Cytotoxicity of Dental Implants: The Effects of Ultrastructural Elements

Ebru Cal, DDS, PhD[1]/Vildan Bozok Cetintas, PhD[2]/
Hayal Boyacioglu, PhD[3]/Pelin Güneri, DDS, PhD[4]

Purpose: *In this in vitro study, the purpose was to assess the cytotoxicity profiles of seven commercial dental implant materials by using cell culture methods on an osteoblastic cell line.* ***Materials and Methods:*** *The microstructure of seven commercial dental implants (each given a letter code) was investigated via scanning electron microscopy and energy-dispersive x-ray analysis. Medium extracts were collected on the first and fifth days for each group and tested using MC3T3-E1 cell line. Cytotoxicity was evaluated with Xcelligance System and XTT reagent, and apoptosis was determined by Annexin-V staining. One-way analysis of variance (ANOVA) and Tukey's multiple range tests were used for statistical analyses. In all tests, P was set as .05.* ***Results:*** *ANOVA results disclosed that Ti (P = .001), Na (P = .001), Ca (P = .019), Al (P = .024), and P (P = .020) amounts were significantly different between test materials. Cytotoxicity and apoptosis analyses revealed that implant materials (C) and (E) were the materials with the lowest cell vitality and the highest apoptosis rates among the test materials. Phosphorus was the only element that presented the highest amount in C and E (14.23% and 12.29%, respectively) compared with the other implant materials tested. (F) and (G) had favorable results for all experiments.* ***Conclusion:*** *The results suggest that pure dental implant materials with a lower number of additional elements may possess fewer cytotoxic effects than the other implant materials tested in this study.* Int J Oral Maxillofac Implants 2017;32:1281–1287. doi: 10.11607/jomi.5962

Keywords: *cell culture, cytotoxicity, dental implants, energy-dispersive x-ray analysis, osteoblasts*

Successful implant restoration primarily requires adequate and appropriate osseointegration, which is defined as the direct apposition of bone tissue to the implanted material. Osseointegration starts with the blood clot formation at the wound site, which is accompanied by protein adsorption and adherence of polymorph nuclear leukocyte. Following the fibroblast proliferation into the blood clot, extracellular matrix production is initiated, and within a week, osteoblast-like cells start to form the new bone, which eventually covers the implant surface.[1] Unfortunately, various host-dependent and implant-related factors may influence this bonding process and interfere with the osseointegration process.[2,3] Excessive surgical trauma, infection, or metabolic upset may influence the quantity and quality of the new bone formation on the biomaterial-tissue interface zone, and thus, alter the healing and long-term load-bearing function of the implant.[2,4–6] Additionally, the biomaterial surface chemistry (purity and surface tension for wetting), texture, and topography (roughness) of the material are directly related to the bone formation.[7] Even though the intact bone-titanium interface is established down to the nanoscale, both the initial cellular and molecular activities and environmental/inherent co-factors that determine the tissue response at the bone-implant interface are not yet fully understood.

Accurate assessment of both the physical properties and biocompatibility of any dental material is imperative prior to its introduction to dental clinical practice.[8] Within this context, implant materials entail thorough evaluation with respect to their interaction with the vital tissues, because they may release substances that could negatively influence the implant-host interaction, resulting in allergic reactions and inflammation, leading to nonintegration. The presence of implant particles within the periprosthetic

[1]Professor, Department of Prosthodontics, School of Dentistry, Ege University, Bornova, Izmir, Turkey.
[2]Associate Professor, Department of Medical Biology, School of Medicine, Ege University, Bornova, Izmir, Turkey.
[3]Associate Professor, Department of Statistics, Faculty of Science, Ege University, Bornova, Izmir, Turkey.
[4]Professor, Department of Oral and Maxillofacial Radiology, School of Dentistry, Ege University, Bornova, Izmir, Turkey.

