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Original article

Microsphere controlled drug delivery for local control of tooth movement

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Summary

Background: Because orthodontic tooth movement is dependent upon osteoclast-mediated resorption of alveolar bone adjacent to the pressure side of tooth roots, biologic mediators that regulate osteoclasts can be utilized to control tooth movement.
Objectives: To develop a novel method to locally enhance orthodontic anchorage.
Methods: We encapsulated osteoprotegerin (OPG) in polymer microspheres and tested the effectiveness of microsphere encapsulated versus non-encapsulated OPG for enhancing orthodontic anchorage in a rodent model of tooth movement. A single injection of 1 mg/kg non-encapsulated or microsphere encapsulated OPG was delivered into the palatal mucosa mesial to the first maxillary molar 1 day prior to tooth movement. A positive control group received injections of 5 mg/kg non-encapsulated OPG every 3 days during tooth movement. After 28 days of tooth movement, hemi-maxillae and femurs were dissected. Molar mesial and incisor distal tooth movement was measured using stone casts that were scanned and magnified. Local alveolar, distant femur bone, and tooth root volumes were analyzed by micro computed tomography. Serum OPG levels were measured by ELISA. Osteoclast numbers were quantified by histomorphometry.
Results: The single injection of microsphere encapsulated OPG significantly enhanced orthodontic anchorage, while the single injection of non-encapsulated OPG did not. Injection of encapsulated OPG inhibited molar mesial movement but did not inhibit incisor tooth movement, and did not alter alveolar or femur bone volume fraction, density, or mineral content. Multiple injections of 5 mg/kg non-encapsulated OPG enhanced orthodontic anchorage, but also inhibited incisor retraction and altered alveolar and femur bone quality parameters. Increased OPG levels were found only in animals receiving multiple injections of non-encapsulated 5 mg/kg OPG. Osteoclast numbers were higher upon tooth movement in animals that did not receive OPG. Osteoclast numbers in OPG injected animals were variable within groups.
Conclusions: Microsphere encapsulation of OPG allows for controlled drug release, and enhances site-specific orthodontic anchorage without systemic side effects. With additional refinements, this drug delivery system could be applicable to a broad array of potential biologic orthodontic therapeutics.

Introduction

Orthodontic treatment involves the carefully controlled application of mechanical forces to teeth to obtain an optimal occlusal relationship. Because no fixed intraoral anatomical anchor exists, every applied orthodontic force will cause a counter-action of equal force, which is often accompanied by undesirable tooth movement. Orthodontic anchorage refers to methods for inhibiting these unwanted tooth movements. In practice, orthodontists currently utilize a variety of intraoral and extraoral mechanical methods to enhance orthodontic anchorage and prevent unfavourable tooth movement. Given that tooth movement is regulated at the cellular level by osteoclast activity, biological regulation of osteoclastogenesis may offer a viable option for modulating orthodontic tooth movement and improving anchorage control during orthodontic treatment.

Osteoclasts are regulated via the nuclear factor kappa B ligand (RANKL)/nuclear factor kappa B (RANK)/osteoprotegerin (OPG) ligand-receptor system (1, 2). In humans, injection with recombinant OPG or a monoclonal antibody to RANKL decreases serum markers of bone resorption, reduces fracture incidence, and increases bone mineral density (3–7). While systemic inhibition of osteoclast activity is beneficial for systemic disorders of bone, local inhibition of osteoclasts through controlled delivery of RANKL inhibitors would be useful for situations in which inhibition of bone resorption is desirable at specified locations, such as enhancement of orthodontic anchorage, or treatment of localized osteolytic disease.

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 (8–11), and that manipulation of RANKL or OPG levels can alter the rate of orthodontic tooth movement (12, 13). Pertinent to the current study, injection of recombinant OPG protein was previously shown to significantly enhance posterior orthodontic anchorage during tooth movement and diminish post-orthodontic relapse after tooth movement in rats (14–16). While the investigation utilizing OPG to improve orthodontic anchorage (14) points to the potential use of osteoclast inhibitors for biologic control of orthodontic tooth movement, the study design did not attempt to limit affects to local tissues and tooth movement overall was inhibited. Here, we took advantage of emerging biomaterial technologies for controlled drug delivery, and developed a system for local release of an osteoclast inhibitor (OPG) for enhancing orthodontic anchorage, without inhibiting desirable tooth movement at distant sites or leading to systemic effects on bone.

Materials and methods

Microsphere encapsulation of OPG

Rat recombinant OPG was loaded into PLGA (poly(lactic-co-glycolic acid)) microspheres using a double emulsion technique (17). To calculate the total amount of loaded OPG, 5 mg of loaded spheres were hydrolyzed in 1M NaOH with shaking at room temperature for 2 h. To establish *in vitro* OPG release kinetics, 5 mg of loaded spheres were dispersed in 1× phosphate buffer solution with shaking at 37°C and the solution was collected at designated time points. The samples were pH neutralized then centrifuged to collect supernatant. OPG amounts were measured using a rat OPG ELISA kit per manufacturer instructions (Mybiosource).

Animals

Forty-two male Sprague Dawley rats weighing approximately 360 g were randomly divided into seven groups (n = 6 per group). Rats were housed with a 12-h light and dark cycle, fed standard rat chow, and distilled water *ad libitum*. Three groups had no orthodontic appliances and received a single dose of empty microspheres, 1 mg/kg microsphere encapsulated OPG, or 1 mg/kg non-encapsulated OPG. Three groups had orthodontic appliances and received a single dose of empty microspheres, 1 mg/kg microsphere encapsulated OPG, or 1 mg/kg non-encapsulated OPG. One additional positive control group had orthodontic appliances and received multiple doses of 5 mg/kg non-encapsulated OPG, every 3 days throughout the tooth movement period. Doses of recombinant rat OPG used were based upon previously published results (14, 15) showing that injection of 5 mg/kg non-encapsulated OPG inhibited molar and incisor tooth movement and entered the systemic circulation, and in preliminary studies which showed that a single injection of non-encapsulated 1 mg/kg OPG did not inhibit tooth movement after 28 days of orthodontic force application. All injections were administered into the mucosa adjacent to the mesial surface of the first maxillary molar on experimental day 0, prior to placing orthodontic appliances. All procedures were approved by the University Committee on Use and Care of Animals.

