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BIOCOMPATIBILITY EVALUATION OF SELF-ADHESIVE RESIN CEMENT AND GLASS IONOMER CEMENT ON HUMAN GINGIVAL FIBROBLAST CELLS (COMPARATIVE IN VITRO STUDY)

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ABSTRACT

Background: Dental cements can potentially release substances that negatively affect the surrounding oral soft tissues. Nonetheless, there is a notable shortage of comprehensive studies assessing the biocompatibility and cytotoxic potential of different dental cements.

Aim: To evaluate and compare the biocompatibility of self-adhesive resin cement (Breeze™) and glass ionomer cement (Medicem) using Human Gingival Fibroblasts (HGFs) cell line.

Materials and Methods: HGFs were divided into; **group I**: control group cultured with culture medium only, **group II**: cells were cultured with glass ionomer cement (**Medicem**) and **group III**: cells were cultured with self-adhesive resin cement (**Breeze™**). Biocompatibility was evaluated using hematoxylin and eosin stain, MTT assay, immunohistochemical staining with BAX after 24 and 72 hrs, and qPCR for IL-1B and IL-6 expression after 72 hrs.

Results: Medicem group showed many fibroblasts with intact and regular cell membranes, although some cells displayed necrotic or apoptotic characteristics at both durations. In contrast, Breeze™ group exhibited numerous necrotic cells with ruptured membranes, along with apoptotic cells featuring shrinkage, membrane blebbing and apoptotic body formation, that were notably evident after 72 hrs. MTT assay showed significantly higher cell viability in the Medicem group compared to the Breeze™ group. BAX immunostaining was significantly more evident in the Breeze™ group compared to the Medicem and control groups after both durations. qPCR analysis demonstrated significant downregulation of both genes in the Breeze™ group, which was greater than that observed in the Medicem group.

Conclusions: Glass ionomer cement (Medicem) demonstrated superior biocompatibility compared to self-adhesive resin cement (Breeze™).

KEY WORDS: Biocompatibility, Self-adhesive resin cement, glass ionomer cement, Breeze™ and Medicem.

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INTRODUCTION

The long-term success of dental prosthetic treatment requires appropriate prosthetic care and treatment planning. Periodontics and prosthetic dentistry are closely related because periodontal health affects the longevity of permanent tooth restorations [1-3].

Human gingival fibroblast cells (HGFCs), when come into contact with the cement margins of restorations, they serve as an efficient barrier between the alveolar bone and the oral environment [4].

Successful prostheses depend on the long-term adherence to the tooth structure and the biological compatibility of the materials used, luting agents may release substances that cause post-cementation hypersensitivity or damage to oral health [5].

Glass ionomer cement (GIC) was introduced in 1970 and is still widely used, mostly due to its bacteriostatic properties. It also has many advantages, including its capacity to chemically adhere to the tooth structure, its capacity to release fluoride, having adequate compressive and tensile strength, and biocompatibility [6].

The term "self-adhesive resin cements" refers to cements made of filled polymers that adhere to tooth structure without the need for an additional adhesive or etchant. For aesthetic restorations, these are the recommended adhesive systems [7]. They have many advantages, such as strong adherence to many surfaces, containing less filler particles and methacrylate, being insoluble in the mouth, and coming in a range of colors [8,9]. However, compared to traditional GIC cements, they have a shorter history of clinical usage, and their biocompatibility is a cause for worry [10].

Several studies have investigated the cytotoxic potential of various types of resin cements (RCs), they indicated that RCs induce inflammatory changes, cytotoxicity, genotoxicity, cell necrosis, and apoptosis, when cultured with different cell lines [5,11-14]. Compared to glass ionomer (GI) cement, RCs have been found to induce the production of reactive oxygen species (ROS), cell cycle arrest and reduce cell viability [15, 16]. Additionally, RCs with higher resin content have been reported to exhibit greater cytotoxicity toward fibroblast. Furthermore, inadequate RC polymerization can also cause highly toxic effect that irritate the gingiva [14]. Among the newer adhesive resin cements available on the market, Breeze™ has been demonstrated to exhibit moderate cytotoxic effects on Balb/3T3 cells [17].

Despite the generally reported biocompatibility of GICs, **Marczuk-Kolada et al.** [18], highlighted that the cytotoxicity of various conventional GIC products can vary. Notably, Medicem's biocompatibility has not been specifically studied, prompting its inclusion in our investigation. Additionally, the biocompatibility of Breeze™ has not been thoroughly examined to date. Therefore, this study aimed to evaluate and compare the impact of Medicem GIC and Breeze™ self-adhesive resin cement on human gingival fibroblasts.

MATERIALS AND METHODS

The current in vitro study was approved by the ethical committee of the Faculty of Dentistry, Minia University. (Committee no. 92 decision 666/2022)

Cell culture

Human gingival fibroblasts cell line (HGF-1) (ATCC) (American type culture collection) (CRL2014) was obtained from International Centre for Terrestrial Antarctic Research (ICTAR). Cell culture, staining, viability test and qPCR were conducted in Biology department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

The cells were cultured for 24 hrs and 72 hrs at 37°C in a humidified atmosphere with 5% CO2 in MEM-E medium supplemented with 10% fetal bovine serum (GIBCO, Thermo Fisher Scientific, Waltham, MA, USA) (Jouan SA, Saint-herblain,

Pays de la Loire, France). The cells were maintained in accordance with the manufacturing protocol, which involved decanting the growing medium and washing the cells in phosphate buffer saline (Adwia Pharma-ceuticals, El Sharkeya, Egypt). Then 0.25% trypsin enzyme and 0.05% (v/v) EDTA (GIBCO) were applied to the cells for five minutes at 37 °C. Finally, detached cells were splatted according to need [19].

