

# Matrix Metalloproteinase Levels in Chronic Periapical Lesions and Inflamed Dental Pulp

## SUMMARY

*The aim of this study was to accomplish quantitative measurement of tissue levels of collagenases (MMP-1, 8 and 13) in chronic inflamed tissues (pulp and periapical lesions), as well as to determine the dependence between collagenase level and the degree of tissue destruction of examined material. Chronic periapical tissues were collected by periapical surgery from 50 teeth with clinically and radiographically verified different chronic periapical lesions (20 granulomas, 20 diffuse periapical lesions, 10 cysts). Chronically inflamed pulps were obtained from 20 patients by endodontic treatment. Control group contained vital dental pulps from 10 impacted third molars. For rapid analysis of MMP-1, 8 and 13 collagenase activities in the examined material Chemicon Collagenase Activity Assay Kit was used.*

*The biggest values of concentration of MMPs were registered in chronic diffuse lesions (5.39 ng/mL). Expression and activity of MMPs in normal tissue of pulp from impacted third molars was from 0.00 to 0.02 ng/mL. Different concentrations of collagenases (MMP-1, 8 and 13) in chronic periapical lesions from different inflammation types showed different activity of MMPs, which was confirmed with high significant difference (ANOVA  $F=67.475$ ;  $df=4$ ;  $p=0.000$ ). The concentration of collagenases (MMP-1, 8 and 13) in chronically inflamed pulp was from 0.1-1.28 ng/mL, which means that inflammation process was still localized in pulp tissue only, and periapical connective tissue was not affected.*

*We conformed the destructive role of collagenases (MMP-1, 8 and 13) in inflammation processes, which is directly dependent from the concentration of MMPs in pathologically changed tissue. With respect to conventional methods in everyday dental practice, this study recommended determination of MMPs (proteolytic enzymes) as the most sensitive markers in chronically inflamed tissues, which opens new opportunities to contemporary methods of evaluation and monitoring of inflammation activity in chronic periapical lesions.*

**Keywords:** Chronic Periapical Lesions; Extracellular Matrix; Collagen; Collagenases

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## Introduction

Matrix metalloproteinases (MMPs) are proteolytic enzymes capable of degrading almost all extracellular matrix (ECM) and basement membrane (BM) components<sup>6</sup>. MMPs form a family of structurally related, but genetically distinct endo-peptidases, expressed at low levels in normal tissues, but unregulated during inflammation<sup>1</sup>. Normal embryonic development and tissue remodeling needs a controlled balance between

extracellular matrix synthesis and degradation, as well as a balance between MMPs and their natural inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs)<sup>13,14</sup>. This group of proteolytic enzymes has a role in many normal physiological events, like ovulation, embryo implantation, organ development, angiogenesis, wound healing and bone remodeling<sup>19</sup>.

Disruption of the balance between MMPs and TIMPs contributes to pathophysiological processes, such as many chronic tissue destructive inflammatory and autoimmune

diseases<sup>13,14</sup>. The obvious destructive capability of MMPs initially focused most research onto diseases that involve breakdown of the connective tissues (e.g. rheumatoid arthritis, cancer, periodontal disease and periapical lesions)<sup>19</sup>. Leukocytes, particularly macrophages, are major sources of MMP production. MMPs released by leukocytes play vital roles in allowing leukocytes to extravagate and penetrate tissues, a key event in inflammatory disease<sup>25</sup>.

MMPs are divided according to their substrate specificities and structures to interstitial collagenases, gelatinases, membrane-type MMPs, stromelysins, matrilysins and other MMPs<sup>23</sup>. Collagenases (collagenases-1, 2 and 3; MMPs-1, 8, 13) are named after their unique capacity to degrade fibrillar collagen types I, II, III, V and IX. In oral tissues, this first characterized collagenase, MMP-1 (collagenase-1, interstitial collagenase, fibroblast collagenase), is detected in gingival fibroblasts capable of disrupting ECM collagen. MP-8 (collagenase-2) represents the second collagenase, and was originally synthesized and stored exclusively in intracellular granules of human polymorphonuclear neutrophils (PMNs) in bone marrow<sup>11</sup>. MMP-13 (collagenase-3) was originally cloned from human breast tumor. It is also expressed by hypertrophic chondrocytes, osteoblasts, periosteal cells and fibroblasts during human fetal bone development<sup>5, 20</sup> and postnatal in bone remodeling<sup>20</sup>.

