Effect of appliance reactivation after decay of initial activation on osteoclasts, tooth movement, and root resorption

Gaoman Gu, DDS, PhD; Steven A. Lemery, DDS; Gregory J. King, DMD, DMSc

Abstract: Clinical orthodontists frequently reactivate appliances following decay. Studies of tooth movement and tissue responses following reactivations indicate that linear tooth movement and rapid recruitment of osteoclasts can be achieved if reactivation is timed to coincide with the latter part of the bone remodeling cycle initiated by the first activation. Both can be delayed if reactivations are timed for the early part of the previous cycle. The objective of this study was to examine tooth movement, root resorption, and osteoclast recruitment following appliance reactivation after the first activation had decayed. Bilateral orthodontic appliances were activated with 40 cN in 144 rats to mesially tip the maxillary molars. After 16 days, rats were randomized into two groups of 72. In group 1, appliances were reactivated in precisely the same manner as the first activation. In group 2, appliances were sham-reactivated. Rats were sacrificed at 1, 3, 5, 7, 10, and 14 days. Orthodontic movement was measured cephalometrically; changes in osteoclasts and root resorption were assessed at both compression and tension sites histomorphometrically; tartrate-resistant acid phosphatase (TRAP) was measured in alveolar bone and serum biochemically. Orthodontic tooth movement was linear in group 1, but osteoclasts required 3 to 5 days to appear. There were no group- or time-related differences in root resorption. Bone TRAP levels were elevated in both groups but dropped significantly ($p<0.01$) in group 2 at day 7. Appliance reactivations that followed decay of the first activation produced efficient tooth movement without increased risk of root resorption, but these changes were not accompanied by rapid osteoclast recruitment at compression sites. Timing appliance reactivations for the latter portion of the previous bone remodeling cycle could have significant clinical advantages because the delay period seen in tooth movement following a single activation or short-term reactivation can be avoided.

Key Words: Orthodontic tooth movement, Osteoclasts, Histomorphometry, Alveolar bone, Root resorption, TRAP

Orthodontic treatment consists of numerous appliance reactivations that are usually planned without full appreciation of biological issues. After activation of an appliance, tooth movement follows a three-part pattern consisting of displacement, delay, and movement.[1] This is accompanied by the appearance of osteoclasts at sites of compression.[2] These cells initiate alveolar bone remodeling at these sites,[3] but require 3 to 5 days to appear in rats. This requirement contributes to the delay seen in tooth movement. When a second appliance activation is timed to coincide with either the osteoclast recruitment period (1 day after the initial activation)[4] or the peak expansion of the osteoclast population (4 days after the initial activation),[5] further significant delays occur in the appearance of osteoclasts and the progress of tooth movement. In contrast, osteoclasts appear immediately and tooth movement is linear following appliance reactivation during the period when osteoclasts have cleared from compression sites and bone formation has begun (10 days after the initial activation).[6] These findings suggest that the timing of appliance reactivations may be critical to achieving the most efficient orthodontic treatment (longer intervals between reactivations may be more efficient). Data are currently unavailable on the nature of alveolar

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bone remodeling that may occur when a reactivation is timed to follow complete decay of the initial activation. This is significant in light of observations that osteoclasts can remain in previously compressed sites for several days following appliance removal[7] or decay.[8] Therefore, the purpose of this experiment was to examine the appearance of osteoclasts, tooth movement, and root resorption in rats when appliances are reactivated following decay of the initial activation (16 days after activation). Our null hypothesis was that under these conditions, the kinetics of the osteoclast population, tooth movement, and root resorption will not differ from those following initial appliance activation.

Materials and methods

Animals

One hundred forty-four male Sprague-Dawley rats (35 to 40 days old) were purchased (Charles River Breeding Laboratories, Wilmington, Mass) and acclimatized for 1 week under experimental conditions. During the entire experimental period, all rats were fed a diet of ground laboratory chow and distilled water ad libitum and maintained on a 12-hour light/dark cycle. Four separate sessions were performed for all rats:[1] preparatory, appliance placement/activation, reactivation/sham, and sacrifice. Prior to each session, rats were weighed and anesthetized with ketamine (87 mg/kg) and xylazine (10 mg/kg).

The preparatory session consisted of inserting submucosal implants bilaterally on the palatal side of the molars, bonding cleats onto the occlusal surfaces of the acid-etched maxillary first molars bilaterally using autopolymerizing orthodontic adhesive, extracting the mandibular first and second molars, and pinning the maxillary and mandibular incisors.

