

Histopathological, Histochemical and Histomorphometrical Investigation of the Effects of Different Piezosurgery Tools on Tissues: Experimental Study

Farklı Piezocerrahi Aletlerinin Dokular Üzerine Olan Etkisinin Histopatolojik, Histokimyasal ve Histomorfometrik Olarak İncelenmesi: Deneysel Çalışma

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ABSTRACT Objective: Piezoelectric surgical instruments are widely used in medicine and dentistry. Devices with new features are produced day by day and offered to surgeons. The aim of this study is to evaluate the effects of new and old generation piezosurgery instruments and conventional rotary hand tools on bone after osteotomy histopathologically, histochemically and histomorphometrically. **Material and Methods:** In the parietal bones of 12 rabbits, bone defects with a diameter of 8 mm were created with two different piezosurgery tools and a conventional rotary tool. Animals were sacrificed on the 7th and 21st days and evaluated histopathologically, histochemically and histomorphometrically the amounts of new bone formation, sequestration, connective tissue, fibrin and cartilage and percentages of full-empty area in the total area. **Results:** In the histopathological examinations, the least sequestra formation was observed in the new generation piezosurgery group. In the histomorphometric analysis, it was determined that there was a statistically significant difference between the mean of new bone and total area measurements in all groups on the 7th day ($p<0.05$) but there was no statistically significant difference between the mean and medians of the values in all groups on the 21st day ($p>0.05$). **Conclusion:** As a result of the histopathological evaluation, it was determined that filling (both bone and connective tissue) in the defect areas in the new generation piezosurgery group was higher in the short term follow up compared to other groups, but there was no significant difference between the groups in the midterm follow up.

Keywords: Bone healing;
oral and maxillofacial surgery;
osteotomy; piezoelectric surgery; rabbit

ÖZET Amaç: Piezoelektrik cerrahi aletleri tıpta ve diş hekimliğinde oldukça yaygın olarak kullanılmaktadır. Günbegün yeni özelliklere sahip cihazlar üretilmekte ve piyasaya sürülmektedir. Bu çalışmanın amacı; yeni ve eski nesil piezocerrahi aletleri ile konvansiyonel döner el aletinin osteotomi sonrası kemik üzerindeki etkilerini histopatolojik, histomorfometrik ve histokimyasal olarak değerlendirmektir. **Gereç ve Yöntemler:** On iki adet tavşanın parietal kemiklerinde, 2 farklı piezocerrahi aleti ve geleneksel bir döner alet ile 8 mm çapında kemik defektleri oluşturuldu. Hayvanlar 7 ve 21. günlerde kurban edilerek; histopatolojik, histokimyasal ve histomorfometrik olarak yeni kemik oluşumu, sekestr, bağ doku, fibrin ve kartilaj miktarları ile toplam alan içerisindeki dolu-boş alan yüzdeleri değerlendirildi. **Bulgular:** Histopatolojik incelemelerde, en az sekestr oluşumu yeni nesil piezocerrahi grubunda gözlemlendi. Histomorfometrik analizde, 7. günde tüm gruplarda yeni kemik oluşum miktarı ile toplam alan ölçümleri arasında istatistiksel olarak anlamlı fark olduğu belirlendi ($p<0,05$), ancak 21. günde istatistiksel olarak anlamlı fark bulunmadı ($p>0,05$). **Sonuç:** Piezoelektrik cerrahi cihazlarını konu alan birçok çalışmada, piezocerrahi aletlerinin kemik doku iyileşmesinde daha üstün olduğu tespit edilmiştir. Bu çalışmada da histopatolojik, histomorfometrik ve histokimyasal değerlendirme sonucunda yeni nesil piezocerrahi grubunda defekt bölgelerindeki kemik dolununun (hem kemik hem de bağ doku) kısa dönem takipte eski nesil piezocerrahi ve konvansiyonel döner alet gruplarına göre daha yüksek olduğu, ancak uzun dönem takipte gruplar arasında anlamlı fark olmadığı belirlenmiştir.

Anahtar Kelimeler: Kemik iyileşmesi;
oral ve maksillofasial cerrahi; osteotomi;
piezoelektrik cerrahi; tavşan

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Osteotomy is a common procedure in medical and dental treatments. Traditionally, rotary instruments are used for bone surgery. In many surgical branches, the demand for precise and reliable bone-cutting methods has increased.^{1,2}

The piezoelectric surgery technique is designed to complement and, in some cases, replace traditional methods. Piezoelectric is a technique that allows safe and effective osteotomies to be performed using ultrasonic vibrations.³⁻⁵

