Review Article

The Correlation of Replicating Cells and Osteogenesis in the Condyle During Stepwise Advancement

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Abstract: The aim of this study was to quantify the number of replicating mesenchymal cells and to correlate it to the amount of bone formation in the condyle during stepwise advancement of the mandible. Two hundred and fifty female Spraque-Dawley rats, 35 days old, were randomly divided into 10 control groups (n = 5) and 20 experimental groups (n = 10). Fifty rats from the stepwise experimental group relieved a two-mm advancement initially and veneers were added on day 30 with another 1.5 mm advancement. The rats were sacrificed after 3, 7, 14, 21, 30, 33, 37, 44, 51, and 60 days. One hour before death, all rats were injected with bromodeoxyuridine (BrdU) intravenously. Tissue sections of seven μm were cut through the condyle in the sagittal plane and stained with anti-BrdU antibody to evaluate the number of replicating mesenchymal cells. Haematoxylin stain was applied to observe cellular response. The results indicated that during the first advancement, replicating mesenchymal cells in the posterior region of the condyle showed the highest increase on days 7 and 14 when compared with the control. Such an increase preceded the highest level of bone formation between days 30 and 37 of advancement. In response to the second advancement, another increase of replicating cells was evident on day 44, along with a significant increase in bone formation observed on day 60. We concluded that forward positioning of mandible in a stepwise manner delivers a mechanical strain that solicits an increase in the number of replicating mesenchymal cells in the condyle. The increase in the population size of the osteoprogenitor cells subsequently leads to more bone formation. (*Angle Orthod* 2003;73:457–465.)

Key Words: Bone formation; Condyle; Osteoprogenitor cells; Stepwise advancement

INTRODUCTION

Mesenchymal cells give rise not only to embryonic bone but also to the continuous supply of osteogenic cells required for bone remodeling and fracture repair throughout adulthood.[1] This critical role of mesenchymal cells in bone growth gives them a particular importance in the field of growth modification in orthodontics. The importance of these mesenchymal cells lie in their high self-renewal capacity and their potential to produce a whole host of differentiated cells, in particular osteoblasts and chondroblasts, which are the precursors of bone and cartilage, respectively.[2]

During natural growth of the mandible, the amount of replicating cells in the posterior region of the condyle was observed to be significantly higher compared with the anterior and middle regions.[3] Interestingly, the amount of bone formation in the condyle was measured to be more in the posterior region when compared with its middle and anterior counterparts. Moreover, forward mandibular positioning solicited a greater cellular response in the rat condyle compared with the natural growth especially in the posterior region, whereby a significant increase in the number of replicating mesenchymal cells preceded a significant increase in the amount of newly formed bone.[3,4] Accordingly, a direct correlation exists between the number of mesenchymal cells[3,5] and the amount of bone formed[4] in the condyle. Mesenchymal cells play a critical role in bone

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growth with the size of the population of mesenchymal cells directly influencing the number of osteoblasts available to form bone.[1] The inference is that the more mesenchymal cells in a given site, the more bone is the forming capacity at that site. Such a correlation was the foundation of auto-transplantation of mesenchymal cells for the repair of bone defects when other clinical strategies failed.[6] The number of mesenchymal cells significantly increase when the mandible is positioned forward.[3] Mandibular advancement produces stretching of the posterior fibers and the net effect of this mechanical strain brings about an increase in the number of replicating mesenchymal cells to the site.[7] Therefore, growth of the mandible could be influenced to a greater extent by advancing the mandible forward in a stepwise manner to recruit a greater number of replicating cells to the site. Clinical studies have shown[8] that if a mandibular advancement is periodically positioned forward, then a greater increase in condylar growth and mandibular length could be achieved.

Thus the objectives of our study were

- To identify and quantify the number of replicating mesenchymal cells during stepwise advancement and to compare it with the one-step advancement.
- To identify and correlate the number of mesenchymal cells to the amount of bone formed during stepwise advancement and one-step advancement.

