Does The Mini-İmplant Supported Rapid Maxillary Expansion Cause Less Root Resorption Than Traditional Approaches ?

A Micro-Computed Tomography Study

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Running Title: Does the mini-implant supported RME cause less resorpsion?

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DOES THE MİNİ-İMPLANT SUPPORTED RAPİD MAXİLLARY EXPANSİON CAUSE LESS ROOT RESORPTİON THAN TRADİTİONAL APPROACHES ?

A MİCRO-COMPUTED TOMOGRAPHY STUDY

ABSRACT

Objectives: This study aims to evaluate the volume, amount, and localization of root resorption in the upper first premolars after four different rapid maxillary expansion (RME) appliances using microcomputed tomography (micro CT).

Methods: The study consists of 20 patients who required RME and extraction of the upper first premolars. The patients were divided into four groups: miniimplant supported hybrid RME appliance, hyrax RME appliance, acrylic bonded RME appliance, and full-coverage RME appliance. The same activation protocol (one activation daily) was performed in all the groups. For each group, 10 premolars and totally 40 premolars were scanned with the micro-CT (SkyScan). The reconstructed 3D images of each root sample were divided into six regions. The resorption craters on these six different root surfaces were analyzed by special CTAn (SkyScan) software for direct volumetric measurements. For the statistical assessment, the Kruskal–Wallis one-way analysis of variance and the Mann–Whitney U test with Bonferroni adjustment were used.

Results: The hybrid expansion appliance had the lowest volume of root resorption and number of craters ($P < 0.001$). No significant difference was found in the other group in terms of total root resorption ($P > 0.05$). Except the hybrid group, more resorption was observed on the buccal surface than on the lingual surface in all groups ($P < 0.05$).

Conclusions: All expansion appliances caused root resorption in the upper first premolar teeth. The resorption craters were generally concentrated on the buccal surface. Minimal root resorption was detected with the mini-screw-supported hybrid appliance after RME.

Keywords: Rapid Maxillary Expansion, Micro-CT, Root Resorption

INTRODUCTION

Rapid maxillary expansion (RME) is the main treatment modality to solve maxillary deficiency. This technique implements heavy forces to the teeth and supporting structures. RME has the desirable effects of opening the medial palatal suture and expanding the maxilla, but it has undesirable effects. Histological and radiography studies reported that the heavy forces produced by RME [1] induce and cause external root resorption on the anchor teeth.[2-7] Clinicians can use various RME appliances to widen the maxilla, such as tooth-borne and tooth tissue-borne banded or bonded appliances. [1-9] In previous studies, conventional RME appliances and the combination of Hyrax, Haas, and cap splint appliances were compared with each other from the point of root resorption. [2,3-7,10] However, whether tooth-borne or tooth tissue-borne appliances should be preferred to cause less root resorption remains unclear. Recently, the use of mini-implant-supported RME appliances to minimize tipping and the undesirable effects of the expansion on the teeth have become common. The first molars were selected as the posterior anchorage unit and the rugae area as the anterior anchorage unit because of the insufficiency of appropriate bone in the posterior region. [11] However, studies on the effect on the root resorption of mini-implant-supported hybrid RME appliances compared with conventional appliances are limited. [7-12]

Root resorption can be assessed quantitatively by histological and radiographic methods. However, 2D radiographic techniques are limited to measuring only the amount of root apex loss and are highly incorrect because of magnification errors. Moreover, histological studies are technique sensitive, and as material is lost during histological sectioning, the quantitative measurement of root resorption is undependable.[13] By contrast, with the development in 3D imaging techniques; the examination of resorption craters in 3D configurations with high-dimensional resolutions is possible with micro computed-tomography (Micro-CT). Moreover, the high-precision volumetric measurement of root resorption craters can be performed with computer software. [13] Micro-CT has been used as an imaging and evaluation technique in different fields of dentistry [14-18] and to investigate root resorption caused by orthodontic tooth movement.[13,14] However, root resorption after the use of different RME appliances has not been evaluated with Micro-CT in the literature.

Thus, in this study, we evaluated the root resorption volume and the amount of root resorption craters on the upper first premolars after mini-screw-supported hybrid, hyrax, acrylic bonded, and full-coverage expansion appliances using Micro-CT.

