Periodontal bone response under the influence of Cr(VI)

Y. KUZENKO, A. ROMANYUK, A. KOROBCHANSKAYA, L. KARPENKO

Sumy State University, Department of Pathological Anatomy, Sumy, Ukraine

SOUHRN

Kuzenko Y., Romanyuk A.,.Korobchanskaya A, Karpenko L: **Reakce periodontální tkáně na chrom**

Cíl práce: Studovat reakci alveolární kostní tkáně na chrom.

Metodika: Autoři hodnotili histologické a elektronmikroskopické nálezy Ca, P, K, Cr u dvaceti samců laboratorních potkanů o váze 300–325 gramů.

Výsledky: Rozdíly mezi normální periodontální tkání a po šedesáti dnech experimentu byly významné. Destrukce alveolární kostní tkáně může postupovat až do situace, kdy zub není na svém místě fixován a vzniká komplexní porucha kostní tkáně. Ačkoli se zánětlivá reakce šíří do hloubky, nemusí být v alveolarní kosti přítomna, což dokumentuje chybění známek osteoklastické aktivity v histologickém vzorku. Nejnižší hladiny P a K byly zjištěny v šedesátém dni experimentu ($P < 0,01$). Takto dlouhá doba může ovlivnit minerály v kosti při chronickém příjmu chromu.

Závěry: Chronická intoxikace chromem vede u pokusných zvířat k závažné destrukci kolagenu, apoptóze kostní tkáně a hyperplázii epitelu. Neléčená chronická expozice chromu pomalu progreduje v chronickou periodontitidu s destrukcí hlubších tkání. Během šedesáti dnů dochází k resorpci alveolární kosti a k definitivní ztrátě zubu.

Klíčová slova: chrom, samci bílých potkanů, reakce periodontální kosti

SUMMARY

Kuzenko Y., Romanyuk A., Korobchanskaya A., Karpenko L: **Periodontal bone response under the influence of Cr(VI)**

Object: The object of this study has analyzed alveolar bone reponse under the influence chromium (VI).

Methods: 20 male albino rats (300–325 g) have been evaluated for histological and scanning electron microscopy of Ca, P, K, Cr.

Results: The interaction between normal periodontal tissues and 60 days experiment was strongly significant. Destruction may progress until tooth support becomes inadequate and more complex patterns of bone loss develop. Despite the deep extension of inflammation it may be absent in the alveolar bone, which may also lack any sign of osteoclastic activity histologically. The lowest levels of P and K were observed in 60 days of experiment ($P < 0.01$). 60 days level may affect the mineral status of bone and chronic incoming of chromium.

Conclusion: Chronic chrome (VI) intoxication is characterized by severe chronic destruction of collagen, apoptotic, bone changes, and epithelial hyperplasia. Untreated chronic chrome (VI) intoxication slowly progresses to chronic periodontitis in which the destruction of deep tissues develops. Within 60 days, progressive resorption of alveolar bone occurs and the tooth ultimately gets detached.

Keywords: chromium, male albino rats, periodontal bone response

Osteologický bulletin 2014;19(1):23–27

Adresa: MUDr. Yevhen Kuzenko, Sumy State University, Department of Pathological Anatomy 2, Rymskogo-Korsakova st., Sumy, 40007, Ukraine, e-mail: kuzenko_yevhen@rambler.ru

Došlo do redakce: 6. 12. 2013
Přijato k tisku: 7. 4. 2014

Introduction

Chronic inflammation and degeneration of the teeth supporting tissues, that results in teeth loss is a common condition. Besides inflammation, other diseases such as leukemia and scurvy, are associated with gingival swelling. Pregnancy, puberty and use of drugs like PHENYTOIN are also associated with periodontal disease more often than not. The disease begins as chronic marginal gingivitis, secondary to bacterial plaques around the teeth such as due to calculus (tartar) on the tooth surface impacted food, uncontrolled diabetes, tooth-decay and ill-fitting dental appliances. The gingival sulcus acts as convenient site for lodgment of food debris and bacterial plaque leading to formation of periodontal pocket from which purulent discharge can be expressed by digital pressure [1].

