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Cytotoxic effect of elements released clinically from gold and CAD-CAM fabricated ceramic crowns

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Abstract

Objectives: The aim of this study was to investigate the cytotoxic effects of elements released from gold and CAD-CAM fabricated ceramic crowns.
Materials and methods: According to the determination of elements released from gold alloy[1] and CAD-CAM fabricated ceramic[2] crowns into saliva of fixed prosthodontic patients by using inductively coupled plasma mass spectroscopy, similar amounts of elements (Au, Pd, Ag, Zn, Cu, Al, Si) were prepared as salt solutions. A well without any tested element was used as a negative control. These salt solutions were tested for cytotoxicity by culturing mouse L-929 fibroblasts for a 7-day period of incubation. Then, the percentage of viable cells for each element was measured using trypan blue exclusion assay. The data ($n = 5$) were statistically analyzed by ANOVA/Tukey test ($p < 0.05$).
Results: The lowest percentage of viable cells (Mean ± SD) was evident with Zn and Cu released from gold crowns indicating that they are the most toxic elements. Ag was found to be intermediate in cytotoxic effect. Au, Pd, Al, Si were found to be the least cytotoxic elements.
Conclusion: Zn and Cu released from gold alloy full crowns showed evidence of prominent cytotoxic effect on fibroblasts cell cultures.
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Keywords: Cytotoxicity; Gold; Ceramic; Ions; Fibroblasts

1. Introduction

Human natural teeth could be subjected to damage or loss due to different reasons, such as caries, trauma, or oral pathology. Full coverage restorations are an important solution to restore badly damaged or

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missing teeth. Cast gold crowns have been very successful for restoration especially in those patients in whom placement of gold crown would not result in an unaesthetic display of metal [1]. However, the patient demands for tooth-colored restorations have led to increased use of ceramics for dental restorations, prompting a dramatic increase in the all-ceramic materials available commercially. The CEREC 3D computer-aided design/computer-aided manufacturing (CAD/CAM) system, which uses optical data for milling solid blocks of ceramics, became an important all-ceramic choice. The principal advantage of this system is its ability to provide tooth-colored restoration in one appointment without provisional restorations [2].

Whatever the restoration material, it is always in close proximity to oral tissues for an extended period. In the oral environment, biodegradation of the dental material occurs because of chemical/physical destruction by saliva, wear and erosion caused by food, chewing and bacterial activity [3]. Therefore, it is important to evaluate the material's reactivity in the oral cavity, which is governed by electro-chemical reaction kinetics and thermo-dynamic principles. This means that when a material is placed in the oral cavity, the material-saliva system is driven toward a state of thermo-dynamic equilibrium. At equilibrium, the material either stays stable in its elemental form or converts to its ionic form [4]. Thus, the initially uncharged elements inside the material lose electrons and become positively charged ions as they are released into solution (saliva). Biodegradation or corrosion is a chemical property that has effects on other material properties, such as esthetics, strength and biocompatibility. From a biocompatibility standpoint, the biodegradation of a material indicates that some of the elements are available to affect the tissues around it [5].

The term biocompatibility refers to the ability of a material to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, while generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy [6]. Therefore, cytotoxicity is an important component of biocompatibility. Different previous studies have tested the cytotoxicity of full coverage restorative alloys such as gold alloy [7–10] while limited studies have evaluated the cytotoxicity of all-ceramic materials [11,12]. Previous studies tested the cytotoxicity of gold and ceramics by placing solid specimens of these materials in cell culture medium. However, clinically, the release of elemental ions from full coverage restorative materials into the oral cavity occurs with a resultant formation of organo-metallic and metallic salts such as chlorides and sulfides during corrosion. Accordingly, other cytotoxicity studies for gold [10,13] and CAD-CAM fabricated ceramics [10], used the salt solutions made from elements in amounts equal to what were released into a cell culture medium, sodium chloride solution, or acidic medium (lactic acid). To date, there is no study that tested the cytotoxic effect of these materials using amounts equal to what are clinically released in the patients' saliva. Therefore, to be more relevant to what occurs in the oral environment, the goal of this study was to evaluate, *in vitro*, the cytotoxicity of elemental ions on fibroblast cell lines at the concentrations released in the oral environment from the two commonly used crown materials (Type IV gold alloy[1] and CAD-CAM fabricated ceramic[2]).

