Original Article

Effect of incisal loading during orthodontic treatment in adults: *A randomized control trial*

Pornputthi Puttaravuttiporn[a]; Mutita Wongsuwanlert[b]; Chairat Charoemratrote[c]; Steven J. Lindauer[d]; Chidchanok Leethanakul[c]

ABSTRACT

Objective: To measure the changes in tooth mobility, alveolar bone, and receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) in the gingival crevicular fluid (GCF) during orthodontic treatment to regain incisal function in the presence and absence of biting exercises.

Materials and Methods: Thirty-six females (42.3 ± 6.5 years old) with periodontally compromised upper incisors received orthodontic treatment to obtain ideal incisor relationships. Eighteen subjects in the experimental biting exercise group were instructed to bite a soft plastic roll for 5 min/d; the 18 control subjects were not given plastic rolls. Alveolar bone thickness, height, and density around the upper incisors were assessed at three root levels using cone-beam computed tomography. GCF was collected at the labial and palatal sites of the upper incisors at pretreatment (T0), end of treatment (T1), 1 month after T1 (T2), and 7 months after T1 (T3). RANKL/OPG was determined using enzyme-linked immunosorbent assays.

Results: Labial and palatal bone thickness significantly increased (>twofold) from T1 to T3 in the experimental group at all three root levels (all $P < .05$). Bone thickness correlated negatively with RANKL/OPG ratio between T1 and T2 ($P < .05$). Tooth mobility, bone height, and density were not significantly different between T1 and T3.

Conclusions: Biting exercises significantly increased bone thickness but did not affect tooth mobility, bone height, or density. The RANKL/OPG ratio decreased 1 month after treatment (T2) and correlated with increased bone thickness. (ClinicalTrials.in.th TCTR20170625001). (*Angle Orthod.* 2018;88:35–44.)

KEY WORDS: RANK ligand; Osteoprotegerin; Periodontal disease(s)/periodontitis; Bone remodeling; Cone-beam computed tomography; Exercise therapy

INTRODUCTION

Mechanical stimuli are necessary for periodontal tissue/bone maintenance and remodeling.[1] In animal studies, occlusal hypofunction decreased cancellous bone mass and inhibited cortical bone formation,[2] whereas rehabilitation of masticatory function improved alveolar bone architecture.[3] Therefore, mechanical loading is important for alveolar bone homeostasis and maintenance of alveolar process structure and mass. However, no study has quantified the effects of rehabilitation of masticatory function on alveolar bone in humans. Pathologic tooth migration (PTM) frequently occurs in patients with periodontitis and can result in occlusal hypofunction, especially of the anterior teeth due to proclination of the maxillary incisors and absence of incisal stops with the lower anterior teeth.[4] When occlusal contact is restored with orthodontic

[a] PhD Candidate, Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

[b] Lecturer, Periodontic Section, Department of Conservative Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

[c] Associate Professor, Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

[d] Professor and Chair, Department of Orthodontics, School of Dentistry, Virginia Commonwealth University, Richmond, Va.

Corresponding author: Dr Chidchanok Leethanakul, DDS, MSc, PhD, Associate Professor and Chair, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand (e-mail: nokleethanakul@yahoo.com)

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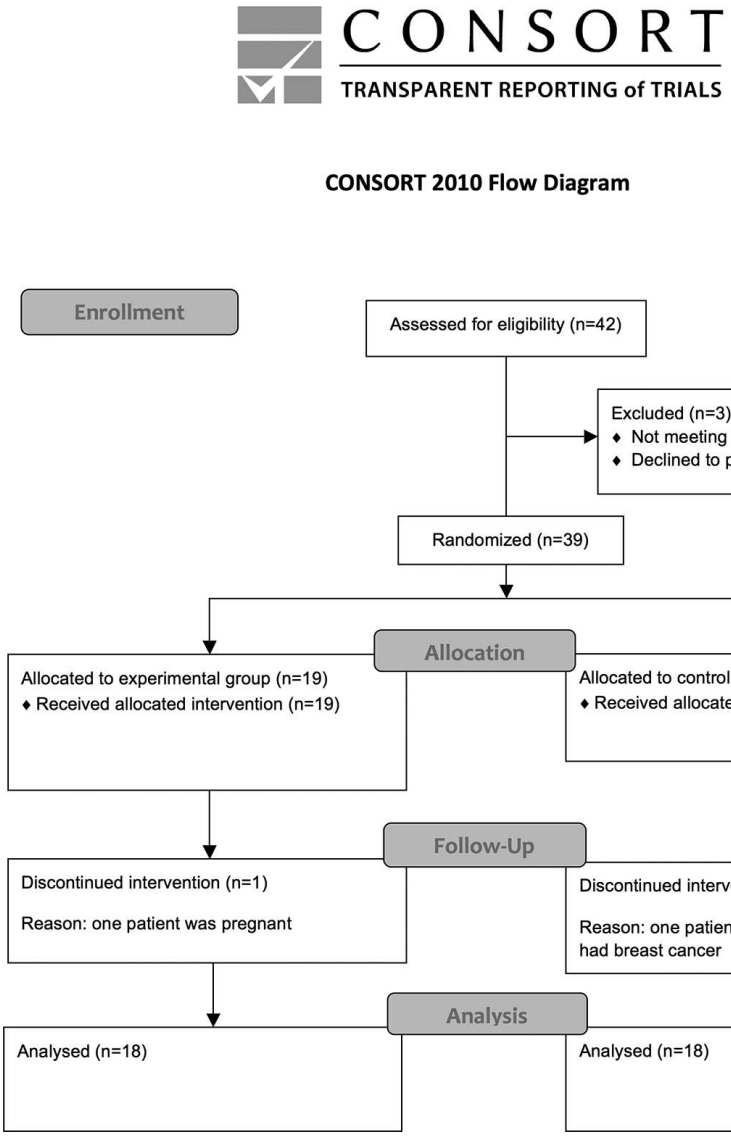


