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Comparative analysis of root canal changes after preparation with three systems using Cone-Beam Computed Tomography



Analisi comparativa alla CBCT delle modificazioni canalari dopo la preparazione con tre diversi sistemi

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KEYWORDS

Cone-Beam Computed Tomography;
Canal curvature;
Canal transportation;
Revo-S[®];
HEROShaper[®] and
ProTaper[®].

Abstract

Introduction: The aim of this study was to investigate the morphological changes in the root canal trajectory on extracted teeth after preparation with Endoflare/Revo-S[®], Endoflare/HeroShaper[®] and ProTaper[®] using Cone-Beam Computed Tomography (CBCT).

Methods: 39 root canals with similar curvatures were divided into three homogeneous groups ($n = 13$). Root canals in Group 1 were shaped with Endoflare/Revo-S[®]; Group 2 with Endoflare/Hero Shaper[®], and Group 3 with ProTaper[®]. All specimens were scanned pre- and postoperatively using the Kodak[®] 9000C 3D imaging system. Changes in both degree and position of the root canal

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PAROLE CHIAVE

Tomografia computerizzata a fascio conico;
 Curvature canale;
 Trasporto canale;
 Revo-S[®];
 HEROShaper[®] and ProTaper[®].

curvature were assessed. Canal transportation was calculated for each slice by comparing the position of the root canal centroid before and after instrumentation. Statistical analysis was carried out by the non-parametric Kruskal–Wallis test ($p < 0.05$), and Mann–Whitney test applying the Bonferroni correction ($p < 0.05$).

Results: The mean of curvature degree decreases significantly ($p < 0.003$) for each group, with no statistical differences between the three groups. Mean canal transportation scores ranged from 52 μm (Revo-S[®]) to 85 μm (ProTaper[®]) in the apical third; 51 μm (Revo-S[®]) to 87 μm (ProTaper[®]) in the middle third, and 77 μm (HEROShaper[®]) to 119 μm (ProTaper[®]) in the cervical third. In the apical and the middle parts, Revo-S[®] produced statistically less transportation than HEROShaper[®] (respectively $p = 0.01708$, $p = 0.01328$) and ProTaper[®] (respectively $p = 0.02402$, $p = 0.0202$).

Conclusion: All instruments produced a small curvature deviation and mild canal transportation. Revo-S[®] resulted in less transportation in the apical and middle thirds.

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Riassunto

Obiettivi: Lo scopo di questo studio è stato quello di studiare le alterazioni morfologiche della traiettoria canale su denti estratti dopo la preparazione con Endoflare/Revo-S[®], Endoflare/HeroShaper[®] e ProTaper[®] utilizzando tomografia computerizzata a fascio conico (CBCT).

Materiali e metodi: Metodi: 39 canali radicolari con curvature simili sono stati suddivisi in tre gruppi omogenei ($n = 13$). I canali radicolari del gruppo 1 sono state sagomati con Endoflare/Revo-S[®], quelli del gruppo 2 con Endoflare/Hero Shaper[®] e quelli del Gruppo 3 con ProTaper[®]. Tutti i campioni sono stati sottoposti a scansione CBCT prima e dopo la preparazione canale utilizzando il sistema di imaging 3D Kodak[®] 9000C. Sono stati valutati sia i cambiamenti del grado e della posizione della curvatura canale che il trasporto del canale, confrontando la posizione del canale radicolare centroide prima e dopo strumentazione. L'analisi statistica è stata effettuata utilizzando il test non parametrico di Kruskal–Wallis ($p < 0,05$) e il test di Mann–Whitney applicando la correzione di Bonferroni ($p < 0,05$).

