

## Interleukin-17 modulates uPA and MMP2 expression in human periodontal ligament mesenchymal stem cells: involvement of the ERK1/2 MAPK pathway

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**Abstract:** Periodontal disease is a chronic infection of periodontal tissue characterized by extracellular matrix (ECM) degradation due to increased expression of plasminogen activators and matrix metalloproteinases (MMPs) and various proinflammatory cytokines, including interleukin (IL)-17. Successful regeneration of damaged periodontal tissues depends on the proper functionality of periodontal ligament mesenchymal stem cells (PDLMSCs), especially the production of extracellular matrix proteases. We investigated the influence of IL-17 on ECM remodeling through modulation of urokinase-type plasminogen activator (uPA) and MMP2/MMP9 expression in human PDLMSCs at mRNA, protein and activity levels using by RT-PCR, Western blotting and zymography, respectively. Investigation of the involvement of MAPKs in these processes in PDLMSCs was determined by Western blotting, as well as by utilizing specific p38 and MEK1/2 inhibitors. Our results show that IL-17 activates MAPK signaling in PDLMSCs. Moreover, IL-17 had no effect on MMP9 expression, but it stimulated uPA and MMP2 gene and protein expression in PDLMSCs through the activation of the ERK1/2 MAPK signaling pathway. The obtained data suggest that IL-17 contributes to ECM degradation in the periodontal ligament by stimulating uPA and MMP2 expression and activity in PDLMSCs. This information is important for understanding periodontal disease development and defining future directions of its treatment.

**Keywords:** interleukin-17; periodontal ligament mesenchymal stem cells (PDLMSC); urokinase-type plasminogen activator (uPA); metalloproteinase (MMP); extracellular signal-regulated kinase (ERK)1/2; mitogen-activated protein kinase (MAPK) signaling pathway

## INTRODUCTION

Periodontal disease is a progressive and degenerative disease of the periodontium, teeth-supporting tissue that is composed of the gingiva (gums), periodontal ligament (PDL), tooth cementum and alveolar bone, which leads to the loss of connective tissue and bone support and consequently, teeth loss [1]. The presence of a small subpopulation of mesenchymal stem cells (MSCs) within the human periodontal ligament, named periodontal ligament mesenchymal stem cells (PDLMSCs), is of special importance for maintaining periodontal tissue structure. The isolation and

identification of human PDLMSCs has enabled their application in reconstructive dentistry and tissue engineering due to their ability to repair degenerative PDL tissues through differentiation into fibroblasts, osteoblasts or cementoblasts in response to a specific microenvironment [2].

Plasminogen activators and matrix metalloproteinases (MMPs) are proteolytic enzymes responsible for the degradation of extracellular matrix (ECM) components, facilitating cell migration and the development of a new tissue [3]. However, following stimulation by periodontal pathogens, the increased

expression of inflammatory cytokines and reactive oxygen species (ROS) can lead to the overexpression of MMPs and subsequently to accelerated matrix degradation associated with periodontitis [4-6]. The principal MMPs involved in the digestion of collagen in bone and periodontal ligament are gelatinases, MMP-2 and MMP-9 [5]. The role of plasminogen activators, including urokinase-type plasminogen activator (uPA) in the pathology of periodontal diseases is insufficiently examined. Recently, it was determined that deficiency in plasminogen activators leads to the elongation of mouse bones and increased bone mass, which points to the importance of the plasminogen system in bone matrix degradation [7]. As regards periodontal disease, in a recent study it was proposed that uPA contributes to periodontal tissue degradation directly and indirectly through the activation of latent forms of MMPs [8].

Interleukin (IL)-17 is a proinflammatory cytokine produced by T helper type 17 (Th17) cells that is, beside its important role in the regulation of hematopoietic stem cell growth [9,10] involved in the pathology of various autoimmune and inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and periodontitis [11,12]. In addition to Th17 cells, recent research confirmed other sources of IL-17, predominantly among effector immune cells, such as CD8+ T lymphocytes,  $\gamma\delta$  T lymphocytes, (natural killer (NK) cells, natural killer T (NKT) cells, lymphoid tissue inducer (LTi) cells, as well as macrophages and granulocytes [13,14]. Also, non-immune cells produce some members of the IL-17 family, such as muscle cells, adipocytes, and neurons [15].

IL-17 was shown to stimulate proliferation and differentiation of human MSCs supporting the maintenance of bone homeostasis [16]. Previous studies have reported the presence of the cell type that secretes IL-17, Th17 cells in aberrant periodontal tissue [17], as well as the involvement of IL-17 in bone destruction, inflammatory root resorption and orthodontic tooth movement mediated by the remodeling of alveolar bone, and PDL osteogenic potential [18-20].

