In vitro Comparative Tests About the Biocompatibility of Some Dental Alloys

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We evaluated the biocompatibility of four types of commercial alloys (two CoCr alloys and two NiCr alloys) used to make dental bridges. For the cell biology tests, a human osteosarcome type culture cell line MG63 (American Type Culture Collection) was used. Taking into account the results obtained, it can be said that the best results in terms of cell proliferation were observed for the Ni-Cr / Solibond N alloy closely followed by Co-Cr / Heraenium CE, then Co-Cr / Solibond C and Ni-Cr / Kera N, while cell viability tests revealed that the Co-Cr / Heraenium CE alloy exhibits the best biocompatibility, followed by Ni-Cr / Kera N, Co-Cr / Solibond C and Ni-Cr / Solibond N.

Keywords: biocompatibility, dental alloys, dental bridge

The metal-ceramic fixed prosthetic restorations which reestablish the continuity of the dental arches in the case of a reduced partial edentation can have both dental and implant support [1]. Among the requirements for acceptable dental material are included their quality and performances, their proper composition, the good mechanical properties, the corrosion resistance in the case of dental alloys and their biocompatibility [2,3]. A material is considered as biocompatible when it does not harm nor create toxic reactions or systemic side effects. For saliva and dental tissues media, which a dental restoration comes into contact it is of extremely importance the proper knowledge of the changes which can occur in time at the materials level [4]. Interactions of dental cast alloys with the oral tissues take place by different mechanisms leading to bacterial adherence promotion, toxic and subtoxic effects and allergy. Whereas bacterial adhesion promotion may be avoided by adequate oral hygiene measures, the other mechanisms may lead to clinically adverse local reactions due to the metal presence [5].

Experimental part

We evaluated the biocompatibility of four types of commercial alloys (two CoCr alloys and two NiCr alloys) (coded in table 1) used to make dental bridges.

Samples were obtained by casting in the form of discs with a thickness of 2 mm and a diameter of 14 mm. These were subsequently processed to obtain surfaces without asperities or oxides from the production process, and *in vitro* tests were performed according to ISO 10993-5. The tests were carried out at the Nicolae Simionescu Institute of Cell Biology and Pathology.

Table 1
CODING THE SAMPLES USED IN BIOCOMPATIBILITY TESTS

No.	Material / Trade name	Coding
1	Aliaj Co-Cr/Heraenium CE	CCH
2	Aliaj Co-Cr / Solibond C	CCS
3	Aliaj Ni-Cr / Kera N	NCK
4	Aliaj Ni-Cr / Solibond N	NCS

For the cell biology tests, a human osteosarcome type culture cell line MG63 (American Type Culture Collection) was used. Samples were sterilized for 70 h in ethanol 70% v/v, then rinsed / washed with sterilized distilled water. After this step the samples were immersed in the culture medium for 24 h. After being brought to the desired temperature, the cells were cultured for 6 days in Dulbecco Eagle's Modified (DMEM) medium with 1% glucose supplemented with 10% fetal bovine serum and antibiotics containing 100 U/L streptomycin and 100 U/L neomycin. Cell samples were maintained under sterile conditions (according to ISO 10993) in an incubator at 37°C in a 5% CO_2 environment and a high relative humidity (> 95%). The tests were carried out with a Ti_6Al_4V alloy as a control sample, and the values obtained on the investigated alloys were reported on the results on Ti_6Al_4V alloy (*p* values).

The cell colonization capacity on the surface of the experimental samples was monitored by fluorescence microscopy. The labeling protocol consisted in washing with isotonic phosphate buffer (PBS), then fixed in 2% formaldehyde and permeabilized with 0.2% Triton ™ X-100 in PBS for 5 minutes at 4°C. Subsequently, the experimental samples were washed with PBS for 10 min and labeled with Hoechst 33342 (0.2 μg / mL) for 15 min to be able to label the nuclei and labeled with fluorescein isothiocyanate-conjugated phaliodine solution (FITC) for the detection of these actin filaments being involved in cellular spread.