MATERIALS AND METHODS

Two hundred and fifty rats were randomly divided into 20 experimental groups (n = 10 animals) and 10 control groups (n = 5 animals). One hundred rats were randomly selected to wear the one-step bite-jumping appliance and 100 to wear the stepwise bite-jumping appliance. Each of the groups was divided into 10 subgroups to be sacrificed on days 3, 7, 14, 21, 30, 33, 37, 44, 51, and 60. Fifty rats from the stepwise experimental group had the stepwise veneer advancement added on day 30. The one-step appliance rats had 3.5 mm advancement from the incisal edge in the sagittal plane and three mm inferior displacement of the mandible. The stepwise rats had two mm of initial advancement, and veneers were added on day 30 with another 1.5 mm of advancement. The animals wore the appliances full time because they were cemented in place using the method described by Rabie et al.[9]

To detect active cell proliferation, the rats were injected intravenously with a thymidine analog—bromodeoxyurindine (BrdU)—at a dose of 20 mg per kg body weight one hour before sacrifice. Sections were made from each condyle and were immunostained with BrdU. Leica Qwin computer system was used to analyze and compare the results between the control and the experimental rats on the specific days.

TABLE 1. Results of Method Error for Digitization of Replicating Cells with Computer-Assisted Image Analysis System

Mean of the Differences	SD of the Differences	*P*	Size of Method Error (mm^2)
0.158	0.254	.08	0.204

Detection of actively replicating cells

Actively replicating cells were detected using Monoclonal Anti-BrdU (Sigma Code B2531, 1:15 diluted with normal rabbit serum) according to the manufacturer's instructions. The distinctive brown stain produced allowed observation of the actively replicating cells.

Quantitative analysis

The number of replicating cells and the amount of new bone formation in the anterior, middle, and posterior regions of the condyle were measured with a true color RGB (red-green-blue) computer-assisted imaging analyzing system with Leica Qwin Pro software (Version 2.2). The system acquires high-definition digital images of the specimen, and features from the acquired images are selected by the operator and recognized by color, shape, and contrast. The expression of replicating cells and bone formation were quantified by measuring the area of signals under a fixed measuring frame. The data was processed with GraphPad InStat (Version 3.00, GraphPad Software Inc, San Diego, California) for both *t*-test and analysis of variance with Bonferroni multiple comparisons test. The size of the method error in digitizing the replicating cells area was calculated by the formula $\pm \sqrt{\Sigma d^2/2n}$, where d is the difference between the two registrations of a pair and n the number of double registrations. Ten of 250 sections were randomly drawn and digitized on two separate occasions. A paired *t*-test was also performed to compare the two registrations. Hypothesis testing indicated no significant difference among the duplicate registrations ($P = .08$; Table 1) of the 10 randomly sections.

RESULTS

The current study shows that the replicating cells in the posterior region of the condyle increased significantly both when the mandible was advanced in a single step manner and in a stepwise manner when compared with their level of replication during natural growth (Figure 1). One step resulted in a significant increase in the number of replicating cells ($P < .001$) on the initial days of advancement (days 3, 7, 14, and 21), followed by a lower level of replicating cells from days 30 to 60 ($P < .05$) (Figure 1). On the other hand, in the stepwise group, there was a significant increase in the number of replicating cells ($P < .01$) in response to the second advancement, whereas the one-step group showed a decline.

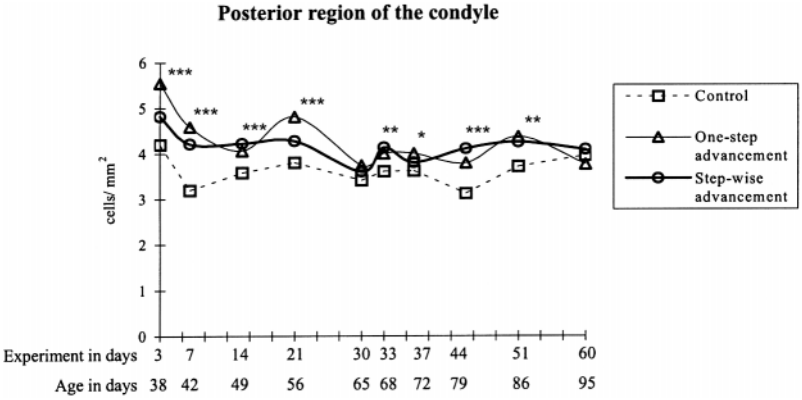

FIGURE 1. The temporal pattern of replicating cells in the posterior regions of the condyle from day 3 to day 60 in the control group, one-step advancement, and stepwise advancement.

