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Periodontal clinico-morphological changes in patients wearing old nickel–chromium and copper alloys bridges

LUMINIŢA DĂGUCI[1)], CONSTANTIN DĂGUCI[2)], CRISTIANA IULIA DUMITRESCU[3)], CĂTĂLINA FARCAŞIU[4)], DANIELA IOANA TĂRLUNGEANU[5)], MARILENA BĂTĂIOSU[6)], MAGDALENA NATALIA DINA[7)], CLAUDIU MĂRGĂRITESCU[8)], MIHAELA JANA ŢUCULINĂ[9)], OANA-CELLA ANDREI[5)]

[1)]*Department of Prosthodontics, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania*

[2)]*Department of Prevention of Oro-Dental Diseases, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania*

[3)]*Department of Pharmacology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*

[4)]*Department of Pedodontics, Faculty of Dentistry, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[5)]*Department of Removable Prosthodontics, Faculty of Dentistry, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[6)]*Department of Pedodontics, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania*

[7)]*Department of Dental Techniques, Faculty of Midwifery and Nursing, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*

[8)]*Department of Pathology, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania*

[9)]*Department of Endodontics, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Elderly population frequently presents more than one prosthetic restoration realized from different types of dental alloys which, in time, suffer various alterations in the oral environment. Metallic ions are released in saliva due to its electrolytic qualities, interacting with the contact tissues. Studies regarding cytotoxicity of dental alloys are providing contradictory results. Besides biocompatibility, the microbial factor is also greatly influencing the long-term success of the prosthetic rehabilitation. This study's aim was to assess the response of the gingival tissue to nickel–chromium (Ni–Cr) and copper (Cu)-based dental casting alloys from fixed dentures present in many patients from Romania. Gingival samples were taken from 124 patients wearing fixed dental restorations made from these two types of alloys from injured areas surrounding the abutment teeth; histological specimens were prepared, fixed in 10% neutral buffered formalin, paraffin-embedded and stained with Hematoxylin–Eosin (HE). Histological analysis showed the existence of a chronic inflammatory infiltrate in the gingival chorion, necrosis areas, and vascular congestion. Various morphological alterations appeared, depending on the intensity of the inflammation and the immune response. The surface epithelium suffered a hyperplasic reaction, either limited to acanthosis or involving the whole epithelium, the release of the Cu^{2+} and Ni^{2+} ions from the dental alloys used in bridges and crowns being responsible for inducing gingival hyperplasia and a chronic inflammation in the areas situated around the abutment teeth. The immunohistochemical study allowed us to observe an increased number of positive cluster of differentiation 3 (CD3) T-lymphocytes in periodontium, proving that the cellular immune response is rapid and intense.

Keywords: nickel–chromium, copper-based dental alloys, bridge, periodontal changes.

Introduction

Dental alloys are used since a long time in prosthetic restoration; research papers support or, on the contrary, disapprove their use. Still, studies showed that a lot of European patients successfully wear fixed prosthetic restorations, such as crowns or bridges that are entirely or partially made from these alloys [1, 2]. The use of dental alloys on a large scale is justified by their favorable characteristics, accessible price, and physical properties that are making them function in various types of prosthetic restorations [3]. Their most important characteristic regarding clinical applications is biocompatibility; the alloy must be inert, non-toxic, non-mutagenic, non-irritating and non-allergenic [4]. The biocompatibility of an alloy is not only given by its corrosive properties [5, 6], but it is also influenced by the erosion produced in contact with food, during mastication, and by the microbial activity [7]. As a result of all these factors interacting, the alloys release metallic ions that are inducing a long-term cytotoxicity. Data in literature regarding the toxic effects of dental alloys emphasize the risk of exposing the adjacent tissues of the prosthetic restorations to high levels of metallic ions. Previous studies reported that metallic prosthetic restorations can cause responses from the surrounding tissues, such as gingivitis or marginal periodontitis [8]. Other authors reported that the degree of these responses is significantly correlated with the metals in the composition of the dental alloy [9–11].