With respect to other literature findings that underline the role of collagenases (MMP-1, -8, -13) in chronic periapical lesions, the **aims** of this study were formulated as follows:

- to accomplish quantitative measurement of tissue levels of collagenases (MMP-1, -8, -13) in chronically inflamed tissues: chronic periapical lesions and chronically inflamed dental pulps with enzyme method;
- to determine the dependence between levels of collagenases (MMP-1, -8, -13) and the degree of tissue destruction of the examined material (periapical tissue and pulp).

## Materials and Methods

Examination material was collected on the basis of clinical diagnosis after thoroughly realizing dental histories and clinical investigation and analysis of radiological findings.

Chronic periapical tissues were collected by periapical surgery from 50 teeth with clinically and radiographically verified different chronic periapical lesions (20 granulomas, 20 diffuse periapical lesions, 10 cysts). Chronically inflamed pulps were obtained from 20 patients during endodontic treatment. 10 normal pulps that were obtained by extirpation of the pulp of impacted third molars after surgery used as control specimens.

Examination material was frozen at -80°C as soon as possible and stored until the analysis, but not longer than 6 months.

### Laboratory Analysis

For rapid quantitative analysis of MMP-1, -8, -13 collagenase activities in all the examined material (chronic periapical tissues, chronically inflamed pulps and normal pulp tissue) Chemicon Collagenase Activity Assay Kit (ECM710) was used. The colorimetric kit is designed in order to achieve quick, convenient and sensitive evaluation of MMP-1, -8 and -13 collagenase activities in a 96-well microplate format. Biotinylated, native triple helical Type I collagen was used as a substrate and was cleaved from the activated MMP-1, -8, -13 enzymes.

Each sample was macerated in Phosphate-Buffered Saline - PBS (1.5 ml) and then homogenized samples were centrifuged with Eppendorf-Centrifuge at 10,000 g for 10 min. The supernatant was detected in new test tubes and used for following analysis. In homogenized mixture with Bradford micromethod using series of 5 standards of bovine-serum albumin, and then measuring the absorbance on 450nm with spectrophotometer, concentration of total proteins was determined. With interpolation from the standard curve, concentration of the proteins in samples was measured. A microplate reader (Anthos ht III) was used to measure the absorbance at 450nm. Values of the absorbance of each standard were corrected according to protein concentration.

By adding 100µL of Stop Solution to each well, the bright yellow convert to bright blue coloured product and the enzyme reaction was stopped. The MMPs concentration of each sample was normalized *versus* concentration of proteins in each sample. Standard curve was designed with soft wear program Curve Expert 1.3. With interpolation of the values, MMP-1, -8, -13 collagenase concentrations were calculated.

Statistical analysis for comparison of values to define the significance between specimens of the examined material used descriptive and analytical statistical methods from the programme Stat Soft Statistic 6.0.

## Results

MMP-1, -8, -13 collagenase concentrations in the specimens of the examined material varied from undetectable values, increasing proportionally with the clinical picture of the inflammation and propagation of the infection from the root canal to the periapical tissue (Tab. 1).

Expression and activity of MMPs in normal tissue was very low. The measured concentration of collagenases (MMP-1, -8, -13) in normal tissue of pulp from impacted third molars was from 0.00 ng/mL to 0.02 ng/mL. The concentration of collagenases (MMP-1, -8,

-13) in chronically inflamed pulp tissue was from 0.1-1.28 ng/mL, which means that inflammation process is still localized only in the pulp tissue and had not affected periapical connective tissue. In the specimens of patients with chronic periapical granulomas, the concentrations of collagenases (MMP-1, -8, -13) in most of the cases (85%, 17 specimens) were in borders from 0.1-0.99 ng/mL.

