ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Immunohistochemical aspects of apoptosis in gingival mucosa with papilloma and condyloma acuminata

MONICA SCRIECIU[1)], VERONICA MERCUŢ[1)], RĂZVAN MERCUŢ[2)], MARINA OLIMPIA AMĂRĂSCU[3)], SANDA MIHAELA POPESCU[4)], ANCA MIHAELA PREDESCU[5)], ILEANA MONICA BANIŢĂ[5)]

[1)]*Department of Prosthetic Dentistry, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Plastic Surgery, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Dental Technology, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Oral Rehabilitation, University of Medicine and Pharmacy of Craiova, Romania*
[5)]*Department of Histology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

The oral mucosa is a component of the oral ecosystem, which can be aggressed by corrosion products released from the dental alloys used in prosthetic dentistry therapy. The purpose of this study was to compare the *in vivo* effect of nickel and copper compounds on the oral mucosa cells, including their ability to induce cell death, by analyzing the cytochrome c (cyt. c) immunohistochemical expression. Gingival mucosa fragments obtained from the subjects with dentures manufactured by nickel or copper casting alloys were processed through the histological technique of paraffin inclusion. The sections obtained were stained by usually histological methods in order to highlight the histopathological lesions and also analyzed using the immunohistochemical technique in order to study the cyt. c expression. The papillomatosis lesions were observed in the gingival mucosa fragments obtained from the subjects with nickel-based alloy dentures and the condyloma acuminata lesions were observed in those obtained from the subjects with copper-based alloy dentures. The cyt. c immunohistochemical expression was different in the epithelial layer of two types of mucosal fragments but it was the same in their lamina propria connective tissue. We can conclude that the two types of metal alloys have different effects on the adjacent gingival mucosa.

Keywords: gingival mucosa, papilloma, condyloma acuminata, apoptosis, cytochrome c.

Introduction

The oral ecosystem contains an important heterogeneity of tissue types such as mucosa, the gums, the teeth and the tongue and also biological fluids such as the saliva and the crevicular fluid. Each of these represents ecological niches, which are available for colonization by oral micro-organisms [1]. In the oral ecosystem, there are rapid changes in temperature, changes in the composition and pH of saliva, variations of microbial saprophyte flora. From time to time, these changes occur in the same individual, but differ from one individual to another.

The metal dental alloys used in prosthetic dentistry therapy are placed in the oral cavity for a long period of time, for years or even permanently. A protective oxide film is formed on the surface of the metal dental prosthesis inserted into the mouth to stop its corrosion [2]. The oxide film can be altered when changes occur in the oral ecosystem [3]. The metal alloys may suffer various forms of corrosion [4, 5] releasing elements such as nickel, chromium or copper [2] and [6]. The biochemical, bio- or microbiological corrosion of metal alloys is due to the metabolic activity of microorganisms using the metal as the culture medium. Bio-corrosion usually occurs in metallic structures, in contact with backwaters or implanted in living organisms [7].

The corrosion of dental metal casting alloys placed into the oral cavity in the watery saliva is a continuous one. Therefore, the release of corrosion products is carried out for a long period [8].

The adverse effects of the oral mucosa upon the dental metal alloys are probably due to the release of metal ions by corrosion [9]. The toxic and carcinogenic effect of nickel compounds, including their oxidative mechanisms on human and animal cells has been well documented [10, 11]. Many studies have shown that copper oxide nanoparticles can induce cytotoxicity, DNA alterations and apoptotic events in human cell cultures [12, 13].

Apoptosis is an essential (last) phase in the life cycle, a process that removes unwanted cells. The dysregulation of apoptosis highlights many diseases and physiopathological alterations.

When the cells detect the apoptotic stimuli, such as DNA damage or metabolic stress, the intrinsic apoptotic pathway is triggered and cyt. c is released from the mitochondria into the cytosol where are engaged to a strategic combat to promote or to counteract the activation of caspases and cell death [14].

The purpose of this study was to compare *in vivo* effect of nickel and copper compounds on the oral mucosa cells, including their ability to induce cell death, by studying immunohistochemical expression of cyt. c.

Materials and Methods

Subjects

The participants in the study were selected from those who came in the Prosthetic Dental Clinic within the Faculty of Dentistry, University of Medicine and Pharmacy of

Craiova, Romania, between July 2013 and June 2014. They were informed about the study and signed an informed consent. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

The inclusion criteria: adult subjects, aged over 21 years, who had fixed dentures older than five years. The fixed dentures of the study participants were made of nickel-based metal alloys and copper-based metal alloys.

The exclusion criteria: there were excluded the smoking participants, those with allergic disorders, those with associated diseases of the oral mucosa, those with general diseases and those who worked in a toxic environment.

