

The effect of recasting on biological properties of Ni-Cr dental alloy

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SUMMARY

Introduction Increases in market prices of gold over the last 20 years have led to expansion of basic dental alloys, which, primarily due to their good mechanical properties and acceptable prices, have found their place in everyday dental practice. However, within the procedure of making dental prosthetic restorations, the alloys are melted and cast, which leads to changes in their physical, mechanical and biological properties.

Objective The objective of the study was to test biocompatibility of a Ni-Cr dental alloy (WIRON 99) depending on the number of melting and casting processes.

Methods The working method included the testing of cytotoxicity of the alloy obtained by casting after one, after four, and after eight successive processes of melting. Cytotoxicity of samples was tested by means of a 24-hour and a three-day cytotoxicity test, done on L929 fibroblasts.

Results A repeatedly melted and cast alloy shows a reduced biocompatibility and causes specific responses of the tissues in the surrounding area. Since the cytotoxic effect is more significant in the extended contact with the culture cells, a three-day cytotoxicity test showed discrete changes which were the indicator of cell growth inhibition in the cell culture.

Conclusion The obtained results confirm the working hypothesis that repeated alloy melting and casting will decrease biocompatibility of dental alloys and will lead to specific responses of the tissue in the surrounding area.

Keywords: biocompatibility; dental alloys; cytotoxicity

INTRODUCTION

Despite many weaknesses, metals are irreplaceable constructive materials in everyday dental practice. Metals are also present in all living organisms and have significant roles – they are structural elements, stabilizers of biological structures, components of control mechanisms in nerves and muscles, and above all they are enzyme activators and redox-system components. The essential metals are those metals that are necessary for human life, and without which there is no normal cell functioning. Metal cations, with their electrophilicity, stabilize all electronrich functional groups thus maintaining the stability of molecules (DNA, RNA, structural proteins, enzymes, hormones, receptors), and due to their chemical potency they are often in the center of activities of cell processes. Other metals, which are not essential, can cause an increase in toxic manifestations even in case their intake is only slightly higher than that in the natural surroundings. A cation is a toxicity carrier and a compound or an element in the given environment will be as much toxic as the amount of cations chemically released. A cation reacts with molecules in the bio-environment, i.e. with certain functional groups towards which it shows affinity (carboxylic, amino, hydroxyl, sulphhydryl, imidazole functional group, etc.) [1, 2].

One of the mechanisms for revealing toxicity of metals is by explaining their interac-

tion with bioelements. Cations that enter this interaction are similar enough to a certain bioelement, with regard to their physical and chemical properties, and therefore can come into reaction with a bio-ligand instead of it, and yet they are different enough to lead to weakening or ending the function of the cell process in which they are involved. However, cations can react even in situations where metals, i.e. bioelements do not have a specific role, if the appropriate chemical conditions have been provided. These reactions are not usually in function of the cell system and they cause disorders [3].

Once absorbed, the metal remains in the organism until being excreted. In contrast to the majority of organic compounds which are eliminated from the tissue by means of metabolic degradation, metals are indestructible as elements and therefore they have the power of cumulation, which leads to the occurrence of chronic effects in the organism. Excretion is the only way of elimination of metals from the organism. Accumulation in tissues does not always necessarily result in a toxic effect (e.g. lead is deposited in bones in an inert form).

Microscopic observations of thus formed metal materials reveal their microstructural texture. Microstructure determines mechanical, chemical and other properties of dental materials. Microstructure, which is seen through the microscope on the polished and etched surface, is composed of elements. When

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making castings of precious dental alloys, the aim is to achieve a fine grained microstructure [4].

Ageing is a universal process. Ageing of materials in dentistry can be defined as fatigue of materials, hardening, corrosion, degradation, sorption, and deformation. During the process of definite formation, constructive elements are exposed to some changes, either in the laboratory (*in vitro*) or in the mouth (*in vivo*). Those changes can be spontaneous or deliberately caused in order to improve physical and chemical properties or to passivate the surfaces. Over time, in the mouth, changes in temperature or the pH value, the effect of mastication and other factors may result in changes in constructive materials. Understanding the ageing of materials requires knowledge of the theory of ageing, which takes place in multi-phase solutions. The consequence of changes in the constructive material itself can be dissolution of the material, release of ions or another way in which the material influences the surrounding tissue, either causing or not causing the reaction [5].