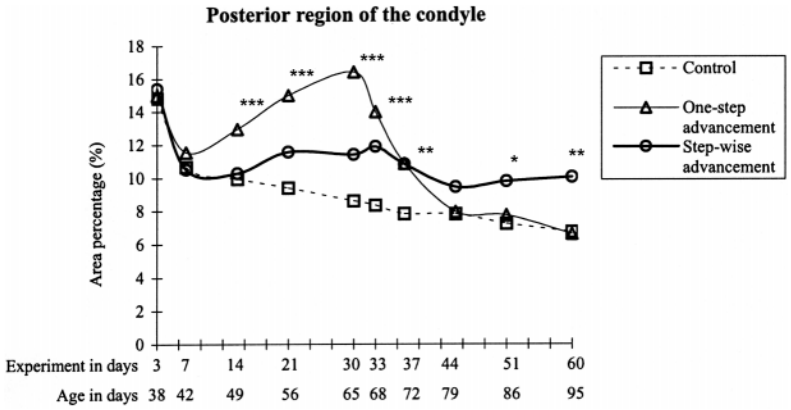

FIGURE 2. The temporal pattern of bone formation in the posterior regions of the condyle from day 3 to day 60 in the control group, one-step advancement, and stepwise advancement.[4]

The amount of bone formed in response to the one-step advancement showed a significant increase ($P < .0001$) on days 14, 21, 30, and 33 followed by a decline to levels equal to that formed during natural growth (Figure 2).

The stepwise group, on the other hand, showed a significant amount of newly formed bone in response to the second advancement on days 33, 37, 44, 51, and 60 (Figure 2, Appendix Table 2.1).

DISCUSSION

Generally mesenchymal cells are pleuropotential progenitor cells that possess multilineage developmental potential to differentiate into chondrogenic or osteogenic cells.[10] Many researchers have found that undifferentiated mesenchymal cells in the condyle serve as the main source of replicating cells for growth of the condylar cartilage.[11–13] Hence, mesenchymal cells play a critical role in the field of growth modification in orthodontics.

The size of the mesenchymal cell population directly affects the number of osteoblasts available to form bone.[1] This indicated that the more the mesenchymal cells in a given site, the more is the bone-forming capacity at the site. Therefore, it was important to determine the number of mesenchymal cells in the condyle when the mandible is positioned forward in a stepwise manner and to compare it to the number present during a single-step advancement. Thus, we can provide a better understanding of the tissue responses to different clinical modalities of treatment of class-II growth modification.

Results of the present study demonstrated that the stepwise advancement produces a different pattern of replicating mesenchymal cells when compared with single-step advancement (Figures 1 and 3). During the initial step of the stepwise advancement, the replicating mesenchymal cells in the posterior region of the condyle showed the highest level of increase when compared with controls on days 7 (31%) followed by day 14 (18%) (Figure 1). Such an increase preceded the highest level of new bone that was formed between days 30 and 37 of advancement (Figure 2). Such a temporal pattern could be explained on the basis of osteogenic lineage. Mesenchymal cells differentiate into chondrocytes, which engage in cartilage matrix formation.[10] Cartilage is then invaded by blood vessels, and endochondral ossification begins.[14] Therefore, the first advancement

