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Impact of pharmacologic inhibition of tooth movement on periodontal and tooth root tissues during orthodontic force application

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Abstract

Objective: The goal of this study was to investigate potential negative sequelae of orthodontic force application ±delivery of an osteoclast inhibitor, recombinant osteoprotegerin protein (OPG-Fc), on periodontal tissues.

Setting and Sample Population: Sprague Dawley rats from a commercial supplier were investigated in a laboratory setting.

Materials and Methods: Rats were randomly divided into four groups (n = 7 each): one group with no orthodontic appliances and injected once prior to the experimental period with empty polymer microspheres, one group with orthodontic appliances and injected once with empty microspheres, one group with orthodontic appliances and injected once with polymer microspheres containing 1 mg/kg of OPG-Fc, and one group with orthodontic appliances and injected with non-encapsulated 5 mg/kg of OPG-Fc every 3 days during the experimental period. The animals were euthanized after 28 days of tooth movement for histomorphometric analyses.

Results: Root resorption, PDL area and widths were similar in animals without appliances and animals with appliances plus high-dose OPG-Fc. PDL blood vessels were compressed and decreased in number in all animals that received orthodontic appliances, regardless of OPG-Fc. Hyalinization was significantly increased only in animals with orthodontic appliances plus multiple injections of 5 mg/kg non-encapsulated OPG-Fc when compared to animals without appliances.

Conclusions: Results of this study indicate that while pharmacological modulation of tooth movement through osteoclast inhibition is feasible when delivered in a locally controlled low-dose manner, high-dose levels that completely prevent tooth movement through bone may decrease local blood flow and increase the incidence of hyalinization.

KEYWORDS
periodontal ligament, root resorption, tooth movement, vascularization

1 | INTRODUCTION

Orthodontic tooth movement is possible because bone modelling activity is responsive to the application of mechanical forces to a tooth.[1,2] The biological response to mechanical tooth loading results in bone resorption in areas of compression (areas where the tooth is being moved within the periodontal ligament space towards the bone) and bone formation in areas of tension (areas where the tooth is being moved away from the bone). At the cellular level, tooth movement is mediated by the activity of bone-forming

osteoblasts and bone-resorbing osteoclasts. Uncoupled osteoclast activity (resorption) at the compression sites and osteoblast activity (deposition) at tension sites causes an overall relocation of the tooth socket, allowing the tooth to move through the bone in the direction of force application.[3] Cellular responsive and reparative processes result in the biologic adaptation of the teeth and surrounding periodontal ligament and bone under orthodontic forces.[4,5]

Orthodontic treatment requires the careful and controlled application of forces to relocate teeth into their most ideal functional and aesthetic positions. Accomplishing efficient and effective movement of teeth can be challenging, owing to Newton's third law of motion, which states that for every applied force there is an equal and opposite reactive force. During the course of orthodontic treatment, forces that are reactive to our applied forces are often clinically undesirable. The concept of anchorage refers to methods to control and avoid these unwanted reactive forces. Throughout the history of orthodontics, the management of orthodontic anchorage has relied on strategic positioning of the dental units and use of auxiliary mechanical appliances, both intraoral and extraoral.[6,7] However, in many cases these strategies require patient compliance and/or offer less control than desired, so there is a continuing search for novel and predictable sources of anchorage control.[8-11]

