Toxicological Risks of the Cobalt–Chromium Alloys in Dentistry

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Cobalt–chromium (Co-Cr) alloys have been used for a long period of time in dentistry, but several risk factors remain involved. The toxicological risk of Co-Cr dental alloys is actually a sensitive subject with the European regulatory changes, namely regulation (EU) 2017/745 and annex VI to the CLP regulation (EC) 1972/2008. Studies assessing Co-Cr dental alloys' biocompatibility are urgently needed.

cobalt | cobalt–chromium alloys | dental | mucosa | oral | biocompatibility

1. Introduction

In dentistry, cobalt–chromium (Co-Cr) alloys have been used for a long period of time. While they mainly contain Co and Cr, other metals such as manganese (Mn), molybdenum (Mo), or nickel (Ni) are also present [1]. For patients who have lost teeth, these Co-Cr alloys are a common material for removable dental prostheses. These applications are designed to replace missing teeth with artificial teeth supported by a metal alloy [2]. Co-Cr is relatively cheap, features good strength and stiffness, and shows suitable longevity. All of these qualities make it a good material for dental prosthesis production. These alloys are also widely used for orthodontics with brackets, arch wires, and bands.

Co-Cr alloys are also known for their good cytocompatibility parameters (e.g., passivation layer) [3], but several risk factors remain involved. The primary concerns are a resulting increased sensitization, allergic reaction, and oral cavity inflammation. It is hence important to analyze the toxicological data of Co-Cr alloys, especially bearing in mind that the use of medical Co-Cr alloys has been questioned in recent years. In 2020, there was a radical change around the regulation of metallic Co, which in Europe has become a carcinogenic, mutagenic, or toxic-for-reproduction (CMR) substance [4]. It now belongs to category 1B of CMR substances (carcinogenic 1B, mutagenic 2, toxic for reproduction 1B), and its use is limited to a specific concentration of 0.1% in the final product. With a Co concentration lying between 35 and 65%, this has a direct impact on the application of Co-Cr dental alloys [4].

It has been shown that Co-Cr alloys induce low irritation and sensitization levels, ensuring low risks for allergic reactions [1]. Various pure or base metal alloys still have risks of causing allergic reactions in the oral cavity. They can also induce non-adequate immune responses. One of the most common side effects of using these prosthetics is the increased sensitization [5]. Biological responses to dental alloys are affected by the susceptibility of metals to corrosion and ionic release. Although usually metal ions might be washed away by saliva, the removable prostheses are used for a longer period of time with close contact to the oral cavity, decreasing the organism's

capacity to ensure its physiological functions. That is especially evident with an upper jaw prosthesis, where the palate might not come in contact with saliva. As a result, released metal ions will congregate under the metal frame, inducing local irritation and further oral cavity problems [2].

2. Cytotoxicity of Co-Cr Alloys Based on In Vitro Studies

Ganbold et al. [6] conducted an in vitro experiment in order to assess human adipose-derived stem cell (hADSC) behavior on a three-dimensional (3D)-printed Co-Cr alloys in comparison to a Ni-Cr alloy. Cell morphology was examined by a field emission scanning electron microscope, cell proliferation with a bromodeoxyuridine assay kit, and cell viability with a water-soluble tetrazolium salt assay kit. The Ni-Cr alloy was associated with significantly lower cell proliferation and viability in comparison to the Co-Cr ones. Proliferation for the Ni-Cr group presented an OD (optical density) value of 0.23. For all Co-Cr alloys, OD values were higher (0.38 for casting group, 0.33 for milling group, and 0.42 for 3D group). It revealed that Co-Cr alloys are more cytocompatible than Ni-Cr alloys. Comăneanu et al. [7] examined cytocompatibility of different Ni-Cr (N1, N2, N3) and Co-Cr (C1, C2, C3) alloys. While all materials exhibited moderate to high cytocompatibility, higher content of Co-Cr was associated with better cell adhesion. Cytocompatibility of alloys was summarized in the following descending order: C1 > C3 > N2 > N3 > C2 > N1.