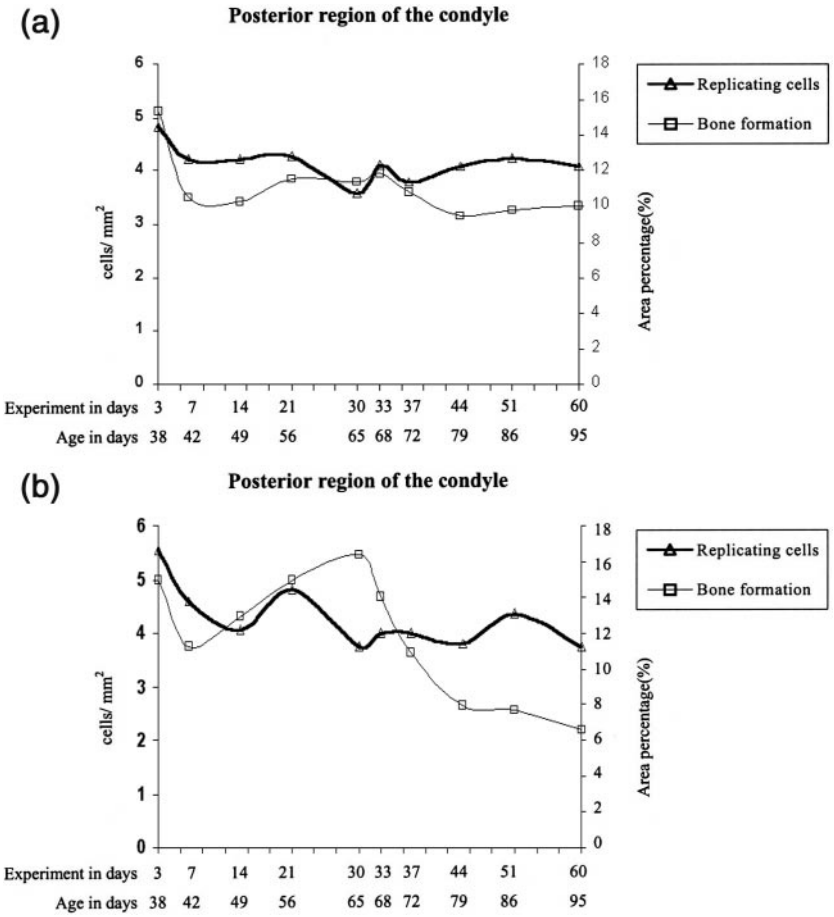

FIGURE 3. (A) Comparison between replicating cells and bone formation in the posterior regions of the condyle from day 3 to day 60 in stepwise advancement. (B) Comparison between replicating cells and bone formation in the posterior regions of the condyle from day 3 to day 60 in single-step advancement.

resulted in significant increase in the number of replicating mesenchymal cells between days 7 and 21 of advancement followed by a significant increase in new bone formation between days 30 and 37 of advancement (Figure 3).

On day 30 of the experiment, the second advancement took place, and it resulted in a similar pattern to that noted during the first advancement (Figures 1 and 3). The highest level of replicating mesenchymal cells was reached 7 and 14 days after each advancement, that is, a 31.61% increase after the first advancement and a 31.45% increase after the second advancement. The levels of bone formation triggered by the second advancement between day 30 and 60 showed a significant increase when compared with both one-step and natural growth group (Figure 2). These results again support and demonstrate that the number of replicating mesenchymal cells in a given site is directly proportional to the bone formation at that site.

It is important to interpret the clinical results of stepwise advancement in the light of the present data. Du et al[7] reported a greater increase in skeletal growth of the mandible when mandibular advancement was carried out in a stepwise manner. The current results provide a good explanation where mechanical strain produced by mandibular advancement solicits an increase in the number of replicating mesenchymal cells, which increases the size of the osteoprogenitor cell population and subsequently leads to more bone. This response is repeated, to a lesser extent, with the subsequent advancements where the amount of bone formation 60 days after advancement was 49% more than that found during natural growth and during one-step advancement (Figure 2).

