## **Original Article**

# Age-Related Changes of Periodontal Ligament Surface Areas during Force Application

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## ABSTRACT

**Objective:** To investigate the age-dependent morphology of the periodontal ligament (PDL) tissue and changes in its surface area (SA) during force application provided with a standardized orthodontic setup for a period of 12 weeks in young and adult rats.

**Methods:** Two groups of 30 rats, age 6 weeks and 9 to 12 months, were used. Orthodontic appliances were placed to move the maxillary molars mesially with the contralateral sides used as controls. At 1, 2, 4, 8, and 12 weeks, groups of animals were killed. The PDL SA and the PDL SA ratio between pressure and tension regions were determined.

**Results:** An age-related decrease in the PDL SA was noted at control sides. Significant changes during the experimental period occurred only at experimental sides: The PDL SA was smaller at pressure than at tension regions only at week 1 in young rats; in adult rats, the difference between the two regions was significant at week 8. These changes were confirmed by the morphologic disorganization of the PDL and alterations in the PDL SA ratio.

**Conclusions:** During force application, the PDL at the pressure regions became disorganized and subsequently was reorganized, as is shown by the histologic changes and SA of the PDL over time. This process occurred earlier and was more prominent in young rats; it occurred later and was more prolonged in adult animals.

**KEY WORDS:** Periodontal ligament (PDL); Surface area (SA); Age; Rats; Orthodontics; Tooth movement

## INTRODUCTION

The periodontal ligament (PDL) primarily provides a supportive function by attaching the tooth to the sur-

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Accepted: December 2007. Submitted: August 2007. © 2008 by The EH Angle Education and Research Foundation, Inc. rounding alveolar bone proper. It also serves a major remodeling function that is enabled by cells that deposit and resorb all tissues that make up the attachment apparatus (ie, bone, cementum, and the periodontal ligament).<sup>1</sup>

The application of an orthodontic force generates biomechanical stress in the PDL and the alveolar bone; this force triggers biologic reactions that result in tooth movement. Studies have suggested that deformation of the PDL might be the key stimulus for initiation of orthodontic tooth movement.<sup>2,3</sup> However, the literature shows that much effort has been placed on alveolar bone remodeling, and little is known about changes that occur in the PDL during tooth movement. Moreover, the possible influence of age-related changes of the PDL on its morphology and reactions during force application has not been investigated.

The morphology of the PDL changes as age increases. PDL width is often small and irregular in old animals.<sup>4</sup> The maximum shear stress and stiffness of the rat molar PDL and its stress-relaxation curve decrease with age.<sup>5,6</sup> In old animals, (1) collagen fibers, which are primarily responsible for the mechanical properties of the PDL in rat molar teeth, are less well organized<sup>7,8</sup>; (2) the production of collagenous fibers shows an age-related decrease<sup>9</sup>; and (3) the functional characteristics of periodontal tissue cells have been altered by the aging process.<sup>10</sup> Proliferative activity of the PDL cells is significantly greater in young rats<sup>11</sup> and in young humans.<sup>12,13</sup> Periodontal fibroblasts also exhibit age-related changes.<sup>14</sup> However, to date, no detailed quantitative findings have revealed age-dependent effects on the histology of the PDL itself.

Periodontal tissue, the PDL in particular, plays a significant role in bone remodeling at the PDL–alveolar bone interface during orthodontic force application. The PDL width has often been used as an indicator of the morphology of periodontal space.<sup>15–17</sup> However, PDL width varies at different parts of the roots,<sup>18</sup> and changes that occur at each part may depend on the nature of tooth movement. The PDL surface area (SA) along the long axis of the root is a reflection of the average amount of periodontal ligament between root cementum and alveolar wall, which is a matter of significant importance to orthodontic tooth movement. Therefore, investigators used the PDL SA as the indicator.

The aim of the present study was to investigate the age-dependent morphology of the PDL tissue and changes in the PDL SA during force application with a standardized orthodontic setup provided over a period of 12 weeks in young and adult rats.

#### MATERIALS AND METHODS

#### **Experimental Tooth Movement**

Two groups of 30 male Wistar rats, aged 6 weeks and 9 to 12 months, were used as experimental animals. These animals were acclimatized for at least 1 week before the experiment was begun, were housed under normal laboratory conditions, and were fed powdered laboratory rat chow (Sniff, Soest, The Netherlands) and water ad libitum. Ethical permission for the study was obtained from Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

A split-mouth design was used with the experimental side randomly chosen and the contralateral side used as the control. A standardized orthodontic appliance was placed after general anesthesia had been induced at a dosage of 2.7 mL/kg body weight with an intraperitoneal injection of FFM mix, which contained fentanyl citrate 0.079 mg/mL, fluanisone 2.5 mg/mL (Janssen Animal Health, Beerse, Belgium), and midazolam 2.5 mg/mL (Rosteoclasthe, Mijdrecht, The Netherlands). The appliance has been described extensively elsewhere.<sup>19</sup> A transverse hole was drilled through the alveolar bone and through both maxillary incisors at the midroot level. A preformed ligature wire that enclosed all three maxillary molars was bonded



**Figure 1.** An overview of the study section ( $\times$ 4). At each study section, three roots were selected for measurements: two roots from the first molar (the middle and distal roots—the most mesial root was not used because it is oblique to the occlusal plane) and one from the second molar (the mesial root).

on the experimental side. A coil spring (GAC, Bohemia, New York, USA) was attached to a ligature wire that was put through the hole to move the molar unit to the mesial with a force of 10 cN. At 1, 2, 4, and 8 weeks, five or six rats from both age groups were killed for immunohistochemical evaluation; the remaining eight or nine animals underwent this process at 12 weeks.