Tooth movement model

Orthodontic forces were applied for 28 days using a previously established rodent model of orthodontic tooth movement (14, 18). Animals were placed under inhalational anesthesia with 3 per cent isoflurane for the placement of the orthodontic appliance. A nickel-titanium spring calibrated to provide 25 g of force was ligated from the maxillary first molar to the ipsilateral incisor. Mandibular incisors were reduced weekly to diminish appliance breakage. During the course of the experiment, loose springs were repaired as needed and appliances were readjusted as needed due to maxillary incisor eruption. Stone models made from polyvinylsiloxane impressions were scanned at 1200 dpi and magnified 300× using *Adobe Photoshop* software to measure tooth movement (14). 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.

Serum analysis

Serum was drawn from the lateral tail vein. A commercially available kit (MyBioSource) was used to quantify circulating levels of OPG at days 0, 1, 14, and 28.

Micro computed tomography

To quantify local alveolar and distant long bone changes, dissected hemi-maxillae and femurs were fixed and transferred to ethanol, then scanned at an 18 µm resolution. Analyses were conducted using *Microview version 2.2* software (GE Healthcare Pre-Clinical Imaging) and established algorithms (19, 20). Alveolar bone was analyzed in the maxillary first molar furcation region according to a previously established protocol (15, 21). Cortical long bone analysis was performed at the mid-diaphyseal shaft and trabecular long bone analysis was performed at the proximal metaphysis of the femur.

To quantify root resorption, tooth root volumes were quantified by micro CT. Individual maxillary molar roots were outlined. Individual tooth root volumes were calculated and summed for each tooth, to assess the total root loss for each tooth.

Histomorphometry

To quantify osteoclast numbers, dissected hemi-maxillae were fixed in 4 per cent paraformaldehyde, decalcified in EDTA then embedded

in paraffin. About 6 um axial sections containing the five roots of the maxillary first molar were stained by immunohistochemistry. Briefly, sections were permeabilized in 0.025 per cent Triton X-100, blocked with 1 per cent 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 counter stain. Multinucleated trap5b positive cells were counted within intra-radicular bone of the maxillary first molar (n = 8 per group).

Statistical analyses

Descriptive statistics (mean, standard deviation) were calculated for all measurements in all animals. Comparisons between groups were made using analysis of variance (ANOVA). Differences with $P < 0.05$ were considered to be significant.

Results

Microsphere release kinetics

Approximately 96 of 167 µg (57%) of OPG from the PLGA 75–25 and approximately 131 of 167 µg (78%) from PLGA 50–50 microspheres was released within 1800 h of encapsulation (Figure 1). The release profile of OPG from the PLGA 75–25 microspheres was deemed more suitable for sustained release of OPG. Therefore, OPG was encapsulated in 75–25 PLGA microspheres for the *in vivo* study.

Animals

One animal died during the appliance placement procedure due to an adverse reaction to anaesthesia and a replacement rat was used. All other animals tolerated procedures with no discernable effect on health or ability to thrive. There were no significant differences in weight gain among the groups at the end of the experiment.

Tooth movement

Local delivery of a single injection of microsphere encapsulated 1 mg/kg OPG significantly reduced mesial molar tooth movement at days 14, 21, and 28, when compared with the single injection of empty microspheres (Figure 2E). In contrast, there was no significant reduction in molar movement when animals received a single injection of non-encapsulated 1 mg/kg OPG, at any time point. As expected based upon previously published data (14), multiple injections of non-encapsulated 5 mg/kg OPG significantly inhibited molar movement, when compared with the empty microsphere group on days 14, 21, and 28. Multiple local injections of non-encapsulated 5 mg/kg OPG also inhibited tooth movement to a significantly greater extent than a single injection of microsphere encapsulated 1 mg/kg OPC-Fc at days 14, 21, and 28. At day 28, molars in animals receiving a single injection of empty microspheres moved 0.8 ± 0.1 mm, molars in animals receiving a single injection of non-encapsulated 1 mg/ml OPG moved 0.8 ± 0.1 mm (no reduction compared to empty microspheres), molars in animals receiving a single injection of microsphere encapsulated 1 mg/ml OPG moved 0.6 ± 0.1 mm (26% reduction relative to empty microspheres), and molars in animals receiving multiple injections of non-encapsulated 5 mg/ml OPG moved 0.2 ± 0.1 mm (75% reduction relative to single injection of empty microspheres; 67% reduction relative to single injection of microsphere encapsulated 1 mg/kg).

To determine if local injections delivered immediately adjacent to the mesial surface of the first molar tooth had more distant effects on tooth movement, we also measured incisor retraction. There was no reduction in incisor retraction when animals received a single injection of 1 mg/kg encapsulated or non-encapsulated OPG, when compared with animals injected that received empty microspheres (Figure 2F). In contrast, incisor retraction was significantly reduced in animals that received multiple injections of 5 mg/kg non- encapsulated OPG, when compared with any of the other three groups.

