

The effect of single-file reciprocating systems on Substance P and Calcitonin gene-related peptide expression in human periodontal ligament

J. Caviedes-Bucheli¹, J. O. Moreno¹, C. P. Carreño¹, R. Delgado¹, D. J. Garcia¹, J. Solano¹, E. Diaz² & H. R. Munoz³

¹School of Dentistry, Universidad Santo Tomas, Floridablanca Bucaramanga, Santander; ²Universidad de La Sabana, Bogota, Colombia; and ³Postgraduate Endodontics Department, School of Dentistry, Universidad de San Carlos de Guatemala, Guatemala City, Guatemala

Abstract

Caviedes-Bucheli J, Moreno JO, Carreño CP, Delgado R, Garcia DJ, Solano J, Diaz E, Munoz HR.

The effect of single-file reciprocating systems on Substance P and Calcitonin gene-related peptide expression in human periodontal ligament. *International Endodontic Journal*, **46**, 419–426, 2013.

Aim To quantify the effect of two single-file reciprocating root canal preparation systems on Substance P (SP) and Calcitonin gene-related peptide (CGRP) expression in healthy human periodontal ligament (PDL).

Methodology Forty PDL samples were obtained from healthy premolars where extraction was indicated for orthodontic reasons. Prior to extraction, 20 of these premolars were divided equally in two groups, and then, root canals were prepared using one of two different single-file systems: WaveOne and Reciproc. Ten premolars were prepared with hand files and served as a positive control group. The remaining 10 premolars were extracted without treatment and served as a negative control group. All PDL samples were processed, and SP and CGRP were measured by radioimmunoassay.

Results Greater SP and CGRP expression were found in the hand instrumentation group (1.220 pmol SP

and 0.084 pmol CGRP per mg of PDL), followed by the WaveOne group (0.908 pmol SP and 0.046 pmol CGRP per mg of PDL) and the Reciproc group (0.511 pmol SP and 0.022 pmol CGRP per mg of PDL). The lower SP and CGRP values were associated with the intact control group (0.453 pmol SP and 0.018 pmol CGRP per mg of PDL). The Kruskal–Wallis test revealed significant differences between groups ($P < 0.001$). *Post hoc* Tukey HSD tests revealed significant differences in SP and CGRP expression between intact teeth in the control group and all the other groups ($P < 0.001$) except with the Reciproc group ($P = 0.165$ and $P = 0.42$ for SP and CGRP, respectively). Hand instrumentation was associated with significant differences with all the other groups ($P < 0.001$). Differences between the WaveOne and Reciproc groups were also significant ($P < 0.001$).

Conclusion Substance P and CGRP expression in PDL cells increased when teeth were prepared with WaveOne as well as with hand instrumentation. Reciproc maintained SP and CGRP levels in line with the negative control group.

Keywords: Calcitonin gene-related peptide, human periodontal ligament, neurogenic inflammation, single-file reciprocating systems, Substance P.

Received 5 June 2012; accepted 5 September 2012

Introduction

One of the greatest challenges during root canal treatment is to clean and shape the root canal system, preserving its anatomy, particularly the form

Correspondence: Javier Caviedes-Bucheli, Cra 7 No. 40-62 Building 26, Bogotá, Colombia (e-mail: javiercaviedes@gmail.com).

and position of the apical foramen, avoiding extrusion of irritants from the root canal to the periapical tissue, such as dentine debris, necrotic tissue, bacteria and/or filling materials (Peters 2004). This irritation to the periodontal ligament (PDL) could lead to an antigen–antibody reaction generating an inflammatory response, clinically known as symptomatic apical periodontitis (Siqueira *et al.* 2002).

Different incidence rates for post-treatment symptomatic apical periodontitis, ranging from 2% to 30% have been reported (Georgopoulou *et al.* 1986, DiRenzo *et al.* 2002, Siqueira *et al.* 2002). This wide range is conditioned by the type of study, pre-operative clinical conditions, treatment protocol and individual response to treatment (Glennon *et al.* 2004).