Metal alloys are used in fabrication of numerous fixed restorations and orthodontic appliances. Each alloy is accompanied by the information on its exact composition provided by the manufacturer. In everyday dental practice, it is usual that, due to economic reasons, a previously recast alloy is not thrown away, but 50% of the new (not recast) alloy is added to it. This can lead to serious changes in microstructure and consequently to changes of the biological values of the dental alloy. The matter of the precise chemical composition of castings obtained in this way still remains unclear. This generates another, more important issue – what are the biological properties of castings obtained in this manner? An important fact is that prosthetic restorations remain in the patient's mouth for many years and therefore it is crucial to examine the biocompatibility of dental alloys.

OBJECTIVE

The aim of this study was to examine biocompatibility of one commonly used Ni-Cr dental alloy depending on the number of melting and casting processes. The starting point in this study was the working hypothesis that repeatedly melted and cast alloy changes its chemical composition, which affects physical and mechanical properties of the alloy. Modified physical and mechanical properties have an adverse effect on the surrounding area where the dental restoration is placed and this further leads to unfavorable reactions and brings into question the biological quality of such a restoration.

METHODS

A dental alloy used in this research has a wide variety of applications in dental practice. The experiment included several *in vitro* tests.

Test samples of dental Ni-Cr alloy were prepared in the dental-technical laboratory of the Clinic for Dental Prosthetics, Faculty of Dental Medicine in Belgrade. Compo-

sition of the alloy was as follows: Ni – 65%; Cr – 22.5%; Mo – 9.5%; Nb, Si, Fe, Cr, C. Alloy samples were shaped as discs, 5 mm in diameter and 1 mm thick, and 10 mm in diameter and 1 mm thick. All samples were cast in the induction appliance for dental alloy casting. The number of alloy samples and controls was six.

The samples of the tested dental alloy were marked as follows: W1 – the alloy melted once in the induction appliance and cast in the dental-technical laboratory; W4 – the alloy melted four times in the induction appliance and cast in the dental-technical laboratory; W8 – the alloy melted eight times in the induction appliance and cast in the dental-technical laboratory.

For the cell culture, a complete culture medium was used, which contained RPMI 1640 medium (Sigma-Aldrich, Hamburg, Germany), 10% fetal veal serum (Galenika a.d., Belgrade, Serbia), 2 mM glutamine (Sigma-Aldrich), 100 IU/ml penicillin (Galenika a.d.) and 0.5% streptomycin (Galenika a.d.). Fibroblast cells (NCTC, clone L929) originating from the adipose mouse tissue were used. The cells were obtained from Hammersmith Hospital (London, U.K., Department of Immunology).

As a negative control, a material that does not cause a cytotoxic reaction was used – glass slides for histological preparations, cut with a diamond needle, 5 mm in diameter and 1 mm thick.

As a positive control, a preparation confirmed to lead to cytotoxic changes was applied – a sterile water solution of phenol in 4% concentration, which was applied on a sterile filter of 5 mm in diameter.

As another negative control in the experiment, a culture of fibroblast cells was used without any test material.

Cytotoxicity tests were done in a standard way, and thereafter the qualitative and quantitative test results were interpreted after a 24-hour and a three-day cytotoxicity test.

Qualitative test for estimation of cytotoxicity

A cytotoxic effect was estimated on the basis of morphological characteristics of cell cultures, using the ISO 10993-5:1992 qualitative test [6]. Degenerative changes, such as cell vacuolization, cell fragmentation and lysis, detaching of cells from the base, changes in the cell volume, were analyzed and recorded. Cells around the placed implant were separately analyzed (sample test). The results were interpreted in such a way that the changes in normal morphological characteristics of cells were numerically expressed with the following index of changes: 0 – no changes; 1 – mild changes; 2 – moderate changes; 3 – serious changes.

Quantitative test for estimation of cytotoxicity

The percentage of necrotic cells in the culture (percentage of cytotoxicity) was used to define the cytotoxicity ISO 7405:1997 [7]. Corresponding indexes were calculated by means of numerical indicators and on the basis of this calculation the results were interpreted as follows: index

0 – no cytotoxic effect; index 1 – mild cytotoxicity; index 2 and 3 – moderate cytotoxicity; index 4 and 5 – serious cytotoxicity.

RESULTS

Effect of dental alloy samples on the fibroblast L929 cell culture in a 24-hour test

The first aim of the *in vitro* testing was to check a possible cytotoxic effect of dental alloys on the fibroblast cell line (L929) in the culture. The experiments were done observing corresponding ISO standards for testing cytotoxicity.

Results of the qualitative test

The results showed that none of the tested samples of the dental alloy, either once or multiple times cast, demonstrated a detectable cytotoxic effect. The index of changes in the cell cultures in the presence of dental alloys and negative controls (glass or the culture of L929 cells which was cultivated only in presence of the medium) was marked as 0. As it was expected, a positive control (a filter paper sample soaked in phenol) exhibited the full cytotoxicity (cytotoxicity index 3).

