means that the setting reaction of alkasite begins when the powder and liquid are mixed and can be accelerated further with additional light-curing. The cytotoxic effects of fluoride-releasing materials can also be affected by the amount of fluoride released [18,19].Egil [27] reported that the amount of fluoride releasedby alkasite was lower than that released by HVGIC. Therefore, both the shorter polymerization time andthe less amount of ions releasedmight be effective in maintaining the cytotoxicity of alkasite.

The xCELLigenceassay performed in this study revealed that the cytotoxicity of the composite increased after 72 hours,which is in agreement with the findings of previous studies [28,29]; thiscould be attributed to an increase in monomer release with degradation over time.

The present study used MTT and xCELLigenceassays to investigate the cytotoxic effects of restorative dental materials on human DPSCs. The MTTassay is considered one of the most reliable biocompatibility assays because of its rapid results and sensitivity, although the use of end-point qualitative measures of cell fitness is a major limitation [22,30,31].The key advantage of the recently developed real-time cell analyzer system (xCELLigence) is that it allows theobservation of biological events every 15 minutes, thus providing moreinformation than the previous methods [29,31].To the best of our knowledge, very few studies to date have evaluated the cytotoxicity of dental materials using both assays and, although they yielded similar results in the present study, the findings of the xCELLigenceassay were considered to be more accurate and detailed.

The cytotoxicity of alkasite material was higher than that of any of the other materials assessed in this study.Therefore, our first hypothesis was confirmed and our second hypothesis was refuted. The formation of a partial barrier to protect the pulp in deep cavities with increased dentin permeability can reduce the cytotoxic potential of dental materials. In addition to the mechanical properties of dental

materials, an understanding of its cytotoxic effects is essential to allow appropriate material selection and increase treatment success.

Limitations

This study had several limitations. Although the *in vitro* tests indicated successful results, they cannot exactly mimic *in vivo* conditions. Under clinical conditions, the thickness of dentin remaining in the cavity and the formation of a barrier using a cavity liner may alter the cytotoxic effects of materials on the dental pulp by protecting it. Furthermore, the experimentdurationwas 72 hours; the results may change with longer durations. Therefore, future studies should consider *in vivo* conditions and longer time periods.

Conclusion

Alkasite demonstrated acceptable cytotoxicity on DPSCs after a 72-hour period but were more cytotoxic than HVGIC and composite that demonstrated similar cytotoxicity. The MTT and xCELLigence assays yielded similar results. Considering the other mechanical and chemical properties of the alkasite restorative material, these findings suggest that it is clinically suitable for the restoration of cavities that are not in close proximity to the dental pulp.

Abbreviations

HVGIC: High-viscosity glass ionomer cement; DPSC: Dental pulp stem cells; MTT: Methyl-thiazole-diphenyl-tetrazolium; DMEM:Dulbecco's modified Eagle's medium.

Declarations

Acknowledgments

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Authors' contributions

B.S.C. and T.E. conceived the ideas and revised the manuscript; B.S.C., T.E., C.D. and N.E.O. collected and analyzed the data; B.S.C. and T.E. and led the writing; C.D. and N.E.O. reviewed and criticized the manuscript. All authors approved submission. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and analyzed in this study are included within the article or available from the corresponding author on reasonable request.

Ethics approval

This study was approved by the Ethics Committee of Biruni University (2019/34-12) and was conducted in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

Not applicable.

Competing of interests

The authors declare no competing of interests.