Ultrasonic piezoelectric devices, which have been used frequently by surgeons in recent years, are more reliable and effective in studies comparing with other devices. The reason for this situation is that the piezoelectric device can only work on mineralized tissues, making smooth and precise incisions, protecting vital neurovascular tissues and obtaining a better view in the surgical field, as well as providing a safe and precise osteotomy without osteonecrotic damage by micrometric and selective cutting.³

With the advancement in technology, more comfortable and satisfactory results were obtained in surgery branches with the use of the ultrasonic devices in osteotomies.⁶

The strength of the older generation of piezosurgical instruments drops when they encounter a harder bone mass than they can tolerate. The surgeon increases the force applied to make a faster bone cut. This causes an increase in heat that can cause necrosis of the bone. To eliminate this disadvantage, a new generation of piezosurgical instruments has been developed that do not lose their strength and speed, no matter how dense bone mass they encounter. Because these devices are stronger and faster, it is expected that bone necrosis, which will occur due to heat, will be greater.

The purpose of this article is to compare the dynamics of bone healing in experimental osteotomies using 2 piezosurgical devices with different output power (new generation Acteon Piezotome Cube-A, old generation France and EMS Piezon Master Surgery, Switzerland-E) and a conventional rotary instrument (C), in an *in vivo* rabbit model.

MATERIAL AND METHODS

This research was confirmed by Gazi University Animal Experiments Local Ethics Committee (Ankara, Türkiye, date: November 22, 2018, no: 66332047-604.01.02). The experimental part of the project was conducted in the research laboratory of Gazi University Laboratory of Animal Breeding and Experimental Research Center. Histopathological, histochemical and histomorphometric examinations were done at Gazi University Faculty of Dentistry, Department of Oral Pathology. All animals were exposed to humane treatment in accordance with the "Guide for the Care and Use of Laboratory Animals."

Twelve adult New Zealand-type white male rabbits, 3-4 months old, weighing 2.5 kg on average, were used. The physiological needs of rabbits were met under the control of veterinarians. Environmental factors were adjusted not to cause stress in rabbits. Experimental animals placed 2 pieces in each cage. During the study, tap water and dry pellet feed were given to the rabbits. Any antibiotics or painkillers were not given to the rabbits.

Two study groups were prepared;

1. Group: 6 rabbits were sacrificed on the 7th day after the surgery.
2. Group: 6 rabbits were sacrificed on the 21st day after the surgery.

25-35 mg/kg Ketamine HCl (Alfamine 10%, Alfasan) and 5 mg/kg Xylazine HCL (Basilazine 2%, Bavet) was given intramuscularly to the rabbits. All surgical procedures were performed under sterile surgical conditions. The skull of each animal was shaved and the corresponding area was wiped with povidone iodine. Breathing, muscle movements and painful stimulus response, after sufficient time has passed, the skin incision was prepared about 2.5 cm long along the midline of the calvaria. The incision was continued by taking bone contact and the parietal bones were reached. After skin retraction bone defects 8 mm in diameter were performed under saline irrigation with E in the right parietal bone, with A in the left parietal bone, with C in the midline behind these 2 defects. After performing the defects, the surgical site was cleaned with physiological saline. After

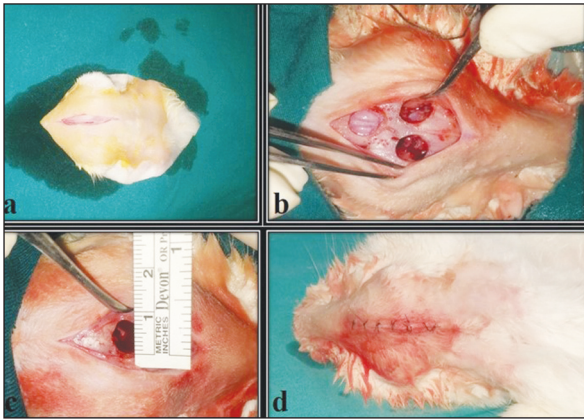


FIGURE 1: a) Incision line b,c) Defect zones, d) After operation.

bleeding control was achieved, the periosteum and skin were closed primarily with resorbable 4/0 catgut sutures and the wound area was wiped with povidone iodine (Figure 1).

Group 1 were sacrificed on the 7th postoperative day, and the 2nd group were sacrificed on the 21st postoperative day by intramuscular high-dose ketamine injection. Sacrificed animals were decapitated and fixed in formalin solution (10% buffered). The samples obtained by excising the calvarial regions containing the defect with a mini saw were fixed in 10% buffered formalin solution for 24-72 hours and then decalcified in formic acid (10% solution). Samples with completed decalcifications were embedded in paraffin blocks following the standart tissue follow-up plan. Sequences of 4 μ m were excerpted with 2 tissue sections on each slide (Surgipath, X-tra Adhesive Microslides, Illinois, USA).