Preparation of the tested dental cements:

Medicem glass ionomer and Breeze™ self-adhesive resin cements were purchased from a dental store in Egypt. Cements were prepared in accordance with the manufacturer's guidelines, for Breeze™ was chemically cured and Medicem glass ionomer was each tested cement was added to DMEM and then added by micropipette to the human gingival fibroblasts for further testing, starting with 10 mg/ml, then arranged for 2-fold dilution as follow (10.5,2.5,1.25,0,625), and so on till 12 dilutions.

The cultured HGFs were classified into three groups:

Group I (control group): cells were cultured with culture medium only.

Group II: Human gingival fibroblast cell line cultured with glass ionomer cement (Medicem).

Group III: human gingival fibroblast cell line cultured with self-adhesive resin cement (Breeze ™).

Biocompatibility was evaluated for the three groups using the following:

Hematoxylin and eosin staining for routine examination:

The cells of the three groups were placed on three methanol-clean slides for each treatment after 24 and 72 hours. Following methanol fixation, air drying, and rehydration at decreasing alcohol concentrations (100%, 90%, 75%, and 50%). Next, the slides were cleaned for 5 minutes by distilled water, then submerged in filtered hematoxylin stain for 3 minutes and again washed with distilled water twice. After that, slides were submerged in filtered eosin stain for five seconds, washed by distilled water and left to dry. The dried slides were dipped in xylene, mounted with Canada balsam, then coverslips were placed and allowed to dry [20].

Slides were examined and photographed with a digital camera (Canon, Japan) attached to a light microscope.

MTT assay for assessment of cell viability and proliferation:

Cytotoxicity of Medicem and Breeze™ was determined using (3- [4,5-dimethylthiazol 2-yl]-2.5-diphenyl tetrazolium bromide) MTT assay, in which the vitality of cells is determined through their ability to cleave the yellow tetrazolium salt in a purple formazan dye.

Growth media was removed from 96-well microtiter plates that had been pre-cultured with HGF cells. Medicem and Breeze™ were applied in double-fold serial dilutions to HGFs, with untreated wells acting as the negative control. Following incubation, the plates were cleaned three times using 250 µl of PBS per well. Fifty µl of MTT solution (0.5 mg/ml) were added to each well, and plates were incubated for further 4 h at 37 °C. Plates were washed with PBS three times, followed by the dissolution of the blue formazan using 50 µl/well DMSO (Sigma Aldrich, USA), then shaking the plates for 10 min at room temperature. Using an ELISA plate reader (Biotek, ELX-800-USA), the optical density (OD) was measured at 570 nm. GraphPad Prism software version 5 (S. Diego, USA) was used to calculate the percentage of cellular viability and determine the half maximal inhibitory concentration (IC50), which is the concentration that results in 50% inhibition of cellular growth

after 24- and 72-hour exposure to (Medicem glass ionomer, and Breeze™ self-adhesive resin cement) compared to the untreated control cells [21].

Immunohistochemistry

BAX immunolocalization was employed as a marker for cell apoptosis [22] after 24 and 72 hours of cement exposure. For ten minutes, the adhering fixed slides were incubated in 1% H2O2 in PBS containing 0.1% sodium azide to decrease the activity of the inherent levels of peroxidase. After that, slides were drained and exposed to a suitable dilution (1:200) of BAX antibody in an antibody dilution buffer in a humidified chamber overnight at 4°C. After five minutes of PBS-T washing, slides were incubated for one hour with a secondary antibody combined with horseradish peroxidase. Following two cycles of washing, the slides were incubated in the dark with a solution of horseradish peroxidase substrate, which produces a dark stain that can be seen in a light microscope. Slides were then photographed and subjected to a microscopic examination. The software ImageJ was used for image analysis [23].

Real-time qPCR for expression of IL-6 and IL-1β

Reverse transcription of RNA, or real-time PCR, is used to transform RNA into cDNA. Total RNA was extracted from the negative control, Breeze™, and Medicim-exposed fibroblast cell lines using the iScript™ One-Step RT-PCR Kit with SYBR® Green according to the manufacturer's instructions. The concentration of the extracted RNA was measured with a Beckman dual spectrophotometer (Beckman Instruments, Ramsey, MN, USA). Real-time PCR was used to measure the expression levels of (IL1β: F 5'-CCACAGACCTTCCAGGAGAATG-3', IL1β: R 5'-GTGCAGTTCAGTGATCGTACAGG-3', IL6: F 5'-TACCACTTCACAAGTCGGAGGC-3', IL6: R 5'-CTGCAAGTGCATCATCGTTGTTC-3', and GAPDH: F 5'- ACCACCCTGTTGCTGTAGCCAA-3', GAPDH; R 5'-ACCACCCTGTTGCTGTAGCCAA-3'). The reaction step included an enzyme activation step lasting 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C, 20 seconds at 55°C, finally the amplification step lasting 30 seconds at 72°C. The average critical threshold (CT) values of the housekeeping gene b-actin were used to standardize changes in the expression of each target gene.

