Orthodontic bone remodeling in relation to appliance decay

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The movement of teeth in the alveolar process requires bone turnover. Histomorphometric and biochemical data are available which demonstrate that turnover in the paradental alveolar bone of orthodontically-treated teeth is not balanced in the short-term but, instead, is characterized by tandem periods of activation, resorption, reversal and formation. These bone turnover dynamics occur at both sites of pressure and tension with bone accretion or removal being controlled by the relative amount of bone formation or resorption occurring at each site.[1,2]

Data are currently unavailable on the nature of any further alveolar bone turnover that may occur following the first cycle of alveolar bone remodeling after an initial appliance activation, or how this may relate to the loss of appliance activity. It is possible that the preexisting pattern of distal drift immediately returns (i.e., relapse) or, in the light of data which show that short-term forces can stimulate significant tooth movement for a period in the absence of continued force,[3] that the alveolar bone remodeling pattern initiated by an initial appliance activation persists. Improving our understanding of alveolar

Abstract

This study examined alveolar bone turnover and orthodontic tooth movement after appliance decay. One group of rats (N=54) received orthodontic force (40 g initial activation) while the other (N=36) was sham-treated. Groups of six rats were sacrificed at various times following activation. Tooth movement and appliance decay were monitored cephalometrically, and bone turnover was monitored locally by histomorphometry and phosphatase chemistries and systemically by serum phosphatase and osteocalcin changes. A significant association was found between spring forces assessed by direct measurement and by cephalometric images ($R^2 = 0.784$; $p=0.02$). The cephalometric method indicated appliances were at least 93% deactivated by day 16. Tooth movement continued beyond the point of appliance decay ($p<0.001$). This was accompanied by a dramatic decline in osteoblast surface ($p<0.0001$) and an increase in osteoclast surface to control levels ($p<0.001$). A significant peak in bone formation rate was also noted around appliance decay ($p=0.005$). Serum acid phosphatase and osteocalcin also increased after appliance decay ($p<0.05$), but alkaline phosphatase did not. Bone acid phosphatase was characterized by a peak after appliance decay ($p=0.0004$), but alkaline phosphatase remained depressed ($p<0.0001$). These data demonstrate that significant amounts of alveolar bone turnover continue for an indeterminant period following appliance decay.

Key Words

Tooth movement • Bone turnover • Remodeling • Appliance decay

Submitted: April 1994 **Revised and accepted:** August 1994 Angle Orthod 1994;65(2):129-140.

bone turnover during this critical period of appliance decay could lead to better appliance reactivation and relapse prevention strategies.

A rodent model has been developed to activate orthodontic tipping appliances with precise initial forces.[4] However, methods are not currently available to monitor the decay of these appliances. Such a method is an important prerequisite to achieving the primary goal of this study.

Serum osteocalcin is known to reflect bone formation.[5] Since serum alkaline phosphatase, another systemic marker for bone formation, has been shown to have validity for monitoring bone formation during orthodontic tooth movement,[2] it would also be useful to know if osteocalcin can be used as a similar marker. Assessing the usefulness of serum osteocalcin as a marker for bone formation in the rat tooth movement model is a secondary goal of this study.

Therefore, the rationales for this study are: 1) to develop and characterize means to monitor orthodontic appliance decay in the rodent model; 2) to investigate histomorphometrically, biochemically and cephalometrically the time course of changes in alveolar bone turnover and orthodontic tooth movement with respect to decay; and 3) to assess the usefulness of serum osteocalcin as a means to monitor the biological response in orthodontic tooth movement. The hypothesis being tested postulates that orthodontic tooth movement and alveolar bone turnover will continue following the first remodeling cycle and after appliances have lost most of their initial activation.

Materials and methods

Animal manipulations: Ninety adult male Sprague-Dawley strain rats (89-94 days old) were purchased from Charles River Breeding Laboratories (Wilmington, Mass). They were shipped by air freight and acclimatized for at least 2 weeks under experimental conditions including housing in plastic cages, feeding a diet of ground laboratory chow and distilled water *ad libitum* and being maintained on a standard 12-hour light/dark cycle. All animal manipulations, including sacrifices, were done at the same time of day.

Weights were recorded and anesthesia was obtained using intramuscular injections of ketamine (87 mg/kg) and xylazine (13 mg/kg). Modified orthodontic cleats were bonded bilaterally to the occlusal surfaces of all maxillary first molars and short lengths of barbed broach were inserted subperiosteally on the palate lingual to the maxillary first molars as stable cephalometric superpositional landmarks.[4] All four incisors were pinned using a modification of the method described by Beertsen et al.[6] in order to prevent further eruption, to provide a reproducible anterior landmark and to eliminate movement of the anchorage. The lower incisors were reduced slightly to prevent appliance damage and the mandibular first molars were extracted. The animals were then allowed to recover for three weeks, monitoring wound healing and weight gain.

For appliance activations, the animals were positioned in a head restraint and orthodontic springs were placed as previously described.[4] Briefly, one end of a length of closed coil spring (7 mm length of Unitek Hi T coil with a wire diameter of .006" and an arbor diameter of .022") was ligated to the molar cleat while the other was attached to a 40 gram suspended weight. The anterior end of the coil was then bonded with autocuring methacrylate to the acid-etched lateral surface of the maxillary incisors followed by removal of the weight and excess wire. This method assured a precise initial orthodontic force designed to tip the maxillary first molars to the mesial and equivalent decay rates among appliances.