Correspondence to: Dr Ebru Cal, Ege University School of Dentistry, Department of Prosthodontics, 35100 Bornova, Izmir, Turkey. Fax: +90 2323880325. Email: ebrucal68@gmail.com

Table 1 Manufacturers and Titanium Grades of Implant Materials Tested in This Study

Code	Brand	Titanium grade	Manufacturer	Size	Lot No.
A	GC Implant Aadva	Grade V	GC Corporation	4.0 Regular 10 mm	1306061
B	Blue Sky Bio	Grade V	Blue Sky Bio	4.1 × 10 mm	13-0112
C	Nobel Replace	Grade IV	Nobel Biocare	3.5 × 10 mm	495735
D	Screw Plant	Grade V	Implant Direct	3.7 × 10 mm	103710
E	Implance	Grade IV	AGS Medikal	4.3 × 10 mm	120713
F	Nucleoss	Grade IV	Şanlılar Tıbbi Cihazlar Medikal Kimya San.Tic.	3.8 × 1 mm	1402552
G	Roxolid	Ti-Zr Alloy	Straumann	3.3 × 1 mm	021.2510 JE418

environment that initiate adverse biologic reactions and lead to progressive bone loss and deterioration of osseointegration have already been documented.[9–11] In dental implants, due to their mechanical strength and chemical stability, titanium (Ti) is one of the most widely used biomaterials.[5,12] Titanium materials, either commercially pure titanium, ASTM Grades 1 through 4, or Ti-based alloys, are considered to be the most biologically compatible materials.[2] However, the titanium-alloy implants (Ti4Al-4V), which are generally preferred for their higher fatigue strength compared with pure titanium implants, generated higher levels of particles than stainless steel (316L) or chromium cobalt (Co-Cr) materials.[13,14]

The suppression of normal osteoblastic process by the particles or reactive inflammatory mediators has been reported in the literature.[11,15–17] Phagocytosis of the released particles by the macrophages is accompanied with the stimulation of the multipotential mesenchymal stem cells that are located within the bone marrow cavity.[11] This cascade develops further with reduction of the osteoblast progenitor population, decrease of the proliferation rates, and osteogenic activities of mesenchymal stem cells, leading to eventual failure of osseointegration.[9,11]

The aim of this in vitro study was to determine the cytotoxicity profiles of seven commercially available dental implant materials by using cell culture methods with osteoblastic cell line as a model of osteoblasts.

MATERIALS AND METHODS

The microstructure of seven commercial dental implants (Table 1) was investigated by using scanning electron microscopy and energy-dispersive x-ray analysis. A scanning electron microscope (Leo Evo-40 VPX; Leo Electron Microscopy) was operated at 20-kV acceleration voltage, and energy-dispersive x-ray spectroscopy (EDX; Bruker 125 eV) was used for atomic concentration examination and phase distribution.

Medium Extracts of Implant Materials

Seven different implant materials were tested in this study. Three sterile samples were used for each group, and prior to the tests, they were further sterilized by using ultraviolet light. A 0.1 g/mL volume of extraction was determined according to the ISO 10993-12 standards for irregularly shaped devices. Materials were immersed in minimum essential medium (MEM)-α containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and L-glutamine. They were incubated in the highly humidified atmosphere containing 5% CO_2 at 37°C. Medium extracts were collected and filtered on the first and fifth days and stored at –80°C.

Cell Culture

MC3T3-E1 cell line was obtained from the German Collection of Microorganisms and Cell Culture (DSMZ). Cells were grown in a highly humidified atmosphere containing 5% CO_2 at 37°C. Culture medium MEM-α was supplemented with 10% FBS, 1% penicillin-streptomycin, and L-glutamine. The medium was changed every 3 days, and cells were passaged after trypsinization.

Cytotoxicity Assay

Cytotoxicity was evaluated using two different methods: Xcelligance System and XTT Cell Proliferation Kit (Roche Applied Science).

The Xcelligance System (Roche Applied Science) was used to monitor cell proliferation in real time without the incorporation of labels. This system measures electrical impedance across microelectrodes integrated on the bottom of tissue culture e-plates.[18] To determine the optimal cell density, MC3T3-E1 cells were seeded as 7,500; 15,000; 30,000; 60,000; 120,000; 240,000; and 480,000 cells per well in the e-plate. After 96-hour incubation, 15,000 cells/well was indicated as the optimal cell density. For cytotoxicity assays, 15,000 cells/well were seeded in 200 µL medium. After 24-hour incubation, 100 µL medium was aspirated, and cells were treated with 100 µL medium extracts of implant materials. Cell impedance was measured

Table 2 Phase Dispersion Analysis Results of the Test Materials and the Significance of the Differences Among the Constituents of the Implants

Material code	Ti	Na	Cl	Ca	Al	P	K	S	Cr	Mg	Si	Zr
A	83.76	6.05		0.49	8.28	0.06	0.51	0.26	0.59			
B	61.43	22.97	6.52	0.36	7.54	0.31	0.22	0.18	0.48			
C	79.74	5.34				14.23	0.69					
D	83.24	7.87		1.34	5.59	0.10	0.51	0.55	0.81			
E	58.79	3.42		24.36	0.00	12.29	0.80			0.22	0.10	
F	93.69	5.78		0.53								
G	83.33	7.36		0.83	1.55							6.94
P value	**.001**	**.001**	–	**.019**	**.024**	**.020**	.399	.311	.459	–	–	–

Statistically significant differences are in bold.

every 15 minutes for 190 hours. All experiments were performed in triplicate. The obtained data were evaluated with the Xcelligance software (ACEA Biosciences).