After a 3-week period for weight recovery and alveolar bone remodeling, the appliance was activated in each rat. Rats were mounted in a head-holding device. One end of a 7-mm length of closed coil spring was ligated to the bilateral molar cleats, while the other was attached to a suspended weight of 40 grams. The spring was then bonded to the lateral surfaces of the maxillary incisors and the weight was removed.

Sixteen days following the initial activation, cephalometric radiographs were taken and the rats were divided into two equal groups: reactivation and sham reactivation. Reactivation was performed by removing the initial spring and replacing it with a new one of similar design and activation. The sham group underwent the same procedure except the spring was not removed or replaced. In previous work, similar appliances with precisely the same initial loads were shown to have decayed after 16 days.[8]

Each group was subdivided into six time-points, with 12 rats in each (day 1, 3, 5, 7, 10, and 14 following reactivation or sham). At these time-points, a second cephalometric radiograph was taken, blood was obtained via cardiac puncture for analysis of serum phosphatase, and the rats were sacrificed by decapitation. For example, rats at the day 3 time-point of the reactivation group had their appliances activated initially 19 days prior to sacrifice and reactivated 3 days prior to sacrifice. Those in the sham group had their appliances activated initially 19 days prior to sacrifice and sham-reactivated 3 days prior to sacrifice. After sacrifice, the maxillae were dissected. One hemimaxilla from each rat was frozen in liquid nitrogen for biochemical analysis; the other was fixed and prepared for undecalcified histology as previously described.[2]

Measurement of tooth movement

Tooth movement was assessed cephalometrically by determining the change in molar location along the spring vector between two times (reactivation or sham reactivation and sacrifice), using the submucosal palatal implants as superimpositional landmarks.[1] Four independent cephalograms were taken at each of the two times. The means of four independent determinations of molar position were taken and the mean of left and right molar position was calculated for each animal. This was used to represent the tooth movement in each animal. The 95% confidence limits of this method were ±23 μm.

Histomorphometry

The embedded samples were sectioned (4 mm thick) longitudinally using a Reichert/Jung Leica SM 2500S Microtome (Leica Inc, Deerfield, Ill). Each section contained the crown, root, and adjacent alveolar bone of the first molar. These sections were stained according to the Von Kossa method with a tetrachrome counterstain, and measured at 400x. The alveolar bone surface adjacent to the periodontal ligament space of each distal buccal root was measured using a light microscope equipped with a camera lucida tube and digitizing tablet interfaced with a computer. These microscopic fields permitted the measurement of cells on the ligament surface and those on the marrow surface, but did not permit measurement of cells from more distant sites in the alveolar bone. Therefore, this study does not address bone remodeling in the latter sites. A Bioquant image system (R&M Biometrics, Nashville, Tenn) was used for all histomorphometry. Previous studies of the initial displacement of the distobuccal root using these mechanics[2] have indicated that the

center of rotation of the root is at the apex, with the entire mesial surface representing a gradient of compression and the entire distal surface one of tension. Therefore, compression measurements were made from the mesial alveolar crest to the root apex and tension measurements from the distal alveolar crest to the apex.

The parameters measured were osteoclast number (number per trabecular bone surface, 0.874), percent osteoclast surface (osteoclastic surface per total trabecular bone surface, 0.992), and percent of the root surface with resorption craters (crater surface per total root surface, 0.932). The numbers in parentheses represent the R^2 from two independent determinations (n=10) made several days apart. Reproducibility of approximately 90% was considered to be acceptable.

Biochemical analyses

Biochemical analyses were conducted as previously described.[9] The crowns of the first molars and all extraneous tissue were removed in the frozen state with continuous reimmersion in liquid nitrogen. Samples were homogenized for 10 seconds using a triturator with a prefrozen capsule and metal pestle. Homogenates were extracted overnight in a Triton buffer at 4ºC. Supernatants were analyzed for tartrate-resistant acid phosphatase (TRAP) in acid/tartrate buffer using a commercially available phosphatase colorimetric assay kit (Sigma Diagnostics, St. Louis, Mo).[10] The phosphatase readings were performed at 414 nM using a Titertek Multiskan R MC (Flow Laboratories, Inc, Mclean, Va). Serum phosphatase values were expressed in Sigma units, and the alveolar bone phosphatase values were calculated as Sigma units per μg of protein.