Forster et al. [8] and Gălăţeanu et al. [9] observed that Co-Cr exhibited good cell proliferation. Forster et al. [8] studied attachment and proliferation rate of cultured human epithelial cells on polished lithium-disilicate, yttrium-modified zirconium dioxide, and Co-Cr alloys. Cell attachment (24 h) and proliferation (72 h) were investigated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and AlamarBlue® assays. All surfaces exhibited significant cell proliferation in comparison to the control group. Li-disilicate and zirconia exhibited the highest and lowest cell attachment, respectively. It revealed that all restorative materials, including Co-Cr, were equally suitable for subgingival restorations, but Li-disilicate had the best cytocompatibility. Although Co-Cr did not have the highest preferable parameters, it was still within the range. Regarding the study of Gălăţeanu et al. [9], they examined electrochemical behavior of two Co-Cr dental alloys (Wirobond 280 and Wirobond C also containing gallium and Mn). Their electrochemical aspects were examined in the artificial Erikson saliva at temperatures between 25 and 55 °C by potentiodynamic polarization and electrochemical impedance spectroscopy. Results showed that Wirobond 280 exhibited a greater cell viability and was associated with smaller levels of intracellular reactive oxygen species (ROS).

McGinley et al. [10] tested cytocompatibility and effect of Ni-Cr alloys on human-derived oral mucosa. Cytocompatibility was assessed by histological analysis for cell viability parameters, inflammatory cytokine expression, oxidative stress responses, and cellular toxicity. Co-Cr had significantly better cytocompatibility than the Ni-Cr alloy. Oral mucosal models treated by Ni-Cr alloy were associated with (i) significant reductions in cell viability and (ii) increases in oxidative stress, inflammatory cytokine expression, and cellular toxicity (in comparison to untreated oral mucosal models). With a continuous 72-hour observation, the higher Ni levels in the alloys generated the higher toxicity. In the following year, McGinley et al. [11] also provided more supportive evidence for Co-Cr better cytocompatibility. In this new study, each alloy was exposed to a 3D human-derived oral mucosal

model for 2–72 h. Immersion solutions of Ni-Cr base-metal alloy showed significantly lower cytocompatibility than Co-Cr alloys. In comparison to controls, Ni alloy was associated with significantly decreased cell viability, increased oxidative stress, inflammatory cytokine expression, and cellular toxicity levels. The Co-Cr alloy did not increase oxidative stress or cellular toxicity when compared to controls. These findings directly supported the good Co-Cr cytocompatibility as opposed to Ni-Cr.

Good Co-Cr cytocompatibility was also confirmed by Puskar et al. [12]. The aim of the study was to determine the cytotoxicity of the direct metal laser-sintered (DMLS) and cast Co-Cr-Mo dental alloy on human MRC-5 fibroblast cells. This in vitro study suggested that Co-Cr-Mo alloy did not have a cytotoxic effect and could be used for application in dentistry. Cytotoxic effect was not observed in either conventionally cast Co-Cr-Mo alloys. In another in vitro study conducted by Rusu et al. [13], the cytotoxicity of Ni-Cr and the Co-Cr alloys was studied on pure cell line dermal fibroblasts and of those obtained from skin biopsies. The corresponding results highlighted their non-cytotoxic effect. For example, after 7 days of inoculation, the cells did not detach from the plate and then grew well in contact with both alloys.

On the other hand, an in vitro analysis of the effects of Co-Cr alloys on human gingival fibroblasts (HGF) and osteoblasts by Kim et al. [14] provided evidence that Co-Cr alloys might exhibit cytotoxicity. Cytotoxic and inflammatory effects of Co-Cr alloys were investigated through the activation of NF-E2-related factor 2 (Nrf2)/antioxidant response element (ARE). The alloys were revealed to be cytotoxic to HGF and osteoblasts. It significantly increased ROS production, upregulated pro-inflammatory cytokines, and increased levels of inflammatory mediators (iNOS-derived nitrite oxide and COX-2-derived prostaglandin E2).

According to Imirzalioglu et al. [15], cytotoxicity of alloys was affected by recasting. The effect of repeated casting on gingival fibroblast cytotoxicity was analyzed by an in vitro study. Three disks were selected, namely high noble gold–platinum (Au-Pt, n = 60) alloy and two base metal alloys (Ni-Cr and Cr-Co, n = 20). Cytotoxic effects were examined on human gingival fibroblast with a MTT colorimetric assay. Recasting significantly increased ionic release in both Co-Cr and Ni-Cr alloys. Ni-Cr alloys were associated with higher cytotoxicity, especially after recasting Ni-Cr alloys. Other factors such as composition must therefore be considered.

