CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

COLLAGEN TYPE II GENE EXPRESSION IN MESENCHYMAL STEM CELLS DELIVERED FROM RATS FEEDING WITH LEAD ACETATE II

Hossein.Rafiei [1]*, Mehrdad Shariati [2], Jina Khayatzadeh [3], Sima Afsharnezhad [4], Majid Farhoodi [5]

[1]PhD candidate, Department of Biology, Faculty of Sciences, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
[2]Associate Professor, Department of Biology, Kazerun Branch, Islamic Azad University, Kazerun, Iran.
[3]Assistant professor, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
[4]Assistant professor, Department of Biochemistry, Mashhad Branch, Medical Innovation Research Center of Islamic Azad University, Mashhad, Iran.
[5]Assistant professor, Razi Vaccine & Serum Research Institute (RVSRI), Mashhad, Iran.

Abstract:

Background and Objectives: *One of the most harmful heavy metals with deleterious effects on tissues is lead (Pb). In this project, the effects of lead acetate II oral doses on Collagen type II gene expression in mesenchymal stem cells (MSCs) in chondrogenic media were studied. Collagen type II is one of the most plentiful differentiated proteins of the in extracellular matrix (ECM) in cartilage.*

Methods: *Twelve male Wistar rats were randomly divided into 3 groups. Except libitum feed, the first group were fed orally by 50 ppm lead acetate II, the second group were fed orally by 100 ppm lead acetate II while the control group was fed by distilled water for 2 months. After killing, the femoral bone marrow mesenchymal stem cells (BM-MSCs) were isolated then cultured in chondrogenic medium. Finally the gene expression of Collagen type II was evaluated by real time PCR.*

Results: *After 2 passages, Population Doubling Time of MSCs in the second group was more than the first while the control group was the least. Real time PCR demonstrated that after feeding rats bone marrow with lead acetate II, Collagen type II gene expression in MSCs in chondrogenic medium decreased significantly in the second group compared with the first, while Collagen type II gene expression in the control group was the most.*

Conclusion: *It seems that accumulation of lead acetate II with the doses 50 and 100 ppm in rat bone marrow in femur lead to reduce Collagen type II gene expression in MSCs in chondrogenic medium thus, public health is a priority.*

Kew words: *Bone Marrow, lead acetate, Mesenchymal Stem Cells, Real-Time PCR, Collagen type II*

Corresponding author:
Hossein.Rafiei,
Department of Biology,
Faculty of Sciences,
Shiraz Branch, Islamic Azad University,
Shiraz, Iran00989155032834

QR code

Please cite this article in press as Hossein.Rafiei et al, ***Collagen Type Ii Gene Expression In Mesenchymal Stem Cells Delivered From Rats Feeding With Lead Acetate II*** *, Indo Am. J. P. Sci, 2017; 4(06).*

INTRODUCTION:

Although the standard amount of lead in water in World health organization (WHO) report is 10 ppm, in many regions level are higher. There are many factors that cause water pollution such as human activities particularly industrial contaminants and presence of lead components adjacency underground aquifer layer. At higher doses than WHO report, some tissues like brain, liver, kidney, reproductive system, cartilage and bone maybe damaged (1, 2). Lead is absorbed in different ways such as breathing, skin and mouth. In the oral way, the absorption of this heavy metal is higher, therefore it can easily enter into blood and consequently into soft tissues and bone. Also long-term toxicity is caused by lead accumulation in bone (3, 4).

One of the greatest interests in biology is adult stem cells. They are recognized by their self-renewal capacity and much plasticity. With these properties, they are able to procreate various mature progenitor cells retaining actively processes of homeostasis by replacing the damaged cells of tissues (5-8). Therefore, they can treat numerous degenerative disorders such as bone and cartilage diseases, various types of aggressive cancers, heart failures, muscular degenerative disorders, chronic liver, type 1 and 2 diabetes, Parkinson and Alzheimer (9, 10). Some tissues such as bone marrow have the source of stem cells with specific niches (8).