References

1. Sidhu SK. Glass-ionomer cement restorative materials: a sticky subject. Aust Dent J. 2011;56(Suppl 1):23-30.
2. Mousavinasab MS. Biocompatibility of composite resins. J Dent Res. 2011;8:21-29.
3. Demirci M, Tuncer S, Uysal O, Yucel T. The Reasons for Replacement of Amalgam Restorations. Turkiye Klinikleri J Dental Sci. 2008;14(3):147-155.
4. Jackson RD, Morgan M. The new posterior resins and simplified placement technique. J Am Dent Assos. 2000;131:375-383.
5. Khoroushi M, Keshani F. A review of glass-ionomers: From conventional glass-ionomer to bioactive glass-ionomer. Dent Res J (Isfahan). 2013;10(4):411-420.
6. Nicholson JW, Croll TP. Glass-ionomer cements in restorative dentistry. Quintessence Int. 1997;28:705-714.
7. Markovic DL, Petrovic BB, Peric TO. Fluoride content and recharge ability of five glass-ionomer dental materials. BMC Oral Health. 2008;8:21.
8. Mann JS, Sharma S, Maurya S, Suman A. Cention N: a rewiev. Int J Cur Res. 2018;10(5):69111-69112.
9. Naz, F, Khan AS, Khader MA, Gelban LO, Mausa NM, Asiri RS, et al. Comparative evaluation of mechanical and physical properties of a new bulk-fill alkasite with conventional restorative materials. Saudi Dent J. 2020. https://doi.org/10.1016/j.sdentj.2020.04.012.
10. Uzun IH, Bayındır F. Testing procedures for biocompatibility of dental materials. GÜ Diş Hek Fak Derg. 2011;28(2): 115-122.

11. Moharamzadeh K, Brook IM, Noort RV. Biocompability of resin-based dental materials. Materials. 2009;2:514-548.
12. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, et al. Effects of HEMA and TEGDMA on the in vitro odontojenic differentiation potential of human pulp stem/progenitor cells derived from deciduous teeth. Dent Mater. 2011;27:608-617.
13. Huang F, Chang Y. Cytotoxicity of resin-based restorative materials on human pulp cell cultures. Oral Surg Oral Med Oral Pathol. 2002;94(3):361-365.
14. Huang Q, Huang S, Linang X, Quin W, Liu F. The antibacterial, cytotoxic, and flexural properties of a composite resin containing a quaternary ammonium monomer. J Prosthet Dent. 2018;120(4):609-616.
15. Cosgun A, Bolgul B, Duran N. In vitro Investigation of Antimicrobial Effects, Nanohardness, and Cytotoxicity of Different Glass Ionomer Restorative Materials in Dentistry. Niger J Clin Pract. 2019;22(3):422-431.
16. Ersahan S, Oktay EA, Sabuncuoğlu FA, Karaoğlanoğlu S, Aydın N, Suloğlu AK. Evaluation of the cytotoxicity of contemporary glass-ionomer cements on mouse fibroblasts and human dental pulp cells. Eur Arch Paediatr Dent. 2020;21(3):321-328.
17. Schubert A, Ziegler C, Bernhard A, Bürgers A, Miosge N. Cytotoxic effects to mouse and human gingival fibroblasts of a nanohybrid ormocer versus dimethacrylate-based composites. Clin Oral Invest. 2019;23(1):133-139.
18. Taghizadehghalehjoughi A, Ok E, Kamalak H. Investigation of the cytotoxic effect of composite materils on gingival fibroblast cells. Turkiye Klinikleri J Dental Sci 2019;25(3):310-318.
19. Awad MM, Alsheri T, Alqarni AM. Evaluation of the Bond Strength and Cytotoxicity of Alkasite Restorative Material. Sci. 2020;10(6175):1-12. doi:10.3390/app10186175.
20. Da Silva EJ, Santos CC, Zaia AA. Long-term cytotoxic effects of contemporary root canal sealers. J Appl Oral Sci. 2013;21:43–47.
21. Croll, TP, Nicholson JW. Glass ionomer cements in pediatric dentistry: review of the literature. Pediatr Dent. 2002;24:423-429.
22. Marczuk-Kolada G, Łuczaj-Cepowicz E, Pawinska M, Holownia A. Evaluation of the cytotoxicity of selected conventional glass ionomer cements on human gingival fibroblasts. Adv Clin Exp Med. 2017;26(7):1041–1045.
23. Celik N, Binnetoglu D, Ozakar Ilday N, Hacimuftuoglu A, Seven N. The cytotoxic and oxidative effects of restorative materials in cultured human gingival fibroblasts, Drug Chem Toxicol. 2019;1-6. doi: 10.1080/01480545.2019.162026.
24. Nicholson JW, Czarnecka B. The biocompability of resin-modified glass ionomer cements for dentistry. Dent Mater. 2008;24:1702–1708.
25. Demirci T, Gurbuz T, Sengul F. Cytotoxicity of dental resin composites: an in vitro study. J Dent Fac Atatürk Uni. 2014;24(1):10-15.