Rats were randomly divided into two groups. One group (N=54) received the orthodontic force at a level of 40 grams while the other (N=36) was sham-treated, receiving all procedures except spring placement. The spring was excluded to allow normal distal drift of the molars. In the 40-gram group the animals were subdivided into nine groups of six animals each and were sacrificed at 1, 3, 5, 7, 10, 14, 16, 18, and 21 days. In the sham-treated group, the animals were subdivided into six groups and were sacrificed at 1, 3, 5, 7, 10, and 14 days. Controls for the longest three times were not included in this study because, from previous studies,[1,2] it was known that no significant changes in any parameters occurred beyond the first 5 days. However, slight differences were recorded among controls during the first 5 days. These were attributed to animal manipulations required to place the appliances. Previous studies have also indicated that the first six time intervals adequately produce and monitor tooth movement kinetics which exhibit characteristic changes including instantaneous movement, a delay period and late tooth movement.[4] Although we did not measure appliance decay in previous studies, it was our impression that these appliances were largely deactivated by day 14. Thus, the last three time intervals in the 40-gram group were chosen to

study the alveolar bone turnover changes and tooth movements which occur subsequent to the loss of appliance activity and the completion of the bone remodeling cycle.

All rats were injected intraperitoneally with tetracycline derivatives (15 mg/kg) on two separate occasions. Oxytetracycline hydrochloride (Terramycin; Pfizer, Inc., Brooklyn, NY) was administered 5 days prior to sacrifice. After a 3-day period with no tetracycline administration, all rats were injected again with demeclocycline (Declomycin, Lederle Laboratories, Pearl River, NY) 2 days before sacrifice. This regimen resulted in the deposition of a double tetracycline label at bone surfaces that were actively mineralizing throughout the injection period and facilitated the measurement of dynamic histomorphometric parameters.[7] In reporting dynamic histomorphometric parameters, it was necessary to adjust the sacrifice times to the actual times when labeling occurred. Therefore, mean labeling days were calculated by subtracting the days of tetracycline injections from the days of sacrifice, and calculating the mean labeling day.

Animals were euthanized under anesthesia at each of the time intervals by exsanguination from the inferior vena cava and decapitation. Maxillae were dissected into halves. One hemimaxilla was frozen in liquid nitrogen. The other was fixed in 10% formalin for 24 hours, followed by dehydration in increasing concentrations of ethanol and embedding undecalcified in methyl methacrylate.[7]

Histomorphometry: The embedded samples were sectioned parallel to the long axis of the first molars at 4 μm thicknesses with an AO Autocut/Jung 1150 Microtome and mounted on 1% gelatinized slides. Alternate sections were stained according to the Von Kossa method with a tetrachrome counterstain. All surface-based parameters were quantified on these sections using a semiautomatic image analyzer (Bioquant, R & M Biometrics, Nashville, Tenn) coupled to a photomicroscope equipped with epifluorescence (Nikon Labophot). All tetracycline-derived parameters were measured on similarly mounted, unstained sections cut at 6 μm thicknesses.

Paradental and interradicular alveolar bone immediately adjacent to the periodontal ligament of distobuccal and middle-buccal maxillary first molar roots was sampled as follows: Parasagittal sections were selected by choosing those demonstrating a radicular pulp to the apical third of the root; multiple non-overlapping fields at 400X were then sampled from each side of the root. The bone parameters measured are defined according to Parfitt et al;[8] they were chosen to best represent bone formation activity as well as bone and root resorption. Osteoblast and osteoclast surfaces were calculated by dividing the osteoclast or osteoblast perimeter by the total bone perimeter. The mineral apposition rate was calculated by dividing the average interlabel thickness by the labeling interval (i.e., 3 days). The mineralization surface was calculated by dividing the total double-labeled perimeter and half the single-labeled perimeter by the total bone perimeter. The bone formation rate was calculated by dividing the product of the mineral apposition rate and mineralizing surface by the total bone perimeter.

Orthodontic tooth movement and appliance activities

Extraoral cephalometric radiographic views were taken from the superior using a specially designed cephalostat previously described.[4] Four independent cephalograms were taken at each of two times: prior to spring placement and at sacrifice. Five cephalometric landmarks were located on projections of these radiographs using acetate tracing paper. These included the molar cleat, the anterior end of the spring, the posterior end of the spring, several adjacent barbs on the subperiosteal implant and the incisor pin. These landmarks were then digitized using a computer. Tooth movement was determined along the incisor-molar vector, superimposing on the implant and calibrating magnification by using the known interbarb distance. Spring lengths were determined by measuring the distance between the anterior and posterior spring landmarks. The mean of four independent determinations of tooth movement and spring length was calculated for each animal.

In order to determine force magnitude from the cephalometric radiographs of spring lengths in the above-described animals, 28 appliances with initial activations ranging up to 40 grams were placed on 15 additional rats. Cephalograms were taken on these to calibrate spring image versus activity. Data on the initial appliance activities and the images of initial spring lengths were compared for each of the springs and an algorithm was generated which best described the kinetics. The error of this method of force determination was then assessed using a series of two independent determinations from the same rat.

