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Research Article / Araştırma Makalesi

Cytotoxicity Evaluation of Different Types of CAD/CAM Blocks

Farklı CAD/CAM Blokların Sitotoksisite Değerlendirmesi

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ABSTRACT

Aim: The aim of this study is to determine the cytotoxic effects of Computer Aided Design (CAD) and Computer Aided Manufacturing (CAM) blocks produced by different companies on human peripheral blood lymphocytes by MTT assay.

Methods: Six different CAD/CAM materials were investigated: feldspar ceramic VM (Vitablocks Mark II), resin nano-ceramic LU (Lava Ultimate), hybrid ceramic C (Cerasmart), leucite-reinforced ceramic LRF (GC LRF), zirconia-reinforced lithium silicate ceramic VS (Vita Suprinity), polymer-infiltrated ceramic-network VE (Vita Enamic). A total of 36 disc-shaped samples (Ø: 5 mm; h: 2 mm) were prepared from commercial blanks and blocks. Cell proliferation and cytotoxicity were assessed at 24h and 48h using MTT assay. The data were statistically evaluated with the Two-way ANOVA test ($p<0.05$).

Results: MTT viability data at 24 h showed that group VM and LU blocks were mildly cytotoxic, but there was no statistically significant difference when compared with other groups ($p>0.05$). It was determined that all blocks caused cell proliferation after 48 h of exposure ($p>0.05$). It has been shown that all blocks whose cytotoxic effects were investigated did not cause any toxic effects (except VM and LU for 24 h) at different application times (24 h and 48 h).

Conclusion: Based on the results obtained and the limitations of the current in vitro study, the tested materials were not cytotoxic. Only VM and LU caused negligible cytotoxicity at 24-hour exposure.

Keywords: human peripheral lymphocytes, cytotoxicity, CAD/CAM blocks

ÖZ

Amaç: Bu çalışmanın amacı, farklı firmalar tarafından üretilen Computer Aided Design (CAD) ve Computer Aided Manufacturing (CAM) bloklarının insan periferik kan lenfositleri üzerindeki sitotoksik etkilerini MTT testi ile belirlemektir.

Yöntem: Altı farklı CAD/CAM materyali araştırıldı: feldspat seramik VM (Vitablocks Mark II), rezin esaslı nano-seramik LU (Lava Ultimate), hibrit seramik C (Cerasmart), lösitle güçlendirilmiş cam seramik LRF (GC LRF), zirkonya ile güçlendirilmiş lityum silikat seramik VS (Vita Suprinity), polimer infiltrasyonu ile güçlendirilmiş cam seramik VE (Vita Enamic). CAD/CAM bloklardan toplam 36 disk şeklinde numune (Ø: 5 mm; h: 2 mm) hazırlandı. Hücre proliferasyonu ve sitotoksisite, MTT testi kullanılarak 24. ve 48. saatlerde değerlendirildi. Veriler İki Yönlü ANOVA testi ile istatistiksel olarak değerlendirildi ($p < 0.05$).

Bulgular: 24 saatteki MTT canlılık verileri, grup VM ve LU bloklarının hafif sitotoksik olduğunu gösterdi, ancak diğer gruplarla karşılaştırıldığında istatistiksel olarak anlamlı bir fark yoktu ($p>0.05$). Tüm blokların 48 saat sonra hücre çoğalmasına neden olduğu belirlendi ($p>0.05$). Sitotoksik etkileri araştırılan tüm blokların farklı uygulama sürelerinde (24 saat ve 48 saat) herhangi bir toksik etkiye (24 saat VM ve LU hariç) neden olmadığı gösterildi.

Sonuç: Elde edilen sonuçlara ve mevcut in vitro çalışmanın sınırlamalarına dayanarak, test edilen materyaller sitotoksik değildi. 24 saatlik sonuçlarda VM ve LU ihmal edilebilir sitotoksisiteye neden oldu.

Anahtar Kelimeler: insan periferik lenfositleri, sitotoksisite, CAD/CAM blokları

1. INTRODUCTION

In the field of modern dentistry, the development and application of CAD/CAM was one of the most exciting progresses in the process of designing, analyzing and manifacturing fixed prostheses including inlays, crowns as well as implant abutments etc.[1-3] In the milling process of materials used for all ceramic and long lasting interim prostheses is enabled by some factors of the system such as flexibility, speed, precision and efficiency. As various new materials exist for CAD/CAM systems, deciding upon the choice material clinically becomes more difficult.[4,5]