Another important issue in the present study is the pattern of bone formation in response to single-step advancement vs stepwise advancement (Figure 2). In the single-step advancement group, the posterior region of the condyle contained twice as many replicating mesenchymal cells as those present in response to the initial advancement in the stepwise group. During the stepwise advancement, the level of new bone formation in response to the initial advancement was half as much as the level of new bone formed in the one-step advancement (Figure 2). This could be due to the difference between the 3.5 mm advancement in the one-step group vs two mm initial advancement in the stepwise group. These results lend support to the earlier work by

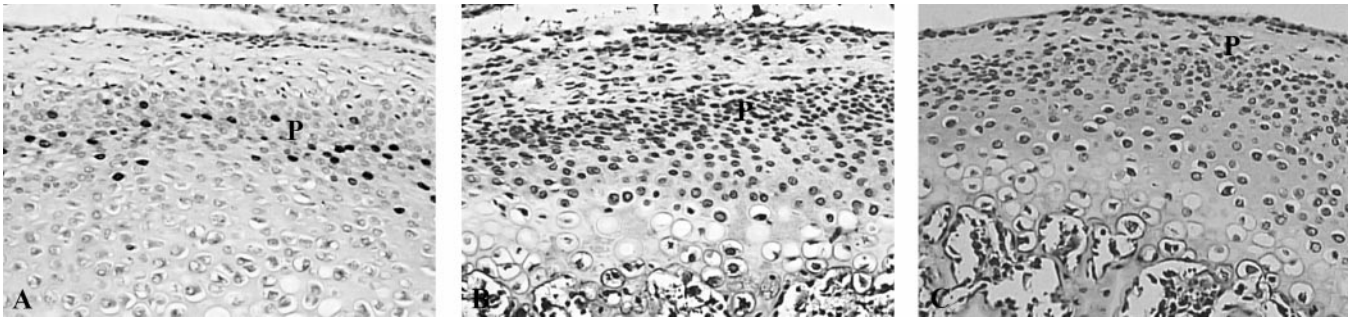

FIGURE 4. Photomicrographs showing cellular changes in the posterior region of condyle (BrdU immunostaining and hematoxylin stain; original magnification, ×360). (A) Control group, (B) one-step advancement, and (C) stepwise advancement on day 7; Proliferative zone (P).

Frost[15] where he postulated that a prerequisite for bone formation to occur is that the mechanical strain must first surpass a minimum threshold value.[15]

The maximum level of bone formation in the single-step advancement group was reached 30 days after advancement followed by a decline to levels equal to those expressed during natural growth between days 44 and 60 (Figure 2). Such a pattern could be explained on the basis that, in the single step advancement, the differentiation of mesenchymal cells to chondroblasts or osteoblasts curtails the population size because, once differentiated, they loose their replication ability.[16] Therefore, they go back to the levels of bone formation expressed during natural growth from day 44 to day 60. In contrast, the second advancement in the stepwise manner recruits more blood vessels[14] leading to more mesenchymal cells because of yet another cycle of mechanical stimulation leading to considerably more new bone when compared with both single-step advancement and natural growth.[4]

CONCLUSIONS

Stepwise advancement leads to a significant increase in the number of replicating mesenchymal cells that is closely related to the increase in the level of bone formation, thus increasing the growth potential of the condyle. This study provided an explanation of some tissue responses to stepwise advancement of the mandible to correct class-II malocclusion.

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APPENDIX

TABLE 1.1. Comparison of Amount of New Replicating Cells in One-Step Advancement Groups (OS) and Stepwise Advancement Groups (SW) vs that in Control Groups at Day 3 through 60

Day	Control (Cells Per Area)	OS (Cells Per Area)	SW (Cells Per Area) Analysis of Variance	Bonferroni Multiple Comparisons Test
3 (38 days old)	4.20	5.55	4.82***	Control vs OS***
				Control vs SW*
				OS vs SW*
7	3.20	4.59	4.21***	Control vs OS***
				Control vs SW***
				OS vs SW*
14	3.58	4.06	4.22***	Control vs OS***
				Control vs SW**
				OS vs SW*
21	3.80	4.81	4.27***	Control vs OS***
				Control vs SW**
				OS vs SW**
30	3.41	3.74	3.60	Control vs OS
				Control vs SW
				OS vs SW
33	3.61	4.00	4.13**	Control vs OS*
				Control vs SW**
				OS vs SW
37	3.62	4.00	3.80*	Control vs OS
				Control vs SW*
				OS vs SW
44	3.12	3.79	4.10***	Control vs OS**
				Control vs SW***
				OS vs SW
51	3.71	4.37	4.25**	Control vs OS**
				Control vs SW*
				OS vs SW
60	3.95	3.76	4.08	Control vs OS
				Control vs SW
				OS vs SW