MATERIAL AND METHODS

This blinded and randomized clinical study was conducted in the Department of Orthodontics, İnönü University after obtaining the ethical approval from the human ethics committee of İnönü University (2014/23). Twenty patients (12 female and 8 male) within the 12–15 age range were selected (Table 1). All patients had maxillary constriction, maxillary dental crowding, and orthodontic indication for both RME and the extraction of first premolars. Patients with any systemic disease, craniofacial anomaly, parafunctional habits, bruxism, history of trauma, and filling or root canal treatment in the upper premolar teeth were not included in the study. The randomization was made at the start of the study with prepared random number tables. One researcher evaluated the patients and the other author did the enrolling. Patients were divided into four groups: mini-implant supported hybrid, hyrax, acrylic bonded, and full-coverage appliances. Each group consisted of the 10 maxillary first premolars of 5 patients. To determine the sample size for each group, a power analysis was carried out based on an alpha significance level of 0.05 and beta value of 0.1 to achieve 80% power to detect an average difference of 0.5 mm^3 (± 0.20 mm^3) in resorption craters on root surfaces from the different groups (version 3.0.10, G*Power; Franz Faul Universidad, Kiel, Germany). [14] The power analysis showed that at least eight samples for each group were required. For this reason; each group consisted of 5 patients (10 maxillary first premolars teeth).

In the hybrid group (Fig. 1a), two 2 mm × 9 mm mini-screws (PSM Benefit; Tuttlingen, Germany) were inserted bilaterally, perpendicular into the anterior palate, to the second and third palatal rugae and about 2–3 mm paramedian to the suture. Bands (3M Unitek; Monrovia, USA) fitted to the upper first molars and laboratory abutments were attached to the mini-screw heads. The rings (PSM, Tuttlingen, Germany) of two standard Benefit systems were screwed onto the laboratory analogs on the cast. Hyrax screws and bands and hyrax ring connections were provided by laser welding. The hybrid appliance was bonded to the teeth with a multi-cure glass ionomer (3M Unitek). In the hyrax group, the expansion appliance was used with bands on the first premolars and first molars (Fig. 1b). In the acrylic bonded group, the bonded expansion appliance with acrylic coverage was used. The acrylic part of the appliance extended over the occlusal and middle third of the vestibular surfaces of all posterior teeth (Fig. 1c). In the full-coverage group, the modified tooth tissue-borne and full occlusal coverage bonded acrylic cap splint RME appliance was used (Fig. 1d).

The hyrax, acrylic bonded, and full-coverage expansion appliances were bonded to the teeth with a glass ionomer luting cement (Ketac Cem, 3M ESPE). Afterward, the activation was continued once every day until the desired expansion was achieved. Active treatment was followed by a three-month retention period for all patients. There are no patients excluded from this study because RME procedure was successful in all groups. After the retention phase, the same oral surgeon extracted the left and right premolars at the Oral Surgery Department. The teeth were washed with the isotonic solution without applying pressure and touching the root surfaces and then disinfected for 30 min with 70% alcohol. The teeth were stored in sterile tubes containing distilled water.

Micro-CT Analysis

All samples were scanned with an X-ray desktop microtomographer (SkyScan 1172; Bruker, Kontich, Belgium). The Micro-CT system was set to 100 kV and 100 mA with the aid of a 0.5 mm Al + Cu filter. During scanning, the teeth were rotated 360° around the vertical axis with a single rotation step at 0.40. The scanned data were transformed into images with NRecon (Version 1.6.9.4, SkyScan). Then, the reconstructed 3D images were viewed and processed using the data analysis software CTAn (Version 1.13.5.1, SkyScan). The resorption crater volumes on the root surface were analyzed in six regions. The entire root surface was divided vertically into three sections: cervical, middle, and apical. Each section was then divided into two sub-regions: buccal and lingual (Fig. 2). These regions are called buccal cervical, lingual cervical, buccal middle, lingual middle, buccal apical, and lingual apical. In the reconstructed 3D images of each sample, the cross-sectional images showing the whole resorption craters on the buccal or lingual surface of the tooth were located (Fig. 3). Then, the region of interest was drawn for each sampled image. In the second phase, the thresholding of the lesion density was defined. Lastly, through custom processing, the lesion depth in each selected image was measured.