In conclusion, in vitro studies showed that Co-Cr alloys have good cytocompatibility, which is highlighted by cell adhesion and proliferation as well as a non-cytotoxic effect. However, there are some exceptions. Alloy recasting may increase elemental release in Co-Cr alloys associated with higher cytotoxicity.

3. Cytotoxicity of Co-Cr Alloys Based on in Vivo Studies

Seldén et al. [16] measured the effect of cobalt chromium molybdenum (Co-Cr-Mo) exposure to lung disorders. The 37 participants were dental technicians with at least 5 y of exposure to dust from Co-Cr-Mo alloys. All participants agreed to undergo radiography to examine their lung condition. Aligning with previous studies that have shown toxic Co-Cr effects, Seldén et al. concluded that the dust from Co-Cr-Mo dental constructions can cause pneumoconiosis. Six participants in total exhibited radiological parameters associated with Co-Cr-Mo alloys.

Additionally, the authors found that subjects from an environment with local exhaust ventilation showed better results at the end of the study. In a follow-up study, Seldén et al. [17] investigated the effect of Co-Cr-Mo exposure to lung disorders for three patients with confirmed pneumoconiosis cases in dental technicians. Pneumoconiosis was associated with inorganic dusts resulting from the handling of Co-Cr-Mo dental alloys. Such results are important to analyze because it would reveal toxic Co-Cr effects. However, the primary causes for the reported cases cannot be exclusively reduced to alloys because patients already had lung problems. Moreover, the scope of these studies was not comprehensive enough to draw conclusions. One cannot dismiss the fact that the study is 25 years old, as the results are strongly influenced by the lack of local exhaust ventilation in laboratories. Nowadays, local exhaust ventilation is mandatory in all laboratories.

In a recent study, Yu et al. [18] examined the in vivo biocompatibility of four different crown materials, namely Co-Cr, Au-Pt, titanium (Ti), and zirconium (Zr). Twelve months after use (n = 196), probing depth (PD) and gingival crevicular fluid (GCF) volumes for all groups were significantly higher when compared to the control group. Zr- and Ti-based materials showed the best results. The Ti group had the highest concentration of osteoprotegerin (OPG), and Ti and Zr groups had smallest concentrations of receptor activator of nuclear factor kappa-B ligand (RANKL) and calcium ion as well as the smallest RANKL/OPG ratio. In conclusion, the Co-Cr biocompatibility was considered as poor.

Thus far, various studies have provided evidence that Ti is preferred over Co and Ni in terms of cytotoxicity, and the Ni exhibited the least-suitable parameters. Martín-Cameán et al. [19] compared the ionic release of aluminum (Al), copper (Cu), Cr, Mn, Ni, Ti, and vanadium (V) in oral mucosa cells from dental implants. The patients wore conventional orthodontic appliances (brackets, arch wires, and bands) and were additionally treated with mini-screws. A control group was added to the study. Few released traces of Co and V were observed. For other metals, the following order has been established: Cr < Ni < Ti < Cu < Al. Significant differences in comparison to the control group were observed in Ni release for orthodontic and orthodontic + mini-screw groups as well as in Cu release for the orthodontic group. However, mini-screws alone were not associated with a significant increase of metal release in all cases. These results suggested that Co, Cr, and Ni were released faster than Ti.

In a 5-year in vivo study, Baričević et al. [20] observed the genotoxicity of Co-Cr-Mo and Ni-Cr alloys when exposed to contact with oral cavity. Genotoxicity was examined using alkaline comet assay, and the cell viability was assessed with trypan blue exclusion test on 30 patients wearing prosthodontic appliances and 25 controls. Comet assay parameters (tail length and percentage DNA in the tail) were significantly higher in the group wearing prosthodontic appliances (both Co-Cr-Mo and Ni-Cr alloys) in comparison to the control group. The mean of tail length was 13.13 for the control group and 15.85 for the group wearing prosthodontic appliances, independently of the material used, while the percentage DNA in the tail was 0.36 for the control group and 2.07 for the experimental group. The results showed that metal ions released by both alloys could cause DNA damage of oral mucosa cells.