Risultati: Il grado di curvatura è risultato diminuito significativamente in tutti i gruppi ($p < 0,003$), senza evidenziare però differenze statisticamente significative tra i tre gruppi. I valori di trasporto canale medio variavano da 52 μm (Revo-S[®]) a 85 μm (ProTaper[®]) nel terzo apicale, da 51 μm (Revo-S[®]) a 87 μm (ProTaper[®]) nel terzo medio e da 77 μm (HEROShaper[®]) a 119 μm (ProTaper[®]) nel terzo cervicale. Nel terzo apicale e medio i Revo-S[®] hanno determinato statisticamente meno trasporto degli HEROShaper[®] (rispettivamente $p = 0,01708$, $P = 0,01328$) e dei ProTaper[®] (rispettivamente $p = 0,02402$, $P = 0,0202$).

Conclusioni: Tutti gli strumenti hanno prodotto una piccola modificazione della curvatura e un leggero trasporto canale. Gli strumenti Revo S[®] ha dimostrato un minor trasporto nei terzi apicale e medio.

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Introduction

Canal shaping is a critical aspect of endodontic treatment. It influences the outcome of the subsequent phases of irrigation, root canal filling and therefore, the success of the endodontic treatment. Once the canal is shaped, it should have a uniformly tapered funnel shape, increasing in diameter from the apical foramen to the coronal orifice. This shape enhances the efficiency of the irrigation and allows the placement of an effective tooth filling.¹

The development of Nickel–Titanium (Ni–Ti) rotary instrumentation has been a great technological advance. These instruments enable root canals to be shaped with fewer procedural errors.^{2,3} Procedural errors such as transportation and loss of working length were mainly associated

with the use of stainless-steel files, which had insufficient flexibility.^{4,5} Ni–Ti rotary instruments also work faster thus reducing operating time.²

A number of techniques are currently available to evaluate canal transportation and centring ability of instruments during root canal preparation. Micro-Computed Tomography seems to be a promising tool for root canal anatomy studies but this technique is time-consuming and not indicated for chairside use. Recently, Cone-Beam Computed Tomography (CBCT) has become available for clinicians and many endodontic applications have been identified.^{6,7} The aim of this study was to investigate the morphological changes in the root canal trajectory after preparation with Endoflare/Revo-S[®], Endoflare/HeroShaper[®] and ProTaper[®] using CBCT.

Materials and methods

Thirty nine root canals with completely formed apices were selected from a pool of teeth that have been extracted for periodontal or orthodontic purposes (agreement DC-2014-2262). Teeth were stored in a 0.5% chloramines solution and access cavities were prepared by using round burs and Endo-Z burs (Maillefer Dentsply). Canal length and patency were determined with size 10 MMC files (Micro-Mega). Then, distribution of samples was performed after the first imaging prior to instrumentation.

A cut from the 3D reconstruction in the main axis of the curvature of the selected canal was identified. The degree of curvature was determined by a modified version of the Berbert and Nishiyama method (Fig. 1), initially described for a 2D image.^{8,9} The teeth were randomised into three homogeneous groups. Each group was shaped with a Ni–Ti rotary system: Revo-S[®], HEROshaper[®] and ProTaper[®] according to the manufacturers' guidelines.

Group 1 was prepared using Endoflare[®] for coronal flaring and Revo-S[®] up to SU (size 25, taper 6%).

Group 2 was shaped using Endoflare[®] for coronal flaring and HeroShaper[®] up to size 30 (taper 4%).

Group 3 was prepared using SX[®] for coronal flaring and Protaper[®] up to F2 (size 25, taper 8%).

