## High-Frequency Acceleration: Therapeutic Tool to Preserve Bone following Tooth Extractions

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M. Alikhani<sup>1,2,3,4</sup>, J.A. Lopez<sup>1,2</sup>, H. Alabdullah<sup>1</sup>, T. Vongthongleur<sup>1</sup>, C. Sangsuwon<sup>1</sup>, M. Alikhani<sup>1</sup>, S. Alansari<sup>1,2</sup>, S.M. Oliveira<sup>1,5</sup>, J.M. Nervina<sup>1,2</sup>, and C.C. Teixeira<sup>1,2,6</sup>

## Appendix

Comparison of "No Treatment" Control with "Static" Control

Appendix Tables 1 through 4 and Appendix Figure 2 show a sample of data collected for the "no treatment" control

Appendix Tab	ole I. Bone V	Vas Analyzed	by Micro–computed
Tomography as	described in t	the Materials	and Methods Section.

	28	d
Parameter	No Treatment	Static
BV/TV, %	32 ± 2.9	33 ± 4.6
Tb.Th, mm	0.17 ± 0.03	0.16 ± 0.02
Tb.N, I/mm	$2.02 \pm 0.2$	2.06 ± 0.21
Tb.Sp, mm	0.32 ± 0.04	0.33 ± 0.05
TMD, mg/mL	781 ± 34	774 ± 46

BV/TV, ratio of bone volume/total volume; Tb.N, trabecular number; Tb.Sp, trabecular spacing; Tb.Th, trabecular thickness; TMD, tissue mineral density.

At day 28, micro–computed tomography quantification was completed for hemimaxillae of animals that did not receive any treatment (no treatment) other than the molar extraction and animals that received a static force 5 min/d (static). Each value represents the mean  $\pm$  SEM of 5 animals.

Appendix Table 2. Expression of Bone Formation Markers at Day 14.

Marker	No Treatment	Static
Col I	6.6 ± 0.6	6.2 ± 0.6
Osteopontin	5.1 ± 0.5	5.8 ± 0.6
Osteocalcin	6.8 ± 0.5	6.1 ± 0.5

At day 14, hemimaxillae of animals that did not receive any treatment (no treatment) other than the molar extraction and animals that received a static force 5 min/d (static) were evaluated by reverse transcription polymerase chain reaction. Data are presented as fold change in comparison with day 0 for "no treatment" control and "static" control. No significant differences found between groups (P < 0.05). compared with "static" control. Micro–computed tomography quantification, gene expression, and alkaline phosphatase activity data show that there is no statistical difference between these 2 control groups. Therefore, for simplification, the "no treatment" control results were not presented throughout the manuscript.

## Appendix Table 3. Expression of Inflammatory Markers at Day 14.

Marker	No Treatment	Static
ILI	2.2 ± 0.3	2.6 ± 0.2
IL6	1.9 ± 0.4	2.1 ± 0.2
TNF	$3.3 \pm 0.2$	3.1 ± 0.1
CCL2	$2.4 \pm 0.3$	$2.5 \pm 0.3$
CCL5	$1.4 \pm 0.1$	1.7 ± 0.2

At day 14, hemimaxillae of animals that did not receive any treatment (no treatment) other than the molar extraction and animals that received a static force 5 min/d (static) were evaluated by reverse transcription polymerase chain reaction. Data are presented as fold change in comparison with day 0 for "no treatment" control and "static" control. No significant differences found between groups (P < 0.05).

<sup>1</sup>Consortium for Translational Orthodontic Research, New York University College of Dentistry, New York, NY, USA

<sup>2</sup>Department of Orthodontics, New York University College of Dentistry, New York, NY, USA

<sup>3</sup>Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA, USA

<sup>4</sup>Department of Oral and Maxillofacial Surgery, New York University College of Dentistry, New York, NY, USA

<sup>5</sup>Department of Mechanical Engineering, Institute Polytechnic of Viseu, Viseu, Portugal

<sup>6</sup>Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, New York, NY, USA

## **Corresponding Author:**

C.C. Teixeira, New York University, 345 East 24th Street, New York, NY 10010, USA.

Email: cristina.teixeira@nyu.edu



**Appendix Figure 1.** Schematic of apparatus developed to deliver high-frequency accelerations to the maxillary molar. The rat head was immobilized and the mouth kept open standing on a stable platform. A silicone head at the end of mechanical convertor delivers the high-frequency acceleration to the occlusal surface of the second molar for the high-frequency acceleration group animals. A tightening dial in the standing rod allows adjustment of the head to lightly touch the tooth with minimal load. In the static group, the rod was allowed to just touch the molar occlusal surface without delivering high-frequency acceleration, frequency, and load was performed on dry and wet rat skulls.

Appendix	Table 4	<ul> <li>Expression</li> </ul>	of Osteoclastogenesis	Regulators at
Day 28.				

Regulator	No Treatment	Static
RANKL	3.6 ± 0.2	3.8 ± 0.2
OPG	$2.5 \pm 0.2$	2.4 ± 0.1
RANK	1.4 ± 0.1	1.2 ± 0.1

At day 28, hemimaxillae of animals that did not receive any treatment (no treatment) other than the molar extraction and animals that received a static force 5 min/d (static) were evaluated by reverse transcription polymerase chain reaction. Data are presented as fold change in comparison with day 0 for "no treatment" control and "static" control. No significant differences found between groups (P < 0.05)



**Appendix Figure 2.** Alkaline phosphatase (ALP) activity was measured as described in the Materials and Methods section. Cell extracts were collected for "no treatment" control, "static" control, and "HFA" (high-frequency acceleration) animals for analysis of ALP activity at different time points: days 0, 3, 7, 14, and 28. At days 7, 14, and 28, all groups are significantly different from ALP at day 0 (0 d). \*Significantly different from ALP for other groups at same time point (P < 0.05).