Nine participants of both sexes aged between 47 and 63 years were selected from rural and urban areas. The period of using fixed dentures in the studied subjects was between 7 and 15 years. Three-study participants worn nickel-based alloy fixed dentures and six subjects worn copper-based alloy fixed dentures. The selected subjects presented diseases at least one tooth of a fixed denture. From the subjects that required the extraction of one of the fixed denture teeth, the fragments of gingival mucosa were obtained through excision. The excision of gingival mucosa was performed to smooth the edges of the extraction wound and to prevent the extraction complications. The gingival mucosa was in contact with the metal of the fixed dentures before being removed.

Methods

The gingival mucosa fragments were fixed in 10% formalin and then it were processed through the histological technique of paraffin embedding.

For the histological examination, the obtained sections were stained with Hematoxylin–Eosin (HE) and examined by light microscopy.

Immunohistochemistry

Serial sections were dewaxed and incubated for 30 minutes with 0.3% hydrogen peroxide in methanol, in order to block the endogenous peroxidase. Blocking of non-specific binding was made with non-immune serum 1:75 diluted in phosphate-buffered saline (PBS) for 20 minutes at room temperature. Sections were incubated with the primary mouse monoclonal antibody anti-cytochrome c 1:50 (Santa Cruz Biotechnology Inc.), in a moist chamber for 12 hours at 4^0C.

After excess reagent removing, sections were further incubated with biotinylated secondary antibody (horse anti-mouse) diluted 1:200 for 30 minutes. After washing with PBS, further incubation was carried out with avidin–biotin–peroxidase complex (Vector Laboratory) 1:100 for 60 minutes. Peroxidase activity was revealed with 3,3'-diaminobenzidine (Sigma Chemical Co.). The sections were counterstained with Mayer's Hematoxylin and mounted in Eukit. The examination was performed with a Nikon microscope.

Results

The microscopic assessment showed polymorphic histological lesions of gingival mucosa, depending on the type of dental metal alloy used in the construction of dentures.

Papillomatosis lesions of gingival mucosa were observed in the subjects with nickel based alloy fixed dentures. The histopathological examination revealed a stratified squamous epithelium with acanthosis, increased connective papillae and pronounced parakeratosis (Figure 1).

In the upper third of the epithelium, there were frequent highlighted koilocytes. Thick collagen fibers and nodular inflammatory infiltrate were observed in the connective tissue of the lamina propria. We also notice the epithelium–lamina propria junction presented corrugations of the papillifera type. The condyloma acuminata of gingival mucoasa was observed in the subjects with copper-based alloy fixed dentures. In this situation, the histopathological examination showed a squamous epithelium with pronounced acanthosis and discrete parakeratosis. An area with epithelial cells and koilocytes was observed in the superficial lamina propria in one of the fragments with condyloma acuminata. An intense karyolysis and karyopyknosis phenomenon was noticed in these structures (Figure 2). The koilocytes cells, showing a perinuclear luminous area and a hyperchromic nucleus, were scattered throughout the entire thickness of the epithelium. The epithelium–lamina propria junction was infiltrated with diffusely scattered mononuclear inflammatory elements. In the superficial lamina propria, we also notice parallel collagen fibers and lymphocytic inflammatory infiltrate.

The cyt. c antigen immunohistochemical expression revealed a different reaction in the epithelium from gingival fragments with papillomatosis compared to the gingival samples with condyloma acuminata. The same reactivity was observed in the lamina propria of both types of mucosal fragments (Tables 1 and 2).

Table 1 – ***Cytochrome c immunohistochemical expression in gingival mucosa with papillomatosis***

No.	Histological localization	Antigen type	Highlighted cells	Results
1.	Gingival epithelium	Cytochrome c	Apoptotic cells	▪ absent reaction in the superficial and basal epithelial layers; ▪ positive reaction in the intermediate epithelial layer with increased intensity in the upper areas.
2.	Lamina propria	Cytochrome c	Apoptotic cells	▪ intensely positive reaction in the connective papillae and the upper lamina propria; ▪ inconstant positive reaction in the deep lamina propria.

Table 2 – ***Cytochrome c immunohistochemical expression in gingival mucosa with condyloma acuminata***

No.	Histological localization	Antigen type	Highlighted cells	Results
1.	Gingival epithelium	Cytochrome c	Apoptotic cells	▪ absent reaction in the superficial and basal epithelial layers; ▪ positive reaction in the intermediate epithelial layer with increased intensity in the upper areas.
2.	Lamina propria	Cytochrome c	Apoptotic cells	▪ intensely positive reaction in the connective papillae and the upper lamina propria; ▪ inconstant positive reaction in the deep lamina propria.