STATISTICAL ANALYSIS

The data acquired in the research were analyzed using the SPSS 25.0 (IBM, USA) program. Descriptive statistical methods (number, percentage, mean, standard deviation, minimum and maximum) were used while rating the data. Besides, the normal distribution of the data used was analyzed with the Shapiro Wilk and Kolmogorov Smirnov tests. The homogeneity of variance was analyzed with the Levene test and the methods were determined. Parametric tests were wielded for normally distributed measurements, and non-parametric tests were wielded for non-normally distributed measurements. In the comparison of quan-

titative data, the discrepancy between the 2 groups is independent sample t (independent t-test) for normally dismantled measurements, Mann-Whitney U test for non-normally dismantled measurements, F test (ANOVA) for normally dismantled measurements in comparisons of more than 2 group means, non-normally distributed measurements. Kruskal Wallis H test was used for measurements. Bonferroni analysis was used to analyze which group caused the difference between the groups. Chi-square analysis was done to dismantle whether there is a statistically significant connection between categorical variables.

RESULTS

HISTOPATHOLOGICAL FINDINGS

Day 7 Evaluations

In all samples, the host bone was vital and the defect areas were observed to heal towards the center. The best bone filling was seen in the defects opened with A, filled with vascularized cellular connective tissue and fibrin exudate. Almost half of the defects opened with E have a similar appearance. In most of the defects opened with C, large cavities were seen in places. All specimens have osteoid formation, but the least bone sequestra is found in defects opened with A. Resorption and osteoclast were not observed in the defects opened A, but were seen in one of the defects opened with E and in 2 of the defects opened with the C (Figure 2).

Day 21 Evaluations

In all samples, the host bone was vital and the defect areas were observed to heal towards the center. It was observed that all of the defects opened with A were filled with fibroosseous structure (new bone and connective tissue). Necrosis was observed in one of the defects opened with the E, and fibroosseous structure was observed at different rates in other defects other than this defect. Necrosis was observed in one of the defects opened with C, and defects other than this defect had fibrocellular connective tissue, congested vascular structures, occasional free bleeding areas and less new bone compared to other groups. All specimens have osteoid formation except for specimens with necrosis. No bone sequestra was observed

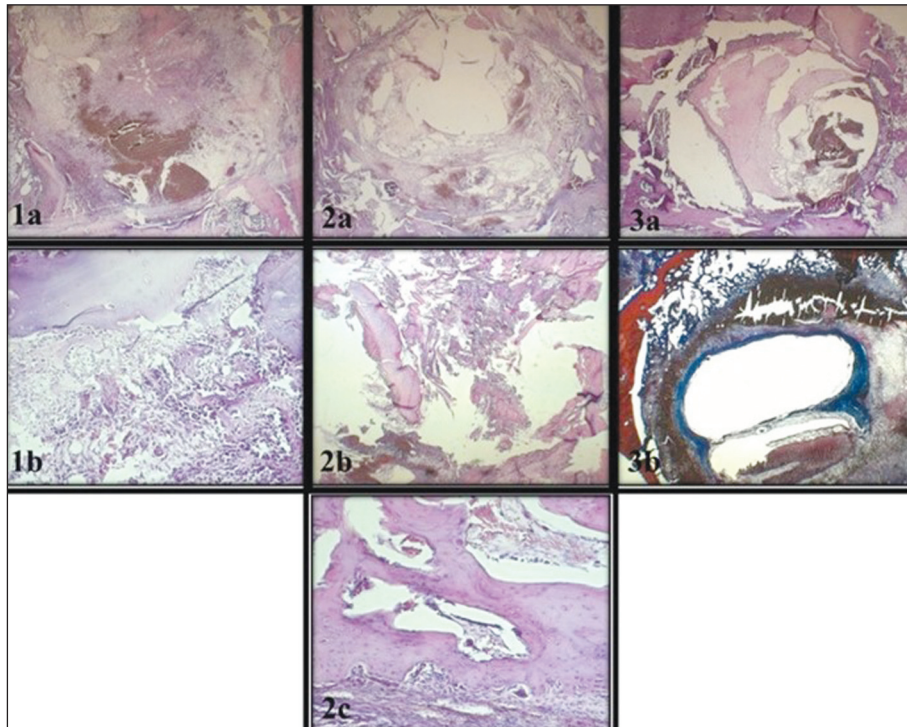


FIGURE 2: Day 7 evaluations; A group left column, E middle column, C group right column. **1a)** Defect area. [Hematoxylin&eosin (H&E) x20], **1b)** New bone and connective tissue in host bone continuity. (H&E x200) **2a)** Defect area. (H&E x20), **2b)** Bone sequestrants in the defect area (H&E x20), **2c)** Resorption areas and osteoclasts in the host bone (H&E x200), **3a)** Defect area (H&E x20), **3b)** Limited connective tissue and new bone formation in the defect zone (Trichrome histochemistry x20).

in the defects opened with A. Most of the defects opened with E have bone sequestra and multinucleated giant cells around them. Most of the defects opened with C have bone sequestra and necrotic debris around them. Abscess finding was only seen in one of the defects opened with A. There is also one resorption in defects opened with E and C (Figure 3).

