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AAO Foundation Final Report Form
(a/o 6/30/2018)

Type of Award: 2019 Orthodontic Faculty Development Fellowship Award

Name of Principal Investigator: Wei Huang

Institution: Rutgers University

Title of Project: Building a non-invasive and locally induced reversible osteopenia model for accelerating orthodontic tooth movement through the latest technology of controlled-release drug delivery with poly (lactic-co-glycolic acid) (PLGA) nanoparticles

Period of AAOF Support: 07-01-19 to 06-30-20 with NCE approved through 06-30-22

Amount of Funding: $20,000

Summary/Abstract: How to shorten the lengthy orthodontic treatment time has a been a Holy Grail of orthodontic research and clinical practice, as it comes with invaluable benefits of reducing complications, cutting down costs and increasing care access. Decreased bone density of the jaws has been shown to lead to accelerated orthodontic tooth movement (OTM) both in clinical case reports and animal studies. In this study, we utilized the advanced understanding of the molecular regulators of osteoporosis and investigated the effect of local injection of two molecules, VEGF and BMP2, on the rate of OTM and the bone mineral density. We showed that OTM in rats using an ultra-light force of 4cN was measurable after one week and evident after two weeks. After two weeks of OTM, the local alveolar bone mineral density (BMD) around the tooth decreased significantly compared to the control side with no appliances. Local injection of VEGF showed no effect on the rate of OTM or the BMD's decreased amount. Local injection of BMP2 possibly had an accelerating effect on OTM in rats. In addition, we developed a novel BMD analyzing method utilizing a special expansion feature of the imaging software VivoQuant to analyze the BMD of a definable area of bone surrounding the roots of each tooth.

Introduction: Malocclusion is one of the most prevalent dentofacial conditions that can significantly compromise oral health and craniofacial development. Although 50-75% of the U.S. population present with moderate to severe malocclusion, only a very small percentage receives orthodontic treatment. The lengthy treatment itself (~1.5-2.5 years) is a major factor driving the cost beyond the reach of many families and shutting the door to many adult patients with its prolonged undesirable social and professional impacts. It can also increase the risk of many complications such as root resorption, etc. Thus, shortening the orthodontic treatment time holds the ultimate key to faster, cheaper and more accessible orthodontic care. Decreased jawbone mass and increased bone remodeling has been shown to significantly shorten orthodontic treatment time both through clinical case reports and animal studies. In this study, we investigated the effect of two locally injected osteoporosis-causing mediators, VEGF and BMP2, on the rate of orthodontic tooth movement (OTM) and the alveolar bone density.

Results:

- ***OTM using an ultra-light force of 4cN was measurable in rats after one week***

Temporary skeletal anchorage (TAD) device-assisted OTM was carried out in eight 12-week old Sprague Dawley male rats. Custom-made mini-TADs (Figure 1A) were placed approximately 1mm distal to the right incisor. A custom-made mini-NiTi coil-spring (Figure 1B) capable of generating ultra-light force of 4cN (Figure 1C) was ligated to the right first molar and bonded to the TAD (Figure 1D). Rats were sacrificed after 9 days and 14 days of OTM. As shown in Table 1, tooth movement was measurable through μCT analysis on day 9 with an average of 82.7μm gap between the first and second molars. On day 14, tooth movement amount increased to a more prominent level of 264.5μm. Due to the scan resolution, a distance of less than 100μm is less reliable. Therefore, 14 days of tooth movement was chosen as the experimental time point for this study.

- ***Local injection of VEGF has no effect on the rate of OTM in rats***

Rats were injected with 10μl recombinant VEGF (0.5μg/μl) mesial to the first molar immediately after the placement of the OTM appliances and then every three days afterwards for two weeks. Control groups had an injection of 10μl of PBS every three days. As shown in Figure 2A, the rate of OTM, as suggested by the distance between the first and second molars, was smaller in the VEGF-injected group compared to controls. However, there was no statistical significance between the two groups. This indicates that local injection of VEGF had no effect on the rate of OTM in rats. To assess the first molar tipping after OTM, the angle between the long axis of the mesial root and the plane perpendicular to occlusal plane was measured. As shown in Figure 2B, the experiment side had on average a 5^{o} increase in angulation compared to the control side in PBS-injected group, indicating more mesial tipping of the first molar after OTM. The VEGF-injected group had a smaller increase in the angulation but there was no statistical significance between the VEGF-injected and PBS-injected groups.

- ***Local injection of VEGF has similar effect to controls on the alveolar bone density changes around the tooth in rats***

We further analyzed the bone mineral density (BMD) of the first molar inter-radicular area. The experiment right side consistently showed statistically significantly reduced BMD compared to the control left side within the same rat. This was observed for both PBS-injected and VEGF-injected groups (Figure 2C, 2D). When the difference of BMD between the right and left side of the same rat was compared, there was no statistically significant difference between PBS-injected and VEGF-injected groups (Figure 2E). This indicates that the decrease of BMD on the experiment right side compared to the control left side was similar in both PBS-injected and VEGF-injected groups.

