**VIA MEDICA**

O R I G I N A L   A R T I C L E

# Periodontal ligament regulatory role in experimental diabetic rat model of periodontium remodelling

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*Background: Diabetes, among multiple systemic harmful health issues, also may deteriorate normal regenerative and reparative functions of periodontium. The aim of this research was to study the role of periodontal ligament in tissue remodelling under the orthodontic appliance stimulation in two rat experimental models (healthy Wistar rats and Goto-Kakizaki, rodent model of non-obese type 2 diabetes).*

*Materials and methods: Four groups of rats were defined: Wistar (WI; n = 8) and Goto-Kakizaki (GK; n = 8) control groups without orthodontic appliances, and Wistar (n = 16) and Goto-Kakizaki (n = 16) appliance groups with orthodontic appliances. After 42 days, rats were sacrificed and histopathology descriptive analysis about periodontal ligament and adjacent structures was performed as well as cellularity of periodontal ligament and Kappa curvature of tooth roots were measured.*

*Results: Goto-Kakizaki control rats showed statistically significantly higher cellularity in comparison with Wistar control rats (p < 0.001). Both applied groups (WI 44.63 ± 6.68; GK 79.58 ± 10.06) also showed statistically significantly higher cellularity (p < 0.001) in comparison with control groups (WI 34.48 ± ± 6.92; GK 45.31 ± 11.18). Applied groups (WI 0.197 ± 0.2; GK 0.126 ± 0.083) had statistically significant higher values of Kappa curvature in comparison with control groups (WI 0.023 ± 0.011; GK 0.037 ± 0.011) (WI appliance vs. WI control: p < 0.001; GK appliance vs. GK control: p < 0.05). Agitated periodontal ligament caused different degrees of cementoclasia and additionally dentinoclasia, altering the natural root curvature.*

*Conclusions: Although not significantly different (WI and GK appliance groups) according to degree of molar roots odontoclasia, higher cellularity of agitated GK periodontal ligament could be influencing factor for, as previously reported, elevated osteoclast mobilization and possible prolonged periodontium reconstitution.* (Folia Morphol 2022; 81, 4: 1031–1041)

**Key words: periodontal ligament, fibroblast, orthodontic appliance, tooth root, remodelling, periodontium**

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## INTRODUCTION

Orthodontic tooth movement (OTM) is a consequence of biological tissue responses to applying a force on the tooth. The biological response of different tissues (dental and paradental tissues, periodontal ligament, alveolar bone, gingiva) depends on many factors as a force magnitude, direction and duration of the force, local and systemic factors as individual differences, application of drugs, presence of systemic diseases etc.

Periodontal ligament (PDL) is the mechanosensory structure, which responds on applied force by remodeling itself also influencing on other structures of periodontium to self-reconstruction, which is finally manifest as directed translation of teeth through the bone. The PDL is a dense fibrous connective tissue structure that consists of collagenous fibre bundles, cells, neural and vascular components and tissue fluids. Its primary function is to support the teeth in their sockets while allowing teeth to withstand considerable chewing forces. These force-induced strains alter the PDL's vascularity and blood flow, resulting in local synthesis and release of various key molecules, such as neurotransmitters, cytokines, growth factors, colony-stimulating factors, and arachidonic acid metabolites. These molecules can evoke many cellular responses by various cell types in and around teeth, providing a favourable microenvironment for tissue deposition or resorption [7, 15].

In response to the deformation, fibroblasts and osteoblasts in the PDL as well as osteocytes in the bone are activated. A combination of PDL remodelling, and the localised apposition and resorption of alveolar bone enables the tooth to move [13].

It has been shown that fibroblast may be involved in the differentiation of osteoclasts and also play an important role in bone modelling due to mechanical loading. When there is application of the force the production of osteoclastogenesis stimulating molecules as receptor activator of nuclear factor $\kappa$B ligand (RANKL) are increased probably leading to increased osteoclast formation [21]. It was also found RANKL deletion in periodontal ligament cells significantly decreased the amount of OTM and reduced number of osteoclasts formed on the compression side after subjecting the teeth to orthodontic force [28].

Biological mechanisms of OTM could be influenced by different local and systemic factors, among them also metabolic disorders, such as diabetes mellitus (DM) characterised by chronic hyperglycaemia. In our previous study [18] we showed that the amount of OTM in rats with type 2 DM was not compromised after 42 days of experiment. The slight increase in bone resorption, diminished bone formation and decreased alveolar bone volume were observed. However, some other studies suggest that DM induces a decrease in osteoclast numbers and also diminishes differentiation of osteoblasts leading to reduced bone remodelling [25]. The duration of orthodontic force in this experiment lasted for 48 hours which is not long enough to study the linear phase of OTM where bone and PDL remodelling take place and reach the maximum capacity [17].

Diabetes induces changes in PDL fibroblasts gene expression that can enhance neutrophil recruitment and bone resorption which could be explained by high glucose induced nuclear factor kappa B (NF-$\kappa$B) activation, which play very important role in the mechanism of OTM [29].

It is widely known that root resorption (RR) is one of the unwanted side effects of OTM. Its mechanism is not exactly known. RR is associated with the bone modelling around the tooth. Patients' factors such as genetics and external factors including trauma are also thought to be associated with increased RR [1, 12, 16].

Increased incidence and severity of RR was found in patients undergoing comprehensive orthodontic therapy. It was also shown that heavy force application produced more RR than light force application [27]. There is not a lot of knowledge of the influence of diabetes on the amount of RR after OTM. Arita et al. [2] showed less amount of root resorption and less OTM after 2 weeks of experiment.

