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CYTOTOXICITY OF INDIRECT RESTORATIVE MATERIALS ON FIBROBLAST CELLS: *IN-VITRO* STUDY

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ABSTRACT

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Introduction The aim of this study was to assess the cytotoxicity profiles of eight different fixed prosthetic restoration materials [Gold-based alloy (A), Chromium-Cobalt alloy (B) and Nickel-Chromium alloy (C), fiber-reinforced-resin-blocks (D), resin-nano-ceramic (E), lithium-disilicate-glass-ceramics (F), monolithic-zirconia (G) and feldspathic-ceramic (H)] by using cell culture methods on the L929 mouse fibroblast cells.

Methodology 36 disc-shaped samples of each test material were prepared (5x2mm, N=288). After sterilization, discs were placed in EMEM and incubated at 37°C. Mediums were collected and filtered from each of four samples in 1st and 7th days. After 24hours incubation, cells were treated with 100 µl medium extracts of materials. Viability of cells was measured after 48 hours. Cytotoxicity was assessed with XTT and xCELLigence tests. Apoptosis was analysed using Annexin-V/PI staining. All data were statistically analysed with One-way ANOVA and Tukey's multiple range tests ($p<0.05$).

Results Considering the cell viability and apoptosis rate significant differences were found after the 1st and 7th days of incubation periods for each material group ($p<0.05$). Among the material groups significant differences were observed ($p<0.05$). F group showed the lowest cell viability and showed highest apoptosis rate ($p<0.05$). Along the entire test period, E group showed the highest cell viability and lowest apoptosis rate ($p<0.05$).

Conclusion All fixed restoration materials investigated in the study exposed various levels of cytotoxicity, with significant differences among the test groups.

KEYWORDS

Dental Alloys; CAD-CAM Materials; Cytotoxicity; Apoptosis; Fibroblasts.

1. INTRODUCTION

In parallel with the improvements in dental technology and material science, innovative materials are being developed to be used in fixed prosthetic restorations, which are in direct contact with bone, connective tissue or oral epithelium. Dental casting alloys that play an important role in restorative dentistry are widely used as restorative materials in dental applications [1]. Recently, the use of cast alloys has become very limited due to the improvements of full ceramic restorations and more durable resin-based composites. Nevertheless, for fixed prosthetic restorations, dental alloys have continued to be used as the primary material [2]. In general, alloys usually include at least four and often six or more metals,

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Table 1. The codes, brand names, compositions and manufacturers of the materials.

Code	Brand	Material type	Chemical composition	Manufacturer
A	PX Premium Bio	Au alloy	Au 86.5%, Pt 11.5%, Zn 1.4%, Rh, Fe, Mn, Ta <1%	PX Dental SA, Marin, Switzerland
B	Strabond CoS	Cr-Co alloy	Co 59%, Cr 25%, W 9.5%, Mo 3.5%, Si %1, C, Fe, Mn, N <1%	Scheftner Dental Alloys, Germany
C	MoguCera N	Ni-Cr alloy	Ni 62%, Cr 24%, Mo 11%, Si 1.6%, Mn <1%	Scheftner Dental Alloys, Germany
D	TriLor®	Fiber reinforced resin block	Epoxy resin matrix and multidirectional integrated fiberglass	Bioloren, S.r.l., Italy
E	Lava Ultimate	Resin nano ceramic	Matrix: Bis-GMA, UDMA, Bis-EMA, TEGDMA Filler: SiO_2, ZrO_2, aggregated ZrO_2/SiO_2 cluster (80wt%)	3M-ESPE, Seefeld, Germany
F	Rosetta SuperMill	Lithium disilicate glass-ceramic	Li_2O 10-15%, SiO_2 71-80%, B_2O_3 0-6%, P_2O_5 2-5%, Al_2O_3 2-5%, other oxides and colorants 5-12%	Hass Corporation, Korea
G	InCoris TZI	Monolithic zirconia	ZrO_2+HfO_2+Y_2O_3 ≥99.0%, Y_2O_3 5.4%, Al_2O_3<0.35%, F_2O_3 < 0.01%, other oxides < 0.2%	Sirona Dental Systems GmbH, Bensheim, Germany
H	CEREC locks	Feldspathic ceramic	SiO_2 56-64%, Al_2O_3 20-23%, Na_2O 6-9%, K_2O 6-8%, CaO 0.3-0.6%, TiO_2 0.0-0.1%	Sirona Dental Systems GmbH, Bensheim, Germany