Statistical methods

Statistical analysis was conducted using IBM-SPSS (Statistical Package for Social Science) version 24 (Standard version, NY, USA. 2016). Quantitative variables were presented as mean, median, standard deviation (SD), and range. The Shapiro-Wilk test was used to investigate the data normality, and accordingly, comparison of quantitative data was conducted using the one-way ANOVA test for normally distributed data or the Kruskal-Wallis test for non-normally distributed data, and post-hoc analysis with Tukey's test was used for pairwise comparisons. A p-value <0.05 was considered significant.

RESULTS

Histological examination results:

Cells of **group I (control)** appeared normal with regular, intact cellular and nuclear membranes, along with normally distributed chromatin after both 24 and 72 hrs **(Fig. 1A&B)**.

In group II (Medicem): After 24 hours, most fibroblasts showed regular and intact nuclear and cellular membranes; however, a smaller group of fibroblasts showed apoptotic characteristics like shrinkage and membrane blebbing, as well as fragmentation into apoptotic bodies, indicating late apoptosis. Some cells also appeared swollen, indicating necrosis. In addition, normal mitotic figures were detected. Following a 72-hour culture with Medicem, more cells were swollen and necrotic with peripheral chromatin condensation, besides, other group of cells showed apoptotic features **(Fig. 1 C&D)**.

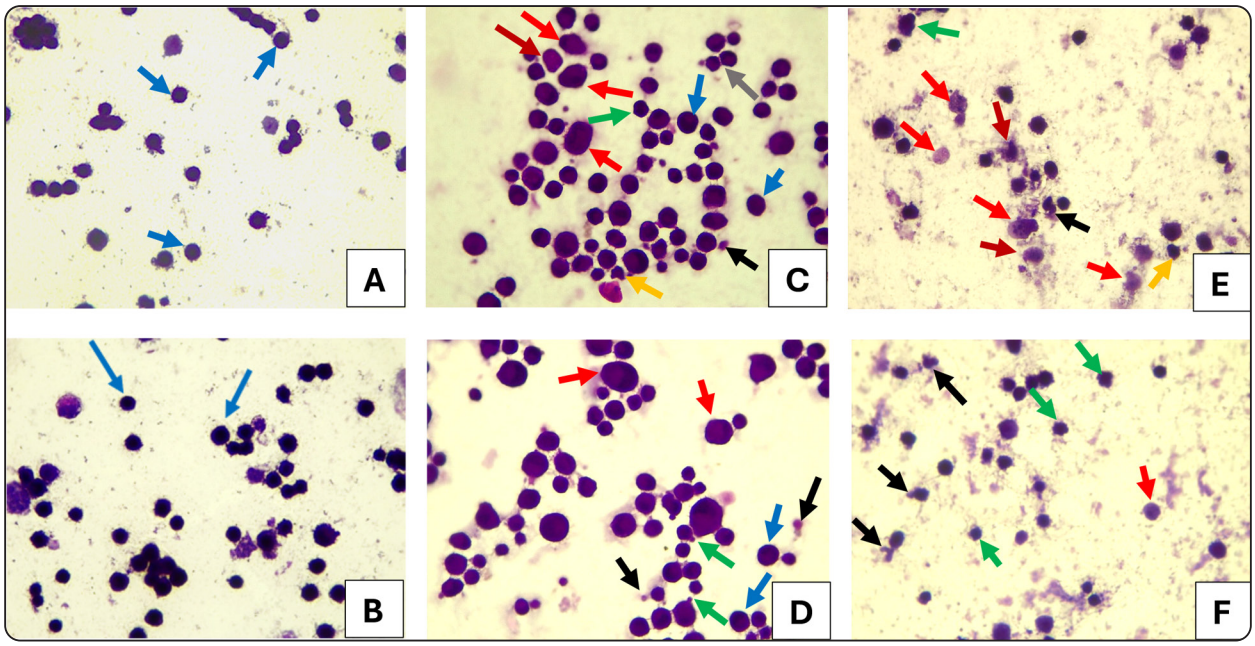
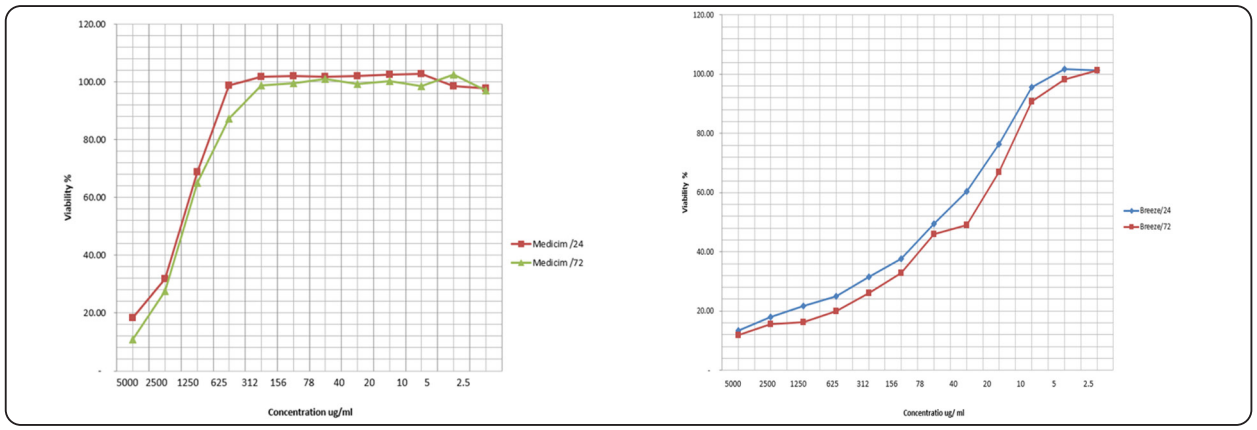