Concentration of collagenases in diffuse periapical lesions was in borders from 3-4.99 ng/mL (45%, 9 specimens). Concentration of the MMPs in all 10 specimens with clinical diagnosis of radicular cyst was in borders from 0.1-0.99 ng/mL. Similar concentration of collagenases from specimens with chronically inflamed pulp (85%, 17 specimens) was found.

Table 1. MMP-1, -8 and -13 collagenase concentrations (ng/mL) in the specimens of the examination material

Clinical Diagnosis	N	Min	Max	Median	$\bar{X}$	SD
Chronic Granulomatous Parodontitis	20	0.05	0.95	0.44	0.46	± 0.29
Chronic Diffuse Parodontitis	20	1.15	5.39	4.12	3.63	± 1.46
Radicular Cyst	10	0.10	0.64	0.19	0.25	± 0.16
Chronic Pulpitis	20	0.1	1.28	0.35	0.47	± 0.38
Dens Impactus	10	0.00	0.02	0.01	0.01	± 0.009

The highest values of the concentration of collagenases (MMP-1, -8, -13) between chronic periapical processes with different clinical diagnosis were detected in the inflamed tissue of the patients with diffuse periapical lesions (5.39 ng/mL). Different concentration of collagenases (MMP-1, -8, -13) in chronic periapical lesions from different inflammation type was found, which was statistically significant ( $p < 0.05$ ). The difference between various clinical diagnoses analyzed from the median values of collagenase (MMP-1, -8, 13) concentration was statistically significant (ANOVA  $F=67.475$ ,  $df=4$ ,  $p < 0.01$ ). High statistical significance was confirmed with Kruskal-Wallis test ( $\chi^2=59.363$ ,  $df=4$ ,  $p < 0.01$ ), as well as, with Mediana test ( $\chi^2=37.400$ ,  $df=4$ ,  $p < 0.01$ ).

Assessment of the median values of collagenase (MMP-1, -8, -13) concentration between different clinical diagnoses is presented in figures 1-4 and with box-plot diagram in figure 5.

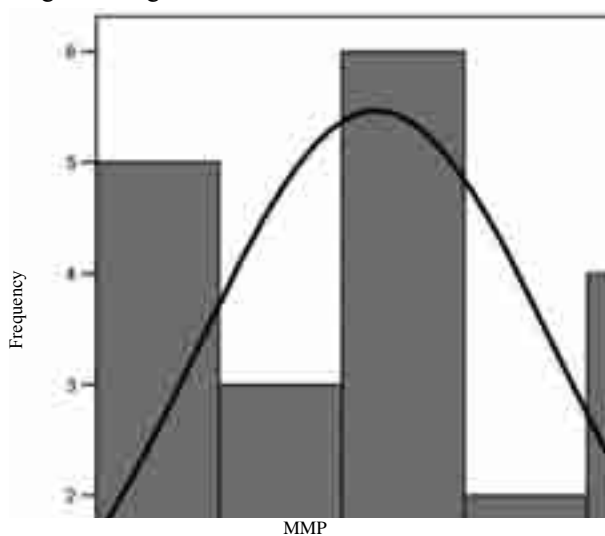


Figure 1. Median values of MMP-1, -8, -13 collagenase concentrations in chronic periapical granulomas