Validity was measured as follows: A series of nine rats were sacrificed 24 hours after initial coil

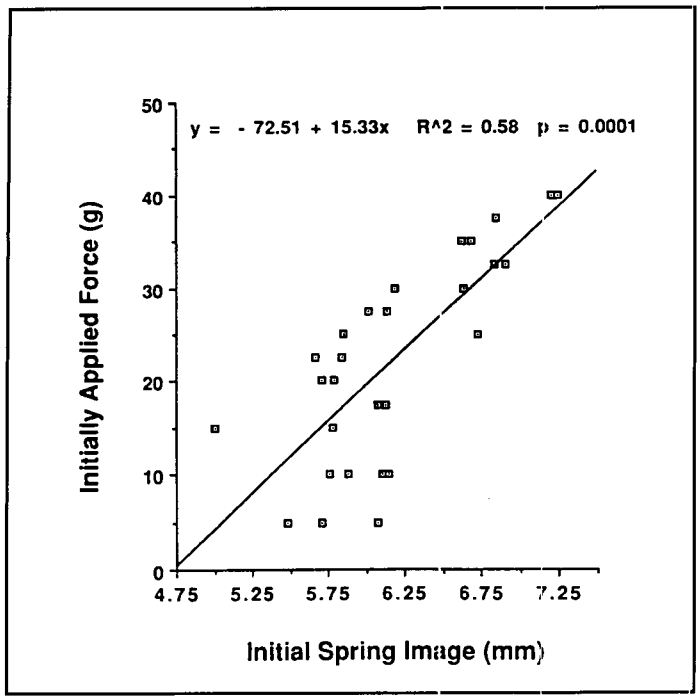

Figure 1

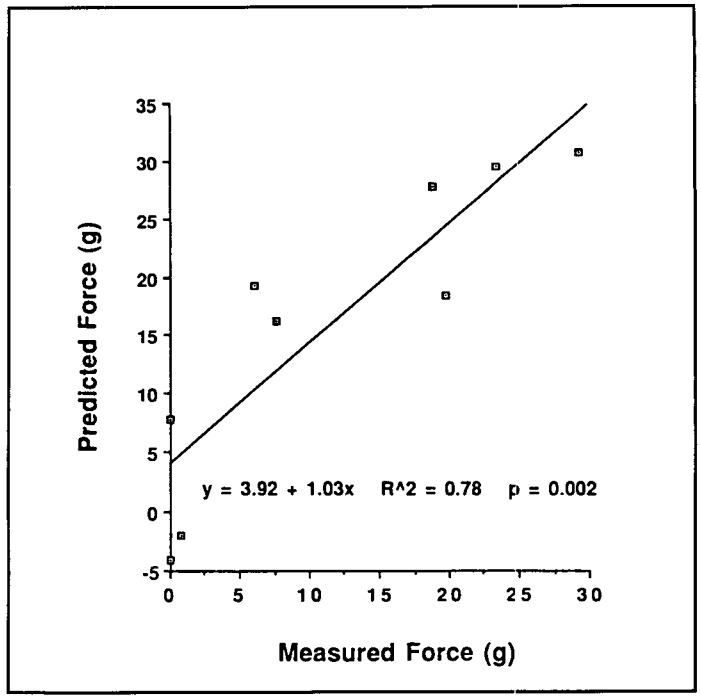

Figure 2

Figure 1
Correlation between the initial applied force and the initial spring image on the cephalogram (N=28)

Figure 2
Correlation between force levels from coil springs after 1 day of tooth movement measured directly from defleshed skulls and predicted from cephalometric images by algorithm from Figure 1 (N=9).

placement. This interval was chosen because a major tooth deflection is known to occur during this period, but little further movement occurs thereafter until an interval between 7 and 10 days.[1] Therefore, the appliance would be significantly deactivated during this first 24 hours, allowing the opportunity to determine this day 1 appliance activity by algorithmic prediction and direct measurement. Direct measurement was achieved by decapitation of the animals after sacrifice, defleshing the skulls using dermistic beetles, and mounting the skulls in the beam of an optical comparator, oriented in such a way that the coils were perpendicular to the beam. The latter was determined by positioning the skull so that both ends of the coil were in focus on the enlarged image of the comparator. After this image was traced on acetate paper, the coil was detached from the molar cleat. This was followed by determining the amount of suspended weight required to re-deflect the coil to its predetached image length, superimposing the comparator image on the acetate tracing. The predicted and measured 24-hour forces were then compared using linear regression analysis.

Phosphatases: Acid phosphatase and alkaline phosphatase activities were assayed in sera and bone using commercially available quantitative, colorimetric kits (Sigma Diagnostics, St. Louis, Mo).[9,10] The phosphatase readings were performed at 414 nm using a Titertek Multiskan® MC (Flow Laboratories, Inc, McLean, Va). Serum phosphatase values were expressed in Sigma units; the bone values were calculated as Sigma units per microgram of protein. A Sigma unit is defined as the amount of enzyme activity that will liberate one uM of p-nitrophenol/hour under test conditions described by Bessey et al.[9] The protein colorimetric assays were done with a bovine serum albumin standard and the Coomassie Blue dye method[11] at 595 nm using an Ultrospec 4050® spectrophotometer (LKB Biochrom Ltd., Cambridge, England).

Alveolar bone phosphatase concentrations were assayed from extracts that were prepared as follows. While maintaining the hemimaxilla in the frozen state with repeated reimmersion in liquid nitrogen, the tooth crowns and extraneous tissues were removed from the first molars with a diamond disc attached to a straight handpiece. The final samples consisted of the roots of the maxillary first molars and their supporting tissues. These samples were homogenized for 60 seconds using three equal bursts by trituration in amalgam capsules equipped with metal pellets and pre-frozen with liquid nitrogen. The resulting powder was washed from the capsule into a test tube using 2 mL of Triton buffer (0.1% Triton X-100, 0.3 M KCl, 0.05 M Tris-acetate; pH 7.5) and extracted overnight at 4°C with continuous stirring. The extracts were centrifuged at 4,000 rpm for 20 minutes at 4°C, and the supernatant was retrieved and stored in aliquots at 4°C. These aqueous samples were then diluted 1:10 with Triton buffer for acid phosphatase and tartrate-resistant acid phos-

