Local delivery of recombinant osteoprotegerin enhances postorthodontic tooth stability

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ORIGINAL RESEARCH

Local Delivery of Recombinant Osteoprotegerin Enhances Postorthodontic Tooth Stability

James Bradley Hudson · Nan Hatch · Takayuki Hayami · Jae M. Shin · Marina Stolina · Paul J. Kostenuik · Sunil Kapila

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Abstract Relapse after orthodontic tooth movement is a significant problem in orthodontics. The purpose of this study was to examine the efficacy of the osteoclast inhibitor osteoprotegerin-Fc (OPG-Fc) for inhibiting postorthodontic relapse. Rat maxillary molars were moved mesially and allowed to relapse for 24 days. Low-dose (1 mg/kg) or high-dose (5 mg/kg) OPG-Fc or saline was injected adjacent to the molars during relapse. Tooth movement, micro-CT, histologic bone quality, and serum OPG and TRAP-5b were measured. OPG-Fc injections significantly diminished postorthodontic relapse from 63% (0.78/1.20 mm) of total movement in vehicle control rats to 31% (0.31/1.00 mm) in low-dose and 24% (0.28/1.16 mm) in high-dose OPG-Fc groups 24 days after appliance removal. Normalization of bone and periodontal tissues occurred as early as 8 and 16 days in the high- and low-dose OPG-Fc-treated groups, respectively, while the vehicle-treated group showed only partial tissue recovery 24 days following tooth movement. After 24 days of relapse, there was complete recovery to pre-tooth-movement values for bone volume fraction (BVF) and tissue mineral density (TMD) in both the low- and high-dose OPG-Fc groups, while BVF recovered only partially and TMD did not recover in the vehicle control group. Greatly elevated serum OPG levels and reduced serum TRAP-5b levels in OPG-Fc-treated animals indicated systemic exposure to locally injected drug. The profound decrease in postorthodontic relapse by local OPG-Fc administration indicates that osteoclasts are critical to bone maturation following tooth movement and points to the potential pharmacologic use of OPG-Fc or other RANKL inhibitors for orthodontic retention.

Keywords Osteoclast · OPG · TRAP-5b · Orthodontic treatment · Orthodontic relapse · Micro-computed tomography

M. Stolina and P. J. Kostenuik are employees of Amgen and own Amgen stock. All other authors have stated that they have no conflict of interest.

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"Orthodontic relapse" refers to the tendency of teeth to regress toward their original locations after being moved through bone with orthodontic forces. It is observed clinically as the movement of teeth relative to each other from their aligned positions. Posttreatment relapse is a significant limitation of orthodontic treatment due to its high rate of occurrence, adverse effects on tooth alignment, and

additional financial burden on patients. Furthermore, the classic approaches to orthodontic retention primarily involve the use of fixed or removable mechanical retainers whose caveats include reliance on patient compliance and the long duration of retention required to ensure stability. The high frequency of orthodontic relapse and the need for long-term patient compliance-based retention make it important to understand its causes for the purpose of developing innovative methods to enhance posttreatment stability of teeth. While the specific causes of orthodontic relapse remain poorly understood, traditional theories implicate factors such as imbalances in muscle and soft tissue pressures [1, 2]; forces exerted by stretched periodontal and gingival connective tissue fibers [3–6]; changes in tooth inclination [7, 8], arch dimensions [9], or facial growth [10, 11]; and potentially ongoing bone turnover [12]. Of these variables, the current knowledge of bone biology, and particularly its responses to mechanical forces, may provide cues toward understanding how bone undergoes posttreatment normalization, thereby aiding in therapeutic strategies to enhance postorthodontic stability.

Orthodontic tooth movement involves osteoclast-mediated resorption of alveolar bone adjacent to the pressure side of the tooth roots [4, 13–15]. This bone resorption allows for tooth movement in the direction of the compressive force beyond the original constraints of the tooth socket. Simultaneously, new bone is deposited by osteoblasts on the opposite side of the tooth roots in the area from which the tooth was moved [13]. After completion of orthodontic treatment, this new woven bone is purportedly remodeled into mature bone, which is more organized and of higher strength and mineral content than newly formed bone and therefore potentially less susceptible to resorption. The new woven bone is thought to mature through remodeling, the first step of which is the removal of immature bone by osteoclasts, followed by its replacement with lamellar bone. It is likely that the initial resorptive activity of this immature bone might permit relapse by creating a temporary void adjacent to the periodontal ligament (PDL) neighboring the tooth roots, which are under rebound compression [16]. Thus, mechanisms that enhance bone maturation or diminish its resorption by osteoclasts are likely to aid in minimizing posttreatment relapse.

The key regulator of osteoclast differentiation and activation is the receptor activator of nuclear factor $\kappa\beta$ (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) receptor/ligand system [17]. RANKL, which is found on the surface of osteoblasts and PDL cells, stimulates osteoclast activity by binding to its receptor, RANK, a transmembrane protein found on mature osteoclasts and their precursors [18]. The binding of RANKL to RANK is critical for osteoclast formation, activation, and survival involved in bone catabolism [19]. Conversely, OPG is a soluble protein that binds to RANKL and functions as a competitive inhibitor of RANK [20] such that the binding of OPG to RANKL inhibits osteoclastogenesis and bone resorption [20–22].

That OPG could be a suitable adjunct for orthodontic retention is suggested by findings showing that administration of OPG-Fc results in a rapid and sustained decrease in bone surface osteoclasts as well as increases bone mineral density [23] and rescues the estrogen [24] or androgen [25] deficiency-induced osteoporosis in rats. Furthermore, both OPG and a monoclonal antibody to RANKL (denosumab), which functions similarly to OPG by blocking RANKL binding to RANK, decrease bone resorption in postmenopausal women [26, 27]. Besides its systemic effects, OPG also functions to restore bone locally in lipopolysaccharide-induced periodontitis [28] and decreases the rate of tooth movement and osteoclastogenesis, while increasing alveolar bone volume in an animal model of orthodontic tooth movement [29, 30]. Conversely, local RANKL gene delivery in rats results in locally increased RANKL expression, osteoclastogenesis, and enhanced rates of tooth movement [31]. In the context of postorthodontic relapse, it is known that new bone which forms behind orthodontically mobilized tooth roots likely serves as a barrier that resists, but does not eliminate, relapse [16]. By inhibiting the initial turnover of this new bone, OPG might allow it to rapidly mineralize in a manner that better resists compressive forces from the neighboring tooth roots. This postulate together with the known role of the RANK/RANKL/OPG axis in orthodontic tooth movement [28–30] suggests that manipulation of osteoclast activity via local delivery of OPG could enhance postorthodontic tooth stability. Here, we utilized a rodent model of orthodontic tooth relapse to investigate whether local delivery of recombinant rat OPG-Fc inhibits alveolar osteoclast activity and postorthodontic relapse. In addition, we utilized micro-computed tomography (CT) and histology to gain a better understanding of the little understood biological processes of bone maturation that may contribute to postorthodontic relapse.

Materials and Methods

Animals

All experiments were performed following approval from the University of Michigan Committee on Use and Care of Animals (protocol 10104). The study design, animal numbers, and OPG doses were based on findings of preliminary studies performed with the relapse model utilized in the current study and/or published studies [28]. A total of 50 young adult, male Sprague-Dawley rats weighing approximately 350 g were used for the study. The

experiment was divided into two phases: initial tooth movement (days 1–28), during which only tooth-movement measurements were taken, and the tooth relapse phase (days 28–52), during which injections of OPG-Fc or phosphate-buffered saline (PBS) vehicle were administered and the various procedures described below and in Fig. 1 were performed.

Forty-two rats, divided into three groups of 14 each, were subjected to orthodontic forces for 28 days, after which the forces were removed and volumetrically equivalent injections (60 μL) of 5.0 mg/kg (high dose) OPG-Fc (rat recombinant OPG; Amgen, Thousand Oaks, CA), 1.0 mg/kg (low dose) OPG-Fc, or PBS vehicle were administered. Four other animals received no orthodontic appliances or injections and were killed as baseline pre-tooth-movement controls. Four additional animals were killed at day 28 of the experiment as post-tooth-movement controls (henceforth referred to as day 0 of the relapse phase). During the relapse phase, three groups of six animals each served as the control, low-dose OPG-Fc, and high-dose OPG-Fc groups, which were observed for the entire 24 day period of relapse. These rats were killed at day 24 post-tooth movement for histology and micro-CT. Additional animals were killed at days 8 and 16 postremoval of appliances ($n = 4$ in each group at each time point) for histologic analysis. Rats were anesthetized by intraperitoneal administration of ketamine (87 mg/kg) and xylazine (10 mg/kg) for appliance placement and all subsequent procedures and killed by CO_2 inhalation.