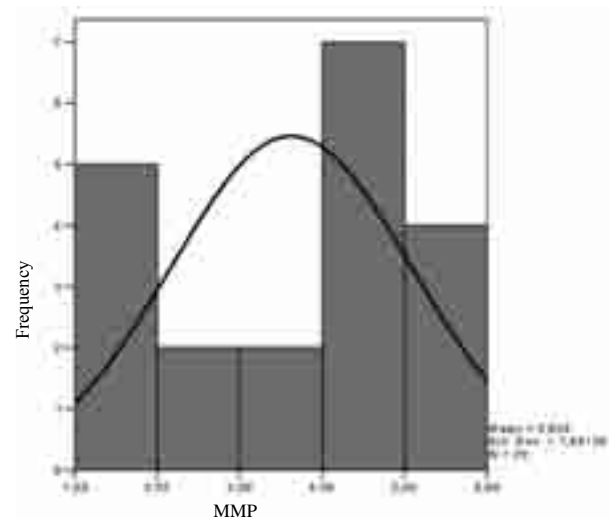


Figure 2. Median values of MMP-1, -8, -13 collagenase concentrations in diffuse periapical lesions

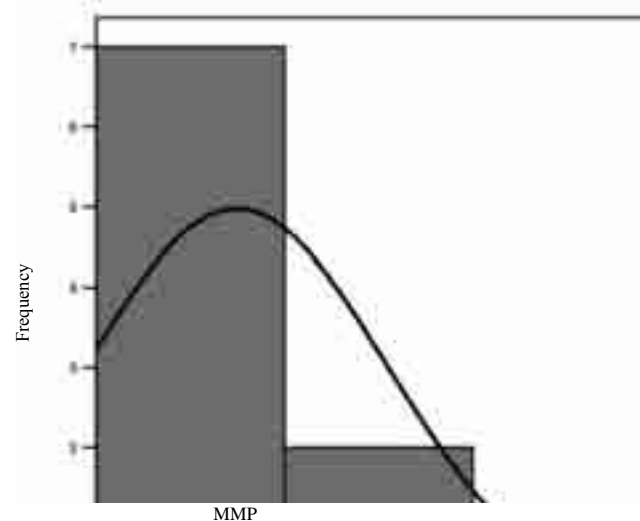


Figure 3. Median values of MMP-1, -8, -13 collagenase concentrations in radicular cysts

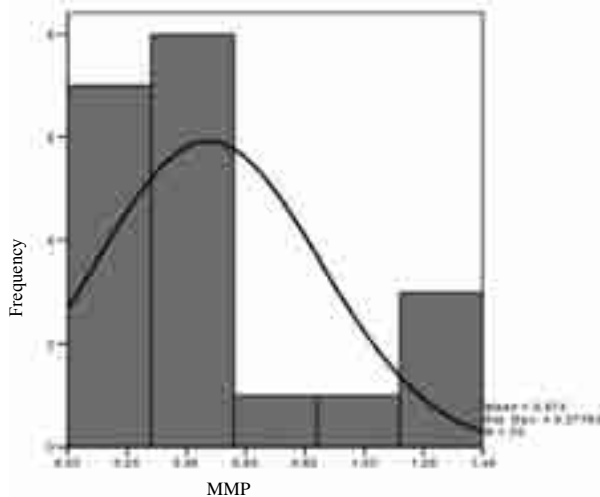


Figure 4. Median values of MMP-1, -8, -13 collagenase concentrations in inflamed pulp tissues

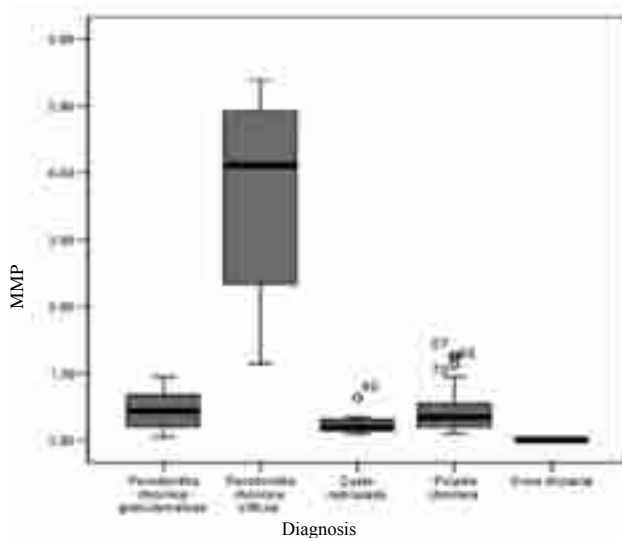


Figure 5. Box-plot diagram of median values of MMP-1, -8, -13 collagenase concentrations in different clinical diagnoses

