Periodontal tissue reaction during orthodontic relapse in rat molars

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SUMMARY Relapse after orthodontic tooth movement (OTM) is an undesirable outcome that involves a number of factors. This study investigated the remodelling of the alveolar bone and related periodontal structures during orthodontic relapse in rat molars. The maxillary right first molars of 35 Wistar rats were moved mesially by a fixed orthodontic appliance for 10 days and the contralateral molars served as controls. The appliances were removed and six animals killed. The molars were allowed to relapse, and the remaining animals were sacrificed at 1, 3, 5, 7, 14, and 21 days. The jaws were sectioned and stained with haematoxylin and eosin and tartrate-resistant acid phosphatase (TRAP). One day after appliance removal, the molars relapsed to a mean 62.5 per cent of the achieved OTM and then steadily relapsed to 86.1 per cent at 21 days. The number of osteoclasts situated along the alveolar bone of the first molars was highest at the end of active treatment and significantly decreased during the relapse period. In the OTM group, osteoclasts were most numerous in the pressure side of the periodontal ligament (PDL). As the molars relapsed over time, the osteoclast distribution shifted, and after 7 days of relapse, TRAP-positive cells were registered in previous pressure and tension sides of the first molars. After 21 days, these cells were concentrated in the distal parts of the PDL of all three maxillary right molars. These results indicate that orthodontic relapse in the rat model occurs rapidly and remodelling of the alveolar bone and PDL plays a central role in the relapse processes of both actively moved and adjacent teeth.

Introduction

Orthodontic relapse is a complex problem, with many factors potentially governing the outcome. Studies have shown that stability and relapse following orthodontic treatment are unpredictable, with a relapse tendency of 33–90 per cent after at least 10 years post-treatment (Little et al., 1988; Olive and Basford, 2003). Permanent retention has been advocated as the only way to ensure long-term post-treatment stability (Little et al., 1988).

Numerous studies have investigated the response of the dental supporting tissues to orthodontic force, and it has been reported that orthodontic tooth movement (OTM) is achieved following remodelling of the alveolar bone and a reaction of the periodontal ligament (PDL) to mechanical stimuli. Tooth movement occurs in the direction of force when there is a multifaceted bone remodelling response, with bone resorption on the compression side and bone apposition on the tension side of the PDL (Krishnan and Davidovitch, 2009).

Few studies have however been published about the biological mechanisms behind the relapse process or that adequately describe the course of relapse. It has generally been accepted that overstretched supravalveolar connective tissue fibres are responsible for relapse (Reitan, 1967; Parker, 1972). However, results from several histological studies do not support this view and suggest that factors other than the straightening of the supracrestal fibres may participate in the relapse process (Row and Johnson, 1990; Redlich et al., 1996; Lovatt et al., 2008). Yoshida et al. (1999) performed a comprehensive investigation into the cellular roles in relapse processes of rat molars. Their results suggest that remodelling of the principal fibres of the PDL and the surrounding alveolar bone are the main cause of relapse of experimentally moved molars. It is clear that a greater understanding of the relapse process is required in order to prevent this undesirable post-treatment outcome.

The purpose of this study was to investigate the remodelling of the alveolar bone and related periodontal structures, with particular attention to osteoclast activity, during the orthodontic relapse process in rat molars. It was hypothesized that orthodontic relapse and OTM would display a similar cellular pattern; specifically, the osteoclast population would be increased in areas of compression and decreased in areas of tension.

Materials and methods

Animals and experimental procedure

The material comprised 35 six-week-old (body weight 180–200 g) male Wistar rats (HanTac:WH, Taconic,
Danish). The animals were housed in Makrolon polycarbonate cages and fed a standard pellet diet (SDS R1E, Special Diets Services; Witham, Essex, UK) with tap water ad libitum. Standard 12 hour light–dark cycles, temperature 21 ± 2°C, and humidity 50 ± 15 per cent were maintained. Animals were acclimatized for 1 week before experimentation start date. The study was conducted in accordance with the animal welfare act and was authorized by the Norwegian Animal Research Authority.