Abbreviations: Au: gold; Pt: platinum; Zn: zinc; Rh: rhodium, Fe: iron; Mn: manganese; Ta: tantalum; Cr: chromium; Co: cobalt; W: tungsten; Mo: molybdenum; Ni: nickel; Si: silicon; Bis-GMA: bisphenol A-glycidyl methacrylate; UDMA: urethane dimethacrylate; Bis-EMA: ethoxylated bisphenol A-glycol dimethacrylate; TEGDMA: triethylene glycol dimethacrylate; SiO_2: silicon dioxide; ZrO_2: zirconium dioxide; Li_2O: lithium oxide; B_2O_3: Boron trioxide; P_2O_5: Phosphorus pentoxide; Al_2O_3: aluminium oxide; ZrO_2: zirconium dioxide; HfO_2: hafnium dioxide, Y_2O_3: yttrium Oxide; F_2O_3: ferric oxide; Na_2O: sodium oxide; K_2O: potassium oxide; CaO: calcium oxide; TiO_2: titanium dioxide.

but metallurgically, considering the periodic table various elements can be used in dental alloys and they are even more complex materials. The intricacy and variety of these alloys complicate to understand their biocompatibility, since the body can be affected by any element released from the alloy [1]. According to the American Dental Association (ADA) (1986), dental cast alloys can be: (1) high noble alloys (≥60% Gold (Au), Platinum (Pt), Palladium (Pd) and ≥40% Au), (2) noble alloys (≥25% Au, Pt, Pd) and (3) predominantly base metal alloys (<25% Au) [3]. Dental alloys are defined by their composition, but composition can be explained in two ways, either as in the alloy percent of the number of atoms of each element (atomic percentage = at%) or percentage of weight (wt%) of elements. Even though the alloy manufacturers and standards organizations describe an alloy's composition by weight percentage, atomic percentage of these materials determine their biological properties [1]. In recent decades, esthetic and durable restorations have been designed and produced with computer-aided design and computer-aided manufacturing (CAD/CAM) technology. Feldspathic ceramics, glass ceramics containing leucite and lithium disilicate or yttrium tetragonal zirconia polycrystals are the examples of CAD/CAM high quality CAD/CAM ceramic materials. Recently, nano-hybrid ceramics, zirconia-reinforced lithium silicate ceramics, composites and glass-fiber composites have been added to the previous CAD/CAM materials [4,5]. Glass-fiber-composite technology has been introduced to the dental practice for well over 20 years. Fiber reinforced composite materials like TRILOR are alternative materials for permanent and temporary dental restorations. Copings, substructures, frameworks for anterior or posterior crowns, bridges, telescopic restorations, bar attachments on implants and drilling guide for implant surgery are among the indications for these materials [6].

Since dental materials used in fixed prosthodontics are contact with oral tissues, the biocompatibility of these materials is very critical and dentists, especially prosthodontics, should focus on dental biomaterials [7]. The release of copper or nickel from cast alloys has been suggested as the main (toxic) cause of oral tissue reactions, such as gingival inflammation [8]. In order to study the cytotoxicity of restorative materials, various in vitro systems including organ cultures and cells in culture have been utilized. However, for in-vitro toxicity test of dental materials, the most commonly used biological system is the cell culture method. Two different types of cells are generally preferred; permanent cell lines derived from type-culture collections (L929 or 3T3 mouse fibroblasts), and primary cells derived from mucosal or gingival explants and established in each individual laboratory. However, permanent cell lines