The aim of this research was to determine the extent of the morphologic transformation of periodontal ligament under the orthodontic appliance stimulation in two rat experimental models (healthy Wistar rats and Goto-Kakizaki rats, rodent model of non-obese type 2 DM), and to discuss influences of agitated periodontal ligament fibroblasts (PDLFs) on other structures of remodelling periodontium.

## MATERIALS AND METHODS

This study was performed on 24 male Wistar rats (WI) (314 ± 10 g, 13–14 weeks old), representing healthy controls, and 24 male Goto-Kakizaki rats (GK) (320 ± 9 g, 13–14 weeks old), which were used as the type 2 DM animal model. After overnight fasting,

glucose levels were measured in blood samples taken from the tail vein. The inclusion criterion for the GK rats was a blood glucose level of > 150 mg/dL.

Four groups of animals were defined. In the WI (n = 8) and GK (n = 8) control groups, no orthodontic appliances were placed. The animals in the WI (n = 16) and GK (n = 16) appliance groups were fitted with orthodontic appliances. The orthodontic appliance consisted of a superelastic closed-coil spring (25 cN, 0.15--mm wire diameter; Dentsply GAC International, York, Pa). The coil spring was placed with a stainless steel ligature between the maxillary left first and second molars and the incisors [22]. The orthodontic appliance was placed in and replaced (every 7 days) on animal under general anaesthesia — mixture of ketamine (50 mg/kg of body weight; Bioketan; VetoquinolBiowet, Gorzow Wielkopolski, Poland) and medetomidin hydrochloride (67 mg/kg of body weight; Domitorp; Pfizer, Brooklyn, NY, USA) injected intraperitoneally. On day 42, all animals in all groups were sacrificed. The dissected maxillary bone specimens with 3 molars were fixed with phosphate-buffered 4% paraformaldehyde (pH, 7.2–7.4) for 24 hours and decalcified in an ethylene diamine tetraacetic acid (EDTA)-water solution for 12 days. The samples were than routinely processed to paraffin blocks, with cutting plane orientated perpendicular to the occlusal plane of the molars. Paraffin sections 7 mm thick were deparaffinized and stained with haematoxylin and eosin.

For histomorphometry, microphotographs of the microscopic slides were captured on light microscope Olympus BX50 (Olympus, Japan) equipped with digital camera Leica DFC 295 (Leica Microsystems, Germany) under the total magnification of ×100. Morphometric analysis of digitalised microphotographs was done in image analysis software, ImageJ (v1.53c, Fiji, Wayne Rasband, NIH, USA).

Quantification of periodontal ligament cell number was done on roots' outer and inner aspects, using the point-counting method (ImageJ plugin CellCounter), where in selected region of interest of previously measured area, all fibroblastoid cells of periodontal ligament, transectioned through nucleus, were selected (tagged and counted), and peripherally positioned cementoblasts, osteoblasts and osteoclasts were excluded. The final periodontal ligament cellularity was calculated per equivalent area of 10,000 micrometers square ($\mu m^2$). Obtained values were relativised, and expressed as number of nuclei per 10,000 $\mu m^2$.

Aiming to evidence deformities of root surfaces toward periodontal ligament, resulted from possibly present cementoclasia and dentinoclasia, curvature analysis of outer and inner molar root contours was done by plugin Kappa v2.0.0 of ImageJ 1.53c (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, US). Initial curves of the contours were traced in B-Spline input (open type), passed through Data Fitting Algorithm (Point Distance Minimisation), and the resulting values of Average Curvature ($\mu m^{-1}$) were used for further statistical analysis.

### Ethical considerations

All animal procedures and the study protocol were approved by the Veterinary Administration of the Republic of Slovenia (number 34401-62/2008/9) and were in accordance with the Guide for the Care and Use of Laboratory Animals.

### Statistical analysis

Statistical analysis was performed in IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.), and comparison between measured locations was done using one-way ANOVA, Student's t-test or Mann-Whitney U test (depends on normality of values in groups) with the level of significance at $p < 0.05$. The Shapiro-Wilk test was a test of normality. As a post-hoc test during one-way ANOVA, Tukey test was used.
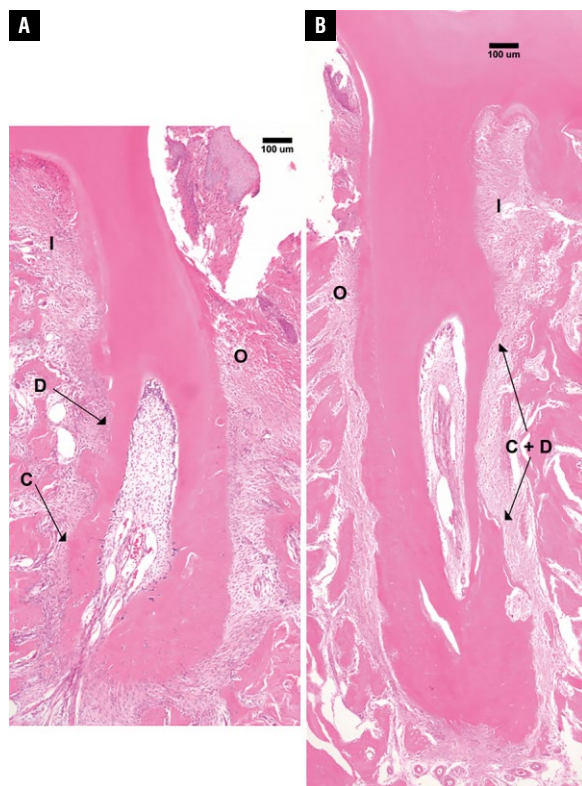
## RESULTS

