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

Yijin Ren; Jaap C. Maltha; Lets Stokroos; Robert S. B. Liem; Anne Marie Kuijpers-Jagtman

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 surrounding 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).1

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.2,3 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.4 The maximum shear stress and stiffness of the rat molar PDL and its stress-relaxation curve decrease with age.5,6 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; (2) the production of collagenous fibers shows an age-related decrease; and (3) the functional characteristics of periodontal tissue cells have been altered by the aging process. Proliferative activity of the PDL cells is significantly greater in young rats and in young humans. Periodontal fibroblasts also exhibit age-related changes. 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. However, PDL width varies at different parts of the roots, 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 FFmix, 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 and was not the focus of the present study.

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, Braunschweig, 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 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 (×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 μm.

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 × 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
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 × 10⁴ μm²) rats than in adult rats (11.2 × 10⁴ μm²). 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.
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.

ACKNOWLEDGMENTS

The authors thank Mr. G. Poelen and Ms. D. Smale for their skillful assistance in the Central Animal Laboratory, Ms. WWHPA Meijer and Ms. MPAC Helmich for the histological work, Mr. REM van Rheden for the immunohistochemical work, Ms. WWHPA Meijer and Ms. MPAC Helmich for the histological work, and the bonding material by Kuraray Europe GmbH (Düsseldorf, Germany).

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