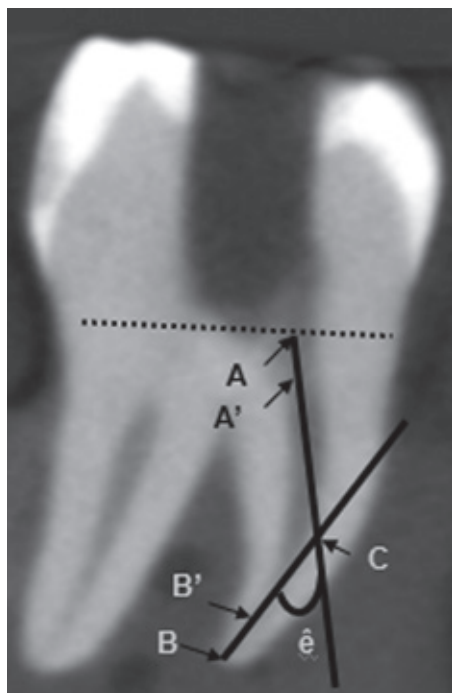


Figure 1 Modified version of the Berbert and Nishiyama method. From the tangent to the pulp floor, point A is placed at the centre of the canal entrance and point A' is placed about 1 mm from A on the path of the canal. These two points form a straight line. Then point B is placed at the apical foramen and point B' about 1 mm from B on the path of the canal. These last two points form a second straight line. These two lines intersect at point C, and a ratio of the distances AC/CB represent the coefficient of the position of the curvature. Angle ϵ represents the degree of curvature.

For all groups irrigation with a 3% NaOCL solution was performed after each files passage (at least 32 ml). Then, a 14% EDTA flush flow (2 ml for 1 min) was performed to remove the smear layer and was followed by a 3% NaOCL irrigation and a distilled water final rinse.

The 3D acquisitions were performed using Cone-Beam Computed Tomography in small fields, 3D 9000C (Kodak[®], University Hospital of Rennes-France). For analysis, the teeth were subjected to two acquisitions: the first one was performed after the opening of the pulp chamber and the second once the procedure was completed. Precise repositioning of pre- and post-preparation images was ensured by a custom-made mounting device. Acquisition parameters were, for cutting: 76 μm , 70 kV, 3.2 mA, 10.77, and, for exposure: 69 mGy/cm². The spatial resolution is 76 μm . During acquisition, the CBCT imaging system data plate stores 4–5 teeth samples. The file was then divided into several files containing a stack of slices corresponding to only one sample. This file, in the TIFF format, can be opened and analysed with ImageJ[®] software (<http://imagej.nih.gov/ij/>). This program provides opportunities for analysis and many important functions.

Trajectory analysis: degree and position of curvature

On a cut provided from the data obtained using a 3D reconstruction, the degree and position of curvature were calculated using the modified Berbert and Nishiyama technique.⁸ From the tangent to the pulp floor, point A is placed at the centre of the canal entrance and point A' is placed about 1 mm from A on the path of the canal. These two points form a straight line. Then point B is placed at the apical foramen and point B' about 1 mm from B on the path of the canal. These last two points form a second straight line. These two lines intersect at point C, and a ratio of the distances AC/CB represent the coefficient of the position of the curvature. Angle ϵ represents the degree of curvature (Fig. 1).

Trajectory analysis: root canal transportation

To investigate the canal trajectory along the entire root length, the Image J software was used. Stacks of images in the grey scale were processed in order to determine the root canal transportation. This transportation is determined by comparing the coordinates of the geometric centre, "centroid" of the canal before and after instrumentation. The displacement of the canal centre is then established (Fig. 2) by calculating the difference between the relative position of the centroids before and after treatment after setting the centroid of the root itself as reference.

For a given sample, a thresholding of the root was first performed in order to determine the geometric centre of the root (x and y coordinates of the piecewise zone for each cut), which remains identical before and after instrumentation. This step was necessary in order to eliminate the bias of a possible skewing while repositioning the teeth according to a fixed landmark. Therefore, the coordinates of the root centroid were used as the reference point.

Then, a second thresholding was performed to determine the coordinates of the canal's geometric centre before and



Figure 2 Transportation of the position of the centroid of the canal before and after preparation.

after instrumentation. For each canal, the values were automatically calculated for the whole stack according to the piecewise zone: first for the root contour, then for the canal. For each cut, the processed values represent the coordinates of the geometric centre of the root contour and the canal before and after instrumentation. All these series of values related to the geometric centres are then listed in Excel tables. In order to eliminate the bias related to the repositioning of the root within the landmarks, the measurement based on the contours of the root, before and after instrumentation, remains unchanged, and is used as a reference. The measurement consists in subtracting the coordinates of the canal centroid from this reference. These new values, calculated according to the root contours: coordinates of the canal centroid before (“xPRE” and “yPRE”) and after (“xPOST” and “yPOST”) instrumentation are used to assess root canal transportation.

