The influence of low-level laser on orthodontic relapse in rats

Tanya J. Franzen*‡, Sherif E. Zahra**‡, Abbadi El-Kadi** and Vaska Vandevska-Radunovic*

*Department of Orthodontics, Institute of Clinical Dentistry, University of Oslo, Oslo, Norway and **Department of Orthodontics, Suez Canal University, Ismailia, Egypt

‡These authors have contributed equally to this work.

Summary

Objectives: This study evaluated the effect of low-level laser therapy (LLLT) on the tendency of rat molars to relapse following orthodontic tooth movement (OTM).

Material and methods: Maxillary rat molars were moved mesially for 10 days. Animals were randomly assigned to group I (non-irradiated) or II (irradiation with LLLT). Appliances were removed, and the molars allowed to relapse for 1, 3, 5, 7, 14, or 21 days; rats in group II received LLLT according to a protocol. Bone density of periapical alveolar bone was measured using radiographs and Digora software. Dental supporting structures were examined histologically with haematoxylin and eosin and tartrate-resistant acid phosphatase.

Results: In both groups, first molar relapse was rapid 1 day after the end of active treatment; by 21 days percentage relapse was measured as 86.1 per cent in group I, and 72.22 per cent in group II. Osteoclast number was highest at the end of active OTM, and thereafter successively decreased during the relapse phase in both groups. Decrease in number, and redistribution of osteoclasts occurred more rapidly in the non-irradiated than the LLLT group. Whilst molar relapse was generally less and osteoclast numbers generally higher in group II compared to group I, the differences were not significant. There was no significant difference in bone density between the two groups.

Conclusions: These results indicate that LLLT may reduce the relapse tendency, possibly due in part to bone formation in previous tension areas, and to redistribution of osteoclasts following removal of orthodontic force. The role of LLLT in the prevention of orthodontic relapse requires further study.

Introduction

The biological mechanism of relapse of orthodontically moved teeth appears to be similar to that of orthodontic tooth movement (OTM), and relapse will occur rapidly in the absence of sufficient retention (1, 2). In rats, the mean relapse 1 day after removal of orthodontic appliances ranged from 62.5 per cent to 73.3 per cent, with the rate of relapse decreasing gradually over time (1, 3, 4). In humans, Edman Tynelius et al. (5) stated that the major part of relapse took place during the first year of retention, whilst Kuijpers–Jagtman (6) reported that almost 50 per cent of the relapse occurred within the first 2 years of retention.

This tendency toward rapid relapse has generated the interest to develop methods to reduce or prevent this undesirable change. Various systemically and locally administrated pharmacologic agents have been reported to reduce the amount of relapse in animal models, including bisphosphonate (7), osteoprotegerin (8, 9), simvastatin (10), relaxin (11), and bone morphogenetic proteins (12). The mechanisms of action are varied, but relapse is ultimately decreased by modification of the remodelling process of the dental supporting tissues. Any technique which could alter the normal biologic process following relapse could possibly be used as an adjunct to retention. Low-level
laser therapy (LLLT) has been used widely in dentistry; it is a non-invasive tool with various reported bio-stimulatory effects and could therefore be utilized to aid retention (13).

Following a literature review of the influence of LLLT on OTM in both animal and human studies, Torri and Weber (14) found that most authors report that LLLT increases the rate of OTM. Similarly, using meta-analysis of randomized control trials of LLLT use on human subjects, Ge et al. (15) concluded that LLLT could accelerate OTM. Previous studies have observed that LLLT may stimulate the velocity of OTM via increased expression of several key molecules such as RANK and RANKL (16, 17), M-CSF and c-fms (18), MMP-9, cathepsin K, and alpha(v) beta(3) integrin (19). As a result of these molecular reactions, the effects of LLLT biostimulation may be increased bone remodelling, with increased collagen synthesis, bone formation and mineralization, cellular proliferation and differentiation, and angiogenesis (13, 17, 20, 21). Despite the common finding of increased OTM, Goulart et al. (22) observed that a high dose of laser irradiation retarded OTM, and it has been suggested that LLLT may inhibit relapse due to accelerated bone regeneration (13, 23). However, Kim et al. (24) concluded that LLLT would only aid retention if a retainer was in place during the irradiation therapy, otherwise rate of relapse would be accelerated.

