In vitro analysis of the biocompatibility of metallic alloys for dental use

In vitro analýza biokompatibility kovových zliatin pre použitie v zubnom lekárstve

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ABSTRACT

The study was to evaluate the biocompatibility of metal alloys used in dentistry, as subjected to different corrosion attacks according to ISO 10271:2011 and ISO 22674:2016. We prepared cultures from pulp fibroblasts isolated from fresh dental pulp of third molars. Samples of 4 metal alloys, 1 cm2 diameter each, were brought in contact with pulp fibroblasts and analysed in intervals of 24 hours for 7 days to examine the cytotoxic effects by qualitative methods. Alloys A and C did not show any alteration of the qualitative parameters. Alloys D and E showed a slight zone of inhibition from Day 2. By Day 7, alloys A and C had a slight zone of inhibition, with no suffering at the cytoplasmic or cell membrane. Alloy D caused a significant decrease of cell colonies with degeneration, lysis and cytoplasmic granules. The concentrations of ions released after the attack of corrosion were primarily Co (alloy E 379 ng/cm2), Cr (alloy E 62ng/cm2), Fe (alloy A 551ng/cm2) and Si (alloy C 526 ng/cm2). The study offers significant insights for further research of innovation. It provides substantial information about this type of products in terms of quality and market impact .

Keywords: Biocompatibility, dental alloys, corrosion.

ABSTRAKT

Cieľom tejto analýzy bolo zhodnotiť biokompatibilitu kovových zliatin, používaných v zubnom lekárstve, pri vystavení rôznym korozívnym atakom podľa ISO 10271:2011 a ISO 22674:2014. Na analýzu sme si pripravili bunkové kultúry pozostávajúce z fibroblastov zubnej drene. Tieto fibroblasty boli izolované z čerstvej zubnej drene po extrakcii tretích molárov. Vzorky 4 kovových zliatin o priemere 1cm^2 boli vystavené kontaktu s fibroblastami zubnej drene a následne analyzované v časovom intervale 24 hodín po dobu 7 dní kvôli kvalitatívnemu posúdeniu cytotoxického vplyvu. Zliatiny A a C nespôsobili žiadnu zmenu kvalitatívnych parametrov. Zliatiny D a E vykazovali miernu zónu inhibície, bez poškodenia cytoplazmy a bunkovej membrány. Zliatina D spôsobila signifikantný pokles bunkových kolónií, lýzu a vznik cytoplazmatických granúl. Koncentrácie iónov uvoľnených po korozívnom ataku boli namerané nasledovne: Co (zliatina E 379 ng/cm^2), Cr (zliatina E 62 ng/cm^2), Fe (zliatina A 551 ng/cm^2) a Si (zliatina C 526 ng/cm^2). Táto štúdia prináša dôležité poznatky pre ďalší výskum a inovácie. V súčasnosti taktiež poskytuje informácie o tomto druhu produktov z hľadiska ich kvality a dopadu na trh.

Kľúčové slová: biokompatibilita, dentálne zliatiny, korózia

1. Introduction

The oral cavity has some peculiarities regarding the evaluation of the biocompatibility of metallic materials, such as bacterial contamination, temperature, cyclic loading which undergo the materials on stress, hardly comparable to those of other biological districts [4-5]. The presence of a strong cell turnover, a highly reactive cellular and humoral immune responses make it a less predictable tissue [6].

The biocompatibility of dental materials is a problem of great importance in dentistry. The substantial interest is linked to the fact that obtained results of the biocompatibility of these materials are sometimes contradictory [11]. Animal studies have shown that biomaterials may have different effects on the oral cavity. There are different factors, such as bacterial contamination, the presence of cellular and humoral immune responsiveness, making it difficult to control evaluation of the biocompatibility of these materials not only in experimental systems but also in the clinical field [15]. The materials which come into contact with tissues give rise to a complex series of reactions that can alter the interactions between various cellular components. These biological reactions, which evoke the release of chemotactic factors and/or growth factors, can cause a massive immediate and chronic inflammatory response. In both cases the appearance of neutrophils and macrophages in the area of tissue surrounding the implant was noted. It was proved that most of these phenomena are determined and guided by monocyte chemotactic factor (MCF) and by the macrophage inhibition factor (MIF) [19-21].