Statistical analysis

Statistical analysis was performed with the software StatXact 7.0-Cytel-USA using the Wilcoxon and Kruskal–Wallis test with a threshold value of $p = 0.05$. The Kruskal–Wallis test is then completed by Mann–Whitney tests using the Bonferroni correction to assess differences between groups.

Results

Degree and position of curvature

The mean degree of curvature respectively decreases of 7.07° for the Revo-S, 6.92° for the HeroShaper and 9° for the Protaper (Table 1). The difference between before and

after instrumentation shows a statistically significant deviation of curvature for all groups ($p < 0.003$). In addition, there is no statistically significant difference among the three systems ($p > 0.6$).

In contrast, the mean coefficient of curvature position before and after instrumentation shows no statistically significant difference ($p > 0.5$) for all systems (Table 1).

The position variation of the curvature among the three systems did not differ significantly ($p > 0.7$), with similar averages of low values.

Canal transportation

In the apical third, the mean transportation was about $53 \mu\text{m}$ for the Revo-S[®] group, $77 \mu\text{m}$ for the HERO Shaper[®] and $85 \mu\text{m}$ for the ProTaper[®] with statistically significant differences between Revo-S[®] and HERO Shaper[®] ($p = 0.01708$) and ProTaper[®] ($p = 0.02402$) (Fig. 3).

In the middle third, statistically significant differences were found between Revo-S[®] and HeroShaper[®] system ($p = 0.01328$) as well as between Revo-S[®] and ProTaper[®] ($p = 0.0202$). The mean transportation values are about $51 \mu\text{m}$ for the Revo-S[®] group, $69 \mu\text{m}$ for HeroShaper[®] and $87 \mu\text{m}$ for ProTaper[®] (Fig. 4).

In the coronal third, the mean transportation values were about $77 \mu\text{m}$, $94 \mu\text{m}$ and $119 \mu\text{m}$ for HEROShaper[®], Revo-S[®] and ProTaper[®] respectively with no statistically significant differences ($p > 0.05$) (Fig. 5).

Discussion

The aim of this *in vitro* study was to assess and compare the shaping ability of three Ni–Ti rotary systems, Revo-S[®], HeroShaper[®] and ProTaper[®] with a simple imaging system which can be used *in vivo*.

In terms of degree of curvature, a statistically significant ($p < 0.003$) root canal straightening (6.92 – 9°) after preparation is shown for each group. Although no statistically significant difference is demonstrated between the three systems, the ProTaper[®] group records a mean straightening which is higher than the other groups (9°). These results are in agreement with the already published data of the literature: in 2007, Yang et al.¹⁰ using a modified Bramante technique, compared root canal preparations with ProTaper[®] and HEROShaper[®] and the results of this study showed more straightening of the curvature for the ProTaper[®] system for both the degree and the radius of curvature.

The compilation of all cross-sections performed every $76 \mu\text{m}$ using CBCT enables the Image J software[®] to render an accurate analysis of the actual shape changes of the canal.

Table 1 Variations of the curvature according to the modified version of the Berbert and Nishiyama method.

	Variation of curvature in degrees (means \pm SD)	Variation of position of the curvature (AC/CB ratio) (means \pm SD)
HERO Shaper [®]	$6.92^\circ \pm 3.90^*$	-0.08 ± 0.76
Revo-S [®]	$7.07^\circ \pm 5.11^*$	0.00 ± 0.65
Pro Taper [®]	$9.00^\circ \pm 6.19^*$	0.04 ± 1.1

* Represents a statistically significant difference between the angle of curvature before and after treatment.