CAD/CAM blocks may consist of different materials including composite resins, feldspathic glass ceramics, yttrium tetragonal zirconia polycrystals, aluminum-oxide, leucite-reinforced glass ceramics or lithium disilicate glass ceramics.[6-8] Recently, nano-hybrid ceramic blocks have been developed as an alternative to ceramic blocks. These blocks are ceramics integrating with the polymer network that polymerizes at higher temperature and pressure.[9,10] Zirconia-reinforced lithium silicate ceramic is another original CAD/CAM material which displays not only the mechanical characteristics of zirconia, but also the optical structure of glass ceramics as well.[10-12]

Moreover, the technology of CAD/CAM is utilised with the aim of producing long lasting interim prostheses from polymer materials with high density. Manifacturing those materials requires controlled polymerization performed under optimum pressure and temperature. Considered as bio-inert materials, dental ceramic materials are attributed as conventional restoration material.[13]

Despite the fact that dental ceramics are classified as chemically inert materials, it would be wrong to attribute a specific property of a ceramic as a general quality of all ceramics.[14]

Such factors as the temperature of the environment and the period to which the ceramics are exposed may influence their chemical behaviour in a negative way as well as other factors such as the diverse constituents and microstructures of the ceramics and the corrosive properties.

Oral environment is considered to be corrosive due to reasons including the structure and pH of saliva, pH of foods, plaque amount and the availability of abdominal acids.[15] The possible increase in the release of potential toxic inorganic ions from dental ceramics results from deterioration of chemical stability. Even though CAD/CAM blocks display high degradation resistance, it becomes possible to emit toxic components like elemental ions from CAD/CAM fabricated ceramics which may have an impact upon oral cavity in the patients' saliva.[16-18]

Material composition is a quite effective factor in cell adhesion, as

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well. The primary monomers used in the systems of CAD/CAM are constituted by A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethyleneglycol dimethacrylate (TEGDMA), and ethoxylated bisphenol-A dimethacrylate (Bis-EMA).[19]

The term biocompatibility is defined as the potential of a material to function in such a way that no intolerable local or systemic effects occur. Along with this, the most appropriate and beneficial response of the cell or tissue is generated in that particular situation as well as optimization of the clinically relevant performance of treatment.[20] Circulating between blood and peripheral lympoid tissue until encountering antigens, immune cells are forced to proliferate and differentiate which mounts an inflammatory response. Reaching the blood circulation of the products separated from the biomaterials placed in the body affects the lymphocytes and their mechanisms.[21] Considering the fact that CAD/CAM materials placed in the body have different chemical structures and the residual monomers released from these materials, their interaction with cells involved in body defense creates a gap in the literature.

Therefore, this study aimed to determine the biocompatibility of six newly introduced CAD/CAM block materials with different ingredients and production techniques and their effects on human peripheral lymphocyte cell proliferation and cytotoxicity. The null hypotheses are no difference in the proliferation of the human peripheral lymphocyte cell in contact with the CAD/CAM blocks at different exposure times.

2. Materials and Methods

2.1. Chemicals and Instruments

Six CAD/CAM materials groups were used: feldspar ceramic VM (Vitablocks Mark II; VITA Zahnfabrik, Germany), resin nano-ceramic LU (Lava Ultimate; 3M ESPE, Seefeld, Germany), hybrid ceramic C (Cerasmart; GC, America), leucite-reinforced ceramic LRF (GC LRF; GC,America), zirconia-reinforced lithium silicate ceramic VS (Vita Suprinity; VITA Zahnfabrik, Germany), polymer-infiltrated ceramic-network VE (Vita Enamic; VITA Zahnfabrik, Germany) the manufacturers' details are summarised in **Table 1**. Chemicals used for experiments Phosphate Buffered Saline (PBS), Fetal Bovine Serum (FBS), Antibiotic Antimycotic Solution, Histopaque-1077, Dulbecco's Modified Eagle's Medium (DMEM) (Sigma), Dimethylsulfoxide (DMSO) (Sigma-Aldrich) and MTT (Acros) were purchased commercially and used without any purification. In addition, Thermoscientific-Multiskan Sky Microplate Spectrophotometer, HETTICH EBA 200 centrifuge device, Nüve BM 101 Water bath, J.P. Selecta Digiheat drying and sterilization oven, ISOLAB vortex mixer, Bandelin Sonorex RK-106 ultrasonic bath and Panasonic MCO 170AICUVH-PE CO2 Incubator were used.