HISTOCHEMICAL EVALUATIONS

Collagenization in connective tissue and bone formed in the defect area was evaluated with trichrome staining. From the 7th to the 21st day in both experimental groups, it was observed that the collagen started from the thin short fibrillar structure and matured into thick dense bands. On the 7th day, it was observed that the extracellular matrix and fine fibrillar collagen were stained in light blue, and myofibroblasts and young fibroblasts were red. It was observed that the new bone formed on the 21st day was stained blue-dark blue, and the mature bone and bone sequestrants were stained red. There was no discrepancy between the groups in terms of staining patterns.

HISTOMORPHOMETRIC EVALUATIONS

A total of 36 defects were created in the project, but 4 defects were not evaluated histomorphometrically due to low section quality. Evaluated 17 defects are included in Group I and 15 defects in Group II. Histomorphometrically, the variables of new bone, sequestra, connective tissue, fibrin, total area, cartilage, filled area, empty area, percent filled and percent empty were evaluated and descriptive statistics for these values are given in Table 1.

For analyze whether there is a statistically significant discrepancy between the groups in E, C, A independent sample t-test was performed for normally distributed data and Mann-Whitney U analysis was performed for data that did not have a normal distribution, and the values are given in Table 2, Table 3, Table 4.

According to the analysis results; it was specified that there was a statistically significant discrepancy between the averages of the new bone, fibrin, empty area, percent filled, and percent empty measurements on the 7th and 21st days for E ($p < 0.05$).

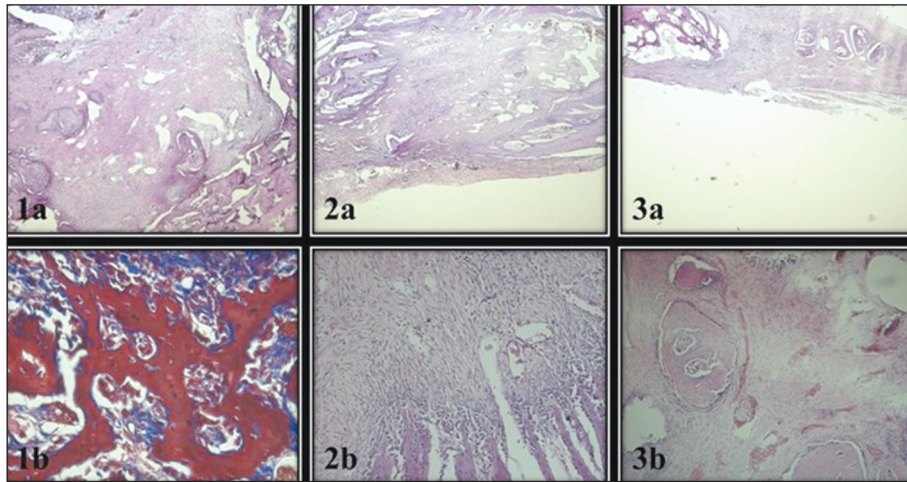


FIGURE 3: Day 21 evaluations; A group left column, E middle column, C group right column. **1a)** Defect area. (H&E x20), **1b)** Newly formed bone (osteoid) in connective tissue at high magnification (Trichrome histochemistry x400), **2a)** Defect area (H&E x20), **2b)** New bone, congested vascular structures and mild mononuclear inflammatory cells in the fibrocellular connective tissue (H&E x200), **3a)** Defect area (H&E x20), **3b)** New connective tissue, congested vascular structures, free bleeding areas, new bone in the defect zone (H&E x40).

TABLE 1: Descriptive statistics of parameters on day 7 and day 21.

		n	Minimum	Maximum	Average	Standard deviation
Day 7	New bone	17	0	117109	47856.18	36178.33
	Sequestra	17	0	57866	16889.06	20799.58
	Connective tissue	17	92092	826246	267172.35	177435.02
	Fibrin	17	73887	820212	521508.82	179577.97
	Total area	17	506116	1127256	853426.41	141290.40
	Cartilage	17	0	0	0	0
	Filled area	17	101134.00	857281.00	315028.53	178371.25
	Empty area	17	73887.00	820212.00	538397.88	176381.66
	Percent filled	17	0.11	0.92	0.37	0.18
	Percent empty	17	0.08	0.89	0.63	0.18
Day 21	New bone	15	9308	406006	158203.60	133374.01
	Sequestra	15	0	25307	9174.00	8479.19
	Connective tissue	15	72088	602100	272282.67	149349.15
	Fibrin	15	37660	462091	142310.27	129665.63
	Total area	15	174352	1332032	581970.53	313959.22
	Cartilage	2	50614	313942	182278.00	186201.01
	Filled area	15	136692	969076	430486.27	235283.33
	Empty area	15	37660	473291	151484.27	131399.32
	Percent filled	15	0.42	0.90	0.75	0.12
	Percent empty	15	0.10	0.58	0.25	0.12