#### **Material Preparation**

The rats received an overdose of anesthetic prior to the time of sacrifice. They were then perfused with 4% paraformaldehyde solution in 0.1 M PBS at 37°C. The maxillae were dissected and immersed in 4% paraformaldehyde for 24 hours at 4°C, then were rinsed in 0.1 M Phosphate buffered saline (PBS). After decalcification in 10% ethylenediaminetetraacetic acid (EDTA) and paraffin embedding, serial parasagittal 7 µm sections were cut. Every 25th section was collected on SuperFrost/Plus slides (Menzel-Gläser, Braunschwieg, Germany) and was stained with hematoxylin and eosin (H&E) for general tissue survey purposes. Additional sections were stained with ED1 antibody (Instruchemie, Delfzijl, The Netherlands) for evaluation of the morphology of osteoclasts. The method has been described extensively elsewhere<sup>20</sup> and was not the focus of the present study.

## PDL SA Measuring Protocol

Three roots (the distal root of the first molar and the mesial and distal roots of the second molar) per section were chosen for the study (Figure 1). Three sections with the longest study roots were selected for each animal and were scanned (Soft Imaging System [SIS] 3.2, Klausdorf, Germany). The borders of the PDL space were highlighted (Quantimet 520, Leica, Cambridge, England), and the PDL space was divided into mesial and distal regions by a line through the



**Figure 2.** Histologic changes in the periodontal ligament (PDL) during orthodontic force application ( $\times$ 40). In all cases, the left side was the alveolar bone, and the right side was the teeth (a, c, d, g, j, k, l, and p have been reverted to have the same orientation as the rest). Columns from left to right: weeks 1, 2, 8, and 12. Rows from up to down: young rats' tension regions, young pressure, adult tension, adult pressure. Arrows in a–d, i–l indicate spherical to slightly flattened osteoblasts; arrows in e–h, m–p indicate osteoclasts (close to alveolar bone surface, cytoplasm stained as dark brown). Bar length, 50  $\mu$ m.

long axis of the root that represented pressure or tension regions, depending on the direction of tooth movement. The cervical boundary of the PDL space was defined at the cementodentin junction. The PDL SA was defined as the SA of the PDL space, as was described earlier. At each region, the PDL SA was measured. To exclude possible effects caused by the orientation of histologic sections, the ratio of the PDL SA between pressure and tension regions was calculated for comparison between experimental and control sides. Before the orthodontic appliance was placed, the ratio was assumed to be 1.

## Statistics

For each study region, the mean of the nine measurements (3 roots  $\times$  3 sections) of the PDL SA and its ratio was calculated for each individual animal. Distributions of the data were checked with the D'Agostino-Pearson normality test. When data did not pass the normality test, values for the median, 25%

and 75% percentiles, and minimum and maximum were calculated. Data were further analyzed with the Kruskal-Wallis nonparametric (analysis of variance [ANOVA]) test; this was followed by the Tukey-Kramer multiple comparison tests. Mann-Whitney *U*-tests were used for comparison between pressure and tension regions and between experimental and control sides. Significant differences were recognized when P > .05.

## RESULTS

At the experimental side, teeth were moved to the mesial; thus the mesial was the pressure region and the distal the tension region. At the control side, teeth were undergoing distal drift; therefore, the distal was the pressure region and the mesial the tension region.

## Histologic Changes in the PDL Space

The PDL showed alterations in morphology over time (Figure 2). The PDL at the tension regions was



**Figure 3.** The periodontal ligament (PDL) surface area (SA) during orthodontic force application. exp-t indicates experimental tension regions; exp-p, experimental pressure regions; con-t, control tension regions; con-p, control pressure regions. \* P > .05; \*\* P > .01.

abundant in collagen fibers, spindle-shaped PDL cells, and spherical to slightly flattened osteoblasts and remained rather intact over time (Figure 2a–2d, 2i–2l). At the pressure regions, the PDL in young rats showed obvious disorganization at week 1 (Figure 2e), remained disorganized at week 2 (Figure 2f), and were reorganized and rearranged at weeks 8 and 12 (Figure 2g, 2h); in adult rats, the PDL started to become disorganized at week 2 (Figure 2n), showed obvious disorganization at week 8 (Figure 2o), and remained in a disturbed status at week 12 (Figure 2p). Disorganization of the PDL tended to be more intensive in the vicinity of osteoclasts (close to the alveolar bone surface, the cytoplasm stained as dark brown).