In adults, the chondrocytes are only cell types of articular cartilage occupying more than 5% of cartilage volume but 95% of this tissue volume is composed of extra cellular matrix (ECM). *Collagen type II* is the backbone of structure in this matrix. Fifty percent of cartilage protein and 80-90% of articular cartilage collagen are *Collagen type II* forming fibrils. The network of *Collagen type II* fibrils can entrap proteoglycan aggregate providing tensile strength for cartilage tissue (11, 12). Many joint treatments have been performed based on prevention of the *Collagen type II* degeneration or the reforming and the restoration of cartilage by mesenchymal stem cells (MSCs) (12, 13). *Collagen type II* plays an important role in these processes. In this research, we investigated on the level of *Collagen type II* gene expression in isolated BM-MSCs in a chondrogenic medium after feeding rats with lead acetate II for 2 months.

MATERIALS AND METHODS:

Animals and Housing:

For this original research, twelve male Wistar rats were purchased from Razi Vaccine and Serum Research Institute of Mashhad Maintained in standard condition comprising standard cages with 12:12 hour interval light/ dark cycle at 25° C. The animals were divided randomly into 3 groups: the control group was fed by distilled water with libitum feed, the first group were fed with a dose of 50 ppm lead acetate II (Gibco Grand Island, NY) dissolved in distilled water along with libitum feed and the second group were fed with a dose of 100 ppm lead acetate II dissolved in distilled water with libitum feed. All groups were treated for 2 months. The research was in accordance with institutional animal care and use committee (IACUC). The studies on animals were done under ethical committee of Razi Vaccine and Serum Research Institute of Mashhad. We selected 50 and 100 ppm lead acetate II because some mines (like Forumad mine in Iran) have several time of WHO lead dose (1, 14) where some animals live there.

BM-MSCs Isolation and Incubation:

After two months of treatment, the rats were anesthetized by diethyl ether and the mesenchymal stem cells of their femurs were isolated under sterile condition then cultured in Dulbecco's Modified Eagle Medium (DMEM) and incubated at 37°C and 5% CO2 (15).

Population Doubling Time of mesenchymal stem cells:

During the culture period (first and second passage), Population Doubling Time (PDT) were calculated base on following formula:

PDT = CT/(log N/N0)×3.31

CT, N0 and N were respectively the period of cell culture, the number of the cells at the beginning of culture and the number of the cells at the end of culture (16).

Chondrogenic Culture:

After 2 passages, the medium of MSCs was changed by chondrogenic medium. This medium consisted of DMEM, 1.25 mg/ml bovine serum albumin, 50 µg/ml ascorbic 2-phosphate, 100 nM dexamethasone, 500 ng/ml bone morphogenetic protein-6, 10 ng/ml transforming growth factor ß3 and 50 µg/ml ITS. The cells were incubated at 37° C and %5 CO2 for 21 days. The period of chondrogenesis for mesenchymal stem cells was reported 21 days. The bone medium was replaced every two days (17). We extracted RNA from 3×10^6 cells of control, first and second groups at the end of the 21st day (N= 8 for each group).

RNA Isolation:

For each group, 3×106 cells were utilized for RNA extraction (N=8 for each group). Total RNA was obtained from the culture cells and the culture pellets by TRIzol reagent (Invitrogen) base on the manufacturer's protocol. Synthesis of cDNA was

done for 1 h at 42°C in a mixture of reaction including 5 µg total RNA, 100 U ReverTra Ace, 2 µl 10 mM dNTPs, 1 µl 5 pM oligo (dT) and 4µl 5×RT buffer regulated with RNase-free water for 20 µl of total volume (18).

The forward and reverse primer sequences of *Collagen type II* were respectively 5´-GTGGAAGAGCGGTGACTAC-3´ and 5´-TAGGTGATGTTCTGGGAGC-3´. Also, 5´-CCTTCATTGACCTTCACTACATGGTCTA -3´ and 5´-TGGAAGATGGTGATGGCCTTTCCATTG -3´ were respectively as the forward and reverse primers of GAPDH (19, 20). For *Collagen type II*, PCR method was done by BioRad system (Herc`ules, CA, US). The condition of PCR was respectively, 95ºC for 5 min for initial denaturation, followed by 40 amplification cycles. Each cycle comprised: denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and the step of an additional extension at 72°C for 5 min. The conditions for GAPDH were similar.