In this tooth movement model, orthodontic anchorage refers to the ability to minimize mesial movement of molars without inhibiting distal movement/retraction of incisors. The ratio of incisor to molar tooth movement was not significantly different between animals that were injected with empty microspheres and animals that received a single injection of 1 mg/kg non-encapsulated OPG. In contrast, the ratio of incisor to molar tooth movement was significantly and on average 1.4 times greater in animals that that received a single injection of 1 mg/kg encapsulated OPG, when compared with animals that received empty microspheres. The ratio of incisor to molar tooth movement was significantly and on average 2.3 times greater in animals that received multiple injections of 5 mg/kg non-encapsulated OPG, when compared with animals that received empty microspheres. The ratio of incisor to molar tooth movement was significantly and on average 1.7 times greater in animals that received multiple injections of 5 mg/kg non-encapsulated OPG, when compared with animals that received a single injection of 1 mg/kg non-encapsulated OPG (Figure 2G).

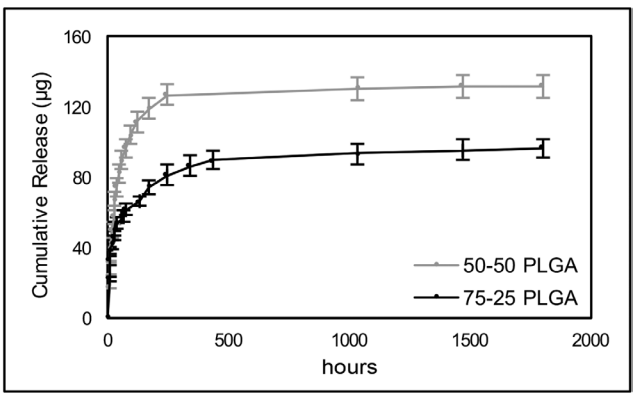

Figure 1. OPG release profile from microspheres. *In vitro* release of OPG from PLGA 50/50 and PLGA 75/25 microspheres was quantified over a 4-week period (n = 3 samples per time point and sphere type).

Serum OPG levels

The only group that showed increased serum OPG was the multiple injection, non-encapsulated 5 mg/kg OPG group (Figure 3). No differences were seen between the groups in serum OPG levels on days 1 and 14 of the experimental period. Serum OPG levels in animals that received a single injection of empty microspheres, non-encapsulated 1 mg/kg OPG or microsphere encapsulated 1 mg/kg OPG were relatively flat throughout the duration of the study. Serum OPG levels in animals that received multiple injections of non-encapsulated 5 mg/kg OPG were statistically greater than that found in any of the three other groups at day 28.

Local alveolar bone micro CT analyses

Bone within the furcation area of the maxillary first molar was analyzed by micro CT to evaluate effects of tooth movement, the locally delivered drug and/or microspheres on local alveolar bone after tooth movement (Table 1). As expected, tooth movement significantly decreased all parameters of alveolar bone quality and quantity when