26. Al-Qathami H, Balto H, Al-Nazhan S, Siddiqui Y. Effect of root perforation repair materials on morphology and attachment behaviour of human PDL fibroblasts invitro. Saudi Dent J. 2004;16:113-117.
27. Egil E. Long Term Fluoride Release of Newly Developed Alkasite Based Restorative Material. Erzincan Univ J Sci Tech. 2020;13(3),1281-1288.
28. Arslan Malkoç M, Demir N, Şengun A, Bozkurt SB, Hakkı SS. Cytotoxicity evaluation of luting resin cements on bovine dental pulp-derived cells (bDPCs) by real-time cell analysis. Dent Mater J. 2015;34(2):154–160.
29. Atilan Yavuz S, Surmelioglu D. Evaluation of Cytotoxicity of Different Universal Bonds Using the Xcelligence System. Cumhuriyet Dent J. 2020;23:4:371-381.
30. Siquiera PC, Magalhes APR, Pires WC, Pereira FC, Silveira-Lacerda EP et al. Cytotoxicity of glass ionomer cements containing silver nanoparticles. J Clin Exp Dent. 2015;7(5):e622-7.
31. Urcan E, Haertel U, Styllou M, Hickel R, Scherthan H,Reichl FX. Real-time xCELLigence impedance analysis of the cytotoxicity of dental composite components on human gingival fibroblasts. Dent Mater. 2010;26(1):51–58.

Tables

Table 1. Dental materials examined in the study

Materials	Type	Manufacturer	LOT Number	Components (a description of abbreviations)
Gradia Direct, Posterior	Composite	GC Europe, Belgium	1908202	UDMA, co-monomer matrix, silica, pre-polymerized fillers, fluoro-alumino-silicate glass
Equia Forte	High-vicosity glass ionomer	GC Europe, Belgium	2008191	Strontium fluoro-alumino-silicate glass, polyacrylic acid powder, Pigment Polyacrylic acid, Distilled water, Polybasic carboxylic acid
Cention N	Alkasite	Ivoclar Vivadent, Liechtenstein	W96066	UDMA, DCP, Aromatic aliphatic-UDMA, PEG-400 DMA, Barium aluminium silicate glass, Ytterbium trifluoride, Isofiller, Calcium barium aluminium fluorosilicate glass, calciumfluoro silicate glass
UDMA = urethane dimethacrylate, DCP = dicalcium phosphate, PEG-400 DMA = polyethylene glycol-400 dimethacrylate				

Table 2. Change in DPSC proliferation by time, observed using MTT

	CTRL		Alkasite			HVGIC			Composite		
Time	**Total amount of cells/µm²**	**Viability (%)**	**Total amount of cells/µm²**	**Viability (%)**	**p value**	**Total amount of cells/µm²**	**Viability (%)**	**p value**	**Total amount of cells/µm²**	**Viability (%)**	**p value**
24 h	1.467	100	1.305	88.9	0.136	1.73	117.9	0.277	1.46	99.5	0.9888
72 h	1.484	100	1.339	90.2	0.079	1.33	89.6	0.259	1.396	94.1	0.2731

Two-way ANOVA test *$p<0.05$

Table 3. Change in DPSC proliferation by time, observed using xCELLigence system

Time	**CTRL**		**Alkasite**		**p value**	**HVGIC**		**p value**	**Composite**		**p value**
24-hour impedance values	0.5139	0.6915	0.3094	0.3406	**0.0064***	1.0345	0.9573	**0.0007***	0.7203	0.847	0.059
72-hour impedance values	0.5961	0.6027	0.154	0.1497	**0.0003***	0.4867	0.4662	0.2329	0.4592	0.4662	0.1699