Quantitative Real-time Polymerase Chain Reaction:

The number of molecules of cDNA in the samples of transcription was detected by analysis of real-time PCR using a modified method along with QuantiTect SYBR Green PCR kit an Icycler iQ Multicolor Real-time PCR Detection System (Bio- Rad). The cDNA content was normalized using by substracting numbers of GAPDH cycle from target gene (ΔCt = tagret gene Ct – GAPDH Ct). The level of gene expression was measured by $2^{-(\Delta\Delta Ct)}$ formula (21). On days 7, 14 and 21, the gene expression of *Collagen type II* were compared between the first, the second and the control groups.

Statistical Analysis:

The significant differences between the first, the second and control groups were surveyed at P< 0.005 by Anova and Dunnett t-test. Also comparison between days was done (P< 0.005).

RESULTS:

PDT of the first, the second and the control groups were respectively 52.2×10^5 ±2, 48.14×10^5 ±2 and 45.92×10^5 ±2 showing in fig 1.

The first repeat of real time PCR showed that *Collagen type II* gene expression of the first group was almost one-third of the expression in the control, while the expression of the second group was less than one-tenth of the control (P <0.005). *Collagen type II* gene expression of the second group was one-fourth of the first (P <0.005). The second and third repeats of real time PCR were almost similar to the first repeat showing the table (N = 8). Also, the median of *Collagen type II* gene expression in three groups is shown in fig 2.

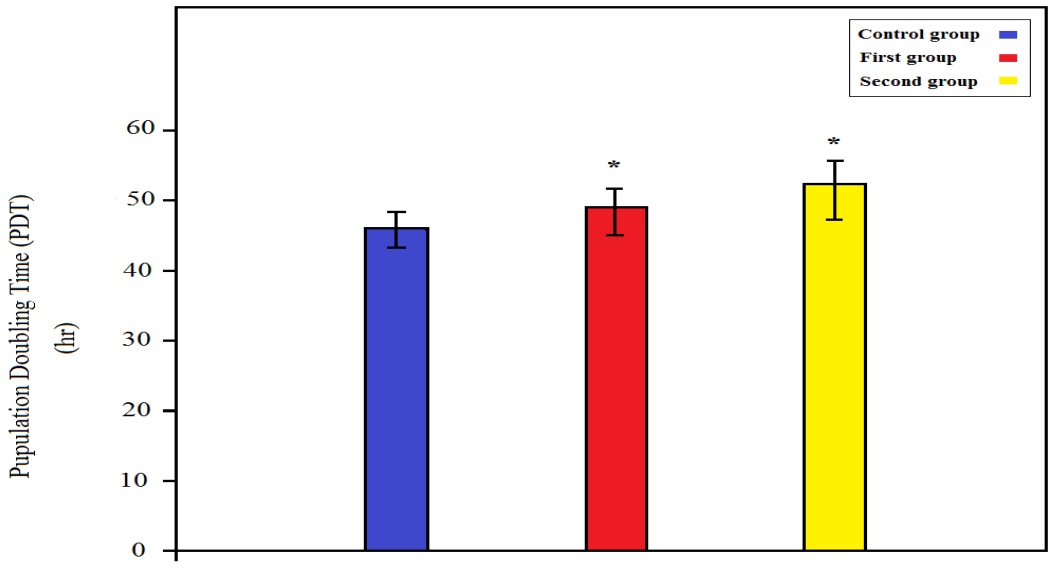

Fig 1: Comparison between PDT median of MSCs in the first, the second and control groups after 2 passages in DMEM culture medium (N= 8) (P <0.005).