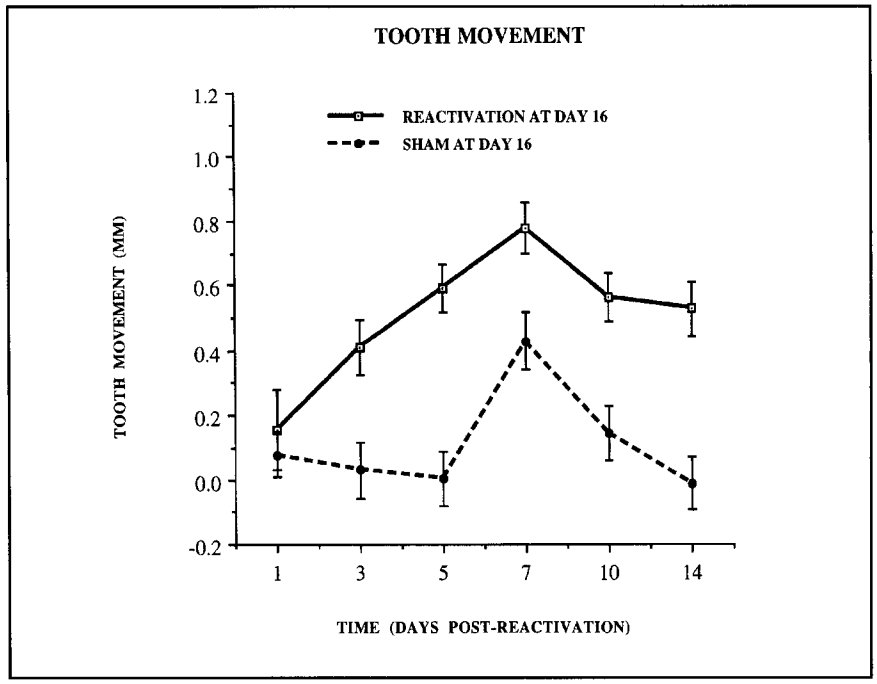

Figure 1
Cumulative orthodontic tooth movement as function of time following completed first activation, and reactivation after decay (day 16). Each point represents the mean of 9 to 12 rats at each time-point of reactivation. Vertical bars represent SEM. Horizontal dotted lines represent mean of each parameter from sham reactivation. Sham group did not have significant time-related changes. There was significant time related change in the reactivated group, with day 7 different from day 1 ($p<0.01$). There were significant group-related changes in the reactivated groups at days 5, 7, 10, and 14, different from sham groups with days 1, 3, 5, 10, and 14, and at day 3 with days 5 and 14 ($p<0.01$).

Data handling and statistical analyses

Means and standard errors of the mean were calculated for each time-point. Intra- and intergroup comparisons were performed by ANOVA to determine the differences in each parameter across time, and a Tukey/Kramer HSD test was used to test for pairwise differences when ANOVA indicated that significant differences existed ($p<0.05$).

Results

Cumulative orthodontic tooth movement following a completed first activation and reactivation after decay (day 16) showed a linear mesial movement (Figure 1). The classical three-part tooth movement kinetics characterized by initial displacement, delay, and secondary movement was not evident in this group. Between the first and seventh days following appliance reactivation, first molars were displaced mesially 0.78 ± 0.12 mm ($p<0.01$). In the sham group, there were no significant time-related changes in tooth position until day 7, when the molars transiently moved mesially 0.43 ± 0.08 mm ($p<0.01$). Tooth movement in the reactivated group was significantly greater than that in the sham group at days 5, 7, 10, and 14 ($p<0.01$).

In the reactivated group, both osteoclast number and surface percent were significantly elevated at compression sites by day 3 ($p<0.01$), then slowly returned to baseline by day 7 (Figures 2A and 3A). Neither the number of osteoclasts nor the surface percent at the