For the XTT Cell Proliferation Assay, MC3T3-E1 cells were plated at 1×10^5 cells/well in a 96-well plate and incubated for 24 hours. After the culture medium was aspirated, 100 µL of medium extract of each implant material was pipetted immediately into each well containing MC3T3-E1 cells. Each assay was run in triplicate. Formazan formation was quantified spectrophotometrically at 450 nm using a microplate reader (Thermo) after 48 hours of incubation.

Apoptosis Assay

MC3T3 cells were seeded into a 96-well plate at a density of 15,000 cells/well. After 24-hour incubation, 100 µL medium was aspirated, and cells were treated with 100 µL medium extracts of implant materials. Cells were harvested after 48 hours and washed with phosphate-buffered saline (PBS) and analyzed using Annexin V FITC/PI Apoptosis Detection kit (Roche Applied Science) with a bench top flow cytometer instrument (BD Biosciences).

Statistical Analyses

Data from Xcelligance were analyzed using the instrument's real-time cell analyzer (RTCA) software. Cell indexes were normalized at 24 hours, and slope values were calculated. Cell viability was calculated from spectrophotometric measurements of XTT assay. One-way analysis of variance (ANOVA) and Tukey's multiple range tests were used for statistical analyses. In all tests, $P < .05$ was considered significant.

RESULTS

Phase dispersion analysis revealed the constituents of the test materials (Table 2). B had the most elements within its structure (nine different elements), and F included only three elements (Table 2).

In this study, the values provided by the cell survival rates were measured using Xcelligance and XTT tests (Figs 1 and 2 and Table 3), and the apoptotic cell rates (early apoptosis, late apoptosis, and necrosis) were obtained by apoptosis analysis (Fig 3 and Table 3).

The baseline (day 1 medium extracts) measurements revealed that F provided the highest cell viability values with both Xcelligance system (65.61 ± 4.46) and XTT (84.00 ± 3.61). It was followed by G, with an Xcelligance system viability value of 58.11 ± 4.05 and XTT value of 79.00 ± 2.65, and by B, with an Xcelligance value of 30.77 ± 1.81 and XTT value of 76.87 ± 6.45. In both systems, C and E formed the lowest cell viability group, respectively ($C_{Xcelligance}$ = 19.98 ± 1.22, C_{XTT} = 59.52 ± 8.85; $E_{Xcelligance}$ = 19.10 ± 1.17, E_{XTT} = 36.43 ± 6.38) (Table 3).

On day 5, D had an improved effect on cell survival and had the highest viability value, both with Xcelligance (69.65 ± 2.91) and XTT (89.16 ± 5.11). F (52.76 ± 3.73; 71.33 ± 5.13), A (45.42 ± 2.19; 70.01 ± 6.0), and G (53.70 ± 2.45; 70.00 ± 5.57) revealed a similar order with the Xcelligance and XTT methods. In both groups, B (27.09 ± 1.31; 54.41 ± 6.76), C (20.81 ± 1.06; 51.20 ± 13.89), and E (13.54 ± 1.03; 28.14 ± 5.17) followed these test materials.

Considering the 5-day observation period, D had the highest cell survival values with XTT and Xcelligance among the tested materials, whereas E revealed the lowest cell viability results, both with XTT and Xcelligance tests.

On day 1, apoptosis was mostly induced by E (38.55 ± 1.03), D (34.50 ± 0.70), and C (30.95 ± 1.52), respectively. B (25.88 ± 0.89) and A (24.54 ± 0.75) had similar apoptosis induction rates and were followed by G (21.35 ± 0.57) and F (12.77 ± 0.94) (Fig 4).

At the end of the observation period on day 5, F (18.90 ± 1.34), G (19.17 ± 1.44), and A (20.28 ± 0.46) provided apoptosis values comparable to the control group, whereas B (33.24 ± 0.69) and E (34.45 ± 0.83) constituted the group with the highest apoptosis rate.