Orthodontic Tooth Movement and Relapse

A previously described rat tooth-movement model [29, 32] was modified to develop a model of orthodontic relapse. For the tooth-movement phase of this model, mesial molar force was delivered bilaterally with closed coil nickel titanium springs (extra-light Sentalloy®; GAC, Bohemia, NY) calibrated to provide 50 g of force, which produces significant molar movement in the rat [29, 32]. Springs were fastened to the maxillary first molars and ipsilateral incisor with 0.010 stainless-steel ligatures tied around the teeth and secured in small grooves prepared on each tooth (Fig. 1). Ligatures were bonded to the teeth with composite (Transbond XT Light Cure Adhesive Paste; 3 M Unitek, Monrovia, CA). Mandibular incisors were reduced on a weekly basis to prevent breakage of the appliance. After 28 days of tooth movement, appliances were removed and relapse was allowed to occur for 24 more days (Fig. 1).

Local Administration and Doses of OPG-Fc

The doses of recombinant rat OPG-Fc used were as determined from previous studies [29] and through preliminary investigations. Volumetrically equivalent injections (60 μL) of low-dose (1.0 mg/kg) OPG-Fc, high-dose (5.0 mg/kg) OPG-Fc, or PBS were administered at appliance removal (day 28), at 2 days after appliance removal (days 30 and 32), and every 4 days thereafter until the end of the relapse observation period (day 52) (Fig. 1). OPG-Fc

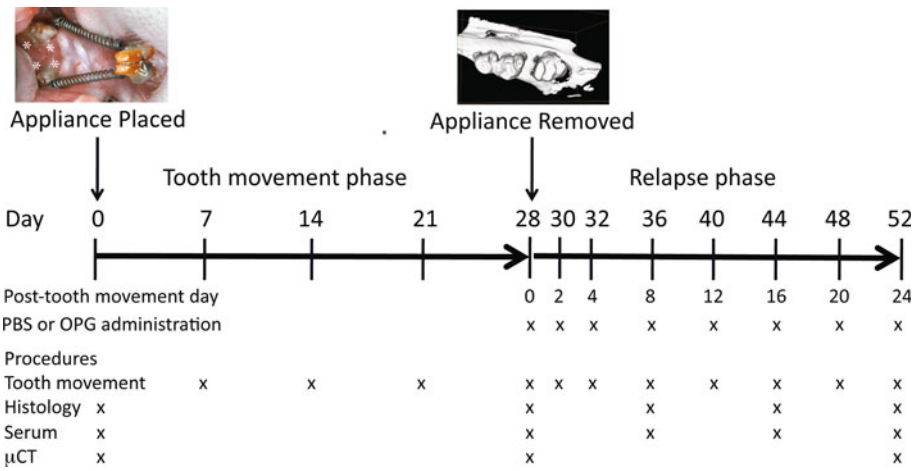

Fig. 1 Study design and rodent model of orthodontic tooth movement and relapse. Closed coil nickel titanium springs calibrated to deliver 50 g of force were placed bilaterally between incisor and molar teeth (*upper left panel*) during the tooth-movement phase. Appliances were removed after 28 days and the molars allowed to relapse for an additional 24 days (relapse phase). During this relapse phase, PBS, low-dose (1 mg/kg) OPG-Fc, or high-dose (5 mg/kg) OPG-Fc was administered near the molars (*asterisks in upper left panel*) at appliance removal and at specified time points. Upper-arch impressions were taken for tooth-movement measurements at the indicated time points during active tooth-movement and relapse phases. Additionally, micro-CT imaging was performed, and tissue and serum samples were retrieved at specified time points for determination of bone quality, periodontal histology, and systemic concentrations of TRAP-5b and OPG. An untreated group of four rats were maintained without springs for 28 days, after which they were killed, blood was retrieved for OPG and TRAP5b assays, and tissues were utilized for baseline control histologic and micro-CT analyses

or PBS was administered into the palatal mucosa in proximity of the mesiopalatal and distopalatal surfaces of each maxillary first molar with 33 gauge microneedles (Hamilton, Reno, NV).

Tooth-Movement Measurements

Tooth movement was measured as previously described, with minor modifications [29]. Precise stone models (Jade Stone; Whip Mix, Louisville, KY) were fabricated from polyvinylsiloxane (Dimension Garant 2L Quick; 3M ESPE, St. Paul, MN) impressions of the maxillary teeth. The occlusal surfaces together with a 100 mm ruler placed next to the casts were scanned (Epson Expression 10000XL; Epson, Long Beach, CA) at 1,200 dpi and magnified $\times 300$ using Adobe Photoshop (CS3; Adobe Systems, San Jose, CA). Since the focus of this study was to determine the postorthodontic changes in interdental relationships, the movement of the first molar was measured relative to that of the third molar rather than to skeletal structures. This approach minimizes the effects of maxillary growth and other physiologic changes in determining the magnitude of postorthodontic dental relapse. Molar movement was measured to the nearest 0.02 mm by a single blinded, calibrated examiner from the distal groove of the maxillary first molar to the distal surface of the maxillary third molar using the measuring tool in the software. The ruler on each scan was also measured to calibrate the measurements from the casts.

To determine whether local administration of OPG-Fc also affected the stability of other teeth, incisor movement was measured by determining the distance from the center of the facial surface of the maxillary incisor at the gingival margin to the distal surface of the maxillary third molar. The amount of incisor relapse was calculated as the difference in relative position of incisors to third molars between the immediate post-tooth-movement measurement and that at end of the relapse phase.

The error of method for tooth-movement measurements was determined by two repeat measurements of first molar movement on 36 teeth taken 2 weeks apart. Bland-Altman analysis was performed with a resulting coefficient of repeatability of 0.045 mm. Most (85%) of the repeat measurements were smaller than this coefficient, which shows high reliability in the measurement of tooth movement. The higher limit of agreement was 0.041 mm and the lower limit of agreement was -0.048 mm, which means that there was no fixed or systematic bias.

Micro-CT Analysis

Block tissue biopsies of the right and left hemimaxillae were harvested, immediately fixed in 10% formalin for 48 h, and transferred to 70% ethanol. Micro-CT analysis was performed to quantify the alveolar bone surrounding the maxillary first molars. Rat hemimaxillae were scanned with a polychromatic cone beam micro-CT system (GE Healthcare, London, ON, Canada). Scans were reconstructed at a voxel mesh size of 18 μm^3 and 3-D digital reconstructions generated for each of the specimens (Fig. 5a). GE Healthcare Microview Analysis + software was used to rotate and crop the 3-D images into a standard orientation. A standard threshold was applied to each sample to distinguish the levels of mineralization in the various hard tissues present, such as bone and tooth roots. An optimal threshold value was calculated for each sample, and the individual thresholds were averaged to give a representative threshold value that was used on all images.

A region of interest (ROI) for alveolar bone surrounding the five maxillary first molar roots was defined by a single, blinded, and calibrated investigator on all of the samples using a previously established protocol (Fig. 5a) [33]. This ROI was chosen because of its previously verified high level of reproducibility and because it includes the highly relevant region of bone directly investing the five roots of the orthodontically moved teeth, thus permitting quantification of the recently remodeled bone tissue [34]. The bone volume fraction (BVF), defined as bone volume/total volume of the ROI, and tissue mineral density (TMD), defined as mineral content/volume of the ROI using an auto threshold for mineralization, were determined using a previously established algorithm [34].