It has been demonstrated that PDL inflammation has a neurogenic component; where nerve fibres control vascular tone and immune response through neuropeptide actions, such as Substance P (SP) and Calcitonin gene-related peptide (CGRP) (Stashenko *et al.* 1998). These neuropeptides could be released from C-type nerve fibres present in the PDL when stimulated by extrusion of irritants from the root canal, leading to vasodilation, plasma extravasation, immune system activation, chemotaxis and upregulation of macrophage, lymphocytes and mast cell actions (Caviedes-Bucheli *et al.* 2008a). All these biological effects are observed during post-treatment symptomatic apical periodontitis, where the severity of the PDL inflammation is directly correlated with the extent of tissue damage and the mechanical stress exerted on the tooth (Caviedes-Bucheli *et al.* 2010, 2011a).

It has been demonstrated that all root canal preparation techniques cause some extrusion of irritants from root canal to the periapical tissues; which could vary depending on the characteristics of the instruments, the type of movement and the technique used (Al-Omari & Dummer 1995, Reddy & Hicks 1998, Ferraz *et al.* 2001, Tanalp *et al.* 2006). Traditionally, root canal preparation was carried out with stainless-steel hand files. However, instruments designed with a square or triangular cross-section and the filing movement (in which the instrument acts like a piston) tend to pump debris and irritants to the periapical tissue (Ferraz *et al.* 2001, Tanalp *et al.* 2006, Leonardi *et al.* 2007). Moreover, stainless-steel files have an increased incidence of canal transportation, and several instruments are necessary to obtain an adequate shape in the canal (Kuhn *et al.* 1997).

To overcome these disadvantages, NiTi rotary files were introduced. They gained popularity due to their

flexibility and greater variety of tapers, which allow preparation of a root canal with a reduced number of instruments in a shorter period of time (Reddy & Hicks 1998, Ferraz *et al.* 2001). Cross-sections of rotary files were also modified to reduce the amount of debris extruded to the periapical tissue. However, studies have shown that these instruments still extrude dentine debris and tend to make round preparations leaving portions of the canal unprepared, modifying original root canal anatomy (Al-Omari & Dummer 1995, Kuhn *et al.* 1997, Reddy & Hicks 1998, Ferraz *et al.* 2001, Schäfer & Vlassis 2004, Veltri *et al.* 2005, Tanalp *et al.* 2006, Leonardi *et al.* 2007). This could be explained due to the different physical characteristics of the instruments, such as cross-section, core diameter, rake and helicoidal angles, distance between flutes and tip design (Kuhn *et al.* 1997, Schäfer & Vlassis 2004).

Recently, WaveOne (Dentsply Maillefer, Ballaigues, Switzerland) and Reciproc (VDW, Munich, Germany) were introduced. These are single-file reciprocating (alternate rotation) root canal preparation systems that alternate different values of counter clockwise (CCW) and clockwise (CW) rotation movements, allowing the file to rotate 360° after performing a series of reciprocating movements. The greater CCW movement advances the instrument, engaging and cutting dentine, whilst the shorter CW movement disengages the instrument from the dentine before it can lock into the canal, trying to resemble the 'balanced-force' concept. This alternate rotation action combined with a brushing motion against dentinal walls allows the instrument to contact a considerable portion of canal walls creating uniform shapes both bucco-lingually and mesio-distally (Webber *et al.* 2011, Yared 2011).

These systems are designed to prepare the entire root canal with only one instrument, significantly reducing the amount of time needed to prepare a canal, which could reduce the mechanical stress exerted over the tooth during preparation. The cross-section designs, the helicoidal angles and the tip of these instruments are also designed to avoid extrusion of dentine debris into the periapical tissues (Webber *et al.* 2011, Yared 2011).

A previous study suggested that root canal preparation with hand files and some rotary preparation systems may trigger a neurogenic inflammation response in the PDL, and that SP and CGRP play an important role during this inflammatory process (Caviedes-Bucheli *et al.* 2010). Therefore, the purpose of this study

was to quantify and compare the effect of two new single-file reciprocating root canal preparation systems on SP and CGRP expression in healthy human PDL. The null hypothesis was that there are no significant differences in SP and CGRP expression in PDL cells from teeth prepared with single-file reciprocating root canal preparation systems when compared with baseline levels.

