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High Matrix Metalloproteinase Activity is a Hallmark of Periapical Granulomas

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Abstract

Introduction—Inability to distinguish periapical cysts from granulomas prior to performing root canal treatment leads to uncertainty in treatment outcomes, because cysts have lower healing rates. Searching for differential expression of molecules within cysts or granulomas could provide information with regard to the identity of the lesion or suggest mechanistic differences that may form the basis for future therapeutic intervention. Thus, we investigated whether granulomas and cysts exhibit differential expression of extracellular matrix (ECM) molecules.

Methods—Human periapical granulomas, periapical cysts, and healthy periodontal ligament tissues were used to investigate the differential expression of ECM molecules by microarray analysis. Since matrix metalloproteinases (MMP) showed the highest differential expression in the microarray analysis, MMPs were further examined by *in situ* zymography and immunohistochemistry. Data were analyzed using one-way ANOVA followed by Tukey test.

Results—We observed that cysts and granulomas differentially expressed several ECM molecules, especially those from the matrix metalloproteinase (MMP) family. Compared to cysts, granulomas exhibited higher MMP enzymatic activity in areas stained for MMP-9. These areas were composed of polymorphonuclear cells (PMNs), in contrast to cysts. Similarly, MMP-13 was expressed by a greater number of cells in granulomas compared to cysts.

Conclusion—Our findings indicate that high enzymatic MMP activity in PMNs together with MMP-9 and MMP-13 stained cells could be a molecular signature of granulomas, unlike periapical cysts.

Keywords

periapical cyst; periapical granuloma; periodontal ligament; extracellular matrix components; matrix metalloproteinases

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INTRODUCTION

Periapical disease represents the progression of a bacterial infection from the dental pulp to apical foramen that results in a localized inflammatory response concomitant with bone resorption (1–4). Periapical lesions include granulomas and cysts, and both are thought to represent different stages of the same inflammatory process (5–7).

Differential diagnosis of granulomas and cysts using radiographic analysis is problematic. Although preliminary studies have proposed that CT scans and ultrasound with power Doppler flowmetry can provide an additional diagnostic tool in Endodontics (8–10), it is widely accepted that histologic evaluation is necessary to confirm diagnosis (4,11–14). However, microscopic examination can only be performed after the periapical disease is removed, which is a limitation, since in most cases root canal treatment is performed without removing the lesion. This inability to identify the status of periapical disease makes treatment outcomes unpredictable because cysts exhibit lower healing rates and generally require additional surgical treatment (6,15–18).

Identifying extracellular matrix molecules (ECM) specific to human periapical cysts or granulomas can provide information to potentially discriminate between these lesions. Specific proteins present in the extracellular matrix and their respective receptors may offer the basis to develop novel approaches aiming to detect disease biomarkers and therefore to improve diagnosis for cysts and granulomas prior to performing root canal treatment. Possible likely candidates as biomarkers for periapical inflammation include proteases that are responsible for ECM degradation such as matrix metalloproteinases (MMPs). MMPs are a family of metal-dependent endopeptidases, which are secreted as inactive proenzymes (zymogens) and activated in tissues by cleavage of the propeptide (19–23). Although MMPs have been reported in periapical lesions (11,24–30), a direct comparison of MMPs in cysts and granulomas has not been undertaken. Since MMPs must be activated to exert their function, it is also important to localize areas of proteinase activity within lesions. Thus, the aim of this study was to compare the expression of several ECM molecules and cell membrane receptors within different cellular components from human periapical granulomas and cysts. In addition, we sought to examine the presence and activity of MMPs in these tissues because of their reported involvement in these lesions, and because our initial data revealed differential expression of this class of proteins in these tissues.

MATERIAL AND METHODS

Collection of samples

Formalin-fixed paraffin-embedded sections from 10 periapical granulomas and 10 periapical cysts were obtained from the archives of the Oral Pathology Biopsy Service at the University of Michigan School of Dentistry after Institutional Review Board approval. Periodontal ligament (PDL) tissues obtained from the middle third of the dental root of extracted healthy teeth, after Institutional Review Board approval, were used as controls.

Histological examination of the samples

Histological examination was performed by a pathologist on hematoxylin-eosin stained slides of tissues from the apical regions of non vital teeth. Periapical cysts were selected based on the presence of granulation tissue with lining stratified squamous epithelium. Periapical granulomas were selected based on the presence of granulation tissue without lining epithelium (16,17,31). Fibroblast-like cells were characterized by their spindle shaped morphology,

mononuclear inflammatory cells were identified as cells with a large single nucleus, and PMNs were identified by their multilobed nuclei.

RNA extraction and microarray analyses

Paraffin-embedded tissue sections were deparaffinized, exposed to protease digestion and immersed in Trizol® Reagent (Invitrogen Corporation, Carlsbad, CA) for RNA extraction. Extracted RNA was purified using the RNEasy Micro Kit (Qiagen, Valencia, CA). The quality and concentration of isolated RNA was evaluated using a capillary electrophoresis system in an RNA 6000 Pico LabChip (Agilent 2100 BioAnalyzer, Agilent Technologies Inc, Santa Clara, CA). Analysis of expression of ECM molecules (Table 1) in tissues was performed using focused cDNA microarrays (SuperArray, Bioscience Corporation, Frederick, MD). Ten micrograms of total RNA were used as a template to generate biotin-16-dUTP labeled cDNA probes. cDNA probes were denatured and hybridized at 60°C with the SuperArray membranes, which were developed using chemiluminescence. Gene expression was evaluated by densitometric analysis of the membrane spots. Comparison among groups was performed on a gene-by-gene basis after normalization by β-actin mRNA expression.