It was specified that there was a statistically significant discrepancy between the mean values of fibrin, empty space, full percent and empty percent measurements on the 7th and 21st days for C (p<0.05).

It was specified that there was a statistically significant discrepancy between the mean values of fib-

rin, total area and free space measurements on the 7th and 21st days for A (p<0.05).

To compare the measurements of the variables on the 7th and 21st days according to the instruments, one-way ANOVA (analysis of variance) was performed when the data had normal distribution, and Kruskal Wallis H analysis was performed when it did

TABLE 2: Comparison of the variables according to the 7th and 21st days of E.

		n	Average	Standard deviation	Median	Test statistic	p value
New bone	Day 7	5	9688.20	14895.09	0.00	-3.471*	0.017*
	Day 21	6	158952.17	104060.29	153741.00		
Sequestra	Day 7	5	36971.00	22221.17	41241.00	2.311*	0.067
	Day 21	6	12364.83	9356.75	11177.50		
Connective tissue	Day 7	5	252370.60	126642.08	186673.00	-0.697*	0.504
	Day 21	6	309831.17	143432.66	347771.50		
Fibrin	Day 7	5	427354.40	84881.68	443680.00	5.741*	0.000*
	Day 21	6	109946.17	96126.14	73085.00		
Total area	Day 7	5	726384.20	141291.64	718578.00	1.053*	0.320
	Day 21	6	591094.33	254981.70	622995.00		
Filled area	Day 7	5	262058.80	121409.85	186673.00	-1.917*	0.088
	Day 21	6	468783.33	212859.70	451822.50		
Empty area	Day 7	5	464325.40	93778.09	501537.00	6.030*	0.000*
	Day 21	6	122311.00	93568.49	88946.00		
Percent filled	Day 7	5	0.35	0.11	0.3688	-6.691*	0.000*
	Day 21	6	0.80	0.11	0.8280		
Percent empty	Day 7	5	0.65	0.11	0.6312	6.691*	0.000*
	Day 21	6	0.20	0.11	0.1720		

Test statistic: *t (independent sample t).

TABLE 3: Comparison of the variables according to the 7th and 21st days of C.

		n	Average	Standard deviation	Median	Test statistic	p value
New bone	Day 7	6	63729.00	40141.15	70652.50	-0.989*	0.356
	Day 21	6	140741.33	196074.26	35274.00		
Sequestra	Day 7	6	10091.50	16772.78	0.00	6.000**	0.418
	Day 21	6	12871.33	2008.85	12314.00		
Connective tissue	Day 7	6	239838.33	128242.73	206471.50	-1.342*	0.221
	Day 21	6	382114.00	193663.77	306910.00		
Fibrin	Day 7	6	617470.33	135456.61	617844.00	2.909*	0.023*
	Day 21	6	290461.67	206400.80	347856.00		
Total area	Day 7	6	931129.17	113504.31	914689.00	0.522*	0.618
	Day 21	6	826188.33	500572.99	815475.00		
Filled area	Day 7	6	303567.33	148583.75	264743.50	-0.943*	0.434
	Day 21	6	522855.33	388761.79	342184.00		
Empty area	Day 7	6	627561.83	133109.07	637784.50	2.911*	0.023*
	Day 21	6	303333.00	206334.75	362956.00		
Percent filled	Day 7	6	0.32	0.14	0.30	-2.843*	0.025*
	Day 21	6	0.64	0.19	0.72		
Percent empty	Day 7	6	0.68	0.14	0.69	2.843*	0.025*
	Day 21	6	0.36	0.19	0.27		

Test statistic: *t (independent sample t); **U (Mann-Whitney U); *p<0.05.

not have a normal distribution. In case there is a discrepancy between the groups, Bonferroni analysis was performed to determine between which groups or from which group the difference originated, and the values are given in [Table 5](#), [Table 6](#).

Accordingly, it was specified that there was a statistically significant discrepancy between the mean of new bone and total area measurements measured by E, C and A, and the medians of sequester measurements on the 7th day (p<0.05).