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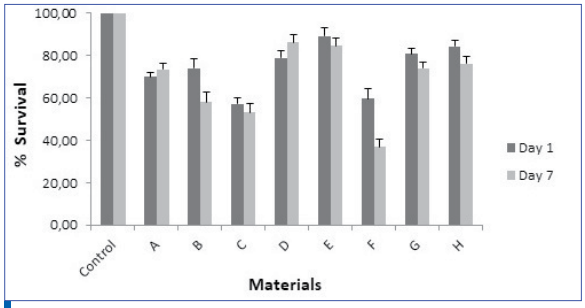

Figure 1. Mean and standard deviation (SD) values of cell viability comparison among the 1st and 7th day medium extracts of restorative materials obtained from XTT assay.

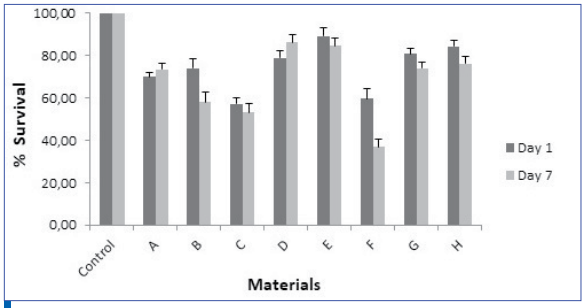

Figure 2. Mean and SD values of cell viability comparison among the 1st and 7th day medium extracts of restorative materials obtained from xCELLigence assay.

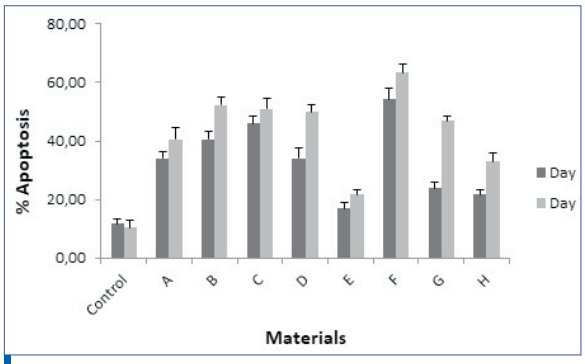

Figure 3. Apoptosis analysis for 1st and 7th day medium extracts of restorative materials. Bar graphs showing the percentage of cell populations (early apoptosis, late apoptosis and necrosis) in treated cells.

are preferred since they are well defined and easily available [9]. Prior to introduction to dental clinical practice, physical property and biocompatibility accuracy assessment of dental materials is imperative [7]. Within this context, restorative materials entail thorough evaluation with respect to their interaction with the vital tissues, because they may release substances resulting in allergic reactions and inflammation [8]. The aim of the study was to assess the cytotoxic and apoptotic effects of eight indirect restorative materials by using XTT cell proliferation assay, xCELLigence real-time cell analysis system and Annexin-V PI staining on fibroblast cells.

2. MATERIALS AND METHODS

Three metal framework restoration materials [Gold-based alloy (A), Chromium-Cobalt alloy (B) and Nickel-Chromium alloy (C)] and five metal-free CAD/CAM materials [fiber-reinforced-resin-blocks (D), resin-nano-ceramic (E), lithium-disilicate-reinforced-ceramics (F), monolithic-zirconia (G) and feldspathic-ceramics (H)] were studied. They are shown in Table 1. 36 disc-shaped specimens (h=2mm, Ø=5mm) were prepared in accordance with ISO 10993–5: Tests for Cytotoxicity - In Vitro Methods [10] for each material group. For the A, B and C groups; the disc-shaped wax patterns (2x5mm) were produced with conventional lost-wax technique using an induction-casting machine (Argonocaster-C, Shofu, Japan). The casting method was performed under the pressure of argon gas and vacuum suction, and afterwards standard dental laboratory procedures were performed.