The periodontal ligaments of non-appliance groups (WI and GK), as expected, were composed of regular fibrous connective tissue, characterised by regularly distributed filamentous components of extracellular matrix, and among them, fibroblastic cells noticeable by their uniformly ovoid nuclei. There was a visual impression of higher cellularity in PDLs of GK rats. The curvatures and contours of outer and inner (interradicular) aspects of teeth root were anatomically smooth, and occasionally discontinued by section of accessory root foramina (Fig. 1). Figure 1 shows the histological preparation of the tooth root in the control groups. A higher cellularity of PDL was observed on in GK rats (Fig. 1B) compared to WI rats (Fig. 1A). The difference in cellularity between the outer and inner side of the tooth root existed and varied from discrete to more noticeable, and was in the domain of individual characteristics. On some preparations there was a higher cellularity of PDL on
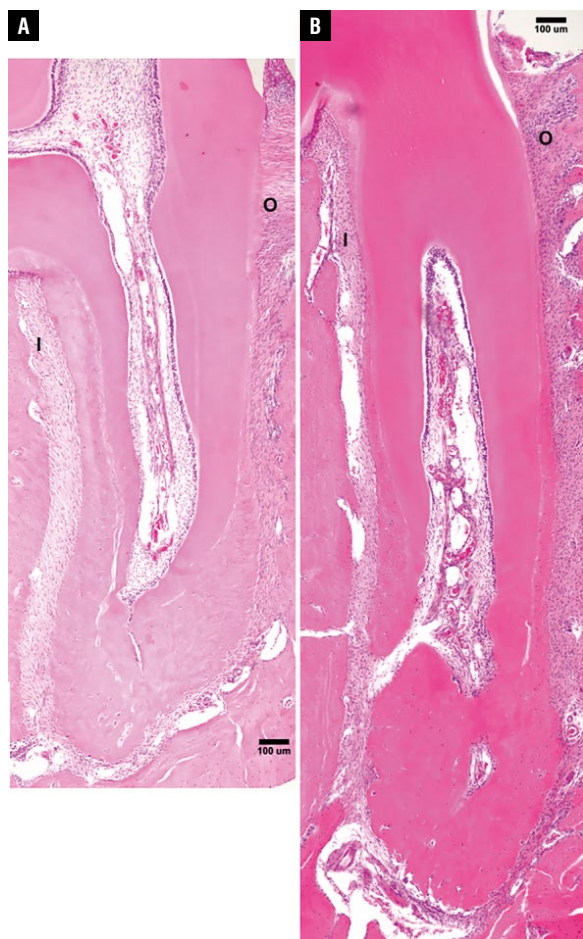
**Figure 1.** Presentation of tooth roots and periodontal ligament from Wistar rats (**A**) and Goto-Kakizaki rats (**B**) control groups (accessory root foramen on its inner aspect); haematoxylin and eosin ×100; I — inner side of the root; O — outer side of the root.



**Figure 2.** Presentation of cementoclasia and dentinoclasia in tooth roots in Wistar rats (**A**) and Goto-Kakizaki rats (**B**) appliance groups; haematoxylin and eosin ×100; I — inner side of the root; O — outer side of the root; C — cementoclasia; D — dentinoclasia.

the inner and on some on the outer side of the tooth root. These differences can also be seen in Figure 7, but the statistical analysis did not reveal a statistically significant difference in these values.

Histologic evaluation of the appliance groups (WI and GK) revealed that the volume of alveolar bone was diminished compared to controls, PDL region was widened, extending borderless into the broadened vascular channels of neighbouring alveolar bone, and different degrees of cementoclasia and additionally dentinoclasia were noticed, altering the natural root curvature, appearing as cavities in mineralised tissues of root, filled with mesenchyme like transformed PDL tissue (Fig. 2). Figure 2 shows the process of penetration of agitated PDL through cementum and then through dentine in the appliance group. In the example of WI rats (Fig. 2A), a thinning of the cementum and the dentine on the inner side of the tooth root

is observable in few areas. In the example of GK rats (Fig. 2B), severe cementoclasia and dentinoclasia can be seen in some cases, which drastically thin the root of the tooth from the inside. The picture shows the absence of inflammatory cells, which indicates that this process of destruction of tooth tissue is not mediated by a local inflammatory reaction.