The aim of this study was to evaluate the biocompatibility of metal alloys for use in dentistry subjected to different corrosion attacks according to ISO 10271:2011 and ISO 22674:2016 by using in vitro tests and prepare culture medium specified for selected cell line, which consisted of fibroblasts isolated and pulled from fresh dental pulp of third molars extracted for orthodontic reasons.

2. Materials and Methods

Samples of 4 metal alloys for dental use, 1 cm^2 diameter each, were prepared according to ISO 10993-5 and ISO 10993-12. These samples had both surfaces flat (upper and lower) and were sterilized in an autoclave at 121°C for 20 minutes. Every dental alloy had a different corrosion attack according to ISO 10271:2011 and ISO 22674:2016.

A direct contact test, that allows qualitative assessment of cytotoxicity, was conducted. Pipette, with a known aliquot of the continuously stirred cell suspension, was placed into each of a sufficient number of vessels for direct exposure to the test sample.

Cells were distributed evenly over the surface of each vessel by rotation, the culture was incubated at (37±1) °C in CO_2 5 % until the cultures had grown to sub confluency.

The sub confluency and morphology of the cultures was verified with a microscope before the start of the test.

Petri dishes with cultures based on pulp fibroblasts have been prepared: the papilla of a germ of a lower third molar (extracted for orthodontic reasons) was isolated and the same papilla was afterwards collected in Petri dishes under sterile laminar flow hood. The dental pulp was shredded using a sterile scalpel into the chips of 3 – 5 mm. These chips were then transferred to a new plate with 5 ml of RPMI-1640 supplemented with 10% fetal bovine serum, sodium pyruvate 100X, 100X L-glutamine, amino acids 100X 200X non-essential and beta-mercaptoethanol previously conditioned at 37° C in a CO_2 atmosphere. After 24 hours from sowing, 1 – 2 ml of fresh medium were added, so that the cells formed a continuous but thin layer. This procedure was repeated 2 times during the week. The fibroblast cells were usually parallel to each other and the confluence in monolayer was reached after 3 weeks.

During the study multiple tests of corrosion attack on samples of alloys (considered by convention called ACDE) according to the protocol standardized in ISO 10271:2001 and 22674:2006 were performed.

Cultures were documented photographically, observed in time intervals of 24 hours for 7 days with the inverted optical microscope (Diaphot-TMD, Nikon. 10X objective) to evaluate the response to growth and cell morphology and to assess the cytotoxic effects by qualitative means.

The parameters considered were:

- Malformation, degeneration, suffering or lysis of cells on the surface of the entire monolayer.
- Reduction of the density of the cell layer, reduction of cell growth.
- Presence of intracytoplasmic granules, vacuolization.

Also, reactivity grades were evaluated:

0 None: No detectable zone around or under specimen.

1 Slight: Some malformed or degenerated cells under specimen.

2 Mild: Zone limited to area under specimen.

3 Moderate: Zone extending specimen size to 1 cm.

4 Severe: : Zone extending farther than 1 cm beyond specimen.

3. Results

Alloys A and C did not show any alteration of the first 3 parameters observed and taken into account up to 5th day of observation [Fig.1]. Alloys D and E showed a slight zone of inhibition from the 2nd day, no alterations in cell number or structure [Fig. 2-3]. To 7th day alloys A and C had a slight zone of inhibition, with no suffering at the cytoplasmic or cell membrane [Fig. 4]. The alloy E had a huge zone of inhibition with the cells distant from the sample without cytoplasmic degeneration [Fig. 5]. The alloy D caused a significant decrease in the concentration of cell colonies and the remainder showed degeneration, lysis and cytoplasmic granules [Fig. 6].