TABLE 4: Comparison of the variables according to the 7th and 21st days of A.

		n	Average	Standard deviation	Median	Test statistic	p value
New bone	Day 7	6	63790.00	18324.65	64867.50	-1.630	0.162
	Day 21	6	166186.17	152779.15	110927.50		
Sequestra	Day 7	6	6951.67	11437.09	0.00	17.000**	0.858
	Day 21	6	4134.50	7826.35	909.00		
Connective tissue	Day 7	6	306841.17	261658.96	216088.50	10.000**	0.200
	Day 21	6	179818.50	87693.28	168539.00		
Fibrin	Day 7	6	504009.33	242485.29	522732.00	3.934	0.003*
	Day 21	6	100598.67	65437.65	67758.50		
Total area	Day 7	6	881592.17	103395.75	877391.50	4.143	0.004*
	Day 21	6	450737.83	232809.77	480976.50		
Filled area	Day 7	6	370631.17	247526.47	288408.50	17.000**	0.873
	Day 21	6	346004.67	183836.19	362396.00		
Empty area	Day 7	6	510961.00	242630.89	536203.50	3.935	0.003*
	Day 21	6	104733.17	71278.29	67758.50		
Percent filled	Day 7	6	0.42	0.26	0.3649	6.000**	0.055
	Day 21	6	0.76	0.07	0.7489		
Percent empty	Day 7	6	0.58	0.26	0.6351	6.000**	0.055
	Day 21	6	0.24	0.07	0.2511		

Test statistic: *t (independent sample t); **U (Mann-Whitney U); *p<0.05.

TABLE 5: Comparisons of instruments for variables at 7th day.

		n	Average	Standard deviation	Median	Test statistic	p value	Bonferroni
New bone	E	5	9688.20	14895.09	0.00	6.800*	0.009*	1<2
	C	6	63729.00	40141.15	70652.50			1<3
	A	6	63790.00	18324.65	64867.50			
Sequestra	E	5	36971.00	22221.17	41241.00	6.585**	0.037*	3<1
	C	6	10091.50	16772.78	0.00			2<1
	A	6	6951.67	11437.09	0.00			
Connective tissue	E	5	252370.60	126642.08	186673.00	0.171**	0.918	-
	C	6	239838.33	128242.73	206471.50			
	A	6	306841.17	261658.96	216088.50			
Fibrin	E	5	427354.40	84881.68	443680.00	1.712*	0.216	-
	C	6	617470.33	135456.61	617844.00			
	A	6	504009.33	242485.29	522732.00			
Total area	E	5	726384.20	141291.64	718578.00	4.308*	0.035*	1<2
	C	6	931129.17	113504.31	914689.00			
	A	6	881592.17	103395.75	877391.50			
Filled area	E	5	262058.80	121409.85	186673.00	0.626**	0.731	-
	C	6	303567.33	148583.75	264743.50			
	A	6	370631.17	247526.47	288408.50			
Empty area	E	5	464325.40	93778.09	501537.00	1.334*	0.295	-
	C	6	627561.83	133109.07	637784.50			
	A	6	510961.00	242630.89	536203.50			
Percent filled	E	5	0.35	0.11	0.37	0.254**	0.881	-
	C	6	0.32	0.14	0.30			
	A	6	0.42	0.26	0.36			
Percent empty	E	5	0.65	0.11	0.63	0.254**	0.881	-
	C	6	0.68	0.14	0.70			
	A	6	0.58	0.26	0.64			

Test statistic: *F (one-way ANOVA); **X2 (Kruskal Wallis H); *p<0.05.

TABLE 6: Comparisons of instruments for variables at 21th day.

		n	Average	Standard deviation	Median	Test statistic	p value
New bone	E	6	158952.17	104060.29	153741.00	0.031*	0.969
	C	6	140741.33	196074.26	35274.00		
	A	6	166186.17	152779.15	110927.50		
Sequestra	E	6	12364.83	9356.75	11177.50	4.009**	0.135
	C	6	12871.33	2008.85	12314.00		
	A	6	4134.50	7826.35	909.00		
Connective tissue	E	6	309831.17	143432.66	347771.50	2.661*	0.111
	C	6	382114.00	193663.77	306910.00		
	A	6	179818.50	87693.28	168539.00		
Fibrin	E	6	109946.17	96126.14	73085.00	3.242*	0.075
	C	6	290461.67	206400.80	347856.00		
	A	6	100598.67	65437.65	67758.50		
Total area	E	6	591094.33	254981.70	622995.00	1.546*	0.253
	C	6	826188.33	500572.99	815475.00		
	A	6	450737.83	232809.77	480976.50		
Filled area	E	6	468783.33	212859.70	451822.50	0.664*	0.533
	C	6	522855.33	388761.79	342184.00		
	A	6	346004.67	183836.19	362396.00		
Empty area	E	6	122311.00	93568.49	88946.00	3.398*	0.068
	C	6	303333.00	206334.75	362956.00		
	A	6	104733.17	71278.29	67758.50		
Percent filled	E	6	0.80	0.11	0.83	1.838*	0.201
	C	6	0.64	0.19	0.73		
	A	6	0.76	0.07	0.75		
Percent empty	E	6	0.20	0.11	0.17	1.838*	0.201
	C	6	0.36	0.19	0.27		
	A	6	0.24	0.07	0.25		

Test statistic: *F (one-way ANOVA); **X² (Kruskal Wallis H); *p<0.05.