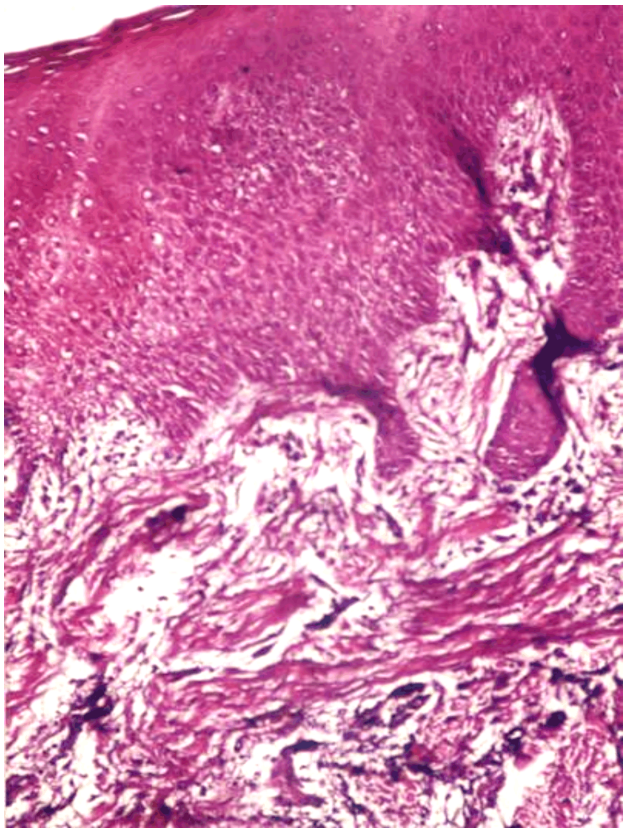

Figure 1 – *Gingival mucosa papillomatosis. HE staining, ×100.*

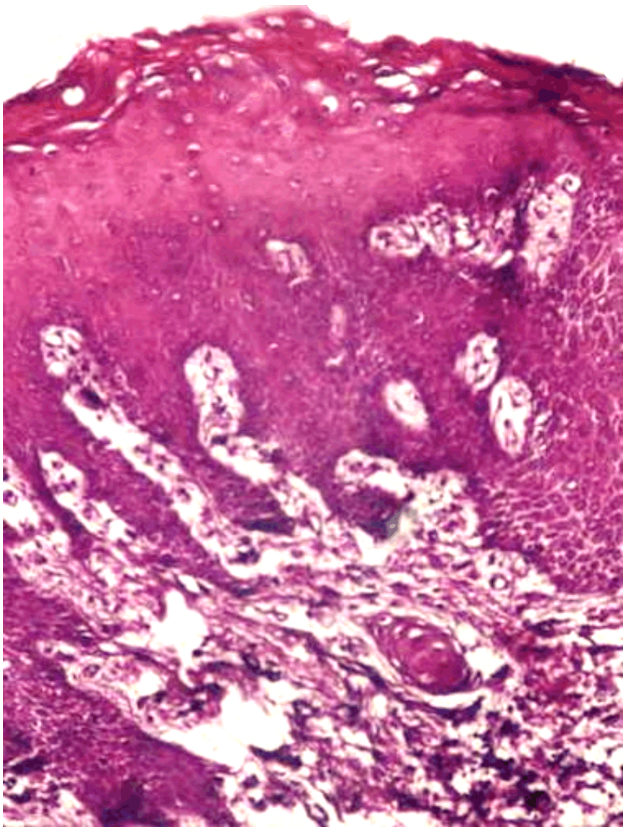

Figure 2 – *Gingival mucosa condyloma acuminata with koilocytes in an epithelial area located in the connective tissue. HE staining, ×100.*

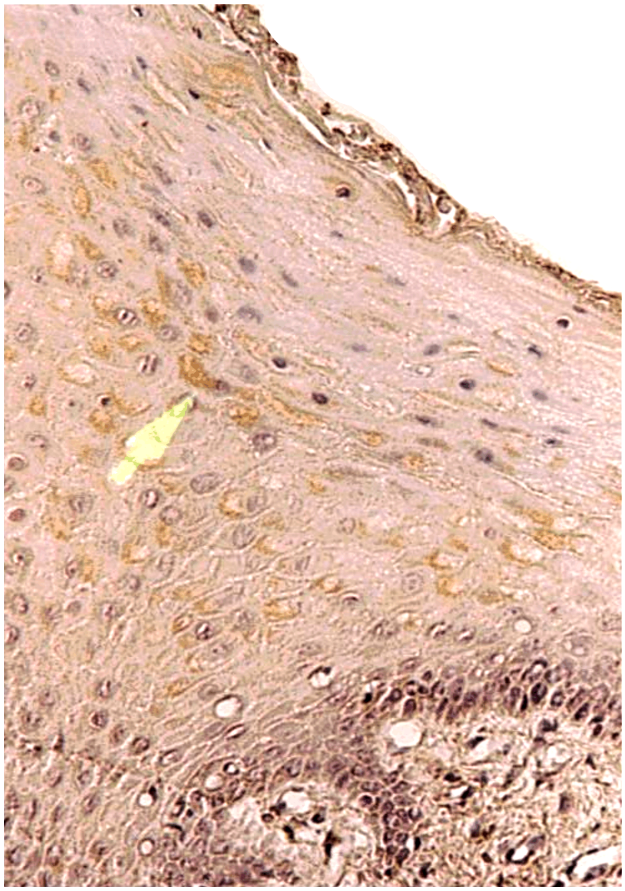

Figure 3 – *Papillomatosis gingival mucosa with apoptotic anucleate cells, ×200.*