- ***Local injection of BMP2 possibly has an accelerating effect on OTM in rats***

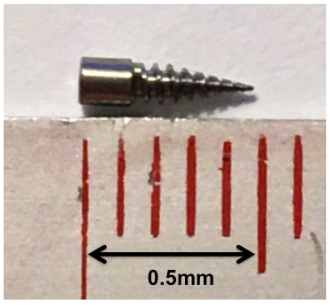
Rats were injected with 3.5μg/10μl of BMP2 mesial to the first molar immediately after the placement of the OTM appliances and then every three days afterwards for two weeks. Control groups had an injection of 10μl of PBS every three days. Unfortunately, many of the rats in this group were lost throughout the experiment due to various reasons, such as coil spring being broken likely from rat chewing. Only one rat was successfully analyzed. As shown in Figure 3, the distance between the first and second molars was much bigger in the BMP2-injected group compared to controls. More rats will be needed to repeat this experiment to reach statistical power and a more conclusive result.

- ***A novel bone expansion method to analyze BMD around the roots of a tooth.***

Micro-CT images of the rat maxilla were processed using a customized algorithm based on an expanded tooth method. All three molars were digitally segmented and then the boundaries of the segmented teeth with periodontal ligament (PDL) were digitally expanded to encompass 0.5mm of the alveolar bone that extended beyond the PDL space. The alveolar bone area that was not covered by the region of interest (ROI) defined by the expanded teeth was then digitally deleted. The volumes and density of the bone (Figure 4A) and non-bone space (Figure 4B) of the ROI were then analyzed. As shown in Figure 4C, compared to the control side, the tooth movement side of the teeth showed decreased BMD surrounding the root as indicated by decreased voxel intensity as well as decreased bone area as indicated by decreased frequency. As bone density surrounding the roots likely is the most relevant bone area determining the rate of tooth movement, our novel method allowed the analysis of an objectively defined area of alveolar bone exactly surrounding each root of a tooth.

Figures and Tables:

A.



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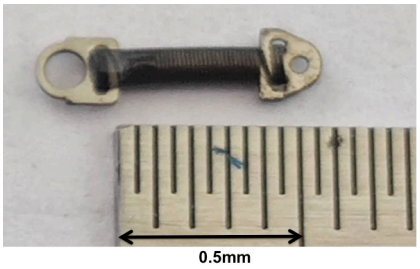

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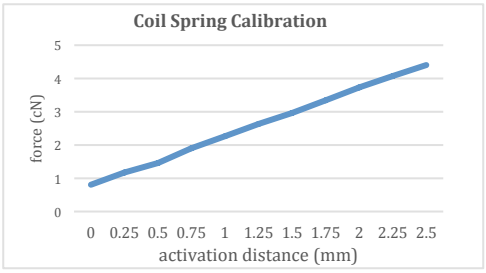

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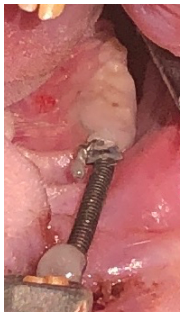
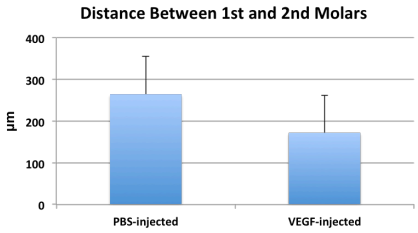


Figure 1. Experimental design and custom-made mini-TAD and ultra light force mini-coil springs. A. Custom-made stainless steel mini-TAD; B. Custom-made mini-NiTi coil spring. The two ends of the coil spring have different designs to allow for mini-screw connection on one end (bigger hole) and steel ligature tie connection on the other (smaller hole); C. Coil spring showed a linear relationship between activation distance and force. Coil springs were activated for 2mm in rats generating around 4cN of force; D. OTM appliance in place in the rat mouth.

Table 1. Distance between first and second molars after 9 and 14 days of OTM

OTM	Average Distance	Standard Deviation
D9	82.7	9.1
D14	264.5	116.0

A.



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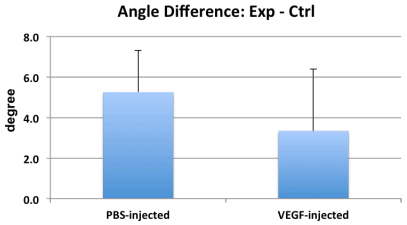
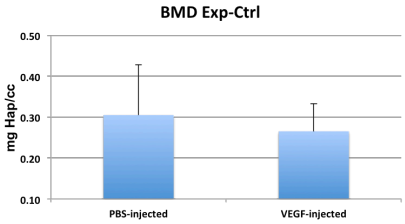


C.

Bone Mineral Density	Exp-Ctrl Average (mg Hap/cc)	Exp-Ctrl Standard Deviation	Paired t-test *p*
PBS-Injected	0.305	0.124	0.016*
VEGF-Injected	0.268	0.068	0.021*

E.