Figure 1. CONSORT 2010 flow diagram.

assistance, patients regain the biting function of the front teeth.

Cone-beam computed tomography (CBCT) can be used to estimate alveolar bone changes in three dimensions. However, alveolar bone remodeling is a gradual, continuous process that can only be detected by CBCT several months after the process begins. Early changes in alveolar bone can be monitored using biomarkers. Collection of gingival crevicular fluid (GCF) is a convenient, noninvasive method used to investigate bone remodeling biomarkers during orthodontic treatment. Receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) play critical roles in bone remodeling,[5] and their ratio in GCF has been shown to increase during orthodontic tooth movement.[6] However, RANKL and OPG changes and relationships with alveolar bone changes observed on CBCT during oral rehabilitation have not been investigated.

This randomized clinical experimental study investigated the changes in tooth mobility and alveolar bone after establishing incisor function in the presence and absence of biting exercises. Relationships between tooth mobility, alveolar bone changes and RANKL/OPG ratio were examined. The main goal of this study was to test the hypothesis that restoring function would significantly change the alveolar bone status of periodontally compromised teeth.

MATERIALS AND METHODS

This study was approved by the Faculty of Dentistry, Prince of Songkla University Ethics Committee (0521.1.03/573). Subjects were recruited at the perio-

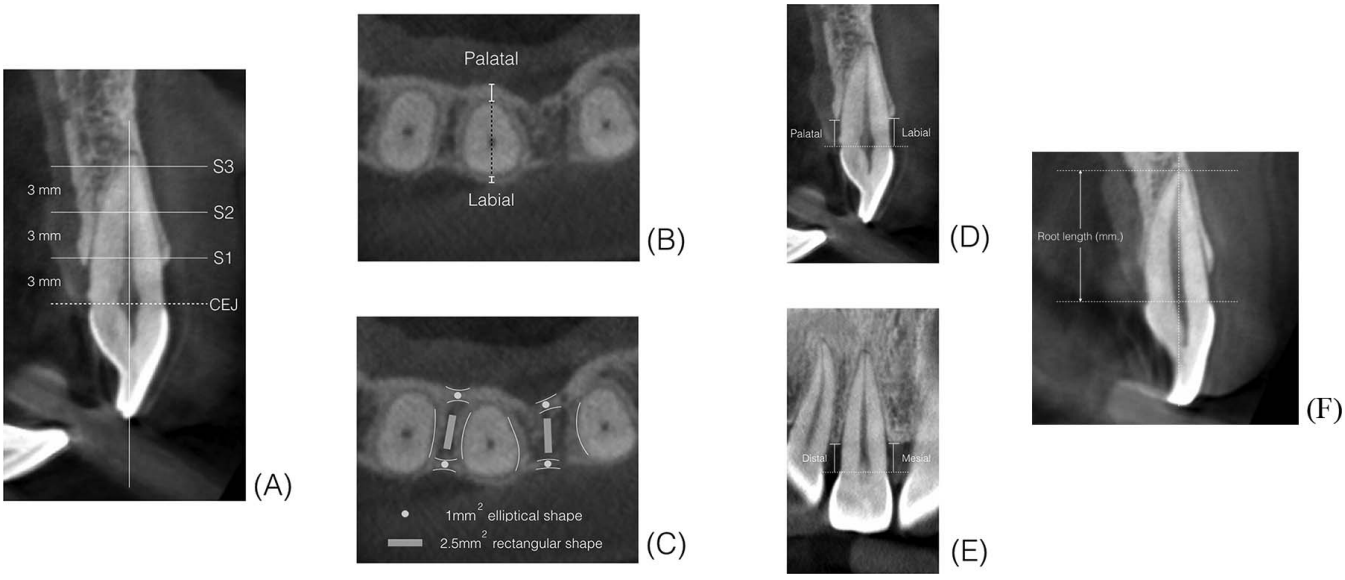


Figure 2. Measurement of (A) bone thickness and density at three levels, (B) bone thickness, (C) cortical and trabecular bone density, (D,E) bone height, and (F) root length on CBCT images.

dontic clinic between March 2014 and February 2015. Inclusion criteria were (1) 3–5 mm radiographic bone loss (as measured from the cementoenamel junction (CEJ) to the alveolar bone crest), (2) in periodontal maintenance phase, and (3) upper incisors exhibiting a horizontal bone loss pattern with PTM and no incisal stop. Patients with initial signs of menopause during the study period[7]; plaque index (PI)[8] or gingival index (GI)[9] >1; bruxism; allergies; pregnancy; systemic diseases; or long-term use of cigarettes, medications, or supplements were excluded.