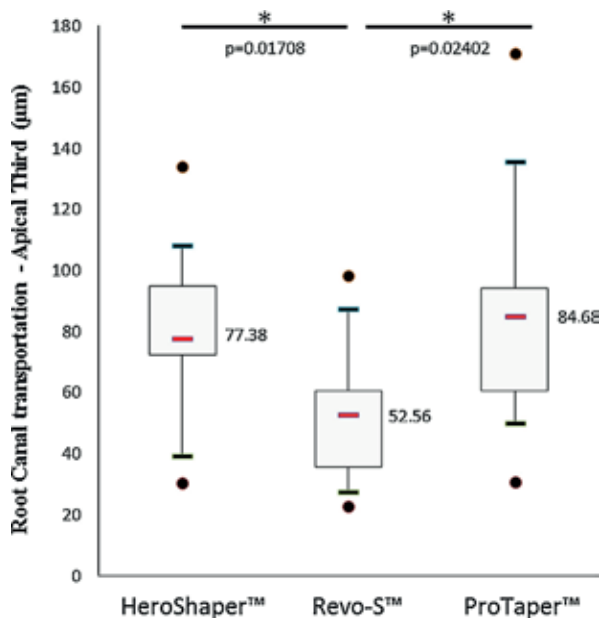


Figure 3 Root canal transportation values in the apical third. Red bar represents median value. Black points represent maximum and minimum values. Black bars represent first and ninth decile and limits of white rectangle represents first and third quartile. * represents a statistically significant difference between groups.

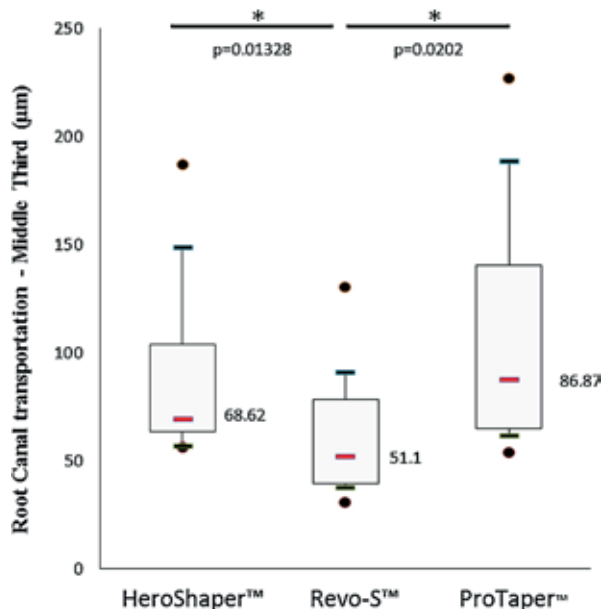


Figure 4 Root canal transportation values in the middle third. Red bar represents median value. Black points represent maximum and minimum values. Black bars represent first and ninth decile and limits of white rectangle represents first and third quartile. * represents a statistically significant difference between groups.

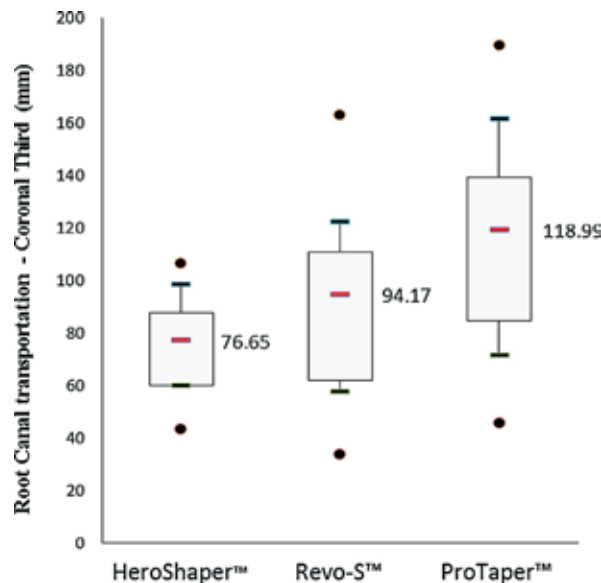


Figure 5 Root canal transportation values in the cervical third. Red bar represents median value. Black points represent maximum and minimum values. Black bars represent first and ninth decile and limits of white rectangle represents first and third quartile.