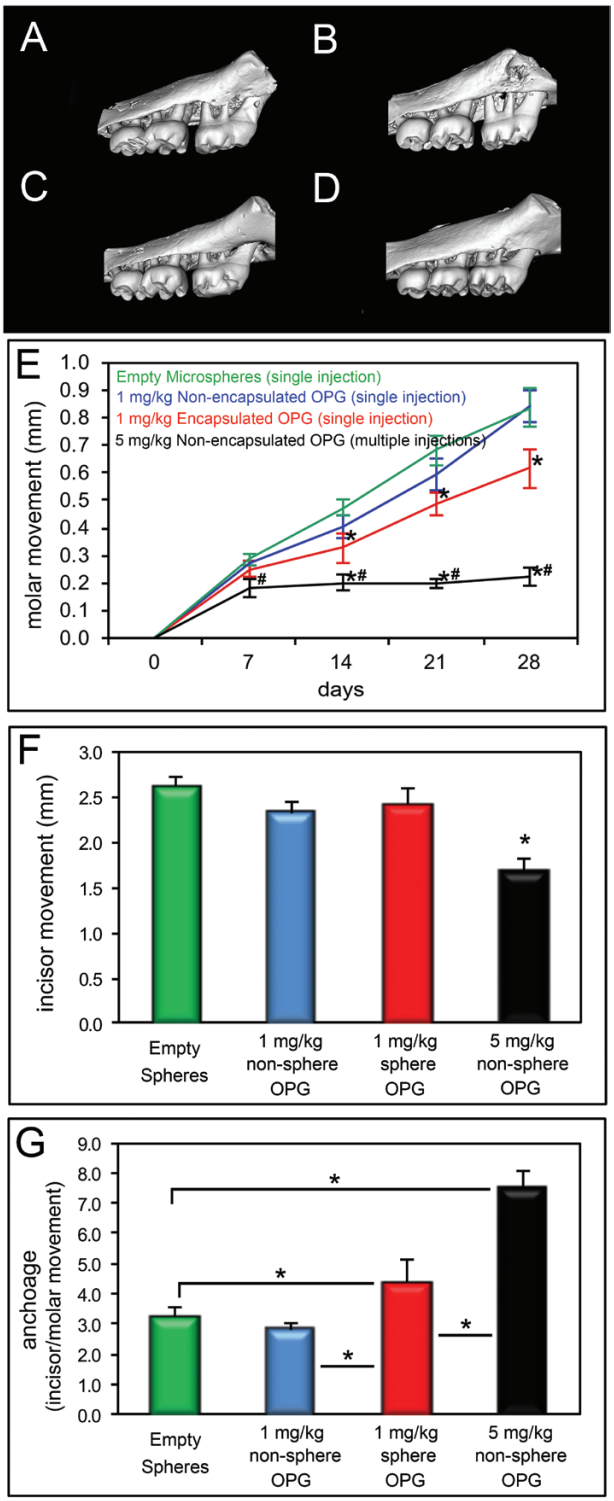

Figure 2. Microsphere encapsulated OPG inhibits molar but not incisor tooth movement. (A–D) Qualitative analysis of molar tooth movement. Micro CT isosurface images of representative samples immediately post tooth movement are shown. (A) A single injection of empty microspheres does not inhibit mesial molar tooth movement. (B) A single injection of non-encapsulated 1 mg/kg OPG does not inhibit mesial molar tooth movement. (C) A single injection of microsphere encapsulated 1 mg/kg OPG inhibits mesial molar tooth movement. (D) Multiple Injections of 5 mg/kg uncoated OPG prevents mesial molar movement. (E)Time course of mesial molar tooth movement over the experimental period. As expected, a single injection of empty microspheres (green) allowed for more than 0.8 mm of mesial molar tooth movement. No significant differences in molar movement were seen in animals that received a single injection of non-encapsulated 1 mg/kg OPG (blue), as compared to empty microsphere injected animals. In contrast, a single injection of microsphere encapsulated 1 mg/kg OPG (red) significantly inhibited molar tooth movement at days 14, 21, and 28. Also as expected, multiple injections of 5 mg/kg non-encapsulated OPG (black) significantly inhibited molar tooth movement at days 14, 21, and 28, as compared to empty microsphere injected animals. Animals that received multiple injections of 5 mg/kg non-encapsulated OPG also showed significantly less molar tooth movement at days 14, 21, and 28, as compared to animals that received a single injection of microsphere encapsulated 1 mg/kg OPG. $^{*}P < 0.05$ versus empty microsphere injected animals. $^{\#}P < 0.05$ versus microsphere encapsulated 1 mg/kg OPG injected animals. (F) Total distal incisor tooth movement. No significant differences in the amount of incisor retraction were found between groups that received a single injection of empty microspheres, a single injection of non-encapsulated 1 mg/kg OPG and a single injection of microsphere encapsulated 1 mg/kg OPG after 28 days of orthodontic tooth movement. Multiple injections of 5 mg/kg OPG significantly inhibited incisor retraction when compared with the control, empty microsphere group. (G) Orthodontic anchorage expressed as incisor distal/molar mesial orthodontic tooth movement at the end of the experimental period. No significant differences in the amount of orthodontic anchorage were found between animals that received a single injection of empty microspheres or a single injection of non-encapsulated 1 mg/kg OPG. Animals that received a single injection of microsphere encapsulated 1 mg/kg OPG had significantly enhanced orthodontic anchorage compared to animals that received a single injection of empty microspheres. Animals that received multiple injections of non-encapsulated 5 mg/kg OPG had significantly enhanced orthodontic anchorage compared to animals that received a single injection of empty microspheres, compared to animals that received a single injection of non-encapsulated 1 mg/kg OPG, and animals that received a single injection of encapsulated 1 mg/kg OPG. Results are expressed means ± standard deviations, $^{*}P < 0.05$ between groups.

compared with animals with no orthodontic appliances. A single injection of non-encapsulated or microsphere encapsulated 1 mg/kg OPG did not alter this effect. Multiple injections of non-encapsulated 5 mg/kg OPG during tooth movement significantly increased all parameters of bone quality and quantity, as compared to animals that were injected singly with empty microspheres, non-encapsulated 1 mg/kg OPG, or microsphere encapsulated 1 mg/kg OPG. No differences were found in quality of intra-radicular bone between animals that were injected singly with empty microspheres, non-encapsulated 1 mg/kg OPG, or microsphere encapsulated 1 mg/kg OPG.

Femur bone micro CT analyses

Cortical bone in the femur mid diaphysis and trabecular bone in the femur distal metaphysis was analyzed by micro CT to evaluate effects of tooth movement, the locally delivered drug and/or microspheres on long bones distant from the site of injection (Supplementary Tables 1 and 2). No differences in femur cortical bone of the mid diaphysis were found between any of the groups regardless of tooth movement, injections or microsphere encapsulation, at the end of tooth movement. In animals that did not undergo tooth movement, the single injection of non-encapsulated 1 mg/kg OPG significantly increased bone volume, trabecular bone volume fraction, trabecular bone surface, trabecular thickness, and trabecular number in the femur distal metaphysis, when compared with animals that did not undergo tooth movement that received a single injection of empty microspheres. The single injection of microsphere encapsulated 1 mg/kg OPG did not alter trabecular bone parameters in animals that did not undergo tooth movement, when compared with animals that received a single injection of empty microspheres. Tooth movement did not alter femur

trabecular bone parameters. In animals undergoing tooth movement, all trabecular bone parameters were significantly different in animals that received multiple injections of 5 mg/kg non-encapsulated OPG, as compared to animals that received a single injection of empty microspheres. Femur trabecular bone volume, volume fraction, bone surface, thickness, and number were significantly increased, while femur trabecular bone spacing was significantly decreased, indicating increased trabecular bone and systemic effects of OPG in these animals. In animals with tooth movement, the single injection of non-encapsulated or microsphere encapsulated 1 mg/kg OPG did not alter trabecular bone parameters, as compared to animals that received a single injection of empty microspheres.

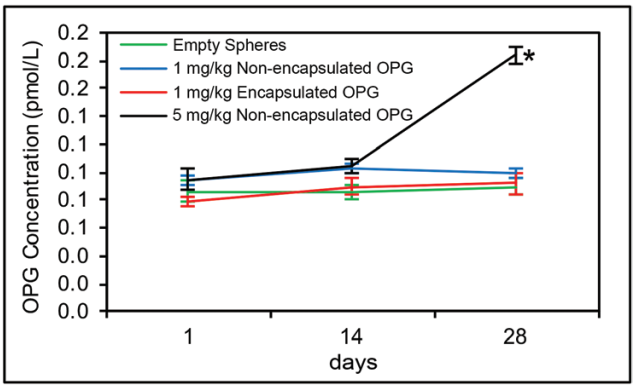

Figure 3. Serum OPG levels. Serum was isolated at indicated time points and OPG was measured by ELISA. No significant differences were found for serum OPG levels in animals that received a single injection of empty microspheres, a single injection of non-encapsulated 1 mg/kg OPG and a single injection of microsphere encapsulated 1 mg/lg OPG. In contrast, multiple injections of 5 mg/kg OPG significantly increased serum OPG levels by day 28, when compared with empty microsphere injected animals. Results are expressed means ± standard deviations, $*P < 0.05$ versus empty microsphere injected animals.