Two-way ANOVA test *$p<0.05$

Figures

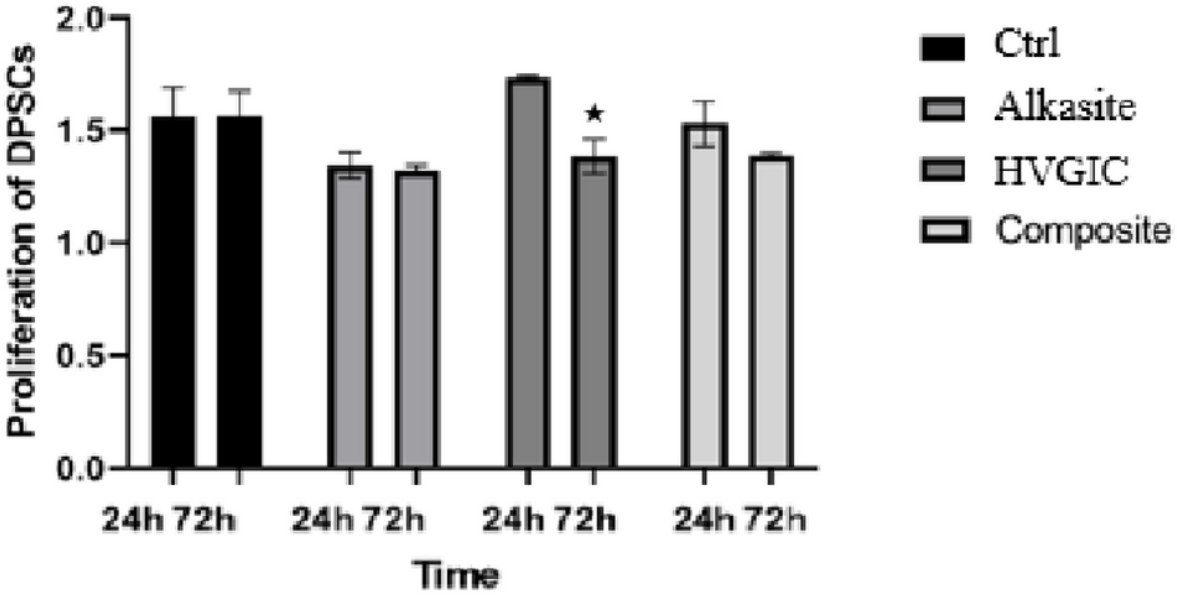

Figure 1. Change in DPSC proliferation, observed using MTT. HVGIC is indicated by the sign (★) of decrease in DPSC proliferation at 72 hours.

