Comparative Assessment of Biocompatibility of NiCr and CoCr Alloys Used in Metal-fused-to-ceramic Technology

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A material is considered as biocompatible when it does not harm nor create toxic reactions or systemic side effects. Most of reactions caused by dental materials are of allergic type, with symptoms pointed out at the oral mucosa level or cutaneous tissue level. 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. The present study assays the biocompatibility of six samples of dental alloys used in metal-fused-to-ceramic technology, three based on NiCr and three based on CoCr. All biocompatibility assays were performed on an osteoblast-like cell line, phenotypically close to osteoblasts (MNNG-Human Osteosarcoma cell line - HOS).

Keywords: NiCr and CoCr alloys, metal-fused-to-ceramic restoration, biomaterial, biocompatibility

The term of biomaterial [1] was for the first time used in a conference organized by Clemson University, USA, when it was defined as „an inert substance from the systemic and pharmaceutical point of view, intended for implants or embedded in living systems."

Another definition of the biomaterial term [2] was given by Black: „a non-living material used in a medical device, in order to interact with biologic systems".

Bruck [3] defines a biomaterial as „a synthetic material or of natural origin, in contact with tissues, blood and biologic fluids, used for reconstruction, diagnosis and therapy, without generating side effects which might affect living bodies or their components".

Williams [4] defines it as follows: „a biomaterial is a substance, another one than medications or a combination of synthetic or natural substances used in different time periods, in order to treat or replace a tissue, organ or human function".

Biocompatibility is the property of a material of being compatible with living bodies.[5,6] Taking into account that biocompatibility represents a complex process, when assessing it we should consider the phenomena produced during the biomaterial interaction with the body [7].

Biocompatibility [8] is a complex concept which takes into consideration all processes which took place between the biomaterial and the living body. By biocompatibility we understand the property of a material to be compatible with living bodies, thus, to be accepted entirely by the body without generating side effects and without being chemically and mechanically deteriorated.

Biomaterials must fulfill the following conditions:

- to be biocompatible, bioinert or biotolerant, that is to carry out their function without producing a toxic effect on living tissues and, at the same time, to produce an adequate response of the body.

- to be stable biochemically – not to suffer degradation processes.

- to have mechanic properties similar to the substituted tissue, in order to take over, in optimal conditions, its mechanical function.

Tests for biocompatibility assessment [9] can be classified into two groups:

-initial assessment tests, represented by tests for sensitivity, irritant potential, cytotoxicity, intra-cutaneous reactivity, genotoxicity, hemo-compatibility, systemic toxicity, sub-chronic toxicity.

-complementary assessment tests, such as tests for chronic toxicity, carcinogenicity, biodegradation, toxicity on reproduction and development.

Table 1 synthesizes interaction mechanisms between a biomaterial and biological environment [9].

Metal alloys based on nickel, chromium and cobalt are widely used in dentistry field [11]. Cobalt-based alloys have a good wear resistance, oxidation resistance, corrosion resistance and are less complex than the nickel-based alloys [10, 11].

Replacement solution of noble alloys with other cheaper led to appearance of the dental alloys from CoCr system [12-15] and NiCr system.

The present study purpose was to assay and compare biocompatibility of six alloys used in metal-fused-to-ceramic technology, produced by worldwide well-known manufacturers.

Experimental part

Among 6 types of alloys, three were based on NiCr (codified N1, N2 and N3) and three were based on CoCr (codified C1, C2 and C3) [16]. Samples were processed as disks of 1mm height and 1 cm diameter. This processing was necessary for in vivo biocompatibility tests, by direct

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Influence of the biomaterial on the body		*Influence of the body on the biomaterial*	
Local effects	**Systemic effects**	**Physico-mechanical effects**	**Biological effects**
1) Interaction with blood: proteins absorption, coagulation, fibrinolysis, proteins and leukocytes adherence, hemolysis	1. Embolisms	1. Abrasive attrition	1.Absorption of substances into tissues
2) Toxicity	2. Hypersensitivity	2.Friction	2.Enzyme degradation
3) Changes of normal healing (foreign body reaction)	3.Releasing metal particles into blood	3.Corrosion	3. Calification
4. Infections	4.Transport of lymphatic particles	4. Corrosion under Tension	
5. Occurrence of tumors		5. Degradation	

Table 1
MAIN MECHANISMS OF BIOMATERIAL _ BIOLOGICAL ENVIRONMENT INTERACTION [10}

contact with human osteosarcoma cells, according to ISO 10993-5. In order to assay cell adherence and proliferation to the metallic sample surfaces, it was necessary to manufacture the samples of the above mentioned dimensions.