Precious metal based dental alloys generally exhibit a superior corrosion resistance, in particular enhanced resistance to pitting and crevice corrosion, compared to non-precious metal based alloys such as cobalt (Co) – chromium (Cr) alloys [3]. Precious metals are not available to much dental

Figure 1
Normal periodontal tissues A – cortical plate, B – transeptal and horizontal fibres, C – periodontal bone, Haematoxylin and Eosin staining (x400 magnification)

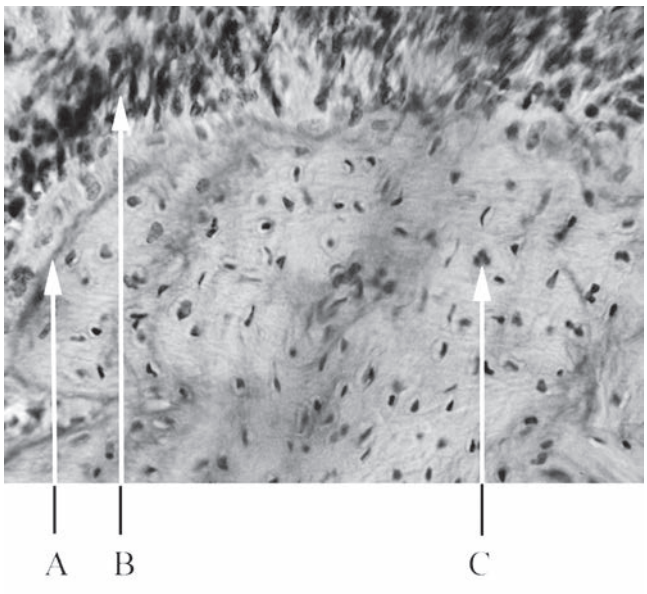
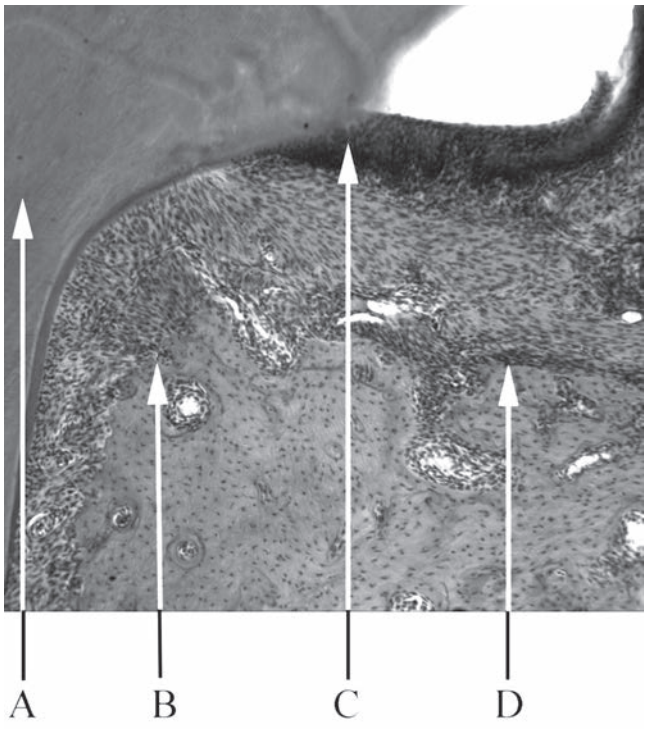


Figure 2
The periodontal tissues of the 20 days experiment A – tooth, B – horizontal bone loss, C – junctional epithelium proliferation, D – cortical plate, Haematoxylin and Eosin staining (x100 magnification)



case. However cobalt-chromium alloys are used for structures in the oral cavity (chronic cobalt-chromium intoxication) Cobalt–chromium (Cr-Co) alloys are commonly so far used for the Third World.

