Expression of Endothelin-1 and Endothelin Receptors in Human Periodontal Ligament Cells from Adolescents and Adults

Ratthakorn Sumetcherngpratya[1], Sirima Petdachai[1] and Supaporn Suttamanatwong[*2]

[1]Department of Orthodontics, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[2]Department of Physiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
[*]Corresponding author, E-mail: supaporn.sut@chula.ac.th

Abstract

Endothelin (ET) is one of the biomolecules that play important role in orthodontic tooth movement which may contribute to the different rate of tooth movement between adolescents and adults. This study aimed to investigate the messenger RNA (mRNA) expression of endothelin-1 (ET-1), endothelin A (ET_A), and endothelin B (ET_B) receptors in human periodontal ligament (PDL) cells from adolescents and adults. The PDL cells were obtained from ten patients, which were categorized by age into 2 groups: adolescent group and adult group. The levels of ET-1, ET_A, and ET_B mRNA expressions were determined by quantitative polymerase chain reaction (qPCR). Expressions of ET-1, $ET_A$and ET_B receptor mRNA in the adolescent group were significantly higher than in the adult group. (p = 0.025, 0.027, and 0.019 respectively). In summary, mRNA expressions of ET-1 and its receptors in PDL cells from adolescents were higher than adults but the role of the ET system on the different rate of tooth movement remained to be elucidated.

Keywords: *Endothelin, Endothelin A receptor, Endothelin B receptor, Periodontal ligament cell*

1. Introduction

Nowadays, adult patients have a high tendency to seek orthodontic treatment and one of the challenges in adult orthodontic treatment is the slower rate of tooth movement than adolescents (Göz, 1990; Kawasaki et al., 2006; Dudic et al., 2013; Vujačić et al., 2017; Alikhani et al., 2018). The differences in biological responses between young and adult patients are one of the factors determining the rate of tooth movement. When orthodontic force is applied to a tooth, the pattern of cellular activity and blood flow in the periodontal ligament (PDL), as well as many mediators and second messengers involving cellular function and differentiation, are changed (Khouw and Goldhaber, 1970; Vanarsdall, 1994). Applying occlusal force in the microvasculature of rat molar PDL can upregulate the endothelin-1 (ET-1), which is a potent vasoconstrictor peptide initially isolated from porcine endothelial cell culture (Yanagisawa et al., 1988; Sims, 2001). ET-1 represents the major isoform among ET families comprising ET-1, ET-2, and ET-3, which are produced in various cells such as endothelial cells, fibroblast, smooth muscle cell, and epithelial cell (Zeballos et al., 1991; Shah, 2007). Moreover, osteoblasts and osteocytes can synthesize ET-1 (Kitten and Andrews, 2001). ET-1 is also localized at the plasma membranes, the cytoplasm, and pale vacuoles of osteoclasts (Sasaki and Hong, 1993).

In the periodontal tissue, ET-1 is present in human gingival keratinocytes (HGK) human gingival fibroblasts (HGF) and human PDL cells (HPL) (Fujioka et al., 2003). One of the ET-1 functions is the synthesis of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α (Murlas et al., 1997; Didier et al., 2003; Liang et al., 2014) involving the bone resorption and tooth movement (Krishnan and Davidovitch, 2006). ET-1 action is evoked via its receptors, endothelin receptor A (ET_A) and endothelin receptor B (ET_B) (Sakurai et al., 1990). Previous studies showed that specific receptor antagonists accelerated or decelerated the rate of tooth movement in rats (Drevenšek et al., 2006; Sprogar et al., 2007). ET dual receptor antagonist enhanced tooth movement (Drevenšek et al., 2006) while selective ET_A antagonist slows the rate of tooth movement in rats (Sprogar et al., 2007). Therefore, ET-1 and its receptors may play a role in the rate of orthodontic tooth movement. However, there is little information about the expression level of ET-1 and its receptors between adolescents and adults. We hypothesized that the messenger RNA (mRNA) expression of ET-1 and its receptors in human PDL cells may change with age and contribute to the different rate of tooth movement in adolescents and adults.

2. Objective

This study aimed to compare the mRNA expressions of ET-1, $ET_{A,}$ and ET_B receptors in human periodontal ligament cells from adolescent and adult patients.