Statistical Analysis

The results were analyzed using the Statistical Package for Social Sciences software (Version 22, SPSS for Windows). Descriptive statistical data (the mean and standard deviation) are presented. Non-parametric statistical tests were conducted to determine the volumes and numbers of resorption craters. The Kruskal–Wallis one-way analysis of variance test, and the Mann–Whitney U test with the Bonferroni correction were performed for statistical assessments. The results were considered significant at $P < 0.05$.

RESULTS

The descriptive data of patients age and appliances activation turns among the groups are showed in Table 1. There is no statistical differences patients' age and activation turns ($P > 0.05$). The descriptive statistical evaluations of the total, buccal, and lingual volumes of root resorption among the groups are presented in Table 2. Among all the groups, the hybrid group had the lowest volume of root resorption values, and it was statistically significant ($P < 0.001$). No statistically significant difference was found among the hyrax, acrylic bonded, and full-coverage groups with respect to the total resorption crater volume ($P > 0.05$). Furthermore, in considering the numerical values, the total volume of root resorption was less in the tooth tissue supported full-coverage group than in the tooth-supported acrylic bonded and hyrax groups (Table 2). And additionally, except the hybrid group ($P > 0.05$) more volume of resorption was found on the buccal surface than on the lingual surface in all the groups ($P < 0.05$).

When the volume of resorption analyzed in six different root surface regions, a statistically significant smaller in hybrid group than the others on the cervical, middle, and apical thirds of the buccal and lingual surfaces ($P<0.001$). Moreover, a significant smaller root resorption volume was observed in the hybrid and full-coverage groups on lingual apical region compare to other groups ($P<0.01$)(Table 3).

The number of root resorption craters according to localization is shown in Tables 4 and 5. Statistically significant differences were found in the hybrid and acrylic bonded groups. The lowest number of crater was detected in the hybrid group ($p < 0.001$) and the highest in the acrylic bonded group ($p < 0.05$) (Table 4).

In the buccal cervical, lingual cervical, buccal middle, lingual middle, and buccal apical regions, the hybrid group significantly had the lowest number of resorption craters. The apical lingual region showed a lower number of resorption craters in the hybrid and full-coverage groups than in the hyrax and acrylic bonded groups ($p < 0.001$) (Table 5)

When the regional differences were evaluated, root resorption volume and the number of craters were significantly increased in the buccal cervical, buccal middle, and buccal apical regions and decreased in the lingual surface of these regions ($p < 0.001$) (Table 6).

When the cervical, middle, and apical regions were analyzed, the differences between the cervical and middle regions were insignificant in terms of root resorption volume ($p > 0.05$), and the apical region showed significantly less root resorption than the cervical and middle regions ($p = 0.001$). The number of root resorption craters was significantly low in the cervical region ($p < 0,01$), and no significant difference was found in the apical and middle region ($p > 0.05$) (Table 6).

DISCUSSION

Root resorption is an unavoidable pathologic consequence of tooth movement, [19] but defining the related risk factors can minimize this effect. These factors result from the biological variable, which is patient related or due to orthodontic mechanics. [13,14,19,20] RME treatment is one of the most common orthodontic procedures. When choosing the design of an RME appliance, the amount of root resorption and the improved safety of RME appliance with respect to root resorption should be considered. To minimize the undesirable effect of tooth-borne and tooth tissue-borne appliances such as tipping, periodontal problems, and root resorption, [8-10] bone-borne appliances have been suggested. [11] The expansion forces of bone-borne appliances can be directly transmitted to the palatal bone, reducing the complications to a minimum. [21] However, the application of bone-supported appliances may lead to invasive flap requirement and technique, risk of root infection or lesion, and asymmetrical enlargement. [21,22] In recent years, mini-implants have received great interest in orthodontics because they are minimally invasive, multi-purpose, and low cost. [11,14] To minimize the invasiveness of the surgical technique, Wilmes et al. [22] introduced the hybrid hyrax, which includes two mini-screws as the anterior anchoring unit and the upper first molar teeth as the posterior anchoring unit.

Root resorption on the first premolar teeth was minimal with the hybrid hyrax RME. The hyrax, acrylic bonded, and full coverage RME appliances caused stronger root resorption on the upper first premolars. The reason for this is that the forces resulting from the expansion were transmitted directly to the teeth, which were anchored with these three appliances. In our study, root resorption was observed in all of the upper premolar teeth, even the unattached premolars to the hybrid appliance. The post-expansion root resorption emerged directly on the supported tooth, or the accumulated residual stresses caused resorption on the unsupported teeth. This finding was supported by previous studies. [3,6,7,10,12] By contrast, in their SEM study, Barber and Sims [4] did not find root resorption in the premolar teeth that were not anchored to the appliance. Perhaps, the measurement technique used Barber and Sims [4] is not as sensitive as the one used Micro CT technique. Resorption may have occurred, but it may not have been detected.