After casting, air particle abrasion with 100µm aluminum oxide particles (80 psi=5.62 kgf/cm^2) was applied to the discs. For polishing process, 400, 600, 1200 and 2000 grit silicon carbide papers were utilized and the polishing was completed with diamond and aluminum oxide pastes. For the D, E, F, G, and F groups, the CAD/CAM blocks were cut using a low-speed diamond saw (Mecatome T180; Presi, Grenoble, France). F and G discs were sintered according to the manufacturer's instructions. Then, OptraFine ceramic polishing system (Ivoclar Vivadent, Schaan, Liechtenstein) was used for polishing the surfaces of the specimens, complying with the manufacturer's instructions. Subsequently, the specimens were placed into an ultrasonic water bath (Whaledent Biosonic Jr, Whaledent International, New York, NY) for 10 minutes and then dried. A total of 288 specimens (n = 36 per test material) were prepared. Each group was divided into three (n =12 per group) randomly; two groups were assessed with the cytotoxicity assays, while the other group was used for the apoptosis assay.

2.1. Preparation of medium extracts

The sterilization process was made with 16 kGy gamma irradiation (Gamma-Pak Sterilization Ind., Tekirdag, Turkey). Then the sterilized disc samples were transferred into 96-well plate and each well was filled with 150 µL Eagle's Minimum Essential Medium (EMEM) containing 10% fetal calf serum (FBS) with 100 U/mL of penicillin-streptomycin. In control group, there were no specimens in the well plate. All plates were incubated in a highly humidified atmosphere containing 5% CO_2 at 37°C; medium extracts of the test materials were collected at 1st and 7th days and were stored in –20°C until cytotoxicity experiments.

2.2. Cytotoxicity assay

L929 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and grown in EMEM culture medium that was supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified atmosphere with 5% CO_2 at 37°C. XTT Cell Proliferation Kit (Roche Applied Science, Basel, Switzerland) and xCELLigence real-time cell analyzer

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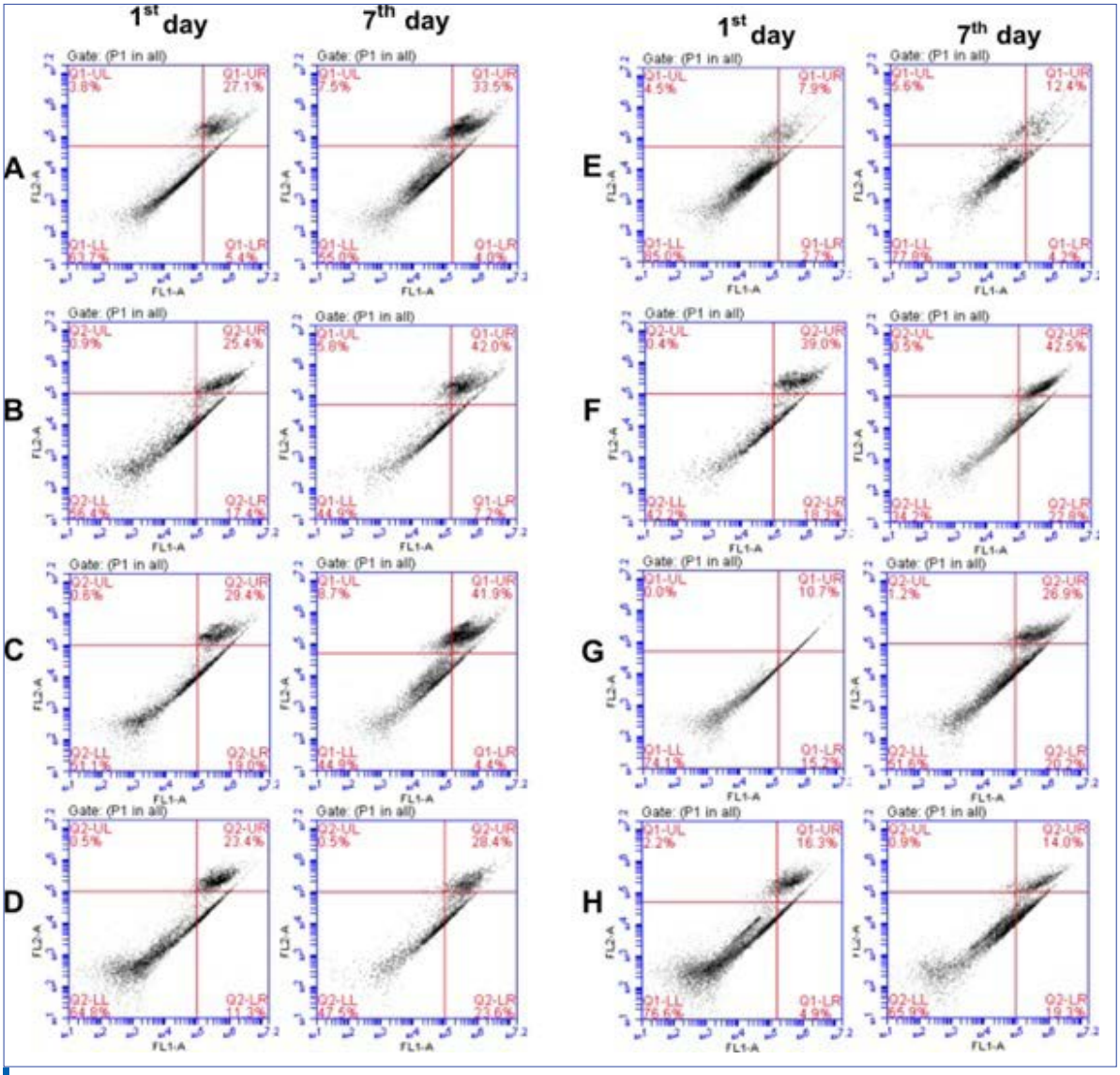