As IL-17 has been reported to regulate the expression of proteolytic enzymes uPA and MMPs in multiple cell types [21-23], the aim of our study was

to investigate whether IL-17 contributes to PDL remodeling and tissue destruction through modulation of uPA and MMP2/MMP9 expression in PDLMSCs. Since it has already been shown that IL-17-induced signaling pathways in various cells involve mitogen-activated protein kinases (MAPKs) [20,24], our goal was also to define the involvement of p38 and ERK1/2 MAPK activation in effects mediated by IL-17 on uPA and MMP2/9 expression in PDLMSCs. The obtained results showed that IL-17 stimulated uPA and MMP2 expression in PDLMSCs, at least in part, through the activation of ERK1/2 MAPK.

## MATERIALS AND METHODS

### Isolation and cultivation of PDLMSCs

Periodontal tissue was isolated after extraction of healthy third molars from 4 patients aged 18-25 years undergoing orthodontic treatment at the Department of Oral Surgery of the Faculty of Dental Medicine (following the approved ethical guidelines set by the Ethics Committee of the Faculty of Dental Medicine, University of Belgrade). Informed consent was obtained from all patients.

As previously described [20], the periodontal ligament (PDL) was gently separated from the tooth root surface, minced into small pieces and cultured in Dulbecco's modified eagle medium (DMEM; Sigma-Aldrich, St. Luis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Capricorn Scientific, Germany), 100 U/mL penicillin and 100  $\mu$ g/mL streptomycin (PAA Laboratories, Fisher Scientific, USA). After reaching first confluence (P0), the cells were detached by 0.05% trypsin with 1 mM ethylenediaminetetraacetic acid (EDTA) (PAA Laboratories) and passaged regularly in growth medium (GM), DMEM with 10% FBS. For this study, passages P3-P6 were used. In order to confirm the mesenchymal origin of isolated cells [25], the expression of positive and negative mesenchymal stromal markers was determined by flow cytometry analyses (CyFlow SL flow cytometer, Partec, Munster, Germany) as reported previously [26]. As an index of stem cell identity, the multilineage differentiation potential of PDLMSCs was confirmed as previously described [26] by culturing cells in a specific differentiation medium that induced

differentiation of these cells into diverse mesenchymal lineages (osteogenic, chondrogenic, adipogenic).

### Zymography assay

The activity of secreted uPA was assayed by zymography using a mini protein system (Bio-Rad, Richmond, CA, USA), as described [27]. Briefly,  $2 \times 10^4$  cells were seeded per well in 24-well plates and cultured in GM until confluence. Confluent PDLMSCs were cultivated for an additional 24 h with recombinant human Il-17 (rhIL-17) in a serum-free medium with or without p38 or MEK1,2 inhibitors. The conditioned medium was diluted 5X with Laemmli sample buffer (10% sodium dodecyl sulfate (SDS), 125 mM Tris-HCl, pH 6.8, 10% glycerol and 0.002% bromophenol blue) and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), after which the gels were washed twice in 2% Triton X-100 for 30 min. To follow the activity of uPA, gels were placed on top of 1% agarose gels containing 0.5% casein and 2  $\mu\text{g}/\text{mL}$  plasminogen and incubated at 37°C for 24 h. uPA-dependent proteolysis of agarose gels was detected as clear bands, for which densitometry was performed using NIH-Image J software.

To determine the activity of secreted MMP-2 [28], the obtained conditioned media were subjected to SDS-PAGE under non-reducing conditions in 8% polyacrylamide gels containing 0.1% gelatin. After washing in 2% Triton X-100 for 30 min, the gels were incubated for 24 h in 100 mM Tris-HCl, pH 8.5, with 10 mM  $\text{CaCl}_2$ . After staining with Coomassie Brilliant blue R-250 for 30 min, MMP-2 activity was visualized as a transparent band inside the stained gel. The digital images were obtained from the scanner and the intensity of the bands was quantified using NIH-Image J software.