In addition to damage to the lateral sides of the tooth root, damage of the apex of the tooth root was also observed in some animals in the appliance group. Figure 3 shows the different degrees of damage of the root apex. Figure 3A shows cementoclasia of the lateral part of the apex in GK rat (arrow). Figure 3B shows root apex damage to a greater extent in GK rat. In this example, a large part of the solid tissue of the tooth root is missing at the level of the apex, on the inner side (Fig. 3B, arrow). The same picture shows sporadic cementoclasia at the level of the lower third of the tooth root on the inner side (Fig. 3B, asterisk). The last figure (Fig. 3C) shows almost completely destroyed apical solid tooth tissue in WI
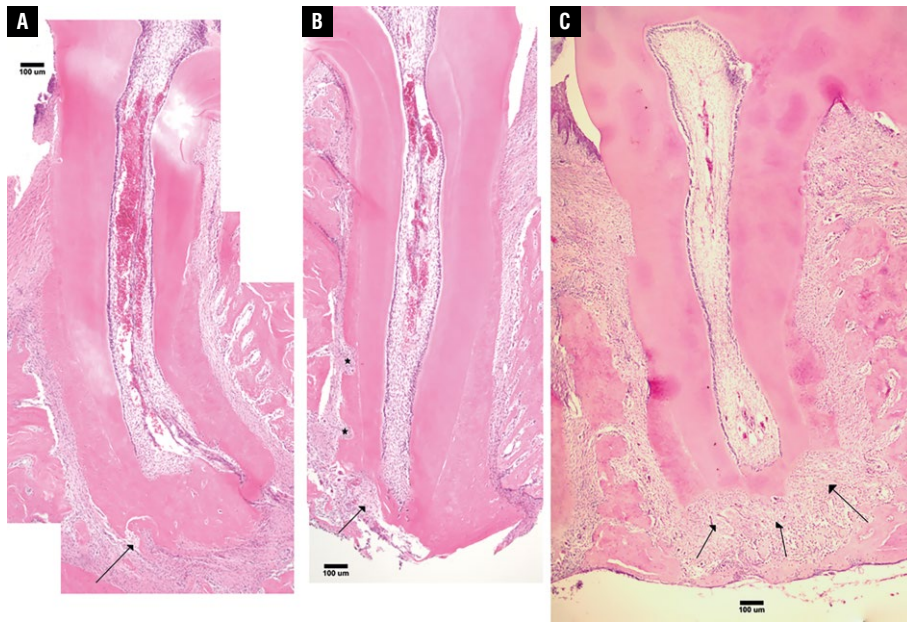
**Figure 3.** Presentation of apical root damage in rats in the appliance group — Goto-Kakizaki rats (**A**, **B**) and Wistar rats (**C**); cementoclasia of the lateral part of the root apex (arrow) (**A**); focal cementoclasia (asterisk), and focal odontoclasia of root apex (arrow) (**B**); advanced odontoclasia of root apex (arrows) (**C**); haematoxylin and eosin ×100.
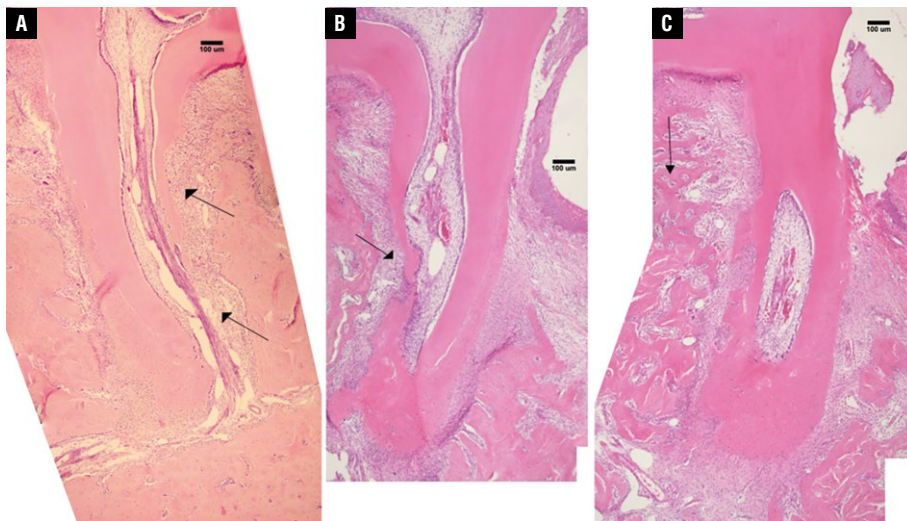


**Figure 4.** Presentation of major root damage (arrows, **A**, **B**) and widening of vascularized tissue between trabeculae of the alveolar bone (arrow, **C**) in the Wistar rats appliance group; haematoxylin and eosin ×100.

appliance group rats (Fig. 3C, arrows). In this case, there was a complete destruction of the cementum, as well as a significant thinning of the dentine at the apical level. At the level of the destroyed solid tissue, an altered PDL can be seen, which migrated and multiplied at this place.

The extent of this lytic morphology in some molars reached two thirds of root wall thickness, or even significant resorption of root apical region. Except

in one case of severe odontoclasia, morphology of dental pulp was unaltered (Figs. 2, 3, 4).

In addition to the partial destruction of cementum and dentine by activated PDL, larger defects were observed on some histopathology preparations. Figure 4 shows the dental preparations of the WI appliance group. Figure 4A shows the complete destruction of the solid tissue of the tooth root in the lower third and on the inner side. In this part, the PDL invades the
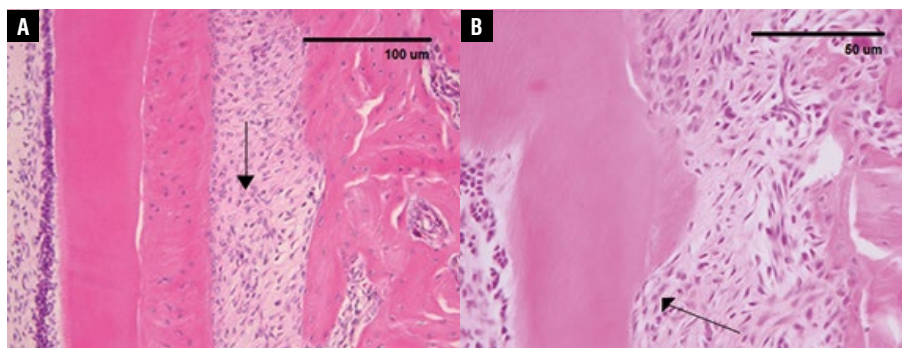
**Figure 5.** Periodontal ligament transformation — early transformation of periodontal ligament (PDL) (**A**) in agitated PDL of Goto-Kakizaki appliance group (interradicular side, arrow): haematoxylin and eosin ×200 (**A**); the same appliance group, mesenchyme like transformation of PDL morphology with cementoclasia (arrow, **B**): haematoxylin and eosin ×400.