Chromium (Cr), in its trivalent form (Cr3+), is an essential nutrient because it is involved in the metabolic pathways for carbohydrates, lipids and proteins. Its most important believed function is the potencialization of insulin action. In human and animal feeds, chromium supplementation is done by addition of chromium piccolinate (CrPic) on food. However, Cr is a heavy metal and it has potential to accumulate in biological tissues and then, the risk of bioaccumulation and biomagnification (when the level of bioaccumulation increases exponentially between trophic levels) exists [2].

A study by Anissian L, Fleury C, Wang JY et al. [4,5,6] showed that short-term exposure to these metal species may affect human bone tissue survival and function. High concentrations of Co 2+, Cr 3+, and Cr 6+ ions are toxic to osteoblasts and reduce cell activity in-vitro. Few data are available on the effect of Cr 6+ ions on bone tissue. A study by Nichols and Puleo [7] showed short-term exposure to Cr ions at sublethal doses resulted in decreased resorptive activity in rat osteoclasts. In contrast, Rousselle et al. found exposure of rabbit osteoclasts to Cr had no effect on rabbit osteoclast function [8]. Sankaramanivel et al. [9] has shown that treatment of rats led to accumulation of chromium in the femur, and associated with reduced systemic assays of alkaline phosphatase and tartrate-resistant acid phosphatase, suggesting an impact on both bone formation and resorption (way of introduction – intraperitoneally with potassium dichromate Cr 6+ over 5 days).

However, the long-term effect of chronic exposure of rats osteoblasts and osteoclasts to these ions at mandibular bone, is unknown. So it is necessary to understand the cellular mechanism and alveolar bone response under the influence of chromium.

In the present work, the effect of chromium ions 6+ (Cr 6+) on bone tissue and collagen fibers was shown.

Methods

The study protocol was according to the provisions "European Community Directive of 24 November 1986 on the maintenance and use of laboratory animals for research purposes". Work implemented within the framework of research – 013U003315.

The subject was 20 experimental male Sprague-Dawley rats that weighed 300–325 g at the beginning of testing (Institute of Pharmacology, Academy of Medical Sciences, Ukraine). They were individually housed in standard cages inside a room maintained on a 12–12-hr light–dark cycle with the light part of the cycle beginning at 7 a.m. Throughout the experiment rats were kept in free water. The study protocol was approved by the Research and Ethics Committee of Sumy State University.

Rats of experimental group – 15 individuals entered potassium bichromate (Sigma, USA) into drinking water in a dose of 0,02 mol/l. The rats of control group (5 individuals) drank ordinary drinking water. Five animals from under skilled group were brought out of experiment in 20, 40 and 60 days after the beginning of introduction of bichromate of potassium.

Microelemental analysis was carried out by scanning electron microscopy (SEM) with energy dispersive spectrophotometer (EDS), the bone specimens were trimmed fi-

xed in 2 percent glutaraldehyde solution (35 μm sagittal sawing were obtained). The prepared samples served to determine the content of the following elements: Ca, P, K, Cr.

The electron beam is finely focused onto the specimen resulting in characteristic X-rays being produced from a microvolume of the sample. These X-rays are detected by an Energy Dispersive Spectrometer (EDS) and the results plotted as a spectrum. Each element has its own 'fingerprint' of peaks which allows both a qualitative and quantitative determination of the elements present in the selected region of the sample. EDS analysis Shown in *Fig. 6*, X-ray. Intensities are measured by counting photons and the precision obtainable is limited by statistical error. For major elements it is usually not difficult to obtain a precision (defined as 2σ) of better than ± 0.01 % (relative), but the overall analytical accuracy is commonly nearer ± 0.09 %.

Bone samples were immersed in 10% paraformaldehyde pH 7.4 fixative at 18 ºC. These were then subsequently demineralized with 17 percent EDTA (S-test UA) solution and dehydrated with increasing concentration of ethanol before being embedded in paraffin.