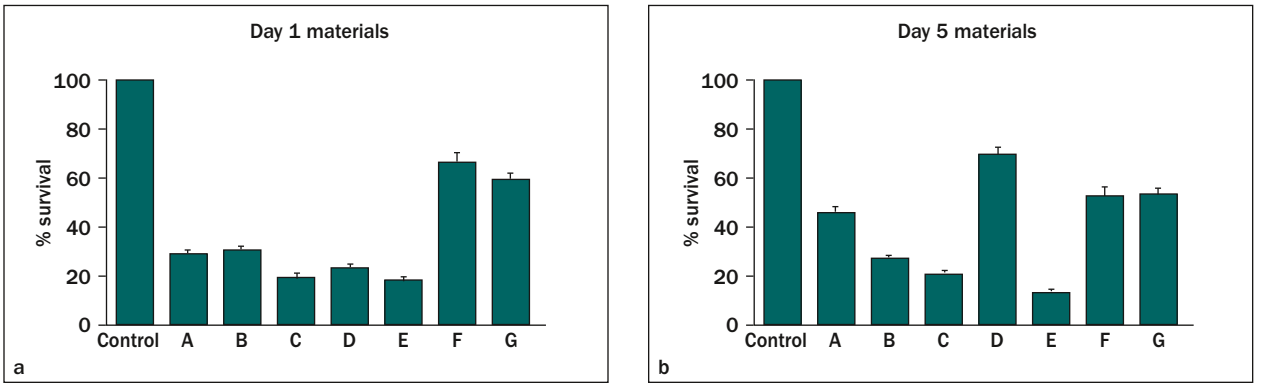


Fig 1 Cell viability comparison among the *(a)* day 1 and *(b)* day 5 medium extracts of implant materials obtained from Xcelligance system.

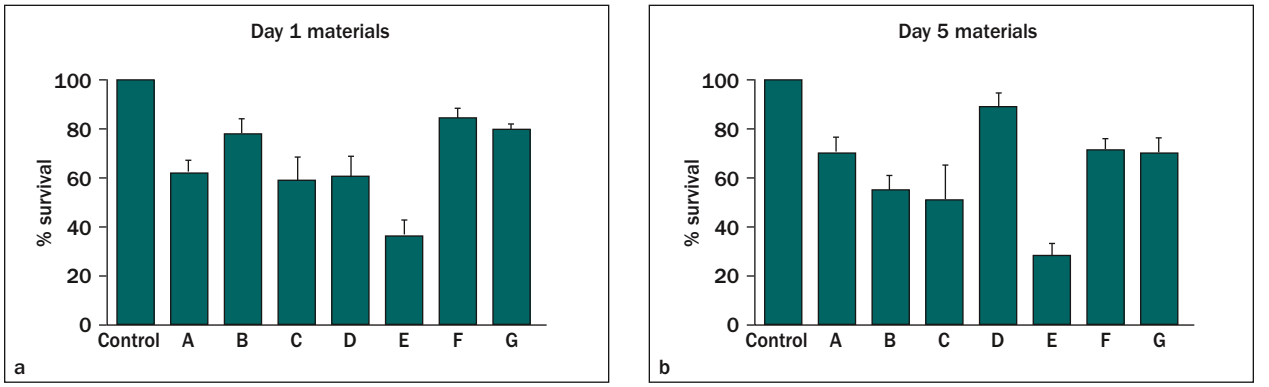


Fig 2 Cell viability comparison among the *(a)* day 1 and *(b)* day 5 medium extracts of implant materials obtained from XTT assay.