The dental, skeletal, and periodontal effects of surgically assisted RME with hybrid and hyrax appliances were compared using cone-beam tomography. The hybrid appliances caused less periodontal risk and dental damage in the first molar teeth, whereas more dental tipping, root resorption, and buccal alveolar bone resorption were reported with the hyrax RME. Similarly, in our study, the hybrid appliances led to less resorption than the hyrax and the other two appliances. [10,12]

There are two main contrasting consequences in the literature with respect to RME appliance type and the effect on root resorption. First, there is no difference between tooth-borne and tooth tissue-borne from the point of root resorption. [2,8] Second, tooth tissue-borne appliances cause less buccal root resorption than tooth-borne appliances because the expansion forces are transmitted not only to the anchor teeth but also to the other supporting tissue segments connected to the tissue-borne appliances. [3,6,7] In this study, although statistically not reflected, less root resorption was seen in the tooth tissue-borne full-coverage appliances than in the tooth-borne acrylic bonded and hyrax appliances. Moreover, more root resorption was observed with the hyrax RME than with the acrylic bonded RME. The reduced occlusal locking and the interferences due to the acrylic occlusal surfaces may be a cause for the appearance of less resorption.

Odenrick et al. [3] suggested that removing the force application point from the teeth would lead to less root resorption. Histological studies on the Haas and hyrax appliances for root resorption showed that tissue-borne Haas appliances led to less buccal root resorption and smaller, shallow resorption lacunas compared with the tooth-borne hyrax appliances. Erverdi et al. [2] found that the tooth-borne and tissue-borne appliances were not different in the amount of root resorption. However, in analyzing the distribution of resorption lacunae, more coronal lacunae were found in the cap splint group because the tooth-supported cap splint appliance caused more tipping to the teeth with lateral forces. In our study, both the resorption volume and the number of resorption craters were found more on the buccal than on the lingual root surface. Depending on the forces applied in the transversal direction in the maxillary expansion, the bending of the teeth to the buccal surface creates more pressure and compression areas on the buccal surface. Therefore, the resorption craters are concentrated on the buccal surface. This finding is supported by previous studies in which more root resorption was detected on the buccal surface after RME . [2-7] When the resorption findings of the cervical, middle, and apical regions were evaluated in this study, the root resorption volume in the cervical and middle regions showed similar values, and the apical region showed significantly less root resorption. Moreover, note that the root surface area of the apical region is less due to the root morphology. Although the number of root resorption craters was significantly less in the cervical region, no significant difference was found between the apical and the middle regions. According to the our micro-CT images and findings, resorption craters were detected wider and fewer on the cervical region, while small and numerous on the middle region. The localization of root resorption after treatment largely depends on the type of orthodontic movement. Especially high pressure areas are more affected by root resorption than stress areas. Under the buccal tipping orthodontic movement more resorption was seen on the buccal-cervical and lingual-apical regions.[13,19] In this study as a result of expansion, wider resorption craters may have been formed on the cervical region due to the buccal tipping of the tooth axes and the stress occurring on the cervical region.

Conventional radiographs may be useful in the detection of severe root resorption. However, the resorption of the mesial, distal, and middle apical regions of the root is difficult to see clearly. [23] In the evaluation of root resorption, 2D conventional radiography is used as a diagnostic tool, but it is not recommended in assessing the amount of root resorption. [24] Techniques that allow the 3D view of the root resorption craters can give more accurate results. As root resorption is a 3D phenomenon, 2D methods such as conventional radiography can lead to underestimation. [12,25,26] The low resolution of the conventional CT is not sufficient for the reconstruction of small objects such as tooth and tooth resorption. With the development of the micro-CT, the vertical resolution capacity has increased to 100–200 µm. In recent years, the resolution increased to 81 µm, to 34–68 µm, and then to 25–15 µm. Currently, a resolution of less than 10 µm has been reached. [15,16] A micro-CT imaging system enables the high-resolution 3D imaging of mineralized tissue. The advantages of this system are as follows: preparing the sample for screening is not necessary, the root surface is not damaged during the scan, it is reproducible, and volumetric data can be obtained from the object being scanned. [14,18]