Table 1 : Real time PCR comparison between collagen type II gene expression in MSCs after culture in chondrogenic medium by livak method (N= 8)

day	First repeat		Second repeat		Third repeat	
Fold difference / Sample	$2^{(-\Delta\Delta Ct)1}$ [2]	$2^{(-\Delta\Delta Ct)2}$ [1]	$2^{(-\Delta\Delta Ct)1}$	$2^{(-\Delta\Delta Ct)2}$	$2^{(-\Delta\Delta Ct)1}$	$2^{(-\Delta\Delta Ct)2}$
Sample 1	0.31± 0.1	0.08± 0.1	0.32± 0.1	0.08± 0.1	0.31± 0.1	0.08± 0.1
Sample 2	0.33± 0.1	0.078± 0.1	0.3± 0.1	0.08± 0.1	0.31± 0.1	0.077± 0.1
Sample 3	0.32± 0.1	0.082± 0.1	0.3± 0.1	0.083± 0.1	0.28± 0.1	0.074± 0.1
Sample 4	0.31± 0.1	0.079± 0.1	0.31± 0.1	0.083± 0.1	0.29± 0.1	0.074± 0.1
Sample 5	0.28± 0.1	0.079± 0.1	0.28± 0.1	0.084± 0.1	0.29± 0.1	0.075± 0.1
Sample 6	0.29± 0.1	0.082± 0.1	0.31± 0.1	0.082± 0.1	0.33± 0.1	0.076± 0.1
Sample 7	0.31± 0.1	0.08± 0.1	0.28± 0.1	0.081± 0.1	0.3± 0.1	0.076± 0.1
Sample 8	0.31± 0.1	0.08± 0.1	0.32± 0.1	0.083± 0.1	0.3± 0.1	0.076± 0.1

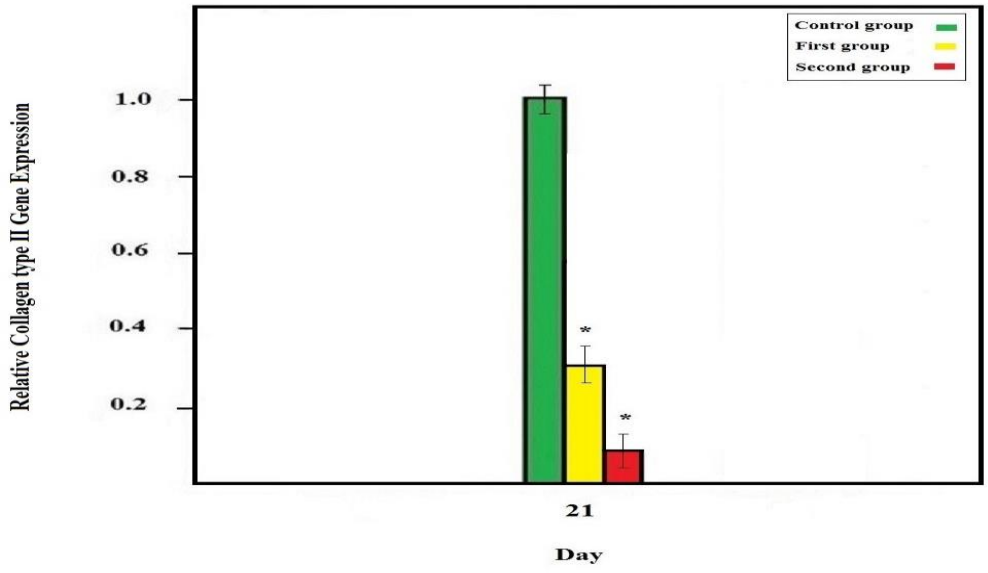

Fig 2: Comparison between Collagen type II gene expression median in MSCs in the first, the second and control groups after 21 days in chondrogenic culture medium (N= 8) (P <0.005).

[1] $2^{(-\Delta\Delta Ct)1}$: Collagen type II gene expression of the first group compare to control group

[2] $2^{(-\Delta\Delta Ct)2}$: Collagen type II gene expression of the second group compare to control group

DISCUSSION:

There is a little information about lead toxicity in MSCs despite of wide investigation. More than 90% of lead accumulates in the bone. So, the main target of lead is bone marrow (22, 23). In this research, the effects of lead on *Collagen type II* gene expression in stem cells of bone marrow was investigated. First of all, the stem cells were isolated from rat exposed to lead acetate II with the dose of 50 and 100 ppm (23). After two passages and increasing stem cells, they were cultured in the chondrogenic medium. On day 21, 3×10^6 cells of each group were utilized for RNA extraction (N= 8 for each group). The cells were induced to chondrocytes using chondrogenic culture medium (24). The expression of *Collagen type II* was enhanced during these days. This gene was transcribed plentifully during chondroblast differentiation and cartilage formation and is substantially secreted to ECM where *Collagen type II* produces the fibrillar network to entrap the proteoglycan and provides tensile strength (25, 26). Many factors can have an affect on *Collagen type II* expression such as TGF-B, ascorbic acid and BMP6 (27, 28, 29). It has been found that much of the concentration of lead in water, air and environment has dangerous effects on various tissues such as bone marrow and stem cells (4, 23). *Collagen type II* expression levels plus might be an important key for lead toxicity of cartilage. Accumulation of this heavy metal in ECM provides a condition to reduce *Collagen type II* gene expression level other related genes in chondrocytes contributing to cartilage destruction. Another reason for *Collagen type II* repression is the high half-life of this element (31, 32). The fig shows that *Collagen type II* was downregulated in the first and second group compare with the control group. Also the expression of this gene in MSCs in the second group was reduced more than the first group. It's unclear how much lead causes a decrease in *Collagen type II* gene expression. It seems that probably lead directly targeted *Collagen type II* gene or impressed on transcriptional factors.

CONCLUSION:

With respect to the ability of lead metal to accumulate in bone, it might impress on BM-MSCs despite of isolation and culturing in chondrogenic medium that can decrease the *Collagen type II* gene expression level. Higher doses (100 ppm) of lead acetate II lead to more significant reduction in this gene than the low dose (50 ppm). It seems that lead can prevent *Collagen type II* gene expression. Also, lead caused to decrease PDT of MSCs. Hence, natural resources conservation and decreasing pollution are priorities for public health.

Acknowledgements:

Also we would like to thank Robert Earl Nickerson and Bahman Rafiei.

We declare that there is no conflict of interests regarding the publication of this paper.

Potential Conflict of Interest:

The authors have no conflicting financial interests.

REFERENCES:

1.World Health Organization. Exposure to lead: A major public health concern. Geneva: WHO. 2010.

2.Otari M, Dabiri R. Assessment of anthropogenic activities on the water quality of froumad plain based on qualitative indicators. Iranian J Earth Sci 2015; 7:14-24.

3.Rader JI, Peeler JT, Mahaffey KR. Comparative toxicity and tissue distribution of lead acetate in weanling and adult rats. Environ Health Perspect 1981; 42:187-195.

4.Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2012; 2(1): 41-46.

5.Wei G, Schubiger G, Harder F, Muller AM. Stem cells plasticity in mammals and transdetermination in Drosophila: common theme? Stem Cells 2000; 18(6):409-14.

6.Woodbury D, Reynolds K, Black IB. Adult bone marrow stromal stem cells express germline, ectodermal, endodermal, and mesodermal genes prior to neurogenesis. J Neurosci 2002; 69(6):908-17.

7.Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem cells 2007; 25 (11):2739-49.

8.Shakoori A, Ahmad A. Cytotoxic and genotoxic effects of arsenic and lead on human adipose derived mesenchymal stem cells (AMSCs). J Stem Cells Regen Med 2013; 9 (2):29-36.

9.Baksh D, Song L, Tuan RS. Adult mesenchymal stem cells: characterization, differentiation, and application in cell and gene therapy. J Cell Mol Med 2004;8(3):301-16.

10.Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008;8(9):726-36.

11.Eyre D. Collagen of articular cartilage. Arthritis Res 2002; 4(1):30-5.

12.Gupta PK, Das AK, Chullikana A, Majumdar AS. Mesenchymal stem cells for cartilage repair in osteoarthritis. Stem Cell Res Ther 2012;3(4):25.