Root resorption

Maxillary first molar tooth root volumes were quantified using micro CT to evaluate effects of tooth movement, the locally delivered drug and/or microspheres on root resorption (Table 2). Trends for increased root resorption were noted in animals that received orthodontic appliances, but no significant differences between groups with versus without appliances were found. In animals that had tooth movement, total root volume was increased in animals that received multiple injections of non-encapsulated 5 mg/kg OPG, as compared to animals that received a single injection of empty microspheres. In animals that had tooth movement, there was no effect of the single injection of non-encapsulated or microsphere encapsulated 1 mg/kg OPG on final root volumes, as compared to animals that received a single injection of empty microspheres.

Osteoclast numbers

Maxillary sections were stained for Trap5b and positive cells were quantified in maxillary first molar intra-radicular alveolar bone to determine effects of tooth movement and/or delivered drug on osteoclast numbers (Figure 4). Osteoclasts numbers were significantly higher in animals with orthodontic appliances and no OPG, as compared to animals without orthodontic appliances. No significant differences were found between animals with orthodontic appliances and either a single dose of encapsulated low dose OPG or multiple injections of non-encapsulated high dose OPG and any other group, primarily due to the high variability found between animals within these two groups. It is worth noting that the variability in animals with orthodontic appliances and multiple injections of non-encapsulated OPG was due to high osteoclast numbers found in one of eight animals (all other animals had very low to no osteoclasts). Variability in animals with orthodontic appliances and a single injection of non-encapsulated OPG was due to high osteoclast numbers found in two of eight animals (all other animals had low to moderate osteoclast numbers).

Table 1. Maxillary molar furcation area bone volume, density, and mineral content. OPG, osteoprotegerin.

	Bone volume (mm^3)	Bone volume fraction	Bone mineral content (mg)	Bone mineral density (mg/cc)	Tissue mineral content (mg)	Tissue mineral density (mg/cc)
No appliances empty microspheres	4.3 ± 0.2	0.66 ± 0.01	5.5 ± 0.2	826 ± 13	4.4 ± 0.2	1013 ± 9
No appliances non-encapsulated 1 mg/kg OPG	4.3 ± 0.1	0.63 ± 0.01	5.7 ± 0.2	846 ± 16	4.5 ± 0.2	1049 ± 14
No appliances encapsulated 1 mg/kg OPG	4.2 ± 0.2	0.63 ± 0.01	5.6 ± 0.3	826 ± 22	4.4 ± 0.3	1028 ± 17
+ Appliances empty microspheres	3.0 ± 0.2*	0.48 ± 0.02*	4.0 ± 0.3*	644 ± 37*	2.8 ± 0.2*	947 ± 24
+ Appliances non-encapsulated 1 mg/kg OPG	3.0 ± 0.2*	0.46 ± 0.03*	4.0 ± 0.2*	598 ± 37*	2.8 ± 0.2*	920 ± 17*
+ Appliances encapsulated 1 mg/kg OPG	3.2 ± 0.2*	0.50 ± 0.02*	4.1 ± 0.3*	639 ± 34*	3.0 ± 03*	919 ± 23*
+ Appliances non-encapsulated 5 mg/kg OPG	4.7 ± 0.2#	0.70 ± 0.01#	6.1 ± 0.3#	900 ± 20#	5.1 ± 0.3#	1076 ± 12#

*Indicates statistical significance when compared with the groups without appliances ($P < 0.05$).

#Indicates statistical significance when compared with empty spheres ($P < 0.05$).

Discussion

Historically the management of orthodontic anchorage to prevent undesirable tooth movement has been predicated on the use of mechanical auxiliary appliances, as well as strategic positioning of the dental units that are not to be moved during treatment, which therefore have the potential to serve as anchorage (22). Yet even with well-designed and utilized orthodontic mechanics, orthodontic anchorage remains a clinical challenge. Because orthodontic tooth movement occurs due to the biological activity of osteoclasts (18, 23), the application of agents that inhibit osteoclasts have the potential to enhance orthodontic anchorage. It was previously shown that recombinant OPG can inhibit molar tooth movement adjacent to the site of injection and enhance orthodontic anchorage when delivered at 5 mg/kg every three days during tooth movement (14). Yet results also showed that when provided at this high dose level, the drug also inhibited incisor tooth movement distant from the site of injection. Injection of recombinant OPG was also previously shown to inhibit relapse of molar and incisor tooth movement after removal of orthodontic appliances when injected distal to the molar tooth at 1 or 5 mg/kg every few days during the relapse period (15). Injection of OPG in this latter study entered the systemic circulation and decreased serum Trap5b levels. Together, these findings demonstrate that bone anticatabolic agents can inhibit tooth movement but must be delivered in a more controlled fashion if they are to be translated into the orthodontic clinic for enhancing orthodontic anchorage without inhibiting overall tooth movement or leading to systemic bone effects.