Figure 2. Annexin V/PI staining of indicated cell groups. Cells were treated with 1st, 3rd and 7th day medium extracts of restorative materials for 48h and then subjected to flow cytometric analysis. (LL: Vital cells, LR: Early apoptosis, UR: Late apoptosis, UL: Necrosis).

(Roche Applied Science, Basel, Switzerland) were used to assess the cytotoxicity of the samples.

For the XTT assay; 3 × 10^4 cells/well were plated in a 96-well plate for 24 hours. The next day, 100 µL of culture medium extract of each test material was pipetted immediately into each well containing L929 cells. Formazan formation was quantified spectrophotometrically at 450 nm with a microplate reader (Thermo, Vantaa, Finland) following 48 hours of incubation. xCELLigence real-time cell analyzer measures electrical impedance across microelectrodes integrated on the bottom of tissue culture e-plates. 3x10^4 cells/well were seeded in 100 µL medium and incubated for 24 hours.

Next day, 100 µL medium extracts of each test materials were added to the wells. Cell impedance was measured every 15 minutes for a period of 3 days. All experiments were applied in triplicate and the data was assessed with the xCELLigence software (ACEA Biosciences). In order to determine the cell viability, the classification used by Sjogren et al [11], was utilized. If cell viability was below 30%, the material was accepted as severely cytotoxic. Moderately cytotoxic materials scored 30–59% cell viability, while slightly cytotoxic materials scored 60–90% and non-cytotoxic materials scored above 90% [11].

2.3. Apoptosis assay

L929 cells were seeded into 96-well plate at a density of 3×10^4 cells/well. Following 24 hours incubation period, 100 µL of the medium was aspirated and the cells were treated with 100 µL medium extracts of test materials. The cells were gathered after 48h of the treatment, washed with phosphate buffered saline (PBS) and assessed with apoptosis detection kit (Annexin V FITC/PI, Roche Applied Science) using BD Accuri C6 Flow Cytometer (BD Biosciences, San Jose, CA).