### Western blotting assay

After collection of PDLMSCs cultivated under the specific treatments described in the Results section, cells were lysed on ice in lysis buffer (50 mM Tris, 150 mM NaCl, 1% NP-40, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM epsilon-aminocaproic acid (EACA), 1 mM  $\text{Na}_3\text{VO}_4$ ) containing 10  $\mu\text{l}/\text{mL}$  protease inhibitor cocktail (Sigma-Aldrich,

St. Louis, MO, USA) for 30 min and centrifuged at 14 000  $\times g$  for 15 min at 4°C. Protein concentrations in the collected supernatants were determined by the bicinchoninic acid (BCA) protein assay (Pierce, IL, USA) and the same amount of proteins from each sample was separated by SDS-PAGE following the electrotransfer onto a nitrocellulose membrane. The membranes were incubated for 1 h in 3% bovine serum albumin (BSA)/Tris-buffered saline (TBS) buffer containing 0.05% Tween 20 and then overnight with the primary antibodies for rabbit anti-uPA (Santa Cruz Biotechnology, TX, USA), mouse anti-MMP2 (Thermo Fisher Scientific), rabbit antiphospho-p38, rabbit anti-p38 $\alpha$ , rabbit anti-phospho-ERK1/2, and rabbit anti-ERK1/2 (all from R&D Systems, Min, USA). After washing the membranes three times for 15 min in TBS buffer containing 0.05% Tween, the membranes were incubated for 1 h at room temperature with secondary anti-rabbit or anti-mouse antibodies conjugated with horseradish peroxidase (HRP) (all purchased from R&D systems). Visualization of the labeled proteins was determined using an enhanced chemiluminescence reagent system from AppliChem (Darmstadt, Germany). Quantification of protein bands by densitometric scanning was observed using NIH-Image J software.

### Semi-quantitative reverse-transcriptase (RT)-PCR assay

After the corresponding cell treatments, total RNA was extracted from PDLMSCs seeded in 6-well plates ( $1 \times 10^5$  cells/well) with TRIzol reagent (AppliChem). The SuperScript First-Strand Synthesis System for RT-PCR from Invitrogen (Carlsbad, CA, USA) and oligo dT as a primer were used to generate the complementary DNA. PCRs were performed using a one-step PCR thermal cycler (Eppendorf, Germany). The primer sets (all purchased from Invitrogen, Carlsbad, CA, USA), annealing temperatures and the number of cycles used for each primer set are listed in Supplementary Table S1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified and served as a control for the amount of cDNA in each sample; densitometry analyses were performed using NIH-Image J software.

## Statistical analysis

The results are presented as the mean±standard error (SE) for at least three independent experiments. To evaluate the significant differences among samples, one-way ANOVA with Tukey's or Dunnett's posteriori tests for multiple comparison was performed in GraphPad Prism 8 software. Gaussian distribution of data was confirmed using the Shapiro-Wilk normality test. A P-value of <0.05 was considered significant.

## RESULTS

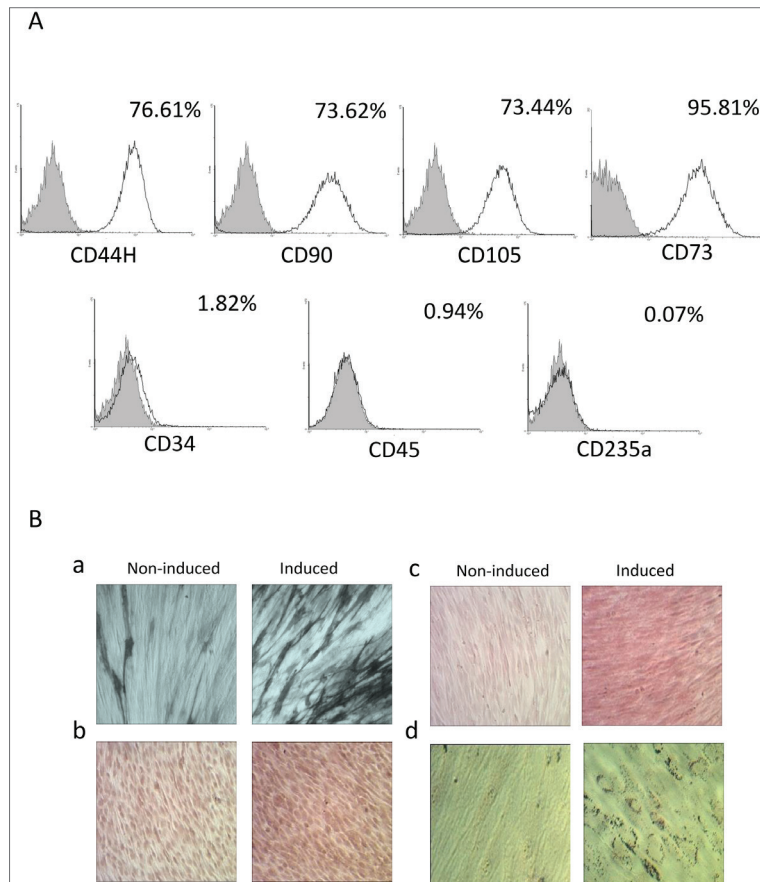
### Phenotypic properties and multilineage differentiation of PDLMSCs