Yildirim and Akin study [7] is very a well-designed investigation, and our results are similar to this study. However, our methodology is very different from this study. Firstly, Yildirim and Akin [7] used an experimental RME appliance; one side of this modified appliance covers the teeth with acrylic, and the other side of the appliance is fixed to the palatal bone with a screw. This design is not suitable for routine clinical using. In our study, we used the four different RME appliances that routinely clinically applied. Consequently, our investigation directly reflected the clinical situation. Secondly, we compared the four different RME appliances, while Yildirim and Akin had only two RME groups . [7]

In this study, root resorption was remarkably low in the hybrid group, and only the upper first premolar teeth with indications of extraction were evaluated as the micro-CT can only perform in vitro studies. Root resorption in the molar teeth, which were the anchorage unit, was not analyzed. A larger sample size and the exception of the first premolars and other posterior teeth should be evaluated from the point of root resorption. According to the results of this study, all of the evaluated RME appliances cause root resorption, but the hybrid may be safer in terms of root resorption.

CONCLUSION

RME with hybrid, hyrax, acrylic bonded, and full-coverage appliances caused root resorption on the upper first premolars. The amount of root resorption was minimal with the hybrid hyrax appliances compared with the other appliances. Generally more resorption was observed on the buccal surface than on the lingual surface in all RME types. Hybrid appliance for RME approach may decrease the amount of potential root resorption, which represents an advantage compared with traditional approaches.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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LEGENDS

Table 1. Distribution of the patients according to groups, mean and standart deviation of the age and activation amount.

Table 2. Comparison of root resorption volumes (mm^3) among the groups and buccal-lingual surfaces.

Table 3. Comparison of resorption volumes (mm^3) of six different surface among the groups.

Table 4. Comparison of root resorption number among the groups and buccal-lingual surfaces.

Table 5. Comparison of resorption number of six different surface among the groups.

Table 6. Comparison of resorption crater total volume (mm^3) and number values measured in six different regions of root surface.

Figure 1. Appliances used in this study **(a)** Mini implant supported hybrid appliance. **(b)** Hyrax appliance. **(c)** Acrylic bonded appliance. **(d)** Full coverage appliance.

Figure 2. Micro-CT image of maxillary first premolar and the seperation of the root vertically three (A) and horizontally two section (B).

Figure 3. CTAn 1.15.4.0 (Skyscan, Kontich, Belgium) software, ısolation of resorption crater from the root surface.

Table 1. Distribution of the patients according to groups, mean and standart deviation of the age and activation amount.

	Patient (n)	Sample (n)	Mean Age ±SD (year)	Activation±SD (Turns)
Hybrid	5	10	12.9±3.9	33.7±7.2
Hyrax	5	10	13.8±0.5	34.2±3.3
Acrylic Bonded	5	10	14.1±0.8	34.6±4.7
Full Coverage	5	10	14.2±0.6	36.6±6.5
P			>0.05	>0.05

n: number of individuals, SD: standard deviation.

Age and activation comparison of groups: According to the Kruskal-Wallis 1 way analysis of variance, there is no statistically significant difference between groups

Table 2. Comparison of root resorption volumes (mm^3) among the groups and buccal-lingual surfaces

Group	Buccal	Lingual	Total	P¥
Hybrid	0.008±0.014 a	0.007±0.010 a	0.015±0.015 a	>0.05
Hyrax	0.190±0.153 b	0.072±0.072 b	0.263±0.180 b	0.023*
Acrylic B	0.164±0.067 b	0.080±0.046 b	0.245±0.088 b	0.031*
Full C	0.124±0.050 b	0.040±0.043 b	0.161±0.072 b	0.012*
P§	0.0001***	0.025*	0.0001***	

p§ Comparison of appliances groups: According to the Kruskal-Wallis 1 way analysis of variance and the Mann-Whitney U test (with Bonferroni correction), there is no statistically significant difference between groups with the same letters

p¥ Comparison of buccal and lingual surfaces: According to Mann-Whitney U test (with Bonferroni correction)

(*P≤0.05 ,**P≤0.01, ***P≤0.001)

Table 3. Comparison of resorption volumes (mm ^{3}) of six different surface among the groups