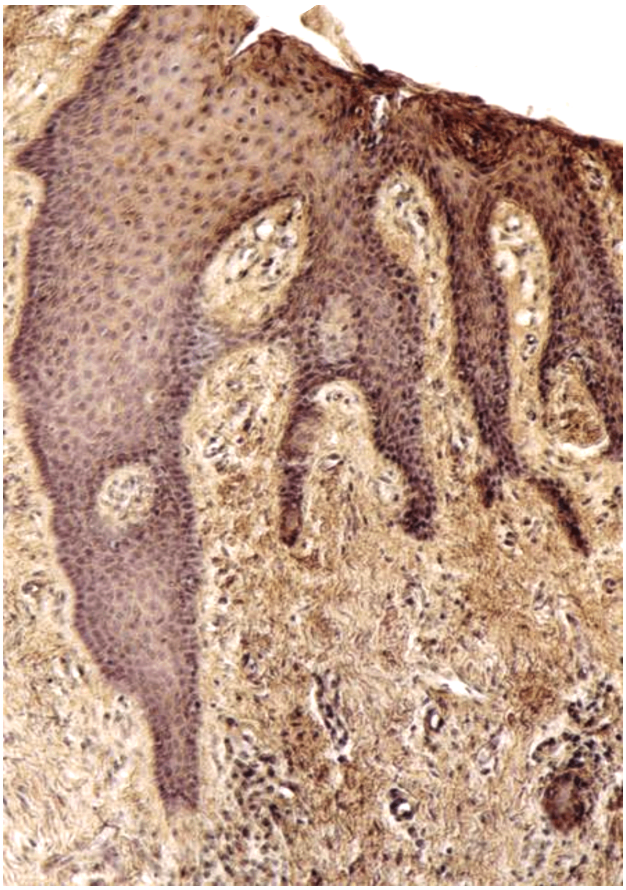

Figure 4 – *Papillomatosis gingival mucosa with positive apoptotic reaction in the connective tissue of papillae and in the superficial lamina propria, ×200.*

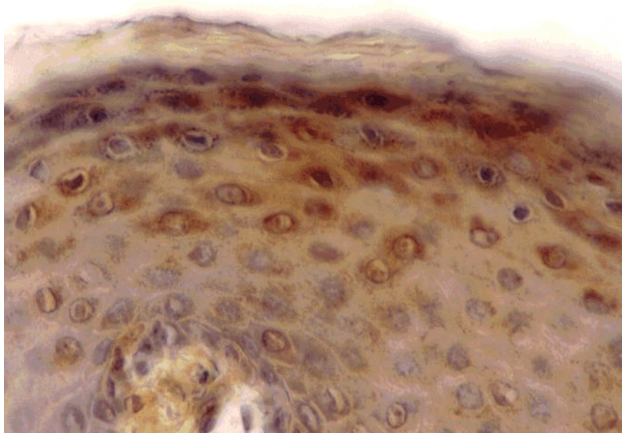

Figure 5 – ***Gingival epithelium with koilocytes and apoptotic cells in different phases of evolution, ×400.***

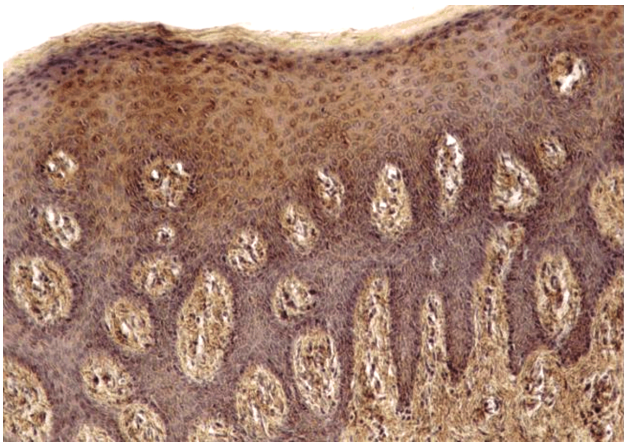

Figure 6 – ***Condyloma acuminata gingival mucosa. Gingival epithelium with acanthosis and intense apoptotic reaction, ×100.***

Anucleated cells with positive reaction for cyt. c and localized in the intermediary layer were found in the epithelium of papillomatosis gingival mucosa fragments.