All the studied techniques produce a shift in the centre of the canal; this confirms the results of the study conducted by Hartmann et al. in 2007.¹¹ In the apical third, the Revo-S[®] system better respected the original canal trajectory than HeroShaper[®] in a statistically significant way ($p = 0.01708$). These data confirm the results of the study of Yang et al. in 2007.¹⁰ Revo-S[®] is also significantly more respectful of the canal path than the ProTaper[®] system ($p = 0.0242$). These results are in agreement with those recorded in the CBCT study conducted by Hashem et al. in 2012.¹²

In the middle third, the Revo-S[®] system causes less canal transportation, with a statistically significant difference compared to the HEROShaper[®] ($p = 0.01328$) and ProTaper[®] ($p = 0.0202$) systems. The highest root canal transportation is located at the cervical third for all groups and ranges from 76 µm to 119 µm. This can be explained by the high taper of the orifice openers that have been used.

The ProTaper[®] group achieves the highest canal transportation for each third. In a similar study conducted in 2013, Elsherief et al.¹³ did not find any statistically significant difference between the amount of transportation induced by Revo-S[®], HEROShaper[®] and ProTaper[®]. They also recorded lower transportation values than in the present study. These contradictory results may be explained by the lower spatial resolution used in the above-mentioned study (125 µm vs. 76 µm). However, in 2011, Ozer using CBCT with a 125 µm spatial resolution recorded the similar values as those reported in the present study for the apical root canal transportation induced by the ProTaper[®] system.¹⁴

The biological samples used in this study represent an adequate model despite the difficulty to obtain perfectly identical groups; however, regarding the degree of curvature, there was no statistically significant difference among these groups. The use of resin blocks would have led to strictly identical conditions but these simulators offer some

drawbacks such as surface texture and hardness of the material, which are very different from that of the tooth. In addition, the circular or oval section of the artificial canal differs from that of a natural canal¹⁵ and this substitution method does not offer any information about remaining dentin thickness after root canal instrumentation.¹⁶

CBCT is now easily available to clinicians and data from the literature have shown that measurements taken from the CBCT images are reliable. Indeed, studies comparing “virtual” measurements to the same measurements following dissection or histological sections show high correlation coefficients. For example, in 2010, Michetti et al.¹⁷ found 93% of correlation between the areas of the canal calculated on histological cross sections and CBCT images, and Kim et al.¹⁸ obtained 94% of correlation in the distance between the apex of the mandibular molar and the mandibular canal measured on both CBCT reconstruction and dissection samples.

In addition, in this *in vitro* study, this non-invasive method allows complementary investigations such as Scanning Electron Microscopic observations of the root canal walls after instrumentation.

Calculation of the root canal transportation was performed on all the sections obtained from the CBCT reconstruction i.e. every 76 µm of the canal. This was achieved through an automated method of calculation using the Image J software[®]. In the literature, the root canal transportation is often studied on a few sections using a manual image analysis.^{11,19,20}

In the present study, the analysis using an automatic threshold technique is based on the greyscale differences of the reconstructed image. The image resolution is about one pixel (76 µm). The threshold determines the shape of the canal and is homothetic to determine the geometric centre.

In very thin root canals, the quality of CBCT images is not sufficient to precisely analyse the apical areas because it does not allow the detection of the canals using this automatic threshold method preoperatively.

Micro-computed tomography remains the gold standard technique for non-destructive *in vitro* studies of root canal trajectory, but it cannot be used for *in vivo* studies. It also requires an extensive protocol for both acquisition and data processing.¹⁵ Nevertheless, in this study, the transportation values recorded after using the Protaper[®] system are really closed to those found in high resolution micro-computed tomography studies.^{21,22}

Conclusion

Under the conditions of this study, all systems tested produce a small deviation of the curvature. Revo-S[®] is significantly more respectful of the canal shape than ProTaper[®] and HEROShaper[®].

Conflict of interest

The authors have no conflicts of interest to declare.