## Discussion

Most literature findings confirm that MMPs are present in inflammatory tissue of pulp and periapical lesions, like in other inflammatory tissues<sup>19,26-28</sup>. According to Vu and Werb<sup>25</sup>, microorganisms and their products may act during the process of tissue inflammation through regulation of production of cytokines in order to increase the expression of MMPs or directly to stimulate the cells to produce MMPs. In chronic periapical lesions, beside elimination of infective material, there is a local destruction of tissue due to proteolytic enzymes release, including collagenases (MMP-1, -8, -13)<sup>26</sup>.

Bacterial endotoxins, always present in radicular cysts, stimulate keratocystic proliferation, and therefore bacterial products activate the production of MMPs<sup>2</sup>. This proteolytic enzyme cascades, in most of the cases,

are involved in degradation of bone matrix, basal membrane and epithelial cell processing during the time of cystic expansion. Degradation and synthesis of ECM components in normal, healthy tissue are in constant balance, and collagenases are expressed at very low levels, being precisely controlled<sup>4</sup>.

MMP-1 can't be detected or is expressed in very low level in normal pulp tissue<sup>16</sup>. Our results confirmed the presence of small concentration of collagenases (MMP-1, -8, -13) in pulp tissues of impacted third molars, which varied from minimal value 0.00 ng/mL to maximal 0.02 ng/mL. This finding is in agreement with that of other authors<sup>4,14,16</sup>.

During chronic inflammation, proliferation of fibroblasts and vascular elements, infiltration of leucocytes, macrophages and plasma cells, play essential role of cell mediator mechanisms involved in chronic periapical lesions<sup>17</sup>. Plasma cells, which enter the inflamed tissue after the PMNs (polymorphnuclear leukocytes), secrete immunoglobulins and express MMP-8 and MMP-13<sup>27</sup>. Monocytes/macrophages express MMP-8 and MMP-13, and these MMPs may work not only extracellularly, taking part in tissue destruction, but also intracellularly, in the phagocytic process<sup>7,15</sup>.

Wahlgren et al<sup>26</sup> detected MMP-8 in the root canal exudates even after root canal was cleaned by chemical and mechanical means, which shows that the MMP-8 must be of periapical origin and that the inflammatory process in the apical area is diminishing slowly but clearly during 3 weeks. Detection of decreased MMP-8 levels during root canal treatment may lead to adjunctive chair-side or point-of-care diagnostic tools similar to the one developed for periodontitis gingival cervical fluid and peri-implant sulcular fluid to evaluate the periapical inflammation process<sup>8,12,19,26</sup>. Chemical compounds, such as chlorhexidine, often used as adjunctive medication in periapical and periodontal treatment, in addition to its antimicrobial properties, exert properties directly *in vitro* and *in vivo* to inhibit the MMPs<sup>3,12,18,26</sup>.

In our study, results from quantitative enzyme method demonstrated that the concentrations of collagenases (MMP-1, -8, -13) in chronically pulp inflammation were significantly higher than those of the control group ( $p < 0.05$ ). In addition, concentrations of collagenases in patients with diagnosed periapical lesions were significantly higher than those of the control group and were higher from the patients with chronic pulp inflammation ( $p < 0.05$ ). Our findings are in agreement with findings of Shin et al<sup>16</sup>, where ELISA results demonstrated that the concentrations of MMP-1, -2 and -3 in the all experimental groups were significantly higher than those of the controls ( $p < 0.05$ ); also, concentration of MMP-1 in the periapical lesion group was higher than that in the chronic pulp group. These authors<sup>16</sup> suggest that MMPs play a role in the progress of pulp inflammation and pulp tissue destruction, which is also our conclusion.