The number of those cells was higher towards the top of the intermediary epithelium layer (Figure 3). The apoptotic intensely positive reaction was observed in the connective tissue of the papillae and in the superficial lamina propria. An unsteady apoptotic positive response and numerous angiogenesis phenomena were observed in the deep lamina propria (Figure 4).

Acanthosis and an intense positive reaction for cyt. c were observed in the upper intermediary epithelium of gingival mucosa fragments with condyloma acuminata. Apoptotic cells were distributed throughout the thickness of the epithelium (Figure 5). The apoptotic cells with different morphological aspects have been observed in the same epithelial layer: cells with cytoplasmic negative reaction for cyt. c, cells with peripheral chromatin condensation process, cells with degradation of the nuclear membrane and with apoptotic intracytoplasmatic corpuscles and cells with diffusely aspect. Regarding the shape, round, oval and fusiform apoptotic cells were observed. The cells with positive reaction for cyt. c, have had variable sizes, especially those of cells with fusiform shape. There were highlighted large round cells with cytoplasmic response for cyt. c and small round cells with intense reaction for cyt. c in the cytoplasm and nucleus. An intense response for cyt. c was observed in the connective tissue of the papillae (Figure 6).

Discussion

Exposure of the gingival mucosa to high concentrations of nickel or copper ions and corrosion products is a mechanism of aggression.

It has been demonstrated that nickel ions can penetrate the cells in a variety of ways [15, 16], to mix with certain biological components [10] in order to alter the function, morphology and ultrastructure of certain cells [17].

In contrast to the uptake of nickel by inhalation and ingestion, the dermis absorption is neglected because nickel ions do not penetrate the intact skin. Soluble nickel salts produced because of corrosion of nickel based-metal alloys may cause dermatitis, by percutaneous absorption [18]. Because both dentures and their adjacent gingival mucosa coexist in the oral cavity, the aggression elements of mucosa are most likely due to bio-corrosion compounds of dental metal alloys. Wataha *et al.* (2001) showed that the distribution of nickel in the tissues was better correlated with pronounced tissue inflammation [19]. Oral keratinocytes are the first tissue target of elements released from dental metal alloys [20]. Maintaining permanent fixed prosthesis in the oral cavity allows the accumulation of these compounds into the surrounding tissues. Therefore, all gingival mucosa structures can be altered. Released nickel ions from the dental metal alloys can have toxic, carcinogenic or immunological effects on the soft tissue of the mouth, due to the placement of these alloys in the oral cavity for a long period of time [21]. Numerous studies have investigated the *in vivo* biocompatibility of fixed orthodontic appliances, assessing the presence of metal ions in the cells of the oral mucosa, cytotoxicity and their potential genotoxic effects. One of these studies confirmed that nickel and cobalt released from fixed orthodontic appliances can induce DNA damage in the oral mucosa cells [22]. Other studies have shown that the corrosion products released from nickel-based alloys could not modify the morphology and viability of cultured human gingival fibroblasts. However, these products caused reductions of cell proliferation. The accelerated corrosion potential and local and systemic exposure of tissue to high levels of corrosion products raises the biocompatibility of these alloys [23]. According with results of this study, similar morphological alterations of the gingival paraprosthetic mucosa were mentioned in our previous studies [24].

Regarding the cytotoxic effect of copper on fibroblasts, Cao *et al.* showed that they become round and eventually detached from the surface of TCP due to the toxicity of copper [25]. Copper-based dental alloy or elements released from these are involved in some allergic reactions such as hypersensitivity reactions or in local chronic toxic reactions [26, 27]. These toxic effects may occur due to a repeated or continuous influence of toxic agents in low concentrations for long periods of time. These reactions are most often located in the area of contact with

the toxic agent [28].The occurrence of the corrosion phenomena of copper-based alloys induces an *in vivo* toxicity, and the metal surface becomes rough with pits and cracks [29].

Corrosion products of copper aluminum alloys are easily removed from the oral cavity through teeth brushing. Benatti *et al.* reported that copper-aluminum alloys suffered corrosion in sulfide solutions and in the oral cavity with poor oral hygiene [30]. Therefore, poor oral hygiene promotes bio-corrosion of copper alloys.

The comparative study of gingival mucosa fragments with papillomatosis and condyloma acuminata showed that the distribution of koilocytes was different in the epithelium layers. Țovaru [31] and Stăniceanu *et al.* [32] have noted that the last years, research increasingly emphasized the role of human papillomavirus (HPV) in the appearance of condyloma acuminata lesions. Țovaru [31] indicated that the types 6 and 11 of HPV are responsible for the appearance of oral condyloma acuminata.