The treated specimens were examined with a Zeiss Axio Observer microscope (Zeiss, Germany) equipped with a suitable filter, and the images were made with an AxioCam MRc 5 camera (Zeiss, Germany) coupled to a microscope. Experimental samples were colonized with cells (cell density of 5 x 103 cells/cm^2). The development of cellular cytoskeleton and the establishment of cell adherence quality have been studied by immuno-fluorescent labeling of proteins (actin). Actin is an important structural protein that provides information about the ability of cells to adhere and spread. This protein also has an important role in cell signaling, proliferation and differentiation.

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Results and discussions

Figure 1 shows the fluorescence microscopy images of human osteosarcoma cell growth on the surface of the experimental samples after 3 and 6 days. According to the images from figure 1, it can be noticed that regardless of the type of material, the cellular activity increases with increasing time, indicating that the investigated materials support cell growth and attachment. It is also noted the presence of osteosarcomas that adhere to the material after 6 days with confluence appearance. At the same time, at 6 days it was observed that the cells showed a fuziform appearance, became denser, and it can be observed cell-cell contact indicating a cellular adhesion effect, demonstrating strong contact with the surface.

However, small differences between the investigated materials were observed. Cell proliferation images after 3 days indicate that better cell density can be observed for CCS samples compared to CCH, with more cells being detected on the surface. After 6 days, CCS and CCH samples show a good cell confluence, with cells covering the entire surface. For NiCr alloys, NCS and NCK samples show similar cell density after 3 days, with cells already showing direct contact between them indicating good adherence. And for these samples, cell density increases with increasing test time, and on 6 days the entire surface of the material is evenly coated with a cell layer. However, given the type of material, it can be observed that cell proliferation after 3 days indicates that NiCr alloys show better proliferation compared to CoCr alloys, and after 6 days all investigated materials show similar results from point of cell density.

In figure 2 the results on the quantification of deoxyribonucleic acid (DNA) are presented. Taking into account the results presented in figure 2 it can be seen that the CCH encoded samples show the best cell proliferation at 3 days followed by NCS, NCK and CCS. It can also be observed that cell proliferation is lower compared to that of 3 days, and in this case the best results were highlighted for NCS samples followed closely by CCH, CCS and NCK. In conclusion, the best in terms of cell proliferation, recording very close values are CCH and NCS samples.

Testing cytotoxicity or cell viability involves examining cell populations of a sample and staining cells to observe cellular behavior. This examination involves the use of several methods. When coloring with specific contrast products, at the microscope we can accurately evaluate cellular behavior. These chemicals are called reagents, which, in contact with the cell membrane, induce chemical reactions, making it possible to evaluate cellular behavior. Cell viability is the determination of the number of live or dead cells in a cell sample. Measurement of cell viability can be used to assess cell viability or lyses as well as rejection of an implanted material or transplanted organ.

In figure 3 are images acquired of the fluorescence microscope following cytotoxicity test on human osteosarcoma cells MG63. As can be seen in figure 3, the investigated materials are not toxic to human osteosarcoma cells MG63, and compared to the rest of the experimental samples, CCH samples showed moderate cellular distress. Although CCH samples slightly affect cell viability, the material is not toxic to osteosarcomas MG63.

The activity of alkaline phosphatase (ALP) was examined at 3 and 6 days after cell culture of the investigated materials. As can be seen in figure 4, ALP activity increases with increasing test time and at 6 days it can be seen that this is approximately twice as high as 3 days for all investigated materials. The best ALP activity was identified for CCH samples followed by NCK, CCS and NCS.