In situ zymography

Five μm-thick tissue sections were immersed in sodium borohydride (1 mg / ml) followed by incubation with a fluorescein isothiocyanate (FITC)-bound gelatin substrate (DQ™ Gelatin, Molecular Probes, Eugene, OR) dissolved in agarose (0.1 mg / ml) for 3 hr at 37°C in a humidified light-protected chamber. Nuclei were counterstained by adding 4'-6-Diamidino-2-phenylindole (DAPI; 0.5 μg / ml) to the incubation medium. Control slides were preincubated in 20 mM ethylene diamine tetraacetic acid (EDTA, Sigma, St Louis, MO) for 1 hr, and then EDTA was added to the incubation medium. Quantification of gelatinolytic activity in the sections was assessed by counting the number of spots of fluorescence in representative areas (40× magnification) and expressed as number of spots of enzymatic activity *per* mm^2.

Immunohistochemistry

Specific MMPs were detected by immunohistochemistry. Tissue sections were quenched in a 6% H_2O_2 methanol solution for 30 min and boiled in 10 mM sodium citrate (pH 6.0) at 93°C for 15 min for antigen retrieval. Nonspecific binding was blocked with 1% bovine serum albumin (Sigma) for 30 min and sections were incubated for 1 hr with primary antibodies for MMP-2 (5 μg / ml; MAB3308, Chemicon, Temecula, CA), MMP-9 (5 μg / ml; MAB3309, Chemicon), and MMP-13 (5 μg / ml; M4052, Sigma). Sections were incubated with secondary antibody followed by streptavidin horseradish peroxidase and 3,3'-diaminobenzidine (DAB500 Chromogen System, Biocare Medical). Tissues were counterstained with Mayer's Hematoxylin and mounted using standard protocols. Negative controls consisted of replacing the primary antibody with mouse or rabbit IgG. The number of positive cells was calculated for each antibody in three representative fields-of-view (100× magnification).

Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey test ($\alpha = 0.05$).

RESULTS

Periapical granulomas exhibit a higher percentage of PMNs compared to cysts

Mononuclear inflammatory cells were the most prevalent cells found in both cysts and granulomas (59.8% ± 19.8 *versus* 43.1% ± 3.8, respectively; $p > 0.05$). Similar quantities of fibroblast-like cells were also observed in both lesions (24.1% ± 8.4 in cysts *versus* 24.6% ± 4.0 in granulomas; $p > 0.05$). In contrast, PMNs were more prevalent in periapical granulomas

compared to cysts (32.1 ± 5.8% *versus* 15.9% ± 7.5 respectively; $p < 0.05$). Inflammatory activity, assessed as a ratio of inflammatory cellular components (PMNs plus mononuclear cells) to fibroblast-like cell count, was similar for both cysts and granulomas (3.2 and 3.3 respectively), however the composition of inflammatory activity was different between the lesions, since PMNs predominated in granulomas. Periapical cysts were surrounded by a stratified squamous epithelium and fibroblast-like cells were observed in the stroma and surrounding the lesions. Similar to cysts, fibroblast-like cells were distributed throughout granuloma lesions but no epithelial cell layer was evident.

ECM genes are differentially expressed in granulomas and cysts compared to healthy PDL

Out of 113 genes, eleven genes in periapical granulomas and 17 genes in cysts showed higher expression levels than in healthy PDL (Figure 1). Of those overexpressed, ADAM metallopeptidase-1 (ADAMTS1), integrin-β4 (ITGB4), integrin-β7 (ITGB7), laminin-α1 (LAMA1), MMP-2 and tissue inhibitor of metalloproteinase-1 (TIMP1) were similarly expressed in cysts and granulomas.

Extracellular matrix protein-1 (ECM1), integrin-α2B (ITGA2B), MMP-10, MMP-7, integrin-αM (ITGAM), and laminin-β2 (LAMB2) genes were more frequently expressed in cysts, followed by a lower expression in granulomas, and the lowest expression was detected in healthy PDL. Osteonectin (SPARC) gene expression was similar in all tissues.

Integrin-α3, integrin-α5, and integrin-β1 in cysts and transforming growth factor β-in granulomas were significantly elevated in lesions compared to healthy PDL. MMP-24 was detected in granulomas and cysts but not in healthy PDL, and ADAM metallopeptidase-13 and secreted phosphoprotein-1 were found exclusively in cysts but not in granulomas or healthy PDL. Catenin-α1, TIMP-3, and selectin-L were found exclusively in healthy PDL but not in periapical lesions, thus these genes were suppressed in the lesions (Table 2). We selected for presentation only those genes which were up or downregulated in cysts and granulomas. Relative expression for genes not modulated or genes not expressed were not reported.

Periapical granulomas exhibit high gelatinolytic activity compared to cysts

Given the high prevalence of MMPs and higher MMP-2 mRNA expression in both cysts and granulomas compared to healthy PDL, the presence of MMP activity in tissue sections was further investigated. *In situ* zymography revealed gelatinolytic activity in both cysts and granulomas. Gelatinolytic activity, detected in the inflammatory cell area, was more intense in granulomas (Figure 2A, 2B) than in cysts (Figure 2C, 2D). Low enzymatic activity was detected in healthy PDL (Figure 2G).