Group	Buccal Cervical	Buccal Middle	Buccal Apical	Lingual Cervical	Lingual Middle	Lingual Apical
Hybrid	0.007±0.012 a	0.000±0.001 a	0.001±0.001 a	0.002±0.004 a	0.002±0.005 a	0.001±0.002 a
Hyrax	0.071±0.049 b	0.090±0.124 b	0.030±0.020 b	0.034±0.050 b	0.030±0.030 b	0.009±0.010 b
Acrylic B.	0.053±0.020 b	0.069±0.042 b	0.041±0.026 b	0.025±0.027 b	0.032±0.027 b	0.024±0.014 c
Full C.	0.051±0.031 b	0.040±0.020 b	0.040±0.025 b	0.016±0.018 b	0.016±0.024 b	0.003±0.007 a
P	0.0001***	0.0001***	0.0001*	0.017*	0.0001***	0.002**

According to the Kruskal-Wallis 1 way analysis of variance and the Mann-Whitney U test (with Bonferroni correction) there is no statistically significant difference between groups with the same letters

(*P≤0.05 ,**P≤0.01, ***P≤0.001)

Table 4. Comparison of root resorption number among the groups and buccal-lingual surfaces

Group	Buccal	Lingual	Total	p¥
Hybrid	1.100±1.200 a	1.700±2.110 a	2.800±2.250 a	>0.05
Hyrax	9.700±4.571 b	5.600±2.412 b	15.300±6.500 b	0.023*
Acrylic B	11.900±4.890 c	8.500±5.300 c	20.400±7.545 c	0.048*
Full C	9.300±1.950 b	3.900±3.250 ab	13.200±4.420 b	0.007**
p§	0.0001***	0.003**	0.0001***	

p§ Comparison of appliances groups: According to the Kruskal-Wallis 1 way analysis of variance and the Mann-Whitney U test (with Bonferroni correction), there is no statistically significant difference between groups with the same letters

p¥ Comparison of buccal and lingual surfaces: According to Mann-Whitney U test (with Bonferroni correction),

(*P≤0.05, **P≤0.01, ***P≤0.001)

Table 5. Comparison of resorption number of six different surface among the groups

Group	Buccal Cervical	Buccal Middle	Buccal Apical	Lingual Cervical	Lingual Middle	Lingual Apical
Hybrid	0.500±0.707 a	0.100±0.316 a	0.500±0.530 a	0.600±0.843 a	0.700±0.160 a	0.400±0.700 a
Hyrax	2.700±1.570 b	3.700±1.890 b	3.300±1.890 b	1.800±1.550 b	2.000±1.054 b	1.800±1.230 b
Acrylic B.	2.500±0.971 b	4.400±2.065 b	5.000±2.830 b	2.300±1.890 b	3.700±3.164 b	2.500±1.581 b
Full C.	2.400±1.505 b	4.200±1.032 b	2.700±1.830 b	1.800±1.316 b	1.400±1.840 b	0.700±0.060 a
P	0.001***	0.001***	0.001***	0.033*	0.017**	0.001***

According to the Kruskal-Wallis 1 way analysis of variance and the Mann-Whitney U test (with Bonferroni correction) there is no statistically significant difference between groups with the same letters.
*: P<0.05 , **: P<0.01 , ***: P<0.001,

Table 6. Comparison of resorption crater total volume and number values measured in six different regions of root surface.

Regions	Volume (Root Resorption)	Numbers (Root Resortion Craters)
Buccal Cervical	0.050±0.051 a	2.290±1.660 a
Lingual Cervical	0.021±0.031 b	1.310±1.361 b
Buccal Middle	0.050±0.055 a	3.130±2.106 c
Lingual Middle	0.025±0.030 b	1.840±1.670 d
Buccal Apical	0.032±0.030 c	3.060±2.365 c
Lingual Apical	0.013±0.015 b	1.680±1.450 bd
P	0.0001***	0.0001***

According to the Kruskal-Wallis 1 way analysis of variance and the Mann-Whitney U test (with Bonferroni correction) there is no statistically significant difference between groups with the same letters.
*: P<0.05 , **: P<0.01 , ***: P<0.001

Figure1.