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 1.2. Comparison of Amount of New Replicating Cells in Protrusion Groups (OS) vs that in Control Groups at Day 3 through 60

Day		Posterior (Cells Per Area)	%
3	Control	4.20	
	OS	5.55	
	Difference	1.35	32.14***
7	Control	3.20	
	OS	4.59	
	Difference	1.39	43.44***
14	Control	3.58	
	OS	4.06	
	Difference	0.48	13.41***
21	Control	3.80	
	OS	4.81	
	Difference	1.01	26.58***
30	Control	3.41	
	OS	3.74	
	Difference	0.33	9.68
33	Control	3.61	
	OS	4.00	
	Difference	0.39	10.80*
37	Control	3.62	
	OS	4.00	
	Difference	0.38	10.50
44	Control	3.12	
	OS	3.79	
	Difference	0.67	21.47**
51	Control	3.71	
	OS	4.37	
	Difference	0.66	17.79**
60	Control	3.95	
	OS	3.76	
	Difference	−0.19	−4.81

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 1.3. Comparison of Amount of New Replicating Cells in Protrusion Groups (SW) vs that in Control Groups at Day 3 through 60

Day		Posterior (Cells Per Area)	%
3	Control	4.200	
	SW	4.819	
	Difference	0.619	14.75*
7	Control	3.200	
	SW	4.211	
	Difference	1.011	31.61***
14	Control	3.580	
	SW	4.221	
	Difference	0.641	17.91**
21	Control	3.800	
	SW	4.273	
	Difference	0.473	12.44**
30	Control	3.410	
	SW	3.596	
	Difference	0.186	5.45
33	Control	3.610	
	SW	4.128	
	Difference	0.518	14.34**
37	Control	3.620	
	SW	3.801	
	Difference	0.181	5.01*
44	Control	3.120	
	SW	4.101	
	Difference	0.981	31.45***
51	Control	3.710	
	SW	4.248	
	Difference	0.538	14.51*
60	Control	3.950	
	SW	4.084	
	Difference	0.134	3.39

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 1.4. Comparison of Amount of New Replicating Cells in One-Step Groups (OS) vs that in Stepwise Groups (SW) at Day 3 through 60

Day		Posterior (Cells Per Area)	%
3	OS	5.550	
	SW	4.819	
	Difference	−0.731	−13.17*
7	OS	4.590	
	SW	4.211	
	Difference	−0.379	−8.25*
14	OS	4.060	
	SW	4.221	
	Difference	0.161	3.97*
21	OS	4.810	
	SW	4.273	
	Difference	−0.537	−11.17**
30	OS	3.740	
	SW	3.596	
	Difference	−0.144	−3.86
33	OS	4.000	
	SW	4.128	
	Difference	0.128	3.19
37	OS	4.000	
	SW	3.801	
	Difference	−0.199	−4.96
44	OS	3.790	
	SW	4.101	
	Difference	0.311	8.21
51	OS	4.370	
	SW	4.248	
	Difference	−0.122	−2.79
60	OS	3.760	
	SW	4.084	
	Difference	0.324	8.61

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 2.1. Comparison of Amount of New Bone Formation in One Step Advancement Groups (OS), Stepwise Advancement Groups (SW) vs that in Control Groups at Day 3 through 60