Results of testing negative control cultures showed a normal morphology of L929 cells, which in a confluent stage of growth exhibited a typical mosaic or cobble pattern. The cells were in close contact with each other, often having a polygonal form or, less frequently, a slightly longitudinal racket-like form. Around the control glass sample (Figure 1), no detectable changes were seen in the morphology of cells in comparison with the cells which were not in immediate contact with the sample.

Figure 2 displays a typical appearance of positive control cultures, which exhibited a maximum cytotoxic effect. The cells within the whole basin area of the cultivation plate demonstrated an altered appearance, such as reduced volume, detachment from the base, fragmentation of cells or their nuclei, and presence of distinct degenerative changes.

In all cultures with dental alloys, either in immediate contact or in zones away from the placed samples, no changes which could differ from the negative controls were noticed.

Results of the quantitative test

Since in cell cultures growing confluent a certain number of cells die spontaneously and nonviable cells can be present, it was necessary for the results of the qualitative analysis to be confirmed by quantitative tests. The results shown in Table 1 indicate that the percentage of nonviable (necrotic) cells in the cultures with dental alloys was very low and ranged within $2.5 \pm 0.5\%$ (sample W1). These values were not statistically significantly different from those of the negative control (the cells in the medium: $2 \pm 0.6\%$;

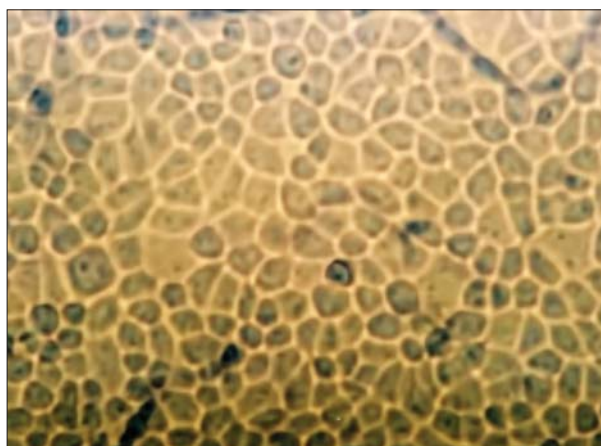


Figure 1. Appearance of L929 cells cultivated for 24 hours in the presence of the control sample (glass); normal morphology of the cells can be noticed ($\times 640$)

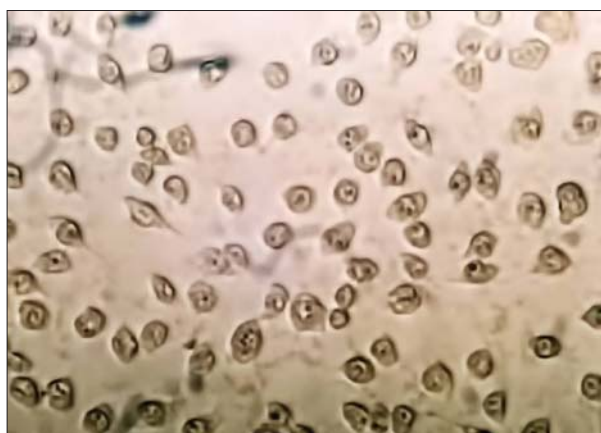


Figure 2. Appearance of L929 cells cultivated for 24 hours in the presence of the positive control; remarkable cytotoxicity is noticed; the index of changes is marked as 3 ($\times 640$)

Table 1. Effect of dental alloys on fibroblast L929 cells in the 24-hour culture (quantitative test)

Type of sample	Percentage of necrotic cells \pm SD
Negative control (medium)	2.0 ± 0.6
Negative control (glass)	2.7 ± 0.5
Positive control	100 ± 0
W1	2.5 ± 0.5
W4	3 ± 0.9
W8	3.3 ± 0.9

Degree of cytotoxicity (cell necrosis) was determined on the basis of ISO-7405:1997.

control glass sample: $2.7 \pm 0.5\%$). As expected, cytotoxicity in the cultures of the positive control was 100% (all cells were necrotic).

Effect of dental alloy samples on L929 cell culture in the three-day test

Since cytotoxic effect of dental alloys is more expressed in extended contact with the tested cells, in the experiments which followed we modified the previous two tests by placing the samples of dental alloys on semi-confluent layers of fibroblast L929, after which the cultivation of cells was performed for three days.