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2.4. Statistical analysis

Three replicated spectrophotometric measurements of XTT assay were completed to calculate the cell viability rates of the samples. Real-time cell analyze data was evaluated with the XCELLigence software. The normality of the data distribution was tested by using the Kolmogorov-Smirnov test.

The data were normally distributed. One-way ANOVA and Tukey's multiple range tests were used with SPSS for Windows (22.0, SPSS Inc., Chicago, IL), $p<0.05$ was considered significant.

3. RESULTS

The survival, early and late apoptosis, and necrosis cell rates of 1st and 7th days medium extracts of eight different prosthetic restoration materials which were obtained by XTT, xCELLigence test (Figs. 1, 2) and apoptosis analysis (Figs. 3, 4) are presented.

3.1. Cytotoxic effects of prosthetic restoration materials

Regarding cell viability, among the material groups statistically significant differences were observed between the 1st day and 7th day medium extracts measurements ($p<0.05$). The lowest cell viability was seen in F group both xCELLigence and XTT experiments ($p<0.05$).

On the 1st day, the highest cell viability values were observed in E and H groups, whereas C and F groups displayed the lowest cell viability with both xCELLigence and XTT tests ($p<0.05$). No significant differences were observed between A and B; and D and G groups ($p>0.05$).

On the 7th day, the cell viability was significantly affected by the material type ($p<0.05$). E group showed the highest viability value and had an enhanced effect on cell survival, while F group displayed the lowest cell viability both with xCELLigence and XTT tests ($p<0.05$).

There were no substantial differences among A, G and H; B and C groups, respectively in all test methods ($p>0.05$).

The cell viability of B, F, G and H groups decreased over time both with XTT and xCELLigence tests ($p<0.05$). However, no significant differences were discerned among A, C, D and E groups regarding time periods ($p>0.05$).

Considering the 7-day observation period, among the tested materials E group showed the highest cell survival values, whereas F group displayed the lowest cell viability, both with xCELLigence and XTT tests (Figs. 1, 2).

Throughout the entire test period, A, D, E, G and H groups continued to exhibit cell viability between 60–90%. Hence, these materials were considered as slightly cytotoxic.

The C and F groups were moderately cytotoxic at all incubation periods (Figs. 1, 2). On the other hand; Group B was slightly cytotoxic on the 1st day but moderately cytotoxic on the 7th day.

3.2. Apoptotic effects of prosthetic restoration materials

Within different incubation periods, apoptosis rates of the prosthetic restoration materials varied significantly ($p<0.05$).

F group presented the highest apoptosis rates for both 1st and 7th days (54.27±3.95; 63.27±2.96) whereas the lowest apoptosis rates were observed in E (17.01±2.02; 21.73±1.55) and H group (21.80±1.71; 33.03±2.66). C and B groups followed with the 45.97±2.59, 40.53±2.83 and 51.03±3.66, 52.36±2.57 apoptosis rates on the 1st and 7th days, respectively (Figs. 3, 4).

4. DISCUSSION

Currently, wide ranges of restorative materials for fixed prosthodontics are available in the market for dental practitioners' use [7]. Dental casting alloys have played a major role in the restorative treatment of the patients, but this role has changed considerably in recent years due to the development of more durable resin-based composites and the improvement of all-ceramic restorations. Nevertheless, alloys will continue to be a commonly used material for fixed prosthetic restorations for the upcoming years [12], despite the fact that their mutual shortcoming remainis the long-term presence of all fixed prosthodontic materials in the oral cavity [7]. Considering that biocompatibility is one of the critical factors affecting the treatment outcome, the biomaterials that are used for partial or complete substitutions of tooth and/or oral tissues should be examined thoroughly before clinical applications [13-15]. Fortunately, increasing development of the innovative materials in dental applications has led to an improved awareness of the biological risks and restrictions of these materials. Monitoring the cell viability is crucial for biomedical study both from a systematic view to comprehend the biochemical and molecular pathways regulating cell viability, and from a therapeutic approach to acquire agents that modulate cell viability [16].

