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AAO Foundation Final Report

Type of Award: Biomedical Research Award

Name of Principal Investigator: Nan E. Hatch

Title of Project: Polymer Microsphere Controlled Delivery of Osteoprotegerin for Enhancing Orthodontic Anchorage

Period of AAOF Support: 07-01-16 to 12-31-17

Amount of Funding: $30,000

Summary/Abstract:

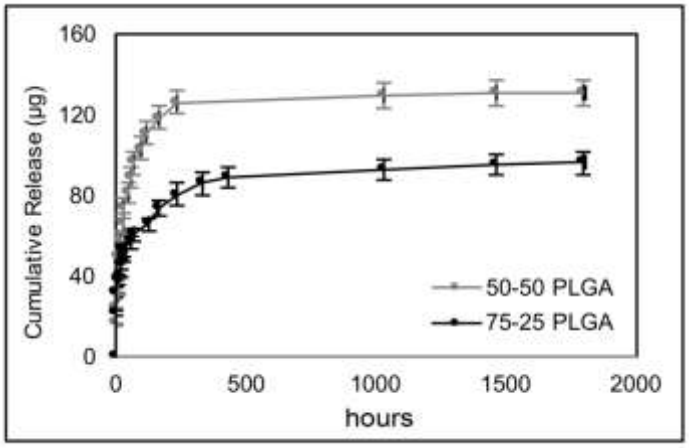
Because orthodontic tooth movement is dependent upon osteoclast-mediated resorption of alveolar bone adjacent to the pressure side of tooth roots, biologic mediators that regulate osteoclasts can be utilized to control tooth movement. The goal of this study was to develop a novel method to locally enhance orthodontic anchorage, without leading to systemic effects. We encapsulated recombinant osteoprotegerin (OPG) in PLGA polymer microspheres and tested the effectiveness of microsphere encapsulated vs. non-encapsulated OPG for enhancing orthodontic anchorage in a previously established 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 one day prior to 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 and distant femur bone were analyzed by micro computed tomography. Serum levels of OPG were measured by ELISA. 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. Polymer microsphere encapsulation of OPG allows for controlled drug release, and enhances site-specific orthodontic anchorage without systemic side effects. With additional refinements, this oral drug delivery system could be applicable to a broad array of potential biologic orthodontic therapeutics.

Response to the following questions:

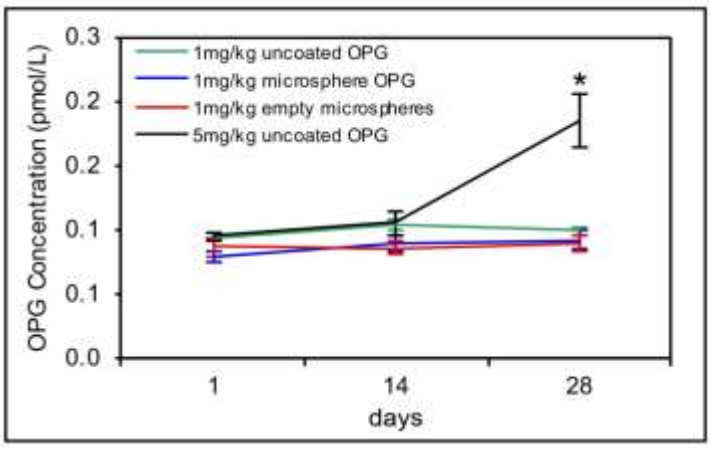
1. Were the original, specific aims of the proposal realized?

We have completed most, but not all of the proposed aims. We have 6 months left on the originally planned timeline to complete all of the aims. We have completed microsphere encapsulation of recombinant osteoprotegerin protein (OPG), measured release kinetics in vitro, completed animals experiments to provide data to determine if encapsulated OPG can be used to enhance orthodontic anchorage in a rodent model of tooth movement without negative side effects. We have also measured and analyzed molar and incisor tooth movement measurements, serum OPG levels, micro CT tooth root volumes, micro CT alveolar bone parameters and micro CT femur bone parameters. The only aim left to complete is histology. All samples have been fixed and embedded for histology and this portion of the study is ongoing.