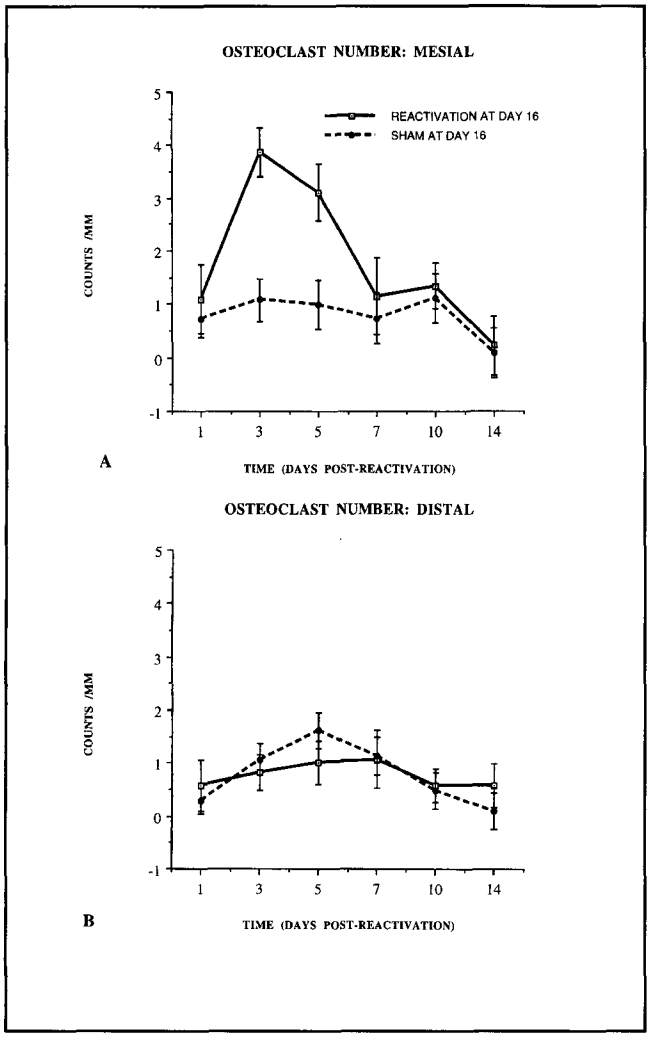

Figure 2A-B
Changes in number of osteoclasts per surface of alveolar bone at (A) orthodontic compression and (B) tension sites as functions of time following a completed first activation and reactivation after its decay (i.e., day 16). Each point represents the mean of 9 to 12 rats. Vertical bars represent SEM.
A: Sham group did not have significant time-related changes. There were significant time related changes in the reactivated group, with day 3 different from days 1, 10, and 14 ($p<0.01$). There were significant group-related changes in the reactivated group at day 3, different from the sham group with all days observed ($p<0.01$).
B: No significant time- or group-related changes were seen on the tension side.