Thin sections were obtained from paraffin embedded blocks of each bone samples and were stained according to the methods specific for Van Gieson staining and Hematoxylin and eosin (H&E Sigma, USA) staining respectively.

Data were analysed using STATISTIKA 8.0 software, user version STA862D175437Q. Results were presented as mean values (± SD). The K-S test was used in order to evaluate the normality of the data. Also, the Student method was used to perform simple comparative analysis A value of $p < 0.05$ was considered significant.

Figure 3
The periodontal tissues of the 40 days experiment A – transeptal and horizontal fibres with superimposed edema,
B – horizontal bone loss, C – cement tooth , D – intrabony pocketing with superimposed edema Haematoxylin and Eosin staining

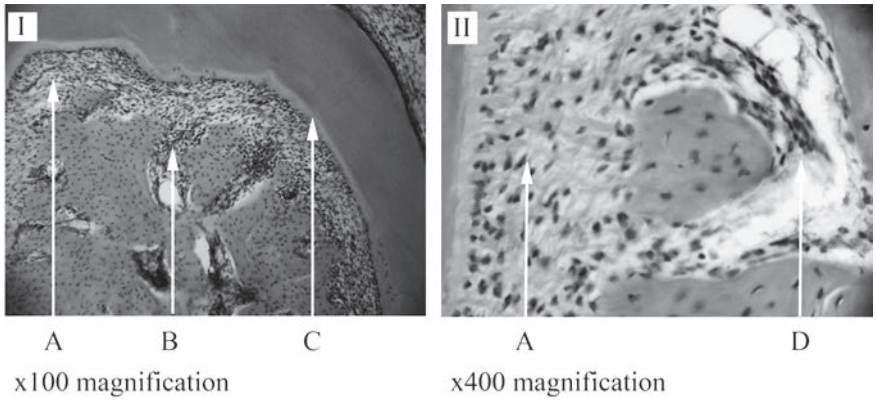
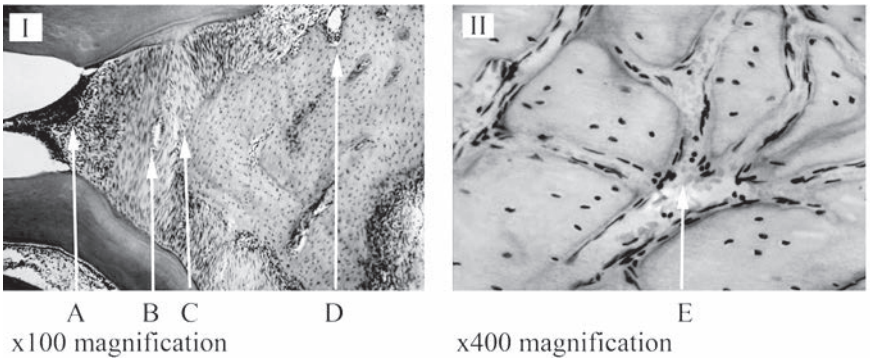


Figure 4
The periodontal tissues of the 60 days experiment A – root word migration of epithelial attachment, B – destruction of periodontal ligament, C – horizontal bone loss, D – intrabony pocketing with superimposed edema,
E – stasis in the capillaries. Haematoxylin and Eosin staining