This investigation aimed to examine the effect of LLLT on orthodontic relapse tendencies in a rodent model. It was hypothesized that the biostimulatory effects of LLLT on the dental supporting tissues may minimize relapse after OTM.

Material and methods
Animals and experimental procedure
A total of 65 male 6-week-old Wistar rats (HanTac:WH, Tacom, Ry, Denmark), body weight 180–200 g, were used. The animals were housed in the Laboratory Animal Unit at The Norwegian institute of Public Health according to a protocol approved by the Norwegian Animal Research Authority, in compliance with the Animal Welfare Act. Body weight of each rat was monitored throughout the experimental period; no significant weight loss was recorded. Four animals were excluded from the study due to appliance complications (final number = 61 rats).

The rats were randomly assigned to group I (non-irradiated) or group II (irradiation with LLLT). The maxillary right first molars of all rats were moved mesially for 10 days using a chrome alloy closed coil spring (0.008 × 0.030, Ormco, California, USA) ligated to the mesial aspect of the first molar and an incisor band (Figure 1). Activation force was approximately 0.5 N, with no reactivation during treatment. Force magnitude was calibrated by a Correx dynamometer (Haag-Streit, Bern, Switzerland). Appliance placement was performed under anaesthesia by intraperitoneal injection of Ketalar 10 mg/ml (Pfizer AS, Lysaker, Norway)/Midazolam 5 mg/ml (Alpharma, Actavis Norway AS, Skøyen, Norway), at a dose of 100 mg/kg body weight/5 mg/kg body weight. All other procedures in the investigation were performed under isoflurane inhalation anaesthesia (Forene, Abbot Scandinavia AB, Sweden).

After 10 days of experimental OTM, appliances were removed, and tooth movement determined using a feeler gauge (Mitutoyo Co., Kawasaki, Japan) with a minimum measurable distance of 0.05 mm. Measurements were performed twice by one operator, with no observed variation in the recordings.

In group I, six rats were sacrificed immediately following appliance removal (I: A0). The remaining animals were killed 1 (I:R1) (n = 4), 3 (I:R3) (n = 4), 5 (I:R5) (n = 6), 7 (I:R7) (n = 5), 14 (I:R14) (n = 5), and 21 (I:R21) (n = 5) days following appliance removal. In this group, a split-mouth design was employed, the right half of the maxilla of each animal was experimental and the contralateral sides (left) served as the control group (C).

In group II, the rats were irradiated with LLLT according to varying protocols, and were sacrificed at the same time points as group I (Figure 2). All group II rats received LLLT on the day of appliance removal, and 1 day later six rats were killed (II:R1) (n = 6). Rats killed after 3 (II:R3) (n = 4), 5 (II:R5) (n = 4) and 7 (II:R7) (n = 5) days were irradiated every second day following end of OTM, whilst those killed after 14 (II:R14) (n = 4) and 21 (II:R21) (n = 3) days were irradiated every third day. Thus, the rats in group II, sacrificed at 1, 3, 5, 7, 14, and 21 days received one, two, three, four, five, and seven doses of irradiation, respectively.

Animals were killed by intracardiac perfusion with 10 per cent formalin, following isoflurane inhalation anaesthesia. Measurement of tooth movement was performed again in all rats on the day of sacrifice.

Figure 1. Appliance in situ in rat model. Experimental tooth movement was achieved by mesial movement of the upper right first molar by approximately 0.5 N activation of a closed coil spring.
Laser exposure
A photon-plus, gallium-aluminium-arsenide (GaAlAs) diode laser device (Rønvig Dental A/S, Daugaard, Denmark) was used, providing a continuous wavelength of 830 nm and a power output of 75 mW. The laser beam was delivered by a probe (18 mm diameter), with spot size 0.13 cm², and intact power density of approximately 550 mW/cm². The probe was in light contact with the first molar from the occlusal and lingual sides due to accessibility. Each animal received 3 J/session. Exposure time was 17 seconds, providing an energy density of approximately 23 J/cm². These conditions were determined based on previous experiments which demonstrated accelerated bone remodelling in bone defects in rats following laser irradiation at energy densities of approximately 20–25 J/cm² (21, 25).