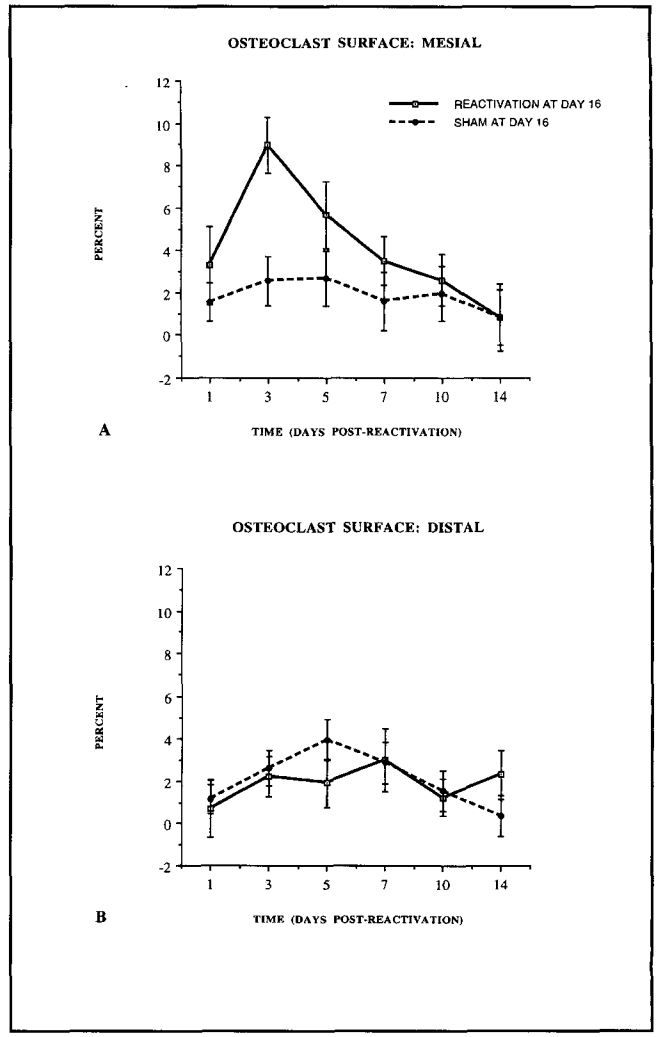

Figure 3A-B
Changes in total alveolar bone surface occupied by all osteoclasts at (A) orthodontic compression and (B) tension sites as function of time following a completed first activation and reactivation after its decay (day 16). Each point represents the mean of 9 to 12 rats. Vertical bars represent SEM.
A: Sham group did not have significant time-related changes. There were significant time related changes in the reactivated group, with day 3 different from days 10 and 14 ($p<0.01$). There were significant group-related changes in the reactivated group at day 3, different from the sham group for all observed days ($p<0.01$).
B: No significant time- or group-related changes were seen on the tension side.

tension sites changed (Figures 2B and 3B).

Root resorption was pronounced at the compression sites in both reactivated and sham groups (Figure 4A). However, significant differences could not be detected over time in either group at either compression or tension sites (Figure 4B).

In the reactivation group, there were no significant differences over time in either serum or alveolar bone TRAP (Figure 5A-B). However, bone TRAP levels in the sham group dropped significantly at day 7 ($p<0.01$).

Discussion

The finding that orthodontic tooth movement is linear following a day 16 reactivation is similar to a previous report following a day 10 appliance reactivation.[6] These findings suggest that timing appliance reactivations for the latter portion of the previous alveolar bone re-

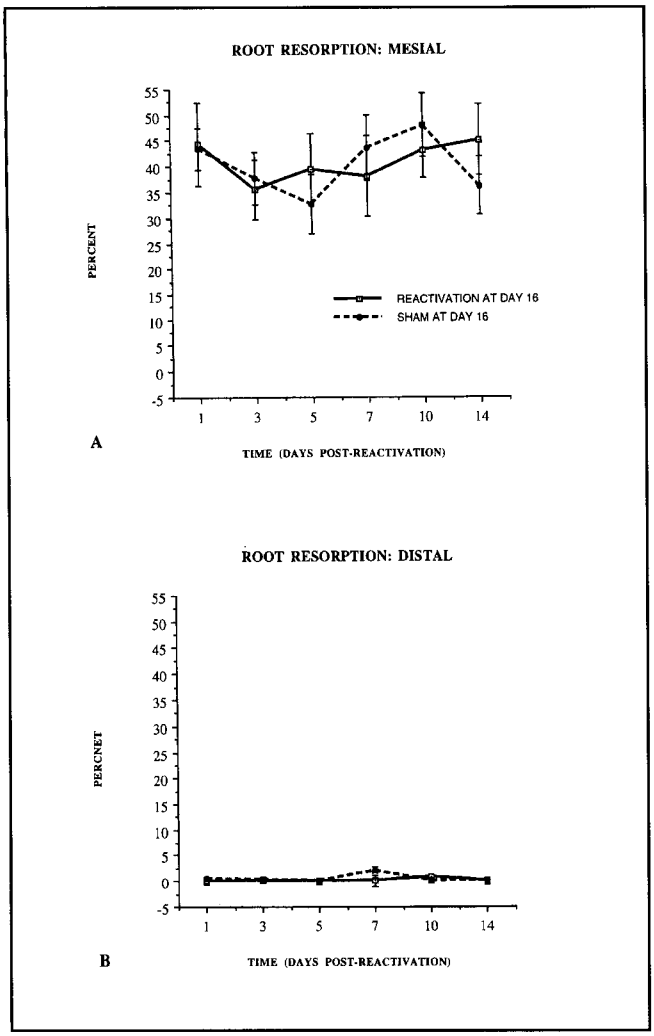

Figure 4A-B
Root resorption at (A) orthodontic compression and (B) tension sites as function of time following a completed first activation and reactivation after its decay (day 16). Each point represents the mean of 9 to 12 rats. Vertical bars represent SEM. No significant time- or group-related changes were seen on either side.