Figure 1

See image above for figure legend

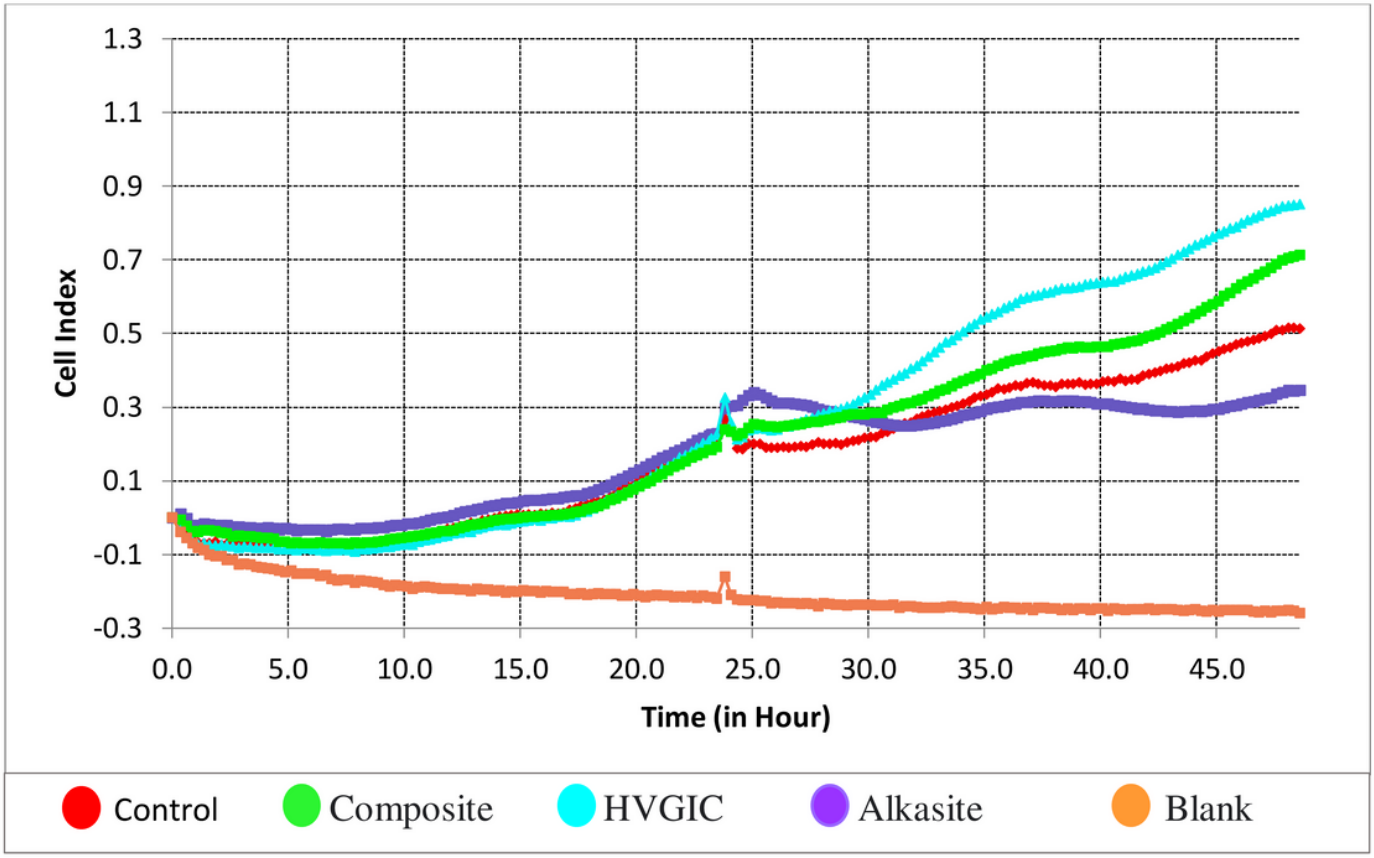

Figure 2. Cell index values of DPSCs with xCELLigence RTCA-DP system (Std. Deviation; Control: 0.002333; Composite: 0.2269; HVGIC: 0.3673; Alkasite: 0.1224).

Figure 2

See image above for figure legend

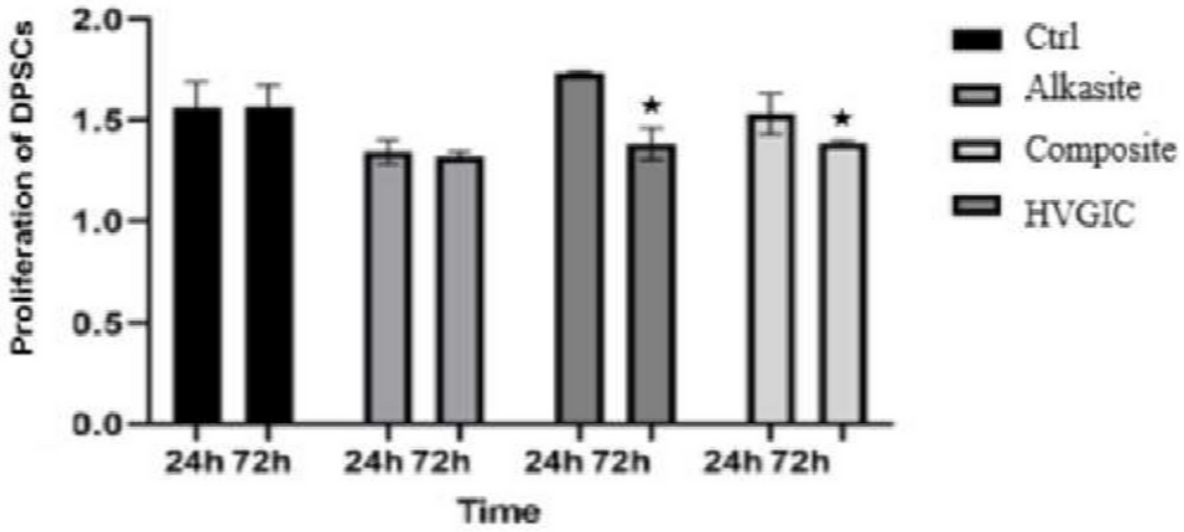

Figure 3. Change in DPSC proliferation, observed using the xCELLigence RTCA-DP system. Significant change in cell proliferation of HVGIC and composite at 72 hours compared to 24 hours is indicated by the sign (★).

Figure 3

See image above for figure legend