A moderate effect size (0.44 mm change in cortical bone thickness) was assumed for power analysis.[10] A total sample size of 34 was required to detect this effect size with 80% power at $\alpha = 0.05$. The periodontal examination and maintenance program (providing confirmation of PI and GI ≤ 1) was done by a periodontist (M.W.) every 3 months. Orthodontic treatment involved placing preadjusted edgewise appliances (Roth system; Ormco Corp, Orange, Calif) with 0.018 $\times$ 0.025-inch slots on the incisors and 0.022 $\times$ 0.028-inch slots on the canines and posterior teeth. A series of 0.012-inch, 0.016-inch, 0.016 $\times$ 0.016-inch nickel-titanium, 0.016 $\times$ 0.016-inch stainless steel, and 0.016 $\times$ 0.022-inch titanium molybdenum alloy archwires was used for alignment. Treatment continued until normal overjet, overbite, and interincisal angulation were obtained.[11] To retain tooth positions, 0.016 $\times$ 0.016-inch stainless steel archwires were placed. All subjects completed modified biting frequency questionnaires[12] daily for 1 month. Randomization was accomplished following CONSORT 2010 guidelines (Figure 1). This parallel-group randomized clinical trial had a 1:1 allocation ratio. Randomization was performed by assigning numbers from a random number table. Patients were blinded to the allocation sequence. The experimental group was instructed to bite gently on a plastic roll (Chewies Aligner; Dentsply Raintree Essix, York, Pa) positioned between the upper and lower incisors for 5 min/d for 7 months and complete an additional recording on the biting questionnaire.

GCF was collected at the labial and palatal sites of the upper incisors at pretreatment (T0), end of treatment (T1), and 1 month (T2) and 7 months after

Table 1. Frequency Distributions of the Degree of Tooth Mobility at Each Time Point in the Experimental and Control Groups and Differences in the Degree of Tooth Mobility Between Groups at Each Time Point

	Frequency Distributions									
	Experimental Group				Control Group				Differences Between Group[a]	
Mobility Score	T0	T1	T2	T3	T0	T1	T2	T3	Chi-Square	Asymp Sig
0	3	–	–	9	2	–	–	6	0.007	0.931
1	7	14	14	7	9	13	13	10	0.144	0.704
2	8	4	4	2	7	5	5	2	0.144	0.704
3	-	–	–	–	–	–	–	–	0.702	0.402

[a] Chi-square test; * $P < .05$.

Table 2. Mean and Mean Differences in Alveolar Bone Thickness, Height and Density, Root Length, and RANKL/OPG Between the Experimental and Control Groups at T0, T1, and T3

	T0				T0 (E–C)			T1			
	E		C					E		C	
	Mean	SD	Mean	SD	Mean Diff	SD	*P*	Mean	SD	Mean	SD
Bone thickness (mm)											
Labial											
S1	0.4	0.5	0.7	0.3	−0.2	0.2	.195	0.4	0.5	0.6	0.4
S2	0.5	0.7	0.5	0.4	0.2	0.2	.962	0.4	0.5	0.4	0.5
S3	0.4	0.4	0.3	0.3	0.1	0.1	.197	0.3	0.4	0.3	0.6
Palatal											
S1	1.0	0.9	0.8	0.6	0.1	0.2	.822	0.7	0.7	0.6	0.7
S2	1.7	1.2	1.7	0.9	−0.1	0.3	.486	1.4	1.1	1.5	1.2
S3	2.9	1.3	2.8	1.2	−0.1	0.4	.764	2.7	1.3	2.8	1.5
Bone height (mm)											
Mesial	4.0	2.6	2.9	1.3	1.3	0.7	.261	3.7	2.1	2.8	0.7
Distal	4.1	2.2	2.9	0.9	1.3	0.6	.159	3.8	1.8	2.9	0.7
Labial	3.5	1.2	2.1	0.7	0.2	0.3	.962	3.9	1.8	2	0.7
Palatal	3.0	1.9	2.3	1.4	0.8	0.6	.164	3.5	1.9	3.4	2.4
Bone density (HU)											
Cortical											
Mesiolabial											
S1	2139	1646	2175	718	−273	296	.924	875	679	1164	316
S2	3002	1038	2133	727	−53	265	.800	1063	268	1164	237
S3	3369	755	2176	771	448	221	.043*	1357	293	1368	324
Mesiopalatal											
S1	2098	1608	1884	889	57	347	.874	860	656	983	338
S2	3039	1031	1982	743	486	285	.054	1206	200	1264	327
S3	3335	745	2028	697	654	243	.011*	1346	223	1343	284
Distolabial											
S1	2193	1695	2064	729	135	324	.106	753	588	1313	291
S2	3299	742	2129	745	500	258	.066	1181	187	1184	320
S3	3417	801	2166	847	739	267	.022*	1248	234	1290	453
Distopalatal											
S1	2116	1617	1882	952	144	391	.391	862	682	1061	386
S2	3043	1034	2074	716	283	246	.065	1256	253	1313	270
S3	3330	737	2042	775	641	239	.039*	1354	255	1321	317
Trabecular											
Mesial											
S1	1928	1469	1734	798	190	321	.319	315	284	479	307
S2	2700	870	1649	671	251	258	.569	391	137	477	247
S3	2898	562	1670	608	544	197	.033*	316	189	475	238
Distal											
S1	1813	1370	1638	768	138	289	.056	307	318	417	221
S2	2679	855	1621	710	492	260	.168	354	273	307	172
S3	2819	518	1570	662	827	224	.040*	528	336	330	183
Root length (mm)	12.6	1.0	12.7	1.7	−0.1	0.5	.892	11.2	3.1	11.9	1.6
RANKL/OPG											
Labial	0.32	0.27	0.39	0.18	−0.09	0.08	.288	0.64	0.52	0.55	0.17
Palatal	0.21	0.89	0.25	0.22	−0.05	0.07	.260	0.33	0.17	0.44	0.28