Studies shown that some metals, such as copper (Cu), nickel (Ni) and cobalt (Co), have an increased allergenic potential, but the real risk of using these materials is not fully known, therefore their conclusion was that the use of their respective alloys must be prudent [7]. Researches made for some elements that are present in the alloys' composition found them as being mutagenic, while others,

such as beryllium (Be) and cadmium (Cd), are known to have a carcinogenetic effect; still, no evidence-based studies to demonstrate any carcinogenetic effect of dental casting alloys have been found [12, 13], while their cytotoxicity is clinically observed in a polymorphic way, depending on the biocompatibility of the material used for the fixed prosthetic rehabilitation as much as on their design [14] and in terms of a correct relation that has to be established between the contours of the preparation and the periodontium [15].

Aim

In this study, our aim was to investigate the effects of biodegradation of the Ni–chromium (Cr) and Cu-based casting alloys used in old fixed prosthetic restorations with subgingival margins on the health of marginal adjacent periodontium.

Patients, Materials and Methods

We selected a number of 124 patients, males and females, aged between 55 and 70 years old, from urban and rural areas, that came to the Department of Prosthodontics of the Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania, between 2016 and 2019. All selected patients were wearing fixed prosthetic restorations that had metallic components made from alloys containing either Ni–Cr or Cu. All fixed prosthetic restorations were in service for more than 10 years and had subgingival margins. In order to improve the accuracy of the study, patients with diabetes, oral mucosa lesions, smokers and those who worked in a toxic environment were excluded from the study. A number of 92 patients remained in the study, 54 women and 38 men. From the total number of patients, 57 had fixed restorations made from Cu alloy, while 23 had fixed restorations made from Ni–Cr alloy and 12 had multiple fixed restorations, including both Ni–Cr and Cu alloy ones. All patients have signed the informed consent, both for the prosthetic treatment and for being included in the study. Their fixed prosthetic restorations were removed, samples were collected from the gingival areas around the abutments and prepared for the histological and immunohistochemical (IHC) analysis and a new treatment plan was made and fulfilled in a later stage. In this preliminary study we aimed to analyze the effect of biodegradation of the alloys in the oral environment and the release of the metallic ions, and the influence that these ions have on the gingival tissues and the periodontium through the mechanism of galvanic corrosion. Clinical examination was followed by the removal of the old crowns and bridges. At clinical examination, patients presented different degrees of gingival hyperplasia, congestion, and bleeding on probing in areas surrounding the abutment teeth, loss of attachment and periodontal pockets, and also gingival recessions.

We collected 4 mm gingival samples from areas surrounding the abutment teeth by punch biopsy technique, under local anesthesia. The samples were fixed in 10% neutral buffered formalin solution for 24 hours and prepared by paraffin embedding. A part of the serial sections obtained with the microtome were first stained with Hematoxylin–Eosin (HE) and examined by electron microscopy; the rest were immunohistochemically processed through immunoenzymatically procedure using soluble substances Labeled Streptavidin–Biotin detection method using Horseradish Peroxidase (LSAB/HRP) and DAKO Universal LSAB2 System/HRP Kit. As primary antibodies were used anti-cluster of differentiation (CD) 3 (clone F7.2.38, 1:25 dilution, Dako), anti-CD20 (clone L26, 1:50 dilution, Dako), and anti-CD68 (clone KP1, 1:100 dilution). Previously, the samples were antigenically unmasked using Tris–ethylenediaminetetraacetic acid (EDTA) buffer solution pH in the microwave for 20 minutes, then the endogenous peroxidase was blocked with 3% hydrogen peroxide and the non-specific binding sites with 8% bovine serum albumin (BSA) solution, respectively. The visualization was done with 3,3'-diaminobenzidine (DAB) and the counterstaining with Mayer's Hematoxylin. Negative external controls in which primary antibodies were removed were used in order to validate the reactions. To quantify the ratio of CD3+ T-lymphocytes/CD20+ B-lymphocytes, the two lymphocyte populations were individually assessed at the ×20 objective lens, about 100 cells in the sites with the most intense IHC signal.