Fig. (1): Photomicrographs of human gingival fibroblasts for the three groups. **(A&B): group I** (control group) after 24 hrs (A) and 72 hrs (B) showing normal cell morphology with intact cellular and nuclear membrane (blue arrows). **(C): group II** (Medicem) after **24hrs**, showing normal cells with regular outline (blue arrow), normal mitotic figure (grey arrow), swollen necrotic cells with peripheral chromatin condensation (red arrow), karyolysis (dark red arrow), cells with shrunken apoptotic nuclei (orange arrow), membrane blebbing (green arrow), apoptotic bodies (black arrow). **(D): group II** after **72 hrs** showing normal cells with regular outline (blue arrow), swollen necrotic cells with peripheral chromatin condensation (red arrow), membrane blebbing (green arrow), apoptotic bodies (black arrow). **(E): group III** (Breeze™) after **24 hrs**, showing karyolysis (red arrow), ruptured cellular membrane (dark red arrow), shrunken pyknotic nuclei (orange arrow), membrane blebbing (green arrow) and apoptotic bodies (black arrow). **(F): group III** after **72 hrs**, showing shrunken and pyknotic nuclei (green arrow), numerous apoptotic bodies (black arrow) and karyolysis of many nuclei (red arrow).

While in **group III (Breeze™)**, many necrotic and apoptotic cells were visible. After 24 hrs of exposure to Breeze, numerous cells showed karyolysis and ruptured cell membranes, indicating necrosis, other cells displayed signs of early apoptosis as being shrunken or late apoptosis as nuclear fragmentation, membrane blebbing, and apoptotic bodies formation. After 72 hrs of adding Breeze™ to the cultured cells, most cells displayed characteristics of late apoptosis; they were shrunken with pyknotic and hyperchromatic nuclei, as well as many apoptotic bodies formation. Additionally, several cells exhibited nuclear karyolysis **(Fig. 1 E&F)**.

MTT assay results:

MTT assay results showed that **Group II (Medicim)** had a concentration dependent decrease in viability after 24 hrs and 72 hrs; however, there was an insignificant difference ($P > 0.05$) in viability between 24 hr (85.6 ± 8.6) and 72 hr (82.3 ± 9.1) durations **(Line graph 1)**. On the other hand, **Group III (Breeze™)** showed time and concentration dependent viability as viability increased relative to decreased Breeze concentration or duration; it showed a significantly decreased value ($p< 0.001$) of viability of 72 hr (47.9 ± 9.7) post treatment than that calculated after 24 hr (52.7 ± 9.7) post treatment **(Line graph 2)**. Additionally, the viability percentage was significantly higher in the Medicem group at most concentrations at 24 and 72 hours, according to data comparing the Breeze™ **(table 1, Fig. 2)**.



Line graph (1) Viability percent in group II (Medicem) at 24 hrs and 72hrs.

Line graph (2) Viability percent in group III (BreezeTM) at 24 hrs and 72 hrs.

TABLE (1) Mean and P value of Viability in the three groups after 24hrs and 72hrs:

	Control (1) (n = 8)	**Medicem (2) (n = 12)**	**Breeze ™ (3) (n = 12)**	**P-value**
At 24 hours	100 ± 3.2	85.60 ± 8.6	52.67 ± 9.7	= 0.002*
P-value	1 vs 2 = 0.239	2 vs 3 = 0.007	1 vs 3 < 0.001	
At 72 hours	100 ± 3.2	82.34 ± 9.1	47.88 ± 9.7	= 0.001*
P-value	1 vs 2 = 0.165	2 vs 3 = 0.006	1 vs 3 < 0.001	
P-value within group	------	> 0.052	< 0.001	

ANOVA test was used to compare the difference in Mean between groups
Post-hoc test with Bonferroni Corrections was used to compare the mean difference between groups.
****Paired Sample t-test was used to compare Mean within Group.***

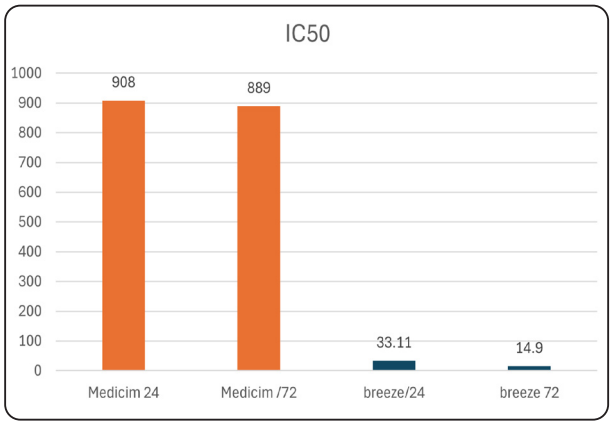


Fig. (2) IC50 of group II (Medicem) and group III (BreezeTM) at 24hrs and 72hrs

Immunohistochemical staining results:

Immuno stained cells in **the control group (I)** showed negative nuclear and cytoplasmic immunoreaction for BAX **(Fig 3. A&B)**.