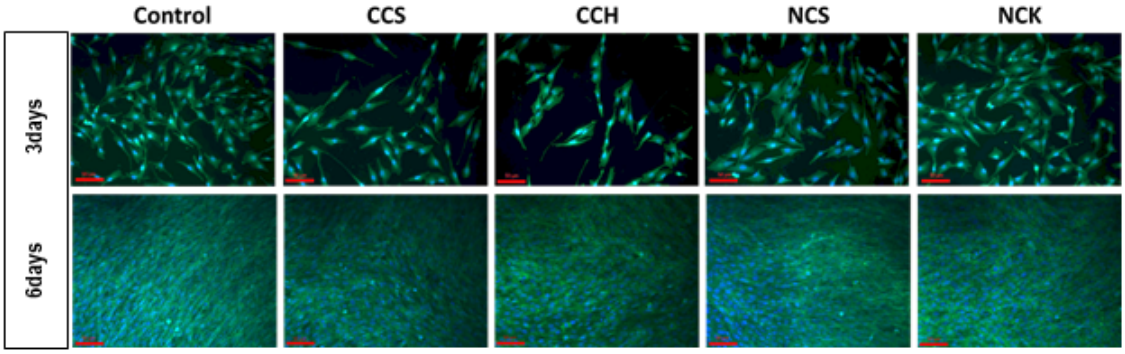

Fig 1. Proliferation of human osteosarcoma cells MG63 grown for 3 and 6 days on metallic alloys [cytoschelet actina - green coloration, nuclei (Hoechst contrast substance) - blue coloration]

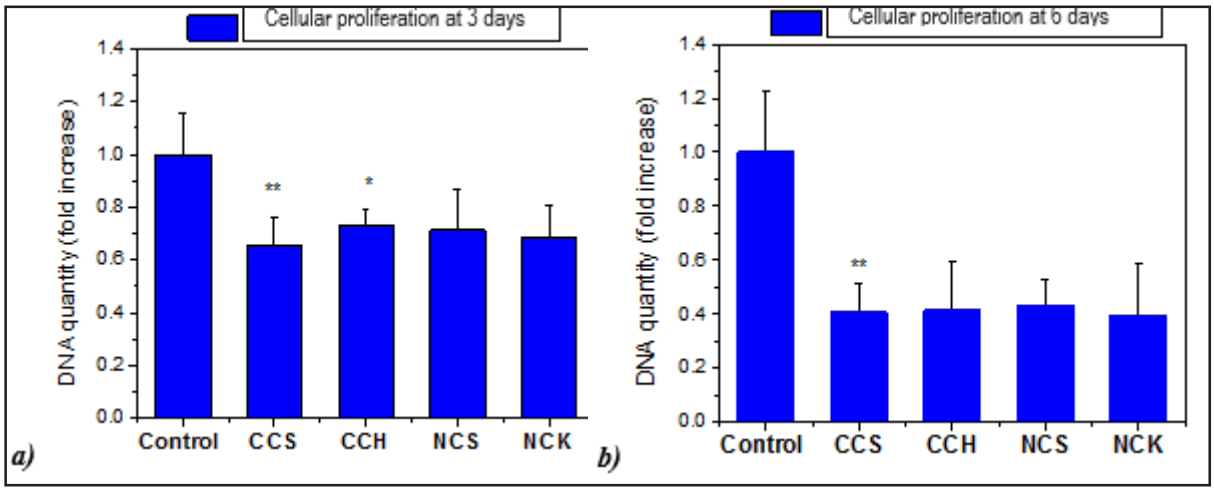

Fig 2. Quantification of DNA following cell proliferation assays for (a) 3 days and (b) 6 days
(* $p \leq 0.05$; ** $p \leq 0.01$)

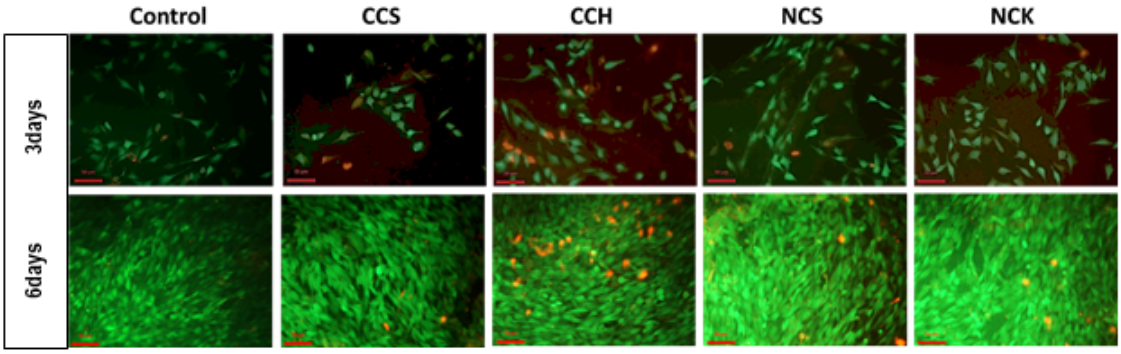

Fig 3. Fluorescence microscopy images obtained from cytotoxicity tests of human osteosarcoma cells MG63 (dead cells - colored in red, viable cells - colored in green)