Histological preparation
Preparation was performed as outlined by Franzen et al. (1). Briefly, following perfusion the maxillae were removed, post-fixed in 10 per cent formalin, demineralized in 10 per cent ethylene diamine tetra-acetate, then embedded in paraffin for histological analysis. Parasagittal sections parallel to the long axis of the first molars were cut at 7 µm and mounted on 3-aminopropyltriethoxysilane coated glass slides. The slide displaying the greatest length of the mesio-palatal root and four adjacent slides were alternatively stained with haematoxylin & eosin (H&E) and tartrate resistant acid phosphatase (TRAP) (in total 12 H&E sections and 15 TRAP sections per animal). The TRAP staining procedure followed the protocol outlined by Brudvik and Rygh (26) using 1 per cent aqueous green counterstain.

Histological analysis
Dental supporting structures of the molars were evaluated in the light microscope. Under high magnification (x100) osteoclasts were counted on the most mesial and most distal roots of the first 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, following inter-operator calibration. The final count was designated to be the mean of these counts.

Bone density—densitometric analysis
Prior to demineralization, standardized radiographs of the right and left maxillary molars of all rats were taken at a focus-film distance of 40 cm, with focus perpendicular to the film-object plane, using a Trophy ETX X-ray machine (Trophy Radiologie, Croissy Beaubourg, France), operating at 70 kV, 10 mA for 0.6 seconds. The bone density was evaluated at two periapical areas; mesial and distal to the distal root of the first molar. Mean bone density was measured using Digora software, version 1.51 (Soredex Corporation, Tuusula, Finland). A high definition window mode was chosen in order to delineate the outline of the roots of the first molar. Images were analysed and the mean bone density was measured using Hounsfield units (HU).

Statistical analysis
Data are presented as mean values ± SD. Relapse was calculated as a percentage per group. Group values were evaluated by independent or paired t tests, or one-way analysis of variance where appropriate. Results were considered statistically significant at the P < 0.05 level. Statistical analysis was carried out using the Sigmaplot 12 program (Systat Software Inc., San Jose, California, USA).

Results
OTM and relapse
Following 10 days of orthodontic force application, all treated first molars demonstrated measurable mesial tooth movement, whilst no tooth movement of the untreated contralateral first molars was detected. The mean OTM for group I was 0.19 ± 0.10 mm, and for group II was 0.15 ± 0.09 mm.

In both groups all appliance-treated molars experienced relapse in a distal direction (Figure 3A); relapsing rapidly 1 day after the end of active treatment (group I: 62.5 ± 14.43%; group II: 54.17 ± 10.21%), with a subsequent reduction in relapse rate (µmd⁻¹) (Figure 3B). By 21 days, the first molars in group I had relapsed a mean 86.11 ± 12.73% of their achieved OTM and those in group II had relapsed 72.22 ± 25.46%. Whilst the molars in group II relapsed less than those in group I at each experimental time point, the differences were not significant.
Osteoclast cell count

No statistically significant differences in osteoclast numbers were found between the mesial and distal roots when comparing groups I and II (Figure 4A and 4B). The irradiated samples mirrored the pattern of the osteoclast number count seen in the non-irradiated samples following appliance removal. In both groups an increase in osteoclast number following OTM was noted; this decreased over the experimental time period following appliance removal. Although not significant, the irradiated molars displayed increased numbers of osteoclasts in nearly all experimental groups compared to the non-irradiated molars. After active tooth movement, the number of osteoclasts found along the mesial roots were generally higher than that found in the distal roots in both groups I and II; the only significant differences between mesial and distal roots were seen in I:R5, II:R3, II:R7 and II:R14 (Figure 4C and 4D).

Histological examination

Non-irradiated group I

In the control group (C), TRAP-positive multinucleated cells were seen in Howship’s lacunae along the alveolar bone wall opposite the distal aspects of all molar roots, suggesting physiological distal drift. Following ten days of active OTM (A0) TRAP-positive cells were located on the bone surfaces corresponding to pressure areas 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 molar, also opposite the mesial aspects of the second and third molars. Some TRAP-positive multinucleated cells were observed in remnants of hylalnized tissue and in root resorption lacunae on root dentine. There was stretching of transseptal fibres and elongation of periodontal ligament (PDL) fibres in tension areas. One day after appliance removal the histological appearance was similar to group A0, however, multinucleated cells were now seen on the alveolar bone surface facing both the mesial and the distal aspects of the first molar roots.