Prior sample incubation with cells, the assessed samples were sterilized by ultra-sonication in 70% ethylic alcohol for 5 min, followed by incubation for 24 h in ethanol 70%, phosphate buffer saline solution (PBS) washing and another 24 h in culture medium with no fetal calf serum (FCS). The samples prepared with cells were maintained in sterile conditions (according to ISO 10993) in humid (>95%) 5% CO_2 atmosphere in an incubator at 37°C.

Samples were placed in a 24 well sterile plate (Corning) in about 2 mL complete culture medium. Prior to medium addition, around each piece agarose solution was poured and let harden in order to allow for selective cell growth only in contact with the metal material surface. Over this construction we applied 1 mL of complete culture medium with 2 x 10^5 cells.

Biocompatibility assays were performed on MNNG-Human Osteosarcoma cell line (HOS). The cells were purchased from CLS (Eppelheim, Germany). Immediately after shipment the cells were thawed, washed in a complete culture medium (MEM - Minimal Eagle Medium - supplemented by 10% heat-inactivated fetal calf serum - FCS, 2% L-glutamine and 1% penicillin streptomycin), centrifuged at 300g for 5 min and re-suspended in 10 mL of complete culture environment. Afterwards, they sub-cultivated in 80 mL sterile culture flasks; cell confluence was reached in about 48 h. Following this step, the cell culture medium was removed and cell layer washed by PBS and detached with trypsin-EDTA.

In each experiment 3 mL of trypsin per flask were added and while the flask was incubated for 2-3 min in the incubator, to 37°C under 5% CO_2 atmosphere. Following incubation, the cell detachment was evaluated by a phase contrast microscope. Trypsin inactivation was performed by adding 7 mL of complete culture medium. Following gentle mixing and transfer into 15 mL tubes, the extracted volume was centrifuged at 300g for 5 min.

The supernatant was removed and the resulted cell pellet was re-suspended in a volume of 1 mL of complete medium while cells were stained by Trypan blue and counted by a Countess (Invitrogen) system, thus obtaining the appropriate number of viable cells.

In the present study we have used:

- 24 well plates in order to perform proliferation and contact inhibition assays at the material surface.
- a fluorophore–1‰ tetracycline solution.
- TissueGnostics imaging system for whole surface sample imaging – on an inverted motorized stage microscope. .

The fluorescence filter used was the usual one for FITC. To assess the cell adherence on sample surface, samples were gently washed by 1 mL sterile PBS for three times, using a 100-1000 μL monochannel pipette.

Results and discussions

The imaging results in the following figures represent the overall aspect of the entire scanned surface for each material and detailed images (up to 1000 magnification) from different areas of each tested sample.

Microscopic images obtained by optical fluorescence technique reflect the general and detailed aspects for cell proliferation on each sample.

As can be seen from figures 1 and 2 the sample N1 presents a good biocompatibility. HOS cells proliferate well and adhere moderately to the working surface of the material, realizing a layer with a confluence of about 50%. On the sample borders, proliferation was more reduced in certain areas.