Table 1. Listed of CAD/CAM block materials

CAD/CAM material	Type of Material	Compounds(%)	Manufacturer
Vita Suprinity	Zirconia reinforced lithium silicate glass ceramic	Glass ceramic, zirconia (approximately 10% by weight) (SiO2, Li2O, K2O, P2O5, Al2O3, ZrO2)	VITA Zahnfabrik, Bad Säckingen, Germany
Vita Enamic	Polymer-infiltrated ceramic-network (PICN)	86% wt feldspathic based ceramic network 14% wt acrylate polymer network (UDMA + TEGDMA)	VITA Zahnfabrik, Bad Säckingen, Germany
Vita Mark II	Feldspar-reinforced aluminosilicate glass	<20% wt feldspathic particles >80% wt glass matrix	VITA Zahnfabrik, Bad Säckingen, Germany
Cerasmart	Resin-based composite	71% wt silica and barium glass nanoparticles 29% wt resin matrix (Bis-MEPP,UDMA,DMA)	GC, America
Lava Ultimate	Nano-particulate pre-polymerized resin composite	80% wt nanoceramic (SiO2, ZrO2, aggregated ZrO2 / SiO2 cluster) 20% wt highly cross linked polymer matrix (Bis-GMA, UDMA, Bis-EMA, TEGDMA)	3M ESPE, Seefeld, Germany
GC LRF	Leucite reinforced ceramic	Glass, oxide, chemicals	GC, America

2.2. Preparation of CAD/CAM Blocks.

A total of 36 disc-shaped samples (h=2 mm, O=5 mm) for each material group were prepared according to ISO 10993–5: Cytotoxicity Tests - In Vitro Methods.[22] CAD/CAM blocks for all groups were cut in the determined sections with the aid of slow-speed diamond saw (Isomet, Buehler, USA). Samples were treated on an automatic polishing machine with a range of metallographic sandpapers (i.e. #240, #400, #800, #1200) (Buehler, Lake Bluff, USA). This process was carried out at 300 rpm, from both sides, and under continuous irrigation. In addition, The polishing of the sample surfaces was performed by Optrafine polishing system (Ivoclar Vivadent, Schaan, Liechtenstein) in accordance with the instructions of each manufacturer. The preparation phase of the samples was continued by cleaning with distilled water in an ultrasonic bath for 1 minute. Sterilization of the samples was carried out by exposing them to UV light for 30 minutes.

2.3. MTT Analysis

Lymphocytes were isolated from a human peripheral blood sample and were dispersed in centrifuge tubes in DMEM medium (supplemented with 10% v/v fetal bovine serum and 1% v/v penicillin/streptomycin/amphotericin B) in a biosafety cabin. The lymphocytes cells were seeded on a 96-well plate with 10^5 cells/well and it was incubated 24 h. After the incubation period was complete, the CAD/CAM blocks were placed in the wells. The cells were incubated at 37°C for 24 h and 48 h in the incubator. When the incubation periods were completed, 10 µL of MTT reagent was added to each well. The plate was gently mixed on the shaker. The cells were incubated at 37°C another 4 h. Then, the medium in the well was completely removed and 200 µL DMSO was added to each well. It was kept in the incubator at 37°C for about 18 h to dissolve the formazan crystals. The absorbance values were measured by spectrophotometer at 570 nm.[23] All experiments were carried out in triplicate and the relative Cell Viability (CV) percentage related to the control was calculated by following equation (CV (%) = ODtest/ODcontrol × 100), where the ODtest is the mean of absorbances of the test samples and ODcontrol is the mean of absorbances of the control.

The classification used by Sjögren et al.[24] was used to determine cell viability. If cell viability was below 30%, the material was considered severe cytotoxic. Moderately cytotoxic materials scored 30–59% cell viability, mild cytotoxic materials 60–90%, and non-cytotoxic materials over 90%.

The statistical analysis was carried out with IBM SPSS statistics package program (v.18.0, IBM, Armonk, New York, USA). Two-way ANOVA (Tukey) was used to assess whether all blocks were significantly different from each other and from the cell control group. Statistically significant level was accepted as 95% ($p<0.05$).

3. Results

When the data obtained were examined, it was determined that almost all blocks caused cell proliferation even in 24 h application (**Table 2**). It was determined that GC LRF Block, GC CERA Smart, Vita Enamic and Vita Suprinity blocks caused 37.14 %, 31.71 %, 22.74 % and 9.62% cell proliferation at 24 h application, respectively. 15.99 % and 4.51% cell death caused by Vita Mark II and Lava Ultimate blocks can be considered as slightly cytotoxic materials. All blocks caused cell proliferation at 48 h application time. In particular, Vita Enamic block provided lymphocyte cells to increase almost 2 times. Although Vita Suprinity caused the lowest cell proliferation at 24 h application, this block provided the second most important cell proliferation with a rate of 102.75% at 48 h application. GC CERA Smart, GC LRF and Vita Mark II blocks induced more than 50% cell proliferation (**Fig 1**). When all results were evaluated, it was determined that the blocks used in this study did not cause a significant cytotoxic effect on lymphocyte cells, and even caused cell proliferation in long-term exposure. In this study, cytotoxicity and cell proliferation did not differ significantly between materials ($p>0.05$). As a result, the null hypothesis was accepted.