2. Materials and methods

2.1. Preparation of salt solutions

Sterile salt solutions (stocks) of seven different cations (salts) were made from Ag^{+} [Ag_2SO_4], Zn^{2+} [$ZnCl_2$], Si^{4+} [$SiCl_4$] and Au^{3+} [$AuCl_3$],[3] Cu^{+} [CuCl], Pd^{2+} [$PdCl_2$], and Al^{3+} [$AlCl_3$].[4] They were diluted in distilled water at concentration ranges similar to the concentrations of elements found to be released from gold alloy and CAD-CAM fabricated ceramic crowns into an individual's saliva. These concentrations were determined by using inductively coupled plasma mass spectroscopy (Table 1) [14]. The measured amounts of elemental ions in the previous research [14] were expressed in ppb (μg/L), and recorded at three months and six months after crowns placement. In the present study, the highest amounts (Mean) released from the crowns (the amount recorded at six months was the highest for all elements except for Zn which had the highest concentration after three months) were used. These amounts were Au (24.55), Pd (26.74), Ag (46.76), Zn (1048), Cu (63.59), Al (233.1) and Si (9455.5).

[1] Ney-Oro 60, Dentsply, USA.
[2] CEREC 3D, Sirona, Germany using IPS Empress CAD blocks, Ivoclar-Vivadent, Germany.
[3] Acros Organics, New Jersey.
[4] Sigma–Aldrich, St. Louis, MO.

Table 1
Elements used and their corresponding materials.

Material	Manufacturer	Product	Released elements concentrations (ppb)
Type IV gold alloy	Dentsply, Konstanz (Germany)	Ney-Oro 60	Zn (1048), Cu (63.59), Ag (46.76), Pd (26.74), Au (24.55)
Machinable Ceramic	Ivoclar-Vivadent, Liechtenstein (Germany)	IPS Empress CAD blocks	Si (9455.5), Al (233.1)

2.2. Preparation and culturing of fibroblasts

L-929 mouse fibroblasts[5] were used for the cytotoxicity testing. These cells were selected based on their common use in dental materials cytotoxicity tests [9,13]. Furthermore, these cells retain two important criteria: (1) they are contact inhibited as they become more dense in culture, and (2) they are non-tumorogenic. These cells were maintained in cell-culture medium consisting of Dulbecco's Modified Eagle's Medium [DMEM][6] supplemented with 10% fetal-bovine serum,[7] gentamycin (10 ug/ml),[7] penicillin/streptomycin (125 units/ml).[6] Antibiotics were added to inhibit growth of microorganisms, and all procedures were performed aseptically. All incubations were carried out in an incubator in a humid atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were passaged by trypsinization (0.05% Trypsin-EDTA).[6] After counting the cells with a hemocytometer,[8] cells were plated (seeded) into sterile 6-well tissue culture plates[9] at 30,000 cells/cm^2 in 3 ml of cell-culture medium. The test salt solutions were added immediately (one test material per well) and incubated at 37 °C for one week. Cell viability was recorded by means of trypan blue exclusion assay. Live and dead cells were counted with a hemocytometer, see below.

2.3. Trypan blue exclusion assay

After discarding the test material and medium, the cells in each well were rinsed three times with 1 ml phosphate buffer solution (PBS) to remove traces of previous cell-culture medium. Next, 0.2 ml trypsin was added and incubated for 10 min to remove the cells from the bottom of the wells. The cells were suspended in 0.5 ml Dulbecco's Modified Eagle's Medium. Then, 0.1 ml of the suspended cells was mixed with 1.0 ml of a mixture composed of equal amounts of trypan blue stain and phosphate buffer solution. The dead cells allowed the stain to enter their membranes, coloring their cytoplasm blue. The live cells excluded the stain, remaining clear. A small amount of the stained cell suspension (10 µl) was placed onto a hemocytometer slide and covered with a coverslip. The viable cells were counted with a light microscope at 100×. Four counts were made from each well. Each was counted on the same hemocytometer slide, two being on the top half corners of the slide and the others being obtained from the bottom half corners of the slide. The average was calculated and the percentage of viable cells was determined using the following formula:

$$\text{Percentage of viable cells} = (A/B) \times 100$$

where A = viable cells in the experimental well, and B = viable cells in the control.

Each test was run in five replicas. A well without any tested materials was used as a negative control. The statistical analysis was conducted by ANOVA/Tukey HSD posthoc test with a significance set at $p < 0.05$.

3. Results

Light microscopic examination of the cells after staining with trypan blue demonstrated variable degrees

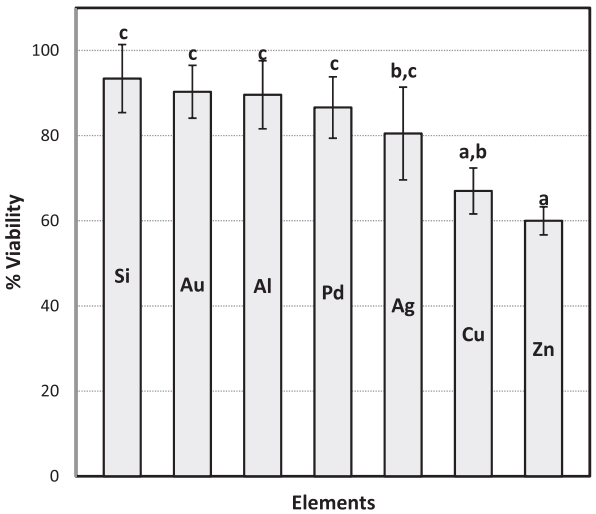

Fig. 1. Comparison of average % cell viability for the tested elements. Identical letters indicate no statistical difference ($p < 0.05$, Tukey HSD test).