Advancements in the understanding of the cellular process of tooth movement have led to the concept of biological modulation of tooth movement. Specifically, there is potential for a pharmacological approach to inhibit the recruitment and differentiation of bone-resorbing osteoclasts. Osteoclasts are regulated via the nuclear factor kappa B ligand (RANKL)/nuclear factor kappa B (RANK)/osteoprotegerin (OPG) ligand-receptor system.[12,13] That orthodontic tooth movement is mediated by RANKL, and OPG is evidenced by previous findings showing that compressive orthodontic forces increase RANKL expression in rodents and in humans,[14-16] and that manipulation of RANKL or OPG levels can alter the rate of orthodontic tooth movement.[17-19] Pertinent to the current study, previous studies showed that delivery of OPG-Fc (recombinant osteoprotegerin protein) at a 5 mg/kg dose locally injected every few days during tooth movement completely inhibited tooth movement beyond the original tooth socket for 28 days.[19,20] In contrast, a single local injection of 1 mg/kg OPF-Fc when encapsulated in polymer microspheres locally inhibited tooth movement by 26% and enhanced anchorage by 40% when compared to the tooth movement seen in control animals.[20] While this bone catabolic approach may provide the ability to pharmacologically control the movement of specific teeth, the impact of interrupting the biological process of osteoclast recruitment and activation while applying orthodontic forces to teeth is unclear. In this study, we sought to directly determine if pharmacological anchorage with OPG-Fc leads to negative sequelae such as damage to the tooth roots and/or PDL structure utilizing tissues from a previous OPG-Fc tooth movement study.[20]

2 | MATERIALS AND METHODS

2.1 | Animals and orthodontic appliance

As described previously,[20] thirty-two male Sprague Dawley rats weighing approximately 360 g were randomly divided into four groups (n = 7 per group). The rats were housed with a 12-hour light and dark cycle and were fed a diet consisting of standard powdered rat chow (Harlan Laboratories) plus distilled water ad libitum. All animals were acclimatized for at least 3 days before the start of the experiment. In this study, we used tissues from a group that received twice-weekly injections of high-dose 5.0 mg/kg non-encapsulated rat OPG-Fc (Amgen Inc) plus orthodontic appliances, a group that received a single injection of 1.0 mg/kg OPG-Fc encapsulated in PLGA polymer microspheres plus orthodontic appliances, a group that received a single injection of empty PLGA polymer microspheres plus orthodontic appliances, and a negative control group had no orthodontic appliances and received a single injection of empty PLGA polymer microspheres. Injections were administered into the palatal mucosa adjacent to the mesial surface of the maxillary first molar teeth using 33-gauge microneedles 1 day prior to appliance placement. Animals were euthanized after 28 days of tooth movement. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

2.2 | Appliance placement

As previously described,[3,19,20] mesial force was delivered to the maxillary first molars by ligation of closed coil nickel-titanium springs calibrated to provide 25 cN of force between the maxillary first molar and ipsilateral maxillary central incisor with 0.010-inch stainless steel ligatures and composite. Mandibular incisors were reduced weekly to ensure that they were out of occlusion and not at risk of breaking the springs. Loose springs were repaired, and springs were adjusted as needed to accommodate for continuing maxillary incisors eruption.

2.3 | Measurement of tooth movement

As previously reported, tooth movement was measured every 7 days by stone models made from polyvinylsiloxane impressions.[20] Models were scanned adjacent to a 100-mm ruler then magnified and measured using Adobe Photoshop software. Molar mesial movement was measured from the distal groove of the maxillary first molar to the distal surface of the maxillary third molar. Incisor distal movement was measured from the facial surface of the maxillary incisor at the gingival margin to the distal surface of the maxillary third molar.

2.4 | Biopsy harvest and histological preparation

Following euthanasia, hemi-maxillae were dissected, fixed in 10% formalin then decalcified in 10% EDTA. After confirmation of decalcification by radiographic imaging (Faxitron), samples were serial dehydrated then paraffin-embedded. Six-micrometre axial sections were taken from the coronal third of the root (within 300 μm apical

to the furcation) to enable visualization of all five first molar roots as well as their pulp chambers and the inter-radicular alveolar bone investing the roots. This allowed simultaneous visualization and comparison of treatment effects on tooth root structure, PDL and surrounding bone in all roots of the maxillary first molar.

2.5 | Descriptive histology

Sections were stained with haematoxylin and eosin (H&E) for descriptive histology. Microscopy and imaging focused on identifying the presence and location of root resorption, presence and location of hyalinized tissue, presence and morphology of blood vessels, presence and location of PDL disruption, description of inter-radicular bone quality and characterization of the PDL.