Table 2. The Cell viability and cell proliferation percentages (%).

Materials	Time	Cell Viability (%)
Cell Control	24 h	100.00
	48 h	100.00
Vita Suprinity (VS)	24 h	109.62
	48 h	202.75
Vita Enamic (VE)	24 h	122.74
	48 h	261.71
Vita Mark II (VM)	24 h	84.01
	48 h	167.38
GC Cerasmart (C)	24 h	131.71
	48 h	178.57
Lava Ultimate (LU)	24 h	95.49
	48 h	169.53
GC LRF Block (LRF)	24 h	137.14
	48 h	170.12

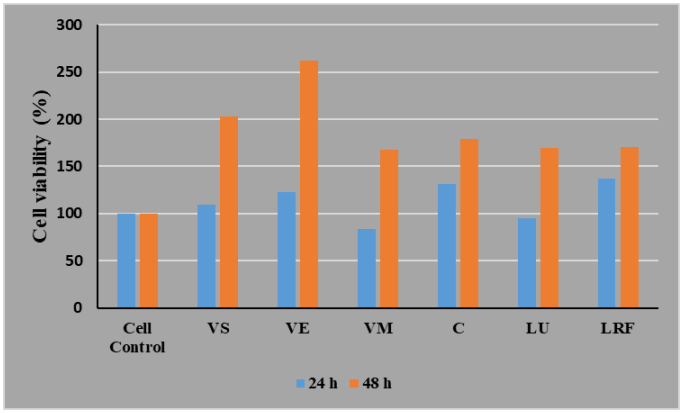

Figure 1. Cell viability (%) results of different CAD/CAM blocks.

4. Discussion

The dental ceramics used in the present study were selected due to the fact that they are contemporary and their chemical content differs from each other (**Table 1**). During the process of evaluating dental materials, physical and mechanical properties are mostly targeted, while biological properties remain in the background. However, in recent years, the necessity of evaluating the biocompatibility of newly developed materials before clinical applications has been emphasized.[25,26]

Limited number of studies have evaluated the cytotoxicity of all-ceramic materials.[27,28] Taking into account that the composition of materials is an effective factor in cell adhesion, in the current study, products from each material group including feldspar ceramic VM (Vitablocks Mark II), hybrid ceramic C (Cerasmart), resin nano-ceramic LU (Lava Ultimate), zirconia-reinforced lithium silicate ceramic VS (Vita Suprinity), leucite-reinforced ceramic LRF (GC LRF) have been utilised.

Grenade et al.[29] investigated the effect of titanium, zirconium and lithium disilicate ceramics on the viability, number and cell coverage of fibroblasts and keratinocytes and found that zirconium and titanium surfaces were better tolerated by cells. Consistent with these results, in our study, a significant increase in cell proliferation was observed in Vita Suprunity. Through these studies[30,31] pressable all-ceramic crown material (IPS Empress-1) and infiltrated all-ceramic crown material (In-Ceram) were reported to have only mild suppression of cell function in vitro at acceptable levels. However, our result did not show agreement with the findings by Messer et al.[32] They compared discs of pressable all-ceramic material (lithium disilicate pressable materials [Empress-2 and Stylepress], conventional feldspathic veneer porcelains [Duceragold and Vita Omega] and pressable leucite-based material [Empress-1]). Both lithium disilicate materials decreased mitochondrial activity dependent upon aging and Empress-2, which was initially severely cytotoxic, turned out to be more cytotoxic again following the process of polishing. The current study found that high cell proliferation was observed in leucite-reinforced material (GC LRF) and zirconia-reinforced lithium silicate ceramic (Vita Suprinity). The dynamics of cytotoxicity may be different when small differences in material composition (i.e percentage of Zr) or processing are considered, which may be attributed to the distinctive material compositions in different brands. Moreover, the difference in cell proliferation in the first 24 hours between Vita Enamic and Vita Mark II may be due to the proportional difference in feldspar content and preparation technique.

In a previous study which shared similarity with the current study, a greater fibroblasts growth rate that was cultured not only on ground but lithium disilicate and zirconia discs that had previously undergone the polishing process as well was higher than the cells grown on feldspathic ceramics in the 24 hours.[33] This finding is compatible with the present study in that cell death took place in the first 24 h when it was subject to feldspathic ceramic.