Results

A total of 198 dental crowns and bridges were removed. Most of these, a number of 134 (67.67%), were made from alloys containing Cu (Figure 1, A–C); 64 were made from Ni–Cr alloys (32.33%) (Figure 2, A and B). After the removal of the old fixed prosthetic restorations, at the clinical examination patients presented different degrees of gingival hyperplasia, congestion and bleeding on probing, loss of attachment and periodontal pockets in areas surrounding the abutment teeth. On the biopsy fragments collected from patients with Cu alloys crowns and bridges, we noticed the presence of a slight acanthosis in the epithelium, the elongation and the thickening of the epithelial ridges, cytoplasmic vacuolization mainly in the keratinocytes within the superficial layers and a slight parakeratosis (Figure 3A). At the level of the gingival chorion, we noticed the presence of a chronic inflammatory reaction, especially at the top of the epithelial ridges and in the deep gingival chorion (Figure 3B). The inflammatory infiltrate had a diffuse character and the tendency towards the pseudofollicular aggregation was much less observed. From the cellular point of view, the lymphocytes and the plasma cells were predominant (Figure 3B). By far, the dominant cell population was that of CD3+ T-lymphocytes, that were present both in contact with the epithelium and especially in the pseudofollicular aggregation sites (Figure 4A). The next population is that of CD20+ B-lymphocytes (Figure 4B) that are rare in the diffuse infiltrate and more numerous in the pseudofollicular aggregates. To these, numerous plasma cells are added and very rare CD68+ macrophages (Figure 4C). The ratio between the two CD3+ T-lymphocyte/CD20+ B-lymphocyte populations was 1.5:1 at the level of pseudofollicular aggregates, and the ratio varied between 2:1 and 3:1 in the diffuse infiltrates.

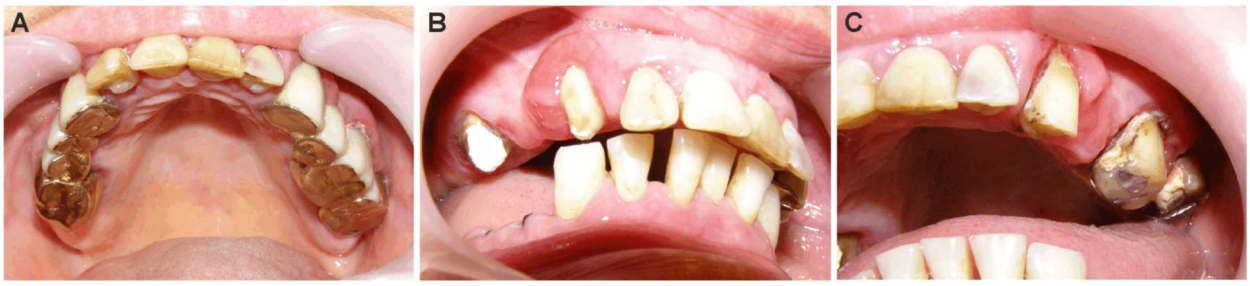

Figure 1 – *(A) Patient with bridge made from Cu-based alloy; (B and C) Clinical aspect of the bridges in situ and of gingival areas surrounding the abutment teeth after removal.*

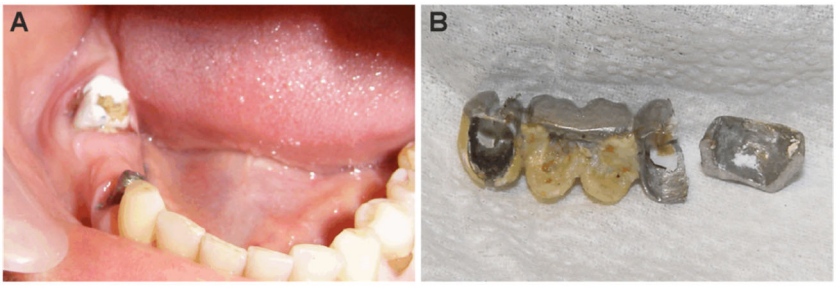

Figure 2 – *(A) Patient with bridge made from Ni–Cr alloy; (B) Clinical aspect of the gingival areas surrounding the abutment teeth and of the bridge after removal.*