Group II (Medicem), After 24 hrs of culture with Medicem, some cells displayed mild cytoplasmic immunoreactivity and others showed weak to strong nuclear immunoreactivity to BAX. After 72 hrs of treatment with Medicem, more cells showed positive BAX staining, ranging from weak to moderate nuclear and cytoplasmic immunoreactivity **(Fig. 3 C&D)**.

In **Group III (Breeze)**, nearly all fibroblasts showed positive staining for BAX after 24 hrs of culture with Breeze ™, most cells had moderate nuclear and cytoplasmic immunoreactivity, and some showed intense nuclear immunoreactivity for BAX. After 72hrs of treatment with Breeze™, almost all cells were strongly stained with BAX **(Fig. 3 E&F).**

Statistical analysis for the measured area percentage of BAX immunoreactivity using the ImageJ software at both time intervals, revealed that after 24 hrs of culture the difference was statistically insignificant (p=0.051) for Medicem group, while it was statistically significant for Breeze™. As compared to the control group.

By comparing experimental groups, the area percentage was lowest in Medicem group (2.27 ± 0.1) and highest in Breeze™ group (12.5 ± 1.7) for 24 hrs duration. The difference between control and both experimental groups was statistically significant (p<0.001). Within experimental groups, the area percentage increased after 72 hrs; Medicem group recorded (3.58 ± 0.1) and Breeze™ recorded (27.6 ± 1.9). However, the increase was statistically insignificant for Medicem (P = 0.06) while it was significant for the Breeze™ group (p<0.001), and by comparing the mean of area percentage for all groups after 72 hrs, the difference was statistically significant (p<0.001). Pairwise comparison revealed also significant differences (p<0.001).

Finally, the Breeze™ group recorded the highest area percentage at 72 hours, while the Medicem group had the lowest at 24 hours **(table 2, Fig. 4)**.

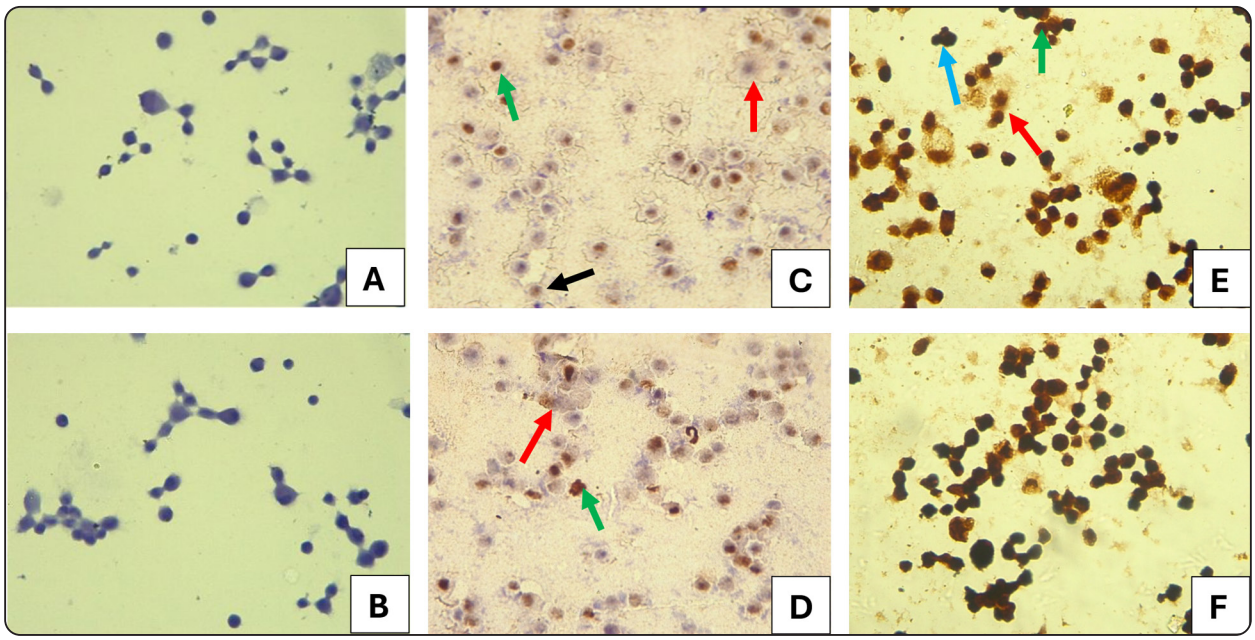


Fig. (3) Photomicrographs of immunohistochemical staining with BAX for **(A&B): group I** (control group) after 24 hrs and 72 hrs showing negative immunoreaction for BAX. **(C): group II** (Medicem) after **24 hrs** showing some cells with weak (black arrow), moderate nuclear (green arrow) and mild cytoplasmic (red arrow) immunoreactivity for BAX**. (D): group II** (Medicem) after **72 hrs** showing more cells with moderate nuclear (green arrow) and cytoplasmic (red arrow) immunoreactivity for BAX**. (E): group III** (Breeze™) after **24 hrs** most cells with moderate nuclear (green arrow), and cytoplasmic (red arrow) immunoreactivity, many cells showed intense nuclear immunostaining (blue arrow) for BAX. **(F): group III** (Breeze™) after **72 hrs**, nearly all cells were intensely stained by BAX.