Flow cytometry analyses revealed high expression of surface markers related to MSCs, such as CD44, CD90, CD105 and CD73 (Fig. 1A), and negative expression of hematopoietic stem cell markers, with less than 2% of PDLMSCs expressing CD34 (1.82%

positive), CD45 (0.94% positive) or CD235a (0.07% positive) antigens (Fig. 1A). Also, PDLMSCs showed the potential to differentiate into cells of osteogenic, chondrogenic and adipogenic lineage since a high level of alkaline phosphatase (ALP) activity, calcium and proteoglycan deposition and lipid droplets were observed in PDLMSCs cultured in specific differentiation media compared with cells cultured in regular GM (Fig. 1B).

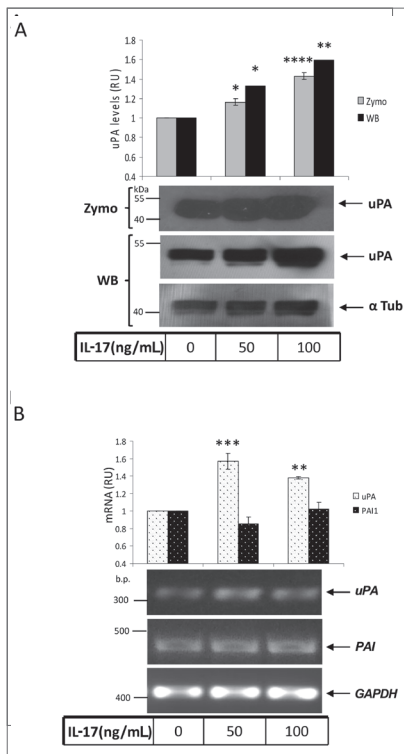
### IL-17 stimulates uPA and MMP2 expression in PDLMSCs

Since uPA and MMP2/MMP9 have been shown to be implicated in periodontal tissue degradation [5,8], first we aimed to determine whether IL-17 modulates the expression of these proteolytic enzymes. For this purpose, PDLMSCs were treated with increasing concentrations of IL-17 for 24 h. The activity of uPA secreted into the conditioned media was analyzed by zymography. The obtained results revealed that IL-17 induced a mild but statistically significant dose-dependent increase in uPA production and activity in PDLMSCs (Fig. 2A). Similarly, when uPA expression was evaluated by Western blot analysis, a dose-dependent increase in its protein expression was detected in PDLMSCs following IL-17 stimulation (Fig. 2A). A marked increase in uPA mRNA was determined by RT-PCR analysis in IL-17-treated PDLMSCs compared to the untreated control, while no effect of IL-17 on the expression of the



**Fig. 1.** Phenotypic properties and multilineage differentiation of PDLMSCs. **A** – Representative flow cytometry histograms showing the expression (empty histograms) of selected mesenchymal markers (CD44H, CD90, CD105, CD73) and hematopoietic markers (CD34, CD45, CD235a) in PDLMSCs, with percentages of positive cells based on isotype controls (shaded histograms). **B** – Multilineage differentiation potential of PDLMSCs. Osteogenic differentiation was confirmed by positive staining for ALP activity (**a**) and extracellular matrix mineralization with Alizarin red (**b**). Chondrogenic differentiation of MSCs was confirmed by positive staining of proteoglycans with Safranin O; scale bar 50  $\mu$ m (**c**). Oil Red O staining of intracytoplasmic lipid droplets demonstrated adipogenic differentiation; scale bar 20  $\mu$ m (**d**).