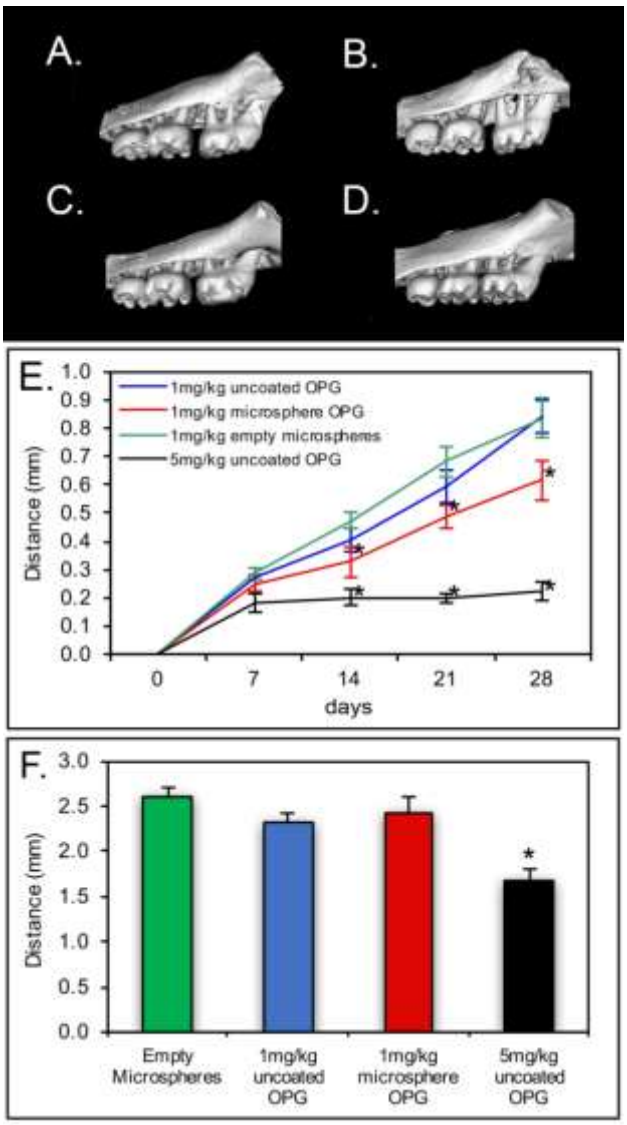
Studies and Results:



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



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/kg OPG. In contrast, multiple injections of 5 mg/kg OPG significantly serum OPG levels by day 28, when compared to empty microsphere injected animals. Results are expressed means +/- standard deviations, *$p<0.05$ vs. empty microsphere injected animals.



Microsphere encapsulated OPG inhibits molar but not incisor tooth movement. **(A-D)** 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 OPG also significantly inhibited molar tooth movement at days 14, 21 and 28, as compared to animals that received a single injection of microsphere encapsulated OPG. **(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 to 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 than animals that received a single injection of empty microspheres. Results are expressed means +/- standard deviations, *$p<0.05$ vs. empty microsphere injected animals.

Maxillary molar furcation area bone volume, density and mineral content.

	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 Uncoated OPG	4.3 +/ -0.1	0.63 +/- 0.01	5.7 +/- 0.2	846 +/- 16	4.5 +/- 0.2	1049 +/- 14
No Appliances Microsphere 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 Uncoated OPG	3.0 +/- 0.2*	0.46 +/- 0.03*	4.0 +/- 0.2*	598 +/- 37*	2.8 +/- 0.2*	920 +/- 17*
+ Appliances Microsphere OPG	3.2 +/- 0.2*	0.50 +/- 0.02*	4.1 +/- 0.3*	639 +/- 34*	3.0 +/- 03*	919 +/- 23*
+ Appliances High Dose Uncoated 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 to the groups without appliances ($p<0.05$).
# Indicates statistical significance when compared to empty spheres ($p<0.05$).

Femur cortical bone analysis

	Cortical Thickness (mm)	Cortical Area (mm^2)	Bone Mineral Content (mg)	Bone Mineral Density (mg/cc)	Tissue Mineral Content (mg)	Tissue Mineral Density (mg/cc)
No Appliances Empty Microspheres	0.36 +/- 0.02	3.5 +/- 0.2	0.12 +/- 0.01	1165 +/- 4	14.1 +/- 0.23	1210 +/- 5
No Appliances Uncoated OPG	0.35 +/- 0.01	3.4 +/- 0.1	0.12 +/- 0.01	1173 +/- 9	13.5 +/- 0.2	1218 +/- 10
No Appliances Microsphere OPG	0.36 +/- 0.02	3.5 +/- 0.2	0.13 +/- 0.01	1186 +/- 9	15.0 +/- 0.5	1231 +/- 9

+ Appliances Empty Microspheres	0.34 +/- 0.01	3.4 +/ 0.1	0.13 +/- 0.01	1170 +/- 8	15.4 +/- 0.5	1213 +/- 8
+ Appliances Uncoated OPG	0.35 +/- 0.01	3.4 +/- 0.1	0.12 +/- 0.01	1171 +/- 7	13.4 +/- 0.3	1216 +/- 6
+ Appliances Microsphere OPG	0.36 +/- 0.01	3.4 +/- 0.1	0.12 +/- 0.01	1192 +/- 10	14.2 +/- 0.4	1237 +/- 10
+ Appliances High Dose Uncoated OPG	0.36 +/- 0.01	3.6 +/- 0.1	0.12 +/- 0.01	1183+/-9	14.1+/- 0.2	1228 +/- 8

No statistical significances between groups for any parameter.