The shape of the DAPI-stained nuclei, indicated that gelatinolytic activity was predominantly in areas with PMNs (Figure 2B). Enzymatic activity was sparse adjacent to mononuclear inflammatory cells and fibroblast-like cells, and absent in the epithelial layer in periapical cysts (data not shown). Sections incubated with EDTA confirmed that proteases responsible for gelatinolytic activity were MMPs (Figure 2E). Absence of autofluorescence was confirmed by incubating tissues with agarose lacking FITC-bound gelatin (Figure 2F).

MMP-2, MMP-9 and MMP-13 are differentially expressed by inflammatory and stromal cells in cysts and granulomas

In situ zymography revealed the presence of generic MMP activity, however it did not reveal the specific MMP profile present in these tissues. Since MMP-2, MMP-9 and MMP-13 use gelatin as a substrate (22,32), immunostaining for these specific MMPs was performed.

Immunopositive cytoplasmic staining for MMP-2 was observed in a higher number of stromal and mononuclear inflammatory cells compared to PMNs ($p < 0.01$) in both granulomas and cysts, without differences between them (Figure 2H, 2I, Figure 4A, 4D). In contrast, MMP-9 and MMP-13 were expressed by a greater number of cells in granulomas compared to cysts ($p < 0.01$) (Figure 2J, 2L, 2M, 2N, Figure 4B, 4C).

MMP-9 was expressed more intensely by inflammatory cells *versus* stromal cells in both granulomas and cysts. Among these, PMNs were predominantly stained in granulomas (Figure 2J), whereas mononuclear cells were stained to a higher percentage in cysts (Figure 2L). Of all PMNs in periapical granulomas, 95.6% were MMP-9 positive, unlike cysts, which exhibited fewer MMP-9 positive PMNs (Figure 4E). MMP-9 expression in granulomas was observed in areas with high levels of enzymatic activity in PMNs (Figure 3).

MMP-13 was observed primarily in mononuclear cells in both lesions, and to a lesser degree in stromal cells (Figure 2M, 2N). A lower percentage of PMNs was stained in granulomas, however this was higher than that in cysts, where most PMNs were not stained ($p < 0.01$) (Figure 4C and 4F).

In periapical cysts, epithelial cells were positively stained for MMP-2, MMP-9, and MMP-13 but no enzymatic activity was detected (data not shown), indicating that these MMPs were inactive. IgG staining controls revealed low non-specific staining (Figure 2O and 2P).

DISCUSSION

Periapical disease pathogenesis involves degradation of several ECM components including collagen, fibronectin, laminin, and small proteoglycans (33). MMPs are likely candidates in this process since these ECM components are known MMP substrates. Using *in situ* zymography, we demonstrated the presence and increased levels of active MMPs in the inflammatory cell zone in periapical granulomas compared to cysts. Although MMP expression has been immunolocalized in human periapical lesions (11,24–28,34), this is the first study to identify areas of MMP enzymatic activity.

Different MMP immunostaining patterns were observed in cysts and granulomas. The minimal epithelial layer in cysts was positively stained for MMP-2, MMP-9, and MMP-13, although it exhibited low MMP enzymatic activity. This may result from the reduced stroma present in this thin epithelial compartment (35). However, the larger stromal compartments of both lesions, where the inflammatory cells are located, exhibited higher MMP enzymatic activity. In lesional stroma, MMP-2 expression was diffuse throughout the ECM, and cells exhibited weak cytoplasmic staining. On the other hand, MMP-9 and MMP-13 staining were localized and highly expressed in the inflammatory cell area, with strong intracellular staining. Interestingly, higher MMP-13 expression and more evident MMP-9 positive staining in PMNs were observed in granulomas compared to cysts.

Simultaneous upregulation of MMP-9 and MMP-13 in granulomas suggests coordinate MMP regulation in the dynamics of periapical disease. Interaction between MMP-9 and MMP-13 could be explained by the ability of MMP-13 to activate MMP-9 (32), thus explaining the more intense enzymatic activity observed in granulomas.

The role of PMNs in periapical disease pathogenesis is well-established (36). Differential expression of MMP-9 in PMNs from granulomas compared to cysts represents an interesting finding, since PMNs uniquely regulate MMP expression. Synthesis of MMP-9 is complete by the time PMNs enter the vasculature, and regulation of this protease is mediated by granule release rather than by transcriptional events (37). Thus, it is not surprising that microarray data did not reveal changes in mRNA expression for MMP-9. Furthermore, MMP activity in general

is regulated post-transcriptionally by proteolytic processing and by tissue inhibitors of metalloproteinases, so changes in MMP activity would not necessarily be reflected at the gene level or in the microarray data for any of the MMPs. However, the mechanism involved in the recruitment of a higher percentage of PMNs positively stained for MMP-9 and the higher enzymatic activity observed in granulomas compared to cysts remains to be investigated.

Previous reports showed that MMP-1 expression is restricted to the lining epithelium, subepithelial macrophages, fibroblasts, and endothelial cells in human periapical cysts (11, 29) and to areas showing active bone resorption during periapical disease development in rats (39). However, we observed MMP-9 and MMP-13 staining more intensely in inflammatory cells in these lesions. Taken together, these findings indicate that specific MMPs are produced in different cell types in periapical lesions, suggesting specific roles for these enzymes in periapical disease pathogenesis. Indeed, recent reports have demonstrated distinct roles for MMPs in periapical disease progression, either stimulating (40) or attenuating lesion development (41). These data and our current findings underscore the importance of characterizing the profile of MMPs involved in periapical lesions prior to using pan-MMP inhibitors therapeutically that can non-specifically inhibit MMPs (23).