3. Materials and methods

3.1 Cell culture

Periodontal ligament fibroblasts were obtained from noncarious bicuspid teeth with healthy periodontium that were extracted for orthodontic reason from 5 healthy subjects without non-parafunctional habit in each group. In the adolescent group (3 females, 2 males), the patients' age ranged from 14 to 20 years old (mean 15.6 years old). In the adult group (2 females, 3 males), the patients' age was 30 years or more (mean 32.6 years old). The sample size was calculated according to the previous study (Vujačić et al., 2017) with a 80% probability power at the 5% significant level by the independent means as follows : $n_1 = [(z_1\text{-}\alpha/2 + z_1\text{-}\beta)^2 (\sigma_1^2 + \sigma_2^2/r)]/\Delta^2$ when $r = n_2/n_1 = 1$, $\alpha = 0.05$, $\beta = 0.2$, σ = standard deviation, $\Delta = \mu_1\text{-}\mu_2$. The written consent forms were obtained from the subjects. The study design and ethical consideration were approved by the ethical committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020-058). PDL cells were obtained exclusively from the middle portion of the tooth roots with a scalpel and placed in 60 mm tissue culture dishes (Thermo Scientific, USA) with Dulbecco's Modified Eagles Medium (DMEM, Sigma, USA) comprising 10% Fetal bovine serum (FBS, Gibco, USA), 1% L-glutamine (Gibco, USA), and 1% Antibiotic-Antimycotic (Gibco, USA) under the humidified atmosphere with 37° C and 5% carbon dioxide incubator. The culture medium was changed every other day. After confluent, PDL cells were passaged in which between third and seventh passages were used in the experiments.

3.2 RNA extraction and reverse transcription

RNA was isolated using the Total RNA Mini Kit (Geneaid, Taiwan). The concentration of the RNA was determined by measuring the absorbance at 260 and 280 nm with a Thermo Scientific NanoDrop™ 2000 Spectrophotometer. Two µg of total RNA of each sample was reverse transcribed to single-strand cDNA using RevertAid Reverse Transcriptase (Thermo Scientific, USA) following the manufacturer's instruction.

3.3 Quantitative polymerase chain reaction (qPCR) assay

ET-1 and its receptors expressions were assessed by real-time qPCR with a total reaction volume of 10 µL consisting of 5 µL of Luna® Universal qPCR Master Mix (New England Biolabs, USA), 0.25 µL of each primer, 2.5 µL of DNA template, and 2 µL of nuclease-free water. The PCR reaction was set at 95° C for 5 minutes followed by 45 cycles for the amplification phase; each consists of denaturation for 30 seconds at 95° C, annealing for 30 seconds at 56° C for GAPDH and 55° C for ET-1 and its receptors, and extension for 30 seconds at 72° C using Roche LightCycler 480. Melting curve analysis was performed to determine the specificity of the PCR product. All data were normalized to the relative quantities of GAPDH. One sample from the adult groups was designated as a reference and the relative gene expression of other samples was calculated as the fold change of the reference sample using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were as followed; ET-1, 5'-GAGAAACCCACTCCCAGTCC-3'(forward) and 5'-GATGTCCAGGTGGCA GAAGT -3' (reverse); ET_A, 5'-TCGGGTTCTATTTCTGTATGCCC-3' (forward) and 5'-TGTTTTTGCCA CTTCTCGACG-3' (reverse); ET_B, 5'-GTCCCAATATCTTGATCGCCAG-3' (forward) and 5'-AAGGCAC CAGCTTACACATCT-3' (reverse); and GAPDH, 5'-TGAACGGGAAGCTCACTGG-3' (forward) and 5'-TCCACCACCCTGTTGCTGTA-3'(reverse).

3.4 Statistical analysis

The experiment was performed in triplicate and repeated twice. Using SPSS version 26, the Shapiro-Wilk test was performed to determine the normality of the data. An independent t-test was used to evaluate the differences of ET-1, ET_A, and ET_B receptor messenger RNA (mRNA) levels between adults and adolescents. The level of statistical significance was set at $p < 0.05$.

4. Results and discussion

4.1 Results

Figure 1 showed that the relative mRNA expression of ET-1 in adolescents (4.11 ± 2.59) was significantly higher than in adults (0.5 ± 0.28) (p = 0.025). Similarly, the expression of the ET_A receptor in adolescents (4.63 ± 3.14) was significantly greater than in adults (0.39 ± 0.34) as shown in Figure 2 (p = 0.027). Moreover, Figure 3 demonstrated that ET_B receptor expression in adolescents (12.42 ± 9.45) was also significantly higher than in adults (0.61 ± 0.33) (p = 0.019). Besides, the highest values of all mRNA expressions were found in the adolescent group.