In groups I:R3, I:R5 and I:R7 relapse of the first molars was evident as new bone formation was sometimes seen in previous compression areas, and osteoclasts were identified along the bone wall in previous tension areas, although in some cases they could still be observed in previous pressure areas. Distal drift of the second and third molars was denoted by TRAP-positive cells on the alveolar bone surface opposite the distal aspects of the roots. After 7 days, the transseptal fibres appeared to be reorganized and appearance was comparable to the control group. In groups I:R14 and I:R21 TRAP-positive cells were now mostly observed opposite the distal surfaces of the roots of the three maxillary right molars (Figure 5A) and there was bone apposition facing the mesial surfaces of the first molar roots. PDL fibres and cells were still irregularly arranged in previous pressure areas.

Irradiated group II

The histological picture observed in the experimental groups was similar to that of the non-irradiated groups, however there appeared to be both an increased number of osteoclasts and a lag in distribution pattern of TRAP-positive osteoclasts. In groups II:R1 and II:R3, around the first molar most osteoclastic activity was seen on the mesial side of the mesial root, although scattered TRAP-positive cells were seen on distal sides of all molar roots. Subsequently, through to 21 days post-appliance removal, TRAP-positive cells were still found on the alveolar bone wall opposite the mesial sides of the first molar mesial roots (Figure 5B), although little evidence of new bone formation was observed in these areas. As the experimental time period increased, more TRAP-positive cells were located on the distal sides of the first molar roots, additionally, new bone formation was seen here. This indicated a delay in relocation of bone resorbing cells, and an increase in bone formation in previous tension areas in the LLLT treated maxillae as compared to the non-irradiated group.

Bone density

The changes in mean bone density of either the mesial or distal sides of the distal roots in the non-irradiated group were not significantly different to those in the irradiated group (Figure 6A and 6B). Moreover, there was no discernible pattern to the changes in bone density over the experimental time period.

Discussion

Clinical and experimental studies have confirmed that long-term stability of orthodontically moved teeth requires the coordination of favourable tissue remodelling, growth development, good treatment result,
and a suitable retention protocol. Any additional method that could be utilized to decrease the relapse of orthodontically treated teeth should be developed on the basis of a comprehensive understanding of both the relapse process itself and the effects of the method in question on the dental supporting tissues after cessation of active orthodontic treatment. This investigation therefore examined the effects of LLLT on the

Figure 4. Number of osteoclasts (mean ± SD) located on the alveolar bone surface of the mesial and distal roots of maxillary first molars in the non-irradiated group I or LLLT irradiated group II at 0, 1, 3, 5, 7, 14, and 21 days following appliance removal. Groups C and A0 were not irradiated, but have been included in figure D to allow for comparison to the irradiated rats in group II. A, B significant differences between A0 and relapse periods of both groups are denoted by $^*P<0.05$. C, D significant differences between mesial and distal roots at each time period are denoted by $^#P<0.05$.

Figure 5. A, B Periodontal tissues of the maxillary first molar mesial root 14 days after appliance removal (R14). A non-irradiated group I (I:R14), B irradiated group II (II:R14). Tartrate-resistant acid phosphatase (TRAP)-positive cells (*) lining the alveolar bone surfaces (ab); A opposite the distal surface of the root (d) in the non-irradiated group, and B opposite both the mesial and distal surfaces in the irradiated group. Large arrow, direction of orthodontic force. A, B TRAP, bar 500 µm. 1, 2, and 3 TRAP, bar 100 µm.
relapse potential of orthodontically moved teeth, with particular attention to osteoclast distribution during the relapse period.

Although LLLT reduced the percentage of relapse, it was non-significant and relapse still occurred rapidly following the removal of orthodontic forces. In both non-irradiated and irradiated groups first molar relapse was rapid 1 day after the end of active treatment; and by 21 days percentage relapse was measured to be 86.11 per cent in the non-irradiated group, and 72.22 per cent in the irradiated group. The causal mechanisms of orthodontic relapse remain relatively unclear, however it would seem to be a complex multifactorial process. Remodelling of the PDL and surrounding alveolar bone is an important element in the relapse process (1, 3, 4). Other potential factors may be normalization of the periodontal vasculature following orthodontic force (27), increase in elasticity of the gingiva that is being retracted and compressed in the direction of the tooth movement (28), and stretching of transseptal fibres (29, 30). However, collagen turnover is high within transseptal fibres and the PDL (31) and therefore stretching of the transseptal fibres is not considered to be an important aetiological factor.