Histological Analysis

Following micro-CT scans, samples were decalcified with 10% vol/vol EDTA for 6 weeks and embedded in paraffin, and 5 μm sagittal sections were obtained through the mesial root of the maxillary first molar and stained with hematoxylin and eosin for descriptive histology. The mesial root was chosen for analysis because it is the largest of the five roots, allows for histological evaluation of the entire root structure, and is commonly used for analysis in tooth-movement studies [29, 35]. Slides were evaluated by an investigator blinded to the identity of the experimental groups. Microscopy was performed using an Olympus BX45 light microscope (Olympus, Central Valley, PA) equipped with a 12.5 megapixel digital camera (Olympus DP72) and images of representative images were captured.

Serum TRAP-5b and OPG Assays

Blood (200 μL) was retrieved from animals at indicated time points through the lateral tail vein with a 27 gauge needle (Fig. 1). Serum was isolated by centrifugation, and 25 μL aliquots were utilized for OPG and TRAP-5b assays.

Serum levels of OPG were measured per the manufacturer's instructions with a luminex-based enzyme-linked immunosorbent assay (ELISA) (Millipore, Billerica, MA). Serum levels of TRAP-5b enzyme were measured with a solid-phase immunofixed enzyme activity assay specific for the determination of osteoclast-derived TRAP-5b (Immunodiagnostic Systems, Fountain Hills, AZ) per the manufacturer's instructions.

Statistical Analysis

Each animal was treated as a unit, and bilateral measures of tooth-dependent parameters were taken for each side of the maxilla and averaged to give a single measure for each animal at each time point. If only one side of the maxilla could be measured due to a damaged or missing sample or a compromised tooth, the value from the available side was used as the measure for that animal. Descriptive statistics (means and standard errors) were calculated for each parameter for all groups. A repeated measures analysis of variance (ANOVA) was used to compare tooth-movement data between the three groups (SAS version 9.1; SAS Institute, Cary, NC). Incisor relapse, BVF, and TMD comparisons were made using one-way ANOVA. Intergroup differences were analyzed with the Bonferroni post-hoc test. Statistical significance was set at $P < 0.05$.

Results

Animal Status

All animals tolerated the various procedures with no discernable effect on their abilities to thrive. Average initial weight of the animals was 364.3 (SD ± 11.0) g, and weight at the time of orthodontic appliance removal was 378.3 (SD ± 12.0) g. There was no difference ($P > 0.66$) in weight gain among the treatment groups during the relapse phase. Mean weights at the end of the relapse phase were 429.8 (SD ± 13.5) g in the vehicle-treated, 427.8 (SD ± 10.9) g in the low-dose OPG-Fc, and 432.0 (SD ± 19.4) g in the high-dose OPG-Fc groups.

OPG Minimizes Postorthodontic Relapse

Upon removal of appliances (day 28) there was no statistically significant difference in the mesial movement of the molars achieved with the orthodontic appliance between the control (1.20 ± 0.17 mm), low-dose OPG-Fc (1.00 ± 0.07 mm), and high-dose OPG-Fc (1.16 ± 0.16 mm) groups (Fig. 2a, b). There was no difference ($P > 0.19$) in the both the linear and percentage relapse measured 2 days post-appliance removal (day 30) between the low-dose OPG-Fc (18.40 ± 3.19%, 0.18/1.00 mm), high-dose OPG-Fc (25.73 ± 2.76%, 0.29/1.16 mm), and control (22.60 ± 2.11%, 0.27/1.20 mm) groups (Fig. 2b, c). Local delivery of low-dose and high-dose OPG-Fc resulted in a significant linear and percentage reduction in distal molar relapse compared with PBS-injected control animals from days 12 to 24 of the relapse phase. Rats in the low-dose group exhibited a minimum of 46% ($P < 0.01$) to a maximum of 52% ($P < 0.001$) less relapse, and the high-dose OPG-Fc group had a minimum of 48% ($P < 0.01$) to a maximum of 60% ($P < 0.001$) lower relapse than control rats over this duration of the experiment. By day 24 of relapse, control rats had 63% (0.78/1.20 mm) reversion of the original tooth movement, which was greater than the 31% (0.31/1.00 mm, $P < 0.001$) and 24% (0.28/1.16 mm, $P < 0.001$) relapse in the low-dose and high-dose OPG-Fc rats, respectively. There were no differences in the magnitude or percent relapse between the low-dose and high-dose OPG-Fc groups at any of the time points ($P > 0.17$).

Incisor relapse at 24 days post-appliance removal was lower in the low-dose OPG-Fc (0.15 ± 0.04 mm, $P < 0.001$) and high-dose OPG-Fc (0.10 ± 0.02 mm, $P < 0.001$) groups compared with the control group (0.67 ± 0.12 mm) (Fig. 2d). However, there was no difference ($P > 0.87$) in incisor relapse between the low-dose and high-dose OPG-Fc groups.

OPG Enhances Bone Regeneration and Decreases Cellular Infiltrate following Orthodontic Tooth Movement

As opposed to control untreated baseline samples, both tension (distal) and compression (mesial) sites post-tooth movement (Figs. 3c, 4c, respectively) showed a widened PDL space, multiple areas of bony lysis, extensive cellular infiltration, and isolated areas of root resorption, which was particularly marked at compression sites. These findings are consistent with the active process of tooth movement and are contrasted with the normal periodontal histology that includes a normal dentine, cementum, cellularity, and width of the PDL and organized bone in the baseline control tissues (Figs. 3b, 4b).

Over the 24 day relapse phase, there was gradual resolution of the cellular infiltrate, increasing amount and maturation of bone, and decreased width of the PDL in all groups of rats (Figs. 3d–l, 4d–l). However, the rates of this trend toward normal varied substantially between the PBS-treated and the two OPG-treated groups, with some differences also being noted between high- and low-dose OPG-Fc samples. Overall, the cellular infiltrate and bone loss were more marked and took longer to resolve on the compression (mesial) side of the root (Fig. 4) than on the tension (distal) side of the root (Fig. 3). PBS control tissues