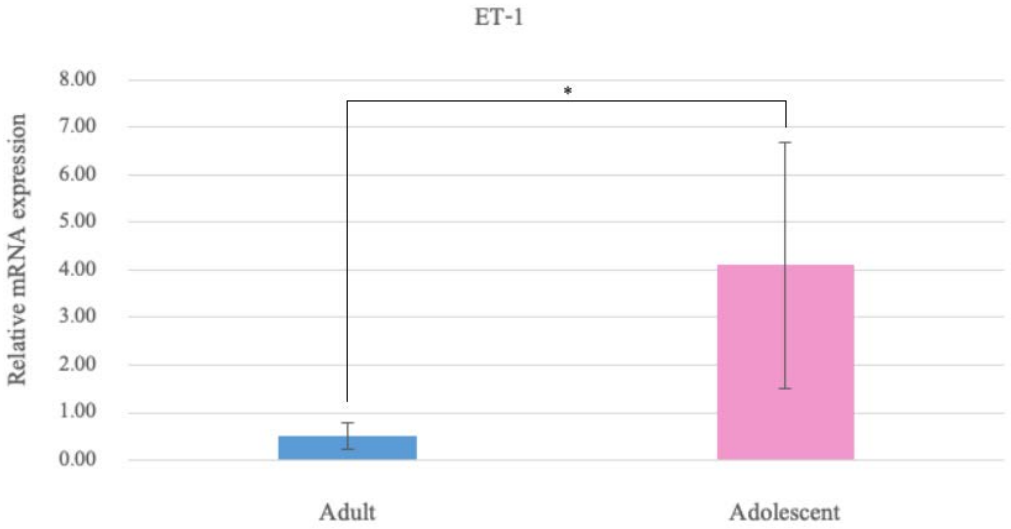

Figure 1 Bar graph of relative mRNA expression of ET-1 in PDL cells from adults and adolescents. Data are mean ± SD (*p < 0.05)

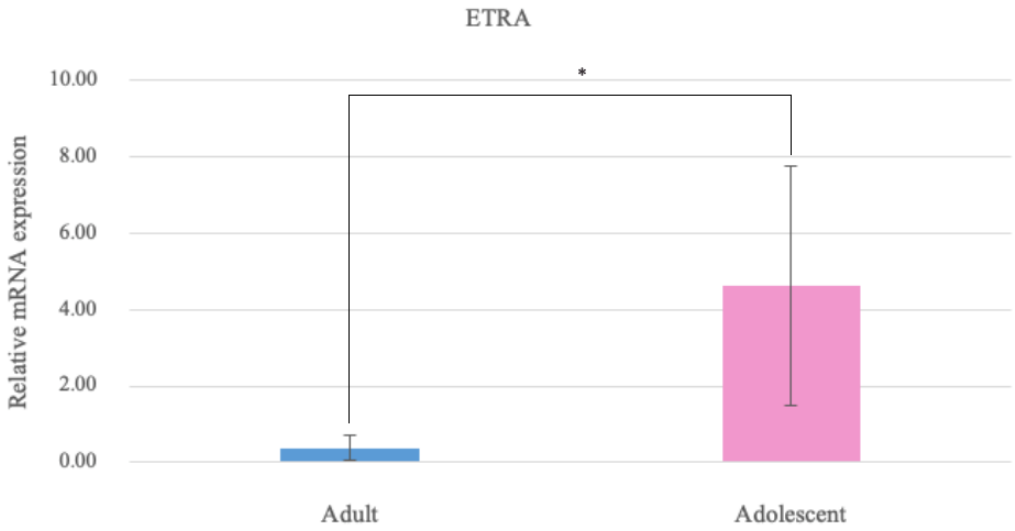

Figure 2 Bar graph of relative mRNA expression of ET_A receptor in PDL cells from adults and adolescents. Data are mean ± SD (*p < 0.05)