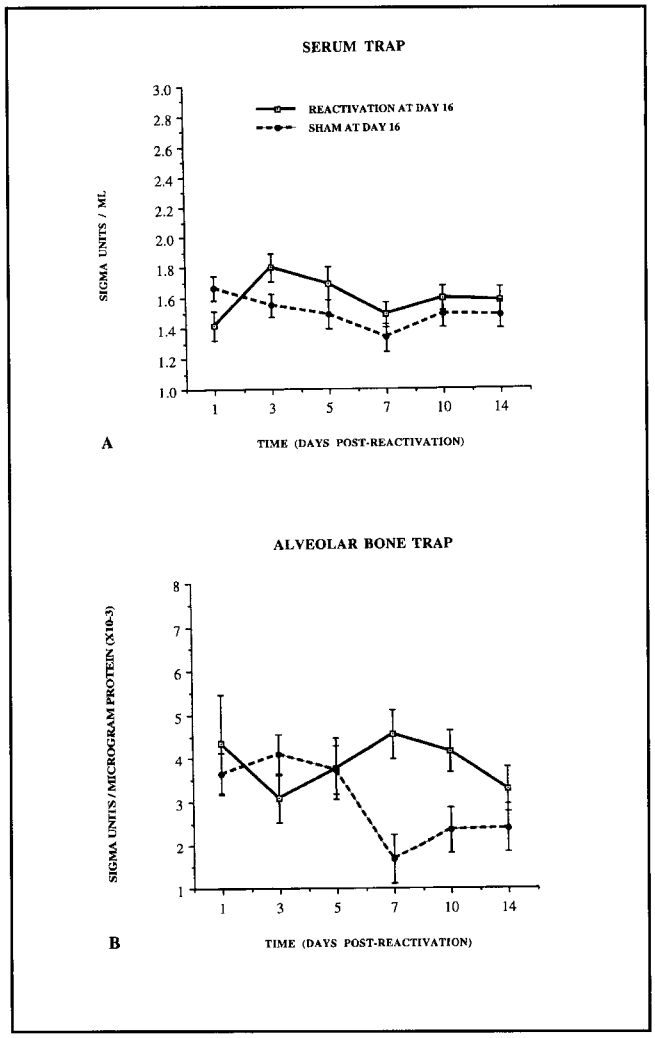

Figure 5A-B
Changes of tartrate-resistant acid phosphatase (TRAP) in (A) serum and (B) alveolar bone (units/mg) as function of time following a completed first activation and reactivation after its decay (day 16). Each point represents the mean of 9 to 12 rats. Vertical bars represent SEM.
A: Neither reactivated nor sham groups had significant time-related changes. There were significant group-related changes in the reactivated group at day 3, different from the sham group at day 7 ($p<0.01$).
B: Neither reactivated nor sham groups had significant time-related changes. There were significant group-related changes in the reactivated group at day 7 ($p<0.01$).

modeling cycle could have significant clinical advantages over doing them earlier,[4,5] because the delay period seen in tooth movement following a single activation or short term reactivation can be avoided. Despite this similarity between the day 10 and day 16 reactivation schedules, the kinetics of tooth movement and osteoclast changes at compression sites are quite different. Osteoclasts required 3 to 5 days to appear at compression sites following the day 16 reactivation, but appeared immediately following a day 10 reactivation. This suggests that changes in bone-resorbing cells alone do not predict tooth movement following appliance reactivation. Bone resorption is a complex process that involves more than the number and size of osteoclasts present at a given site. Other factors that may be significant include the bone-resorbing capacity of individual osteoclasts,[11] osteoclast adhesion to

the bone surface,[12] H^+ ion transport,[13] and enzyme secretion and matrix digestion.[13] Other factors not related to osteoclasts may also play roles, including increased tooth mobility and reduced tissue resistance secondary to ongoing orthodontic tooth movement,[14] increased vascularity of the periodontal ligament,[15] and reductions in alveolar bone density.[16]

The transient mesial movement of molars in the sham group at day 7 suggests that teeth can continue to move in the direction of initial force application despite removal of the force. This is consistent with similar observations following appliance decay[8] and removal[17] using this animal model. Several clinical examples indicate that less than continuous orthodontic force is an effective means for producing tooth relocations. Restorations with deflective interferences cause tooth movement and mobility,[18] and functional extraoral appliances generate orthodontic tooth movement with less than full time wear.[19] The mechanism for this is not well understood, but some have speculated that proteoglycans in bone matrix may have the ability to store strain patterns for an extended period following the removal of a strain.[20,21]

The finding that compression sites are clearly more at risk for root resorption than tension confirms numerous other reports.[22-25] However, a second appliance activation does not stimulate more resorption at these sites. Similar findings were reported following reactivation at days 1[4] and 10,[6] but increased resorption did occur following a reactivation during the peak expansion of the osteoclast population at day 4.[5] This finding suggests there may be some added risk of root resorption when appliances are reactivated in the presence of significant osteoclastic and, presumably odontoclastic, activity.