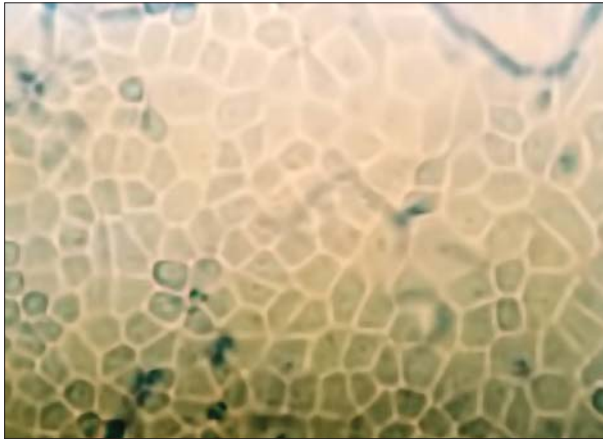


Figure 3. Appearance of L929 cells cultivated for three days in the presence of the Wiron 99 alloy (W4); normal cell morphology is noticed, including a confluent growth as well ($\times 640$)

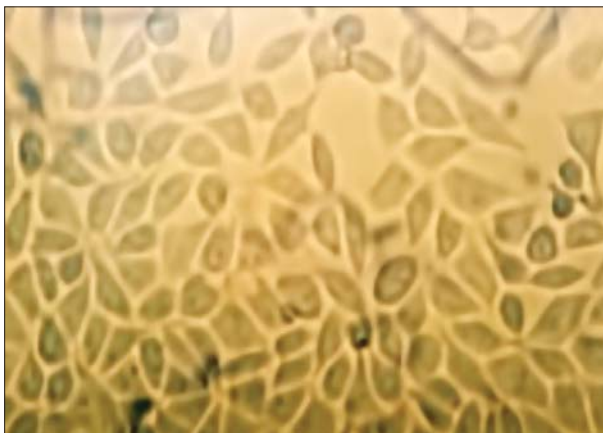


Figure 4. Appearance of L929 cells cultivated for three days in the presence of the Wiron 99 alloy; cell morphology is noticed as normal, but in certain smaller zones next to the explant the cells are of an elongated, racket-like and fibroblastoid form ($\times 640$)

Table 2. Effect of dental alloys on fibroblast L929 cells in the three-day culture (quantitative test)

Type of sample	Percentage of necrotic cells \pm SD
Negative control (medium)	1.8 ± 1.0
Negative control (glass)	8.2 ± 2.3
Positive control	100 ± 0
W1	8.3 ± 2.6
W4	9.5 ± 1.6
W8	10 ± 1.7

Degree of cytotoxicity (cell necrosis) was determined by means of trypan blue dye

Results of the qualitative test

In the negative control (glass) cultures, no morphological changes in L929 cells were noticed, either along the edge of the preparation or along the whole basin surface of the cultivation plate. The appearance of the cells was very similar to the control cultures (medium control). The cell morphology was similar to that of cells in the negative control of 24-hour cultures (Figures 1 and 2), except for periodically present oval-shaped cells which were leaning on the adherent cells. This finding was typical for the long-term tests. Only individual cells in these cultures exhibited degenerative changes in a sense of cytoplasmic vacuolization or volume reduction.

The cultures of Wiron 99 (BEGO GmbH & Co. KG, Bremen, Germany) (W1, W4, W8) exhibited changes which were either discrete (mildly inhibited growth around the alloy samples) or they did not differ from the negative controls (Figures 3 and 4). However, the index of changes for all cultures with dental alloys and for negative controls was marked 0.

Results of the quantitative test

The quantitative test results are given in Table 2. The percentage of necrotic cells ($8.2 \pm 2.3\%$) was significantly higher in the control sample (glass) than in the control without a sample (medium control), where the basal cytotoxicity was $1.8 \pm 1.0\%$. In the cultures with the Wiron 99 samples, no statistically significant changes in percentage of necrotic cells were noticed in comparison to the control (glass). Although the percentage of necrotic cells in the cultures with multiple recasting (W4 and W8) was slightly higher than the one relating to once-cast alloy samples (W1), the differences among them were not statistically significant.

DISCUSSION

The oral cavity is a complex and dynamic environment where constructive dental materials should endure prolonged contact with the saliva (electrolyte), extended pressure of chewing, and contact with various chemical agents originating from food. Normally, every material in such conditions has to undergo some changes in its chemical composition and structure.

During fabrication, dental alloys are thermally treated and enriched with various elements that improve their mechanical properties but also significantly influence the reduction in biocompatibility. As indicators of biocompatibility, the responses of the surrounding tissues (cell viability, changes in cell morphology, changes in cell metabolic activities, etc.) were observed. The cytotoxicity tests which were performed in this study are very reliable [8–11].