Rihet *et al.* [33] showed that the HPV role in causing nuclear damage to the host cells has not been yet defined. The authors showed that HPV viral proteins can bind and inhibit the function of tumor suppressor genes such as p53 proteins. These phenomena can lead to disruption of the cell cycle and so, there results instability of the cellular genome of the host cells.

Apoptosis or programmed cell death is a normal physiological process, in order to maintain tissue homeostasis [34]. There are two types of apoptosis: extracellular apoptosis, determined by extracellular factors, and intracellular apoptosis due to the intracellular factors. Intracellular induced apoptosis is a reaction initiated by the cell in response to stress [35, 36]. Chronic irritation of the cells produces increased levels of various reactive oxygen species (ROS). ROS increases the expression of NADPH oxidase, considered to be a proapoptotic enzyme. Moreover, increased ROS causes the release of apoptotic intracellular signals and formation of apoptotic proteins targeting the mitochondria and the cyt. c releasing into the cytoplasm. Therefore, cyt. c leads to the activation of caspase-3, a protein that plays an important role in the execution of apoptosis and cancer incidence [37–39]. The mitochondrial membrane permeabilization (MMP) was proposed as a "point-of-no-return" during various types of cell death [40]. Both the outer mitochondrial membrane and the inner mitochondrial membrane contribute to MMP [41] and cause the release of cyt. c, apoptosis inducing factor (AIF) and G endonuclease, which are normally located in the mitochondrial intermembrane space [42]. Recent studies showed toxic effects of nickel oxide nanoparticles (NiO NPs) on bacteria and microalgae [43, 44]. NiO NPs induce intracellular production of ROS in dose-dependent manner [45]. Cyt. c increasing in the cell cytosol after nickel treatment was demonstrated by Western blot analysis of cytosolic and mitochondrial fractions. Simultaneously, it was observed a decrease of cyt. c in the mitochondrial fraction indicating a dose-dependent release of cyt. c [46]. Also, a linear increase in the apoptotic cell population was observed with increasing concentrations of copper ions in cultured fibroblasts [25].

In this study, a highly positive apoptotic reaction was observed in the superficial epithelial intermediary layer, in the connective tissue of papillae and in the superficial lamina propria, for both types of gingival mucosal fragments.

Anucleated cells with cytoplasmic positive reaction for cyt. c were observed in the epithelium gingival mucosa with papillomatosis. It is significant that in this case the apoptotic stage of mitochondrial membrane permeabilization is not based on the presence of the nucleus. These data suggest that the phases of decision on life/death cell are clearly different from those that determine cell cycle progression [47]. The process of apoptosis is asynchronous in studied gingival fragments with condyloma acuminata. This idea is supported by the fact that there were detected cells with a negative reaction for cyt. c, cells with positive reaction for cyt. c and high variability of sizes and shapes and various nuclear damage. The same aspects were pointed out in our previous study [48].

These observations lead to the idea that although the apoptosis process is triggered by the same mechanisms, in the same areas of the gingival mucosa, the speed of the apoptotic events is different. If in the papillomatosis of gingival mucosa, in the upper intermediate epithelial layer, the apoptotic cells do not present a nuclear structure, in the case of gingival mucosa fragments with condyloma acuminata there were observed koilocytes and apoptotic cells with various stages of nuclear degradation.

Conclusions

In the gingival mucosa adjacent to fixed dentures made of nickel or copper-based alloys there were observed papillomatosis or condyloma acuminate lesions. In the gingival mucosa fragments with papillomatosis, there was developed a nuclearly independent apoptotic process. In the gingival mucosa, fragments with condyloma acuminata there were highlighted apoptotic cells with variable morphological aspects and various sizes in different stages of evolution. In both types of gingival mucosa fragments, the apoptotic reaction was intensely positive in the connective tissue of the papillae and in the superficial areas of lamina propria.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All authors have equally contributed to this paper.

References

[1] Takahashi N. Microbial ecosystem in the oral cavity: Metabolic diversity in an ecological niche and its relationship with oral diseases. Interface Oral Health Science – Proceedings of the International Symposium for Interface Oral Health, Sendai, Japan, February 2–3, 2005. Int Congr Ser, 2005, 1284:103–112.

[2] Kedici SP, Aksüt AA, Kílíçarslan MA, Bayramoğlu G, Gökdemir K. Corrosion behaviour of dental metals and alloys in different media. J Oral Rehabil, 1998, 25(10):800–808.

[3] Rondelli G, Vicentini B. Evaluation by electrochemical tests of the passive film stability of equiatomic Ni-Ti alloy also in presence of stress-induced martensite. J Biomed Mater Res, 2000, 51(1):47–54.