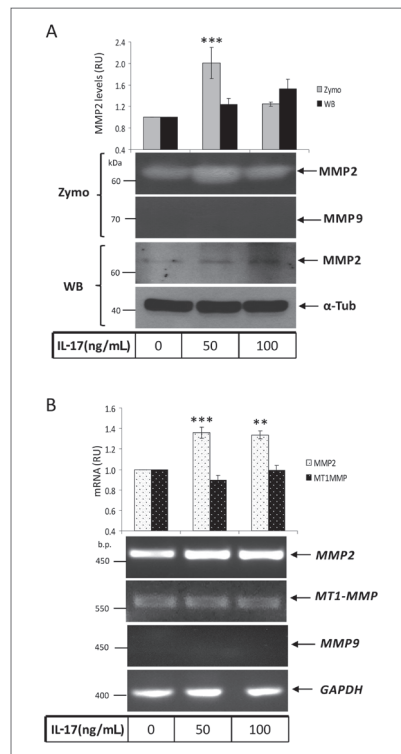




**Fig. 2.** IL-17 stimulates uPA expression in PDLMSCs. Cells were treated for 24 h with 0, 50 and 100 ng/mL IL-17. **A** – uPA activity and protein expression were determined by zymography and Western blotting, respectively, with a tubulin used as a gel-loading control. **B** – uPA and PAI-1 mRNA expression determined by RT-PCR analyses with GAPDH used as a gel-loading control. Densitometric quantification of the bands was expressed relative to untreated cells to which an arbitrary value of 1 was given. RU – relative units. Results from 2-3 independent experiments are shown. Molecular weight standards are indicated in kilodaltons (kDa) for proteins and in base pairs (bp) for PCR products. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . The results from 3 independent experiments are shown and are presented as the mean  $\pm$  SEM.

uPA inhibitor, plasminogen activator inhibitor-1 (PAI-1), was observed (Fig. 2B).

Regarding the involvement of IL-17 in MMP2 and MMP9 expression in PDLMSCs, the results obtained by zymography showed that IL-17 significantly enhanced the activity of MMP2 in the conditioned media of PDLMSCs, with the strongest effect observed at

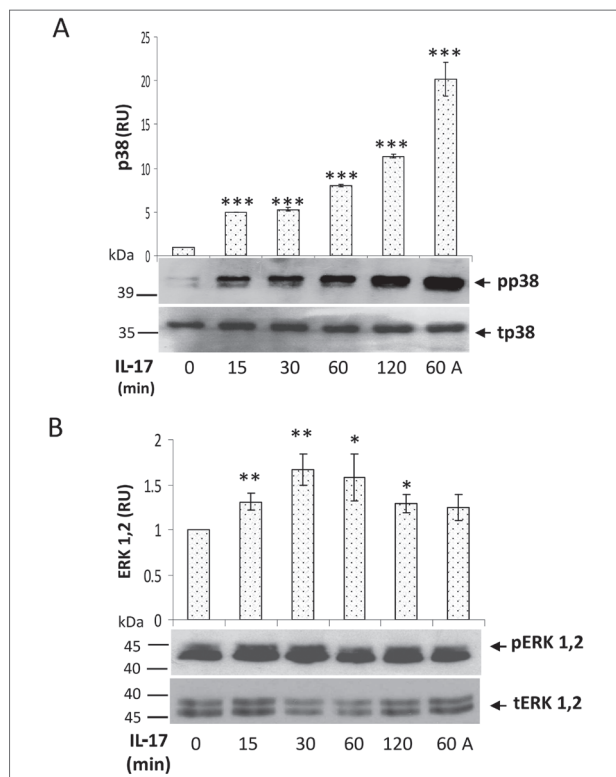


**Fig. 3.** IL-17 stimulates MMP2 expression in PDLMSCs. Cells were treated for 24 h with 0, 50 and 100 ng/ml IL-17. **A** – MMP2 activity and protein expression were determined by zymography and Western blotting, respectively, with a tubulin used as a gel-loading control. **B** – MMP2 and MT1-MMP mRNA expression was determined by RT-PCR analyses, with GAPDH used as a gel-loading control. Densitometric quantification of the bands was expressed relative to untreated cells to which an arbitrary value of 1 was given. The results from 2-3 independent experiments are shown. Molecular weight standards are indicated in kilodaltons (kDa) for proteins and in base pairs (bp) for PCR products. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . The results from 3 independent experiments are shown and presented as the mean  $\pm$  EM.

50 ng/mL (Fig. 3A). In addition, increased expression of MMP2 protein in PDLMSCs treated with IL-17 was demonstrated by Western blotting. These results were further supported by RT-PCR analysis that showed a higher level of MMP2 mRNA expression in IL-17-treated PDLMSCs compared to the control, while no effects of IL-17 on the expression of the MMP2 activator, membrane-type 1 matrix metalloproteinase (MT1-MMP), was observed (Fig. 3B), hence the regulation of MMP2 activity by this enzyme was excluded. Notably, the expression of neither protein nor mRNA MMP9 was detected in untreated or IL-17-treated PDLMSCs (Fig. 3A, B).

### IL-17 activates MAPK signaling in PDLMSCs

We next investigated the signaling events triggered by IL-17 in PDLMSCs. To elucidate whether IL-17 activates p38 and ERK1/2 MAPKs in PDLMSCs, cells were incubated in serum-free medium with IL-17 (100 ng/mL) for different times, and whole cell lysates were subjected to Western blotting. The results of densitometric analyses shown in Fig. 4A demonstrate that IL-17 rapidly induced the phosphorylation of p38 MAPK from 15 min onwards after stimulation, with the highest peak observed when the specific activator of p38 MAPK, anisomycin [29], was added. Increased levels of phosphorylated ERK1/2 were also noted, with peaks of activation observed at 30 and 60 min after the IL-17 treatment (Fig. 4B), although the basic expression of pERK was high.