Our results show that the number of osteoclasts was highest at the end of active treatment and subsequently decreased during the relapse phase in both groups. Fall in number, and redistribution of osteoclasts from the mesial to the distal surfaces of the first molar roots occurred more rapidly in the non-irradiated group than the LLLT group, although differences were not significant. LLLT does not appear to prevent the biological relapse process, but delays redistribution of the TRAP-positive cells along the alveolar bone surfaces, and possibly increases the rate of bone formation. During OTM, some effects of LLLT are stimulation of osteoclast proliferation in the pressure side, and increased bone formation and rate of cellular proliferation in the tension side (16, 20). In this study irradiation was administered to all animals in group II on the day of appliance removal, and osteoclasts were possibly stimulated to proliferate in pressure areas formed during active OTM, as seen by the relatively higher TRAP-positive cell count on the mesial roots in the irradiated group. This would suggest that a LLLT regime used as a therapeutic aid to resist relapse should be started earlier, perhaps prior to debonding of appliances in order to maintain osteoclast presence on the former pressure sides and bone formation on the former tension sides.

Once orthodontic force has been removed, rat molars start to relapse distally. A component of this distal movement is the resumption of physiological distal drift (32). LLLT could possibly stimulate further osteoclast proliferation on the distal surfaces, which could lead to relapse; however, the osteoclasts observed on the distal surfaces during relapse in the irradiated groups in this investigation may only be a result of the resumed drift process. Moreover, stimulated bone formation on the former tension sides may counteract the effect of any osteoclast proliferation in these areas.

Yoshida et al. (33) reported that there was a temporal decrease in bone mineral density (BMD) on the tension side during OTM, with an increase in the amount of OTM in both irradiated and non-irradiated rats. After 7, 14, and 21 days the BMD was significantly greater in irradiated rats compared to the non-irradiated control group. This was suggested to be due to LLLT stimulation of osteogenesis at tension sites balancing osteoclastogenesis and bone resorption at pressure and tension sites. In this investigation no significant difference in bone density between the non-irradiated and the irradiated groups was seen. The employed method of densitometric analysis may not be sensitive enough, possibly due to the small sample area, which may have biased the results, additionally, it is only a two-dimensional measurement, therefore the interpretation of bone density in this study should be given limited weight.

The action of LLLT on OTM has been investigated relatively frequently, even so, the principal mechanisms of action have yet to be clearly determined. Relatively few studies however, have been carried out on the effect of LLLT on orthodontic relapse. Kim et al. (24) studied the effects of LLLT on relapse and retention of rat molars and concluded that LLLT administered with retention facilitated collagen synthesis contributing to faster repair of damaged PDL tissue and better retention, whilst irradiation performed without retention in place would lead to an increased rate of relapse due to increased catabolic metabolism of collagen. The results of the present investigation partially concur with these findings; and it would appear that orthodontically moved teeth should be stabilized by a retainer whilst a period of rapid remodelling is taking place. LLLT application could aid in the remodelling process by increased bone formation and reduction of redistribution of osteoclasts from previous pressure areas, ultimately resulting in less relapse.

LLLT is characterized by many parameters including wavelength, total irradiated time, intensity, and energy density. These parameters must be defined and their effects on relapse studied before LLLT can be implemented as a biologic device to aid regulation of the orthodontic relapse tendency.

Figure 6. Comparison of bone density of the periapical areas of measurement of the first molar at A the mesial side of the distal root in the irradiated and non-irradiated groups, B the distal side of the distal root in the irradiated and non-irradiated groups. Data are represented as mean ± SD.
Conclusion
The results of this study indicate that LLLT may reduce the relapse tendency, possibly due in part to bone formation in previous tension areas, and to a delay in redistribution of osteoclasts following removal of orthodontic force. However, LLLT appears only to decrease orthodontic relapse and not inhibit it, therefore conventional retention must also be employed. More research is required on a molecular and cellular level, and irradiation parameters must be developed before LLLT can be advocated as a biologic tool to reduce the orthodontic relapse tendency.

Funding
Faculty of Dentistry, University of Oslo, and the Ministry of Higher Education, Egypt.

Acknowledgements
The authors wish to thank Dr. Shabaz Yousefi, Marwan Khadra, and Jan Unneberg for technical support.

References