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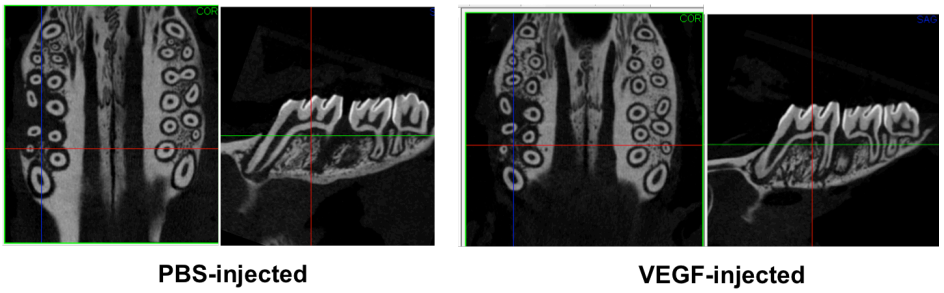
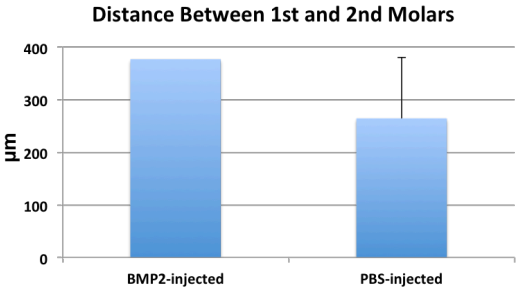


Figure 2. Effect of local injection of VEGF on the rate of tooth movement and alveolar bone density. A. Distance between first and second molars had no statistically significant difference between PBS-injected and VEGF-injected groups (p=0.289); B. First molar angle difference between experimental and control sides had no statistical significance between PBS-injected and VEGF-injected groups (p=0.363); C. BMD of the inter-radicular area of the first molar was decreased in the experiment side compared to the control side. This decease was statistically significant and was observed in both PBS-injected and VEGF-injected groups; D. µCT images showing reduced BMD on the left (experiment) side of the alveolar bone compared to the right (control) side. E. The difference in BMD between the experiment and the control side had no statistical significance between PBS-injected and VEGF-injected groups (p=0.641).

A. B.



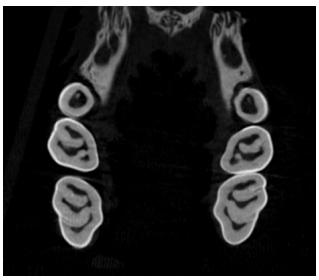
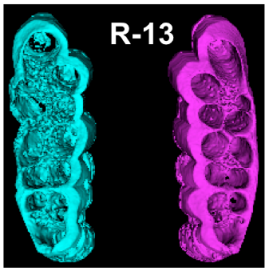
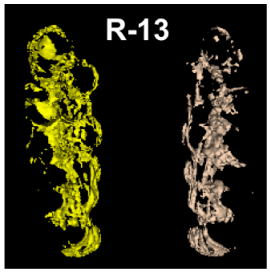


Figure 3. Effect of BMP2 local injection on the rate of tooth movement. A. Distance between first and second molars was larger in the BMP2-injected rat compared to the average of PBS-injected rats. B. µCT image showing the gap between the first and second molars on the experimental side.

A. B. C.





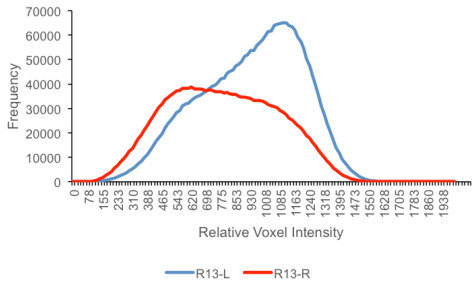


Figure 4. A novel bone expansion method to analyze the BMD in a defined area of bone surrounding roots of teeth. A. Alveolar bone area expanded 0.5mm from each root and PDL. The experiment side of the teeth (cyan) showed less defined socket contour compared to the control side of the teeth (magenta); B. Space within the expanded bone region. More space was seen in the experiment side of the teeth compared to the control side; C. Decreased BMD as represented by the voxel intensity as well as decreased bone area as represented by frequency were observed in the experiment side of the teeth (red) compared to the control side (blue).

Respond to the following questions:

1. Were the original, specific aims of the proposal realized?
   Yes.
2. Were the results published?
   The results have not been published yet. But the manuscript is being prepared and the work will be published in the near future.
3. Have the results of this proposal been presented?
   The results have not been presented yet, but will be in the near future.
4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?

This AAOF funding has been critical to my early career development through supporting an important part of my research activity and helping build the foundation to further my academic career. The result of this funded project will be published in peer-reviewed journals and presented in professional conferences. Based on the result, further research funding application will be carried out.

Accounting for Project: All research funds have been used for research purposes with no leftovers. The financial report has been sent to the AAOF through the accounting department at Rutgers School of Dental Medicine separately.