In this study, we demonstrated that a single injection of polymer microsphere encapsulated OPG at 1 mg/kg inhibits mesial molar tooth movement and enhances orthodontic anchorage for 28 days when provided immediately prior to tooth movement. The microsphere encapsulated OPG injected animals showed 31, 29, and 26 per cent less molar tooth movement when compared with animals that received empty microspheres at days 14, 21, and 28, respectively. Importantly, delivery of OPG via the microspheres did not inhibit incisor retraction. These results indicate that polymer microsphere encapsulation enabled a local effect: inhibition of tooth movement adjacent to the site of injection without inhibition of tooth movement

Table 2. Volumetric tooth root measurements. OPG, osteoprotegerin.

Ortho appliances	OPG dose (mg/kg)	Microsphere encapsulation	Number of injections	Total root volume
No	0	Yes	1	2.5 ± 0.5
No	1	Yes	1	2.7 ± 0.3
No	1	None	1	2.7 ± 0.2
Yes	0	Yes	1	2.3 ± 0.2
Yes	1	Yes	1	2.4 ± 0.3
Yes	1	None	1	2.5 ± 0.3
Yes	5	None	Multiple	2.7 ± 0.3*

No significant differences were found between ± orthodontic appliances.
*Indicates statistical difference compared to empty spheres ($P < 0.05$).

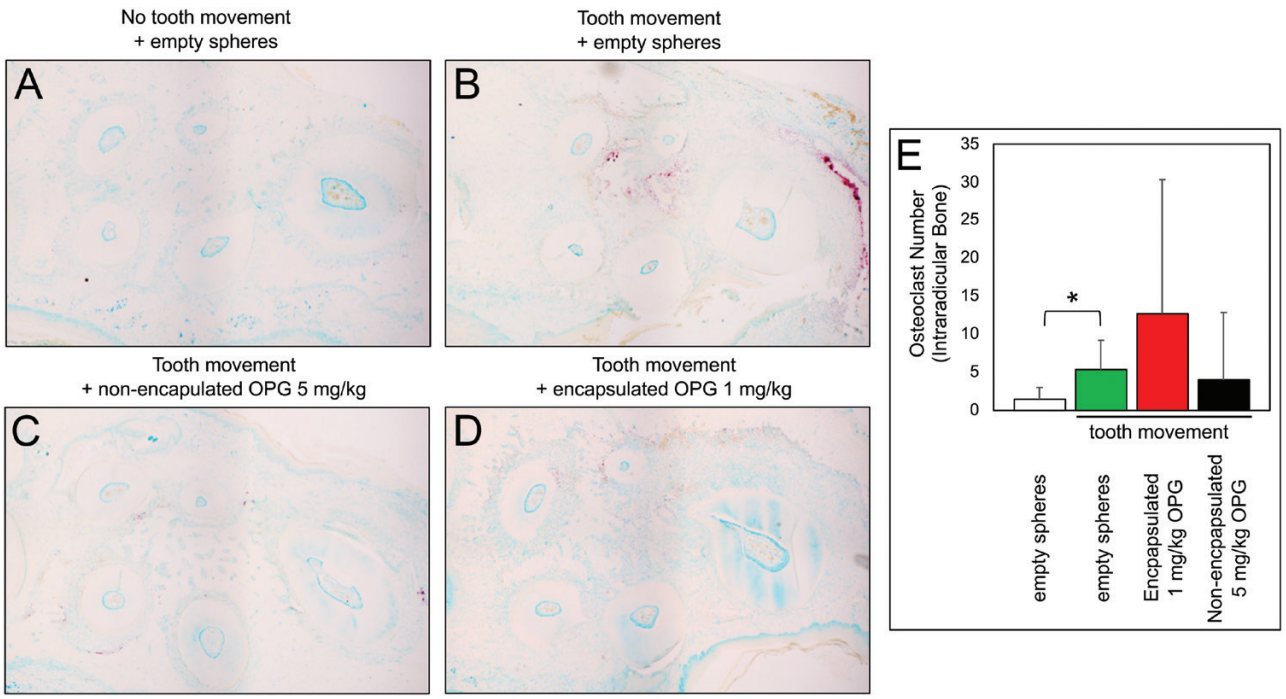

Figure 4. Local tissue osteoclasts. Axial sections of hemi-maxillae at the end of the experimental period were stained for trap5 and multinucleated trap5b positive cells were counted within the intra-radicular bone of the maxillary first molar. (A–D) Axial sections stained for trap5b with toluidine blue counter stain. Note the organized intra-radicular bone with minimal trap5b staining in intra-radicular bone from animals without orthodontic appliances and from animals that received orthodontic appliances plus high dose multiple injections of non-encapsulated OPG. Tissue from animals with orthodontic appliances and no OPG shows less organized intra-radicular bone and strong trap5b staining. Tissue from animals with orthodontic appliances and low dose encapsulated OPG shows moderate levels of trap5b staining. (E) Osteoclast quantification revealed significantly higher numbers of intra-radicular bone osteoclasts in tissue from animals with orthodontic appliances and no OPG, as compared to tissue from animals without orthodontic appliances and no OPG. Osteoclast numbers in tissue from animals with orthodontic appliances and multiple injections of high dose non-encapsulated OPG or a single injection of encapsulated OPG were more variable. $P < 0.05$ versus no tooth movement.

farther away from the site of injection. In contrast, a single dose of non-encapsulated OPG at 1 mg/kg did not inhibit molar or incisor tooth movement. Recombinant OPG protein has a half-life of 6–7 days *in vivo* (24). Increased efficacy and duration of action of OPG for preventing local molar tooth movement upon microsphere encapsulation was likely due to protection from degradation (25, 26).