Using ECM-focused microarrays, we demonstrated that some MMPs are more highly expressed in cysts and granulomas than in healthy PDL. In addition, high gelatinolytic activity was present in granulomas, especially in the inflammatory cell zone, and immunostaining confirmed the presence of MMP-9 and MMP-13 in this process. Recently, gingival crevicular fluid analysis (GCF) revealed that patients with periapical lesions exhibit higher MMP-2 and MMP-9 expression compared to control subjects (29), however the identity of the disease was not further investigated. Since differential MMP-9 and MMP-13 expression was observed between cysts and granulomas in this study, these molecules may serve as molecular markers of the status of periapical disease and could be further explored *in vivo* in combination with GCF analysis to clinically differentiate periapical cysts from granulomas.

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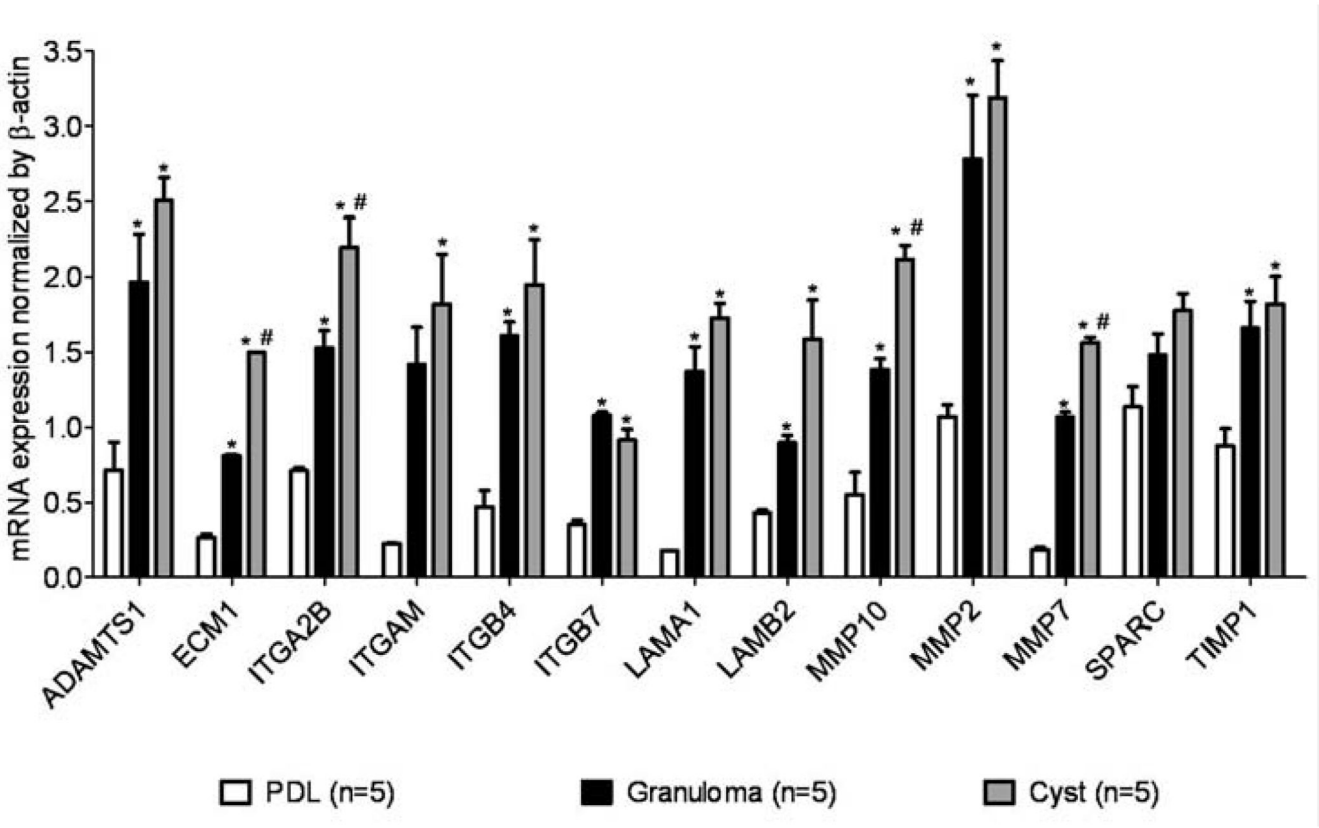

Figure 1.

mRNA expression for human extracellular matrix molecules in periapical granulomas and cysts is different from healthy PDL tissue. For comparison purposes, mRNA expression was normalized to the housekeeping gene β-actin. Graph bars depict mean and standard deviation. * $p < 0.05$ compared to healthy PDL tissue, #$p < 0.05$ compared to periapical granuloma; n = 15.