A later study by Katsoulis et al. [21] led to quite different conclusions. The research was specifically designed to assess the effects of using Ti in removable partial dentures (RPDs) of the Ti6A17Nb-alloy for 10 patients. RPDs were produced from Co-Cr alloy (Remanium GM 800+) and Ti6A17Nb alloy (Girotan L) for comparison purposes.

Patients completed a questionnaire entitled VAS (visual analogue scale) after 1, 3, and 6 months for each RPD. After 6 months, significant biological differences between both alloys were not observed, and patients did not report toxic effects. It means that the Ti6A17Nb-alloy for RPDs could be regarded as equivalent to RPDs in Co-Cr. Hence, it suggests that Co-Cr-alloy was successfully applied at least during 6 months after the installation of RPDs.

In vivo studies provide more controversial results. In some cases, Co-Cr alloys can release metal ions, leading to cytotoxic effects to human oral mucosa.

4. Sensitization and Irritation to Co-Cr Alloys

Könönen et al. [22] performed a 2-year clinical report aimed at investigating the effects of Ti-RPDs. It was previously reported that Co, Ni, and Cr caused hypersensitivity and in some cases gingivitis and stomatitis. Based on this evidence, it was presumed that Ti could be a good alternative for patients who showed an increased sensitization to Co-Cr alloys. In this study, one patient was first treated with Co-Cr framework for the mandible. The patient reported soreness, burning sensation of mucosa, dryness of the mouth, and redness. Patient treatment was hence rapidly replaced with a pure Ti framework. After a use of 1 week, the patient did not have complaints, and no complications occurred further during the next 2 years. The oral mucosa had no signs of irritation. It was concluded that Ti was preferable to Co-Cr alloys for RPD applications. It is important to note that this was a single-patient case report. It is therefore unlikely that this result can provide sufficiently comprehensive conclusions.

Another investigation conducted by Łukomska-Szymańska et al. [23] revealed that Co-Cr alloys did not have protective qualities for the oral cavity, which is important to consider for high-sensitive patients. They analyzed the effect of titanium nitride (TiN) coatings on Co-Cr alloy in framework dentures on human palatal epithelium cytology. The results were compared to two other groups, namely Co-Cr alloy in framework dentures without TiN coating and acrylic dentures. While each prosthesis disturbed palatal epithelium keratinization, Co-Cr alloys were associated with a significantly higher perturbation of keratinization compared to acrylic dentures.

Al-Imam et al. [2] argued that not all irritation and sensitization symptoms are caused by Co-Cr release. The authors examined Co release from 84 used (functional) and 32 new (non-functional) prostheses. During the 1–5-year follow-up on 66 patients, unpleasant symptoms were reported. Notable problems were some signs of inflammation of the oral mucosa (in total for 11 participants), oral candidiasis (2 participants), and ill-fitting prosthesis (16 participants). In addition, all 66 participants had insufficient oral hygiene. However, considering that contact allergy was not spotted, inflammation in 11 participants was related to candidiasis, poor oral hygiene, and ill-fitting prosthesis. Functional prostheses did not release Co, while it was released from 24 non-functional prostheses. This suggests that Co release was associated with manufacturing stage and disappeared within 1 to 5 y. The authors stated that dental prostheses might not be the primary factor for Co exposure leading to sensitization. The evidence that none of the functional prostheses released Co aligns with the hypothesis that it was present only during the fabrication stage for non-functional prosthesis.

In conclusion, sensitization and irritation to Co-Cr still remains an open research area. Though some findings do not provide evidence for such immune reactions, there are a few cases suggesting a correlation between Cr-Co and sensitization. The symptoms that might occur are soreness, burning sensation of mucosa, dryness of the mouth, and redness. In addition, Co-Cr alloys do not have protective qualities for the oral cavity, which might be one of the factors for the increased sensitization.