Femur trabecular bone analysis

	Bone Volume (mm^3)	**Trabecular Bone Volume Fraction**	**Trabecular Bone Surface (mm^2/mm^3)**	**Trabecular Thickness (mm)**	**Trabecular Spacing (mm)**	**Trabecular number (1/mm)**
No Appliances Empty Microspheres	4.9 +/- 0.6	0.19 +/- 0.05	34.6 +/- 3.1	0.06 +/- 0.01	0.26 +/- 0.09	3.28 +/- 0.75
No Appliances Uncoated OPG	7.4 +/-0.8#	0.28 +/- 0.09#	30.6 +/- 3.3#	0.07 +/- 0.01#	0.19 +/- 0.08	4.18 +/- 0.96#
No Appliances Microsphere OPG	5.1 +/- 1.5	0.18 +/- 0.10	34.1 +/- 4.5	0.06 +/- 0.01	0.33 +/- 0.17	2.94 +/- 1.10
+ Appliances Empty Microspheres	5.6 +/- 0.7	0.20 +/- 0.06	33.6 +/- 4.4	0.06 +/- 0.01	0.26 +/- 0.07	3.22 +/- 0.67
+ Appliances Uncoated OPG	6.5 +/- 0.5	0.25 +/- 0.04	32.0 +/- 2.7	0.06 +/- 0.00	0.19 +/- 0.03Ω	3.99 +/- 0.45Ω
+ Appliances Microsphere OPG	5.1 +/- 0.8	0.22 +/- 0.05	32.8 +/- 3.3	0.06 +/- 0.01	0.22 +/- 0.05	3.59 +/- 0.63
+ Appliances High Dose Uncoated OPG	10.5 +/- 1.3#	0.36 +/- 0.07#	28.9 +/- 2.3#	0.07 +/- 0.01#	0.13 +/- 0.03#	5.19 +/- 0.73#

No statistical significances were found comparing groups with/without orthodontic appliances.
# Indicates statistical difference compared to empty spheres ($p<0.05$).

Volumetric tooth root measurements

Ortho Appliances	OPG Dose (mg/kg)	Micro-sphere	Number of Injections	Total Root Volume
No	0	yes	one	2.5 +/- 0.5
No	1	yes	one	2.7 +/- 0.3
No	1	none	one	2.7 +/- 0.2
Yes	0	yes	one	2.3 +/- 0.2
Yes	1	yes	one	2.4 +/- 0.3
Yes	1	none	one	2.5 +/- 0.3
Yes	5	none	multiple	2.7 +/- 0.3#

Trends but no significant differences were found between +/- orthodontic appliances
# Indicates statistical difference compared to empty spheres + orthodontic tooth movement

2. Were the results published?

a. A first manuscript was recently submitted for publication in the Journal of Dental Research.
Title: Microsphere Controlled Drug Delivery for Local Control of Tooth Movement
Authors: Inna Sydorak, Ming Dang, Michael Halcomb, Peter Mah, Sunil Kapila and Nan Hatch

b. AAOF support was acknowledged on this submitted manuscript. AAOF will be acknowledged on all resulting publications.

c. Currently working on publication. Also planning for a 2nd publication when histology work is completed.

3. Have the results of this proposal been presented?

a. I have not presented on this project yet.

b. I plan to present on this project in the future and will acknowledge AAOF support.

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

AAOF funding of this project, and prior research and faculty development projects has been critical for furthering my career as an orthodontic academic. AAOF funding has supported the

generation of data used to apply to other funding sources, produce publications and present at professional orthodontic and scientific meetings. AAOF Funding of this project in particular supported the generation of strong initial data showing that it is possible to achieve a locally limited ability to control tooth movement via biologics. Yet clearly the degree of local anchorage achieved could be further improved. Results of this project will likely result in a continuing research collaboration with an expert in bioengineering and drug delivery (Peter Ma, PhD). We will use results from this study to apply for additional sources of funding to support further refinement of the drug delivery system as well as test other FDA approved bone agents for control of orthodontic tooth movement. We also want to test these systems in a larger animal model.

The AAOF has provided support since the start of my academic career. This support has been absolutely essential for maintaining productivity, morale and a desire to remain invested academically in the profession of orthodontics. I cannot say enough good things about the AAOF! Thank you for your support!

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