Results

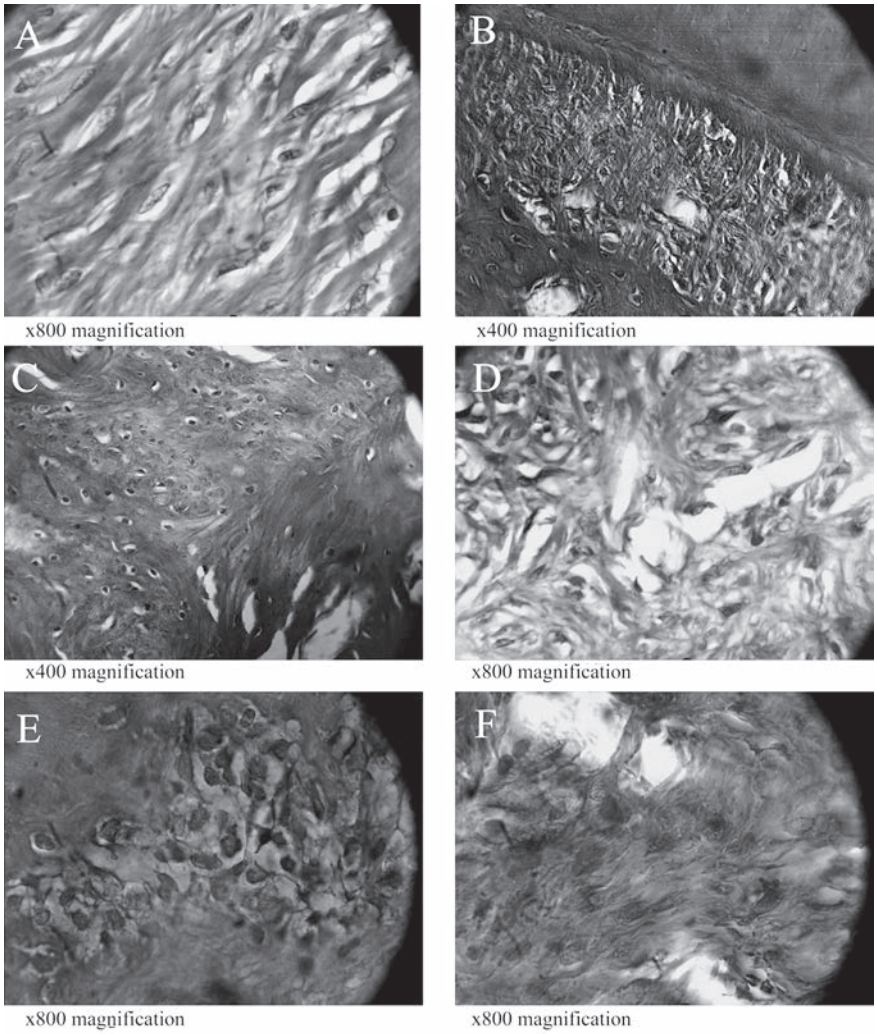
The normal periodontal tissues are shown in *Fig. 1*, *Fig. 5A*. Connective tissue gingival fibres support the gingival margin as a cuff around the tooth. Transeptal fibres join adjacent teeth and, more deeply, horizontal fibres join the tooth to the socket wall (*Figs 1* and *Fig. 5A*).

Table 1
Average concentrations microelement in samples

Microelements	Control mean ± SD	20 days mean ± SD	40 days mean ± SD	60 days mean ± SD
P (%)	10,85±1,01	9,65±3,97**	11,21±0,86**	6,34±1,25**
K (%)	1,11±0,20	1,17±0,43	1,81±0,22	0,15±0,02**
Ca (%)	61,94±2,54	78,06±2,16**	52,01±3,72**	62,04±33,23
Cr (%)	0	0,37±0,15	1,73±0,61***	0,27±0,1***
Ca/P	5,74±0,50	8,90±2,48*	4,63±0,09	10,83±6,90*

* $P < 0.05$ ** $P < 0.01$ ***$P < 0.001$

Figure 5
The normal periodontal tissues and periodontal tissues of the 20, 40, 60 days experiment A – root word migration of epithelial attachment, B – destruction of periodontal ligament, C – horizontal bone loss, D – intrabony pocketing with superimposed edema, E – stasis in the capillaries. F – destruction of the periodontal ligament in mid-root. Van Gieson staining



The results of the 20 days experiment are shown in *Fig. 2*, *Fig. 5B*. Junctional epithelium proliferation with progressive destruction of marginal gingiva (*Fig. 2*) and then of deeper supporting tissues. Influence of Cr(VI) factor acceleration of periodontal fibrous tissue destruction *Fig. 5B* Tissue destruction is arch (horizontal bone loss) the influence of Cr(VI) factor promote more complex patterns of destruction *Fig. 2B*.