Table 3 Mean Cell Viability Ratios and Standard Deviations of Implant Materials

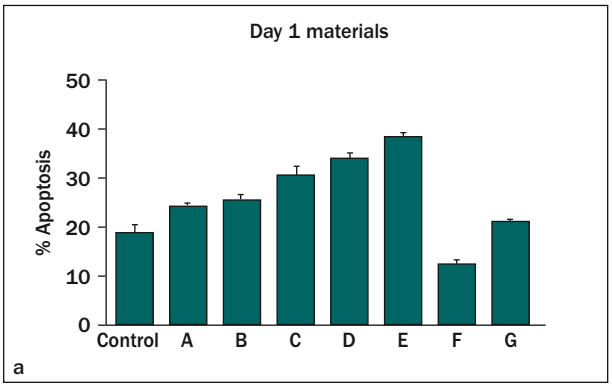
Test duration/ materials	Xcelligance survival (%)	XTT survival (%)	Apoptotic cells (%)
Day 1			
A	29.00 ± 1.72	62.32 ± 4.89	24.54 ± 0.75
B	30.77 ± 1.81	76.87 ± 6.45	25.88 ± 0.89
C	19.98 ± 1.22	59.52 ± 8.85	30.95 ± 1.52
D	23.52 ± 1.41	60.94 ± 7.64	34.50 ± 0.70
E	19.10 ± 1.17	36.43 ± 6.38	38.55 ± 1.03
F	65.61 ± 4.46	84.00 ± 3.61	12.77 ± 0.94
G	58.11 ± 4.05	79.00 ± 2.65	21.35 ± 0.57
Day 5			
A	45.42 ± 2.19	70.01 ± 6.0	20.28 ± 0.46
B	27.09 ± 1.31	54.41 ± 6.76	33.24 ± 0.69
C	20.81 ± 1.06	51.20 ± 13.89	29.01 ± 0.11
D	69.65 ± 2.91	89.16 ± 5.11	29.54 ± 0.52
E	13.54 ± 1.03	28.14 ± 5.17	34.45 ± 0.83
F	52.76 ± 3.73	71.33 ± 5.13	18.90 ± 1.34
G	53.70 ± 2.45	70.00 ± 5.57	19.17 ± 1.44

The moderate apoptosis induction group was formed by D (29.54 ± 0.52) and C (29.01 ± 0.11).

Post hoc analyses revealed that the values obtained with the Xcelligance and XTT methods were significantly different ($P < .05$), except that of the control ($P = 1.000$). ANOVA was used to assess which of the constituents were significantly different between the implant materials. The results disclosed that all implant materials had comparable amounts of K ($P = .399$), S ($P = .311$), and Cr ($P = .459$). On the other hand, Ti ($P = .001$), Na ($P = .001$), Ca ($P = .019$), Al ($P = .024$), and P ($P = .020$) amounts were significantly different between test materials.

DISCUSSION

Cell culture methods are considered to be sensitive, simple, reproducible, and cost-effective means to investigate the toxicity of a material in a simplified system without the effect of confounding variables.[19] Among the cell culture methods, Xcelligance system (ACEA Biosciences) has been used to determine the cytotoxicity of dental materials since 2010.[20–22] The Xcelligance system is a new real-time cell analyzer that uses the electrical impedance through gold



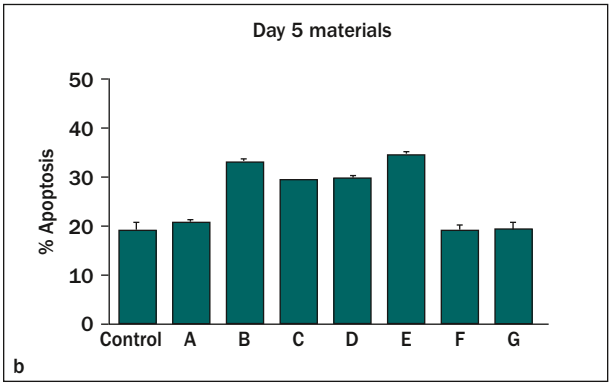


Fig 3 Apoptosis analysis for *(a)* day 1 and *(b)* day 5 medium extracts of implant materials. Bar graphs showing the percentage of cell populations (early apoptosis, late apoptosis, and necrosis) in treated cells.

microelectrodes integrated on the bottom of tissue culture e-plates.[23] These electrodes detect the impedance differences within an electrical circuit and transfer this information to a computer, which converts the impedance measurements into cell index values.[23] In Xcelligance system, the cell index values may be influenced by several parameters, such as cell number, cell size, cell-substrate, or cell-cell attachment.[23] Another commonly used cell culture method is XTT cell proliferation assay, which is a colorimetric assay system that measures formazan products produced by metabolically active cells.[24,25] Apoptosis is defined as the programmed cell death and has a crucial role in the tissue homeostasis.[26] The flow cytometric apoptosis assay identifies cells that have entered the apoptotic pathway, which leads to cell death. These cytotoxicity and apoptosis tests have been widely used to determine the cytotoxicity of dental materials.[13,27]

According to the American Society for Testing and Materials (ASTM), there are six distinct types of titanium available as implant biomaterials. Among these six materials, there are four grades of commercially pure titanium (CpTi) whose mechanical and physical properties are different and related chiefly to the oxygen residuals in the metal. The CpTi materials are called pure Grade I, Grade II, Grade III, and Grade IV