TABLE (2) Mean of area percentage of BAX immunostaining in Medicem, Breeze™ and negative control group at 24 and 72 hours:

Group/area%	Control (1) (n = 8)	Medicem (2) (n = 12)	Breeze ™ (3) (n = 12)	P-value
At 24 hours ***P-value***	0.00 ± 0.0 1 vs 2 = 0.051	2.27 ± 0.1 2 vs 3 < 0.001	12.52 ± 1.7 1 vs 3 < 0.001	< 0.001*
At 72 hours ***P-value***	0.00 ± 0.0 1 vs 2 < 0.001	3.58 ± 0.1 2 vs 3 < 0.001	27.57 ± 1.9 1 vs 3 < 0.001	<0.001*
P-value within same group	1.000	= 0.060	< 0.001	

ANOVA test was used to compare the difference in Mean between groups
*****Post-hoc test with Bonferroni Corrections was used to compare the mean difference between groups***
******Paired Sample t-test was used to compare Mean within Group***

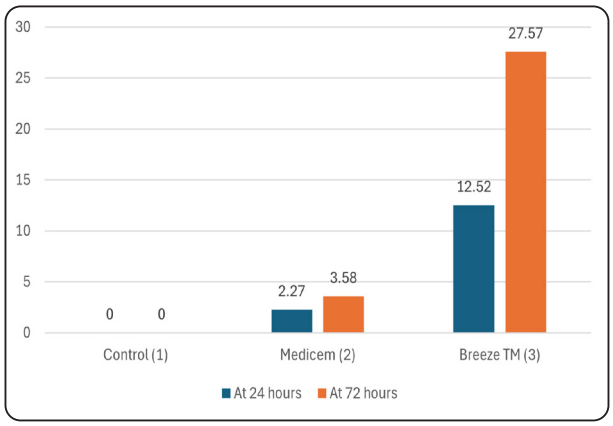


Fig. (4) Mean of area percentage of BAX immunostaining in control and experimental groups at 24 and 72 hrs.

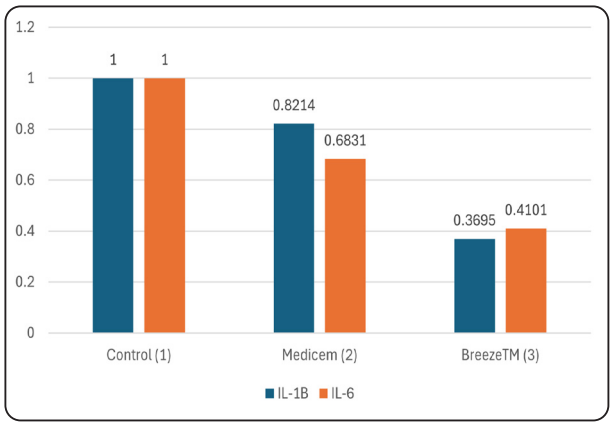


Fig (5) Real -time qPCR for IL-1β and IL-6 for control and experimental groups

RT-PCR results:

In terms of gene expression, compared to group I (control), group II (Medicem-exposed cells) displayed a mild significant (p = 0.04) downregulation (0.8214) of the anti-apoptotic gene IL-1B. Conversely, Group III (Breeze™-treated cells) displayed a highly significant (P<0.001) downregulation (0.3695) as compared to the 0.8214 observed in Medicem-treated cells and the negative control.

Similarly, both groups demonstrated a significant downregulation (p < 0.05) of IL-6 compared to that found in untreated negative control cells, and there was also significant downregulation (p = 0.03) for group III (Breeze™-exposed) cells (0.4101) as compared to group II (Medicem-exposed) cells (0.6831) **(Fig. 5)**.

DISCUSSION

Adhesive luting cements are increasingly used in modern dentistry because they are the most common choice for bonding ceramic restorations, providing excellent mechanical properties, and are very easy to handle with automix application systems [24].

Glass ionomer cement (GIC) is a self-adhesive restorative material with a wide range of applications in adult and pediatric dentistry. Its chemical bonding to the tooth structure, release of fluoride, and combination of biocompatible and bioactive properties are what give it, its strong anti-cariogenic action [25].

When compared to other cements alternatives, self-adhesive resin cements are more extensively

employed due to their adhesive components, which eliminate the need for separate etchants and primers for bonding to dental, metal, or ceramic surfaces [26].

The purpose of this study was to evaluate and compare the biocompatibility of glass ionomer cement (Medicem) and self-adhesive resin cement (Breeze™) on human gingival fibroblasts (HGFs) cell lines.

Biocompatibility was evaluated using four different techniques: histological examination of hematoxylin and eosin-stained slides to examine cell morphology, MTT assay to assess cell viability and proliferation, immunohistochemistry to identify BAX, and finally qPCR for IL-1B and IL-6 expression.

In the current investigation, the cell culture approach was applied on human fibroblasts. It is essential to analyze the biocompatibility of dental products using cell culture, as it is a very dependable and economical assessment method. It has been demonstrated that human gingival fibroblasts are a useful tool for assessing the cytotoxicity of dental biomaterials because of their sensitivity to medications, chemicals, and poisons, as well as because they exist in the mouth cavity [11].