[4] Reclaru L, Meyer JM. Zonal coulometric analysis of the corrosion resistance of dental alloys. J Dent, 1995, 23(5): 301–311.
[5] Canay S, Oktemer M. *In vitro* corrosion behaviour of 13 prosthodontic alloys. Quintessence Int, 1992, 23(4):279–287.
[6] Gil FJ, Sánchez LA, Espías A, Planell JA. *In vitro* corrosion behaviour and metallic ion release of different prosthodontic alloys. Int Dent J, 1999, 49(6):361–367.
[7] ***. Cercetări fundamentale şi aplicative privind realizarea bronzurilor CuNiAl destinate recondiţionării elicelor navale – ELNAV. CEEX 322/06.10.2006.
[8] Yu J, Zhao F, Wen X, Ding Q, Zhang L, Wang G. Apoptosis mechanism of gingival fibroblasts induced by nickel ion contained in dental cast alloys. Biomed Mater Eng, 2012, 22(1–3):151–157.
[9] Wylie CM, Davenport AJ, Cooper PR, Shelton RM. Oral keratinocyte responses to nickel-based dental casting alloys *in vitro*. J Biomater Appl, 2010, 25(3):251–267.
[10] Kasprzak KS, Sunderman FW Jr, Salnikow K. Nickel carcinogenesis. Mutat Res, 2003, 533(1–2):67–97.
[11] Kawanishi S, Oilkawa S, Inoue S, Nishino K. Distinct mechanisms of oxidative DNA damage induced by carcinogenic nickel subsulfide and nickel oxides. Environ Health Perspect, 2002, 110(Suppl 5):789–791.
[12] Ahamed M, Karns M, Goodson M, Rowe J, Hussain S, Schlager J, Hong Y. DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol, 2008, 233(3):404–410.
[13] Ahamed M, Siddiqui MA, Akhtar MJ, Ahmad I, Pant AB, Alhadlaq HA. Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. Biochem Biophys Res Commun, 2010, 396(2):578–583.
[14] Fernández-Checa JC, Garcia-Ruiz C. Apoptosis and mitochondria. In: Dufour JF, Clavien PA (eds). Signaling pathways in liver diseases. Springer-Verlag, Berlin–Heidelberg, 2010, 439–453.
[15] Knöpfel M, Schulthess G, Funk F, Hauser H. Characterization of an integral protein of the brush border membrane mediating the transport of divalent metal ions. Biophys J, 2000, 79(2): 874–884.
[16] Cangul H, Broday L, Salnikow K, Sutherland J, Peng W, Zhang Q, Poltaratsky V, Yee H, Zoroddu MA, Costa M. Molecular mechanisms of nickel carcinogenesis. Toxicol Lett, 2002, 127(1–3):69–75.
[17] Wataha JC. Biocompatibility of dental casting alloys: a review. J Prosthet Dent, 2000, 83(2):223–234.
[18] Denkhaus E, Salnikow K. Nickel essentiality, toxicity, and carcinogenicity. Crit Rev Oncol Hematol, 2002, 42(1):35–56.
[19] Wataha JC, O'Dell NL, Singh BB, Ghazi M, Whitford GM, Lockwood PE. Relating nickel-induced tissue inflammation to nickel release *in vivo*. J Biomed Mater Res, 2001, 58(5): 537–544.
[20] McGinley EL, Moran GP, Fleming GJ. Biocompatibility effects of indirect exposure of base-metal dental casting alloys to a human-derived three-dimensional oral mucosal model. J Dent, 2013, 41(11):1091–1100.
[21] Trombetta D, Mondello MR, Cimino F, Cristani M, Pergolizzi S, Saija A. Toxic effect of nickel in an *in vitro* model of human oral epithelium. Toxicol Lett, 2005, 159(3):219–225.
[22] Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME. *In vivo* study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. Am J Orthod Dentofacial Orthop, 2003, 124(6):687–693; discussion 693–694.
[23] Bumgardner JD, Lucas LC. Corrosion and cell culture evaluations of nickel-chromium dental casting alloys. J Appl Biomater, 1994, 5(3):203–213.
[24] Scrieciu M, Niculescu M, Mercuţ V, Andrei V, Pancă OA. Morphoclinical aspects of the human paraprosthetic gingival mucosa. Rom J Morphol Embryol, 2005, 46(4):335–340.
[25] Cao B, Zheng Y, Xi T, Zhang C, Song W, Burugapalli K, Yang H, Ma Y. Concentration-dependent cytotoxicity of copper ions on mouse fibroblasts *in vitro*: effects of copper ion release from TCu380A *vs.* TCu220C intra-uterine devices. Biomed Microdevices, 2012, 14(4):709–720.
[26] Issa Y, Duxbury AJ, Macfarlane TV, Brunton PA. Oral lichenoid lesions related to dental restorations. Br Dent J, 2005, 198(6):361–366; discussion 549; quiz 372.
[27] Kaaber S. Allergy to dental materials with special reference to the use of amalgam and polymethylmethacrylate. Int Dent J, 1990, 40(6):359–365.