2.6 | Histomorphometry

Further analysis of H&E-stained sections by histomorphometry was performed using ImageJ software (National Institutes of Health) to quantify the root area, the area of root resorption, the root width, the depth of resorption lacunae (resorption width), the area and width of the PDL, the number of PDL blood vessels and the incidence of hyalinization. Roots that were conjoined or otherwise undefined were excluded. Three separate sections from each hemi-maxilla were analysed and then averaged per animal for comparison across groups for each analysis.

Smaller roots are more prone to resorption because they have diminished surface area over which to dissipate applied mechanical forces.[21,22] Therefore, to clarify the greatest potential level of damage sustained by the tooth and surrounding tissues, we chose to examine the roots previously shown to most clearly display the impact of orthodontic force: the buccal/intermediate roots (B) and distobuccal (DB) roots of the maxillary first molar.

To measure the area of the cementum and dentin, the five roots of the maxillary first molar were outlined. If any root resorption had occurred, the original shape of the root was also outlined based on the by estimation. Difference in the area between the original outline and current outline was calculated to determine the total area of root resorption. Furthermore, at the worst area of resorption, the depth of resorption was measured by taking a linear measurement at the deepest area of resorption and this was calculated as a percentage of the average root width in an area of the root without resorption. The area of the PDL was measured by outlining the external edge of the PDL and subtracting the total root area. The width of the PDL was measured by taking the average of three linear measurements from the mesial/compression and distal/tension sides of the PDL. Blood vessels were quantified in the PDL space. Necrotic areas of tissue within the PDL were identified as hyalinization and counted per incidence. (Figure S1).

2.7 | Immunohistochemical quantification of odontoclasts

To quantify odontoclast cells (TRAP-positive cells on root surfaces), tartrate-resistant acid phosphatase (TRAP5b) immunohistochemical staining was performed. Deparaffinized sections were permeabilized in 0.025% Triton X-100, blocked with 1% BSA, then incubated with Trap5b primary antibody (Abcam, ab181468). Sections were stained using horseradish peroxidase-conjugated secondary antibody and a colorimetric substrate (3-amino-9-ethylcarbazole) plus toluidine blue counterstain. Multinucleated, TRAP-5b-positive cells were quantified along mesial and distal root surfaces of the distobuccal and buccal roots of the maxillary first molar.

2.8 | Statistical analysis

Primary outcomes were quantitative measurements of root resorption, PDL area and widths, blood vessel numbers and incidence of hyalinization. Secondary outcomes were qualitative analysis of alveolar bone. Descriptive statistics (mean, standard deviation) for each parameter were calculated for all measurements. Comparisons between groups were made using ANOVA followed by post hoc Student's *t* tests and a Bonferroni correction for multiple comparisons. Statistical significance was established as $P < .05$ ($P < .0125$ with Bonferroni correction). Intra-examiner and inter-examiner errors for the measurements of pulp area, root area (cementum and dentin), root area resorbed, root width (cementum and dentin), root width resorbed, PDL area and PDL width were assessed by two repeat measurements taken on 25% of the samples 2 months apart. Pearson's correlation coefficient analysis demonstrated high average intra-examiner ($r = .99$) and inter-examiner ($r = .99$) reliability. Additionally, because we were surprised to see minimal root resorption in animals with appliances plus multiple injections of 5 mg/kg OPG-Fc, to directly investigate the contribution of tooth movement amount to the root resorption data, linear regressions were derived using previously published measurements of mesial first molar tooth movement.[20]