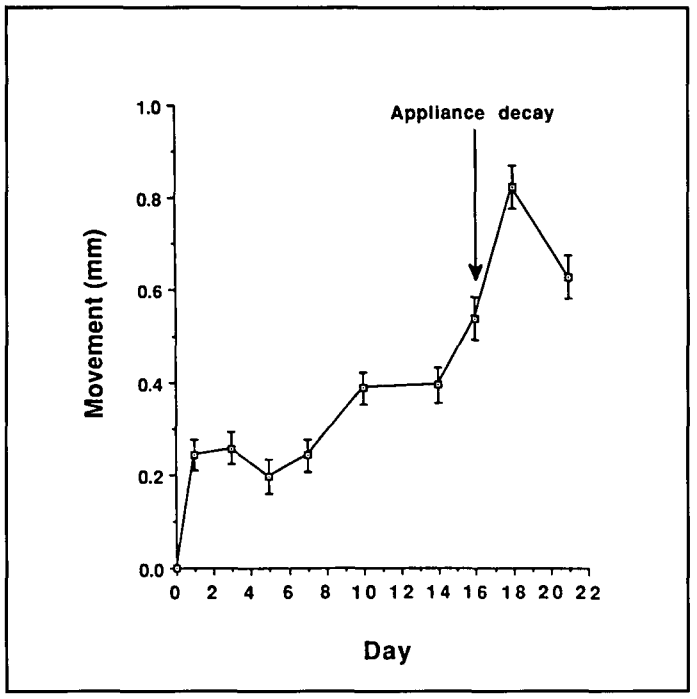

Figure 3

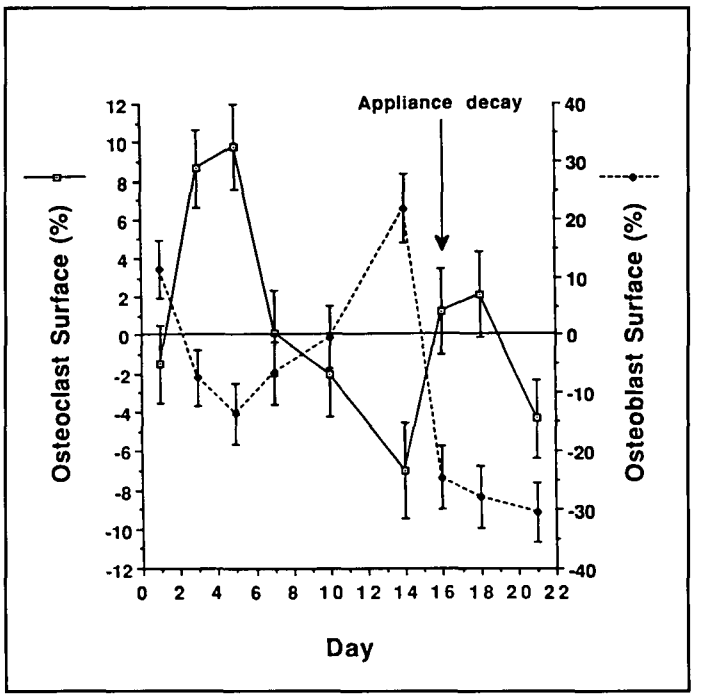

Figure 4

phatase assays. A 100 μL sample of this 1:10 preparation was further diluted 1:2 with additional buffer for the alkaline phosphatase and protein assays.

Osteocalcin: Quantification of serum osteocalcin was performed with a commercially available radioimmunoassay kit (Biomedical Technologies, Inc., Stoughton, Mass). Standard curves were constructed using B/Bo versus nG of osteocalcin standard per mL, where B is defined as [125I] osteocalcin cpm bound to antibody minus nonspecific binding and Bo as that bound to antibody in the absence of unlabeled osteocalcin minus nonspecific binding. Six separate determinations of nonspecific binding and Bo were made for each assay. Total binding was determined for each assay by Bo/total counts added. All unknowns were assayed in triplicate.

Data analysis: Tooth movement for each treated animal was adjusted by subtracting predicted distal drift.[4] For the static and dynamic histomorphometric measures, as well as the bone and serum chemistries, the data for each treated animal were adjusted by subtracting the corresponding mean control value for each day (1 to 14). (Control data available upon request.) Since controls were not included for days 16, 18, and 21, the treated data at 16, 18, and 21 days were adjusted by the mean control data of animals at days 7, 10, and 14. The control animals were not statistically different from each other at these days. The mean and standard error for each parameter in the treated animals were calculated for each timepoint. Analysis of variance (ANOVA) was performed to examine differences in each parameter across time. A Welch ANOVA was performed when variances among the days were not equal. A Tukey/Kramer HSD test was performed to test for pairwise differences when ANOVA indicated that significant differences existed ($p<0.05$). This test protects for type 1 errors from multiple comparisons.

Results

Cephalometric determination of spring force at sacrifice: The association between the cephalometric images of the coils and their activities is shown in Figure 1. The algorithm used to predict force from spring images is also shown. The R^2 for this association equals 0.581 ($P=0.0001$).

There were no systematic errors between trials in the reliability determination ($P=0.25$), and the 95% confidence limits from two independent determinations equalled ± 2.91 grams. Because of this limitation, the point of complete appliance decay cannot be determined with absolute certainty below this level. Since this degree of uncertainty represents the lowest 7% of possible force levels, "appliance decay" is actually the point when appliances have decayed greater than 93%.