Materials and methods

An experimental study was performed according to Colombian Ministry of Health recommendations regarding ethical issues in research involving human tissue; it was approved by the ethics committee of the Universidad Santo Tomas in Bucaramanga, Colombia. Written informed consent was obtained from each patient participating in the study (18–33 years old, healthy, not medicated and nonsmoking human donors). Forty PDL samples were obtained from 20 patients who had two mandibular premolars indicated for extraction for orthodontic reasons. All teeth used were caries- and restoration-free with complete root development determined both visually and radiographically, without signs of periodontal disease or traumatic occlusion and without orthodontic forces. Teeth had only one straight canal [canal curvatures over 20° determined by Pruett technique (Pruett *et al.* 1997) were not included].

For the first 10 patients, teeth from the same subject were randomly assigned to either one of the control groups: (i) Intact-teeth control group; or (ii) Hand Instrumentation. For the remaining 20 patients, teeth from the same subject were randomly assigned to either one of the experimental groups: (iii) WaveOne; or (iv) Reciproc. All teeth were anaesthetised by inferior alveolar nerve block injection of 1.8 mL 4% prilocaine without vasoconstrictor. Adequate pulpal anaesthesia was ascertained with a negative response to an electronic pulp vitality test.

Experimental procedure and sample collection

For the intact-teeth control group, extraction was performed by conventional methods without excessive injury to the PDL 10 min after anaesthetic application. For the rest of the groups, teeth were isolated with rubber dam, cavity accesses were carried out using a Zekrya bur (Dentsply Tulsa Dental, Tulsa, OK, USA) in a high-speed hand piece, canal patency and working length were established using a size 10 K-file

with the aid of an apex locator (Root ZX II; J Morita, Tokyo, Japan) set to 0.5 mm and radiographically confirmed, finally root canals were prepared with the corresponding preparation technique by a single operator to avoid interoperator variation. Files were used only one time and then discarded. Preparation techniques were carried out as follows:

WaveOne Group

The root canal was prepared using one new WaveOne large file (size 40, .08 taper) activated in a WaveOne motor (Dentsply Maillefer, Ballaigues, Switzerland) following the manufacturer's recommendations. The file was used with short up and down motion with slight apical pressure in three cycles, one to prepare each third of the canal (cervical, middle and apical) using an EDTA-containing gel (Glyde; Dentsply Maillefer) as a lubricant. After each cycle, the file was cleaned with wet gauze to remove dentine debris and the EDTA-gel, and the canal was irrigated with 3 mL of 5.25% sodium hypochlorite (NaOCl) using a Monoject syringe with a 30-gauge needle placed 3 mm short of working length to complete a total of 9 mL of NaOCl for each canal. Effective working time of the file inside the canal did not exceed 1 min.

Reciproc Group

The root canal was prepared using one new Reciproc file (size 40, 0.06 taper) activated in a Reciproc motor (VDW) following the manufacturer's recommendations. The file was used with short up and down motion with slight apical pressure in three cycles identically to that described for the WaveOne group. Irrigation volume, EDTA-gel application and effective working time of the file inside the canal were exactly the same as described for the WaveOne group.

Hand Instrumentation Group

The root canal was prepared with hand instrumentation using 0.02 taper Flexofiles (Dentsply Maillefer, Ballaigues, Switzerland) sizes 15–40 to working length with a filing motion using an EDTA-containing gel (Glyde; Dentsply Maillefer) as a lubricant. Canals were irrigated with 1.5 mL of 5.25% NaOCl after each file with a Monoject syringe with a 30-gauge needle placed 3 mm short of working length to complete a total of 9 mL of NaOCl. Effective preparation time of the canal did not exceed 5 min.

Teeth were extracted 10 min later after completing canal preparation with conventional methods without excessive injury to the PDL. After extraction, a size

10 K-file was placed into the canal until its tip exited from the foramen to corroborate apical patency and that all working lengths were at 0.5 mm from the foramen.

Periodontal ligament samples were obtained from the apical 3 mm of the root with a periodontal curette, placed on an Eppendorf tube, snap-frozen in liquid nitrogen and kept at -70°C until use.

Radioimmunoassay

Periodontal ligament samples were defrosted without thermal shock, dried on a filter and weighed on an analytical balance. Neuropeptides were extracted by adding 150 μL of 0.5 mol L^{-1} acetic acid and double boiling in a thermostat bath for 30 min in accordance with previously reported protocols (Caviedes-Bucheli et al. 2005, 2008b, 2009, 2010, 2011a,b).