3 | RESULTS

3.1 | Inter-radicular alveolar bone

Histologic staining revealed differences in the inter-radicular alveolar bone of the four groups (Figure 1). In the group without tooth movement, the inter-radicular alveolar bone displayed organized medullary spaces with well-defined and predominant boney trabeculae and invested the tooth roots. In contrast, the groups with the tooth movement through bone (single injection of empty microspheres or microsphere-encapsulated 1 mg/kg OPG-Fc), the bone contained reduced, unorganized medullary spaces with fewer defined boney trabeculae, a greater proportion of connective tissue and did not invest the tooth roots. Further, in the group that received multiple injections of non-encapsulated 5 mg/kg OPG-Fc, the inter-radicular alveolar bone displayed smaller but fairly organized medullary spaces with well-defined and prominent boney trabeculae and did invest the tooth roots. Overall, the inter-radicular bone appears similar in animals with no orthodontic appliances and in animals that had orthodontic appliances plus multiple injections of high-dose OPG-Fc. Both of these

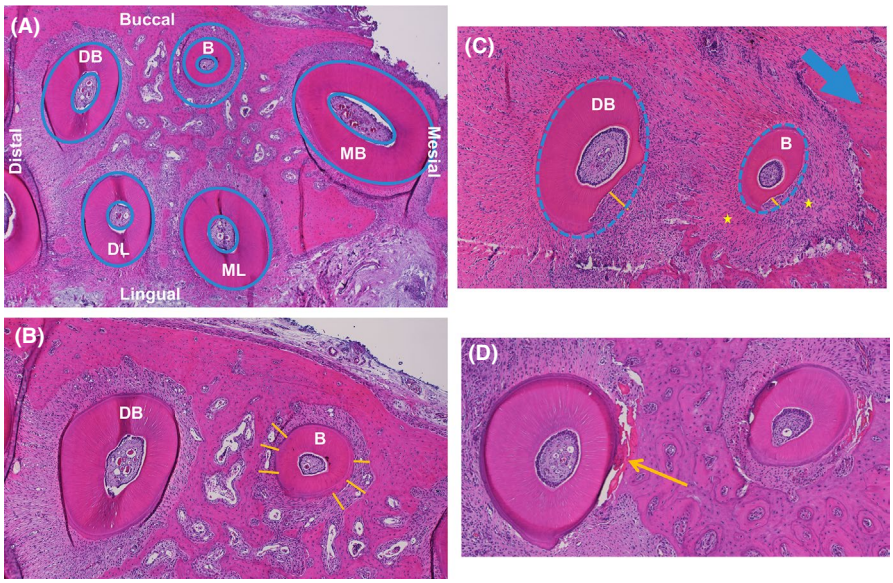

FIGURE 1 Qualitative analysis of alveolar bone, tooth and periodontal ligament tissues. Representative axial sections of five roots of the maxillary first molar tooth are shown in animals with no appliances injected once with empty microspheres (A, no orthodontic force application and no OPG-Fc), with appliances and injected once with empty microspheres (B, orthodontic force application with no OPG-Fc), with appliances and injected every 3 d with non-encapsulated 5 mg/kg OPG-Fc (C, orthodontic force application with high-dose OPG-Fc), with appliances and injected once with microsphere-encapsulated 1 mg/kg OPG-Fc (D, orthodontic force application with low-dose local OPG-Fc) [Colour figure can be viewed at wileyonlinelibrary.com]

groups had no tooth movement beyond the original tooth socket. In addition, the inter-radicular bone appears similar in animals with orthodontic appliances and a single injection of empty spheres and in animals that had orthodontic appliances plus a single injection of spheres containing 1 mg/kg OPG-Fc. Both of these groups had tooth movement beyond the constraints of the original tooth socket.