The validity of the cephalometric determination of spring force is suggested by the strong association between force assessed by direct measurement at day 1 and that predicted from day 1 cephalometric images using the algorithm. This association was linear and highly significant

Figure 3
Orthodontic tooth movement in response to a 40-gram appliance activation, designed to tip the maxillary first molars mesially. Data have been adjusted by subtracting predicted distal drift (0.0077 mm/day). Mean and standard errors of the mean are presented. The point when all appliances had decayed is indicated.

Figure 4
Percentages of osteoblast (right vertical axis; dotted lines) and osteoclast (left vertical axis; solid lines) surface minus control. Mean and standard error of the mean are shown.

Table 1
Dynamic histomorphometric indicators of bone turnover (Mean and standard error of the mean indicated)

Sacrifice Day	Mean Labeling Day	Bone Formation Rate (1)	Mineralizing Surface (%)	Mineral Apposition Rate (2)
5	1.5	0.73 ± 1.71	-3.05 ± 5.83	0.30 ± 0.70
7	3.5	2.94 ± 1.53	15.53 ± 5.22	4.14 ± 0.62**
10	6.5	0.25 ± 1.53	7.17 ± 5.22	-0.11 ± 0.62
14	10.5	0.56 ± 1.71	1.77 ± 5.83	1.29 ± 0.70
16	12.5	2.77 ± 1.53	9.88 ± 5.22	0.84 ± 0.62
18	14.5	9.10 ± 1.53*	15.67 ± 5.22	2.11 ± 0.62
21	17.5	4.69 ± 1.53	5.5 ± 5.22	1.14 ± 0.62

(1)Significant differences existed among days, p = 0.005
(2)Significant differences existed among days, p=0.002
*Day 18 differed from days 5, 10, 14; p<0.05
**Day 7 differed from days 5, 10, 16, and 21; p<0.05

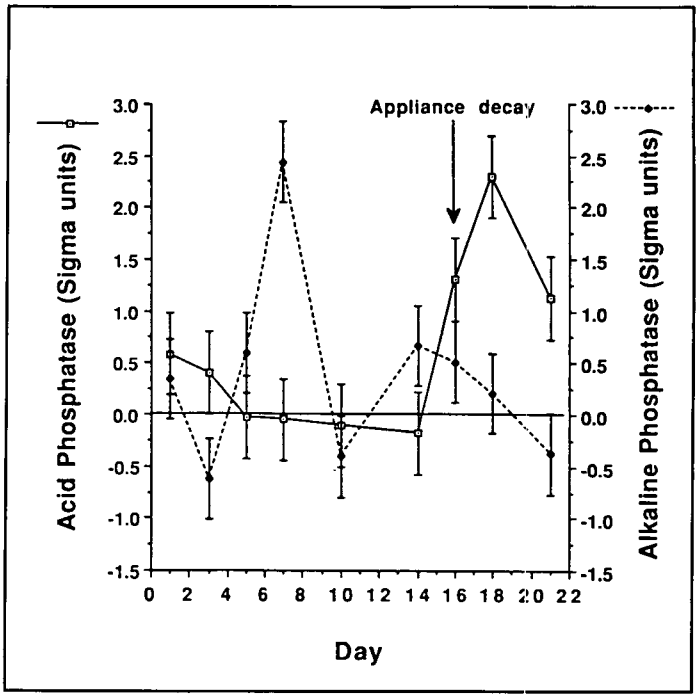

Figure 5

Figure 5
Differences in serum acid (left vertical axis; solid lines) and alkaline (right vertical axis; dotted lines) phosphatase activities across time. Mean and standard error of the mean are shown.

(R^2 =0.784; p=002; Figure 2). Using this method, all appliances were found to be deactivated (± 2.91 grams) at 16 days.

This method proved to be not only valid and reliable, but also easy, because the radiographs were already being taken to monitor tooth movement. All that was required was the addition of the spring length landmarks and the algorithm to the digitization program. The error of this method was determined by calculating the force associated with the error of spring length measurements, which equals 0.119 mm. The error of locating a single landmark cephalometrically using this method was previously found to be 0.06 mm.[4] Since determining a spring's image requires locating two landmarks (i.e., each end of the spring), it is reasonable to find that the error is doubled. The reliability of this approach is acceptable for the purposes of documenting appliance decay in this model as it represents only about 7% of the spring force, which is dissipated over 16 days.

Tooth movement: Changes over time are graphically represented in Figure 3. The Welch ANOVA indicated that significant differences existed in the data set (p<0.001). Pairwise comparisons indicated that there was no significant tooth movement during the intervals between days 1 and 7, between days 10 and 14, and between days 18 and 21. However, significant orthodontic tooth movement occurred between days 7 and 10, as well as between days 14 and 18 (p<0.05).

Static histomorphometric indicators of bone turnover: Bone formation is represented by percent osteoblast surface in Figure 4. ANOVA indicated that significant differences existed across time (p<0.0001); pairwise comparisons indicated that these differences were manifested by a depression at day 5 (versus day 1 and day 14; p<0.05), a peak at day 14 (versus days 3, 5, 7, 16, 18, 20; p<0.05), and a further depression at day 21 (versus days 1, 3, 7, 10, 14; p<0.05).

Data on resorption are represented by percent osteoclast surface in Figure 4. ANOVA indicated that significant differences existed across time (p<.001); pairwise comparisons indicated that these differences were characterized by a peak at days 3 and 5 versus depressions at days 14 and (p<0.05).