[a] E indicates experimental group; C, control group; SD, standard deviation.
* $P < .05$ was considered significant per Mann-Whitney *U*-test.

T1 (T3). Tooth mobility was assessed at all time points using Miller's classification, as Class 0, 1, 2, and 3.[13] CBCT images were obtained at T0, T1, and T3.

CBCT

Changes in alveolar bone were evaluated via CBCT (80 kV, 5 mA, 7.5-second exposure time, 0.125-mm voxel resolution, 80 × 40-mm field of view; Veraviewepocs J Morita MPG, Fushimi-ku, Kyoto, Japan). CBCT data were reconstructed at 0.125-mm increments.

Measurements were taken twice (≥4 weeks apart) by one investigator (P.P.) blinded to groups and time points as previously described for alveolar bone thickness,[14] density,[15] and height.[16] Alveolar bone thickness and density were measured at three levels

Table 2. Extended

T3				T1–T3 (E–C)		
E		C				
Mean	SD	Mean	SD	Mean Diff	SD	*P*
0.3	0.5	0.9	0.3	−0.4	0.1	.001*
0.4	0.5	0.7	0.5	−0.4	0.1	<.001*
0.2	0.3	0.6	0.5	−0.4	0.1	<.001*
0.6	0.7	0.7	0.6	−0.4	0.1	.001*
1.4	1.1	1.6	1.2	−0.3	0.2	.164
2.6	1.4	2.8	1.4	−0.4	0.1	.009*
3.7	2.1	2.8	0.7	0	0.1	.612
3.6	1.8	2.8	0.6	−0.3	0.4	.912
3.6	0.9	1.9	0.6	−0.1	0.5	.569
3.4	1.9	2.9	1.5	0.6	0.4	.874
896	671	1340	326	−208	80	.007*
1261	320	1388	234	−35	103	.681
1412	251	1543	260	−268	114	.021*
890	682	1146	422	−169	131	.062
1313	262	1227	432	144	115	.206
1286	298	1394	309	−167	112	.066
834	670	1340	335	15	104	.800
1217	262	1237	207	−90	90	.327
1305	255	1459	343	−156	103	.146
840	661	1244	327	−249	104	.017*
1359	242	1372	251	28	99	1.000
1367	196	1425	210	−127	87	.129
405	389	769	362	−153	164	.029*
564	342	745	269	−114	89	.114
500	317	767	301	−187	121	.206
320	315	690	380	−281	120	.009*
425	266	494	209	−185	92	.066
488	311	572	313	−378	124	.005*
11.9	1.4	11.9	1.6	0	0.1	.401
0.57	0.25	0.71	0.64	0.04	0.01	<.001*
0.23	0.12	0.36	0.18	0.03	0.01	.015

starting from 3 mm below the CEJ at intervals of 3 mm apically (S1, S2, and S3). Root length was measured from the CEJ to the apex in the sagittal view (Figure 2).

GCF Collection

GCF was collected from all incisors after plaque removal. The teeth were isolated with cotton rolls and gently dried as described by Lu et al.[17] with a slight modification. Sterile paper strips (Periopaper, OraFlow, New York, NY) were inserted into the gingival crevice at midlabial and midpalatal sites and left in situ for 60 seconds to collect GCF. The volume was quantitated using Periotron 8000 (Siemens Medical Systems, Inc, Malvern, Pa). GCF was extracted by placing pooled strips from each site into 180-μL phosphate-buffered saline (pH 7.2). Samples were incubated overnight, shaken gently for 15 minutes at 4°C, and centrifuged (3000 *g*) for 5 minutes at 4°C. The fluids were assayed in duplication using enzyme-linked immunosorbent assays (ELISAs) for RANKL and OPG (Quantikine R&D Systems Inc, Minneapolis, Minn) following the manufacturer's instructions.[6] Patient data were coded so that the examiner was unaware of the group and time point.

Statistical Analysis

The Shapiro-Wilk test was used to examine the normality of distributions of mean alveolar bone thickness, height and density, and RANKL/OPG. Statistical analysis was performed using R software (The Comprehensive R Archive Network, www.r-project.org). Statistical significance was defined as $P < .05$. Changes in alveolar bone thickness, height, and density within and between groups were evaluated using the Kruskal-Wallis and Wilcoxon signed-rank tests, respectively. The Kruskal-Wallis test was used for differences between the central and lateral incisors and RANKL/OPG ratio between groups, Friedman's test was used to evaluate differences in RANKL/OPG ratio at each time point, and Spearman's rank correlation analysis for correlations between changes in RANKL/OPG ratio between time points and changes in alveolar bone.