[28] Issa Y, Brunton P, Waters CM, Watts DC. Cytotoxicity of metal ions to human oligodendroglial cells and human gingival fibroblasts assessed by mitochondrial dehydrogenase activity. Dent Mater, 2008, 24(2):281–287.
[29] Hildebrand G, Schreiber A, Lohrengel M, Strietzel R, Liefeith K. Localized electrochemical investigations of Pd73Cu13.5 ceramic firing dental alloy in DIN-saliva. Corrosion Sci, 2006, 48(11):3629–3645.
[30] Benatti OF, Miranda WG Jr, Muench A. *In vitro* and *in vivo* corrosion evaluation of nickel-chromium- and copper-aluminum-based alloys. J Prosthet Dent, 2000, 84(3):360–363.
[31] Ţovaru Ş. Stomatological medical pathology. Ed. Cerma, Bucharest, 1999, 116–119 (in Romanian).
[32] Stăniceanu F, Costinean Ş, Oprişan A. The oral cavity anatomy and histology, Ed Medicală, Bucharest, 2001, 9–26 (in Romanian).
[33] Rihet S, Bellaich P, Lorenzato M, Bouttens D, Bernard P, Birembaut P, Clavel C. Human papillomaviruses and DNA ploidy in anal condylomata acuminata. Histol Histopathol, 2000, 15(1):79–84.
[34] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer, 1972, 26(4):239–257.
[35] Cotran RS, Kumar V, Collins T. Cellular pathology I: Cell injury and cell death. In: Cotran RS, Kumar V, Collins T. Robbins pathologic basis of disease. 6th edition, Series "Robbins Pathology", W.B. Saunders Co., Philadelphia, 1999, 1–29.
[36] Latenser BA. Apoptotic death in deep partial thickness burns *versus* normal skin of burned patients. J Surg Res, 2008, 146(2):161–163.
[37] Wartenberg M, Wirtz N, Grob A, Niedermeier W, Hescheler J, Peters SC, Sauer H. Direct current electrical fields induce apoptosis in oral mucosa cancer cells by NADPH oxidase-derived reactive oxygen species. Bioelectromagnetics, 2008, 29(1):47–54.
[38] Sauer H, Stanelle R, Hescheler J, Wartenberg M. The DC electrical-field-induced Ca^{2+} response and growth stimulation of multicellular tumor spheroids are mediated by ATP release and purinergic receptor stimulation. J Cell Sci, 2002, 115(Pt 16): 3265–3273.
[39] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 2007, 39(1):44–84.
[40] Kroemer G1, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P, Zhivotovsky B, Blagosklonny MV, Malorni W, Knight RA, Piacentini M, Nagata S, Melino G; Nomenclature Committee on Cell Death. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ, 2005, 12(Suppl 2):1463–1467.
[41] Galluzzi L, Zamzami N, de La Motte Rouge T, Lemaire C, Brenner C, Kroemer G. Methods for the assessment of mitochondrial membrane permeabilization in apoptosis. Apoptosis, 2007, 12(5):803–813.
[42] Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. Physiol Rev, 2007, 87(1):99–163.
[43] Baek YW, An YJ. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb_2O_3) to *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus aureus*. Sci Total Environ, 2011, 409(8):1603–1608.
[44] Gong N, Shao K, Feng W, Lin Z, Liang C, Sun Y. Biotoxicity of nickel oxide nanoparticles and bio-remediation by microalgae *Chlorella vulgaris*. Chemosphere, 2011, 83(4):510–516.
[45] Siddiqui MA, Ahamed M, Ahmad J, Majeed Khan MA, Musarrat J, Al-Khedhairy AA, Alrokayan SA. Nickel oxide nanoparticles induce cytotoxicity, oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin. Food Chem Toxicol, 2012, 50(3–4):641–647.

[46] Wang YF, Shyu HW, Chang YC, Tseng WC, Huang YL, Lin KH, Chou MC, Liu HL, Chen CY. Nickel (II)-induced cytotoxicity and apoptosis in human proximal tubule cells through a ROS- and mitochondria-mediated pathway. Toxicol Appl Pharmacol, 2012, 259(2):177–186.

[47] Jacotot E, Ferri KF, Kroemer G. Apoptosis and cell cycle: distinct checkpoints with overlapping upstream control. Pathol Biol (Paris), 2000, 48(3):271–279.

[48] Monica S, Baniţă M, Niculescu M, Crăiţoiu M. Apoptoza celulară în mucoasa gingivală paraprotetică cu condilom acuminat. Medicina Stomatologică, 2003, 7(2):16–19.

Corresponding author

Monica Scrieciu, Associate Professor, MD, PhD, Department of Prosthetic Dentistry, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40723–516 539, e-mail: scrieciu_monica@yahoo.com

Received: March 30, 2015

Accepted: June 9, 2015