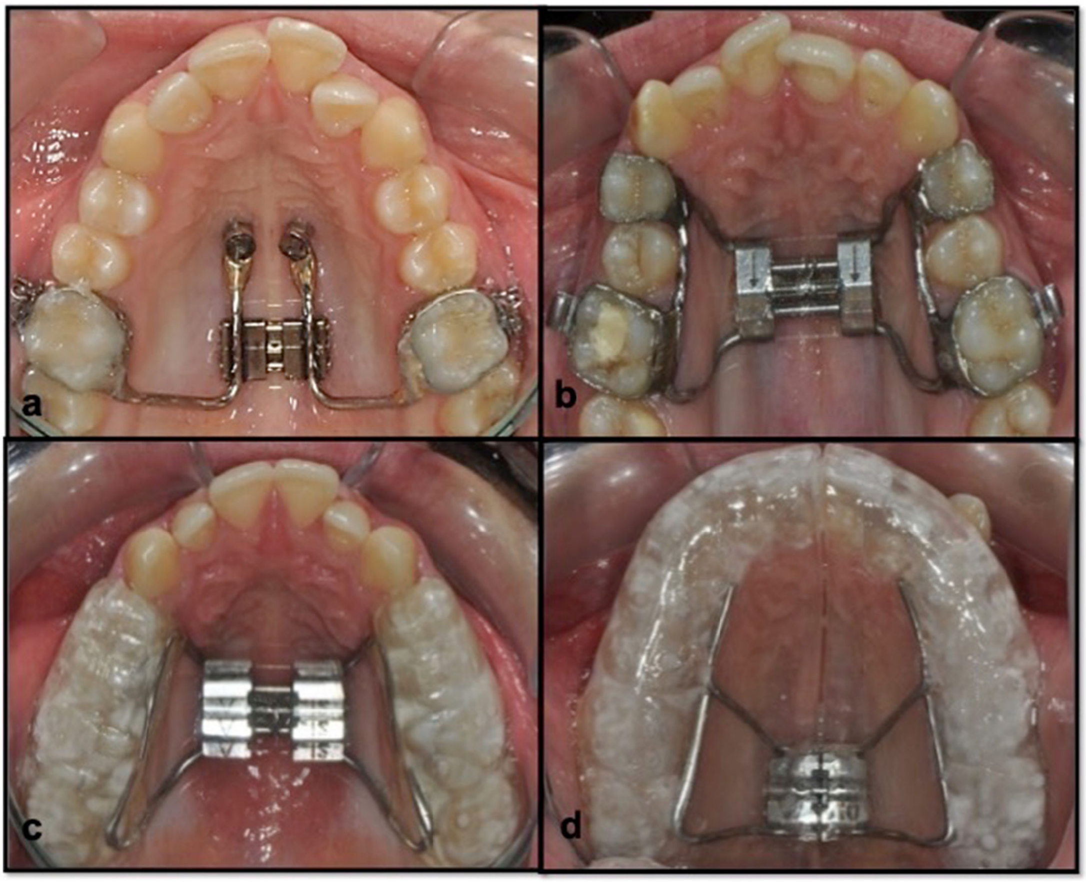


Figure2.