Reproducibility of bone height, thickness, and density measurements was assessed by calculating method error for replicate measurements made at least 4 weeks apart. Bone density measurements showed acceptable reliability (intraclass correlation coefficient[18] = 0.83) and bone height and thickness measurements, good reliability (0.94 to 0.99).

RESULTS

Forty-two female subjects were invited to participate; two declined and were offered alternative treatments; one did not meet the inclusion criteria. The remaining 39 (mean age, 42.3 ± 6.5 years; range, 32–57 years) were included. Twenty and 19 patients were randomized to the control and experimental groups, respectively. Three subjects were later excluded; two became pregnant and one was diagnosed with breast cancer during the study. At T3, each group had 18 subjects.

Mean (±SD) anterior biting frequency was not significantly different between the experimental (58 ±

11%) and control (56 ± 9%) groups (Mann-Whitney *U*-test). Frequency of biting the soft plastic roll in the experimental group was 92.3 ± 8.6%. Mean alveolar bone thickness, height, and density were not normally distributed (Shapiro-Wilk test). Alveolar bone thickness, height, and density, and RANKL/OPG ratio at the labial and palatal aspects were not significantly different between the central and lateral incisors; therefore, only the right central incisor was assessed for each patient.

The number of subjects in each group exhibiting different degrees of mobility at each time point is shown in Table 1. There was no significant difference in tooth mobility between the groups at any time point, though mobility increased in both groups from T0 to T1 and decreased from T1 to T3.

Initial mean differences (T0) in alveolar bone measurements and RANKL/OPG ratio between groups are shown in Table 2. There was a significant difference in alveolar bone density at S3 in all area ($P < .05$). There was no significant difference between the changes in alveolar bone thickness, height, and density, or RANKL/OPG ratio between the experimental and control groups from T0 to T1. The pooled group data revealed significant increases in palatal bone height ($P = .011$) and RANKL/OPG ratio (labial, $P = .002$; palatal, $P = .001$) and decreases in palatal bone thickness (S1, $P = .003$), bone density, and root length ($P < .001$) in both groups between T0 and T1 (Table 3).

With the exception of palatal bone thickness at S2, alveolar bone thickness was significantly increased in the experimental group compared with the control group between T1 and T3. Mesiolabial (S1, $P = .007$; S3, $P = .021$) and distopalatal (S1, $P = .017$) cortical alveolar bone density and mesial (S1, $P = .029$) and distal (S1, $P = .009$; S3, $P = .005$) trabecular bone density significantly increased in the experimental group compared with the control group between T1 and T3 (Table 2). Labial and palatal bone thickness increased significantly between T1 and T3 in the experimental group (labial, $P < .001$; palatal, $P < .01$), but not in the control group (Figure 3).

At T0, fenestrations and dehiscences were present in six, four, and eight cases (at S1, S2, and S3, respectively) in the experimental group and nine, seven, and six cases (at S1, S2, and S3, respectively) in the control group. After orthodontic tooth movement (T1), fenestrations and dehiscences were detected in 7, 8, and 13 cases (at S1, S2, and S3, respectively) in the experimental group and 8, 8, and 10 cases (at S1, S2, and S3, respectively) in the control group. After the biting period (T3), decreased fenestrations and dehiscences were observed in the experimental group compared with the control group (3, 2, and 3 in the experimental group versus 7, 8, and 10 in the control group, respectively).

Table 3. Mean Differences in Thickness, Height and Density of Alveolar Bone, and RANKL, OPG, and RANKL/OPG, T0–T1

	Mean Difference	SD[a]	*P*
Bone thickness (mm)			
Labial			
S1	0.0	0.1	.690
S2	0.1	0.1	.144
S3	0.1	0.1	.117
Palatal			
S1	0.2	0.1	.003*
S2	0.2	0.1	.071
S3	0.0	0.2	.475
Bone height (mm)			
Mesial	0.3	0.2	.177
Distal	0.3	0.2	.179
Labial	–0.2	0.3	.641
Palatal	–0.8	0.3	.011*
Bone density (HU)			
Cortical			
Mesiolabial			
S1	866	114	<.001*
S2	972	133	<.001*
S3	945	117	<.001*
Mesiopalatal			
S1	824	126	<.001*
S2	939	142	<.001*
S3	955	136	<.001*
Distolabial			
S1	974	144	<.001*
S2	1191	135	<.001*
S3	1209	166	<.001*
Distopalatal			
S1	883	133	<.001*
S2	874	123	<.001*
S3	991	139	<.001*
Trabecular			
Mesial			
S1	1339	143	<.001*
S2	1301	132	<.001*
S3	1479	135	<.001*
Distal			
S1	1262	128	<.001*
S2	1526	139	<.001*
S3	1543	122	<.001*
Root length	0.7	0.1	<.001*
RANKL/OPG			
Labial	–0.27	0.08	.002*
Palatal	–0.17	0.05	.001*

[a] SD indicates standard deviation.
* $P < .05$ was considered significant per Wilcoxon signed-rank test.