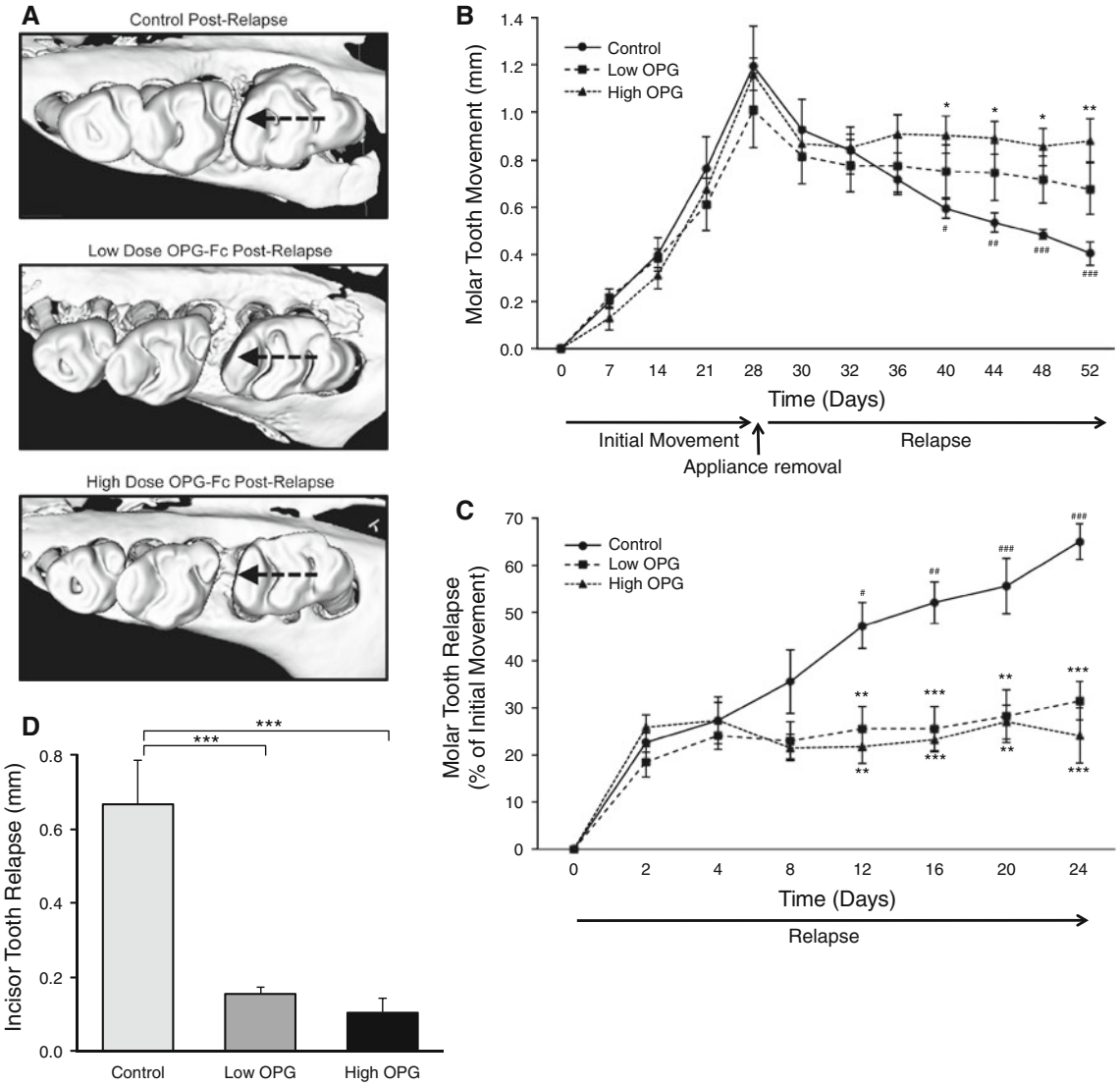

Fig. 2 Local delivery of OPG-Fc inhibits postorthodontic relapse. **a** Axial micro-CT views of representative vehicle, low-dose, and high-dose OPG-Fc-injected animals 24 days after orthodontic appliance removal. Note the difference in interdental distance visible between the first and second molar teeth in vehicle versus OPG-Fc-treated animals. *Arrows with dashed line* indicate the direction of molar distal relapse following appliance removal. **b** Maxillary molar tooth movement and relapse over the entire experimental period show equivalent initial mesial orthodontic tooth movement in the three groups of rats up to 28 days and significantly different relapse distal tooth movement between the vehicle control and the two OPG-Fc-injected groups as well as over time only in the vehicle control group. **c** Molar distal relapse presented as a percentage of initial molar movement shows significant differences between the vehicle control and the low-dose and high-dose OPG-Fc groups. **d** Incisor relapse anterior movement of vehicle control, low-dose OPG-Fc, and high-dose OPG-Fc-injected animals 24 days after orthodontic appliance removal. All results are expressed as means ± SEM. Intergroup comparisons differences are denoted as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Intragroup differences at each time point relative to day 28 of tooth movement or day 0 of relapse are denoted as $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ ($n = 6$ in each group)

showed continued cellular infiltrate and slowly maturing bone from the end of active tooth movement to the end of the experimental period, which also appeared to be more delayed on the compression than tension sites. In contrast, sections from the high-dose OPG-Fc group showed histological features including near normal cellularity, PDL thickness, and bone similar to that in baseline control tissues at both tension and compression sites as early as 8 days post-appliance removal. By 8 days post-tooth movement, sections from the high-dose OPG-Fc rats showed well-organized bone phenotype, which was more advanced at the tension than the compression site. For the low-dose OPG-Fc group the near normalization of the cementum, PDL cellularity and thickness, and bone quality was achieved by 16 days post-appliance removal, while these features were just becoming evident at 24 days after appliance removal in the PBS control group. Bone anatomy and height on both the mesial and intraradicular sites

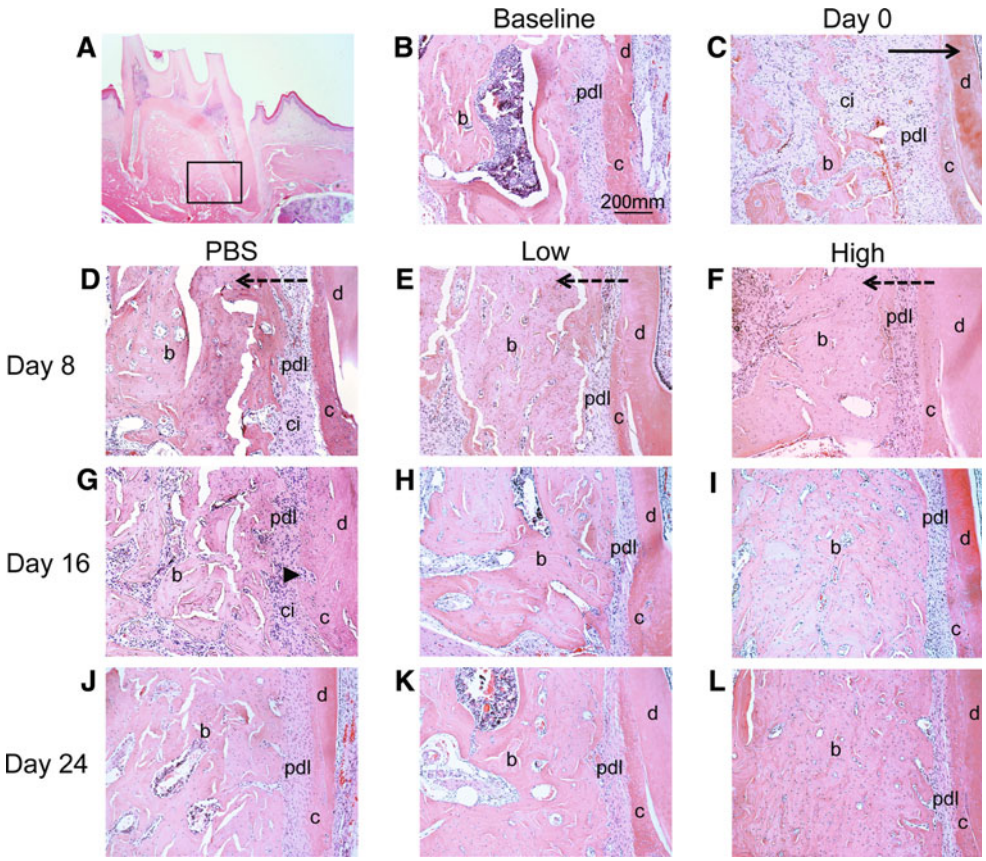

Fig. 3 Photomicrographs of representative hematoxylin and eosin-stained sections of the distal (tension) site of the first molar mesial root at 8, 16, and 24 days of relapse for vehicle control, low-dose OPG-Fc, and high-dose OPG-Fc groups showing more rapid normalization of histology in the OPG-Fc than the vehicle control group. **a** Low-power photomicrograph with *rectangle* showing area represented at higher magnification in remaining panels. Representative photomicrographs of baseline untreated control sample (**b**), immediate post-tooth-movement sample (**c**), vehicle-treated control samples at 8 (**d**), 16 (**g**), and 24 (**j**) days, low-dose OPG-Fc samples at 8 (**e**), 16 (**h**), and 24 (**k**) days; and high-dose OPG-Fc samples at 8 (**f**), 16 (**i**), and 24 (**l**) days. *b* bone, *c* cementum, *d* dentine, *pdl* periodontal ligament, *ci* cellular infiltrate, *arrowhead* root resorption site, *continuous-line arrow* direction of orthodontic tooth movement, *dashed-line arrow* direction of relapse

returned to near normal levels earlier in the OPG-Fc than the PBS-treated groups. The compression sites of the vehicle control group also appeared to have greater areas of root resorption than either the low-dose or high-dose OPG-Fc groups.

OPG Enhances Postorthodontic BVF and TMD

The BVF of supporting alveolar bone was lower in post-tooth-movement (0.481 ± 0.064, $P < 0.01$) than in pre-tooth-movement (0.697 ± 0.010) animals (Fig. 5b). BVF was higher in vehicle control (0.605 ± 0.017, $P < 0.05$), low-dose OPG-Fc (0.714 ± 0.013, $P < 0.001$), and high-dose OPG-Fc (0.725 ± 0.017, $P < 0.001$) animals at day 24 of relapse in comparison with that in immediate post-tooth-movement rats. Also at 24 days of relapse there was no difference ($P > 0.23$) in BVF between baseline animals, control animals, and the two treatment groups. Finally, at the end of the relapse phase, BVF was higher ($P < 0.05$) in low- and high-dose OPG-Fc rats than in vehicle-treated animals.