3.2 | Tooth roots and resorption

Area and depth of root resorption was calculated for the distobuccal (DB) and buccal (B) roots (Table 1). Root resorption area on both DB and B roots was significantly greater in animals with tooth movement through the bone (appliances plus single injection of empty microspheres or microsphere-encapsulated 1 mg/kg OPG-Fc; $P < .005$ vs no appliances or appliances plus 5 mg/kg OPG-Fc). Root resorption depth on DB roots was also significantly greater in animals with tooth movement through the bone (appliances plus single injection of empty microspheres or microsphere-encapsulated 1 mg/kg OPG-Fc; $P < .005$ vs no appliances or appliances plus 5 mg/kg OPG-Fc). Depth of root resorption on B roots was significantly greater in animals with appliances plus the single injection of microsphere-encapsulated 1 mg/kg OPG-Fc; $P < .005$ vs no appliances or appliances plus non-encapsulated 5 mg/kg OPG-Fc). We also compared the depth of root resorption with

TABLE 1 Root resorption of maxillary first molar distobuccal (DB) and buccal (B) roots

	DB root resorption area/total root area (%)	B root resorption area/total root area (%)	DB root mesial width resorbed/total mesial root width (%)	B root mesial width resorbed/total mesial root width (%)
No Appliances Empty Microspheres	.01 ± .02[b,c]	.05 ± .0[c]	.03 ± .07[b,c]	0 ± 0[c]
+AppliancesEmpty Microspheres	.13 ± .06[a]	.14 ± .07	.49 ± .28[a]	.23 ± .25[a]
+Appliances Encapsulated 1 mg/kg OPG	.11 ± .05[a]	.24 ± .09[a]	.53 ± .09[a]	.55 ± .12[a,c]
+Appliances Non-Encapsulated 5 mg/kg OPG	.01 ± .02[b,c]	.02 ± .04[b,c]	0 ± 0[c]	0 ± 0[c]

[a]Indicates statistical significance when compared to without appliances + empty spheres ($P < .05$).
[b]Indicates statistical significance when compared to with appliances + empty spheres ($P < .05$).
[c]Indicates statistical significance when compared to with appliances + OPG spheres ($P < .05$).

the amount of tooth movement by linear regression and found that resorption depth of the distobuccal root significantly correlated by 0.71 ($P < .001$) and of the buccal root by 0.43 ($P < .001$) to amount of tooth movement (Figure S2).

3.3 | Odontoclast numbers

Trap5b-positive multinucleated cells were quantified on mesial and distal tooth root surfaces to determine effects of tooth movement and/or delivered drug on odontoclastic cell numbers (Table 2). Odontoclasts were identified on the distal but not mesial root surface in animals that did not receive orthodontic appliances. In contrast, odontoclasts were identified on the mesial but not distal root surface of all animals that had orthodontic appliances, regardless of drug delivery. On the distobuccal root, we found significantly fewer osteoclasts in animals that received multiple injections of 5 mg/kg OPG-Fc, as compared to those with appliances and empty microspheres, indicative of successful osteoclast inhibition in the high-dose OPG-Fc animals. On the buccal root, we found significantly more osteoclasts in animals that received appliances plus a single injection of 1 mg/kg OPG-Fc, as compared to animal with appliances and empty spheres or appliances with multiple injections of 5 mg/kg OPG-Fc, indicative of successful osteoclast inhibition in the high-dose OPG-Fc animals but not in the low-dose single injection microsphere-encapsulated OPG-Fc animals on this smaller root. It is important to note here that these quantifications were performed after 28 days of tooth movement and no earlier time points were assessed. Osteoclast and odontoclast locations and numbers change dependent upon the stage of tooth movement[3,23,24]; therefore, in future studies it will be important to assess clastic cells and other parameters over time.

3.4 | Periodontal ligament structure

PDL area as well as mesial and distal PDL widths of the buccal (B) roots were measured (Table 3). The PDL widened and became less defined with tooth movement, such that measurements could not be taken on animals that received orthodontic appliances and a single injection of empty microspheres (these animals had the greatest amount of tooth movement). PDL total area increased significantly in animals with orthodontic appliances plus microsphere-encapsulated 1 mg/kg OPG-Fc group as compared to animals with no orthodontic appliances and empty microsphere injection ($P < .005$). These animals had tooth movement with partial anchorage. PDL mesial width was significantly greater in animals with orthodontic appliances and a single injection of 1 mg/kg OPG-Fc than in animals with no orthodontic appliances ($P < .01$) or with orthodontic appliances and multiple injections of non-encapsulated 5 mg/kg OPG-Fc ($P < .01$). No significant difference was seen between any of the groups for PDL distal widths. PDL area and widths of animals with orthodontic appliances and multiple injections of 5 mg/kg OPG-Fc were similar to those seen in animals without orthodontic appliance.