A post hoc test revealed a significant increase in the RANKL/OPG ratio between T0 and T1 at both sites in both groups and between T2 and T3 at the labial site in the experimental group. There was a significant decrease in RANKL/OPG ratio between T1 and T2 at both sites in the experimental group (Figure 4).

The change in labial RANKL/OPG ratio between T1 and T2 correlated negatively with the change in labial

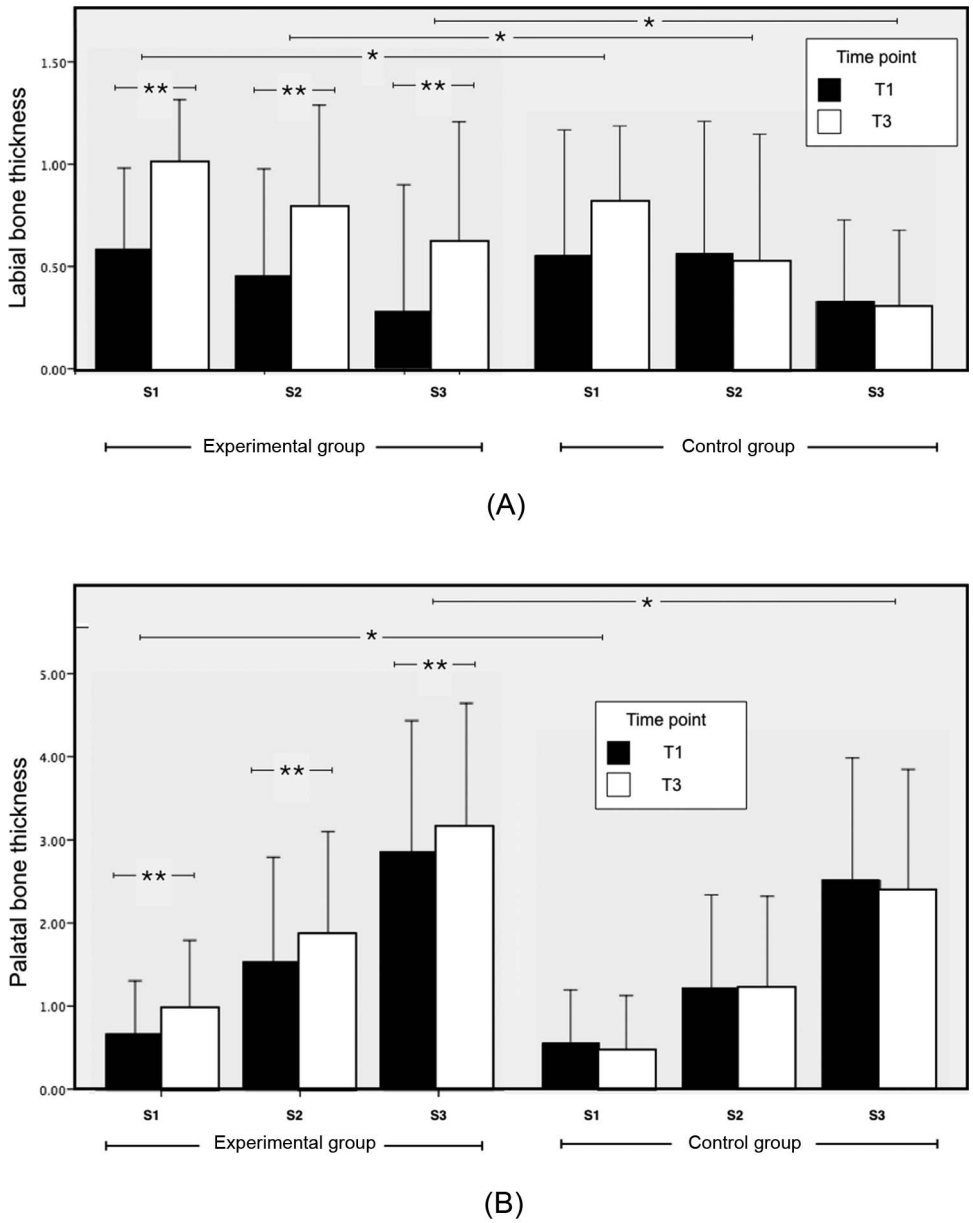
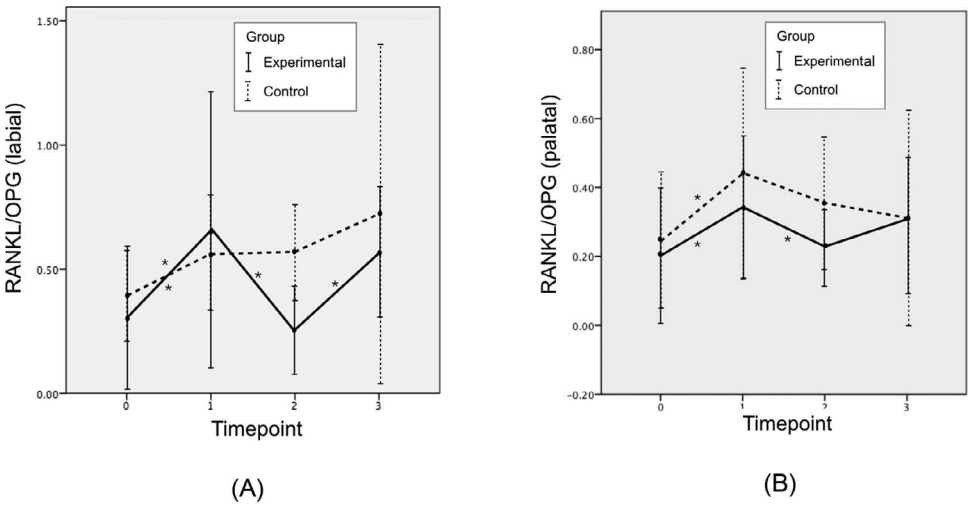