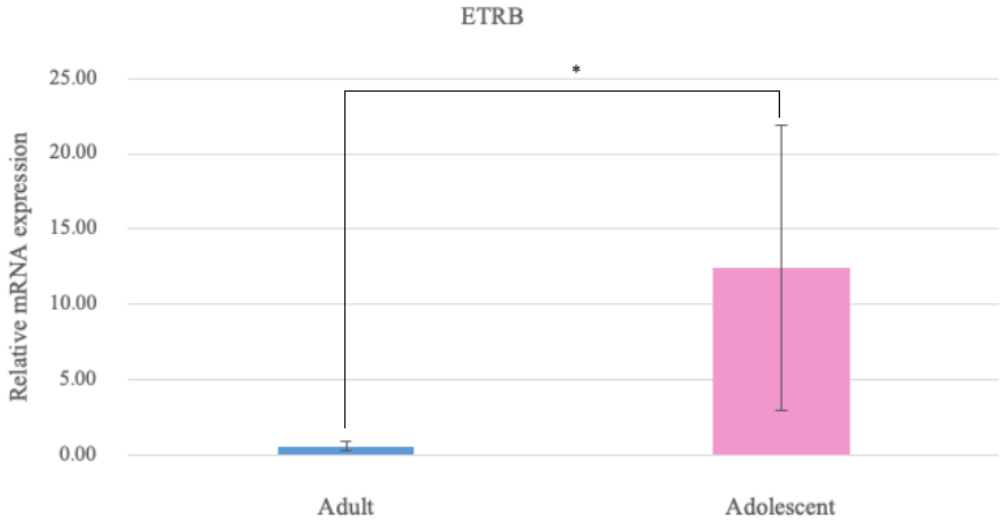

Figure 3 Bar graph of relative mRNA expression of ET_B receptor in PDL cells from adults and adolescents. Data are mean ± SD (*$p < 0.05$)

4.2 Discussion

It is well known that tooth movement in adults is likely to be more time-consuming than in adolescents (Göz, 1990; Kawasaki et al., 2006). Applying force to the tooth has been shown to upregulate ET-1 in the microvasculature of the rat PDL (Sims, 2001). ET-1 has been reported to contribute to inflammatory processes such as gastric mucosal inflammation, intraocular inflammation, and airway inflammation (Shoji et al., 1997; Slomiany et al., 1999; Salmon et al., 2000). In inflamed periodontal tissues, ET-1 upregulated IL-1**β** ~~in~~ in a dose-dependent manner while ET_A or ET_B receptor antagonists downregulated IL-1**β** expression (Rikimaru et al., 2009). In addition, IL-1**β**, IL-6, and TNF-α mRNA and protein levels in both healthy and periodontitis PDL cells were elevated by ET-1 in a dose- and time-dependent manner (Liang et al., 2014). These proinflammatory cytokines regulate the inflammatory response and bone remodeling process, which are the key in orthodontic tooth movement (Ren et al., 2007). Therefore, ET-1 and its receptors may be the factors influencing the rate of tooth movement between adults and adolescents. Our study demonstrated that ET-1 mRNA expression was significantly greater in adolescents than adults, which might affect the proinflammatory cytokine levels resulting in the different rate of tooth movement.

ET-1 action occurs through its receptors, ET_A and ET_B (Sakurai et al., 1990). Endothelin receptors are expressed in many organs including teeth and periodontal tissues. ET_A and ET_B were detected by immunohistochemistry in the developing and mature rat teeth suggesting their roles in tooth morphogenesis and root development (Neuhaus and Byers, 2007). The expression of ET_A mRNA is greater than ET_B in the normal human gingival fibroblasts. ET_A is expressed at a higher level in patients with cyclosporin A-induced gingival overgrowth than normal gingiva. Moreover, cyclosporin A increases ET_A mRNA levels (Tamilselvan et al., 2007). Consequently, patients who have such drug-induced gingival overgrowth may have a large amount of ET_A receptor concentration that can also more likely bind to ET-1. A previous study showed that ET_A and ET_B receptors are weakly expressed in PDL cells by Northern blot analysis (Fujioka et al., 2003). However, using qPCR, our study has confirmed that the ET-1 and its receptor mRNA are expressed in cultured PDL cells from both adults and adolescents. Moreover, our findings revealed that expressions of ET_A and ET_B receptors were significantly higher in adolescents than in adults. One of the main actions of the ET_A receptor is the stimulation of proinflammatory mediator secretion (Shah, 2007). A previous report demonstrated that the selective ET_A antagonist increased alveolar bone volume and decreased osteoclast volume (Sprogar et al., 2008) whereas another study showed that ET_A antagonist slowed the rate of tooth movement in rats (Sprogar et al., 2007). Therefore, it would be possible that the higher the ET_A mRNA level in adolescents, the more pro-inflammatory mediator will be produced causing faster tooth movement. Moreover, the ET_A receptor promotes the receptor activator of nuclear factor-kB ligand (RANKL) production

(Ruest et al., 2016), which is a prerequisite for activation of osteoclastic bone resorption (Teitelbaum, 2000). In rat experiments, the ET_A and ET_B receptor expression levels were varied throughout the phases of tooth movement and significantly increased in the late stage (Sprogar et al., 2008). Furthermore, the blockage of binding between ET-1 and its receptors could control IL-1**β** secretion from the human periodontal ligament fibroblasts and human oral epithelial cell line (Rikimaru et al., 2009). Even though the ET_A and ET_B receptors are involved in the various stages of tooth movement and contribute to regulating the cytokine secretion, little information is known about the downstream consequence of ET_A and ET_B receptor activation in PDL cells. Additional investigation at the post-transcriptional level and the effect of ET-1 treatment on PDL cells such as pro-inflammatory cytokine production still need to be assessed.