Cell culture method is considered as a coordinated, reproducible, and cost-effective technique to investigate the biocompatibility [17]. Dental materials' biocompatibility is commonly investigated with cytotoxicity and apoptosis tests [18]. In the present study, XTT and xCELLigence systems were used to assess the cytotoxicity. The xCELLigence system uses impedance as readout and provides dynamic and real-time monitoring of cellular phenotypic changes. By means of continuous monitoring, this system perceives between various disturbances of cell viability, for instance senescence, cell cycle arrest, and cell death. Additionally, the time perseverance of the xCELLigence system provides determination of the optimal time points to accomplish standard cell viability assays, alongside with other end-point assays to comprehend action mode [16]. XTT cell proliferation is a colorimetric

assay system that measures formazan products produced by metabolically active cells and is used as a common cell culture method [19]. The programmed death of cells is apoptosis, and it is essential for the sustenance of homeostasis. The flow cytometric apoptosis assay is another method that specifies the cells that have started the apoptotic pathway [20,21]. Al-Hiyasat et al. [22], investigated the cytotoxicity of high-noble alloy and base-metal alloys by the di-Methyl Thiazol diphenyl-Tetrazolium (MTT) method on Balb/C 3T3 fibroblasts, and revealed that the difference in the composition of alloys markedly affected their cytotoxicity potential. They stated that the high concentration of chromium (Cr) and molybdenum (Mo) have reduced the cytotoxicity [22]. On the contrary, in the present study, the cell viability was lower in Group C with 11%Mo than Group B with 3.5% Mo in the 1st day of the tests, and also, higher apoptosis rate was observed in Group C. Tai et al. [23], investigated both the corrosion rates and Cr, nickel (Ni) and beryllium (Be) release of alloys in artificial oral environments. According to their study, nickel release was higher than that of chromium. Parallel to their findings, in the present study, on the 7th day tests, the decrease in the cell viability of Group C may be attributed to the Ni release. On the contrary, Schedle et al. [24], stated that Cobalt (Co) ions are more toxic than Ni ions, and the increase in Co content in an alloy would increase the toxicity of the material. In the present study, one of the highest apoptosis induced groups on the first day was the Ni-Cr-Mo alloy group (45.97%). In a similar study where biocompatibility and apoptosis effects of Au, Titanium (Ti) and Ni-Cr alloy on L929 fibroblast cells were investigated, apoptosis was inducted via Caspase-3 and Caspase-9 mRNA expressions increase in Ni-Cr material [25].

Wataha et al. [26], tested different gold alloys for element release into cell-culture medium, and reported that Au and Pd ions generally did not dissolve into the medium, but silver (Ag), copper (Cu) and zinc (Zn) ions were frequently dissolved. In another study, Sjörgen et al. [11], investigated the cytotoxicity of 15 different metals, dental alloys and ceramic materials, and reported that the Au alloy with 0.6% Zn showed moderate cytotoxicity. In the present study, slight cytotoxicity was revealed for the Au alloy with 1.4% Zn content.

Faria et al. [27], investigated the cytotoxicity of Ti6Al4V, CpTi, Ni-Cr and Co-Cr alloys on SCC-9 cell lines with cell viability and quantity. Surprisingly, they found that Co-Cr alloy was cytotoxic but Ni-Cr alloy was not. In the present study, Cr-Co alloy was slightly cytotoxic, as well. Similarly, in-vitro cytotoxic effects of elements released from gold alloy are also reported [7]. Therefore, clinicians should be aware that Au alloy is not completely inert and biocompatible with oral tissues. The clinical relevance of these findings remains unclear and further in-vitro studies, as well as controlled clinical trials, are needed due to possible exceptions.