Figure 3. Mean (±standard deviation) difference in alveolar bone thickness at (A) labial and (B) palatal sites between T1 and T3.

alveolar bone thickness (S1, $P < .001$; S2, $P = .009$) between T1 and T3. The change in palatal RANKL/OPG ratio between T1 to T2 correlated negatively with the change in palatal alveolar bone thickness (S1, $P = .038$; S2, $P = .048$) between T1 and T3 (Table 4).

DISCUSSION

Pathologic tooth migration can result in hypofunctional conditions requiring orthodontic treatment to obtain normal overjet, overbite, interincisal angle, and function. The subjects in this study regained normal function after the anterior teeth were repositioned in occlusion combined with normal biting and eating activity. However, bone thickness increased significantly more in the experimental group instructed to perform biting exercises. Animal studies indicate that occlusal stimuli help to maintain functional alveolar structure and regulate alveolar bone remodeling.[2] Therefore, biting on the front teeth may lead to a functional improvement and stimulate alveolar bone remodeling by decreasing bone resorption, as reflected by the reduced RANKL/OPG ratio. Bone remodeling is



T0, pretreatment; T1, end of treatment, T2, 1 month after T1; T3 7 months after T1
Wlicoxon signed-rank test, *P<0.05

Figure 4. Mean (±standard deviation) RANKL/OPG ratio in GCF in control and experimental groups at (A) labial and (B) palatal sites between T0 and T3.

controlled by the balance between RANK, RANKL, and OPG. The RANKL/OPG ratio increases during orthodontic treatment; orthodontic force induces osteoclastogenesis by upregulating RANKL.[5] The RANKL/OPG ratio increased between T0 and T1 in both groups due to orthodontic treatment. Conversely, a reduced RANKL/OPG ratio was reported to inhibit the terminal stages of osteoclast differentiation, suppress matrix osteoclast activation, and induce apoptosis in human periodontal ligament cells.[19] The reduction in the RANKL/OPG ratio in the experimental group between T1 and T2 may have been due to discontinuation of tooth movement or bone formation in response to the biting exercises. The RANKL/OPG ratio was not significantly different between T1 and T2 in the control group, implying that the decrease in the RANKL/OPG ratio in the experimental group was associated with induction of bone formation. It should be noted that factors that affect the level of RANKL in GCF are gender and the subject's menopause status. These factors have been associated with baseline RANKL levels but not with the RANKL response to orthodontic activation.[7] Accordingly, female subjects whose menopause status changed during the study period were excluded.

Table 4. Correlation Between the RANKL/OPG Ratio and Alveolar Bone Thickness

	Bone Thickness (T1–T3)					
	Labial			Palatal		
	S1	S2	S3	S1	S2	S3
RANKL/OPG						
Labial						
T1–T2						
r	−.555*	−.431*	−.245	−.246	−.396*	−.200
P	<.001	.009	.150	.148	.017	.242
T2–T3						
r	−.188	−.243	−.220	−.189	−.444*	−.317
P	.2773	.154	.197	.271	.007	.059
Palatal						
T1–T2						
r	.215	.069	.097	−.417*	−.332*	.062
P	.209	.689	.574	.038	.048	.718
T2–T3						
r	.311	.332*	.238	.310	.302	.202
P	.064	.048	.162	.066	.074	.239

* $P < .05$ was considered significant per Spearman's rank correlation.

A significant correlation only between RANKL/OPG ratio and bone thickness was observed in this study. Rehabilitation of masticatory function significantly improved alveolar bone architecture, including bone density, in adult rats.[3] These differences may be due to continuous bone remodeling, the difference in baseline bone density between groups, and use of a relatively low-sensitivity measurement technique. We measured bone density in gray scale units from CBCT images and converted them into Hounsfield Units (HUs). However, the conversion process needs to be addressed when comparing mineral density under different conditions.[19] Accordingly, the validity of measuring bone density using CBCT needs to be validated further. In summary, RANKL and OPG may be suitable diagnostic biomarkers for early detection of alveolar bone changes at S1 and S2. However, the correlation between RANKL/OPG ratio and bone thickness was not significant at S3 (labial site, $P = .150$; palatal site, $P = .718$). This may have been due to the fact that S3 was more apical to the gingival crevice from where the