Although the sample size of the present study was calculated at 0.05 level of significance and 80% power based on the mean and standard deviation from the study of Vujačić et al. (Vujačić et al., 2017), it was relatively small in each group. Therefore, it might be preferable to have a larger sample size in the future study.

5. Conclusion

In summary, our study demonstrated that the levels of mRNA expressions of ET-1, ET_A, and ET_B receptors in PDL cells from adolescents were significantly higher than in adults. Consequently, these results suggested the possibility that the ET systems may play a role in the different rates of tooth movement in adolescents and adults.

6. Acknowledgements

The authors are grateful to Dr. Soranun Chantarangsu for her suggestion in the statistical analysis of the data. This study was supported by the Faculty Research Grant, Faculty of Dentistry, Chulalongkorn University.

7. References

Alikhani, M., Chou, M. Y., Khoo, E., Alansari, S., Kwal, R., Elfersi, T., Almansour, A., Sangsuwon, C., Jearah, M. A., & Nervina, J. M. (2018). Age-dependent biologic response to orthodontic forces. *American Journal of Orthodontics and Dentofacial Orthopedics, 153*(5), 632-644.

Didier, N., Romero, I. A., Créminon, C., Wijkhuisen, A., Grassi, J., & Mabondzo, A. (2003). Secretion of interleukin-1β by astrocytes mediates endothelin-1 and tumour necrosis factor-α effects on human brain microvascular endothelial cell permeability. *Journal of Neurochemistry, 86*(1), 246-254.

Drevenšek, M., Sprogar, Š., Boras, I., & Drevenšek, G. (2006). Effects of endothelin antagonist tezosentan on orthodontic tooth movement in rats. *American Journal of Orthodontics and Dentofacial Orthopedics, 129*(4), 555-558.

Dudic, A., Giannopoulou, C., & Kiliaridis, S. (2013). Factors related to the rate of orthodontically induced tooth movement. *American Journal of Orthodontics and Dentofacial Orthopedics, 143*(5), 616-621.

Fujioka, D., Nakamura, S., Yoshino, H., Shinohara, H., Shiba, H., Mizuno, N., Hasegawa, N., Shindoh, N., Uchida, Y., & Tetsuji Ogawa, T. (2003). Expression of endothelins and their receptors in cells from human periodontal tissues. *Journal of Periodontal Research, 38*(3), 269-275.

Göz, G. (1990). The age dependence of the tissue reaction in tooth movements. *Fortschritte der Kieferorthopadie, 51*(1), 4-7.

Kawasaki, K., Takahashi, T., Yamaguchi, M., & Kasai, K. (2006). Effects of aging on RANKL and OPG levels in gingival crevicular fluid during orthodontic tooth movement. *Orthodontics & Craniofacial Research, 9*(3), 137-142.

Khouw, F. E., & Goldhaber, P. (1970). Changes in vasculature of the periodontium associated with tooth movement in the rhesus monkey and dog. *Archives of Oral Biology, 15*(12), 1125-IN1111.

Kitten, A. M., & Andrews, C. J. (2001). Endothelin-1 expression in long-term cultures of fetal rat calvarial osteoblasts: Regulation by BMP-7. *Journal of cellular physiology, 187*(2), 218-225.

Krishnan, V., & Davidovitch, Z. (2006). Cellular, molecular, and tissue-level reactions to orthodontic force. *American Journal of Orthodontics and Dentofacial Orthopedics, 129*(4), 469. e461-469. e432.

Liang, L., Yu, J., Zhou, W., Liu, N., Wang, D. S., & Liu, H. (2014). Endothelin-1 stimulates proinflammatory cytokine expression in human periodontal ligament cells via mitogen-activated protein kinase pathway. *Journal of Periodontology, 85*(4), 618-626.