According to **Ghasemi et al.** [27] cell toxicity research frequently uses the MTT test for assessment of the cellular metabolic activity. In the mitochondria of living cells, MTT is reduced to purple formazan, a color solution is created when the purple formazan is dissolved, and then a spectrophotometer can be used to measure the absorbance of the colored solution.

One of the most reliable and efficient methods for identifying and locating specific antigens in cells and tissue is immunohistochemistry (IHC), which makes use of the specific binding that occurs between an antibody and an antigen. Usually, light microscopy is employed to recognize and assess this antigen [28].

BAX gene (Bcl-2 Associated X-protein) is a pro-apoptotic member of the Bcl-2 gene family, researchers believe that it plays an important role in regulating intrinsic apoptosis [29].

The activity of fibroblasts is heavily influenced by cytokines produced by immune system cells. Notably, fibroblasts themselves can release various cytokines, contributing to local inflammatory and immune responses [30]. Changes in cytokine concentrations, such as interleukin-6 (IL-6) and interleukin-1-β (IL-1-β), are among the most accurate markers of the apoptotic process [31, 32]. Interleukins (ILs) are signaling molecules that regulate cell division, proliferation, and activation. Specifically, IL-1 and IL-6 are produced by macrophages, fibroblasts, and other cell types, playing key roles in immune response and inflammation [30, 33, 34].

In the present study, photomicrographs of fibroblasts stained with H&E and examined under light microscope revealed lesser changes occurred to Medicem group than Breeze™ group. The control group showed intact cellular and nuclear membranes after being cultured for 24 and 72 hours. After being cultured with Medicem for 24 hours, most fibroblasts maintained regular and intact nuclear and cellular membranes, although some fibroblasts appeared swollen or apoptotic. Following a 72-hour culture with Medicem, a greater number of cells exhibited swelling and necrosis along with peripheral chromatin condensation. In contrast, several necrotic and apoptotic cells were visible in the cells cultured with Breeze™ after 24 hours. By 72 hrs, the majority of cells displayed late apoptosis, including the formation of apoptotic bodies, shrunken, pyknotic, and hyperchromatic nuclei. Additionally, some cells demonstrated nuclear karyolysis after prolonged exposure to Breeze™ .

Cellular blebbing is a unique dynamic protrusion that originates from the plasma membrane and can be classified as either apoptotic or non-apoptotic[35]. Apoptosis is generally characterized by distinct morphological changes, such as membrane

blebbing, cellular shrinkage, and fragmentation into apoptotic bodies [36]. On other hand, necrotic cells are characterized by swelling of cellular organelles, rupture of the plasma membrane, and finally lysis of the cell [37]. According to **Takada et al.** [38], karyolysis is the total disintegration of a dying cell's nuclear components.

Our findings regarding the morphological changes seen in Breeze™ group, align with those of **Skośkiewicz-Malinowska et al** [17] who reported that contact with Breeze resulted in altered cell morphology, including lysed, rounded, and vacuolated Balb/3T3 cells. However, in contrast to the sever apoptotic and necrotic changes demonstrated in our study, they reported that Breeze exhibited only moderate cytotoxicity.

Our findings are also consistent with **Reichl et al.** [11], who observed that direct contact between resin cement and human gingival fibroblast, led to necrosis and cell death. They proposed that this outcome could be due to a reduction in cellular glutathione (GSH), the main non-enzymatic antioxidant, caused by the cytotoxic effects of resin monomers, as noted in prior studies. This depletion is associated with increased levels of reactive oxygen species (ROS), which damage biomolecules and ultimately lead to cell death. Furthermore, they reported that the monomer triethyleneglycoldimethacrylate (TEGDMA) adversely affects the mitochondria of gingival fibroblasts and promotes lipid peroxidation, both of which contribute to cell death.

These results are further supported by **Şişmanoğlu et al.** [39] who found that self-adhesive resin reduced the number of fibroblasts. On the other hand, **Lang et al.** [40] found that substances produced by the GIC that came into direct contact with primary human gingival fibroblasts (HGF) altered the PH and had some impact on adhesion and cell function, and these alterations resulted from the GICs' initial acid-base interaction.

Regarding the MTT test, the results showed that Medicem had a greater viability percent than Breeze™ at most concentrations after 24 and 72 hours, with statistically significant difference. In (Medicim) group, the viability was insignificantly changed throughout the duration of 24 and 72 hours. Conversely, Breeze™ demonstrated a viability that was time-and concentration-dependent, where increased viability being associated with lower Breeze™ concentration.

IC50 revealed that Medicem was more biocompatible than Breeze™. These findings align with the study by **Chang et al.** [41], which revealed that cells exposed to self-adhesive resin cement had the lowest cell viability, attributing this to their content of monomers such as hydroxyethyl methacrylate (HEMA) and TEGDMA, which have been demonstrated to be harmful to fibroblast cells at specific doses. Similarly**, Bandarra et al.** [5] who found that there was a pronounced decrease in viability when the cells were exposed to resin cement.

Moreover, **Diemer et al.** [13], highlighted that self-adhesive cements exhibit varied effects on cell viability depending on the evaluation method used. Corroborating our findings, **Ersahan et al.** [42] also found a minimal decrease in viability when cells exposed to GIC. And **Marczuk-Kolada et al.** [18] who found that GIC were less toxic and higher in viability than other cements.