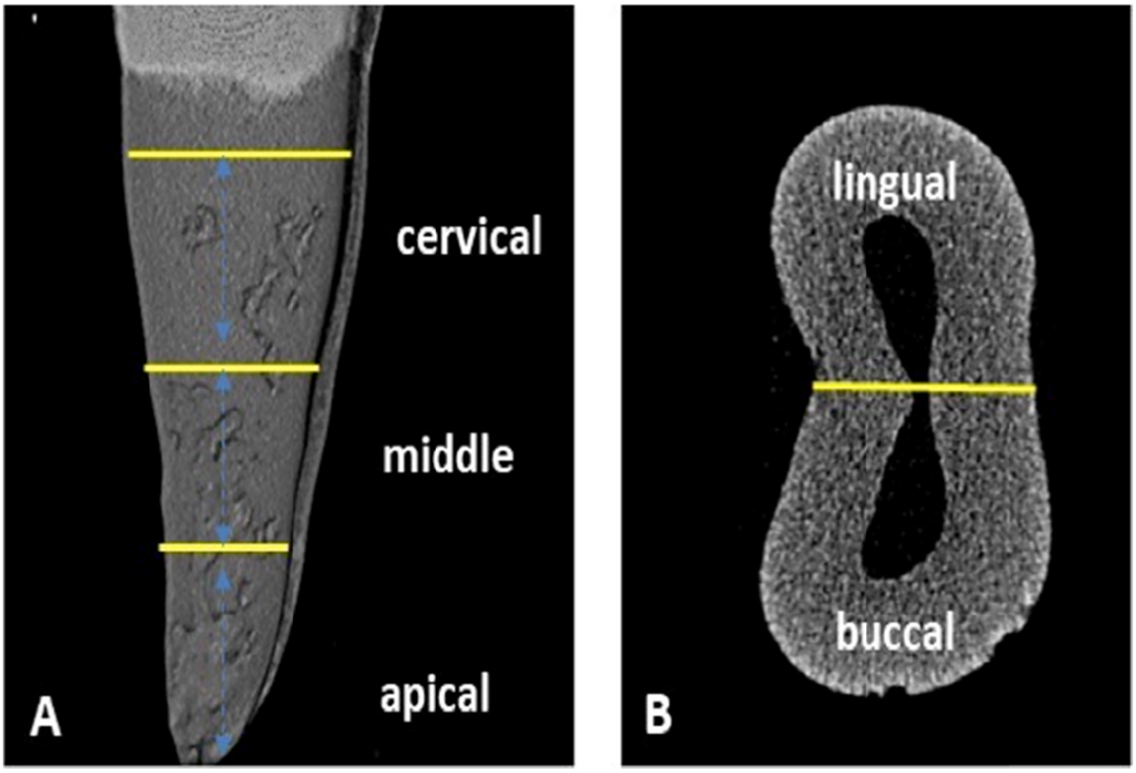
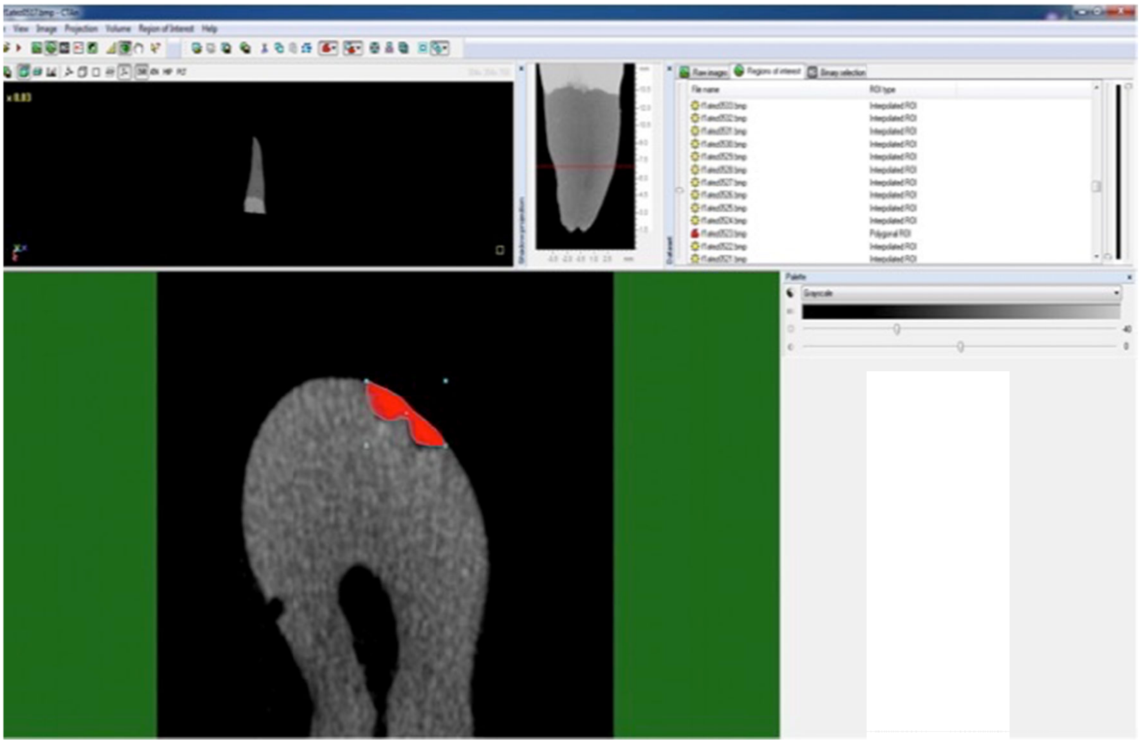


Figure 3



Raw images
Regions of interest
Binary selection
File name
ROI type
Palette
Grayscale