Collagenases (MMP-1 and MMP-8) and gelatinases (MMP-2 and MMP-9) have been detected in cyst fluid and cyst wall extracts<sup>9,22</sup>. Bacterial endotoxin, always present in radicular cysts, stimulate keratinocyte proliferation, and bacterial products activate MMP production<sup>2</sup>. These proteolytic enzyme cascades are most likely involved in the bone matrix degradation, basal membrane and epithelial cell processing during cyst expansion.

In Leonardi et al<sup>10</sup> study, the expression pattern of MMP-13 demonstrates that it was involved in the conversion of a periapical granuloma with epithelium into a radicular cyst. According to these authors, this property was related to the ability of MMP-13 to influence not only the migration of epithelial cell but also the invasion of granulomatous tissue.

MMPs inhibition has also been suggested to decrease bone resorption in pathological conditions and dental caries progression<sup>21</sup>. MMPs inhibition in root canals and periapical tissue may offer new opportunities to root-canal treatment in the future. MMPs are one of the important factors responsible for the kinetics of periapical bone destruction, and therefore it is possible for there to act on periapical bone regeneration after apicoectomy.

## Conclusion

With respect to conventional methods that are present in everyday dental practice, this study opens new opportunities to a contemporary method for diagnostic of the chronic periapical lesions and monitoring of inflammation activity in tissue, based on destructive role of collagenases (MMP-1, -8, 13) in inflammation process, which is directly dependent on concentration of MMP in pathologically changed tissue.

## References

1. Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Bridgedal-Hansen B, DeCarlo A, Engler JA. Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med*, 1993; 4:197-250.
2. Ding Y, Haapasalo M, Kerosuo E, Lounatmaa K, Kotiranta A, Sorsa T. Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Treponema denticola*. *J Clin Periodontol*, 1997; 24:237-248.
3. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases-2,-8 and -9 by chlorhexidine. *Clin Diagn Lab Immunol*, 1999; 6:437-439.
4. Gusman H, Santana RB, Zehnder M. Matrix metalloproteinase levels and gelatinolytic activity in clinically healthy and inflamed human dental pulps. *Eur J Oral Sci*, 2002; 110(5):353-357.
5. Johansson N, Ahonen M, Kähäri VM. Matrix metalloproteinases in tumor invasion. *Cell Mol Life Sci*, 2000; 57:5-15.
6. Kähäri VM, Saarialho-Kere U. Matrix metalloproteinases and their inhibitors in tumour growth and invasion. *Ann Med*, 1999; 31:34-35.
7. Kiili M, Cox SW, Chen HY, Wahlgren J, Maisi P, Eley BM. Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. *J Clin Periodontol*, 2002; 29:224-232.
8. Kiveli-Rajamäki M, Teronen O, Maisi P, Husa V, Tervahartala T, Piriä E, Salo T, Mellanen L, Sorsa T. Laminin-5 gamma2-chain and collagenase-2 (MMP-8) in human peri-implant sulcular fluid. *Clin Oral Impl Res*, 2003; 14:158-165.
9. Kubota Y, Oka S, Nakagawa S, Shirasuna K. Interleukin 1-a enhances type I collagen-induced activation of matrix metalloproteinase-2 in odontogenic keratocyst fibroblasts. *J Dent Res*, 2002; 81:23-27.
10. Leonardi R, Caltabiano CL. Collagenase-3 (MMP-13) is expressed in periapical lesions: an immunohistochemical study. *Int Endod J*, 2005; 38(5):297.
11. Mainardi CL, Hasty DL, Seyer JM, Kang AH. Specific cleavage of human type III collagen by human polymorphonuclear leukocyte elastase. *J Biol Chem*, 1980; 255:12006-10.
12. Mäntylä P, Stenman M, Kinanen D, Tikanoja S, Luoto H, Salo T, Sorsa T. Gingival crevicular fluid collagenase-2 (MMP-8) test stick for chair-side monitoring of periodontitis. *J Periodontol Res*, 2003; 38:436-439.
13. Palosaari H. Matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) in mature human odontoblasts and pulp tissue. The regulation of expression of fibrillar collagens, MMPs and TIMPs by growth factors, transforming growth factor- $\alpha$ 1 (TCF- $\alpha$ 1) and bone morphogenetic protein-2 (BMP-2). Academic dissertation, University of Oulu, Finland, 2003.
14. Palosaari H, Pennington CJ, Larmas M., Edwards DR, Tjäderhane L, Salo T. Expression profile of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in mature human odontoblasts and pulp tissue. *Eur J Oral Sci*, 2003; 111:117-127.
15. Prikk K, Maisi P, Pirila E, Sepper R, Salo T, Wahlgren J, Sorsa T. In vivo collagenase-2 (MMP-8) expression by human bronchial epithelial cells and monocytes/macrophages in bronchiectasis. *J Pathol*, 2001; 194(2):232-238.
16. Shin SJ, Lee JI, Baek SH, Lim SS. Tissue levels of matrix metalloproteinases in pulps and periapical lesions. *Int Endod J*, 2002; 28(4):313-315.
17. Simon JHS. Periapical pathology. In: Cohen S, Burns RC (eds). *Pathways of the Pulp*. 7<sup>th</sup> ed. St.Louis, USA: Mosby, 1998; pp 425-462.
18. Sorsa T, Ding Y, Salo T, Lauhio A, Teronen O, Ingman T. Effects of tetracycline's on neutrophil, gingival and salivary collagenases. A functional and western-blot assessment with special reference to their cellular sources in periodontal diseases. *Ann New York Acad Sci*, 1994; 732:112-131.