5. Type IV Hypersensitivity Reaction to Co-Cr Alloys

Allergies are a common symptom associated with cytotoxicity. Kettelarij et al. [24] investigated the amounts of Co, Cr, and Ni on the skin and in the urine of dental technicians (n = 13) and in the air of their workspaces. The metal dose on skin was investigated with acid wipe sampling and the air exposure by personal air sampling. Co, Cr, and Ni exposures were observed on skin and through the air after 2 h work. Urine samples were analyzed with inductively coupled plasma mass spectrometry. The Co dose on the skin increased significantly. Co was observed in 10 air samples (0.22–155 μg/m^3), Cr in 9 (0.43–71 μg/m^3), and Ni in 4 (0.48–3.7 μg/m^3). After evaluating the results, it was suggested that Co exposure can cause type IV hypersensitivity reactions, commonly called allergic contact dermatitis.

Contact dermatitis evidence was provided by Song et al. [25] after examining reactions to Co in cast dental crowns. A 58-year-old patient wearing crowns developed skin irritation on his hands and feet, which was reported to be a palmoplantar pustulosis-like allergy. Other observed symptoms included redness, pustules, vesicles, and scaly erythema on hands and feet, appearing 1 month after Co-Cr dental application use on molar teeth. The symptoms were persistent and lasted 1 year. Patch testing revealed strong reactions to Co chloride, suggesting that it can cause allergic reaction. Symptoms disappeared in 3 weeks after crown removal, which is in accordance with previous hypotheses.

Though it was concluded that Co exposure could be associated with allergic reactions, the data so far are inconsistent. For example, Al-Imam et al. [2] reached a different conclusion. Together with Co release from used functional and new non-functional prostheses, the authors investigated contact allergy to the alloys. Co release from prostheses was examined with the Co spot test and contact allergy by patch testing. In a 1–5-year study with 66 participants, none of them reported allergic reactions to Co. This study was conducted following previous findings that Co alloys were associated with elicit allergic reactions in Co-allergic patients. However, Al-Imam et al. [2] provided contrary results suggesting that Co prostheses are safe to use in dentistry.

In conclusion, there are no consistent data for Co-Cr-allergizing properties. Some findings clearly support Co-Cr biocompatibility without any allergic reactions though these are not unequivocal. The evident cases of allergic reactions cannot be overlooked. Data inconsistency may be a good reminder for dental practitioners to be cautious of potential allergies when prescribing Co-Cr applications.

6. Conclusions

According to various studies, Co-Cr alloys could rarely cause sensitization, irritation, and allergic reactions except for patients being allergic to Co or Cr. Among the reported side effects, soreness, burning sensation of mucosa, dryness of the mouth, and redness were the most common. In a few cases, contact dermatitis and palmoplantar pustulosis were directly associated with Co-Cr dental applications. The finding that Co-Cr alloys did not have protective qualities for the oral cavity could help to explain some patients' sensitization. As not all patients experienced any of these symptoms, further investigations are still to be performed to understand the causes for these symptoms.

A systematic review of described in vitro and in vivo studies leads to the conclusion that in a descending order, biocompatibility of metal alloys is as follows: Ti > Cr / Co > Ni. In comparison to Ni, Co-Cr alloys exhibit a lower cytotoxicity and a higher cell proliferation and viability. Ti alloys show the best biocompatibility when compared to Ni and Co-Cr alloys. The metal has good resistance to corrosion and displays the lowest risk of allergic reaction and negative immune system responses. This has to be considered knowing that, in general in the included studies, Cr-Co is compared to Ti-based materials in vivo and Ni alloys in vitro.

Thus far, the investigations on biocompatibility and cytotoxicity of Co-Cr alloys for human dental applications have provided inconsistent data. On one hand, one in vitro and one in vivo study reported a correlation to pneumoconiosis, certain cytotoxicity and inflammatory effects, increased ROS production, and levels of inflammatory mediators. On the other hand, most of the in vitro studies did not find cytotoxicity for Co-Cr alloys and rather reported good cell proliferation and adhesion. Considering that data inconsistency complicates an objective evaluation, further specific studies assessing Co-Cr dental alloys' biocompatibility are urgently needed within the current framework of the new European regulations dedicated to Co-Cr alloys [4]. It is therefore important to develop new experimental approaches in vitro to better guide the protocols used in vivo and thus reduce the difference in the observed results.

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