A split-mouth design was used, the right side maxilla of each animal was experimental and the contralateral side (left) maxillae served as internal controls (C). The maxillary right first molars were moved mesially for 10 days by means of a chrome alloy closed coil spring (0.008 × 0.030; Ormco, California, USA) ligated to the mesial aspect of the first molar and the eyelet on an incisor band (Vandevska-Radunovic et al., 1994). The appliances were activated with approximately 0.5 N force with no reactivation during the treatment period. The amount of force applied was monitored using a Correx dynamometer (Haag-Streit, Bern, Switzerland). All procedures were performed under anaesthesia by intraperitoneal injection of Ketalar 10 mg/ml (Pfizer AS, Lysaker, Norway)/Midazolam 5 mg/ml (Alpharma, Actavis Norway AS, Norway), at a dose of 100 mg/kg body weight/5 mg/kg body weight. On the 10th day of experimental OTM, the appliances were removed, and the tooth movement was measured between the distal surface of the first molar and the mesial surface of the second molar using a feeler gauge (Mitutoyo Co., Kawasaki, Japan) with a minimum measurable distance of 0.05 mm. All measurements were performed twice by one operator; no variation was seen in measurement values.

Six rats were sacrificed immediately after appliance removal (group A0). The rest were killed 1 (R1; n = 4), 3 (R3; n = 4), 5 (R5; n = 6), 7 (R7; n = 5), 14 (R14; n = 5), and 21 (R21; n = 5) days following appliance removal. The weight of the animals was recorded on the day of appliance insertion, appliance removal, and prior to sacrifice. All the animals were sacrificed by intracardiac perfusion with 10% formalin and carried out under isoflurane inhalation anaesthesia (Forene, Abbot Scandinavia AB, Sweden).

**Histological preparation**

Following perfusion, the maxillae were removed, post-fixed in 10 per cent formalin for 24 hours at 4°C, and rinsed in phosphate-buffered saline overnight at 4°C. The tissue was demineralized in 10 per cent ethylene diamine tetra-acetate for 6 weeks at 4°C, dehydrated in ascending concentrations of ethanol, and embedded in paraffin for histological analysis. Parasagittal sections parallel to the long axis of the first molars were cut at 7 μm using an Anglia Scientific 0325 sliding microtome and mounted on 3-aminopropyltriethoxysilane-coated glass slides.

The slides displaying the greatest length of the most mesial and most distal roots were used for measurement (in total nine slides with three sections each, with two roots studied on each section per animal). The slides were alternatively stained with haematoxylin and eosin and tartrate-resistant acid phosphatase (TRAP). The TRAP staining procedure followed the protocol outlined by Brudvik and Rygh (1993) using 1 per cent aqueous green counterstain. A negative control medium was used to verify the specificity of the staining.

**Histological analysis**

Dental supporting structures of the first, second, and third molars were evaluated in the light microscope. Under high magnification (×100), osteoclasts were counted on the most mesial and most distal roots of all three molars. Cells were considered to be osteoclasts if they were TRAP-positive, multinucleated, and were located on the bone surface or residing in Howship’s lacunae. Cell counts for each section were blindly performed by two operators (TJF and VVR), after inter-operator calibration. The final count was designated to be the mean of these counts.

PDL width was measured on the mesial side of the most mesial and the distal side of the most distal roots of the first molars (Figure 1) using a Leica DMRBE microscope and Olympus DP50 camera with the cell B soft imaging system (Olympus soft imaging solutions GmbH, Münster, Germany). The width of the PDL was measured at the alveolar crest (cervical) and the apical (lowermost point on the curvature of the root) areas. All counts were performed blindly by one operator (TJF). The method error (Se) of the PDL space width measurements was performed in

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Figure 1 Schematic illustration showing the areas of measurement of the periodontal ligament (PDL) width of the first molar. m1 and m2 = measurement area on the mesial root at the alveolar crest and apical areas, respectively. d1 and d2 = measurement area on the distal root at the alveolar crest and apical areas, respectively.
where

where

Se calculated using Dahlberg’s formula (Dahlberg, 1940):

\[ \text{Se} = \sqrt{\frac{\sum d^2}{2n}} \],

where \( d \) is the difference between the two recordings of the PDL widths and \( n \) the number of double recordings. The Se was 0.679 \( \mu \)m in this analysis.

**Statistical analysis**

Data are presented as mean values ± standard deviation (SD). Relapse was calculated as a percentage per group. Group values were compared using independent tests. To evaluate the differences between the experimental and contralateral control sides, paired \( t \)-tests were performed. Results were considered statistically significant at the \( P < 0.05 \) level. Statistical analysis was carried out using the SigmaPlot 11 program (Systat Software, Inc.).