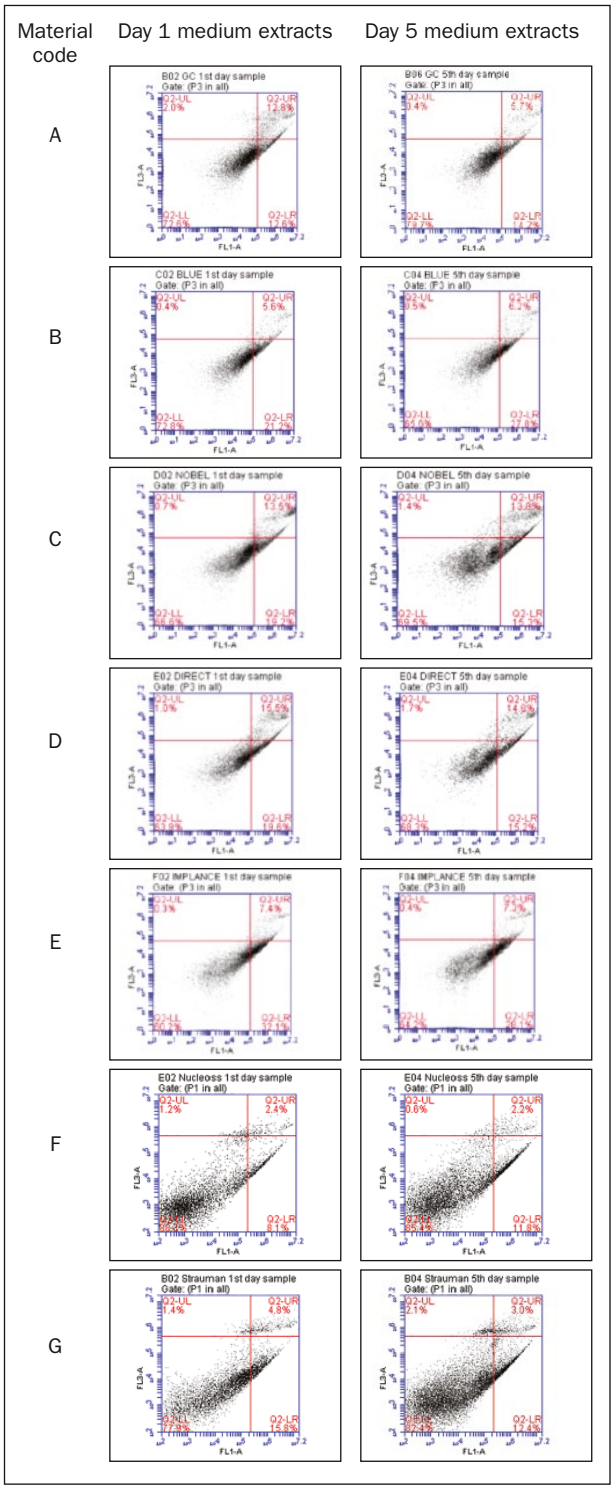


Fig 4 Annexin V/PI staining of indicated cell groups. Cells were treated with day 1 and day 5 medium extracts of implant materials for 48 hours and then subjected to flow cytometric analysis.

titanium. CpTi is also referred to as unalloyed titanium and usually contains some trace elements of carbon, oxygen, nitrogen, and iron. These trace elements markedly improve the mechanical properties of pure titanium and are found in higher amounts from Grade I to Grade IV.[28] The remaining two are titanium alloys (Ti-6Al-4V and Ti-6Al-4V-ELI-extra low interstitial alloys), and these are known as Grade 5, which is the most commonly used alloy.

Dental implants are usually made from CpTi (Grade IV),[6] but to overcome its lower fatigue strength, titanium alloys (Grade V) containing four to eight metals including vanadium, chromium, nickel, aluminum, and iron are used in dentistry.[29] On the other hand, titanium alloys tend to corrode in body fluids[30] and release metallic ions, which may cause side effects such as clinical implant failure, osteolysis, allergic reactions, hypersensitivity, and carcinogenesis.[31] It is cumbersome to assess which dental implant has fewer side effects since they possess complex and diverse structures that are developing continuously.[13]

In the present study, the results of the cytotoxicity and apoptosis tests revealed that C and E were the materials with the lowest cell vitality and the highest apoptosis values among the test materials. When the constituents of the implant materials were taken into consideration in order to detect the origin of this effect, significant differences were noticed between the amounts of Na, Ca, Ti, Al, and P. However, phosphorus (P) was the only element that presented the highest amount in C and E (14.23% and 12.29%, respectively) when compared with the phosphorus amounts of all other implant materials tested.