Our immunohistochemical results for BAX came in line with H&E and MTT results. Following a 24-hour culture with Medicem, BAX expression was detected in a few cells, while after 72 hours, more cells in group II showed positive staining for BAX. Meanwhile, following a 24-hour Breeze™ exposure, almost all fibroblasts in group III exhibited positive staining for BAX; the majority of cells exhibited mild to moderate immunoreactivity. The expression of BAX increased further for 72 hours of culture with Breeze™.

By measuring BAX area percentage, the Breeze™ group was found to record the highest area percentage over the 24 and 72-hour periods, while

the Medicem group was the lowest, and the differences were statistically significant. These results are in accordance with **Duzyo et al.** [43] who found that GIC were less toxic than resin cements using BAX and caspase 9 immunohistochemical staining, they stated that the cytotoxic effects of a substance could be effectively increased by using certain types of monomers, such as propane (Bis-GMA), urethane dimethacrylate (UDMA), 2-hydroxyethylmethacrylate (HEMA), and triethylene glycol dimethacrylate (TEGDMA), which increase oxidative stress in tissues and cells, resulting in cell damage.

These results also were in harmony with the qPCR results for the anti-apoptotic gene IL-1β, which showed slight downregulation for Medicem (0.8) than for Breeze™ (0.3). Similarly, IL-6 exhibited a slight downregulation in Medicem-treated cells (0.6) than in Breeze™-treated cells (0.4). Compared to untreated negative control cells, both groups exhibited significant downregulation ($P<0.05$).

IL-1β is a cytokine that is known to suppress the expression of apoptosis and is essential for the activation of the inflammatory process [29]. On the other hand, IL-6 has been reported to be a pleotropic cytokine capable not only of inducing growth and differentiation but also of modulating cellular apoptosis, and that IL-6 is also capable of preventing apoptosis [30].

Based on our histological, immunohistochemical, MMT assay, and qPCR results, we found that Medicem GIC had less cytotoxic effect and was more biocompatible than Breeze™ adhesive resin cement.

Supporting our findings**, Lan et al.** [44] who indicated that glass ionomer cement was characterized by low cytotoxicity. This cytotoxicity might be attributed to fluoride released, which was found to inhibit protein synthesis, growth, division, and mitochondrial function in cultured human pulp cells, leading to minimally programmed cell death [45]. Furthermore, **Wilson et al.,1971**[46] also reported that there were ions other than fluoride responsible for the cytotoxicity, such as the ions that were released from GIC as F^-, Na+, and Si^+.

Hiraishi et al. [47] reported that GIC has a low setting PH and reported that the materials' acidity may contribute to the luting cements' initial cytotoxic effects. This early acidity, which includes a prolonged period of acidic pH, followed by acidic diffusions from luting cements through dental preparation and cementation operations, might have adverse consequences.

Previous studies also coincided with our results regarding adhesive resin cement cytotoxicity, which was proved to reduce the cell viability owing to reactive oxygen species (ROS) production causing cell cycle arrest [48]. **Hadjichristou et al.** [49] demonstrated that resin cement decreases the biocompatibility because they contain monomers that induce cytotoxicity in pulp and gingival cells, especially TEGDMA, has been shown to be more cytotoxic than other resin monomers, which can cause acute cytotoxicity. In addition, **Bakopoulou et al.** [16] also announced that the cytotoxicity of resin-cement is caused by monomers released from the material, and the more unreacted monomers, the higher the toxic effect will occur. Moreover, **Goldberg et al.** [14] explained that the short-term release of free monomers during the monomer polymer conversion and the long-term release of leachable chemicals caused by erosion and degradation are the two main processes that cause the cytotoxicity of dental cements, and this incomplete RC polymerization increases residual dimethacrylate monomers, which produce intracellular reactive oxygen species (ROS) and induce cellular stress, DNA damage, and cell apoptosis. These monomers include triethyleneglycol dimethacrylate (TEGDMA) and hydroxyethylmethacrylate (HEMA), which, in addition to GDMA, have been proved by **Becher and colleagues** [50] to cause cell death.

Moreover, the morphology of human gingival fibroblasts has been reported to be significantly changed by 2-hydroxyethyl methacrylate (HEMA),

even at low concentrations [51]. also, the mechanical characteristics of resin cement, such as its viscosity, degree of conversion, water absorption, and polymerization shrinkage, have been reported to exert an adverse effect on the tissues surrounding them [52].

De Souza et al. [53] found that hydrophobic monomers Bis-GMA and UDMA are often linked to HEMA, which makes them more hydrophilic. This increased hydrophilicity facilitates the diffusion of these monomers into tissues, potentially leading to tissue damage and cell death.

CONCLUSION

According to the results of the present study, the biocompatibility of GIC (Medicem) was found to be superior to that of adhesive resin cement (Breeze™).

RECOMMENDATIONS:

Taking into account that resin cement has an important role in everyday dental clinical practice, so it is extremely important to encourage the development of less cytotoxic cement and maintain the vitality of the surrounding oral tissues. Further animal studies and clinical trials are needed to reinforce the existing evidence regarding the biological properties of glass ionomer cement. Additionally, comprehensive investigations should be conducted to address and eliminate the release of monomers from resin-based cement, aiming for safer and more biocompatible dental materials.

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