**Fig. 4.** IL-17 activates MAPKs signaling in PDLMSCs. Immunoblot analysis was performed with PDLMSCs stimulated with IL-17 (100 ng/mL) for 15, 30, 60 and 120 min or anisomycin (100 ng/mL), a potent activator of MAPK. Representative blots of Western analyses performed using specific antibodies recognizing phosphorylated or total p38 MAPK (A) and ERK1/2 (B). Time course changes of phosphorylated over total MAPK levels are presented and normalized to the control level at each time point and presented as the mean $\pm$ SEM from three experiments. Molecular weight standards of proteins are indicated in kilodaltons (kDa). \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001. The results from 2-3 independent experiments are shown and presented as the mean $\pm$ SEM.

### The role of p38 and ERK1/2 MAPK activation in IL-17-stimulated uPA and MMP2 expression in PDLMSCs

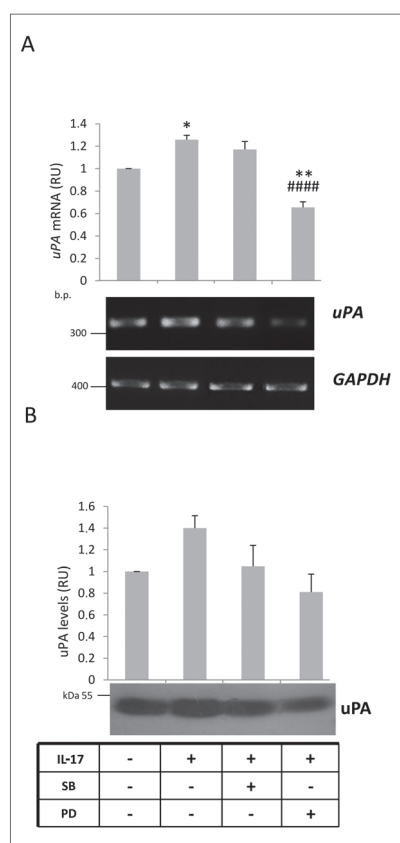
To analyze the involvement of p38 and ERK1/2 MAPK pathways in IL-17-mediated uPA and MMP2 expression in PDLMSCs, specific inhibitors of these signaling pathways were used. Zymography and RT-PCR analyses showed that treatment of cells with SB203580, a p38 inhibitor, had no statistically significant effect on the capacity of IL-17 to increase uPA expression in these cells (Fig. 5A, B). According to these findings, no involvement of p38 MAPK activation was suggested for IL-17-stimulated uPA expression in PDLMSCs. However, treatment of PDLMSCs

with the ERK1/2 inhibitor significantly decreased uPA mRNA expression (Fig. 5A), indicating that the IL-17-induced increase in uPA in PDLMSCs depended on ERK1/2 activation, although *in situ* uPA activity in IL-17-treated cells due to a large variability between samples only followed the trend but not the significance of the results (Fig. 5B).

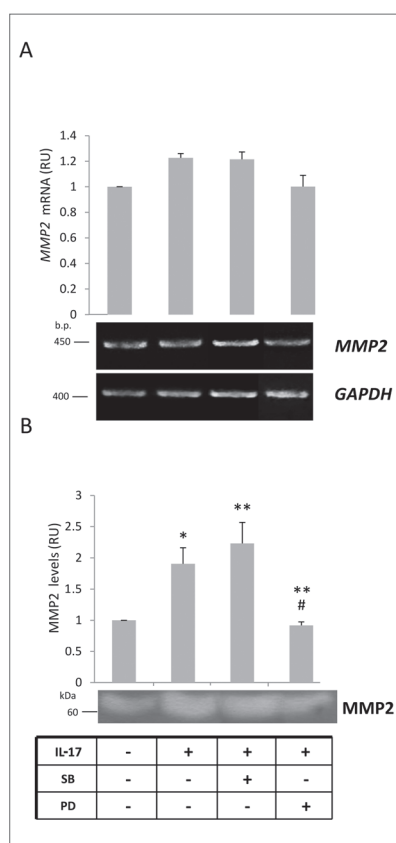
As for the role of p38 and ERK1/2 MAPK pathways in IL-17-induced MMP2 expression in PDLMSCs, the obtained results showed that SB203580 did not affect IL-17-stimulated MMP2 mRNA expression and enzyme activity in PDLMSCs, implying that p38 MAPK activity did not contribute to the IL-17-induced enhancement of MMP2 expression in these cells (Fig. 6A, B). Furthermore, PD98059 treatment demonstrated that ERK1/2 inhibition had no influence on IL-17-induced MMP2 mRNA expression (Fig. 6A), whereas the zymography analyses revealed that this inhibitor markedly hindered the ability of IL-17 to stimulate MMP2 expression in these cells (Fig. 6B). These results suggest the involvement of the ERK1/2 signaling pathway in IL-17-mediated stimulation of MMP2 activity in PDLMSCs.