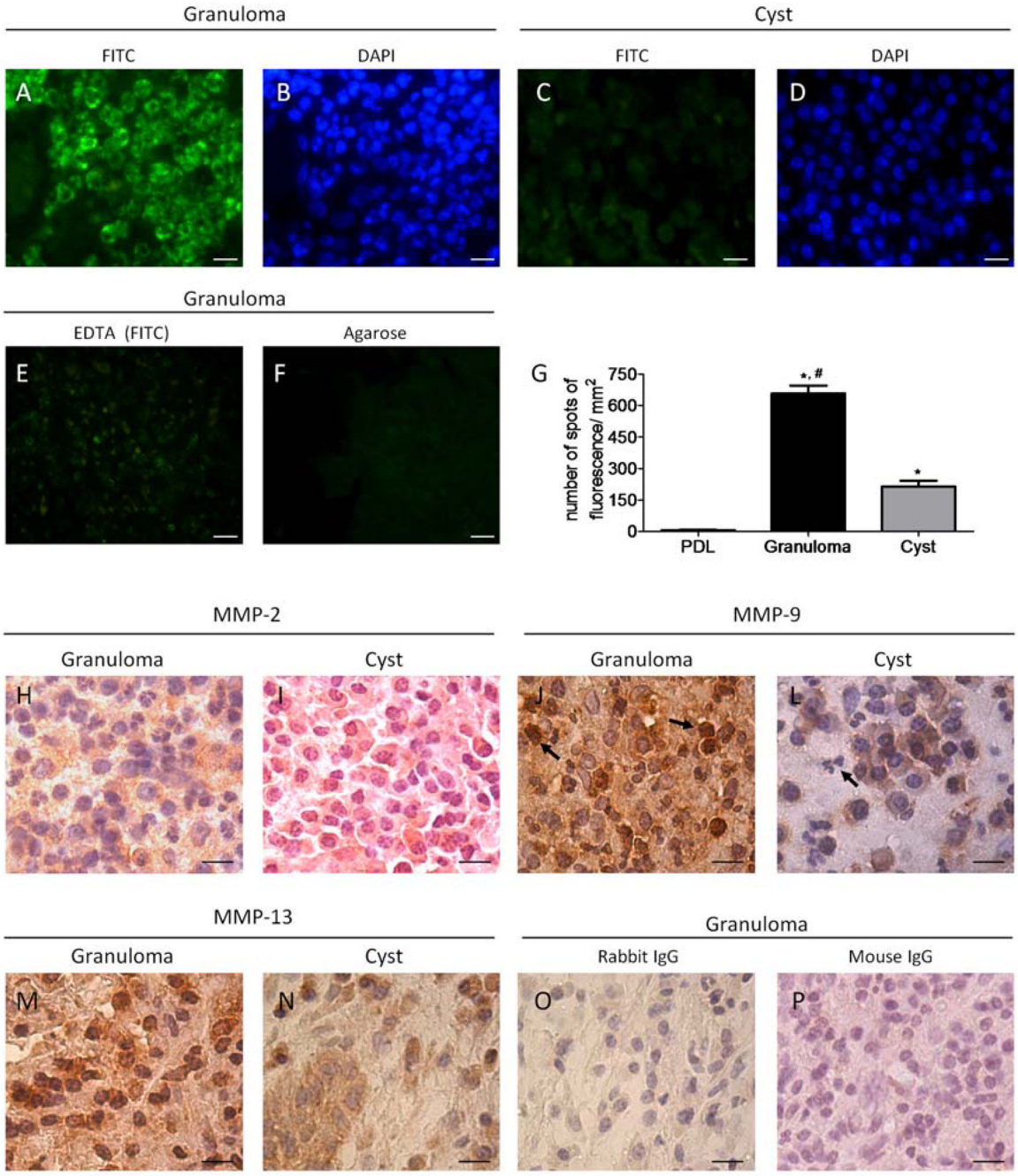

Figure 2.

Matrix metalloproteinase expression and activity are distinct in periapical granulomas and cysts. Areas of gelatinolytic activity were detected by *in situ* zymography and appeared as green spots of fluorescence (**A, C, E, F**); cellular components in the area were visualized by DAPI nuclear staining (**B, D**). Control slides were incubated with EDTA to confirm if gelatinolytic activity was from MMPs (**E**). Levels of autofluorescence in the sections were evaluated by incubating the tissue with agarose without FITC-bound gelatin (**F**). Graphs represent the number of spots of fluorescence per mm^2 in cysts, granulomas, and healthy PDL tissue counted as described in M & M; $*p < 0.05$ compared to healthy PDL tissue, $\#p < 0.05$ compared to periapical cysts (n = 25) (**G**). Immunostaining for MMP-2, MMP-9 and MMP-13

was performed to confirm the profile of MMPs in periapical cysts and granulomas. MMP-2 stained similarly cells in granulomas (**H**) and cysts (**I**) whereas MMP-9 expression was observed in PMN (arrows) in granulomas (**J**) but in not in periapical cysts (**L**). A wide-range of cells was strongly positive for MMP-13 in granulomas (**M**) unlike periapical cysts, which presented faint positive staining in mononuclear cells (**N**). Control staining for slides where the primary antibody was omitted and the slides were incubated with rabbit (**O**) or mouse IgG (**P**) revealed low unspecific staining; bar = 10 μm; magnification 100×.