Dynamic histomorphometric indicators of bone turnover: The dynamic histomorphometric indicators of bone formation are shown in Table 1 where means and standard errors of the mean are given for each parameter by day. The data indicated that significant differences existed across time in bone formation rate (B.F.R.) (p=0.005) and in mineral apposition rate (M.A.R.) (p=0.002), but not in percent mineralizing surface (M.S.) (p=0.2). Pairwise comparisons indicated that differences were manifest in B.F.R. by a peak occurring at mean labeling day 14.5 (versus days 1.5, 6.5, and 10.5; p<0.05). M.A.R. had a significant peak at day 3.5 (versus days 1.5, 6.5, 12.5, and 17.5; p<0.05).

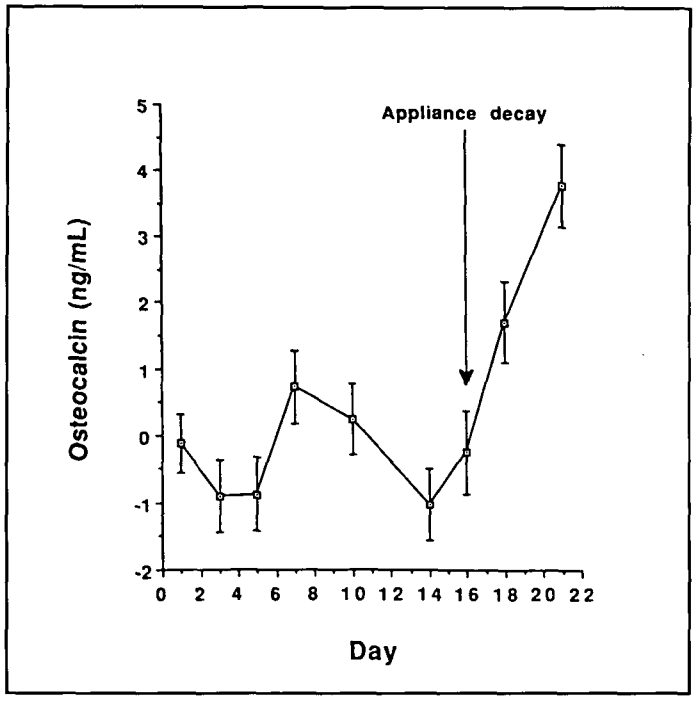

Figure 6

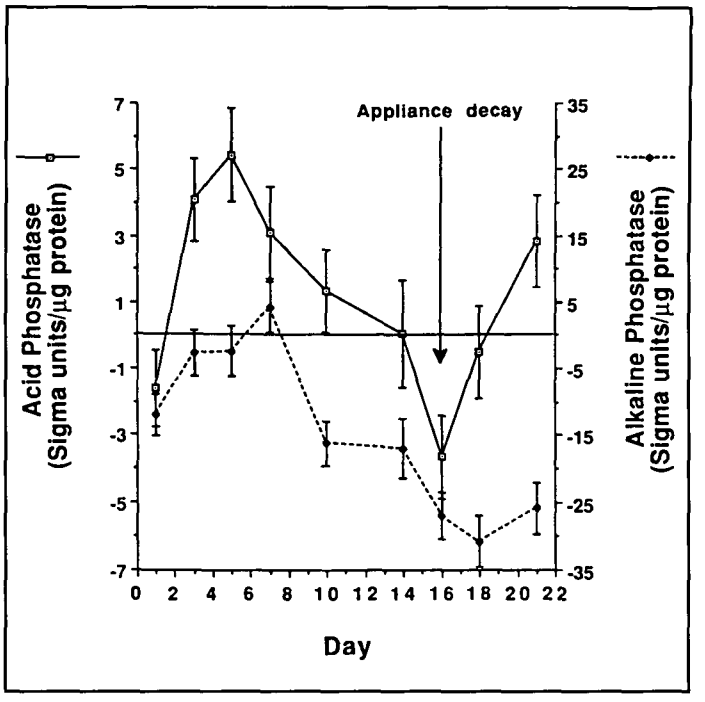

Figure 7

Serum indicators of bone turnover: The serum phosphatase indicators of bone turnover are shown graphically in Figure 5, where serum acid phosphatase values are shown on the left vertical axis and serum alkaline phosphatase on the right vertical axis. The Welch ANOVA indicated that significant differences occurred across time in acid phosphatase ($p<.0001$). These differences were manifested by a peak of activity occurring at day 18 (versus days 3, 5, 7, 10, and 14; $p<0.05$). ANOVA indicated that significant differences existed in alkaline phosphatase across time ($p<0.0001$); pairwise comparisons indicated that a peak in activity occurred at day 7 (versus days 1, 3, 5, 10, 18, and 21; $p<0.05$). In addition, Welch ANOVA indicated that significant differences existed in osteocalcin over time ($p<0.0001$), manifested by a peak of activity at day 21 (versus days 1, 3, 5, 7, 10, and 14; $p<0.05$) (Figure 6).

Chemical indicators of turnover in alveolar bone: Chemical indicators of alveolar bone turnover are represented graphically in Figure 7; acid phosphatase values are on the left vertical axis and alkaline phosphatase values are on the right. ANOVA indicated that significant differences in acid phosphatase existed across time ($p=0.0004$). Pairwise comparisons indicated that these differences were characterized by a peak occurring in acid phosphatase activity at day 5 (versus days 1, 10, 14, 16, 18; $p<0.05$), a depression at day 16 (versus days 3, 5, 7, 10 and 21; $p<0.05$), and a further peak at day 21 (versus days 1 and 16; $p<0.05$). ANOVA indicated differences also existed in alkaline phosphatase across time ($p<0.0001$). Pairwise comparisons indicated that these were characterized by a peak occurring at day 7 (versus days 1, 14, 16, 18 and 21; $p<0.05$) and a depression occurring at day 18 (versus days 1, 3, 5, and 7; $p<0.05$).