## DISCUSSION

PDLMSCs are cells with high proliferation and/or differentiation capacity are irreplaceable in the process of periodontal tissue regeneration. However, if persistent chronic inflammation of periodontal tissue leading to alveolar bone loss exists, the microenvironmental factors become key factors in the process of periodontal tissue degradation. There is increasing evidence indicating that IL-17 is secreted by Th17 cells in human periodontal lesions [17] and that it is associated with the presence and degree of periodontal disease [30,31]. The concentrations of IL-17 in periodontal disease measured in gingival crevicular fluid ranged from 30 to over 150 pg/ $\mu$ L, with mean values between 40 and 100 pg/ $\mu$ L [30,31], whereas in our experimental setting, the observed 50 and 100 ng/mL of IL-17 are in line with *in vivo* conditions. In the present study we investigated whether IL-17 modulates the expression of proteolytic enzymes of the ECM in PDLMSCs. The obtained data demonstrated that IL-17 increases uPA and MMP2 at both mRNA and protein levels, and that these effects are mainly mediated



**Fig. 5.** The role of ERK1/2 MAPKs activation in IL-17-induced uPA expression in PDLMSCs. Cells were incubated for 24 h with 0 and 100 ng/mL IL-17 in the presence or absence of 10  $\mu$ M SB203085 or 25  $\mu$ M PD98059. **A** – Total mRNA was isolated from PDLMSCs and subjected to RT-PCR analysis for *uPA* transcript expression using *GAPDH* as a gel-loading control. **B** – At the same time, conditioned media were collected after these treatments and used for zymography analyses of secreted uPA activity. Densitometric quantification of the bands was expressed relative to untreated cells, to which an arbitrary value of 1 was given. Representative results from three independent experiments are shown. Molecular weight standards are indicated in base pairs (bp) for PCR products. \* $P$ <0.05, \*\* $P$ <0.01 vs untreated cells; #### $P$ <0.0001 vs cells treated with IL-17 alone. The results from 3 independent experiments are shown and presented as the mean $\pm$ SEM.



**Fig. 6.** Involvement of ERK1/2 MAPK activity in IL-17-induced MMP2 expression in PDLMSCs. Cells were incubated for 24 h with 0 and 100 ng/mL IL-17 in the presence or absence of 10  $\mu$ M SB203085 or 25  $\mu$ M PD98059. **A** – Total mRNA was isolated from PDLMSCs and subjected to RT-PCR analysis for *MMP-2* mRNA expression using *GAPDH* as a gel-loading control. **B** – The conditioned media collected after these treatments were used for zymography analyses of secreted MMP-2 activity. Densitometric quantification of the bands is expressed relative to untreated cells to which an arbitrary value of 1 was given. Representative results from 3 independent experiments are shown. Molecular weight standards are indicated in base pairs (bp). \* $P$ <0.05, \*\* $P$ <0.01 vs untreated cells; ## $P$ <0.01 vs cells treated with IL-17. The results from 3 independent experiments are shown and presented as the mean $\pm$ SEM.

through the activation of the ERK/MAPK signaling pathway.

Our previous studies confirmed the presence of stemness markers in PDLMSCs, distinguishing them from PDL fibroblasts [32,33]. Although increased levels of IL-17 have been reported in inflamed periodontal tissues [17], the results of our study for the first time showed that IL-17 induces the expression of both uPA and MMP2 in PDLMSCs. These results are in accordance with previously published data showing that IL-17A can promote the expression of MMP2 in cancer cells [34] and synovial cells in rheumatoid arthritis [35]. IL-17 was shown to downregulate MMP2 expression in human periodontal ligament fibroblasts, indicating distinct roles in different cell types known to be present in periodontal tissue [36]. As for MSCs, increased uPA activity and no MMP9 expression were observed in PDLMSCs after treatment with IL-17, similar to our previous reports in peripheral blood MSCs [37]. At the same time, we showed that the expression of the uPA inhibitor PAI-1 and MMP-2 activator MT1-MMP was not affected by the treatment with IL-17, suggesting that PAI-1 and MT1-MMP are not involved in IL-17-mediated uPA and MMP-2 expression in PDLMSCs.