TABLE 2 Root surface odontoclast numbers of maxillary first molar distobuccal (DB) and buccal (B) roots

	DB root number of osteoclasts per mesial surface	DB root number of osteoclasts per distal surface	B root number of osteoclasts per mesial surface	B root number of osteoclasts per distal surface
No Appliances Empty Microspheres	0	5.5 ± 9	0	6.9 ± 6.5
+Appliances Empty Microspheres	15.3 ± 6.1	0	3.6 ± 6.8	0
+Appliances Encapsulated 1 mg/kg OPG	11.9 ± 9.9	0	17.7 ± 12.2[a,b]	0
+Appliances Non-Encapsulated 5 mg/kg OPG	3.0 ± 3.4[b]	0	3.1 ± 3.1	0

[a]Indicates statistical significance when compared to without appliances + empty spheres ($P < .05$)
[b]Indicates statistical significance when compared to with appliances + empty spheres ($P < .05$).

TABLE 3 PDL width and area of maxillary first molar buccal (B) root

	B root total PDL area (μm^2)	B root mesial PDL width (μm)	B root distal PDL width (μm)	B root mesial + distal PDL width (μm)
No Appliances Empty Microspheres	.35 ± .05[b]	.14 ± .05	.10 ± .02	.24 ± .04[b]
+AppliancesEmpty Microspheres	Undefinable	Undefinable	Undefinable	Undefinable
+Appliances Encapsulated 1 mg/kg OPG	.44 ± .01[a]	.21 ± .05	.16 ± .04	.37 ± .02[a]
+Appliances Non-Encapsulated 5 mg/kg OPG	.37 ± .08	.10 ± .03[b]	.13 ± .05	.24 ± .07[b]

[a]Indicates statistical significance when compared to without appliances + empty spheres ($P < .05$).
[b]Indicates statistical significance when compared to with appliances + OPG spheres ($P < .05$).

TABLE 4 Hyalinization adjacent to maxillary first molar distobuccal (DB) and buccal (B) roots

	Hyalinization present?	Per cent hyalinizationin animal group	*P* value vs no appliances + empty spheres	*P* value vs appliances + empty spheres
No Appliances Empty Microspheres	no	0	ns	ns
+Appliances Empty Microspheres	yes	25	ns	ns
+Appliances Encapsulated 1 mg/kg OPG	yes	50	ns	ns
+ Appliances Non-Encapsulated 5 mg/kg OPG	yes	62.5	$P < .05$	ns

3.5 | Blood vessels

Blood vessels in the total periodontal ligament space of the representative distobuccal (DB) and buccal (B) roots were qualitatively assessed and counted in the entire PDL space (Table 4). Blood vessel compression and the number of blood vessels were decreased in animals that received appliances and empty microspheres (tooth movement), animals that received orthodontic appliances and a single injection of microsphere 1 mg/kg OPG-Fc (tooth movement with partial pharmacologic anchorage) and in animals that received orthodontic appliances and multiple injections of 5 mg/kg OPG-Fc (tooth movement with complete pharmacologic anchorage beyond tooth socket), as compared to those animals that had no orthodontic appliances and empty spheres (no tooth movement) at $P < .005$.

3.6 | Hyalinization

Necrotic/hyalinized tissue was observed only in animals with orthodontic appliances (Table 5). A significantly greater incidence of hyalinization was seen in animals that received multiple injections of 5 mg/kg non-encapsulated OPG-Fc as compared to animals without orthodontic appliances (Fishers exact test, $P < .05$).