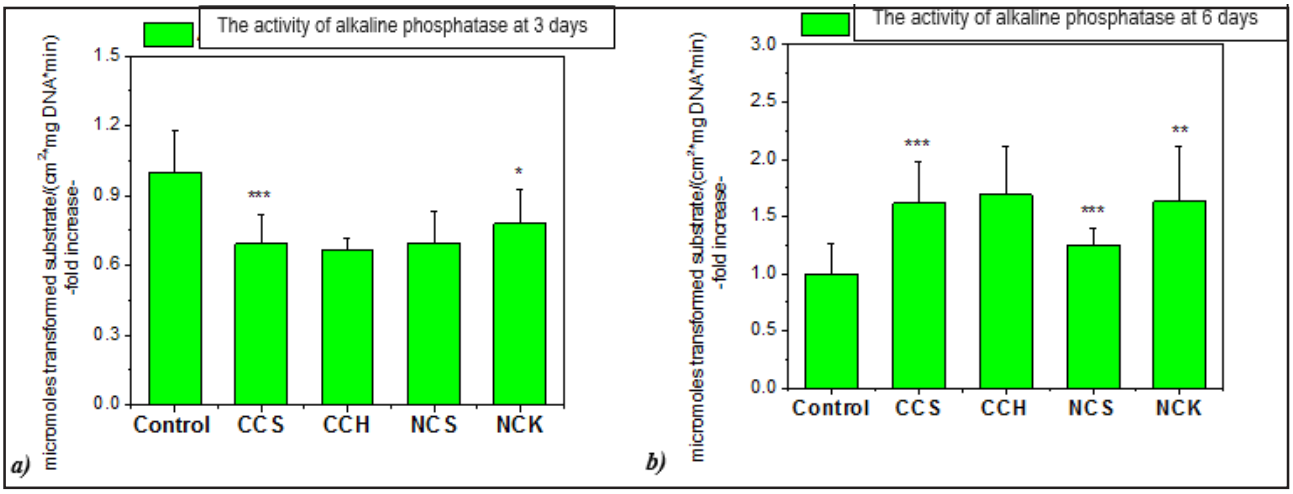

Fig 4. The activity of alkaline phosphatase at (a) 3 and (b) 6 days
(* p≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001)

Multiple studies present the benefits of biomaterial properties, as well as the need to reduce their use failures and the need to optimize their biomechanical performance [6].

Titanium alloys are actually preferred in bioapplications in orthopedics and dental surgery, because of the necessity to enhance mechanical properties [7].

Thus, Ti_6Al_7Nb is increasingly interesting for bioapplications, and various procedures of activation and bioactivation have been developed in the last decade in order to enhance its stability and biocompatibility [8].

Interactions between oral microbes and dental materials may occur [9]. Dental casting alloys are widely used in applications that place them into contact with oral tissues for many years and the practitioners must choose among hundreds of alloy compositions, often without regard to biologic properties [10-12].

In the article realised by Lucchetti et al [13], metal ions released into the oral cavity from dental prosthesis alloys may damage the cellular metabolism or proliferation and cause hypersensitivity or allergies.

Nickel–chromium (NiCr) alloys used in fixed prosthodontics have been associated with type IV Ni-induced hypersensitivity [14], and available data revealed that substances are released from alloys into the surrounding tissues; mainly nickel, zinc, and copper. Some alloys such as nickel–chromium alloy have shown to be cytotoxic *in vitro* [15].

After the studies of Oilo et al [16], fabrication method affects the design, stiffness, microhardness, and microstructure of metalic frameworks.

Conclusions

Taking into account the results obtained, it can be said that the best results in terms of cell proliferation were observed for the NCS alloy closely followed by CCH, then CCS and NCK, while cell viability tests revealed that the CCH alloy exhibits the best biocompatibility, followed by NCK, CCS and NCS.

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