Identification of IL-17-induced signaling pathways in periodontal tissues is another issue that may contribute to the understanding of the mechanisms involved in the effects of IL-17. Published data showed that signaling pathways triggered by IL-17 include mitogen-activated

protein kinases (MAPKs) in different cell types [24]. As expected, our results showed that IL-17 significantly increased the phosphorylation of p38 and ERK1/2 MAPKs in PDLMSCs, which is in accordance with our previous report [20] confirming the activity of IL-17 in intra- and inter-heterogenic populations of PDLMSC as a type of MSC [38]. Further experiments using specific MAPK inhibitors demonstrated that the activation of ERK1/2 MAPKs was essential for IL-17-induced uPA expression in PDLMSCs at both mRNA and protein activity levels, while p38 MAPK activation had no major influence on IL-17-induced uPA expression. The capacity of IL-17 to enhance uPA expression in an ERK1/2 MAPK-dependent way is in agreement with our previously reported result regarding the similar effect of IL-17 in peripheral blood MSC [37].

The results concerning the involvement of MAPKs in the activity of MMPs demonstrated that p38 MAPK was not involved in the IL-17-induced activity of MMP2. When the role of ERK1/2 MAPK activation was analyzed, the obtained data revealed that MAPK was not essential for IL-17-induced MMP2 mRNA expression. However, the effect of IL-17 was markedly reduced in the presence of specific ERK1/2 inhibitor, confirming that the activation of ERK1/2 MAPK was critical for the IL-17-induced increase in MMP2 activity. Although unexpectedly, a different involvement of ERK1/2 activation in IL-17-induced effects on MMP2 mRNA and protein expression can be related to regulation at the post-transcriptional level where MMPs are subjected to a number of regulation points before they acquire their active form [36]. A lack of correlation between the MMP2 mRNA and protein expression and/or activity is established and was previously reported [39].

Evidence for the presence of IL-17 and Th17 cells in human periodontal disease is clear [40]. However, further research into their role is still required, including the underlying mechanisms in IL-17-induced effects in inflammation and/or bone destruction. Previous clinical studies implicated Th17 cells in periodontal lesions as specialized osteoclastogenic lymphocytes associated with the disease progression [17], which links T cell activation to bone resorption [41]. Moreover, the mechanism of amplifying the inflammatory response of PDL fibroblasts by the ability of IL-17 to stimulate their expression of IL-23 through the ERK1/2 MAPK-dependent signaling pathway was also reported [41].

Conversely, MMPs and uPA were also shown to be involved in the remodeling and turnover of periodontal tissue [42]. Namely, MMPs are capable of degrading all components of the ECM, whereas by converting plasminogen into plasmin that degrades fibrin and activates MMPs, uPA and its specific receptor, urokinase-type plasminogen activator receptor (uPAR), they play an important role in processes that require concentrated proteolytic activity [42]. For example, the induction of MMP9 activity by the uPA/plasmin system has been shown in bronchial epithelial cells [43]. However, information on IL-17-induced uPA and MMP2 activity in periodontal tissue is scarce. Higher amounts of uPA and PAI-1 have been detected in diseased periodontal tissue [7], and previous studies have shown the involvement of the plasmin system in periodontal disease development [44,45]. Literature data suggest that MMP2 is probably involved in ECM degradation during the initial phase of lesion development in periodontal disease, as demonstrated in experimentally induced periapical lesions in the rat [5].

The above studies describe the involvement of both IL-17 and ECM molecules in the pathogenesis of periodontal disease. Moreover, the findings presented herein suggest that IL-17 could be involved in its development also by increasing the expression and activity of ECM-degrading proteases in PDLMSCs, and subsequent ECM degradation in the periodontal microenvironment. Further investigation of the role of IL-17 and the underlying mechanisms is needed considering that this cytokine could be a potential target for anticytokine therapy of periodontal disease, a widespread disease that affects millions of people.

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**Conflict of interest disclosure:** The authors declare no conflict of interest.



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## Supplementary Material

The Supplementary Material is available at: [https://www.serbiosoc.org.rs/NewUploads/Uploads/Okic%20Djordjevic%20et%20al\\_7089\\_Supplementary%20Material.pdf](https://www.serbiosoc.org.rs/NewUploads/Uploads/Okic%20Djordjevic%20et%20al_7089_Supplementary%20Material.pdf)