**Results**

**Animals**

There was an overall mean weight loss of 0.025 g ± 14.37 upon appliance removal; however, all rats gained weight by the end of the experimental periods. No significant relationship was found between the amount of OTM and body weight upon appliance removal or at the experiment end points (data not shown).

**OTM and relapse**

Tooth movement in a mesial direction was evident on all appliance treated first molars, with measurable spacing developing between the first and second molars. The mean OTM was 0.188 mm ± 0.099, with some individual differences being seen. There was no correlation between percentage relapse and OTM in any of the relapse groups.

Group A0 was sacrificed after 10 days of OTM; this group demonstrated the effect of OTM on the area of interest prior to relapse. Orthodontic relapse in a distal direction was apparent in all experimental relapse groups following appliance removal, with the tendency towards relapse being rapid initially following cessation of active treatment with only a gradual decrease in relapse rate (\( \mu \)md) towards the end of the experimental period (Figure 2A). One sample \( t \)-tests performed on each experimental group showed statistically significant differences between the mean percentage relapse of each group and group A0. There was a significant change in the relapse percentage from initial to late relapse (Figure 2B). The mean total relapse of the pooled data was 0.141 mm, which was approximately 74 per cent of active OTM.

**Osteoclast cell count**

Osteoclasts were recognized as TRAP-positive multinucleated cells located on or close to bone surfaces. There were no significant differences in TRAP-positive cell counts between different experimental times within control group C. There was an increase in osteoclast number following OTM; this number decreased over time as the first molars relapsed (Figure 3). Independent \( t \)-tests showed a statistically significant decrease (\( P < 0.05 \)) in osteoclast numbers around the mesial root of the first molars in groups R3, R7, R14, and R21 compared to A0. In the distal roots, significant differences were seen between group A0 and relapse groups R3, R14, and R21, between R1 and R14, and between the contralateral controls and relapse groups R3 and R14 (Figure 3). There were no statistically significant differences in osteoclast numbers between the mesial and distal roots.

**Histological evaluation**

**Contralateral side/control (C).** In the contralateral sides, TRAP-positive cells were observed in Howship’s lacunae along the alveolar bone wall opposite the distal aspects of the molar roots, suggesting physiological distal drift. There did not appear to be any difference in the histological picture between contralateral control sides in the different time groups.

![Figure 2](image-url) (A) Relapse rates and (B) mean percentage relapse ± standard error of the mean (SEM) of experimental groups of maxillary first molars at 0, 1, 3, 5, 7, 14, and 21 days following appliance removal. Day 0 corresponds to the 10 days active (A0) group.
Ten days active OTM group (A0). Active root resorption involving dentine was observed along the mesio-coronal half to two-thirds of the mesial and distal roots and the disto-apical half of the mesial roots of the first molars, with TRAP-positive multinucleated cells residing in root resorption lacunae. They were also found on the corresponding alveolar bone surfaces (pressure areas) indicating a tipping movement of the first molars (Figure 4). There was disruption of the PDL tissues in the compression areas and in some specimens, TRAP-positive multinucleated cells were observed infiltrating remnants of hyalinized tissue. In tension zones around the first molars, the PDL fibres appeared to be elongated, with stretching of the transseptal fibres between the first and second molars.

Scattered TRAP-positive cells were observed along the alveolar bone wall opposite the mesial aspects of the second and third molars, indicating ongoing bone resorption and mesial drift of the second and third molars (Figure 4).

One day relapse group (R1). Root resorption was present at the same sites as in group A0, with hyalinized tissue present in some specimens. Multinucleated cells were now observed on the alveolar bone surface facing both the mesial and the distal aspects of the first molar roots (Figure 5). TRAP-positive cells were now mostly seen on the distal sides of mesial and distal roots of the second and third molars. The PDL cells were irregularly arranged, and the appearance of the transseptal fibres at the interdental area between the first and second molars was similar to those in group A0.

Three, five, and seven days relapse groups (R3, R5, and R7). Some specimens still displayed active root resorption in the previous pressure zones of the PDL, indicated by TRAP-positive cells in and around the resorption lacunae, but hyalinized tissue had mostly disappeared from the PDL. In some instances, a chain of osteoblasts was seen in close approximation to osteoid tissue along the alveolar bone wall in tension areas (previous compression areas), denoting deposition of new bone (Figure 6).