TMD of supporting alveolar bone was lower ($P < 0.05$) in post-tooth-movement (932.76 ± 43.58 mg/cc) than in pre-tooth-movement ($1{,}048.22 \pm 6.45$ mg/cc) animals (Fig. 4c). TMD remained significantly diminished ($P < 0.01$) in vehicle-treated animals (933.91 ± 14.81 mg/cc) after 24 days of relapse relative to pre-tooth-movement levels. In contrast, TMD in low-dose and high-dose OPG-Fc-treated animals at 24 days of relapse returned to levels similar to those seen in pre-tooth-movement rats. At the end of the relapse phase, TMD in the high-dose OPG-Fc animals (1021.83 ± 8.90 mg/cc) was greater ($P < 0.05$) than that in post-tooth-movement animals (932.76 ± 43.58 mg/cc). Also at this time point, TMD was greater ($P < 0.05$) in both the low-dose (1015.31 ± 13.05 mg/cc, $P < 0.05$) and high-dose OPG-Fc-injected rats than in vehicle-injected animals.

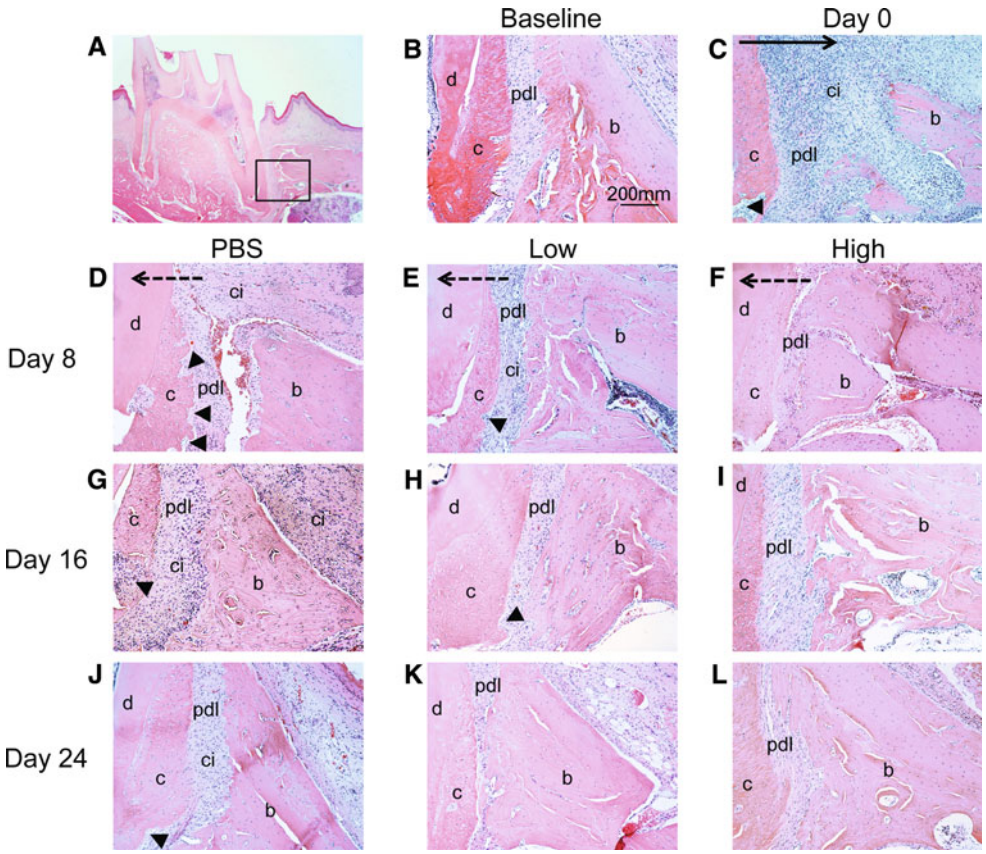

Fig. 4 Photomicrographs of representative hematoxylin and eosin-stained sections of the mesial (compression) site of the first molar mesial root at 8, 16, and 24 days of relapse for vehicle control, low-dose, and high-dose OPG-Fc groups showing more rapid normalization of histology in the OPG-Fc than the vehicle control group. **a** Low-power photomicrograph with *rectangle* showing area represented at higher magnification in remaining panels. Representative photomicrographs of baseline untreated control sample (**b**), immediate post-tooth-movement sample (**c**), vehicle-treated control samples at 8 (**d**), 16 (**g**), and 24 (**j**) days, low-dose OPG-Fc samples at 8 (**e**), 16 (**h**), and 24 (**k**) days, and high-dose OPG-Fc samples at 8 (**f**), 16 (**i**), and 24 (**l**) days. *b* bone, *c* cementum, *d* dentine, *pdl* periodontal ligament, *ci* cellular infiltrate, *arrowhead* root resorption site, *continuous-line arrow* direction of orthodontic tooth movement, *dashed-line arrow* direction of relapse

Local Administration of OPG Modulates Serum TRAP-5b and OPG Levels

Serum concentrations of TRAP-5b at days 8 (4.7 ± 0.6 U/L) and 24 (6.0 ± 1.0 U/L) in PBS-treated rats were similar to, while that in day 16 (6.3 ± 1.5 U/L) rats was greater ($P < 0.05$) than, those in immediate posttreatment rats (4.9 ± 1.2 U/L) (Fig. 6a). In contrast, at days 8, 16, and 24 post-tooth movement, serum TRAP-5b levels were profoundly lower ($P < 0.001$) in the low-dose OPG-Fc-injected (1.0 ± 0.3, 1.0 ± 0.2, 0.8 ± 0.2 U/L, respectively) and high-dose OPG-Fc-injected (0.7 ± 0.3, 0.8 ± 0.4, 1.0 ± 0.5, respectively) animals compared with vehicle-injected animals (4.7 ± 0.6, 6.3 ± 1.5, 6.0 ± 1.0 U/L, respectively). No differences in serum TRAP-5b levels were noted between the low-dose and high-dose OPG-Fc groups at any of the relapse time points ($P > 1.0$).

Concentrations of endogenous serum OPG in control rats progressively and significantly decreased at 24 days (0.7 ± 0.2 ng/m, $P < 0.05$) relative to that at 8 days of the relapse phase (Fig. 6b). As expected, serum levels of OPG in OPG-Fc-treated animals were profoundly increased ($P < 0.001$) at days 8, 16, and 24 following appliance removal in the low-dose OPG-Fc (4,996 ± 794, 4,569 ± 1,011, 4,744 ± 1,095 ng/mL, respectively) and high-dose OPG-Fc (11,844 ± 1,477, 11,732 ± 1,595, 11,039 ± 431 ng/mL, respectively) groups compared with vehicle-injected animals (6.5 ± 3.6, 2.5 ± 2.0, 0.7 ± 0.2 ng/mL, respectively). At days 8, 16, and 24 post-tooth movement, the high-dose OPG-Fc-injected animals also showed higher levels ($P < 0.001$) of OPG in serum compared with low-dose OPG-Fc-injected animals.

Discussion

By regulating osteoclast activity, the RANK/RANKL/OPG axis has ramifications for a wide range of physiologic and