As expected and consistent with previous reports (14), injection of non-encapsulated OPG at 5 mg/ml given every 3 days during tooth movement inhibited both molar and incisor tooth movement, and appeared to prevent molar tooth movement beyond the original constraints of the tooth socket. While this degree of inhibition of tooth movement may be desirable in some instances, injection of the non-encapsulated drug at this frequency and dose level led to entrance into the systemic circulation and undesirable long bone effects. While femur cortical bone remained unchanged, multiple injections of non-encapsulated 5 mg/kg OPG significantly increased trabecular bone volume, bone volume fraction, bone surface, thickness, and number, and significantly decreased trabecular spacing. Importantly, the single injection of microsphere encapsulated 1 mg/kg OPG did not change either femur cortical or trabecular bone parameters, and did not increase serum OPG levels at any point during the experimental period. This data demonstrates that polymer microsphere encapsulation of OPG can be used to obtain local bone anticatabolic effects (evidenced by the inhibition of molar movement) without more distant effects (evidenced by the lack of inhibition of incisor movement, lack of changes in serum OPG levels, and lack of changes in long bones).

Also as expected, tooth movement without drug increased osteoclast numbers in the intra-radicular bone of maxillary first molar teeth. Tooth movement with OPG delivery (either encapsulated or non-encapsulated) led to more variable osteoclast numbers. This variability could reflect individual animal differences in response to the delivered drug during tooth movement, though this seems unlikely given that significant and consistent differences were found for tooth movement in these two groups. It is possible that the variability found in animals with orthodontic appliances and a single injection of encapsulated OPG is due to varying loss of the effect of the injected drug 28 days after drug delivery. In future studies, we will incorporate an earlier time point for osteoclast quantification to monitor changes in osteoclast numbers over time after initial drug delivery and during tooth movement.

Poly(lactic-co-glycolic acid) (PLGA) microspheres are biocompatible and biodegradable (16–29). In this study, we found that a 50–50 lactic to glycolic acid ratio resulted in faster drug release than a 75:25 ratio, which is consistent with previous studies (30). Notably, encapsulation of OPG using the 75–25 ratio significantly enhanced orthodontic anchorage without inhibiting overall tooth movement or entering the systemic circulation, but efficacy for inhibiting tooth movement was not to same degree that could be achieved by injecting very high dose levels of non-encapsulated OPG. There is, therefore, room for improvement. It will be important to determine if polymer encapsulation of OPG allows for local control of osteoclasts while also preventing more distant or systemic effects in a larger animal model, for ultimate translation to humans. Before translation into the clinic, polymer controlled drug delivery for control of tooth movement should also be tested using lower, potentially more relevant orthodontic force levels. Additionally, while our results demonstrate the potential of biomaterials for local control of osteoclasts, future studies should include the use of alternative bone anticatabolic and/or anabolic drugs, and test more recently developed drug delivery strategies for improved efficacy. Biodegradable polymers were recently shown to provide pulsatile delivery of parathyroid hormone for spatially restricted bone regeneration within a scaffold (17). Coating of polymer carriers with cell adhesion molecules can also be utilized to enhance drug delivery (31). Such additional strategies for controlled delivery of bone anticatabolic and/or anabolic agents could be useful for any clinical scenario in which local but not systemic bone effects are desirable including control of orthodontic tooth movement, treatment of periodontal disease and treatment of other local osteolytic lesions.

Conclusions

- Injection of non-encapsulated 5 mg/kg OPG every 3 days during tooth movement significantly inhibited mesial molar movement and anchorage; this level of delivered drug also inhibited incisor tooth movement, entered the systemic circulation and increased alveolar and femur bone volume fraction, density, and mineral content.
- A single injection of microsphere encapsulated 1 mg/kg OPG significantly inhibited mesial molar movement, while a single injection of non-encapsulated 1 mg/kg OPG did not inhibit mesial molar movement.
- A single injection of microsphere encapsulated 1 mg/kg OPG enhanced orthodontic anchorage without inhibiting incisor movement, entering the systemic circulation, or altering alveolar or long bone volume fraction, density or mineral content after 28 days of tooth movement.
- Use of novel drug delivery systems such as microsphere encapsulation may allow for locally limited biologic control of orthodontic tooth movement.

Study limitations

- Studies to assess the effect of injected drug and tooth movement on bones and tooth roots were only performed at the final time point.
- Studies were performed in a rodent model of tooth movement which may not entirely reflect human tooth movement and human responses to bone catabolic agents.
- Studies utilized 25 g of force, which is a high force level for the rodent model.

Supplementary material

Supplementary material is available at *European Journal of Orthodontics* online.

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Conflicts of Interest

None to declare.