pulp tissue, i.e. opens the tooth canal from the lateral side. Above this part, in the middle third, only such a layer of dentine separates the pulp from the PDL. The cementum tissue is completely absent (Fig. 4A, arrows). In the case shown in Figure 4B, the continuity of the thinned solid tissue of the tooth root is maintained from the inside. In addition to thinness, the changes are presented in this part as a pronounced deformation of both the inner and outer contours of this side of the root (Fig. 4B, arrow). Cases like these were extreme.

The physiological response of the surrounding tissue to the changes in the level of the tooth root after the placement of the apparatus was also noticed on the preparations. Alveolar bone follows root remodelling by regeneration, which is clearly seen based on the increase in the number of blood vessels within the alveolar bone. This phenomenon is illustrated in Figure 4C (arrow).

The presence of acute or chronic inflammatory cells infiltrates, as well as hyalinisation of PDL were not detected/were absent. The PDL of appliance groups only partially retained morphology similar to controls (mostly in part with horizontal fibres); however, with variable extent, focally or in extent to one third to half root length side(s), had altered morphology transforming itself into the mesenchyme-like morphology ("agitated periodontal ligament"). The cells of agitated PDL were randomly distributed in myxoid appearance of the connective tissue extracellular matrix, varying in sizes, appeared mostly as stellate, and polygonal elongated, mesenchymal cells, having angulated nuclei, and unlike mesenchymal cells, with acidophilic cytoplasm (Fig. 5). In early transformation of the PDL, fibroblastic cells elongate and their cytoplasm becomes more acidophilic (Fig. 5A, arrow). Figure 5B shows mesenchyme-like transfor-

mation of PDL morphology — loose, myxoid appearance of the connective tissue extracellular matrix and random distribution of cells in varying in sizes, which appeared mostly as stellate, and polygonal elongated, mesenchyme-like cells, having angulated nuclei, however with acidophilic cytoplasm. Cementoclasia and partial dentinoclasia are apparent (Fig. 5B, arrow) adjacent to partially preserved cementum (above). Cementoclasts and dentinoclasts are not observable adjacent to resorbed root surface. Osteoclasts are at the border of alveolar bone tissue.

The resorptive cavities of root tissue, show absence of cementoblasts and are mostly filled with mesenchyme-like tissue of agitated PDL. Occasionally, by the edge of these resorptive cavities, appear larger mononuclear polygonal cells with acidophilic cytoplasm, and only sporadically, the presence of multinuclear cementoclasts and dentinoclasts is detectable (Fig. 6). In appliance groups, other type of multinuclear, resorptive type of macrophagal cells, osteoclasts, are more often seen at the surfaces of alveolar bone tissue (Fig. 5).

By histomorphometric measurement of the cellularity of PDL, the average number of fibroblasts in PDL was determined at 10,000 $\mu m^2$ on both sides of the tooth root. By comparing these values, it was found that there is no statistically significant difference in the cellularity of PDL in any of the examined groups (Table 1).

By comparing the values of PDL cellularity among the examined groups, the existence of statistically significant differences was determined. In the WI appliance group, statistically significantly higher PDL cellularity was recorded compared to the WI control group (p < 0.001). With the same statistical significance, a higher cellularity of PDL was found in the GK
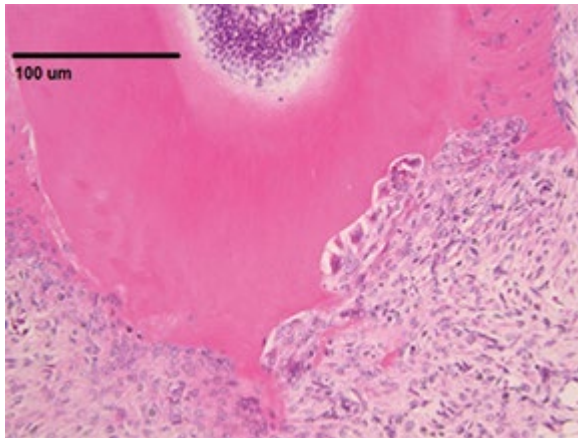
**Figure 6.** Partial resorption of Wistar rat molar root apex: in region of this odontoclastic cavity, the cementum is completely resorbed, and dentinoclasts are apparent in agitated periodontal ligament tissue at the border with partially resorbed dentine; haematoxylin and eosin ×200.

appliance group compared to the GK control group. In addition, an equally high statistically significant difference was found in the comparison of control groups and in comparison of appliance groups. The GK control group had a statistically significantly higher cellularity compared to the WI control group, and GK appliance group had a statistically significantly higher cellularity compared to the WI appliance group (Fig. 7).