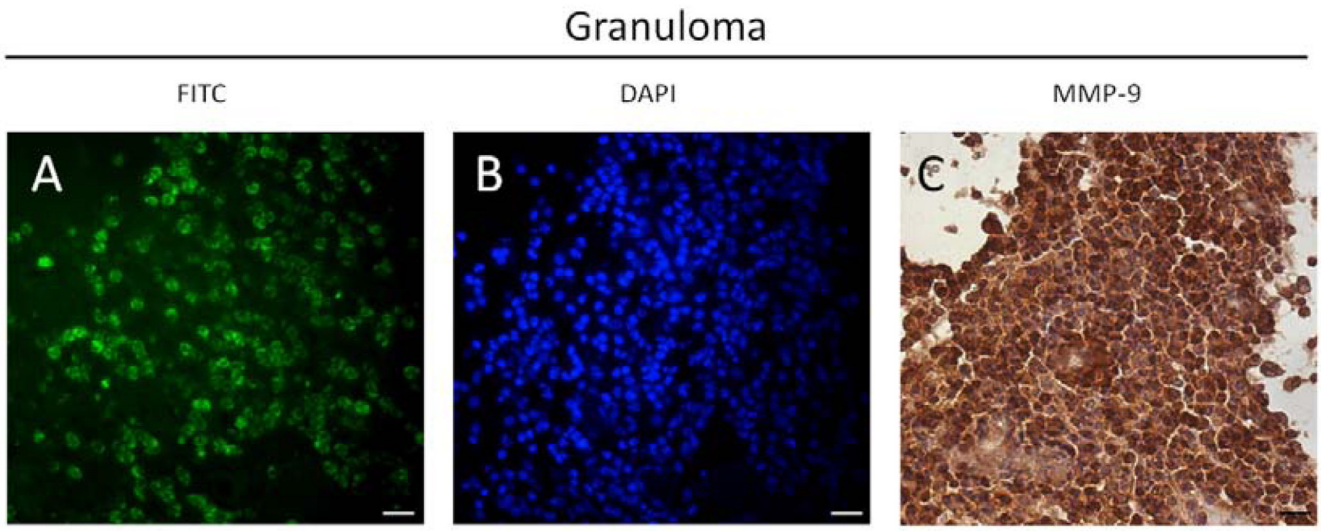

Figure 3.
Areas of MMP enzymatic activity (**A**) in PMN (**B**) in periapical granulomas were co-localized to MMP-9 expression detected by immunostaining (**C**). Bar = 10 μm.

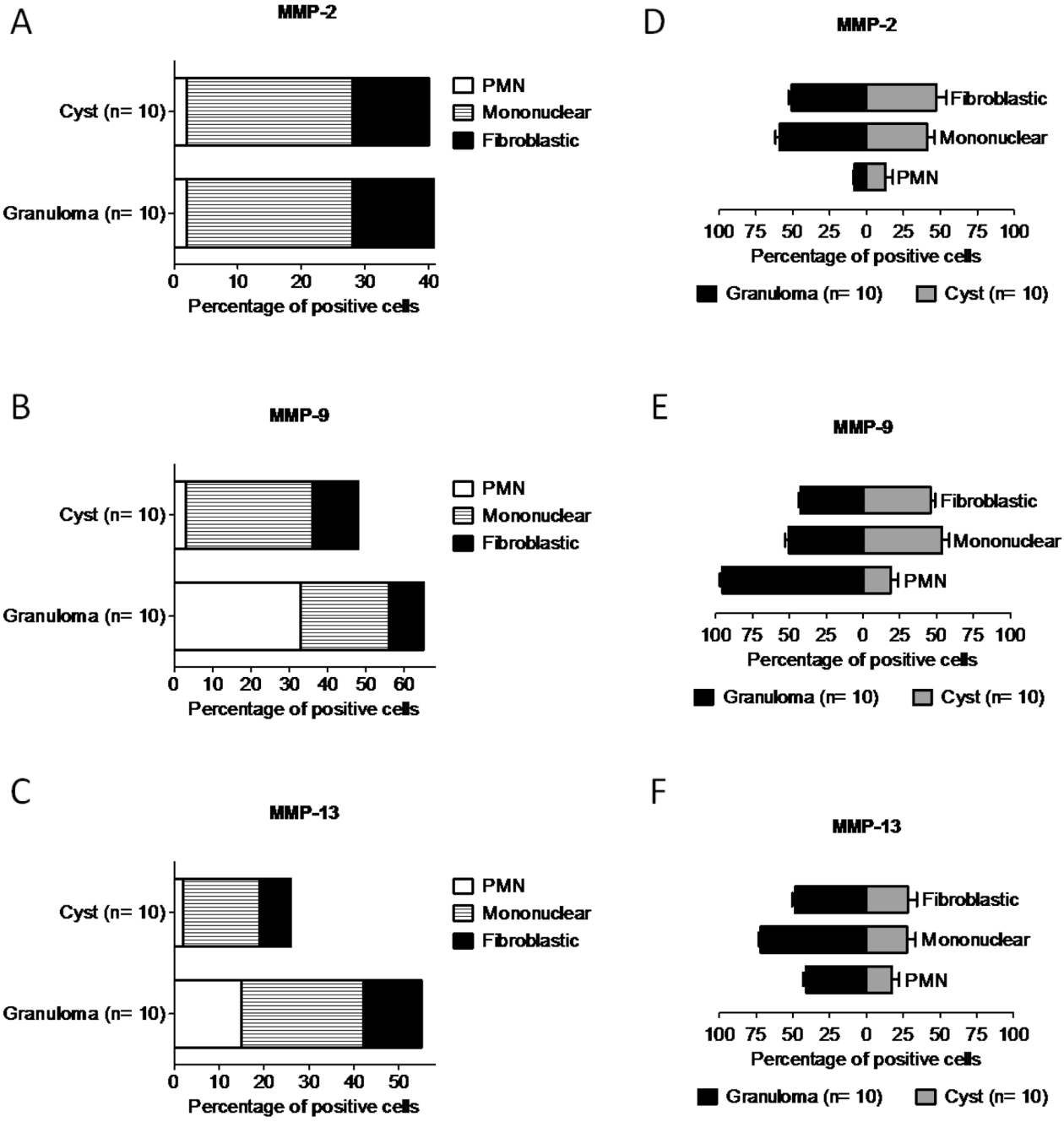

Figure 4.