4 | DISCUSSION

When orthodontic forces exceed the adaptive capacity of the affected tissues, periodontal tissue compression and diminished blood flow may cause cell death, tissue hyalinization and external root resorption.[25,26] Previous preclinical studies showed that pharmacologic inhibition of tooth movement for anchorage by inhibition of osteoclastogenesis is possible,[18-20] yet side effects of continued physical force on the tooth without reparative resolution by osteoclastic bone resorption and migration of the tooth beyond the original tooth socket are unknown. In this study, we sought to determine if pharmacologic inhibition of osteoclastogenesis and tooth movement through bone leads to damage of tooth roots, the periodontal ligament (PDL) and/or alveolar bone by closely examining and measuring tissue sections from a previous rat study of tooth movement with/without controlled low-dose or uncontrolled high-dose recombinant osteoprotegerin protein (OPG-Fc) delivery.[20] Here we compared animals without orthodontic appliances or OPG-Fc (no tooth movement, no drug) with animals that had orthodontic appliances and no OPG-Fc (tooth movement, no drug/no pharmacologic anchorage), animals that had orthodontic appliances and a single injection of 1 mg/kg microsphere-encapsulated OPG-Fc (tooth movement, controlled low-dose drug/partial anchorage) and animals that had orthodontic appliances and multiple injections of non-encapsulated 5 mg/kg OPG-Fc (tooth movement, uncontrolled high-dose drug/complete anchorage beyond original tooth socket).

For some parameters, we found similarity between animals with appliances and high-dose OPG-Fc (complete inhibition of tooth movement through bone) and animals that had no orthodontic appliances (no tooth movement). By histology, alveolar bone appeared similar in these two groups and invested roots of the maxillary first molar, albeit smaller medullary spaces were noted in the animals that had appliances and high-dose drug. The finding of smaller medullary spaces is

TABLE 5 Blood vessels in PDL of maxillary first molar distobuccal (DB) and buccal (B) roots

	PDL Blood vessel appearance	DB root PDL blood vessels	B root PDL blood vessels
No Appliances Empty Microspheres	Normal	$35 \pm 10^{b,c}$	$21 \pm 4^{b,c}$
+AppliancesEmpty Microspheres	Compressed	16 ± 7^{a}	8 ± 4^{a}
+Appliances Encapsulated 1 mg/kg OPG	Compressed	14 ± 5^{a}	13 ± 7^{a}
+ Appliances Non-Encapsulated 5 mg/kg OPG	Compressed	16 ± 3^{a}	7 ± 3^{a}

[a]Indicates statistical significance when compared to without appliances + empty spheres ($P < .05$).
[b]Indicates statistical significance when compared to with appliances + empty spheres ($P < .05$).
[c]Indicates statistical significance when compared to with appliances + OPG spheres ($P < .05$).

consistent with our previous study that used micro-CT to show that alveolar bone in animals with appliances and high-dose OPG-Fc had higher alveolar bone volume and mineral content than animals without tooth movement[20] and numerous other prior findings that showed systemic treatment with OPG-Fc increases bone quantity and quality, including that associated with changing mechanical loads.[27-29] In contrast, inter-radicular alveolar bone was diminished and did not always invest molar tooth roots in animals with tooth movement through bone, regardless of delivered OPG-Fc. Root resorption was similar in animals with appliances and high-dose OPG-Fc and animals that had no orthodontic appliances. Root resorption was increased only in animals with orthodontic appliances and no drug or low-dose microsphere-controlled OPG-Fc (tooth movement with/without partial pharmacologic anchorage). Odontoclast numbers followed a similar pattern except for the fact that no odontoclasts were seen on the mesial surface of animals with no orthodontic appliances but a few odontoclasts were identified on the mesial surface of animals that had appliances plus multiple injections of 5 mg/kg OPG-Fc. Notably, odontoclasts were identified on the distal surface of tooth roots in animals with no orthodontic appliances, likely indicative of the distal drift that occurs normally in rodents.[30-32] Together, these finding suggest that alveolar bone and root resorption are more strongly correlated with tooth movement than with pharmacologic osteoblast inhibition, which linear regression analyses confirmed.