#### The PDL SA During Force Application

Large individual variations in the PDL SA were noted at all regions in young and adult rats. The PDL SA did not change with time at the control sides (Figure 3a, 3b). Therefore, data from the control side were pooled for the two age groups, respectively. The median of the PDL SA of the rat maxillary molars was significantly larger (P > .01) in young ( $13.4 \times 10^4 \mu m^2$ ) rats than in adult rats ( $11.2 \times 10^4 \mu m^2$ ). The Kruskal-Wallis test showed no time-dependent differences for any of the groups. At the experimental side of the young rats, a significant difference between pressure and tension regions was found only at week 1 (P > .05; Figure 3c). The PDL SA at the tension region was 1.5-fold that at the pressure region. In adult rats, it was lower at the pressure region than at the tension region at weeks 2, 4, and 8, but the difference was significant only at week 8 (P > .05; Figure 3d).

#### The Ratio of the PDL SA During Force Application

Large interindividual variations were noted in both groups. Significant changes over time were found with the Kruskal-Wallis test, which was performed in young (P > .01) and adult (P > .05) rats only at the experimental sides. In young rats, the ratio at the experimental side was significantly lower than that at the control side at week 1 (P > .05); in adult rats, the ratio was lower at weeks 2, 4, and 8, and significance was noted only at week 2 (P > .05; Figure 4a, 4b).

## DISCUSSION

The PDL plays an important role in bone remodeling during orthodontic force application. It is well recognized that after orthodontic force application, the general trend is observed as preservation of the amount of PDL, a remarkable process that involves precisely controlled bone resorption and deposition at specific



Figure 4. The ratio of periodontal ligament (PDL) surface area (SA) (pressure/tension) during orthodontic force application. exp indicates experimental side; con, control side.

sites in the paradental tissues.<sup>21</sup> However, limited research has been undertaken to explore the changes in PDL morphology and its surface area during prolonged force application or the effects of age on such changes. This is the first study that has generated detailed quantitative data on the PDL SA of the maxillary molars of young and adult rats during force application over a period of 12 weeks. Results presented here demonstrate that the process of preservation of the PDL space during prolonged force application is affected by age.

The results of the present study are consistent with those of previous studies on the effects of age on PDL width<sup>4</sup> and on changes in PDL width during the early phase of force application in young animals<sup>22</sup>; a time-related decrease in the PDL SA is evident in adult rat molars, as is a decreased or increased SA at compression or tension regions, respectively, at week 1 in young rats. Such a region-related difference in adult rats, however, started a week later, and was not significant until week 8, when a considerable amount of tooth movement and osteoclastic bone resorption occurred, as was also shown in earlier studies.<sup>19,20</sup>

The effects of age on the PDL SA were confirmed by obvious disorganization of the PDL at the respective time points. Previous studies<sup>23,24</sup> may suggest a possible effect of tissue necrosis (hyalinization) on this disorganization, caused by blood flow changes at the compressed PDL. However, whether such an effect is age related remains unclear. The earlier and more prominent disorganization of the PDL in young rats as induced by orthodontic stimuli may be explained by alterations of the elastic property of the PDL associated with age. Experimental studies have shown that oxytalan fibers, the only elastic element in the PDL, are more tortuous and complex in aged rats, and that this is related to considerable loss of elasticity in the PDL.<sup>25</sup> In humans, it has been shown that tooth mobility is significantly reduced in adults. The PDL fiber bundles in adults were shown to be more organized and normal fibroblast turnover was substantially reduced, resulting in alteration of the overall mechanical properties of the PDL.<sup>26</sup>

Large individual variations were noted in the PDL SA and in its ratio in both age groups. In earlier studies performed on the same animals, large interindividual differences were also noted in the velocity of tooth movement and in osteoclast recruitment.<sup>19,20</sup> The focus of the present study was the effect of age on changes in PDL morphology and SA. In young rats at week 1, significant differences between experimental and control sides were found in PDL SA between pressure and tension regions, as well as in the ratio; in adult rats, such differences were found at weeks 8 and 2, respectively. However, in adult rats, disorganization in the PDL and a region-related difference in the PDL SA were already apparent at week 2. Although the statistical significance of the PDL SA and that of its ratio did not completely coincide, both results reveal a relatively late and prolonged remodeling process of the PDL in adult rats.

The authors speculate that disorganization of the PDL is related to osteoclast activity. This theory is based on results that show that the disorganization was significant only at the pressure regions where osteoclasts were engaged in bone resorption, and that this disorganization tended to be more intense in the vicinity of osteoclasts. Our previous study supports this speculation by showing that the number of osteoclasts reached a peak at weeks 1 and 2 in young rats and at weeks 4 and 8 in adult rats.<sup>20</sup> These results taken together suggest that the remodeling of periodontal tissue and that of alveolar bone are closely related at the microscopic level, and that the effect of

age on PDL remodeling was consistent with effects on tooth movement and osteoclast recruitment.

#### CONCLUSIONS

- During orthodontic force application in rats, the PDL at the pressure regions became disorganized and subsequently was reorganized as reflected by the histologic changes seen in the PDL space and by alterations in the PDL SA over time.
- This process occurred earlier and was more prominent in young animals and occurred later and was prolonged in older animals.

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