References

- Schilder H. Cleaning and shaping the root canal. *Dent Clin North Am* 1974;18:269–96.
- Gluskin AH, Brown DC, Buchanan LS. A reconstructed computerized tomographic comparison of Ni–Ti rotary GT files versus traditional instruments in canals shaped by novice operators. *Int Endod J* 2001;34:476–84.
- Young GR, Parashos P, Messer HH. The principles of techniques for cleaning root canals. *Aust Dent J* 2007;52(1 Suppl.):S52–3.
- Morgan LF, Montgomery S. An evaluation of the crown-down pressureless technique. *J Endod* 1984;10:491–8.
- Glickman GN, Dumsha TC. Problems in canal cleaning and shaping. In: Gutmann JL, et al., editors. *Problem solving in Endodontics*. 3rd edn, St Louis: Mosby; 1997. p. 91–122.
- Patel S. New dimensions in endodontic imaging: Part 2. Cone beam computed tomography. *Int Endod J* 2009;42:463–75.
- Patel S, Dawood A, Whaites E, Pitt Ford T. New dimensions in endodontic imaging: Part 1. Conventional and alternative radiographic systems. *Int Endod J* 2009;42:447–62.
- Berbert A, Nishiyama CK. Curvaturas radiculares. Uma nova metodologia para mensuração e localização. *Revista Gaúcha de Odontologia* 1994;42:356–8.
- Bramante CM, Betti LV. Comparative analysis of curved root canal preparation using Nickel–Titanium instruments with or without EDTA. *J Endod* 2000;26:278–80.
- Yang GB, Zhou XD, Zheng XL. Shaping ability of progressive versus constant taper instruments in curved root canals of extraction teeth. *Int Endod J* 2007;40:707–14.
- Hartmann M, Barletta F, Fontanella V, Vanni J. Canal transportation after root canal instrumentation: a comparative study with computed tomography. *J Endod* 2007;33:962–5.
- Hashem AAR, Ghoneim AG, Lufty RA, Foda MY, Omar GAF. Geometric analysis of root canals prepared by our rotary NiTi shaping systems. *J Endod* 2012;38:996–1000.
- Elsherief SM, Zayet MK, Hamouda IM. Cone-beam computed tomography analysis of curved root canals after mechanical preparation with three nickel-titanium rotary instruments. *J Biomed Res* 2013;27:326–35.
- Ozer SY. Comparison of root canal transportation induced by three rotary systems with noncutting tips using computed tomography. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011;111:244–50.
- Peters O. Current challenges and concepts in the preparation of root canal systems: a review. *J Endod* 2004;30:559–67.
- Yun HH, Kim SK. A comparison of the shaping abilities of 4 nickel–titanium rotary instruments in simulated root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;95:228–33.
- Michetti J, Maret D, Mallet JP, Diemer F. Validation of cone-beam computed tomography as a tool to explore root canal. *J Endod* 2010;36:1187–90.
- Kim TS, Caruso JM, Christensen H, Torabinejad M. A comparison of cone-beam computed tomography and direct measurement in the examination of the mandibular canal and adjacent structures. *J Endod* 2010;36:1191–4.
- Gergi R, Rjeily JA, Sader J, Naaman A. Comparison of canal transportation and centering ability of twisted files, Pathfile-ProTaper system, and stainless steel hand K-files by using computed tomography. *J Endod* 2010;36:904–7.
- Sanfelice CM, Da Costa F, So M, Vier-Pelisser F, Bier C, Grecca F. Effects of four instruments on coronal pre-enlargement by using cone beam computed tomography. *J Endod* 2010;36:858–61.
- Loizides AL, Kakavetsos VD, Tzanetakakis GN, Kontakiotis EG, Eliades G. A comparative study of the effects of two nickel-titanium preparation techniques on root canal geometry assessed by microcomputed tomography. *J Endod* 2007;33:1455–9.
- Stern S, Patel S, Foschi F, Sherriff M, Manocci F. Changes in centring and shaping ability using three nickel-titanium instrumentation techniques analysed by micro-computed tomography (µCT). *Int Endod J* 2012;45:514–23.