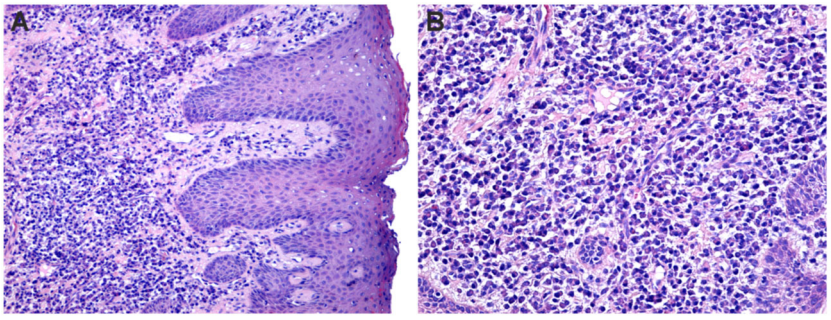

Figure 3 – *(A) Biopsy fragment from patient with Cu alloys bridge. The epithelium showed a slight acanthosis, elongation and thickening of the epithelial ridges, cytoplasmic vacuolization mainly in the keratinocytes in the superficial layers and a slight parakeratosis; (B) At the level of the gingival chorion, a chronic inflammatory reaction was obvious. HE staining: (A) ×100; (B) ×200.*

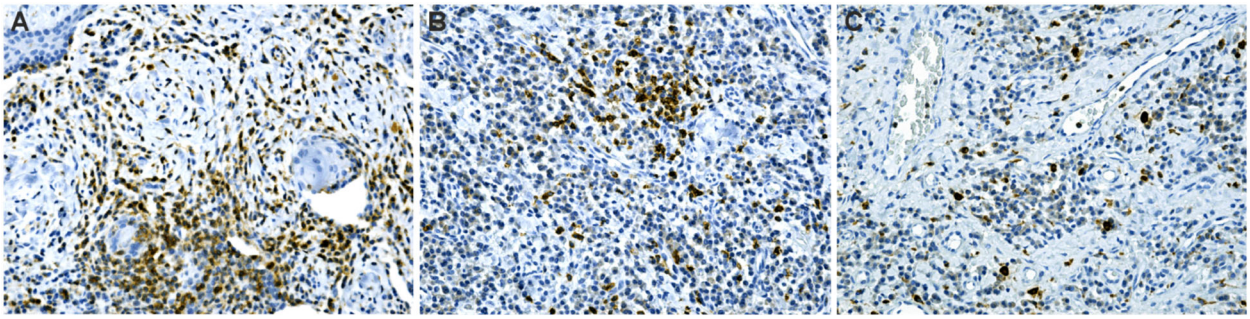

Figure 4 – *Biopsy fragment from patient with Cu alloys bridge (IHC staining – DAB, ×200): (A) CD3+ T-lymphocytes were the most frequently found in the inflammatory infiltrate; (B) CD20+ B-lymphocytes are much less represented compared to T-lymphocytes; (C) The rarest inflammatory cells that were present in these biopsies were CD68+ macrophages. CD: Cluster of differentiation; DAB: 3,3'-Diaminobenzidine; IHC: Immunohistochemical.*

In patients with Ni–Cr alloys bridges, the morphological changes of the epithelium at the level of the biopsies adjacent to the abutment teeth of these bridges were almost similar to those described in patients with Cu alloys bridges. In addition, the hyperplasia was more pronounced, presenting the elongation and the anastomosis of the epithelial ridges, with the formation of "epithelial bridges" and the formation of keratinocytes islands that appear isolated in the underlying chorion similar to morphological changes described in pseudoepitheliomatous hyperplasia (Figure 5). The inflammatory infiltrate from the gingival chorion was lower than in the case of patients with Cu alloys bridges, this being usually present in the papillae and in the deep chorion, being predominantly aggregate and perivascular (Figure 5). CD3+ T-lymphocytes were also predominant, being especially present immediately subepithelial (Figure 6A). CD20+ B-lymphocytes were much fewer, and they predominated in the pseudofollicular aggregates (Figure 6B). The ratio between the two lymphocyte populations varied between 2:1 and 3:1. CD68+ macrophages were also present, but rare and with diffuse distribution in the chorion and in the inflammatory infiltrates (Figure 6C).