The results of the 40 days experiment a shown in *Fig. 3*, *Fig. 5C, D*. Widespread osteoclastic resorption of bone (*Fig. 3-II*) increasing width and depth of pocket to form deep intrabony pocket consists of dilated thin-walled vessels in loose oedematous stroma with superimposed edema (*Fig. 3-II* and *Fig. 5C, D*).

The results of the 60 days experiment a shown in *Fig. 4*, *Fig. 5E, F.* Root ward migration of epithelial attachment (*Fig. 4A*). Stasis in the capillaries (*Fig. 4-II*). Destruction of periodontal ligament fibres and alveolar bone, but osteoclasts are rarely seen (*Fig. 4B, C*).

Gradual root ward progress of destruction leads eventually to loosening of teeth (*Fig. 4D*). Tissue destruction is usually uniform along the arch (horizontal bone loss) but local factors may promote more complex patterns of destruction (*Fig. 5E, F*). Localized destruction of bone around individual teeth (vertical bone loss) may develop or, occasionally, there is a more rapid destruction of periodontal ligament than alveolar bone with extension of pocketing between teeth and bone (intrabony pocketing).

The interaction between normal periodontal tissues and 60 days experiment was strongly significant. Destruction may progress until tooth support becomes inadequate and more complex patterns of bone loss develop. Despite the deep extension of inflammation it may absent in the alveolar bone, which may also lack any sign of osteoclastic activity histologically.

The average content of the micro- and macro-elements under study are shown in *Table 1.*

EDS analyses revealed that inorganic phases of bones were mainly composed of calcium and phosphorus as the major constituents with some minor components such as Cr, and K. The 40 days peak corresponding to chromium in an intermediate product was higher. It can clearly be seen from the *Table 1* that chromium levels increased with statistically significant extent ($P < 0.01$). As for K levels, there was no remarkable difference between the normal and after 40 days ($P > 0.01$) This could result from an excessive accumulation of chromium in hydroxyapatite. The lowest levels of P and K were observed in 60 days of experiment ($P < 0.01$). 60 days level may affect the mineral status of bone and chronic incoming of chromium.

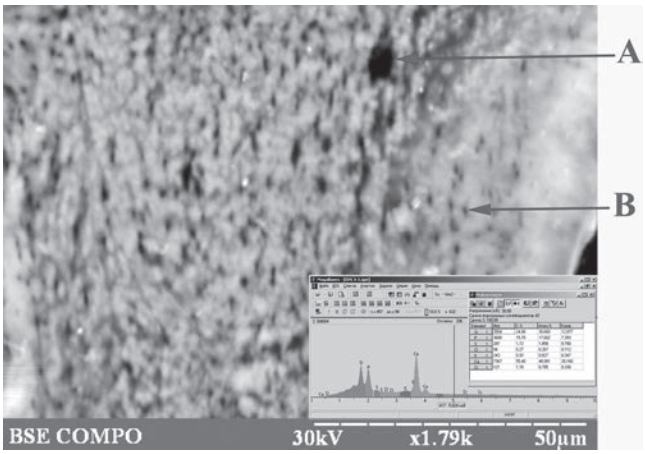
Discussion

In this study we examined the chronic exposure effect of bone tissue. In rat, the absorbed chromium was transferred to the liver where the liver tissue retained 10.9 % of chromium oxide and 51.1 % of sodium chromate. Different chromium absorption of bone tissue depending on the concentration and could be due to the fact that the hexavalent form given orally was reduced to Cr3+ in the acidic environment of the stomach [10]. The removal of hexavalent and trivalent chromiumfrom synthetic solutions has been extensively studied by a number of researchers. According to some investigators, the removal of Cr(VI) occurs through several steps of interfacial reactions.

We found that ions chromium affected on bone cell proliferation and function. Our findings are consistent with studies using animal cells that supraphysiological concentrations of chromium ions induce apoptosis in osteoblast cells in a dose dependent manner [5], and suppress osteoblast synthetic function [4].