Furthermore, zirconia lithium disilicate ceramic was observed to have high proliferation activity in the current study, which possibly caused by the high biocompatibility which was exhibited by these materials' ability to achieve fibroblast adhesion *in vitro*, as stated earlier in the literature upon zirconia.[34] Actually, *in vitro* toxicity effect of lithium disilicate was reported in other studies[35] following two weeks after culture along with the fact that cytotoxicity which could biologically be unacceptable in accordance with current empirical standards applicable for composites and dental alloys was exhibited by lithium disilicate.

The results obtained regarding CAD/CAM nano ceramic resin are in line with a recent study in which prefabricated polymer and nano ceramic resin blocks are realized to be more predictable materials in terms of preserving the periodontal soft tissues.[36] It was reported by Alamoush et al.[37] that a cytotoxic effect was exhibited by polymer-infiltrated reinforced-glass-network (PICN-Vita Enamic) in gingival keratinocytes and human gingivofibroblasts on the third and fifth day, which later showed an increase towards the tenth day. Nevertheless, in another in vitro study, it was revealed that no cytotoxic effects on gingival and pulpal stem cells were possessed by Vita Enamic and other experimental PICN materials which were regarded as quite biocompatible.[38] Moreover, comparable biocompatibility was demonstrated not only with zirconia but also with titanium by an experimental PICN without TEGDMA and a photoinitiator with different filler particles.[39] TEGDMA possesses quite a lot of adverse effects in terms of cytotoxicity and genotoxicity resulting from its small molecular size, which leads to enhancement of diffusion processes.[40,41] In this respect, the cytotoxic effect can account for the presence of TEGDMA, a polymer matrix fragment and a low weight monomer found in commercial PICN (Vita Enamic).[41,42] The cell proliferation in human peripheral lymphocytes was observed to increase at 24h and 48h in the current study displaying a difference in comparison to other studies, which may result from the differences in cell type.

Performing cytotoxicity tests of dental materials is usually conducted on the cells with which they interact. MTT test is one of the fastest and most sensitive tests among biocompatibility tests and can detect low level toxicity differences. It was also preferred in our study. Nonetheless, their impact on blood cells is of great significance when the ions released by these materials and the differences in composition are taken into account. MTT test is one of the fastest and most sensitive tests among biocompatibility tests and was preferred in our study because it can detect low-level toxicity differences.[43]

5. Conclusion

The present study demostrated that, from a biological perspective, all-ceramic groups are advisable on the basis of rapid cell response. This is the first study to determine the effects of CAD/CAM materials on lymphocytes. Due to the inherent limitations of this study due to its *in vitro* nature, further research will be required to understand the longer-term biological advantages and to describe these materials in detail. The limitations of this study are that it is an

in vitro study conducted under laboratory conditions and the results are not directly applicable to clinical practice. However, the findings may provide additional information for clinicians during material selection. Although VM and LU cause negligible cytotoxicity at 24-hour exposure, it is clear that the test materials are not cytotoxic in general. Therefore, these blocks are recommended as biomaterials that can be used safely in dental treatment. In addition, in order to support the results of this study, it is thought that further *in vitro* and *in vivo* studies should be performed using different methods and cell lines on the biocompatibility of these blocks.

Değerlendirme / Peer-Review

İki Dış Hakem / Çift Taraflı Körleme

Etik Beyan / Ethical statement

Tıp Fakültesi Etik Kurulu'ndan etik kurul onayı alındı (Etik No: 2020/15).

Ethics committee approval was obtained from the Ethics Committee of the Faculty of Medicine (Ethics No: 2020/15).

Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur.

It is declared that during the preparation process of this study, scientific and ethical principles were followed and all the studies benefited are stated in the bibliography.

Benzerlik Taraması / Similarity scan

Yapıldı – ithenticate

Etik Bildirim / Ethical statement

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Çıkar Çatışması / Conflict of Interest

Yazarlar çıkar çatışması bildirmemiştir. | The authors have no conflict of interest to declare.

Yazar Katkıları / Author Contributions

Çalışmanın Tasarlanması | Design of Study: FOC (%30), SO (%30), MHA (%20), GBA (20)
Veri Toplanması | Data Acquisition: SO (%40), FOC (%20), , MHA (%20), GBA (%20)
Veri Analizi | Data Analysis: GBA (%30), SO (%30), FOC (%20), MHA (%20)
Makalenin Yazımı | Writing up: SO (%40), FOC (%20), , MHA (%20), GBA (%20)
Makale Gönderimi ve Revizyonu | Submission and Revision: SO (%40), FOC (%20), , MHA (%20), GBA (%20)

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