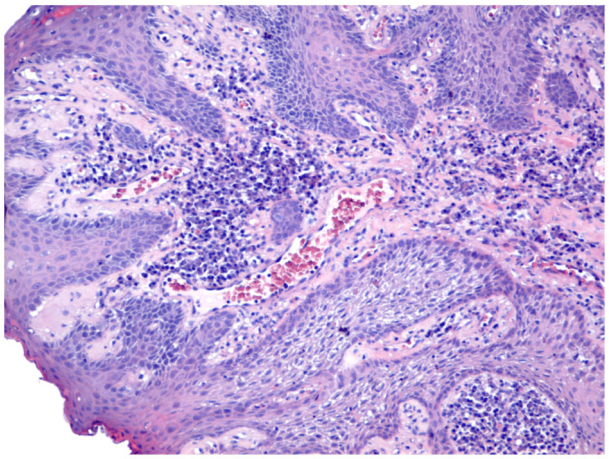

Figure 5 – ***Biopsy fragment from patient with Ni–Cr alloys bridge. Morphological changes similar to pseudo-epitheliomatous hyperplasia were observed. HE staining, ×40.***

Discussions

Periodontal disease is a major cause of edentulism, so the dentists must be able to correctly prevent, diagnose and treat it. One of the major risk factors is incorrectly placing the gingival contour of the fixed prosthetic denture and failing to preserve the periodontium [16]. A study made by Bergman *et al.* found that gingival inflammation is more pronounced in prosthetic devices whose crown margins are subgingival [17]. Other authors also found that periodontal alterations are more linked to the crowns with subgingivally located margins [18]. Silness evaluated the status of the marginal periodontium from the lingual surface of 385 fixed partial dentures and found that the most favorable is the supragingival position of the crown's margin, compared to the subgingival placement [19]. Also, Waerhaug mentioned that subgingival margins are retaining bacterial plaque and are not accessible to the scaling procedures [20].

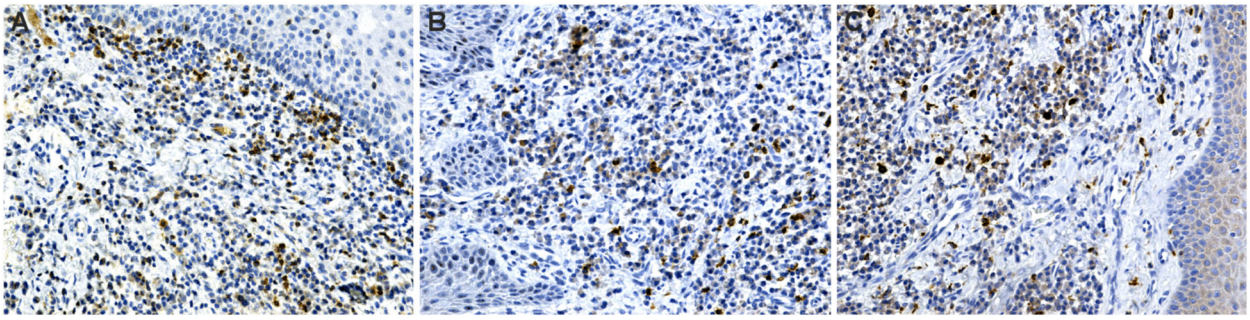

Figure 6 – ***Biopsy fragment from patient with Ni–Cr alloys bridge (IHC staining – DAB, ×200): (A) In the chorion, CD3+ T-lymphocytes were predominant, being especially present immediately subepithelially; (B) CD20+ B-lymphocytes were much fewer, and they predominated in the pseudofollicular aggregates; (C) CD68+ macrophages were rare and with diffuse distribution in the chorion and in the inflammatory infiltrates. CD: Cluster of differentiation; DAB: 3,3'-Diaminobenzidine; IHC: Immunohistochemical.***

Some of the studies in literature reported that incorrect designed fixed prosthetic dentures and poor oral hygiene in elder patients determine, from the histological point of view, papillomatosis and a rich infiltrate with T- and B-lymphocytes, at the level of lamina propria, which have a varied disposition depending on the stage of evolution of the periodontal disease [21]. In our study, the fixed restorations had subgingival margins and presented gingival hyperplasia, congestion and bleeding on probing, loss of attachment and periodontal pockets in areas surrounding the abutment teeth. Few cases presented gingival recessions.