MMPs are differently expressed by several cellular components in periapical lesions. Quantification of cell staining was performed by grouping cells into polymorphonuclear leukocytes (PMN), mononuclear or fibroblast-like cells. The percentage of cells expressing MMP-2, MMP-9 and MMP-13 was calculated in relation to the total amount of cells *per* field-of-view in three representative areas (**A, B, C**). To further estimate the amount of positive cells within the same group of cells, the percentage of PMN, mononuclear and fibroblastic expressing MMP-2 (**D**), MMP-9 (**E**) and MMP-13 (**F**) was calculated; n = 20.

Table 1

Genes evaluated by the Oligo GEArray Human Extracellular Matrix and Adhesion Molecules Microarray

Extracellular Matrix Proteins	
Basement Membrane Constituents	Collagen, type IV, alpha 2
	Collagen, type IV, alpha 3
	Collagen, type IV, alpha 6
	Collagen, type VII, alpha 1
	Collagen, type VIII, alpha 2
	Laminin, alpha 1
	Laminin, alpha 2
	Laminin, alpha 3
	Laminin, alpha 4
	Laminin, alpha 5
	Laminin, beta 1
	Laminin, beta 2
	Laminin, beta 3
	Laminin, gamma 1 (formerly LAMB2)
	Secreted protein, acidic, cysteine-rich (osteonectin)
Collagens and ECM Structural	
Constituents	Collagen, type XI, alpha 1
	Collagen, type XI, alpha 2
	Collagen, type XII, alpha 1
	Collagen, type XIV, alpha 1
	Collagen, type XV, alpha 1
	Collagen, type XVI, alpha 1
	Collagen, type XIII, alpha 1
	Collagen, type XIX, alpha 1
	Collagen, type I, alpha 1
	Collagen, type XXIV, alpha 1
	Collagen, type XXVII, alpha 1
	Collagen, type IV, alpha 2
	Collagen, type IV, alpha 3
	Collagen, type IV, alpha 6
	Collagen, type V, alpha 1
	Collagen, type V, alpha 3
	Collagen, type VI, alpha 1
	Collagen, type VI, alpha 2
	Collagen, type VI, alpha 3
	Collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
Collagens and ECM Structural	
Constituents	
Collagen, type VIII, alpha 1	
	Collagen, type VIII, alpha 2

Extracellular Matrix Proteins	
	Collagen, type IX, alpha 1
	Fibronectin 1
	Kallmann syndrome 1 sequence
	Laminin, alpha 4
ECM Proteases	ADAM metallopeptidase with thrombospondin type 1 motif, 1
	ADAM metallopeptidase with thrombospondin type 1 motif, 13
	ADAM metallopeptidase with thrombospondin type 1 motif, 8
	Matrix metallopeptidase 1 (interstitial collagenase)
	Matrix metallopeptidase 10 (stromelysin 2)
	Matrix metallopeptidase 11 (stromelysin 3)
	Matrix metallopeptidase 12 (macrophage elastase)
	Matrix metallopeptidase 13 (collagenase 3)
	Matrix metallopeptidase 14 (membrane-inserted)
	Matrix metallopeptidase 15 (membrane-inserted)
	Matrix metallopeptidase 16 (membrane-inserted)
	Matrix metallopeptidase 17 (membrane-inserted)
	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
	Matrix metallopeptidase 20 (enamelysin)
	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)
	Matrix metallopeptidase 7 (matrilysin, uterine)
ECM Proteases	Matrix metallopeptidase 8 (neutrophil collagenase)
	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
	Matrix metallopeptidase 24 (membrane-inserted)
	Matrix metallopeptidase 26
	Spastic paraplegia 7 (pure and complicated autosomal recessive)
	TIMP metallopeptidase inhibitor 1
ECM Protease Inhibitors	Collagen, type IV, alpha 3
	Collagen, type VI, alpha 3
	Collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
	Kallmann syndrome 1 sequence
	Sparc/ Osteonectin, cwcv and kazal-like domains proteoglycan
	Thrombospondin 1
	TIMP metallopeptidase inhibitor 1
	TIMP metallopeptidase inhibitor 2
	TIMP metallopeptidase inhibitor 3
Other ECM Molecules	Chondroitin sulphate proteoglycan 2 (versican)
	Connective tissue growth factor
	Extracellular matrix protein 1
	Hyaluronan synthase 1
	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
	Transforming growth factor, beta-induced, 68kDa
	Thrombospondin 2