PDL area and width was not definable due to high alveolar bone loss in animals with appliances and no drug. PDL area was greater in animals with appliances and low-dose microsphere-encapsulated drug than animals without appliances. We interpret this finding to mean that osteoclastic bone resorption to widen the PDL occurs faster and before osteoblastic bone deposition in this rat model of tooth movement. We were surprised to find that PDL area and widths were not different between animals with no orthodontic appliances and animals with orthodontic appliances plus high-dose OPG-Fc/complete drug anchorage. We had anticipated a shift of the tooth within the tooth socket that would be visible via histology and measurable by histomorphometry. It should be noted that our tissue sections were taken after appliance removal and euthanasia, such that a relapse shift within the tooth socket could have occurred in the animals with appliances and complete inhibition of tooth movement through bone. One interpretation of this finding is that osteoclastic bone resorption along the tooth socket wall is required for PDL fibre modelling to occur. While we did not investigate at this level of resolution, it is possible that loss of PDL insertions into alveolar bundle bone requires osteoclast activity and bone resorption.

In contrast to root resorption and PDL area/widths that were similar in animals without orthodontic appliances (no tooth movement) and in animals with appliances plus high-dose uncontrolled OPG-Fc (no tooth movement through bone), vascular and hyalinization differences were seen in animals with appliances plus high-dose OPG-Fc (no tooth movement through bone) when compared to animals without orthodontic appliances (no tooth movement). Those animals that had orthodontic appliances and high-dose OPG-Fc showed compressed blood vessels that were significantly diminished in number, and an increased incidence of hyalinization when compared to animals without orthodontic appliances. These data indicate that despite no differences in PDL area and width and minimal movement of the tooth beyond the original tooth socket, orthodontic force application did effect vascularization and the incidence of hyalinization of PDL tissues in these animals. During experimental tooth movement, it is normal to observe the collapse of blood vessels[4] and our data suggest that force application in combination with pharmacologic inhibition of tooth movement through bone via osteoclast inhibition also leads to this. Reduced vascularization would then lead to increased hyalinization due to diminished blood flow causing cell necrosis. Together, these data suggest that use of osteoclast inhibitors could lead to tissue damage through reduced blood flow. Longer term studies and studies utilizing larger animal models[33,34] will be important to confirm or negate these findings as generalizable and predictable.

It is important to note that we chose to take axial sections in order to visualize all five roots of the maxillary first molar undergoing tooth movement. This led to very low counts of osteo/odontoclasts per section and high variability between groups with orthodontic appliances.[20] In future studies, it will be important to take lateral sections that include the distobuccal and buccal roots to yield more accurate osteoclasts counts per root, as was done in other previous studies.[19] In future studies, it will also be important to obtain data from internal time points, as we may be missing changes that have resolved by the end of the tooth movement period. Hyalinization for example is expected to occur on the pressure side of roots to some degree in all animals undergoing experimental tooth movement.[35] However, studies have suggested that as early as day 9 of induced tooth movement, hyalinized areas have already been resorbed and the periodontal ligament is reorganizing.[36] Earlier and intermediate time points therefore need to be assessed in future studies. Future studies should also investigate utilization of lower force levels.[37]

5 | CONCLUSIONS

Use of osteoclast inhibitors such as OPG-Fc for pharmacologic inhibition of tooth movement to enhance orthodontic anchorage during force application may be limited by tissue necrosis due to limited blood flow to the PDL. Such changes might also be anticipated with use of systemic bone anabolic drugs, such as anti-sclerostin antibody, that have been chose to inhibit tooth movement by inhibiting production of RANKL and osteoclastogenesis.[38] Effects of other biologic mediators of tooth movement are unknown and should be carefully studied in preclinical models for assessment of negative sequelae before translating to patient care.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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