Other authors also found chronic inflammatory symptoms in the periodontium in similar cases [22, 23]. The subgingival placing of the margins has a favorable effect on dental plaque accumulation, generating a destructive inflammatory response, clinically manifested by gingival congestion and hyperplasia, periodontal pockets, or gingival recessions [24]. Maintaining the integrity of the junctional epithelium during preparation or impression phases in clinical situations where a subgingival margin is necessary, represents a crucial factor. In this regard, some studies recommend that the subgingival crown margin should not exceed a depth of 0.5–1 mm, as it is impossible to clinically detect the position of the epithelial junction [25]. Another important factor is the quality of the adaptation, polishing and finishing of the margins, considered by other authors [26] as being more important even than the level of the margins. The danger of existent deficiencies in marginal adaptation, from which some are hard to avoid, is that they are so small that they cannot be easily detected, but are large enough for dental plaque retention; therefore, the most cautious attitude is avoiding the subgingival placement of the margins as frequent as possible, and when that is not possible, maintaining this at the lowest acceptable depth.

A review made by Hasturk *et al.* highlighted the importance of the macrophages in the inflammatory process of the chronic periodontal disease. Thus, the macrophages appear to play a key role as regulators directing inflammation to chronic pathological changes [27]. Other studies also pointed the fact that the role of the macrophages is paramount in chronic inflammation; they intervene in phagocytosis of pathogenic agents, cellular detritus and components of the extracellular matrix but also in development, tissue injury and regeneration [28, 29]. In our study, because the gingival chronic inflammation was present for a long time, the histopathological (HP) analysis showed the association with lymphocyte and macrophage infiltration. At the chorion level, the presence of the macrophages was scarce, their distribution being diffuse in the whole inflammatory infiltrate, respectively in the superficial chorion. Such distribution suggests their involvement in the repair and regeneration during the healing of marginal periodontium injuries. In this analysis, we did

not notice major morphological differences concerning the reactivity of the gingival mucosa to the two types of dental alloys that were investigated. Still, the surface epithelium suffered a hyperplasic reaction, either limited to acanthosis or generalized in all the epithelium, when it took the form of a pseudoepitheliomatous hyperplasia. At the chorion level, we noted the presence of an inflammatory chronic reaction, in which the majority of the cell population was represented by CD3+ T-lymphocytes, being in contact with the elongated epithelial crests or situated at the periphery of the perivascular pseudofollicular aggregates that are diffuse and disseminated in chorion. The ratio of CD3+ T-lymphocyte/CD20+ B-lymphocyte population was clearly in favor of CD3+ T-lymphocytes.

Studies in literature reported that Ni hypersensitivity can be an important issue, especially for patients that need prosthodontic or orthodontic treatment; it is contained in dental casting alloys, in brackets, bands and wires, in various concentrations, from approximately 8% in stainless steel, up to 50–70% in Ni–titanium (Ti) alloys; long-time exposure seems to increase the risk of sensitizing patients, since the initially negative patch tests converted in positive ones after one month of exposure [30–32]. The results obtained by other authors in their studies are contradictory; their conclusion is that the Ni^{2+} ions released by the dental appliances used in orthodontic treatments are in small amounts and do not pose a risk of hypersensitivity [31, 33, 34]. In other studies, evidence was found for the association of the Ni sensitivity and a history of contact allergy, and also for the possibility of a simultaneous response to Ni, Cr and Co that are frequently found in the same alloy; conclusions are also sustaining the possibility of developing mechanisms of oral tolerance that might play a role in modulating the cellular response to Ni [35, 36]. A study made by Rees [37] concluded that the burning sensation accused by patients with hypersensitivity to Ni, in the contact area of the gum with the metal of the prosthetic devices, may be the result of an allergic reaction.