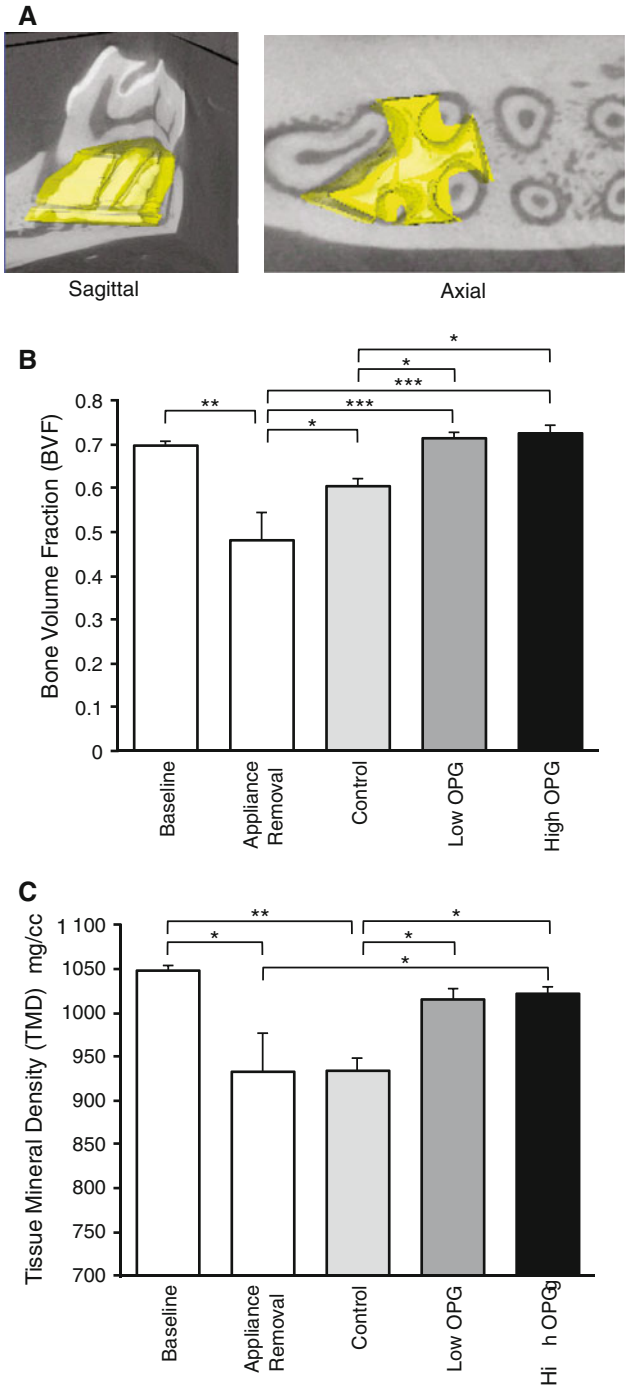

Fig. 5 Local administration of OPG-Fc enhances the normalization of postorthodontic BVF and TMD in alveolar bone investing maxillary first molar roots. **a** Sagittal and axial perspectives of ROI used to quantify BVF and TMD of alveolar bone investing maxillary first molar roots by micro-CT. The ROI includes bone (*yellow*) and excludes tooth roots. BVF (**b**) and TMD (**c**) were significantly reduced at the end of the orthodontic tooth movement and recovered to normal levels at day 24 on administration of low-dose and high-dose OPG-Fc. Results are expressed as means ± SEM. Intergroup differences are denoted as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ ($n = 6$ in each group)

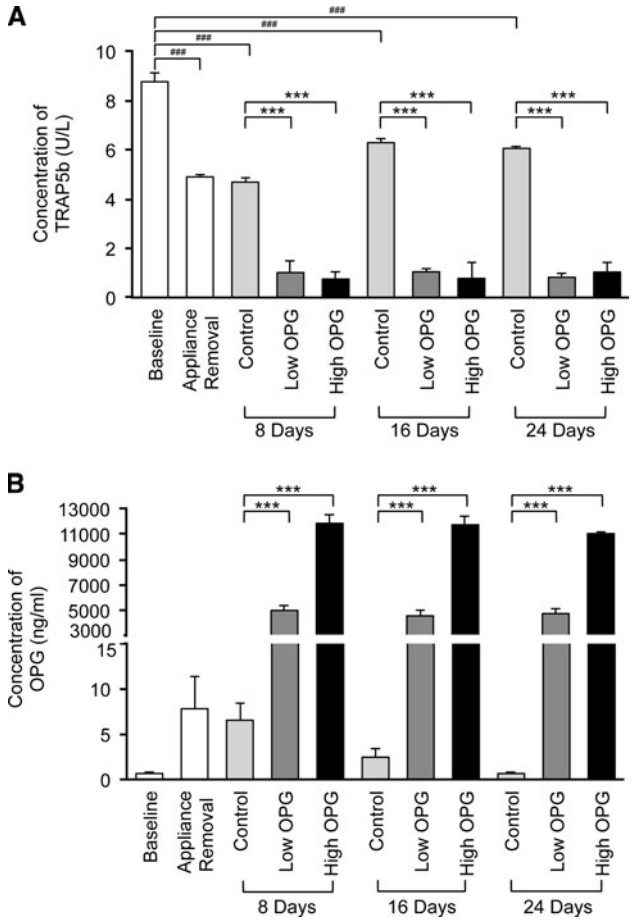

Fig. 6 Local administration of OPG modulates systemic levels of TRAP-5b and OPG. Blood retrieved from animals at indicated time points was utilized for serum TRAP-5b enzyme and OPG protein levels by solid-phase immunofixed enzyme activity assay and ELISA, respectively. **a** Local administration of OPG-Fc resulted in significant decreases in TRAP-5b activity at all time points assayed, reflecting a decrease in osteoclast activity. **b** In contrast, OPG assays that measured both endogenous OPG and exogenous recombinant OPG-Fc showed profound and sustained dose-dependent increases in serum OPG in low- and high-dose OPG-Fc-treated rats relative to PBS-treated control rats at all time points. PBS-treated controls had a temporal decrease in serum OPG levels over the duration of the experiment. All results are expressed as means ± SEM. Intergroup differences are denoted as $***P < 0.001$ ($n = 6$ in each group)

pathologic processes in bone, including osteoporosis, metastatic bone disease, rheumatoid arthritis, fracture healing, and periodontal disease [21, 25–28, 36–42]. OPG has a well-defined bone protective effect and is able to rescue bone mass in a variety of bone catabolic conditions through its central role as an inhibitor of osteoclast differentiation and survival [43]. Thus, administration of OPG (or anti-RANKL antibody) is accompanied by increased bone mass, bone mineral density, and cortical and trabecular bone volumes and by decreased systemic measures of bone turnover and diminished bone surface osteoclast

numbers in a variety of animal models and human conditions [23–27]. Relevant to orthodontics, mechanically mediated cellular responses in the PDL result in increased RANKL expression at compression sites that likely contributes to enhanced osteoclast activity and bone resorption, resulting in tooth movement [44–46]. In addition, OPG gene transfer or local delivery of recombinant OPG profoundly decreases mechanically mediated bone modeling and orthodontic tooth movement by inhibiting osteoclasts [29, 30]. Taken together, these results indicate that manipulation of osteoclast activity via local delivery of RANKL or OPG enhances or inhibits orthodontic tooth movement, respectively.

Our study sought to apply this and other current knowledge of cellular control of bone turnover and the role of OPG in regulating osteoclast activity to mitigate the clinically significant problem of postorthodontic relapse. This immediate post-mechanically mediated bone modeling period is critical for studying the early biologic phenomenon during orthodontic relapse because of the ongoing high rates of bone turnover and cellular activity as well as the unpredictable response of the treated dentition to the withdrawal of orthodontic forces [6, 13, 16]. For this purpose we modified and validated an established rodent model of orthodontic tooth movement to create a post-tooth-movement relapse model, in which mesial molar movement with an orthodontic spring is followed by relapse in the opposite direction upon appliance removal. We also found that OPG-Fc is highly efficacious in minimizing postorthodontic relapse, with its effects being statistically evident by 12 days postorthodontic tooth movement and the differences in relapse progressively increasing over time such that by 24 days relapse was approximately half in the OPG-Fc groups than in the saline-treated rats. Also, in contrast to the 63% relapse in PBS-treated controls, low-dose and high-dose OPG-Fc-treated animals showed profoundly diminished relapse rates of 31 and 24% of the original movement, respectively, at 24 day after appliance removal. More importantly, when the immediate distal rebound of the teeth in the widened PDL space within 2 days of appliance removal, which is similar in all three groups and therefore not attributable to the OPG-mediated biologic phenomenon, was subtracted from the total relapse, the difference in relapse between the sham control and OPG-treated groups was substantially magnified. Thus, this adjusted percentage relapse of 43% in the PBS-treated group was almost 3.5-fold greater than in the low-dose OPG-Fc group (13%) and 40-fold greater than in the high-dose OPG-Fc group (−1.6%). These findings demonstrate for the first time that OPG-mediated osteoclast activity and bone turnover play a critical role in postorthodontic relapse.