Day	Control (Area Percentage)	OS (Area Percentage)	SW (Area Percentage) Analysis of Variance	Bonferroni Multiple Comparisons Test
3 (38 days old)	14.82	14.98	15.40	Control vs OS Control vs SW OS vs SW
7	10.67	11.26	10.51	Control vs OS Control vs SW OS vs SW
14	9.95	12.95	10.27***	Control vs OS Control vs SW*** OS vs SW
21	9.41	14.99	11.55***	Control vs OS Control vs SW*** OS vs SW
30	8.61	16.41	11.40***	Control vs OS* Control vs SW*** OS vs SW*
33	8.34	14.00	11.89***	Control vs OS** Control vs SW*** OS vs SW
37	7.83	10.86	10.82**	Control vs OS** Control vs SW*** OS vs SW
44	7.82	7.94	9.45	Control vs OS Control vs SW OS vs SW
51	7.24	7.73	9.80*	Control vs OS* Control vs SW OS vs SW
60	6.74	6.62	10.05**	Control vs OS** Control vs SW OS vs SW**

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 2.2. Comparison of Amount of New Bone Formation in Protrusion Groups (OS) vs Control Groups at Day 3 through 60

Day		Posterior (Area Percentage)	%
3	Control	14.82	
	OS	14.98	
	Difference	0.15	1.04
7	Control	10.67	
	OS	11.26	
	Difference	0.59	5.58
14	Control	9.95	
	OS	12.95	
	Difference	2.99	30.07***
21	Control	9.41	
	OS	14.99	
	Difference	5.57	59.25***
30	Control	8.61	
	OS	16.41	
	Difference	7.80	90.56***
33	Control	8.34	
	OS	14.00	
	Difference	5.66	67.82***
37	Control	7.83	
	OS	10.86	
	Difference	3.03	38.66***
44	Control	7.82	
	OS	7.94	
	Difference	0.12	1.56
51	Control	7.24	
	OS	7.73	
	Difference	0.49	6.84
60	Control	6.74	
	OS	6.62	
	Difference	−0.12	−1.79

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 2.3. Comparison of Amount of New Bone Formation in Protrusion Groups (SW) vs that in Control Groups at Day 3 through 60

Day		Posterior (Area Percentage)	%
3	Control	14.82	
	SW	15.40	
	Difference	0.58	3.89
7	Control	10.67	
	SW	10.51	
	Difference	−0.16	−1.51
14	Control	9.95	
	SW	10.27	
	Difference	0.32	3.22
21	Control	9.41	
	SW	11.55	
	Difference	2.14	22.77
30	Control	8.61	
	SW	11.40	
	Difference	2.79	32.34*
33	Control	8.34	
	SW	11.89	
	Difference	3.55	42.54**
37	Control	7.83	
	SW	10.82	
	Difference	2.99	38.2**
44	Control	7.82	
	SW	9.45	
	Difference	1.63	20.86
51	Control	7.24	
	SW	9.80	
	Difference	2.57	35.48*
60	Control	6.74	
	SW	10.05	
	Difference	3.31	49.02**

* $P < .05$; ** $P < .01$; *** $P < .001$.

TABLE 2.4. Comparison of Amount of New Bone Formation in One-Step Groups (OS) vs that in Stepwise Groups (SW) at Day 3 through 60

Day		Posterior (Area Percentage)	%
3	OS	14.98	
	SW	15.40	
	Difference	0.42	2.83
7	OS	11.26	
	SW	10.51	
	Difference	−0.76	−6.71
14	OS	12.95	
	SW	10.27	
	Difference	−2.67	−20.64
21	OS	14.99	
	SW	11.55	
	Difference	−3.44	−22.91
30	OS	16.41	
	SW	11.40	
	Difference	−5.01	−30.55*
33	OS	14.00	
	SW	11.89	
	Difference	−2.11	−15.06
37	OS	10.86	
	SW	10.82	
	Difference	−0.04	−0.33
44	OS	7.94	
	SW	9.45	
	Difference	1.51	19.01
51	OS	7.73	
	SW	9.80	
	Difference	2.07	26.8
60	OS	6.62	
	SW	10.05	
	Difference	3.42	51.73**

* $P < .05$; ** $P < .01$; *** $P < .001$.