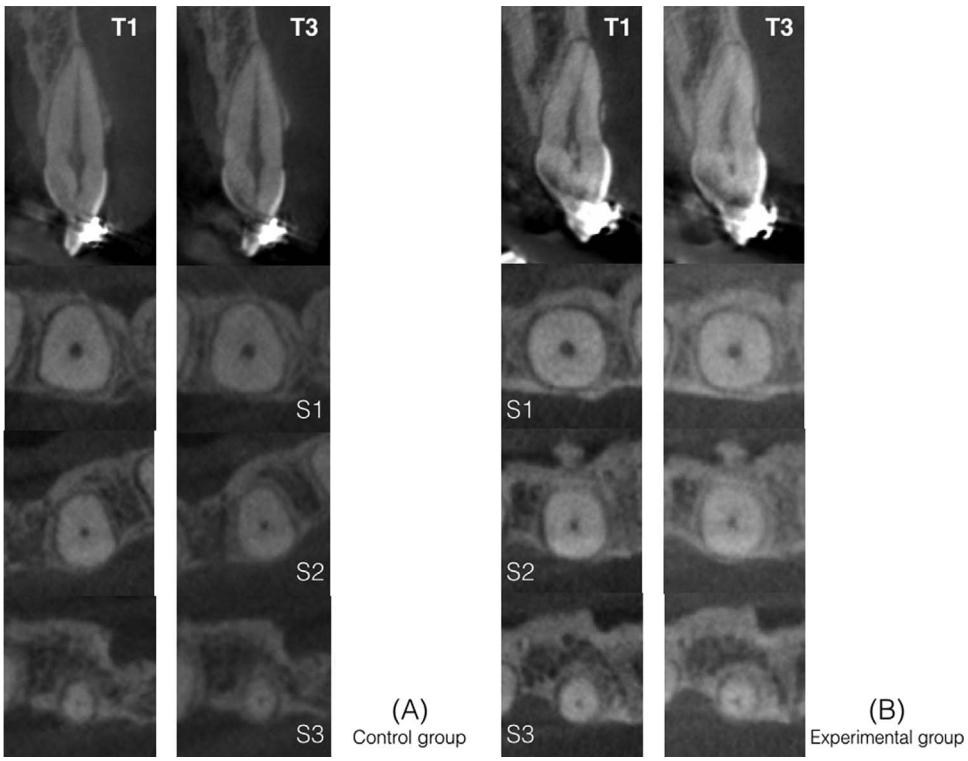


Figure 5. CBCT of treated teeth showing fenestration remaining in a patient from the control group (A) and absence of fenestration in a patient from the experimental group (B) at T1 to T3.

GCF was collected, which was a limitation of this approach.

Orthodontic treatment accompanied by regular periodontal maintenance did not result in decreased alveolar bone height. However, several outcome measures changed between T0 and T1 (Table 2). First, cortical and trabecular bone density decreased between T0 and T1 in both groups. Yu et al.[20] previously demonstrated that alveolar bone density is usually reduced during orthodontic treatment, but recovers by 80% during retention. Second, the extent of root shortening observed (0.7 $\pm$ 0. 6 mm) between T0 and T1 was lower than in previous studies: Baumrind et al.[21] reported root resorption of 1.4 $\pm$ 1.5 mm. The subjects in the current study had marginal alveolar bone loss and were treated carefully using light forces, which may have resulted in less root resorption than in studies employing higher forces. Third, the fenestrations that occurred during orthodontic treatment (T0–T1) were reduced in the experimental group performing biting exercises, but remained in the control group (Figure 5). Consequently, biting exercises can be recommended before debanding, though strict periodontal maintenance is required.[22] Last, the degree of tooth mobility significantly increased between T0 and T1 and decreased between T1 and T3 in both groups. Tanaka et al.[23] reported that teeth could be more mobile during orthodontic treatment, but mobility decreased during retention. Miller's tooth mobility measurement[13] is often used routinely in the clinic, but its accuracy depends on the operator's tactile sense. A tooth mobility measuring device, such as the Periotest, should be considered for future studies.

Bone remodeling occurs continuously, even after tooth movement stops.[24] The alveolar bone changes observed in this study could have been the result of orthodontic bone remodeling or functional rehabilitation. A rest period after orthodontic tooth movement could have been incorporated to allow bone remodeling to have been completed before the biting/no-biting period was started. However, this would have delayed treatment. Therefore, the control group was recruited to compare with subjects having a similar course of tooth movement but without biting exercises.

The limitations of this study should be considered. CBCT is unable to produce sufficiently high-resolution images for fine measurements of bone density, which raises questions about the reliability and accuracy of this method. Second, compliance with prescribed biting exercises was self-reported by the experimental group; methods to measure compliance could be considered (eg, observing changes in roughness of the biting roll material, assessing masticatory muscle activity via electromyography). Finally, the biting area and force were not controlled. However, the soft plastic roll could

be individually modified to ensure simultaneous biting of all incisors and the biting forces could be measured.

CONCLUSIONS

- Biting exercises during orthodontic treatment to restore incisor function induced alveolar bone thickening, but were not associated with significant differences in tooth mobility, bone height, or density compared with subjects who did not perform biting exercises.
- The RANKL/OPG ratio decreased in the month following restoration of occlusal function and correlated negatively with increased bone thickness.

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