Many studies showed that Ni is released from the dental alloys in the oral environment through corrosion and is accumulated in the adjacent tissues, especially in the gingival area, determining hyperplasia and inflammation through its presence and its aggregation at plaque sites [38–41]. Furthermore, the inflammatory reaction of the gingival and periodontal tissues seems to persist after the removal of the bacterial film in which the metal ions have been accumulated [39]; the same ions seem to be responsible not only for stomatitis, but also for triggering remote inflammatory effects, such as general or local contact dermatitis [42–46]. Some results showed that metallic ions released by corrosion from the dental casting alloys are affecting the gingival tissues in a greater degree if the prosthetic dentures are poorly finished [11], but other researchers found that all porcelain fused to metal Ni and a Co based tested alloys were biocompatible in either the as cast or polished condition, while the exceptions were tested casting alloys containing 50–60 wt. % Cu [47]. In a study made by Grivet *et al.*, it was demonstrated that the bacterial adhesion is higher at the level of prosthetic dentures made of alloys with high gold (Au) content, compared to non-precious alloy [48]. An *in vitro* study made by Jia *et al.* using Ni-containing arch wires immersed into a synthetic saliva showed that the amount of Ni^{2+} ions released from all tested arch wires was 700 times lower than the concentration required to elicit cytotoxic reactions in human peripheral blood mononuclear cells from patients with or without sensitivity to Ni [49]. At the same time, it seems that Ni-based dental alloys that have high concentrations of Co or Cr in their composition seem to corrode the most, being also the most potentially toxic [50]. McGinley *et al.* used a full-thickness human-derived oral mucosa model to test the biocompatibility of prosthetic dentures made of metal alloys and found that these alloys appear to affect cell morphology, their metabolic activity having a cytotoxic effect in the end, effects that are most likely due to changes caused by corrosion and release of these metals from the dental alloys [51].

Al-Hiyasat *et al.*, when testing the cytotoxicity of four different Ni–Cr alloys by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method found that alloys with high Cu content (12.3%) had a high cytotoxicity, while the other alloys had lower cytotoxicity as the Cu content was lower, and as the Cr or molybdenum (Mo) content was higher, respectively [52]. Other authors have investigated the biocompatibility of three types of Cu-containing alloys and found changes in the deoxyribonucleic acid (DNA) synthesis, even at low concentrations of Cu and zinc (Zn) [53]. In one of their studies, they demonstrated that Cu dental alloy corrosion products may alter the immune responses in the oral cavity [54]. Other authors have also shown the *in vitro* ability of some metal cations, such as Cu^{2+}, Zn^{2+} and Ni^{2+} to modulate the function of the human immune system [55]. Another study, made on heavy metals like lead (Pb), Ni and Zn, showed that they can stimulate T- and B-lymphocytes through various mechanisms [56].

The results of our HP and IHC investigations regarding the effects of the Cu-based dental casting alloys on the marginal periodontium come to reinforce the idea of the local immunomodulatory effect of released Cu^{2+} ions in the pathogenesis of the induced gingival hyperplasia and inflammatory lesions.

Conclusions

In our study, we did not notice major morphological differences in what concerns the reactivity of the oral gingival mucosa to the two types of dental casting alloys selected in our group of patients. The surface epithelium suffered a hyperplasic reaction, either limited to acanthosis or involving the whole epithelium; the release of the Cu^{2+} and Ni^{2+} ions from the dental alloys used in bridges and crowns being responsible for inducing gingival hyperplasia and a chronic inflammation in the areas situated around the abutment teeth, apparently through their local immuno-modulatory effect.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

Authors #1 (Luminița Dăguci) and #2 (Constantin Dăguci) have equal contributions to this paper.

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Corresponding authors

Marilena Bătăiosu, Associate Professor, DMD, PhD, Department of Pedodontics, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40721–517 492, e-mail: marilena.bataiosu@yahoo.com

Cristiana Iulia Dumitrescu, Lecturer, MD, PhD, Department of Pharmacology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone +40741–210 943, e-mail: dumitrescu.cristiana@gmail.com

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