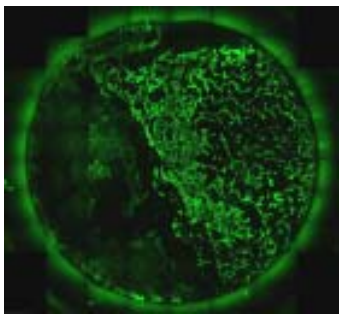

Fig. 1.Overall image of N1 material

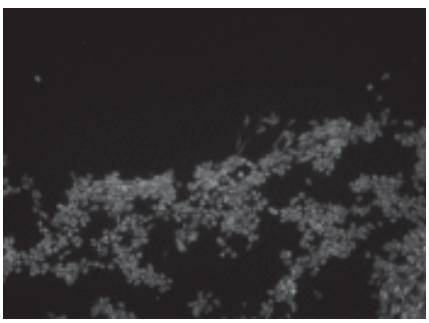

Fig. 2. Details. HOS proliferation on N1 material. Different areas from the sample center. Good proliferation, islanded adherence of about 50% (magnification 1000 x)

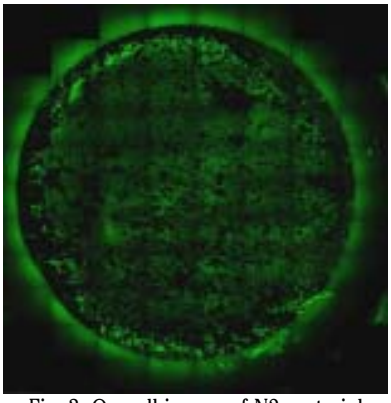

Fig. 3. Overall image of N2 material

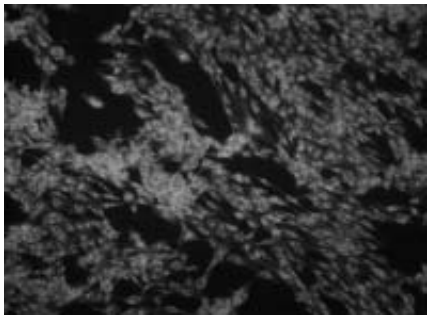

Fig. 4. Details. HOS proliferation on N2 material in the sample center (magnification 1000x).

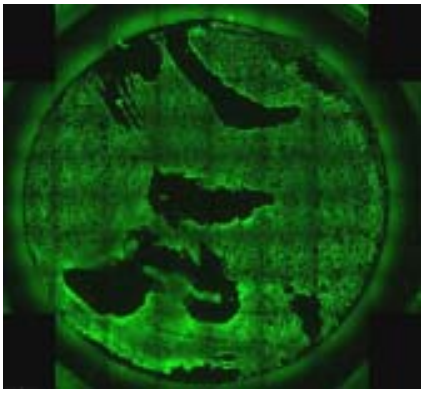

Fig. 5. Overall image of N3 material

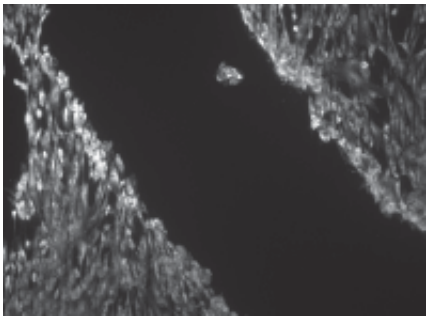

Fig. 6. Details. HOS proliferation on N3 material. Center of the sample. Absence of proliferation areas due to low adherence (magnification 1000x).

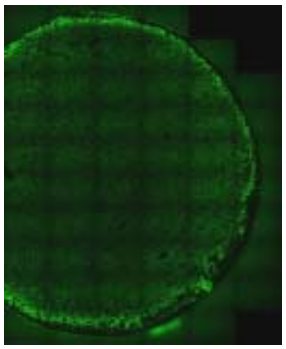

Fig. 7 .Overall image of C1 material

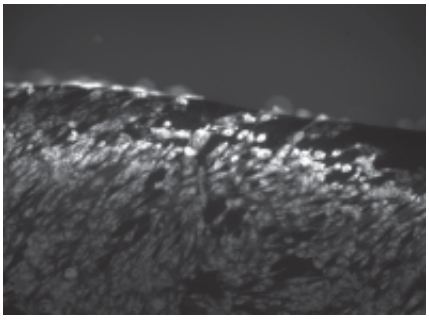

Fig. 8. Details. HOS proliferation on C1 material. Border of the sample. Excellent proliferation (magnification 1000x).