It was specified that there was no statistically significant discrepancy between the mean and medians of the values measured by E, C and A on the 21st day (p>0.05).

DISCUSSION

Ultrasonic devices were used in dentistry mainly in periodontology and endodontics in 1953. Although the ultrasonic osteotomy technique was first identified by Horton et al. in 1975, it didn't become operational up to 2000, when Vercellotti et al. renewed and introduced this soft tissue sparing approach.^{7,8} In the back, substantial experimental efforts have been made to create a better osteotomy and ostectomy device in response to the need for more sensitive and reliable osteotomy and ostectomy in bone surgery compared to conventional devices.⁹ Piezosurgery in-

struments were developed in response to the need for more sensitive and reliable osteotomy and ostectomy compared to conventional rotary instruments. As it is used in many parts of medicine such as orthopedics and neurosurgery, it has become very popular in recent years among the surgical applications of dentistry.

Ultrasonic vibes have been done in osteotomies for over 20st years. Nevertheless, it has been used as an alternative to standard clinical practices in many different surgical fields in experimental applications in recent years. Especially during osteotomy, it is one of its important advantages that it reduces the risk of damaging the connected soft tissues and critical organs.¹⁰ Minimally invasive surgery; it is very important for less tissue trauma and consequently to increase the post-operative comfort of the patients.

According to the literature, it has been observed that clinical studies researching the impressions of piezoelectric surgery instruments on tissue have been carried out. In these studies, inflammatory findings such as postoperative pain, trismus, edema, and paresthesia were evaluated. In these studies, the positive effects of piezosurgical instruments were mentioned.¹¹⁻¹⁴ In clinical studies on humans, biopsy for histopathological examination is not considered ethically appropriate, and experimental animal studies are more accepted in research in this area.¹⁵ This study was carried out on experimental animals, since bone samples were required to follow the degree of bone healing and to make a histopathological evaluation.

Dodde et al., in their experimental study on 18 white New Zealand rabbits; they divided the rabbits into 3 groups and created 5 mm defects in the skulls of the rabbits in the 1st group, 10 mm in the 2nd group and 15 mm in the skulls of the 3rd group.¹⁶ They reported that defects with a diameter of 15 mm could not heal spontaneously in the samples obtained at the 9th and 18th weeks, in computerized tomography and histological examinations, a gap of 3.33 mm remained between the defect edges, and this size defect was the amount of critical size defect for the rabbit model. In this study, short-term follow-up was performed due to the high regeneration capacity of rabbit bones and 8 mm bone defects were created.

Since the bone tissues in the body show different osteogenic activities, they give different responses when there is an interaction in the bone. The mandible and maxilla show intramembranous ossification when evaluated.¹⁷ In this project, it was preferred to study rabbit parietal bones because of its intramembranous ossification, such as the mandibular and maxilla, its ease of transportation, ease of surgical procedure, and its relatively flat structure.

Many graft materials have been tested in animal models for evaluation of their effects on bone healing.^{18,19} Since only device efficiency was investigated in this study, graft material was not used in the defect areas.

In an experimental study conducted by Anesi et al., the effect of 2 different piezosurgical instruments [Piezosurgery® Medical-PM Piezosurgery® – Mectron Medical Technology (Carasco, Italy) and Piezosurgery® Plus-PP Piezosurgery® – Mectron Medical Technology (Carasco, Italy)] and one conventional rotary hand tool (RO) on bone tissue was investigated.²⁰ It has been established that the incision lines created using PP and PM have a higher healing potential compared to the lines created using RO, but there is not much difference, although PP is a more powerful device than PM.

In a study conducted by Ma and colleagues, they aimed to equate bone healing in osteotomies.²¹ In this study, 16 rabbits were randomly divided into 4 groups. Total of three osteotomy techniques, bone healing was observed, which began a week later. The most obvious new bone formation occurred between the 2 and 3rd weeks, and piezoelectric surgery was found to have a faster bone formation and decongestion. However, it has been noted that there is no significant difference between the 3 modalities.