References

1. Simonet, W.S., *et al.* (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell*, 89, 309–319.
2. Kobayashi, Y., Udagawa, N. and Takahashi, N. (2009) Action of RANKL and OPG for osteoclastogenesis. *Critical Reviews in Eukaryotic Gene Expression*, 19, 61–72.
3. Bekker, P.J., Holloway, D., Nakanishi, A., Arrighi, M., Leese, P.T. and Dunstan, C.R. (2001) The effect of a single dose of osteoprotegerin in postmenopausal women. *Journal of Bone and Mineral Research*, 16, 348–360.
4. Bekker, P.J., Holloway, D.L., Rasmussen, A.S., Murphy, R., Martin, S.W., Leese, P.T., Holmes, G.B., Dunstan, C.R. and DePaoli, A.M. (2004) A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. *Journal of Bone and Mineral Research*, 19, 1059–1066.
5. Lewiecki, E.M., Miller, P.D., McClung, M.R., Cohen, S.B., Bolognese, M.A., Liu, Y., Wang, A., Siddhanti, S. and Fitzpatrick, L.A.; AMG 162 Bone Loss Study Group. (2007) Two-year treatment with denosumab (AMG 162) in a randomized phase 2 study of postmenopausal women with low BMD. *Journal of Bone and Mineral Research*, 22, 1832–1841.
6. Cummings, S.R., *et al.*; FREEDOM Trial. (2009) Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *The New England Journal of Medicine*, 361, 756–765.
7. McClung, M.R., Lewiecki, E.M., Geller, M.L., Bolognese, M.A., Peacock, M., Weinstein, R.L., Ding, B., Rockabrand, E., Wagman, R.B. and Miller, P.D. (2013) Effect of denosumab on bone mineral density and biochemical markers of bone turnover: 8-year results of a phase 2 clinical trial. *Osteoporosis International*, 24, 227–235.
8. Kawasaki, K., Takahashi, T., Yamaguchi, M. and Kasai, K. (2006) Effects of aging on RANKL and OPG levels in gingival crevicular fluid during orthodontic tooth movement. *Orthodontics & Craniofacial Research*, 9, 137–142.
9. Nishijima, Y., Yamaguchi, M., Kojima, T., Aihara, N., Nakajima, R. and Kasai, K. (2006) Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. *Orthodontics & Craniofacial Research*, 9, 63–70.
10. Kim, T., Handa, A., Iida, J. and Yoshida, S. (2007) RANKL expression in rat periodontal ligament subjected to a continuous orthodontic force. *Archives of Oral Biology*, 52, 244–250.
11. Yamaguchi, M. (2009) RANK/RANKL/OPG during orthodontic tooth movement. *Orthodontics and Craniofacial Research*, 12, 113–119.
12. Kanzaki, H., Chiba, M., Takahashi, I., Haruyama, N., Nishimura, M. and Mitani, H. (2004) Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement. *Journal of Dental Research*, 83, 920–925.
13. Kanzaki, H., Chiba, M., Arai, K., Takahashi, I., Haruyama, N., Nishimura, M. and Mitani, H. (2006) Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement. *Gene Therapy*, 13, 678–685.
14. Dunn, M.D., Park, C.H., Kostenuik, P.J., Kapila, S. and Giannobile, W.V. (2007) Local delivery of osteoprotegerin inhibits mechanically mediated bone modeling in orthodontic tooth movement. *Bone*, 41, 446–455.
15. Hudson, J.B., Hatch, N., Hayami, T., Shin, J.M., Stolina, M., Kostenuik, P.J. and Kapila, S. (2012) Local delivery of recombinant osteoprotegerin enhances postorthodontic tooth stability. *Calcified Tissue International*, 90, 330–342.
16. Schneider, D.A., Smith, S.M., Campbell, C., Hayami, T., Kapila, S. and Hatch, N.E. (2015) Locally limited inhibition of bone resorption and orthodontic relapse by recombinant osteoprotegerin protein. *Orthodontics & Craniofacial Research*, 18 (Suppl 1), 187–195.
17. Dang, M., Koh, A.J., Jin, X., McCauley, L.K. and Ma, P.X. (2017) Local pulsatile PTH delivery regenerates bone defects via enhanced bone remodeling in a cell-free scaffold. *Biomaterials*, 114, 1–9.
18. King, G.J., Keeling, S.D. and Wronski, T.J. (1991) Histomorphometric study of alveolar bone turnover in orthodontic tooth movement. *Bone*, 12, 401–409.
19. Meganck, J.A., Kozloff, K.M., Thornton, M.M., Broski, S.M. and Goldstein, S.A. (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.
20. Umoh, J.U., Sampaio, A.V., Welch, I., Pitelka, V., Goldberg, H.A., Underhill, T.M. and Holdsworth, D.W. (2009) In vivo micro-CT analysis of bone remodeling in a rat calvarial defect model. *Physics in Medicine and Biology*, 54, 2147–2161.
21. Park, C.H., Abramson, Z.R., Taba, M. Jr, Jin, Q., Chang, J., Kreider, J.M., Goldstein, S.A. and Giannobile, W.V. (2007) Three-dimensional micro-computed tomographic imaging of alveolar bone in experimental bone loss or repair. *Journal of Periodontology*, 78, 273–281.
22. Baker, R.W., Guay, A.H. and Peterson, H.W. Jr. (1972) Current concepts of anchorage management. *The Angle Orthodontist*, 42, 129–138.
23. 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 Odontologica Scandinavica. Supplementum*, 6, 1–240.
24. Body, J.J., *et al.* (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.
25. Wei, G., Jin, Q., Giannobile, W.V. and Ma, P.X. (2006) Nano-fibrous scaffold for controlled delivery of recombinant human PDGF-BB. *Journal of Controlled Release*, 112, 103–110.
26. Wei, G., Jin, Q., Giannobile, W.V. and Ma, P.X. (2007) The enhancement of osteogenesis by nano-fibrous scaffolds incorporating rhBMP-7 nanospheres. *Biomaterials*, 28, 2087–2096.
27. Shive, M.S. and Anderson, J.M. (1997) Biodegradation and biocompatibility of PLA and PLGA microspheres. *Advanced Drug Delivery Reviews*, 28, 5–24.
28. Wei, G., Pettway, G.J., McCauley, L.K. and Ma, P.X. (2004) The release profiles and bioactivity of parathyroid hormone from poly(lactic-co-glycolic acid) microspheres. *Biomaterials*, 25, 345–352.
29. Makadia, H.K. and Siegel, S.J. (2011) Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polymers*, 3, 1377–1397.
30. Chen, Y.J., Chang, H.H., Huang, C.Y., Hung, H.C., Lai, E.H. and Yao, C.C. (2007) A retrospective analysis of the failure rate of three different orthodontic skeletal anchorage systems. *Clinical Oral Implants Research*, 18, 768–775.
31. Tatard, V.M., Venier-Julienne, M.C., Saulnier, P., Prechter, E., Benoit, J.P., Menei, P. and Montero-Menei, C.N. (2005) Pharmacologically active microcarriers: a tool for cell therapy. *Biomaterials*, 26, 3727–3737.