Discussion

The most striking new finding from this study was the observation that, following loss of at least 93% of appliance activity by day 16, a second wave of alveolar bone remodeling begins with an abrupt inhibition of ongoing bone formation and the initiation of a second phase of bone resorption. Biphasic bone remodeling patterns have been noted during orthodontic tooth movement[12,2] and periapical alveolar bone resorption.[13] In normal physiological bone turnover, surfaces are thought to return to quiescence for extended periods following remodeling.[14] However, it is reasonable to suggest that, if the stimuli which initiated the bone remodeling in the first place persist, a second remodeling cycle can be initiated in the same location.

This remodeling pattern is also accompanied by significant orthodontic tooth movement, even after the appliances have substantially decayed. Short-term appliance activations have been reported to produce mesial molar movement in the absence of continued mesial force.[3] It seems clear that very short exposures to mechanical signals can stimulate substantial amounts of bone remodeling.[15-17] Although the precise relationship

Figure 6
Differences in serum osteocalcin across time. Treated data adjusted by subtracting control values for days 1-14 and the mean control values for days 7, 10, and 14 from days 16, 18, and 21. Mean and standard error of the mean are shown.

Figure 7
Differences in alveolar bone acid (left vertical axis; solid lines) and alkaline (right vertical axis; dotted lines) phosphatase activities. Treated data adjusted by subtracting control values. Mean and standard error of the mean are shown.

between frequency/duration and tooth movement remains unclear, it also seems evident that cyclic or pulsating forces can produce tooth movement responses similar to those recorded with continuous forces.[18-21] Moreover, there is abundant anecdotal clinical evidence which indicates that less than continuous forces are an effective means of producing tooth relocations.[22-24] However, because occlusal contacts are integral parts of orthodontic treatment, and because these contacts may not only prevent tooth movement in the direction of the applied force but may even promote tooth movement in other directions, one should adopt some caution when interpreting this finding with respect to clinical treatment.

The link between a remodeling signal and its response is not well understood, but it seems clear that a finite amount of time is required for the complete response to occur.[14] It also is reasonable to postulate that once the process is initiated at a particular site, local mechanisms may carry remodeling events to completion without additional signal. The suggestion has also been made that bone has a "strain memory" which is created by mechanical distortion of its extracellular matrix, possibly proteoglycans.[25,26] Such a mechanism may also explain why cells can continue to be stimulated to facilitate mesial tooth movement after the mechanical signal has been removed.

Another important feature of these data is that alveolar bone remodeling appears to uncouple at these alveolar sites during orthodontic tooth movement. When resorption predominates (around days 3-5 and 16-18), formation is inhibited. When formation peaks around day 14, resorption is depressed. These dynamics should result in alternating periods of net bone loss and accrual. This finding is consistent with the earlier observation of a transient osteopenia in orthodontically-treated paradental alveolar bone.[27] These bone turnover dynamics are distinctly different from the balanced alveolar bone remodeling which has been reported in molar drift.[28,1] The mechanism for this uncoupling is currently unclear. However, interleukin-1 (IL-1) can act to uncouple bone resorption and formation in vivo.[29] IL-1 has also been shown to be elevated in the periodontal ligament during orthodontic tooth movement.[30] This is accompanied by the production of prostaglandins (especially PGE2) by the bone tissue which is , in part, responsible for IL-1 induced bone resorption.[31] Prostaglandins have also been implicated in osteoclast recruitment during orthodontic tooth movement[32] and in the enhancement of the rate of tooth movement in humans.[33] It is also important to note that this uncoupling is a local change, probably regulated by paracrine and autocrine mechanisms. Such changes probably do not impact greatly on the overall skeletal mass, with the uncoupling being a temporal local event without a major overall effect.

The relationship between serum measures of bone remodeling and the histomorphometric data is also noteworthy because of the potential for their use to easily monitor the status of the biological responses to force in a clinical setting by sampling crevicular fluid. Alkaline phosphatase and osteocalcin are considered to be differentiation markers for the osteoblastic phenotype since they are expressed in cells of the osteoblastic lineage in vivo and in vitro. The serum levels of both have been shown to correlate with the rate of bone formation determined histomorphometrically. Therefore they can serve as biochemical indices of osteoblastic activity during bone apposition.[5] In this study, the peaks in serum osteocalcin and the early peak in serum alkaline phosphatase precede the histomorphometric representation of the bone formation wave and, therefore, may be predictive of those events. The late peak in serum osteocalcin may also be predictive of a second formation wave which we speculate would have become evident had the experiment been continued into a fourth week.

The first serum acid phosphatase peak also preceded the histomorphometric resorption peak. However, the second acid phosphatase peak coincided with the cellular changes. The time interval of 3 days required for the activation of bone resorption in the first wave is similar to activation times reported in the rat tooth egression model[34] and that following a single injection of macrophage colony stimulating factor (M-CSF) in mice.[35,36] These findings suggest that similar mechanisms may be required. This is distinct from the significantly shorter activation period (0.5 day) observed in parathyroid hormone (PTH)-stimulated rodent alveolar bone.[37] It has been previously suggested that this difference may be due to the existence of significant numbers of preosteoclasts in the remodeling site in the PTH study as compared with the tooth migration model. Sites of future resorption in the orthodontic model have also been reported to be initially poor in osteoclast precursors. Although there is bone resorption occurring around con-

trol teeth, this is almost exclusively on the opposite (distal) alveolar bone surface from where they appear during tooth movement in this model.[1] However, following the first wave these sites may be rich in preosteoclasts. This could result in a greater degree of coincidence between the serum acid phosphatase peak and the histomorphometric demonstration of resorption for the second wave.