In this research, the cytotoxicity of a Ni-Cr dental alloy was tested. Reference data indicate the disagreement in attitudes among different authors concerning the issue of possible repeated casting of these alloys [12–17]. Knowing the procedure of fabrication of dental restorations, the question which arises is whether the use of previous casting residues is harmful. In practice, up to 50% of an alloy which has not been recast is added to such residues and this mixture is used again in fabrication of dental restorations. It is obvious how important it is to find out whether recasting of the alloy will change its biocompatibility [18–23]. In this research, cytotoxicity was observed after the first, fourth, and eighth recasting of the alloy.

Degradation of the material is the main limiting factor in selection and use of a certain dental alloy and it directly influences the biocompatibility of the alloy. Corrosion is the most common cause of degradation of dental alloys.

One of the signs of corrosion can be discoloration if the outer layer of oxide, sulphide, or chloride is porous.

Many published studies describe the microstructure and properties of precious and basal alloys in their cast form and after a thermal treatment [24–28]. By artificial ageing, i.e. exposing the constructive dental alloys to alternate influences of high and low temperatures and pressures, the conditions close to clinical conditions in the mouth can be created. In this way, changes being the consequence of an ageing process are noticed faster than it is possible in the clinical conditions. A multiple remelted and recast alloy used in fabrication of fixed dental restorations and metal skeletons for partial prosthesis has accelerated ageing of the alloy as a consequence. Alterations which occur due to burning of micro constituents (deoxidants) and binding of some elements affect the alloy microstructure and are responsible for changes in mechanical and other properties of the alloy. Modified physical and mechanical properties of the alloy have an adverse effect on the environment where the dental restoration has been placed, which can cause unfavorable reactions that may affect the response of the surrounding tissue.

Results of this research concerning specific responses of the surrounding tissue, reference data, and known phenomena which are difficult to be controlled (metabolic products of bacteria, enzymes, water, dissolvents of either endogenic or exogenic origin on the one hand, and wear-

ing, dissolving, disintegration, ageing, and degradation of constructive materials on the other hand) explicitly point out that it is best to use the alloy in its original form. This is not the cheapest, but it is the safest method of working with dental alloys.

CONCLUSION

The obtained results confirm the working hypothesis that repeated melting and casting of alloys decreases the biocompatibility of dental alloys. However, reduced biocompatibility is not within the limits of statistical significance and thus all the changes do not have a cytotoxic effect.

Correlation between the number of melting and casting processes and the degree of cytotoxicity was proved. There were some degenerative changes in cell morphology, modified shape of cells and nonconfluent cell growth. Those changes were in direct proportion to the number of recastings, and were more pronounced at the three-day cytotoxicity test due to the prolonged contact between the examined samples and cells in the media.

It is well known from daily practice, as well as from literature, that dental alloys containing nickel represent potential threat to the health of patients and dental technicians. Recommendation of the authors of this paper is to use base dental alloys that do not contain nickel.

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Ефекат претапања на биолошка својства *Ni-Cr* денталне легуре

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КРАТАК САДРЖАЈ

Увод Пораст цене злата на светском тржишту у последњих 20 година довео је до експанзије базних денталних легура, које су пре свега због добрих механичких својстава и приступачних цена нашле примену у свакодневној стоматолошкој пракси. У процедури израде протетских радова легуре се топе и изливају, што доводи до промена њихових физичко-механичких и биолошких својстава.

Циљ рада Циљ рада био је испитивање биокompatбилности једне *Ni-Cr* легуре (*WIRON 99*) у зависности од броја топљења и ливења.

Методе рада У раду је испитивана цитотоксичност легуре добијене изливањем после једног топљења, после четири и после осам узастопних топљења. Цитотоксичност узора-

ка је испитивана помоћу 24-часовног и тродневног теста цитотоксичности на *L929* фибробластима.

Резултати Више пута топљена и ливена легура показала је смањену биокompatбилност, доводећи до специфичних одговора ткива у непосредном окружењу. Будући да је цитотоксични ефекат израженији у продуженом контакту са ћелијама из културе, тродневни тест цитотоксичности је показао дискретне промене које су показатељ инхибиције раста ћелија у култури.

Закључак Добијени резултати потврђују радну хипотезу да више пута понављана топљења и ливења легура смањују биокompatбилност денталних легура и доводе до специфичних одговора ткива у непосредном окружењу.

Кључне речи: биокompatбилност; денталне легуре; цитотоксичност

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