The enhanced postorthodontic stability of the teeth in OPG-Fc- versus PBS-treated rats was accompanied by increased bone regeneration together with a higher quantity and better quality of bone around the roots as evident from micro-CT and histologic analyses. This assessment also revealed several novel facets of post-tooth-movement bone maturation. Thus, immediately post-tooth movement both the quantity of bone (BVF) and the degree of mineralization (TMD) of alveolar bone supporting the molar roots were markedly reduced. This was paralleled histologically by a marked cellular infiltrate and diminished bone levels compared with baseline pre-tooth-movement findings. This indicates that orthodontic tooth movement leads to bone modeling activity, resulting initially in immature alveolar bone formation. Additionally, after 24 days of relapse, the vehicle-treated group showed a partial improvement in BVF but no improvement in TMD compared with the post-tooth-movement values. These findings complement the histologic observations, in which the bone quantity seems to have recovered but likely lacks adequate mineralization, and substantiate the idea that it takes more than 24 days after active tooth movement for newly formed bone to mature and mineralize. In contrast, at 24 days post-appliance removal TMD and BVF in both the low- and high-dose OPG-Fc-treated groups recovered to values similar to those seen pre-tooth movement, indicating that OPG-Fc administration enhances alveolar bone maturation and mineralization after cessation of orthodontic tooth movement.

In addition to the changes in the quality of bone observed by micro-CT at day 24, cross-sectional histologic analysis at 8, 16, and 24 days after appliance removal demonstrated temporal differences in the rates of normalization of bone and periodontal features between the two OPG-Fc-treated groups as well as between these two groups and the PBS-treated control group. Thus, normalization of the bone and PDL occurred in the high-dose and low-dose OPG-Fc-treated groups as early as 8 and 16 days following orthodontic tooth movement, respectively, while these features were just becoming evident at 24 days after appliance removal for the PBS control group. Also, the compression sites had a higher magnitude of cellular infiltration and bony lysis at the end of active tooth movement and a slower resolution of these changes during the relapse phase than the tension sites. Moreover, the continued presence of cellular infiltrate and decreased TMD in saline-treated rats even after 24 days following tooth movement correspond with continued distal movement of the molar and may explain the ongoing relapse in this group throughout the experimental period. This compares with the near normalization of histologic features in the two OPG-treated groups at 8–16 days posttreatment and, consequently, a corresponding significant difference in relapse between the saline- and OPG-Fc-treated groups starting at 12 days post-tooth movement. These findings

taken together indicate that osteoclast activity inhibits bone maturation post-tooth movement and that inhibition of osteoclast activity by local administration of OPG-Fc likely enhances bone maturation, regeneration of the periodontal structures, and diminished cellular infiltrate after orthodontic tooth movement. It is possible that this rapid improvement in bone maturation mediated by OPG-Fc injections could provide a substantial benefit in achieving the desired immediate stability of orthodontic treatment results.

Although currently no other study has examined the process by which OPG contributes to repair or regeneration of intramembranous bone, findings on a model of bone repair in fracture healing which undergoes endochondral ossification provide some insights on the role of OPG in this process. As with orthodontic tooth movement, where RANKL is increased at compression/resorptive sites [47] and OPG levels are elevated at tension/bone formative sites during uncoupled modeling [48], similar observations of temporally distinct phases of RANKL and OPG expression are seen in models of fracture healing. As such, the peak of OPG levels correlates with cartilaginous callus formation and the RANKL peak correlates with remodeling of the callus into bone tissue [37]. Also, chondroclast activity is upregulated in $OPG^{-/-}$ knockout mice, resulting in an increased rate of callus replacement [39]. Conversely, RANKL antibody therapy inhibits the normal sequence of callus remodeling in a mouse long bone fracture model, leading to greater callus size, mineral content, and biomechanical strength [42]. These findings together with ours point to the importance of the OPG/RANKL/RANK axis in distinct models of bone regeneration and maturation during both intramembranous and endochondral bone repair. Our findings also suggest the likelihood that OPG contributes to postorthodontic tooth stabilization by minimizing resorption of immature bone and enhancement of its mineralization following appliance removal.

Besides assessing the local effects of OPG on tooth movement and bone maturation, this study also provides insights on systemic changes in osteoclast activity through serum analyses on TRAP-5b and OPG. Serum TRAP-5b levels were markedly reduced and endogenous OPG concentrations substantially increased immediately post-tooth movement compared with pre-tooth-movement values. These unexpected findings may reflect higher systemic bone turnover present in the younger baseline group compared with that in the 4 week older postorthodontic rats [49]. Serum OPG concentrations gradually decreased in PBS control animals such that at 24 days of relapse these levels returned to those seen prior to tooth movement. Also, serum TRAP-5b levels in PBS-treated rats demonstrated only a partial return to pre-tooth-movement levels over the 24 day relapse phase. These latter results indicate that the body responds to removal of orthodontic appliances by normalizing systemic OPG levels but that this does not immediately result in normalization of systemic osteoclastic activity.

The dramatically increased serum OPG levels and substantially decreased TRAP-5b levels in both OPG-Fc-treated groups compared with vehicle-injected animals at all relapse time points demonstrate that locally injected OPG-Fc protein accesses the systemic circulation and affects systemic osteoclast activity. This suggests that local administration OPG-Fc at the concentrations used in our studies may contribute to a generalized effect on the body. Indeed, this postulate is supported by the profound inhibition of incisor relapse found in both the low-dose and high-dose OPG-Fc groups despite delivery of the protein locally to the first molars. Finally, although high-dose OPG-Fc-injected animals had significantly higher levels of systemic OPG than low-dose animals, both concentrations of OPG-Fc resulted in similarly lowered levels of TRAP-5b in serum and equivalent inhibition of relapse, suggesting a plateauing effect of OPG-Fc in inhibiting osteoclastic activity at OPG-Fc concentrations of 1.0 mg/kg or lower. These findings suggest the need for additional studies to determine the lowest dose of OPG-Fc required for efficacious inhibition of orthodontic relapse.

In conclusion, we have shown for the first time that the regulation of osteoclast activity by the RANK/RANKL/OPG axis immediately following active tooth movement plays a critical role in alveolar bone maturation and postorthodontic stability. While it has previously been shown that modulating osteoclast activity can regulate orthodontic tooth movement, this work provides the first evidence that osteoclasts are also critical for post-tooth-movement relapse and stability. The results of this study also indicate that inhibition of osteoclastogenesis and osteoclast activity by local delivery of recombinant OPG protein could serve as a rational pharmacological approach for enhancing orthodontic retention.

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References

1. Brodie AG (1952) Consideration of musculature in diagnosis, treatment, and retention. Am J Orthod 38:823–835
2. Proffit WR (1978) Equilibrium theory revisited: factors influencing position of the teeth. Angle Orthod 48:175–186
3. Bergstrom K, Jensen R, Martensson B (1973) The effect of superior labial frenectomy in cases with midline diastema. Am J Orthod 63:633–638
4. Reitan K (1951) The initial tissue reaction incident to orthodontic tooth movement as related to the influence of function: an