The tooth movement kinetics demonstrate at least two, and possibly three, periods of delay. The first coincides with the initial displacement of the tooth caused by the appliances and lasts for seven days. The early part of this delay is characterized by peaks in measures of bone resorption (serum acid phosphatase, bone acid phosphatase and percent osteoclast surface). The latter part of the initial delay is characterized by elevations in serum alkaline phosphatase and osteocalcin. The second delay occurs between days 10 and 14. There are no resorptive changes involved, but it is instead characterized by increases in osteoblast surface and bone formation rate. The second period of movement, which occurs between days 14 and 18, is characterized by a second recruitment of osteoclasts followed by a second wave of resorption and inhibition of formation. By day 18, the third delay in tooth movement occurs. This coincides with the beginning of the abatement of the second resorption wave. We conclude from these observations that there is no consistent correlation between the state of tooth movement or delay and histomorphometric or biochemical indicators.

Force-deflection characteristics of closed coil springs are known to be influenced by wire size, lumen diameter, wire type, coil length, pitch angle of the coil, the alloy used and heat treatment.[38-42] Force-deflection correlations have shown that a linear relationship exists over most of the ranges of the coils studied with variations at the lowest and highest deflections.[38,42] It is, therefore, important to realize that these curves have different characteristics when individual coil variables are altered. Thus, the formula reported herein applies only to the conditions described in this paper. In this study, the lowest part of the force-deflection curve tends to underestimate force. This is partially attributed to the presence of residual stresses.[40,43] Certain groups of closed coil springs, including the type used in this study, exhibit a phenomenon termed *initial tension*. This requires that a certain threshold of force must be reached before the spring will begin to deflect. This property is imparted to the spring during manufacture. All samples of Unitek Hi T coil exhibit initial tension.[42] However, some Elgiloy coil springs do not have this property and thus may give more favorable standard curves in this application.

This underestimation in the lowest part of the curve could also be due to changes in the angulation between the coil and the x-ray beam, resulting in foreshortening. The coil images are obviously foreshortened, as indicated by the zero force being shorter than the actual coil (4.75 mm for the former and 7.0 mm for the latter). Bearing in mind that the radiographic image of a coil length is a function not only of its extension but also its angulation, the coil image may be more perpendicular to the beam at 0 force than when loaded. The addition of force would change this orientation to a slightly more foreshortened image, but also extend the coil. At higher forces, coil extension becomes greater than the foreshortening.

The noninvasive prediction of spring forces from cephalometric images of coil lengths also proved to be valid. Seventy-eight percent of the variance can be accounted for with this association ($p=0.002$). Direct measurement of spring deflections from day 1 skulls was used as a "gold standard" after determination that there was no difference between springs in defleshed skulls and immediately after harvesting. Use of the optical comparator allowed the direct measurement of spring deflections at a magnification comparable to that used for the cephalometric predictions.

Bone remodeling waves, as well as coupling and uncoupling, are not well understood, but are thought to be controlled by paracrine and autocrine mechanisms. Biologically active molecules are secreted by the resident cells or released from the bone matrix at appropriate times to regulate ongoing processes. In orthodontic tooth movement, the process is probably initiated by either strain in the tissues,[25,44,45] streaming potentials caused by fluid flow in the tissues,[46,47] microdamage,[48] inflammation,[49] or a combination of these factors.

The preosteoclast pool can be expanded, attracted to the site and activated by a number of molecules, including IL-1, tumor necrosis factor alpha (TNF alpha), M-CSF and metabolites of vitamin D (1,25D3).[50-53,29,36] Many of these factors have also been implicated in orthodontic tooth movement.[30,54,55,33] Although the mechanism of coupling is unclear, it has been postulated that the release of chemotactic[56] and growth factors

from bone matrix by resorption may act to inhibit ongoing bone resorption, recruit and stimulate osteoblasts. Putative factors include transforming growth factor beta (TGF-B), insulin-like growth factor (IGF-I) and platelet-derived growth factor (PDGF).[57-59] From this study, we conclude that orthodontic force stimulates uncoupling of bone remodeling characterized by alternating waves of bone loss and accrual. This remodeling pattern persists for an indeterminate period following appliance decay. There is little correlation between periods of rapid tooth movement or delay and the measured bone remodeling parameters. Serum measures of bone turnover show some promise for monitoring and predicting the biological response in a clinical setting. The cephalometric assessment of spring decay during the course of an experiment has an acceptable level of reliability and validity and is easy to perform. Since orthodontic tooth movement sets up synchronous waves of bone remodeling in large numbers of cells in the paradental alveolar bone, it could serve to assess the roles of selected paracrine and autocrine factors in producing these bone turnover dynamics by monitoring their levels in relation to these responses.

Conclusions

1. The force levels of orthodontic springs can be validly and reliably assessed in the rat using a cephalometric approach and without sacrificing the animal.
2. Significant orthodontic tooth movement continues to occur for several days following appliance decay.
3. Histomorphometric and chemical indicators of bone turnover persist for several days after appliance decay.

Acknowledgments

The authors wish to thank Liz McCoy, Linda Latta, Dr. Bert Hughes, Dr. Alan Ossi, Kirsten Birkett and Kathy Cannon for their assistance.

This work was supported by NIDR Research Award DE 08659I.

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