Phosphorus is an essential element as a component of DNA, RNA, ATP, and the phospholipids, but due to their nerve-hindering effect via acetylcholinesterase blockage, phosphorus-containing organic chemicals are also used as chemical weapons.[32] Organophosphate (OP) nerve agents are designed specifically to cause incapacitation or death in military use and are particularly effective because of their extremely high acute toxicity.[33] Recently, the use of phosphorus in biomedicine has raised concerns about its toxicity. Various concentrations of phosphorus were incubated with human lung epithelial cells for 24 hours, and their cell viabilities were subsequently assessed by using water-soluble tetrazolium salt (WST-8) and methylthiazolyl-diphenyltetrazolium bromide (MTT) assays. The results revealed that toxicologic effects of phosphorus were dose-dependent, with phosphorus reducing cell viabilities to 48% (WST-8) and 34% (MTT) at 50 $\mu g/mL^{-1}$ exposure.[34]

Test material D presented an unexpected result on day 5 with Xcelligance and XTT tests and revealed the highest cell viability among the test materials. However, this acute ascension of cell viability was inconsistent through the test period and was not supported by the results of the apoptosis assay. It is suggested that this may be the result of unforeseen variations of the release pattern of metallic ions of the elements, as acknowledged by Malkoç et al.[13]

In the present study, all cytotoxicity tests disclosed favorable results with F and G, and this finding was attributed to the purity of the materials that can be recognized from the lower number of additional elements.

CONCLUSIONS

In this in vitro study, which partially mimics the in vivo conditions, only two (F and G) of seven commercially available dental implant materials disclosed favorable results on osteoblastic cell line cultures with cell viability and apoptosis tests. Provided that phosphorus (P) was the only element that presented the highest amount in the least favorable implant materials with respect to cell viability, clinicians may be advised to look for the purest implant material with the lowest amount of P among its components.

ACKNOWLEDGMENTS

The authors of this study would like to thank Associate Professor Dr Meral Malkoç for meticulously performing the energy-dispersive x-ray analysis, and Professor Dr Gürcan Eskitaşçıoğlu and all the companies for generously providing the implant materials tested. The authors reported no conflicts of interest related to this study.

REFERENCES

1. Farker AC, Griffin CD. Introduction. In: Corrosion and Degradation of Implant Materials: Second Symposium. American Society for Testing and Materials, STP859-EB, 1985:5–10.
2. Oshida Y, Tuna EB, Aktören O, Gençay K. Dental implant systems. Int J Mol Sci 2010;11:1580–1678.
3. Chambrone L, Mandia J Jr, Shibli JA, Romito GA, Abrahao M. Dental implants installed in irradiated jaws: A systematic review. J Dent Res 2013;92(suppl):s119–s130.
4. Chrcanovic BR, Albrektsson T, Wennerberg A. Diabetes and oral implant failure: A systematic review. J Dent Res 2014;93:859–867.
5. Gardin C, Ferroni L, Bressan E, et al. Adult stem cells properties in terms of commitment, aging and biological safety of grit-blasted and acid-etched Ti dental implants surfaces. Int J Mol Cell Med 2014;3:225–236.
6. Le Guéhennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. Dent Mater 2007;23:844–854.
7. Palmquist A, Omar OM, Esposito M, Lausmaa J, Thomsen P. Titanium oral implants: Surface characteristics, interface biology and clinical outcome. J R Soc Interface 2010;7(suppl):s515–s527.
8. Braden M, Wright PS, Parker S. Soft lining materials—a review. Eur J Prosthodont Restor Dent 1995;3:163–174.