Although dental ceramics are known as chemically inert materials, a specific inert property of a ceramic cannot be attributed as a general feature to all ceramics since different ceramics have distinctive chemical configurations [28]. In addition to the diverse constituents and microstructures of the ceramics and the corrosive properties, the period and the temperature of the environment they are exposed to may negatively affect their chemical behavior [29]. Because of the structure and pH of saliva, pH of foods, plaque amount and the presence of abdominal acids, oral environment is considered corrosive. As a result of deterioration of chemical stability, release of potential toxic inorganic ions from dental ceramics may increase [28]. Milleding et al. [29], investigated the corrosive behaviors of crystal and oxide ceramics in liquid and acidic mediums, and reported that crystal ceramics like Empress was prone to corrosion more than oxide ceramics like zirconia and alumina. Nevertheless, massive loss of ceramics is very hard to investigate technically due to the oxidation of the released elements and the phenomenal precision of atomic absorption methods [30]. In their investigations concerning the cytotoxicity of disilicate materials, Messer et al. [30] and Bracket et al. [31], stated that, regardless of the materials fabrication methods and minor structural differences, Empress 2 is biologically precarious. In a previous study, IPS e.max CAD material was not found toxic [32]. Nevertheless, in the present study, lithium disilicate material Rosetta SuperMill was considered as moderately toxic. This contradiction may be attributed to the distinctive material compositions in different brands.

Y-TZP based materials produced by CAD/CAM systems are introduced to be utilized both in esthetic and load bearing areas. With superior esthetics and physical features, zirconia is the preferred material in current procedures [33]. Shin et al. [34] investigated the cytotoxicity of the zirconia posts cemented with different materials on L929 cells, and reported that zirconia posts alone did not reveal toxicity.

Frese et al. [35] declared that composites with fiber content exhibited minor toxicity. In the present study, fiber reinforced resin material exhibited less toxicity compared to the other materials tested. This finding may be attributed to the controlled polymerization of the CAD/CAM resin materials under optimum pressure and temperature during manufacturing process [32]. The major limitation of this study is that it is an in-vitro study accomplished in laboratory conditions, and the results cannot be directly valid for clinical practice. However, the results may provide additional information for clinicians during material selection. Permanent cell lines from mouse fibroblasts were used in this study, but in future studies primary cells (e.g., gingival fibroblasts) may be preferred due to their better mimicking ability of the oral environment.

5. CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions were drawn:

1) Rosetta SuperMill (Lithium disilicate ceramic material group (F)) revealed the highest apoptosis rate and the lowest cell viability at all incubation periods.

2) Cr-Co alloy material group (B), Ni-Cr alloy material group (C) and F group had moderate cytotoxic effects on the day 7.

3) Au alloy material group (A) showed similar cell viability result with Cr-Co alloy material group (B) on the 1st day, whereas B group showed moderate cytotoxicity at the end of the 7th day.

4) CAD/CAM restorative materials with fiber and resin content had favorable viability results.

5) All fixed restoration materials presented a variable degree of cytotoxicity potential.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AA: Data gathering, analysis and interpretation of the results, manuscript writing. VBC: experimental design, analysis and interpretation of the results. PG: Study design, manuscript proofreading. BBK: sample preparation. EC: Study and experimental design, analysis and interpretation of the results, manuscript proofreading.

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Questions

1. Choose the content of the high noble alloys below:

❑a. <60% Au, Pd;
❑b. ≥60% Au, Pt, Pd and ≥40% Au;
❑c. ≥25% Au, Pt, Pd;
❑d. <25% Au.

2. Which of the following is the element released from cast alloys and shown as the main (toxic) cause of oral tissue reactions such as gingival inflammation?

❑a. Au;
❑b. Ni;
❑c. Cr;
❑d. Co.

3. Which of the following is a colorimetric assay that measures formazan products produced by metabolically active cells and is used as a common cell culture method?

❑a. XTT assay;
❑b. MTT assay;
❑c. xCELLigence system;
❑d. Apoptosis assay.

4. According to the results of this study, which materials showed favorable viability results?

❑a. Cr-Co alloy;
❑b. Lithium disilicate glass-ceramic;
❑c. Fiber reinforced composite material;
❑d. Ni-Cr alloy.