By measuring the Kappa curvature from the inside and outside of the tooth root in the examined groups, lower values were observed in the control compared to the appliance groups. Analysis of Kappa curvature on the inner and outer side of the roots showed that there was no statistically significant difference for the control groups of animals. WI and GK control groups had a normal value distribution, so the Student's t-test was used for this analysis. The WI and GK appliance groups did not have a normal distribution of values, so the Mann-Whitney U test was used to compare their Kappa curvature values. It was found that in the appliance group there was a higher value of Kappa curvature on the inner side of the root, but this difference was statistically significant (p < 0.05) only in GK rats (Table 2).

The difference in average values of Kappa curvature among the experimental groups, regardless of the root side is shown on the Figure 8. Differences in Kappa curvature values between control groups, but also differences between appliance groups were not statistically significant. In contrast, the mean values of Kappa curvature were statistical-

**Table 1.** Average cellularity with standard deviation (average ± standard deviation [A ± SD]) on the inner and outer side of the tooth root in the experimental groups (nuclei per 10,000 $\mu m^2$)

| Animal group | Root side | A ± SD | P |
|---|---|---|---|
| WI control group | Inner | 35.16 ± 8.35 | 1 |
| | Outer | 33.81 ± 5.42 | |
| WI appliance group | Inner | 45.19 ± 8.15 | 1 |
| | Outer | 44.07 ± 5.04 | |
| GK control group | Inner | 43.35 ± 10.09 | 0.993 |
| | Outer | 47.27 ±.12.81 | |
| GK appliance group | Inner | 76.9 ± 9.02 | 0.775 |
| | Outer | 82.27 ± 10.7 | |

WI — Wistar rats; GK — Goto-Kakizaki rats
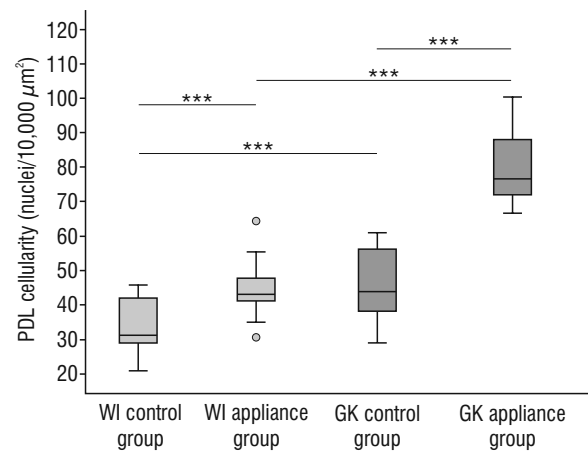


**Figure 7.** Difference in periodontal ligament (PDL) cellularity (nuclei per 10,000 $\mu m^2$) among the experimental groups; ***p < 0.001 (one-way ANOVA; standard error and mean and standard deviation were showed); WI — Wistar rats; GK — Goto-Kakizaki rats.

**Table 2.** Average values of Kappa curvature with standard deviation (A Kappa Curvature ± SD) on the inner and outer side of the tooth root in the experimental groups

| Animal group | Root side | A Kappa Curvature ± SD | P |
|---|---|---|---|
| WI control group | Inner | 0.026 ± 0.013 | 1 |
| | Outer | 0.02 ± 0.009 | |
| WI appliance group | Inner | 0.256 ± 0.249 | 0.093 |
| | Outer | 0.137 ± 0.177 | |
| GK control group | Inner | 0.042 ± 0.009 | 1 |
| | Outer | 0.032 ± 0.116 | |
| GK appliance group | Inner | 0.173 ± 0.07 | 0.007 |
| | Outer | 0.08 ± 0.07 | |

WI — Wistar rats; GK — Goto-Kakizaki rats; SD — standard deviation
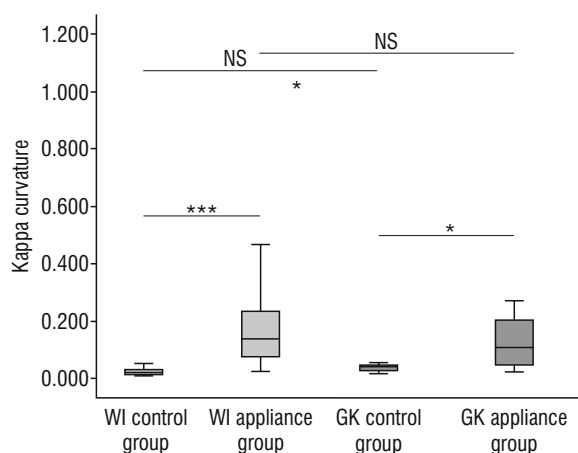
**Figure 8.** Difference in average values of Kappa curvature among the experimental groups; *p < 0.05, ***p < 0.001, NS — non significant (Wistar rats control group vs. Goto-Kakizaki rats control group — one-way ANOVA; the rest of analyses — Mann-Whitney U test; standard error and mean and standard deviation were showed); WI — Wistar rats; GK — Goto-Kakizaki rats.

ly significantly higher in the WI appliance group compared to the WI control group for p < 0.001. Similarly, the mean values of Kappa curvature were higher in the GK appliance group compared to the GK control group, but with lower statistical significance (p < 0.05).