9. Wang ML, Tuli R, Manner PA, Sharkey PF, Hall DJ, Tuan RS. Direct and indirect induction of apoptosis in human mesenchymal stem cells in response to titanium particles. J Orthop Res 2003;21:697–707.
10. Salvati EA, Betts F, Doty SB. Particulate metallic debris in cemented total hip arthroplasty. Clin Orthop Relat Res 1993:160–173.
11. Okafor CC, Haleem-Smith H, Laqueriere P, Manner PA, Tuan RS. Particulate endocytosis mediates biological responses of human mesenchymal stem cells to titanium wear debris. J Orthop Res 2006;24:461–473.
12. Long M, Rack HJ: Titanium alloys in total joint replacement—a materials science perspective. Biomaterials 1998;19:1621–1639.
13. Malkoç S, Öztürk F, Çörekçi B, Bozkurt BS, Hakki SS. Real-time cell analysis of the cytotoxicity of orthodontic mini-implants on human gingival fibroblasts and mouse osteoblasts. Am J Orthod Dentofacial Orthop 2012;141:419–426.
14. Brien WW, Salvati EA, Betts F, et al. Metal levels in cemented total hip arthroplasty. A comparison of well-fixed and loose implants. Clin Orthop Relat Res 1992:66–74.
15. Dean DD, Schwartz Z, Liu Y, et al. The effect of ultra-high molecular weight polyethylene wear debris on MG63 osteosarcoma cells in vitro. J Bone Joint Surg Am 1999;81:452–461.
16. Kwon SY, Takei H, Pioletti DP, et al. Titanium particles inhibit osteoblast adhesion to fibronectin-coated substrates. J Orthop Res 2000;18:203–211.
17. Lohmann CH, Schwartz Z, Köster G, et al. Phagocytosis of wear debris by osteoblasts affects differentiation and local factor production in a manner dependent on particle composition. Biomaterials 2000;21:551–561.
18. Solly K, Wang X, Xu X, Strulovici B, Zheng W. Application of real-time cell electronic sensing (RT-CES) technology to cell-based assays. Assay Drug Dev Technol 2004;2:363–372.
19. Browne RMl. The in vitro assessment of the cytotoxicity of dental materials—does it have a role? Int Endod J 1988;21:50–58.
20. 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;26:51–58.
21. Öztürk F, Malkoc S, Ersöz M, Hakki SS, Bozkurt BS. Real-time cell analysis of the cytotoxicity of the components of orthodontic acrylic materials on gingival fibroblasts. Am J Orthod Dentofacial Orthop 2011;140:e243–e249.
22. Yalçin M, Barutcigil C, Umar I, Bozkurt BS, Hakki SS. Cytotoxicity of hemostatic agents on the human gingival fibroblast. Eur Rev Med Pharmacol Sci 2013;17:984–988.
23. Martinez-Serra J, Gutierrez A, Muñoz-Capó S, et al. xCELLigence system for real-time label-free monitoring of growth and viability of cell lines from hematological malignancies. Onco Targets Ther 2014;7:985–994.
24. Urcan E, Scherthan H, Styllou M, Haertel U, Hickel R, Reichl FX. Induction of DNA double-strand breaks in primary gingival fibroblasts by exposure to dental resin composites. Biomaterials 2010;31:2010–2014.
25. Shehata M, Durner J, Eldenez A, et al. Cytotoxicity and induction of DNA double-strand breaks by components leached from dental composites in primary human gingival fibroblasts. Dent Mater 2013;29:971–979.
26. Tsuda H, Ning Z, Yamaguchi Y, Suzuki N. Programmed cell death and its possible relationship with periodontal disease. J Oral Science 2012;54:137–149.
27. Möller B, Terheyden H, Açil Y, et al. A comparison of biocompatibility and osseointegration of ceramic and titanium implants: An in vivo and in vitro study. Int J Oral Maxillofac Surg 2012;41:638–645.
28. McCracken M. Dental implant materials: Commercially pure titanium and titanium alloys. J Prosthodont 1999;8:40–43.
29. Doruk C, Oztürk F, Ozdemir H, Nalçaci R. Oral and nasal malodor in patients with and without cleft lip and palate who had undergone orthodontic therapy. Cleft Palate Craniofac J 2008;45:481–484.
30. Zaffe D, Bertoldi C, Consolo U. Element release from titanium devices used in oral and maxillofacial surgery. Biomaterials 2003;24:1093–1099.
31. Sedarat C, Harmand MF, Naji A, Nowzari H. In vitro kinetic evaluation of titanium alloy biodegradation. J Periodontal Res 2001;36:269–274.
32. Ohnishi M, Razzaque MS. Dietary and genetic evidence for phosphate toxicity accelerating mammalian aging. FASEB J 2010;24:3562–3571.
33. Munro N. Toxicity of the organophosphate chemical warfare agents GA, GB, and VX: Implications for public protection. Environ Health Perspect 1994;102:18–38.
34. Latiff NM, Teo WZ, Sofer Z, Fisher AC, Pumera M. The cytotoxicity of layered black phosphorus. Chemistry 2015;21:13991–13995.