The previous tension areas showed scattered TRAP-positive cells along the bone wall and in the PDL due to relapse, although osteoclasts could still be observed on bone surfaces on previous pressure areas in some cases. Osteoclasts were now only observed along the alveolar bone opposite the distal aspects of the roots of the second and third molars indicating resumed distal drift. At 3 and 5 days, the PDL cells and fibres were irregularly arranged. After 7 days, the transseptal fibres had a similar appearance to those in the controls.

Fourteen and 21 days relapse groups (R14 and R21). TRAP-positive cells were most abundant opposite

![Figure 3](image_url)  
Figure 3  Number of osteoclasts (means ± SD) located on the alveolar bone surface of the mesial and distal roots of the maxillary first molars of the experimental sides at 0, 1, 3, 5, 7, 14, and 21 days following appliance removal. Day 10 corresponds to the contralateral control group and day 0 to group A0. Significant differences between A0 and relapse periods are noted by *P < 0.05.

![Figure 4](image_url)  
Figure 4  Collated photograph of same section. Periodontal tissues of the maxillary molars after 10 days of tooth movement in direction of large arrow (A0). Tartrate-resistant acid phosphatase (TRAP)-positive cells (asterisk) on root dentine (d), periodontal ligament (PDL), alveolar bone wall, and marrow spaces of the alveolar bone (ab). There is direct resorption of the interdental alveolar bone opposite the mesial surfaces of the roots of all molars. TRAP, bar 500 µm.
the distal surfaces of the distal roots of all three maxillary right molars. There was deposition of bone opposite the mesial surfaces of the roots of the first molars suggesting remodelling following physiological drift in a distal direction (Figure 7). An irregular arrangement of PDL cells and fibres was still observed in the previous pressure areas.

**Width of PDL space**

Following OTM, there was a decrease in PDL width at the alveolar crest level (cervical) and an increase in PDL width apically on the mesial side of the mesial root, while the reverse occurred on the distal side of the distal root. These PDL width measurements are consistent with a mesial tipping displacement of the first molars during experimental OTM (Figure 8A and 8B).

Once the appliances were removed and the first molars began to relapse, the mesial cervical part of the PDL width approximated its pre-treatment width (Figure 8A).
The distal cervical PDL space started to narrow upon appliance removal, while the distal apical PDL space increased slightly. By 21 days post-treatment, the distal PDL appeared to have recovered its normal width, with no significant differences found between measurements in R21 and the contralateral sides (Figure 8B).

**Discussion**

Rats are considered to be a good animal model for studies concerned with bone remodelling and OTM (Frost and Jee, 1992; Ren et al., 2004), though some investigations have found a large inter-individual variability in rats (Verna et al., 1999). In the current study, there were individual variations of percentage relapse and relapse rates, as well as variety in the histology of the OTM within the groups. This indicates that there may be individual variations within factors concerned in the OTM and relapse processes (Reitan, 1960), such as density of alveolar bone, fibrous nature of the PDL, and osteoclast and osteoblast differentiation and recruitment. A split-mouth design was used to provide an internal control for potential confounders such as body weight, stress, tooth movement, and occlusal interference. No significant differences were found in either TRAP-positive cell counts or PDL widths between the contralateral control sides, either within or between the different time groups.

The molars were compressed towards the mesial side during OTM and relapsed towards the distal side. Relapse occurred in all appliance treated first molars following the end of active orthodontic treatment. There was a rapid relapse initially following appliance removal but after
3 days, both relapse rate and the percentage of relapse began to gradually decrease. Previous studies have also shown similar patterns in relapse activity (King et al., 1997; Yoshida et al., 1999; van Leeuwen et al., 2003). Yoshida et al. (1999) found that after 7 days of OTM using the Waldo method, the rat molars relapsed 72.2 per cent after 1 day and 89.9 per cent after 4 days, with relapse rates of 114 and 35.5 μ/m, respectively. These findings correspond favourably to our results of 62.5 and 68.9 per cent relapse and relapse rates of 100 and 31.6 μ/m after 1 and 5 days, respectively, following a 10 day OTM period. van Leeuwen et al. (2003) found a positive correlation between amount of relapse and amount of initial OTM in a dog model; however, in our rat model, these were not correlated.