## DISCUSSION

Mechanically induced remodelling of periodontal ligament by application of orthodontic apparatuses, after 42 days, and in given forces, microscopically could be evidenced by its widespread cellular and tissue transformation of periodontium. Changes are evidenced in 1) resorption of root mineralized tissues of examined molars, 2) transformation of associated PDL tissue as well as 3) changes in morphology of alveolar bone tissue. Mechanically stimulated PDL tissue have lost regularity of dense fibrous tissue, and transformed into low vascularised mesenchyme like connective tissue. Dominant cellular population of PDL regular fibrous tissue, fibroblasts, are transformed in connective tissue cells capable for extracellular matrix (ECM) degradation [9, 23], which are elsewhere nominated as fibroclasts [3, 8]. The synthetic and phagocytic duality of PDL is present even in physiological circumstances, when this cells are involved in renewal of PDL ECM [24].

The remodelling of PDL is based on transformation of periodontal ligament fibrous tissue fibroblasts in resorptive cellular phenotype which is known as fibro-

clasts, which latter could be reverted into previous phenotype. Fibroclast is the cell phenotype which originates from periodontal ligament fibroblast/fibrocyte, and which expresses lytic activities toward ECM, and which reverts into fibroblast phenotype, at the late phase of ligament remodelling. The closest cellular phenotype could be myofibroblast (connective tissue cell of inflammatory/reparatory processes) [3]. Fibroclasts take part in intercellular substance resorption and contain numerous lysosomes. These cells have tremendous potential to resorb tissue components, which is of the greatest significance during orthodontic procedures, taking the measures for reduction of possibility of tooth root resorption, and even root amputation. Actually, each fibroclast show a duality of both synthesis and ECM existing as a functional and possibly transient or reversible state in the fibroblast cycle [8].

This duality of PDL is based also on their mechanosensory properties [14], which as adaptation on different of mechanical forces stimulation, can modulate, and even partially abort synthesis and secretion of different ECM components.

Periodontal ligament fibroblasts are involved in the normal maintenance of ECM, regeneration, and repair of both the ligament and adjacent hard tissues. These cells respond proportionally to different degrees of mechanical stimulation, by which they have a central role in mediating the alveolar bone remodelling that underlies physiological and OTM. Cultivated human PDLFs react to tensional stress altering the biosynthesis of ECM proteins. Lower forces of biaxial deformation (5% biaxial deformation [strain] at a frequency of 30 times/min for 24 h) induce significant increase of collagen I and fibronectin synthesis, and a decrease in tropoelastin production relative to unstretched controls. However, cells exposed to higher (10%) strain exhibited similar responses for fibronectin and tropoelastin while the amount of type I collagen synthesis remained at control levels. These results indicate that mechanical stimulation of PDLFs alters type I collagen, tropoelastin and fibronectin production and that these cells are differentially responsive to varying levels of mechanical stress. The ability of these cells to alter ECM protein synthesis in response to specific magnitudes of tensional stress may in part explain how PDLFs regulate ligament and hard tissue remodelling [14].

Median values of rat molar outer and inner root contour Kappa curvatures mostly differ between compared groups. In the appliance groups of exper-

imental animals (WI and GK), it is apparent presence of numerous deformities of outer and inner contour of root tissue (cementoclasia or even, focally, dentinoclasia) which is documented by higher values of Kappa curvatures. Namely, appliance groups (WI and GK) have about fivefold higher values than molar roots of untreated animals, which is manifestation of PDL agitation, and probably recruiting short-living multinuclear macrophages, odontoclasts (respectively cementoclasts, and dentinoclasts), and less likely by action of fibroclasts — PDLFs differentiation into the resorptive cellular phenotype.

Similarly like in PDL cell number quantification, there is a finesse of statistically significant difference in median values of Kappa curvature between animals of control groups (WI and GK), which may suggest that GK rats have constitutionally different characteristics of PDL compared to Wistar species. The explanation to this notion is that PDL of GK rats may have higher constitutional cellularity, and relatively uneven rate of cementum apposition, or even a possibly inclination toward resorption of a delicate balanced remodelling (secretion and resorption) of periodontal tissues. The similar median values of Kappa curvatures of the root molar contours in the animals of two appliance groups (WI and GK), suggest that the resorptive processes which are performed by PDL fibroclasts aimed toward root tissues (cementum and dentine), and provoked by the orthodontic forces, also may have control mechanisms by which the further tissue damage is restricted and controlled.

The article by Plut et al. [18] demonstrated an evidence about constitutional differences between GK and WI strains of rats (control groups). Namely, GK animals may have a lower rate of osteogenesis/ bone-regenerating processes/turnover (and remodelling also) compared to WI animals, which may be supported by the fact about lesser alveolar bone volume, lower presence (given as surfaces ratios, presence given in per cents) of osteoblasts and osteoclasts in GK animals compared to WI animals. Similarly, in the current research, statistically significantly higher number of PDL cells is present in animals of GK control group compared to WI control. So, the starting positions, associated with capacities for osteogenesis/ osteolysis are quite lesser for GK animals; however, PDL constitution is opposite, being more cellular.

Upon effects of mechanical stimulation caused by orthodontic apparatuses, animals of appliance groups have reduction of alveolar bone volume, as well as rise of histometric values of osteoblast and osteoclast presence. The raise of osteoblast number in appliance groups is not enough high to become statistically different from appropriate control group; however, the increase in osteoblast number in WI group clearly dominates over the elevated number osteoblasts in GK group, indicating the much lesser osteogenic potential in GK rats stimulated by orthodontic forces. The rise of osteoclast number upon orthodontic stimulation is almost the same for WI and GK appliance groups, and osteoclast number increased 2.2 fold in WI appliance group and 5.5 fold in GK appliance group compared to each/their corresponding/respective control. It may be further discussed, that upon constant mechanical orthodontic stimulation, at the 42nd day, bone production lags, and osteolytic processes are more active in GK compared to WI rats.