There was no significant decrease in root resorption over time in either group, suggesting that healing of these lesions was not pronounced. This finding contradicts several reports that root resorption lesions can repair.[26-30] Clearly, there is a repair mechanism capable of reattaching periodontal fibers and re-establishing other tissues; however, the complete repair of gross cratering that was measured in this experiment does not occur.

Phosphatase changes have been described in orthodontically treated tissues. In rats, increased acid phosphatase[31] and decreased alkaline phosphatase[32-34] activities were seen at compression sites up to day 7 after appliance activation. Tartrate-resistant acid phosphatase (TRAP) is an isoenzyme of acid phosphatase secreted by osteoclasts[35] and commonly used as a marker for osteoclastic activity. The most significant finding in this regard was that the activity in bone TRAP was elevated in both groups during the early part of the experiment but diminished in the sham group at day 7. This finding suggests that significant numbers of committed osteoclast lineage cells (TRAP positive) may remain in these tissues for an extended period following a single appliance activation and long after the morphologically recognizable osteoclasts have cleared from the sites. This is further supported by the observation that osteoclasts appear at compression sites immediately following a day 10 reactivation.[6] For this to occur, late-stage osteoclasts must be present in the tissues despite not being recognizable by morphological criteria.

Studies in recent years have shed light on the biochemical and molecular effects of cytokines and growth factors and have shown that these regulatory molecules may mediate bone remodeling and orthodontic tooth movement.[36] However, the complex interrelationship of these molecules is still not clear. Our understanding of the mechanisms involved in bone remodeling is rapidly increasing, as this area attracts much research because of the high incidence of metabolic bone disease in our society. Although studies of adult bone remodeling are of relevance, there is a requirement for increased research directed specifically at biological mechanisms in bone's response to various biomechanical environments. Orthodontic tooth movement offers certain advantages in this regard, since it is an accelerated cycle of cellular division and differentiation, within which events can be readily followed temporally and spatially. However, progress in this field is impeded by the lack of appropriate means of repeating animal studies in humans. Much information has come from studies involving rodents, and species differences must always be taken into account. Larger mammals have also been used, but these studies can be extremely expensive and often do not have sufficient numbers of animals to perform appropriate statistical tests. If the mechanisms of orthodontic tooth movement in humans are to be established at a cellular level, a number of approaches need to be considered in addition to the continued use of animal models. Any effects on tooth movement need to be tracked in three dimensions. Convenient 3-D digitizers are becoming more widely available. In addition, the relevant biological responses need to be followed temporally and correlated with noninvasive measurements, including assays of cytokines, growth factors, and proteins known to regulate bone remodeling and tooth movement in animal models. The use of gingival crevicular fluid to monitor ongoing biological activity shows some

promise for use in human studies as a means of monitoring biological activity in the periodontal ligament clinically.[37-39] Finally, means of monitoring ongoing biomechanics at the level of the individual tooth would be required. Miniature devices that can detect strains and store strain histories are being developed. The convergence of these technologies may eventually provide scientists with the means to conduct these studies in humans, and provide clinicians with the means to use both biological and biomechanical information to achieve the most efficient tooth movement with the least risk.

The question of how these data relate to clinical conditions is certainly quite relevant. Unfortunately, it is difficult to extrapolate from a rodent model of bone remodeling directly to man. This experiment was designed to model the clinical situation wherein an appliance is reactivated following total deactivation. This certainly does occur clinically but, in the absence of good means to monitor appliance activity at the level of the tooth, it is difficult to know for sure when. The bone resorptive portion of the human remodeling cycle lasts about 1 month.[3] Since this phase seems to be over in about 1 week under the conditions of this study, it may be reasonable to speculate that the time frame for humans would be at least four times longer.

Conclusions

Regarding the effects of orthodontic reactivation following appliance decay, the following can be concluded:

1. Orthodontic tooth movement kinetics do not show the delay period commonly seen following a single activation of an appliance or a short-duration reactivation.
2. A second cohort of osteoclasts appears 3 to 5 days after reactivation.
3. Root resorption occurs at compression sites, but no group- or time- related differences are evident
4. TRAP activity remains elevated for an extended period after osteoclasts have been cleared from the tissues.

Acknowledgments

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