Stretching of supraalveolar gingival fibres, the transeptal fibres, in particular, has been suggested as the cause of relapse (Reitan, 1967; Parker, 1972), and surgical fiberotomy has been suggested to support the transeptal fibre theory (Edwards, 1970). Our investigation showed that although transeptal fibres appeared to be normally stretched at 7 days post-treatment, the first molar continued to relapse; this suggests that stretching of the transeptal fibres may not play a central role in the aetiology of relapse. This view is supported by findings of high collagenous protein turnover within transeptal fibres experiencing a tensile force (Row and Johnson, 1990) and ultrastructural analysis revealing torn and disorganized rather than stretched fibres following rotation (Redlich et al., 1996). Both studies question the role of transeptal fibres in relapse and warrant further investigations.

After appliance removal, the teeth began to relapse in the direction of their original position; this reverse tooth movement being accompanied by an alteration in the number and distribution of osteoclasts. The number of osteoclasts declined significantly in both mesial and distal roots of the first molar within 3 days, most probably as a result of apoptosis and/or decreased blood vessel density (Murrell et al., 1996; Noxon et al., 2001). The osteoclast numbers dropped further at 14 days relapse and stabilized over the 14–21 days relapse periods. As the molars relapsed over time, the osteoclast distribution shifted, and after 7 days of relapse, they were registered in both previous pressure and tension sides of the first molars. After 21 days, these cells were concentrated in the distal parts of the PDL of all three maxillary right molars. Thus, by the end of the experimental relapse period, the bone remodelling pattern was similar to that in the contralateral sides. These observations concur with those of King et al. (1997), who reported that alveolar bone remodelling in the direction of force continued for several days following cessation of experimental OTM. Yoshida et al. (1999) suggested that remodelling of the alveolar bone is one of the main causes of relapse of experimentally moved rat molars, a premise supported by the findings in the present study.

Distal drift of adult rat molars has previously been measured at 7.7 μ/m (King et al., 1991), and new bone formation of 6.7 μ/m has been reported during this physiological process (Vignery and Baron, 1980). The measured relapse rate in R21 was 9.9 μ/m which is only slightly more than the previously measured rate of distal drift. This distal rate of movement most likely represents both residual relapse from the initial orthodontic force and resumed physiological distal drift. The low rate of distal molar movement, high percentage relapse, and bone remodelling processes suggest that at 21 days post-treatment, there is little relapse tendency left, rather, the rat molars have stabilized and resumed their normal physiological processes.

Our results confirm that there is a mesial tipping displacement of the first molars upon orthodontic force application, with a reversal of this tipping movement subsequent to appliance removal.

The width of the mesial cervical PDL and the distal apical PDL acted in a similar fashion and began to increase 1 day following removal of orthodontic force. The mesial apical and distal cervical PDL widths however displayed a narrowing from the end of orthodontic treatment to 21 days relapse and demonstrated that these areas of the roots were the tension areas during experimental tooth movement but changed to be considered as the pressure areas during relapse in a distal direction. Our results agree with the findings of Yoshida et al. (1999) that the PDL of the first molar had recovered its normal width in both compression and tension areas by 21 days after appliance removal; however, in our study, the PDL cells and fibres still appeared to be irregularly arranged in the previous pressure areas, implying that remodelling of the PDL did not occur as rapidly as previously suggested (Yoshida et al., 1999). It must be noted that the results for PDL width cannot be considered to be directly comparable to those from other studies since measurements have been taken from different areas, at varying time periods and for different force magnitude of tooth movement.

Conclusions

Orthodontic relapse occurs rapidly once teeth are relieved of orthodontic forces; this stresses the importance of immediate retention following active OTM. TRAP-positive cells change in number and distribution along the alveolar bone of actively moved and adjacent molars during relapse, with resulting bone resorption in the direction of relapse. Simultaneously, there is formation of new bone in the areas opposite the TRAP-positive cell activity. These results support the hypothesis that orthodontic relapse and OTM undergo the same process, namely that osteoclast differentiation increases in compression areas and decreases in tension areas. This indicates that remodelling of the
alveolar bone is an important element in the relapse processes of both actively moved and adjacent teeth.

**Funding**

Faculty of Dentistry, University of Oslo.

**Acknowledgements**

The authors wish to thank Ms Trude Olsen and Mr Dag Marcus Eide for their assistance in the National Lab Animal Centre; Mr Shabaz Yousefi, Ms Rita Greiner-Simonsen, and Osamu Tadokoro for technical support; Jan Unneberg for photographic assistance; and Professor Leif Sandvik for statistical input.

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