It seems that the remodelling of alveolar bone tissue is not relying only on bone ECM osteoclast resorption but also on gradual transformation of bone tissue adjacent to PDL, which is evident in stepwise changes of bone vascular channels content, proportionally related to the distance from the border with PDL.

Both turnover and remodelling are characterised by the coordinated breakdown and synthesis of ECM components. In contrast to remodelling, turnover describes a process in which the structural organization of the tissue remains unchanged. During remodelling the three-dimensional organization of the fibre meshwork is adapted to accommodate for positional changes of the tooth in its socket or changes in functional state. In order to adapt to positional changes of teeth, the fibre systems in the PDL must be broken down. Because the collagen in the PDL appears to form a complex meshwork, not unlike a stretched fishing net, breakdown processes can occur at different sites without compromising tissue integrity. Thus, there is flexibility in the system to permit adaptive changes by breaking down short stretches of collagen fibre bundles or single fibrils while leaving others intact [4].

Periodontal ligament fibroblasts are highly sensitive to mechanical stress and are responsible for the rapid turnover of their extracellular compartment, collagen, which allows a rapid adaptation of the tissue to changing loads, such as in OTM [26]. In molar teeth of rats, PDL is normally constituted by 35% of fibroblastic cells and 51% in dry weight of their secretion product, the collagen fibres [11].

Garant and Cho [10] have studied extensively the process of collagen remodelling by PDLFs. They administered 3H-proline into mice and rats to determine the pattern of collagen synthesis by PDL fibroblasts by using both light microscopy and transmission electron microscopy. They described PDLFs as being elongated, polarized cells, with the nucleus positioned at one end and the cytoplasmic and secretory part at the other pole. Fibroblasts were found to be distributed evenly throughout the rodent PDL, and to migrate between the fibres, interacting during motion with adjacent matrix and cells. This motion appears to be facilitated by cellular microfilaments (actin and myosin), and by attachment to the matrix with glycoproteins (mostly fibronectin). Cho and Garant [6] concluded that in tooth movement, PDLFs in tension sites express the phenotype of actively migrating and matrix-secreting cells.

Normal, intact PDL, under the physiological ranges of mechanical load, appears as robust, highly organized, and morphologically constant structure. However, even in physiological ranges, PDL is a metabolically highly active structure, and its ECM components are under intense process of turnover. Namely, the autoradiographic analyses of tritiated proline metabolized in rats revealed that collagen turnover time in younger animals (6 weeks) varied from 2.45 to 6.42 days, and in older (6 months) ones from 7.08 to 10.87 days [19, 20].

In the previous article of our research team [18] it was shown that the higher values of relative parameter RANKL/OPG in appliance group of GK animals is based on retardation of OPG gene expression. It is emphasized that this phenomenon could be associated with lower alveolar bone volume, and high number of osteoclasts, and lesser production of OPG, which is indicative of lesser potential of bone tissue in GK appliance group animals to control, "brake"/slow down osteolytic process. At the same time, this is in contrast with relations present in appliance group of WI rats, where high level of RANK, as a promoter of osteoclastogenesis, is followed by high values of OPG, which antagonise stimulation of RANK, inhibitory binding to its ligand.

Translated from general model of the physiological regeneration of bone, in alveolar bone remodelling in orthodontic processes, as central regulator of osteoclastogenesis, the significance of osteoblasts is much often highlighted. However, in much complex relationship of teeth and jaw bones, and during regional remodelling induced by orthodontic agitation, the significance of many other structures, primarily of PDL cells (primarily, fibroblasts) have to be taken in consideration, as starters and modulators of specialised populations of multinucleated macrophages [5, 21, 28]. Similarly to osteoblast, periodontal fibroblasts also dictate the homing of precursor cells (from monocyte-macrophage cell lines) of further multinuclear macrophages such as osteoclasts, and additionally, could promote their differentiation into the odontoclasts (cementoclasts and dentinoclasts). The mechanism of mentioned processes is the same for osteoblasts and PDLFs and relies on intercellular communication based on secretion of homing cytokines, and regulated by RANKL and OPG. Presence of cementoclasia and dentinoclasia on appliance group's rat root molars is indication of previous activity of odontoclasts (which are not prominent by numbers, probably because are short-lived), precursors of which are most probably invited in cementum-PDL border, and there influenced (by agitated PDLFs) to differentiate into odontoclasts (cementoclasts and dentinoclasts), which in turn resorb the mineralised tissues of molar roots (up to extremes). It is highly probable, that excess of produced RANKL by agitated PDLFs is responsible for production of additional macrophagal cells (here odontoclasts), which in turn have harmful effect on molar roots.

## CONCLUSIONS

The results of this study confirm the role of the PDL in the remodelling process during OTM. By its transformation, the PDL initiates and modulates the processes of periodontium remodelling. Diabetes, as a cause of metabolically specific substrate, also causes constitutional hypercellularity of the PDL. Although not significantly different (WI and GK appliance groups) according to degree of molar roots odontoclasia, higher cellularity of agitated GK periodontal ligament could be influencing factor for, as previously reported, elevated osteoclast mobilization and possible prolonged periodontium reconstitution. This indicates a reduced ability of the PDL to control its response to mechanical stimulus in diabetic rats. In future the application of the lesser mechanical forces during the orthodontic procedures in diabetic organisms, should be further discussed.

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