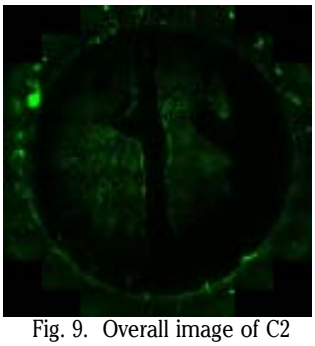

Fig. 9. Overall image of C2 material

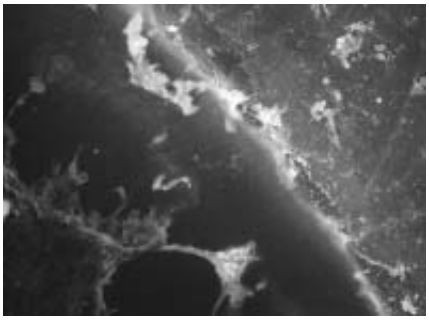

Fig. 10. Details. HOS proliferation on C2 material. Border of the sample. Moderate to reduced proliferation (magnification 1000x).

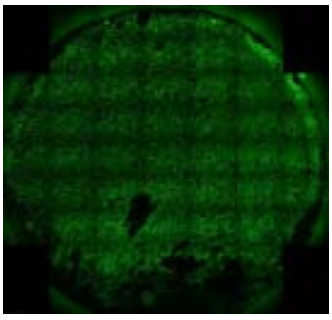

Fig. 11. Overall image of C3 material

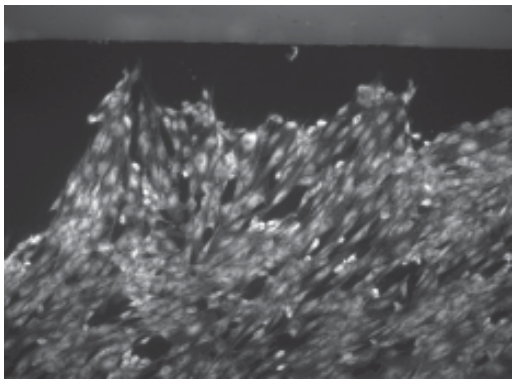

Fig. 12. Details. HOS proliferation on C3 material. Border of the sample. Good proliferation (magnification 1000x).

N2 presents a very good biocompatibility. HOS cells proliferate and adhere well to the working material surface, realizing a layer of confluence of about 80%. In the sample center, the confluence reaches 80%. However, we can notice small areas with proliferation absence even in the sample center.

N3 present good biocompatibility. HOS cells proliferate and adhere variably to the material working surface, realizing a confluence layer of about 65%. Defects are not the result of a manipulation problem, but of cell adherence issues. The areas of proliferation absence appear just on the sample center, while in other regions the proliferation is good.

C1 presents excellent biocompatibility. HOS cells proliferate and adhere uniformly to the working material surface, realizing a layer of confluence of 98%. On the sample borders, the proliferation was slightly reduced.

C2 presents good to moderate biocompatibility. HOS cells proliferate and adhere unevenly to the working material surface, realizing a layer with a confluence of 55%. Peripheral defects are not the result of a manipulation issues but of cell adherence. On all sample borders, proliferation was reduced, with large areas of cellular detachment in about 50% of the inoculated surface.

C3 presents an excellent biocompatibility. HOS cells proliferate and adhere evenly to the working material surface, realizing a layer of confluence of 90%. On the sample borders, proliferation was slightly reduced on the entire sample contour.

Conclusions

The present study shows a comparative study of biocompatibility of NiCr (codified N1, N2 and N3) and CoCr (codified C1, C2 and C3) alloys used in metal-fused-to-ceramic technology.

N1 presents relative adherence, uneven and is a consequence of material quality and surface processing, N2 proliferation is better than in N1.

The material N3 is biocompatible but cell adhesion is relatively reduced on the sample borders. Proliferation was slightly reduced on the sample contour.

The material C1 is excellent from biocompatibility point of view and with represented cell adhesion. C2 is good to moderate from biocompatibility point of view while cell adherence is poor. The material C3 is excellent from the biocompatibility point of view and with very good cellular adherence.

In terms of biocompatibility tests performed, we conclude that the biocompatibility of alloys tested is, in descending order: C1, C3, N2, N3, C2, N1.

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