experimental histologic study on animal and human material. Acta Odontol Scand Suppl 6:1–240
5. Reitan K (1959) Tissue rearrangement during retention of orthodontically rotated teeth. Angle Orthod 29:105–113
6. Reitan K (1969) Principles of retention and avoidance of post-treatment relapse. Am J Orthod 55:776–790
7. Grieve GW (1944) The stability of the treated denture. Am J Orthod Oral Surg 30:171–195
8. Tweed CH (1944) Indications for the extraction of teeth in orthodontic procedure. Am J Orthod 30:405–428
9. Strang RHW (1949) The fallacy of denture expansion as a treatment procedure. Angle Orthod 19:12–22
10. Driscoll-Gilliland J, Buschang PH, Behrents RG (2001) An evaluation of growth and stability in untreated and treated subjects. Am J Orthod Dentofac Orthop 120:588–597
11. Sinclair PM, Little RM (1983) Maturation of untreated normal occlusions. Am J Orthod 83:114–123
12. King GJ, Latta L, Rutenberg J, Ossi A, Keeling SD (1997) Alveolar bone turnover and tooth movement in male rats after removal of orthodontic appliances. Am J Orthod Dentofac Orthop 111:266–275
13. King GJ, Keeling SD, Wronski TJ (1991) Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. Bone 12:401–409
14. Schwarz AM (1932) Tissue changes incident to orthodontic tooth movement. Int J Orthod 18:331–352
15. Stuteville OH (1938) A summary review of tissue changes incident to tooth movement. Angle Orthod 8:1–20
16. Storey E (1973) The nature of tooth movement. Am J Orthod 63:292–314
17. Kobayashi Y, Udagawa N, Takahashi N (2009) Action of RANKL and OPG for osteoclastogenesis. Crit Rev Eukaryot Gene Expr 19:61–72
18. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L (1997) A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390:175–179
19. Grimaud E, Soubigou L, Couillaud S, Coipeau P, Moreau A, Passuti N, Gouin F, Redini F, Heymann D (2003) Receptor activator of nuclear factor kappaB ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. Am J Pathol 163:2021–2031
20. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
21. Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, Van G, Kaufman S, Kostenuik PJ, Lacey DL, Boyle WJ, Simonet WS (2000) Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. J Exp Med 192:463–474
22. Ominsky MS, Li X, Asuncion FJ, Barrero M, Warmington KS, Dwyer D, Stolina M, Geng Z, Grisanti M, Tan HL, Corbin T, McCabe J, Simonet WS, Ke HZ, Kostenuik PJ (2008) RANKL inhibition with osteoprotegerin increases bone strength by improving cortical and trabecular bone architecture in ovariectomized rats. J Bone Miner Res 23:672–682
23. Capparelli C, Morony S, Warmington K, Adamu S, Lacey D, Dunstan CR, Stouch B, Martin S, Kostenuik PJ (2003) Sustained antiresorptive effects after a single treatment with human recombinant osteoprotegerin (OPG): a pharmacodynamic and pharmacokinetic analysis in rats. J Bone Miner Res 18:852–858
24. Kostenuik PJ, Capparelli C, Morony S, Adamu S, Shimamoto G, Shen V, Lacey DL, Dunstan CR (2001) OPG and PTH-(1–34) have additive effects on bone density and mechanical strength in osteopenic ovariectomized rats. Endocrinology 142:4295–4304
25. Li X, Ominsky MS, Stolina M, Warmington KS, Geng Z, Niu QT, Asuncion FJ, Tan HL, Grisanti M, Dwyer D, Adamu S, Ke HZ, Simonet WS, Kostenuik PJ (2009) Increased RANK ligand in bone marrow of orchiectomized rats and prevention of their bone loss by the RANK ligand inhibitor osteoprotegerin. Bone 45:669–676
26. Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR (2001) The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 16:348–360
27. Bekker PJ, Holloway DL, Rasmussen AS, Murphy R, Martin SW, Leese PT, Holmes GB, Dunstan CR, DePaoli AM (2004) A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J Bone Miner Res 19:1059–1066
28. Jin Q, Cirelli JA, Park CH, Sugai JV, Taba M Jr, Kostenuik PJ, Giannobile WV (2007) RANKL inhibition through osteoprotegerin blocks bone loss in experimental periodontitis. J Periodontol 78:1300–1308
29. Dunn MD, Park CH, Kostenuik PJ, Kapila S, Giannobile WV (2007) Local delivery of osteoprotegerin inhibits mechanically mediated bone modeling in orthodontic tooth movement. Bone 41:446–455
30. Kanzaki H, Chiba M, Takahashi I, Haruyama N, Nishimura M, Mitani H (2004) Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement. J Dent Res 83:920–925
31. Kanzaki H, Chiba M, Arai K, Takahashi I, Haruyama N, Nishimura M, Mitani H (2006) Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. Gene Ther 13:678–685
32. King GJ, Fischlschweiger W (1982) The effect of force magnitude on extractable bone resorptive activity and cemental cratering in orthodontic tooth movement. J Dent Res 61:775–779
33. Park CH, Abramson ZR, Taba M Jr, Jin Q, Chang J, Kreider JM, Goldstein SA, Giannobile WV (2007) Three-dimensional micro-computed tomographic imaging of alveolar bone in experimental bone loss or repair. J Periodontol 78:273–281
34. Meganck JA, Kozloff KM, Thornton MM, Broski SM, Goldstein SA (2009) Beam hardening artifacts in micro-computed tomography scanning can be reduced by X-ray beam filtration and the resulting images can be used to accurately measure BMD. Bone 45:1104–1116
35. Sekhavat AR, Mousavizadeh K, Pakshir HR, Aslani FS (2002) Effect of misoprostol, a prostaglandin E_1 analog, on orthodontic tooth movement in rats. Am J Orthod Dentofac Orthop 122: 542–547
36. Body JJ, Greipp P, Coleman RE, Facon T, Geurs F, Fermand JP, Harousseau JL, Lipton A, Mariette X, Williams CD, Nakanishi A, Holloway D, Martin SW, Dunstan CR, Bekker PJ (2003) A phase I study of AMGN-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. Cancer 97:887–892
37. Kon T, Cho TJ, Aizawa T, Yamazaki M, Nooh N, Graves D, Gerstenfeld LC, Einhorn TA (2001) Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. J Bone Miner Res 16:1004–1014
38. Lewiecki EM (2009) Denosumab for joints and bones. Curr Rheumatol Rep 11:196–201
39. Ota N, Takaishi H, Kosaki N, Takito J, Yoda M, Tohmonda T, Kimura T, Okada Y, Yasuda H, Kawaguchi H, Matsumoto M,

Chiba K, Ikegami H, Toyama Y (2009) Accelerated cartilage resorption by chondroclasts during bone fracture healing in osteoprotegerin-deficient mice. Endocrinology 150:4823–4834
40. Smith MR, Saad F, Egerdie B, Szwedowski M, Tammela TL, Ke C, Leder BZ, Goessl C (2009) Effects of denosumab on bone mineral density in men receiving androgen deprivation therapy for prostate cancer. J Urol 182:2670–2675
41. Terpos E, Szydlo R, Apperley JF, Hatjiharissi E, Politou M, Meletis J, Viniou N, Yataganas X, Goldman JM, Rahemtulla A (2003) Soluble receptor activator of nuclear factor kappaB ligand-osteoprotegerin ratio predicts survival in multiple myeloma: proposal for a novel prognostic index. Blood 102: 1064–1069
42. Ulrich-Vinther M, Schwarz EM, Pedersen FS, Soballe K, Andreassen TT (2005) Gene therapy with human osteoprotegerin decreases callus remodeling with limited effects on biomechanical properties. Bone 37:751–758
43. Morony S, Capparelli C, Lee R, Shimamoto G, Boone T, Lacey DL, Dunstan CR (1999) A chimeric form of osteoprotegerin inhibits hypercalcemia and bone resorption induced by IL-1beta, TNF-alpha, PTH, PTHrP, and 1,25$(OH)_2D_3$. J Bone Miner Res 14:1478–1485
44. Kobayashi Y, Hashimoto F, Miyamoto H, Kanaoka K, Miyazaki-Kawashita Y, Nakashima T, Shibata M, Kobayashi K, Kato Y, Sakai H (2000) Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor beta and osteoprotegerin expression. J Bone Miner Res 15:1924–1934
45. Kusumi A, Sakaki H, Kusumi T, Oda M, Narita K, Nakagawa H, Kubota K, Satoh H, Kimura H (2005) Regulation of synthesis of osteoprotegerin and soluble receptor activator of nuclear factor-kappaB ligand in normal human osteoblasts via the p38 mitogen-activated protein kinase pathway by the application of cyclic tensile strain. J Bone Miner Metab 23:373–381
46. Oshiro T, Shiotani A, Shibasaki Y, Sasaki T (2002) Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprotegerin-deficient mice. Anat Rec 266:218–225
47. Yamaguchi M, Aihara N, Kojima T, Kasai K (2006) RANKL increase in compressed periodontal ligament cells from root resorption. J Dent Res 85:751–756
48. Tan L, Ren Y, Wang J, Jiang L, Cheng H, Sandham A, Zhao Z (2009) Osteoprotegerin and ligand of receptor activator of nuclear factor kappaB expression in ovariectomized rats during tooth movement. Angle Orthod 79:292–298
49. Misawa Y, Kageyama T, Moriyama K, Kurihara S, Yagasaki H, Deguchi T, Ozawa H, Sahara N (2007) Effect of age on alveolar bone turnover adjacent to maxillary molar roots in male rats: a histomorphometric study. Arch Oral Biol 52:44–50