Hoigne et al. reported that wound healing and bone consolidation were unproblematic after piezoelectric surgery, and that the healing time was shorter than the traditional healing time.²²

In a study by Esteves et al., they aimed to compare the bone healing dynamics of piezosurgery and conventional drills after osteotomies.²³ As a result, histologically and histomorphometrically; the newly formed bone controlled at the end of the 30th day was found to be almost similar in both groups, except that it was slightly higher in the piezosurgery group.

In an experimental study done by Vercellotti et al. on canine mandibles, wound healing was evaluated after osteotomies with piezoelectric surgery device, diamond and carbide burs.⁵ In osteotomies performed with all 3 methods, an increase in bone level was observed at the end of the 28th day, bone loss was observed in the osteotomy areas made with diamond and carbide burs at the end of the 56th day, while an gain in the bone level was observed in the osteotomy areas performed with the piezoelectric surgical instrument. The outcomes of the project indicated that the bone healing response after osteotomies

with a piezoelectric surgical instrument was better compared to diamond or carbide burs.

The studies of Vercellotti et al., Anesi et al., Ma et al., Hoigne et al., Esteves et al. seem to be quite similar to this study.^{5,20-23} In this study, bone healing was higher on the 7th day in A, E, especially in the A; however, it was specified that there was no significant discrepancy between the groups on the 21st day. Considering the results obtained from the studies, it is statistically understood that the effect of osteotomies performed with A, E on healing in the early period is more successful than C, but that different piezoelectric surgical instruments do not show much superiority to each other. It has been seen that A is more successful in the early period compared to E; however, this was not statistically significant. With this finding, it has been proven once again that piezoelectric surgical instruments are superior to conventional instruments in terms of recovery time.

In another study conducted by Ma et al. 2 years later, it was aimed to utilize and equate the wound healing process after osteotomies with conventional rotary instruments and piezoelectric surgery in a rabbit model.²⁴ Defects prepared by piezosurgery showed a significant reduction in bone debris at week 1 compared to a conventional rotary bone defect. The newly formed bone tissue bridge was observed in the 2nd and 3rd weeks, but no statistically significant result was reached. By week 5, the defects were found to be completely filled with new bone. According to the histomorphometric measurements made in this study, piezoelectric surgery instruments were found to be more successful in terms of bone sequestra. According to the measurements made on the 7th and 21st days, it was statistically determined that the amount of bone sequestra formed by A and E decreased over time, while this value increased in C. It was observed that A was more successful in the early period than E, and this was not statistically significant.

Maurer et al., compared 4 different osteotomy instruments with the histopathological examination of the defects opened in animal models.²⁵ Fresh bone samples of standard size were taken from the rabbit skull. Techniques used; microsaw, Lindemann bur,

ultrasonic osteotome with 2 ends [OT6 (coarse) and OT7 (fine)]. The prepared surfaces were examined by light microscopy, peripheral surface electron microscopy and confocal laser scanning microscopy. In conclusion, micromorphological differences were clearly defined in this study after using various osteotomy techniques. In this study, the degree of roughness of the wound surfaces was not measured. However, as a result of examining the sections under the microscope, it was observed that piezoelectric surgical instruments provide a smoother cutting surface under laboratory conditions.

It is thought that the healing process after the use of osteotome and piezoelectric surgery technique is similar. However, the healing of bone tissue after the use of rotary burs differs greatly from these 2 techniques. Rotary burs cause degeneration of cellular elements at the bone margins, continuity of fibrovascular tissues, and reduced reactivity of osteoblasts and osteoclasts.²⁶ These findings are also supported by the histopathological results of this study. In 2 samples of this study in the control group, resorption areas in the host bone in the defect wall and the presence of osteoclasts in this area were detected. However, osteoid formation was involved in the continuity of the host bone, albeit at a minimal level, in the piezoelectric surgery groups.

CONCLUSION

According to all these data, it can be said that the new generation piezosurgery instruments give more positive results than other instruments. New generation piezosurgical instruments offer a more reliable working environment and cause minimal damage to tissues. The resulting damage also recovers quickly along with the wound healing process. These findings were observed during the surgical procedure and proved histopathologically, histochemically, and histomorphometrically. We think that these results we have obtained should be supported by other studies to be carried out.

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examination of the effects of different piezosurgical instruments on tissues).

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mustafa Öztürk; **Design:** Özgün Yıldırım; **Control/Supervision:** Özgün Yıldırım, Mustafa Öztürk; **Data Collection and/or Processing:** Halil Erhan Ersoy, İpek Atak Seçen, Sibel Elif Gültekin; **Analysis and/or Interpretation:** Özgün Yıldırım; **Literature Review:** Özgün Yıldırım; **Writing the Article:** Özgün Yıldırım; **Critical Review:** Özgün Yıldırım.

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