Hydroxyapatite is component of bone mineral, enamel and dentin [12]. Surface modification of hydroxyapatite by organic molecules or Cr was effective means to manipulate the surface properties of hydroxyapatite. In our point of view, there are two ways to modify the surface of hydroxyapatite by Cr3+. The first method is through surface adsorption. It is known that many polymers and proteins can be firmly adsorbed onto the surface of hydroxyapatite [11]. In our opinion the second approach is to graft Cr3+ through covalent bonding to the hydroxyl groups and replacement of Ca, K which are available on the crystal surface of hydroxyapatite. The hydroxyl group present on the surface of hydroxyapatite seems to be a reactive group of which use can be made to graft Cr3+.

Figure 6
EDS analysis of the periodontal tissues – 40 days experiment (magnification x1790), A – intrabony bone loss, B – osteoblasts places



Conclusion

Chronic chrome (VI) intoxication is characterized by severe chronic destruction of collagen, apoptotic, bone changes, and epithelial hyperplasia. Untreated chronic chrome (VI) intoxication slowly progresses to chronic periodontitis in which the destruction of deeper tissues develops. Within 60 days, progressive resorption of alveolar bone occurs and the tooth ultimately gets detached. It is suggested that the results of this study would be used by anatomy and pathology.

References

1. Rosenstiel SF, Land MF, Fujimoto J. Contemporary fixed prosthodontics. Third edition. Missouri USA, Mosby 2001; p 868.
2. Dallago BS, Lima BF, Mustafa V, McManus C, Paim T, Campeche A, Gomes EF, Louvandini H. Tissue accumulation and urinary excretion of chromium in lambs supplemented with chromium picolinate. Biochem Res 2003;278:487–490.
3. Lucien R, Heinz L, Pierre-Yves E, Andreas B, Christian S. Corrosion behaviour of cobalt–chromium dental alloys doped with precious metals. Biomaterials 2007; 26(21):4358–4365.
4. Anissian L, Stark A, Dahlstrand H, Granberg B, Good V, Bucht E. Cobalt ions influence proliferation and function of human osteoblast-like cells. Acta Orthopedica Scandinavica 2002;73:369–374.
5. Fleury C, Petit A, Mwale F, Antoniou J, Zukor DJ, Tabrizian M, Huk OL. Effect of cobalt and chromium ions on human MG-63 osteoblasts in vitro: morphology, cytotoxicity, and oxidative stress. Biomaterials 2006;27:3351–3360.
6. Wang JY, Wicklund BH, Gustilo RB, Tsukayama DT. Prosthetic metals interfere with the functions of human osteoblast cells in vitro. Clin Orthop Relat Res 1997;26:216–226.
7. Nichols KG, Puleo DA. Effect of metal ions on the formation and function of osteoclastic cells in vitro. J Biomed Mater Res 1997;35:265–271.
8. Rousselle AV, Heymann D, Demais V, Charrier C, Passuti N, Basle MF. Influence of metal ion solutions on rabbit osteoclast activities in vitro. Histol Histopathol 2002;17:1025–1032.
9. Sankaramanivel S, Jeyapriya R, Hemalatha D, Djody S, Arunakaran J, Srinivasan N. Effect of chromium on vertebrae, femur and calvaria of adult male rats. Hum Exp Toxicol 2006;25:311–319.
10. Febel H, Szegedi B, Huszar S. Absorption of inorganic, trivalent and hexavalent chromium following oral and intrajejunal doses in rats. Acta Vet Hung 2001; 49:203–212.
11. Dupraz AMP, de Wijn J, van der Meer SAT, de Groot K. Characterization of silane-treated hydroxyapatite powders for use as filler in biodegradable composites. J of Biomed Mater Res 1996;30:231–238.
12. Bonfield W, Behiri J, Doyle C, Bowman J, Abram J. Hydroxyapatite reinforced polyethylene composites for bone replacement. Biomaterials and Biomechanics 1984;6:421–426.