19. Sorsa T, Tjäderhane, Salo T. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis*, 2000; 10:311-318.
20. Stahle-Bäckdahl M, Sandstedt B, Bruce K, Lindahl A, Jiménez MG, Vega JA, López-Otin C. Collagenase-3 (MMP-13) is expressed during human fetal ossification and re-expressed in postnatal bone remodelling and in rheumatoid arthritis. *Lab Invest*, 1997; 76:717-728.
21. Sulkala M. Matrix metalloproteinases (MMPs) in dentin-pulp complex of healthy and carious teeth. Academic dissertation, University of Oulu, Finland, 2004.
22. Teronen O, Salo T, Laitinen J, Tornwall J, Ylipaavalniemi P, Konttinen YT, Sorsa T. Characterization of interstitial collagenases in jaw cyst wall. *Eur J Oral Sci*, 1995; 103:141-147.
23. Uitto VJ, Airola K, Vaalamo M, Johansson N, Putnins EE, Firth JD, Salonen J, Lopez-Otin C, Saarialho-Kere U, Kähäri VM. Collagenase-3 (matrix metalloproteinase-13) expression is induced in oral mucosal epithelium during chronic inflammation. *Am J Pathol*, 1998; 152:1489-1499.
24. Uitto VJ, Overall C, McCulloch C. Proteolytic host cell enzymes in gingival crevice fluid. *Periodontol 2000*, 2003; 31:91-104.
25. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes and Development*, 2000; 14:2123-2133.
26. Wahlgren J. Matrix metalloproteinases in pulpitis, chronic apical periodontitis and odontogenic jaw cysts. Academic dissertation, University of Helsinki, Finland, 2003.
27. Wahlgren J, Maisi P, Sorsa T, Sutinen M, Tervahartiala T, Pirilä E, Teronen O, Hietanen J, Tjäderhane L, Salo T. Expression and induction of collagenases (MMP-8 and -13) in plasma cells associated with bone-destructive lesions. *J Pathol*, 2001; 194 (2):217-224.
28. Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjäderhane L. Matrix metalloproteinase-8 (MMP-8) in pulpal and periapical inflammation and periapical root-canal exudates. *Int Endod J*, 2002; 35(11):897.

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