The concentrations of ions released after the corrosion attack were mainly Co (higher release: alloy E 379 ng/cm^2), Cr (higher release: alloy E 62ng/cm^2) , Fe (higher release: alloy A 551ng/cm^2) and Si (higher release: alloy C 526 ng/cm^2) and they are not relevant for the maximum concentrations that can be tolerated in the blood for food intake [Table 1-Table 2].

Tables and Schemes

Fig. 1: Alloy A: 5th day
Obr. 1: Zliatina A: 5. deň

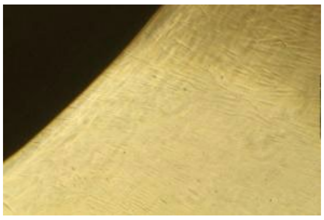

Fig. 2: Alloy D: 2nd day
Obr. 2: Zliatina A: 2. deň

Fig. 3: Alloy E: 2nd day
Obr. 3: Zliatina E: 2. deň

Fig. 4: Alloy A-C: 7th day
Obr. 4: Zliatina A-C: 7. deň

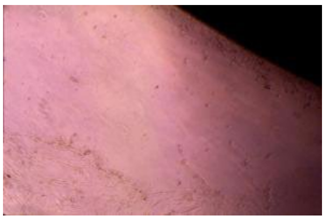

Fig. 5: Alloy E: 7th day
Obr. 5: Zliatina E: 7. deň

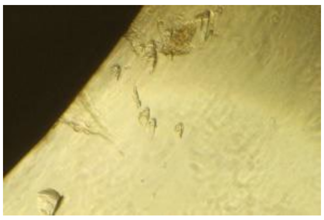

Fig. 6: Alloy D: 7th day
Obr. 6: Zliatina D: 7. deň

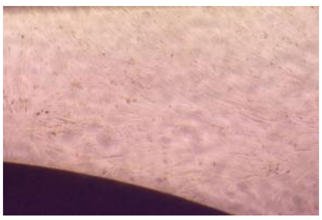

Fig. 7: Alloy C: 7th day
Obr. 7: Zliatina C: 7. deň

Tab. 1: Outcome in corrosion tests. Concentration of ionic release.
Tab. 1: Výsledok korozívnych testov. Koncentrácia uvoľňovania iónov.

	Release in 7 days						
	Co ng/ cm^2	Cr ng/ cm^2	Fe ng/ cm^2	Nb ng/ cm^2	Si ng/ cm^2	W ng/ cm^2	Ta ng/ cm^2
A	306	48	271	5	478	36	5
A1	242	52	551	5	480	5	5
C	276	51	570	5	526	5	5
C1	237	48	443	5	495	5	5
D	360	56	481	5	531	5	5
D1	343	52	487	5	447	5	5
E	379	62	660	5	509	5	5
E1	355	56	376	5	476	5	5

Tab. 2: Outcome in corrosion tests. Concentration in m/m %.
Tab. 2: Výsledok korozívnych testov. Koncentrácia v m/m %.

Sample	concentration m/m %		
	Co	Nb	Ta
A	29	1,1	1,1
C	28,5	2	1
D	28	2	2
E	30	0	0

4. Discussion

The search for reference values for the release of metal ions from dental prosthesis is for many years one of the main areas of research of the biocompatibility evaluation of market surveillance by the regulatory bodies. Research as a set up has remarkable suggestions of innovation for the evaluation of biocompatibility, considering the overlap of assessments of metal alloys for dental use both on free surface and under conditions of localized corrosion. The research allowed to refine some search capabilities that were already present in the academic environment, but which have greater application and development in a bustling area such as dental metallurgy and innovation of products offered on the market.

5. Conclusions

The research has important insights for further research of innovation and at present provides considerable information on such products in terms of quality and impact on the market. The research developed has enabled us to integrate, extend and deepen themes and knowledge in the evaluation of the biocompatibility of dental alloys, already considered in other previous research projects. The research has allowed us to extend and deepen knowledge and issues in the evaluation of the biocompatibility of dental alloys, and also allowed a better and more precise communication with the dental industry, encouraging active collaboration that can be easily repeatable and more productive for the future.

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