Murlas, C. G., Sharma, A. C., Gulati, A., & Najmabadi, F. (1997). Interleukin-1β increases airway epithelial cell mitogenesis partly by stimulating endothelin-1 production. *Lung, 175*(2), 117-126.

Neuhaus, S. J., & Byers, M. R. (2007). Endothelin receptors and endothelin-1 in developing rat teeth. *Archives of Oral Biology, 52*(7), 655-662.

Ren, Y., Hazemeijer, H., de Haan, B., Qu, N., & de Vos, P. (2007). Cytokine profiles in crevicular fluid during orthodontic tooth movement of short and long durations. *Journal of Periodontology, 78*(3), 453-458.

Rikimaru, T., Awano, S., Mineoka, T., Yoshida, A., Ansai, T., & Takehara, T. (2009). Relationship between endothelin-1 and interleukin-1β in inflamed periodontal tissues. *Biomedical Research, 30*(6), 349-355.

Ruest, L. B., Ranjbaran, H., Tong, E. J., Svoboda, K. K., & Feng, J. Q. (2016). Activation of Receptor Activator of Nuclear Factor-κB Ligand and Matrix Metalloproteinase Production in Periodontal Fibroblasts by Endothelin Signaling. *Journal of Periodontology, 87*(1), e1-e8.

Sakurai, T., Yanagisawa, M., Takuwat, Y., Miyazakit, H., Kimura, S., Goto, K., & Masaki, T. (1990). Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature, 348*(6303), 732-735.

Salmon, M., Liu, Y. C., Mak, J. C., Rousell, J., Huang, T. J., Hisada, T., Nicklin, P. L. & Chung, K. F. (2000). Contribution of upregulated airway endothelin-1 expression to airway smooth muscle and epithelial cell DNA synthesis after repeated allergen exposure of sensitized Brown-Norway rats. *American journal of Respiratory Cell and Molecular Biology, 23*(5), 618-625.

Sasaki, T., & Hong, M. H. (1993). Localization of endothelin-1 in the osteoclast. *Microscopy, 42*(3), 193-196.

Shah, R. (2007). Endothelins in health and disease. *European Journal of Internal Medicine, 18*(4), 272-282.

Shoji, N., Oshika, T., & Masuda, K. (1997). Endothelin-1 and intraocular inflammation in pigmented rabbit eyes. *Japanese Journal of Ophthalmology, 41*(3), 150-153.

Sims, M. R. (2001). Endothelin-1 expression in the microvasculature of normal and 3-hour continuously loaded rat molar periodontal ligament. *The European Journal of Orthodontics, 23*(6), 647-662.

Slomiany, B. L., Piotrowski, J. & Slomiany, A. (1999). Involvement of Endothelin-1 in Up-regulation of Gastric Mucosal Inflammatory Responses toHelicobacter pyloriLipopolysaccharide. *Biochemical and Biophysical Research Communications, 258*(1), 17-20.

Sprogar, Š., Volk, J. Drevenšek, M. & Drevenšek, G. (2007). The effects of TBC3214, a selective endothelin ETA receptor antagonist, on orthodontic tooth movement in rats. *The European Journal of Orthodontics, 29*(6), 605-608.

Sprogar, Š., Vaupotic, T., Cör, A., Drevenšek, M., & Drevenšek, G. (2008). The endothelin system mediates bone modeling in the late stage of orthodontic tooth movement in rats. *Bone, 43*(4), 740-747.

Tamilselvan, S., Raju, S. N., Loganathan, D., Kamatchiammal, S., Abraham, G., & Suresh, R. (2007). Endothelin-1 and Its Receptors ETA and ETB in Drug-Induced Gingival Overgrowth. *Journal of Periodontology, 78*(2), 290-295.

Teitelbaum, S. L. (2000). Bone resorption by osteoclasts. *Science, 289*(5484), 1504-1508.

Vanarsdall, R. L. (1994). *Orthodontics: current principles and techniques*: CV Mosby.

Vujačić, A., Konić, A., Pavlović, J., Todorović, V., Vukićević, V., Jevremović, D., &Milošević-Jovčić, N. (2017). Differences in IL-1β and IL-6 levels in the gingival crevicular fluid during acute phase of orthodontic tooth movement between juveniles and young adults. *Vojnosanitetski Pregled, 74*(3), 219-226.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., & Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature, 332*(6163), 411-415.

Zeballos, G. A., An, S. J., & Wu, J. M. (1991). Endothelin-1 secretion by human fibroblasts in culture: effects of cell density and IFN-beta. *Biochemistry International, 25*(5), 845-852.