Extracellular Matrix Proteins	
	Thrombospondin 3
	Thrombospondin 4
	Laminin, beta 4
	Tetranectin (plasminogen binding protein)
	Tenascin C (hexabrachion)
	Vitronectin
Cell Adhesion Molecules	
Transmembrane Molecules	Cadherin 1, type 1, E-cadherin (epithelial)
	CD44 molecule
	Hyaluronan synthase 1
	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
	Integrin, alpha 1
	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
	Integrin, alpha 2b
	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
	Integrin, alpha 6
	Integrin, alpha 7
	Integrin, alpha 8
	Integrin, alpha L
	Integrin, alpha M
	Integrin, alpha V
	Integrin, beta 1
	Integrin, beta 2
	Integrin, beta 3
	Integrin, beta 4
	Integrin, beta 5
	Integrin, beta 6
	Integrin, beta 7
	Integrin, beta 8
	Matrix metallopeptidase 14 (membrane-inserted)
	Matrix metallopeptidase 15 (membrane-inserted)
	Matrix metallopeptidase 16 (membrane-inserted)
	Matrix metallopeptidase 17 (membrane-inserted)
	Matrix metallopeptidase 24 (membrane-inserted)
	Neural cell adhesion molecule 1
	Platelet/endothelial cell adhesion molecule (CD31 antigen)
Transmembrane Molecules	Selectin E (endothelial adhesion molecule 1)
	Selectin L (lymphocyte adhesion molecule 1)
	Selectin P (granule membrane protein 140kDa, antigen CD62)
	Sarcoglycan, epsilon

Extracellular Matrix Proteins	
	Spastic paraplegia 7 (pure and complicated autosomal recessive)
	Vascular cell adhesion molecule 1
Cell-Cell Adhesion	CD44 molecule
	Cadherin 1, type 1, E-cadherin (epithelial)
	Collagen, type XI, alpha 1
	Collagen, type VI, alpha 2
	Collagen, type VIII, alpha 2
	Collagen, type XIX, alpha 1
	Catenin (cadherin-associated protein), delta 1
	Integrin, alpha 8
	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
	Vascular cell adhesion molecule 1
Cell-Matrix Adhesion	ADAM metallopeptidase with thrombospondin type 1 motif, 13
	CD44 molecule
	Integrin, alpha 1
	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
	Integrin, alpha 2b
	Integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)
	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
	Integrin, alpha 6
	Integrin, alpha 7
	Integrin, alpha 8
	Integrin, alpha 9
	Integrin, alpha 10
	Integrin, alpha 11
Cell-Matrix Adhesion	Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
	Integrin, alpha M (complement component 3 receptor 3 subunit)
	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
	Integrin, alpha X
	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
	Integrin, beta 4
	Integrin, beta 5
	Integrin, beta 6
	Integrin, beta 7
	Integrin, beta 8
	Sarcoglycan, epsilon
	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
	Thrombospondin 3
Other Adhesion Molecules	Contactin 1

Extracellular Matrix Proteins
Collagen, type XI, alpha 2
Collagen, type XII, alpha 1
Collagen, type XV, alpha 1
Collagen, type XVI, alpha 1
Collagen, type XVIII, alpha 1
Collagen, type XXIV, alpha 1
Collagen, type XXVII, alpha 1
Collagen, type IV, alpha 3
Collagen, type IV, alpha 6
Collagen, type V, alpha 1
Collagen, type V, alpha 3
Collagen, type VI, alpha 1
Collagen, type VI, alpha 3
Collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
Other Adhesion Molecules Collagen, type VIII, alpha 1
Collagen, type IX, alpha 1
Chondroitin sulphate proteoglycan 2 (versican)
Connective tissue growth factor
Catenin (cadherin-associated protein), alpha 1
Catenin (cadherin-associated protein), beta 1
Catenin (cadherin-associated protein), delta 2
Fibronectin 1
Kallmann syndrome 1 sequence
Laminin, alpha 1
Laminin, alpha 2
Laminin, alpha 3
Laminin, alpha 4
Laminin, alpha 5
Laminin, beta 1
Laminin, beta 2
Laminin, beta 3
Laminin, gamma 1 (formerly LAMB2)
Sparc/ Osteonectin, cwcv and kazal-like domains proteoglycan
Thrombospondin 1
Thrombospondin 2
Thrombospondin 4
Tetranectin (plasminogen binding protein)
Tenascin C (hexabrachion)
Vitronectin

Table 2

Human extracellular matrix molecules mRNA expression in periapical cysts, granulomas, and / or in healthy PDL tissue. mRNA expression was normalized by β-actin

Transcripts	Healthy PDL tissue	Granuloma	Cyst
ADAM metallopeptidase 13	—	—	1.22 ± 0.06
Collagen type XVIII α1	0.67 ± 0.24	—	1.14 ± 0.01
Catenin-α1	0.39 ± 0.11	—	—
Integrin-α3	0.46 ± 0.08	—	1.67 ± 0.01*
Integrin-α5	0.79 ± 0.02	—	1.25 ± 0.01*
Integrin-β1	0.75 ± 0.1	—	2.06 ± 0.01*
Laminin-α5	0.74 ± 0.1	—	1.03 ± 0.01
Matrix metalloproteinase-24	—	1.05 ± 0.32	1.34 ± 0.59
Secreted phosphoprotein-1	—	—	1.38 ± 0.01
Transforming growth factor-β	0.46 ± 0.04	1.01 ± 0.1*	—
Tissue inhibitor of metalloproteinase-3	0.64 ± 0.21	—	—
L-selectin	0.32 ± 0.12	—	—

* $p < 0.05$ compared to healthy PDL tissue