[5] CCL-1, ATCC, Manassas, VA.
[6] Gibco Invitrogen, Carlsbad, CA.
[7] HyClone, Thermo Fisher Scientific Inc., Waltham, MA.
[8] Hausser Scientific, Horsham, Pa.
[9] Becton Dickinson Labware, NJ.

of viability for the different test elements when added to the culture media. The results of the trypan blue viability test are shown in Fig. 1. Statistical analysis revealed that Zn had a significant ($p < 0.05$) higher cytotoxic effect (cell viability = 60%) than all other elements except Cu (67% cell viability), whose cytotoxicity was in turn statistically higher than the remaining elements except Ag (80% cell viability) and Zn.

4. Discussion

The cytotoxic effects of elements contained in gold alloy and CAD-CAM fabricated ceramic crowns were evaluated in the present study by investigating the effects of ions released from them into human saliva in the form of salt solutions. Testing the cytotoxicity using salt solutions has been decided because organometallic and metallic salts form during corrosion of dental materials in the mouth. In addition, anions like chloride or sulfate were not effective when used at low concentrations to form the salt solutions [15–17] which indicated that any recorded effects for the salt solutions were caused by cations alone.

Various levels of cytotoxicity of test elements have been recorded in the present study. The ranking of elements cytotoxicity (most potent to least potent) was: Zn > Cu > Ag > Pd > Al > Au > Si. This ranking agrees with the ranking of a previous study [10] which reported that Zn > Cu > Ag > Al > Pd. In addition, the present study results are in partial accordance with another study made by Schedle et al. (1995) which revealed another order for gold alloy elements: Ag > Cu > Zn > Pd. The differences among the rankings can be attributed to the difference in the amounts of used cations salt solutions. A higher amount of Ag was used in the previous study [13] which allowed Ag to be the most toxic element in that experiment. Because the present study used an amount of metal salt solutions equal to what are released in the mouth [14], the present results are clinically relevant.

From another point of view, the present cytotoxic results from the gold alloy were consistent with their released amounts into patients' saliva [14]. Zn and Cu were the highly released elements from gold crowns, and they were the most cytotoxic elements supporting the idea that it was the material ions that were responsible for its toxicity. However, a higher amount of released ions did not necessarily indicate higher toxicity. Si was released more than Al, but Al had a more cytotoxic effect than Si (non-significant difference). This can be due to a higher inherent biocompatibility of Si.

It has been revealed that Zn was the most toxic element released from gold crowns into the mouth. This finding can explain another finding which is the higher toxic effect of zinc-containing amalgams than Zn-free amalgams [18]. Also, it has been noted that Ag occupied a moderate place order of cytotoxicity. This moderate position was in accordance with another finding which stated that pure Ag metal possessed a moderate cytotoxicity rank between the high toxicity of Cu and low toxicity of Pd [19].

In the present study, the main elements of CAD-CAM fabricated ceramic crowns (Si and Al) resulted in a very high degree of fibroblasts viability. This result agrees to wide extent with another study [9] which showed no evidence of cytotoxicity for pressable all-ceramic crown material (Empress), and those studies [11,12] which reported that pressable all-ceramic crown material (IPS Empress-1) and infiltrated all-ceramic crown material (In-Ceram) had only mild *in vitro* suppression of cell function to levels that would be acceptable on the basis of standards used to evaluate alloys and composites. However, our result disagrees with a study [12] which revealed that pressable all-ceramic material discs (IPS Empress-2: lithium disilicate glass ceramic) had unacceptable cellular toxicity. The contrasting results between the two studies can be due to testing different species of all-ceramic materials (lithium disilicate glass pressable ceramic versus leucite-reinforced glass machinable ceramic). Further studies should focus on factors such as microstructure and porosity to understand the biologic effect of all-ceramic crown materials. Again, because the present study used amounts equal to what were clinically released in the mouth, the present result was assumed to be more clinically relevant.

However, the outcome of the present study must be taken with caution because the trypan blue exclusion assay used in the present study was considered as a legitimate test to measure the end stage cytotoxic effects, rather than some earlier cytotoxic event. Therefore, our future studies will include more measurements dealing with cells functions such as protein fabrication (collagen synthesis), respiratory and digestive cell functions. In the present study, single-salt solutions were investigated for the determination of cytotoxic effects due to an individual element. Further studies should test the combinations of these salts for the detection of synergistic, antagonistic, or additive effects caused by different mixtures of cations.

5. Conclusion

Within the limits of the present study, Zn and Cu released from gold alloy full crowns showed evidence of prominent cytotoxic effect on fibroblasts cell cultures.

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