13.Tiku ML, Madhan B. Preserving the longevity of long-lived type II collagen and its implication for

cartilage therapeutics. Ageing Res Rev 2016;28:62-71.
14.Otari M, Dabiri R. Geochemical and environmental assessment of heavy metals in soils and sediments of Forumad Chromite mine, NE of Iran. J Mining & Environ 2015; 6(2):251-261.
15.Smajilagić A, Aljičević M, Redžić A, Filipović S, Lagumdžija A. Rat bone marrow stem cells isolation and culture as a bone formative experimental system. Bosn J Basic Med Sci 2013;13(1):27-30.
16.Vacanti V, Kong E, Suzuki G, Sato K, Canty JM, Lee T. Phenotypic changes of adult porcine mesenchymal stem cells induced by prolonged passaging in culture. J Cell Physiol 2005;205(2):194-201.
17.Eslaminejad MB, Eftekhari Yazdi P. Mesenchymal stem cells: In vitro differentiation among bone and cartilage cell lineages. Yakhteh Med J 2007; 9(3): 158-169.
18.Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell Tissue Res. 2007; 327 (3):449-62.
19.Baghban Eslaminejad MR, Nazarian H, Taghiyar L. Mesenchymal stem cells with high growth rate in the supernatant medium from rat bone marrow primary culture. jbums 2008; 10(2): 12-22.
20.Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Isolation and multilineage differentiation of bovine bone marrow mesenchymal stem cells. Cell Tissue Res. 2005; 319 (2):243-53.
21.Fei Q, Guo C, Xu X, Gao J, Zhang J, Chen T, et al. Osteogenic growth peptide enhances the proliferation of bone marrow mesenchymal stem cells from osteoprotegerin-deficient mice by CDK2/cyclin A. Acta Biochim Biophys Sin (Shanghai). 2010; 42 (11):801-6.
22.Pounds JG, Long GJ, Rosen JF. Cellular and molecular toxicity of lead in bone. Environ Health Perspect. 1991; 91:17.
23.Adikwu E, Deo O, Geoffrey OB, Enimeya DA. Lead Organ and Tissue Toxicity: Roles of Mitigating Agents (Part 2). British J Pharm Toxicol 2014; 5 (1):1-5.
24.Mauck RL, Yuan X, Tuan RS. Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. Osteoarthritis Cartilage 2006;14(2):179-89.
25.Von der Mark K, Conrad G. Cartilage cell differentiation: review. Clin Orthop Relat Res 1979;(139):185-205.
26.Hubka KM, Dahlin RL, Meretoja VV, Kasper FK, Mikos AG. Enhancing chondrogenic phenotype for cartilage tissue engineering: monoculture and coculture of articular chondrocytes and mesenchymal stem cells. Tissue Eng Part B Rev 2014;20(6):641-54.
27.Galéra P, Vivien D, Pronost S, Bonaventure J, Rédini F, Loyau G, Pujol JP. Transforming growth factor-beta 1 (TGF-beta 1) up-regulation of collagen type II in primary cultures of rabbit articular chondrocytes (RAC) involves increased mRNA levels without affecting mRNA stability and procollagen processing. J Cell Physiol 1992;153(3):596-606.
28.De Haart M, Marijnissen WJ, van Osch GJ, Verhaar JA. Optimization of chondrocyte expansion in culture. Effect of TGF beta-2, bFGF and L-ascorbic acid on bovine articular chondrocytes. Acta Orthop Scand 1999;70(1):55-61.
29.Diekman BO, Estes BT, Guilak F. The effects of BMP6 overexpression on adipose stem cell chondrogenesis: Interactions with dexamethasone and exogenous growth factors. J Biomed Mater Res A 2010;93(3):994-1003.
30.González-Riola J, Hernández ER, Escribano A, Revilla M, Ca-Seco, Villa LF, Rico H. Effect of lead on bone and cartilage in sexually mature rats: a morphometric and histomorphometry study. Environ Res 1997;74(1):91-3.
31.Mestek O, Deyl Z, Miksík I, Novotná J, Pfeifer I, Herget J. Accumulation of lead in tissues after its administration in drinking water to laboratory rats. Physiol Res 1998;47(3):197-202.
32.Zoeger N, Roschger P, Hofstaetter JG, Jokubonis C, Pepponi G, Falkenberg G, Fratzl P, Berzlanovich A, Osterode W, Streli C, Wobrauschek P. Lead accumulation in tidemark of articular cartilage. OsteoArthritis and Cartilage 2006;14(9):906-913.