Substance P and CGRP expression were determined by competition binding assays using a human SP and a human CGRP-RIA kits from Phoenix Peptide Pharmaceutical (Ref. RK-061-05 and RK-015-02; Belmont, CA, USA). Samples were submitted to two different radioimmunoassay (RIA) assays, one for each peptide.

In both RIA assays, a total of 50 μL of each sample solution was incubated in polypropylene tubes at room temperature for 20 h with 100 μL of primary antibody (for each neuropeptide) and 100 μL of different SP (or CGRP) concentrations ($10\text{--}1280\text{ pg mL}^{-1}$). Then, 50 μL of 125I-SP (or 125I-CGRP) was added and left incubate for another 24 h. Bound fractions were precipitated by the addition of 100 μL of a secondary antibody (Goat Anti-Rabbit IgG serum), 100 μL of normal rabbit serum and 500 μL of RIA buffer containing 1% polyethylene glycol 4000. After 2 h of incubation at room temperature, tubes were

spun at 3000 rpm for 45 min at 4°C . The supernatants were decanted, and pellet radioactivity was read on a Gamma Counter (Gamma Assay LS 5500; Beckman, Fullerton, CA, USA). Standard curves of authentic peptide were made in buffers identical to the tissue extracts on semi log graph paper.

Finally, analysis of the binding data assessed the amount of SP and CGRP present in every sample, using the percentage of maximum binding ($B/B_0\%$) calculated for each unknown sample, reading across the graph to the point of intersection with the calibration curve, where the corresponding X-axis coordinate is equivalent to the concentration of peptide in the assayed sample.

Statistical analysis

Values are presented as SP and CGRP concentration in pmol per mg of PDL. Mean, median, standard deviation and maximum/minimum values are presented for each group. Kruskal–Wallis test was performed to establish statistically significant differences between groups ($P < 0.05$). Tukey HSD *post hoc* comparisons were also performed.

Results

Both neuropeptides were found to be present in all PDL samples (Tables 1 and 2). Highest SP levels were observed in the Hand instrumentation group, with a mean value of 1.220 ± 0.085 pmol SP per mg of PDL, followed by the WaveOne group with a mean SP value of 0.908 ± 0.061 pmol SP per mg of PDL. Mean value for the Reciproc group was 0.511 ± 0.048 pmol SP per mg of PDL. Lowest SP levels were observed in the intact-teeth control group samples with a mean value of 0.453 ± 0.039 pmol

Table 1 Substance P (SP) expression in periodontal ligament from healthy human premolars after the root canal preparation with single-file reciprocating systems

	N	Mean	Median	Standard deviation	Minimum	Maximum
Intact teeth*	10	0.453	0.458	0.039	0.386	0.494
Hand instrumentation**	10	1.220	1.200	0.085	1.110	1.360
WaveOne***	10	0.908	0.920	0.061	0.820	0.990
Reciproc***	10	0.511	0.510	0.048	0.440	0.580

Values are presented as SP concentration in pmol per mg of periodontal ligament.

Kruskal–Wallis test revealed significant differences between groups ($P < 0.001$).

*HSD *post hoc* test revealed significant differences with all the other groups ($P < 0.001$) except with the Reciproc group ($P = 0.165$).

**HSD *post hoc* test revealed significant differences with all the other groups ($P < 0.001$).

***Differences between these groups were also significant ($P < 0.001$).

Table 2 Calcitonin gene-related peptide (CGRP) expression in periodontal ligament from healthy human premolars after the root canal preparation with single-file reciprocating systems

	N	Mean	Median	Standard deviation	Minimum	Maximum
Intact teeth*	10	0.018	0.017	0.004	0.011	0.025
Hand instrumentation**	10	0.084	0.083	0.006	0.077	0.092
WaveOne***	10	0.046	0.047	0.006	0.038	0.054
Reciproc***	10	0.022	0.020	0.006	0.015	0.032

Values are presented as CGRP concentration in pmol per mg of periodontal ligament.

Kruskal–Wallis test revealed significant differences between groups ($P < 0.001$).

*HSD *post hoc* test revealed significant differences with all the other groups ($P < 0.001$) except with the Reciproc group ($P = 0.420$).

**HSD *post hoc* test revealed significant differences with all the other groups ($P < 0.001$).

***Differences between these groups were also significant ($P < 0.001$).

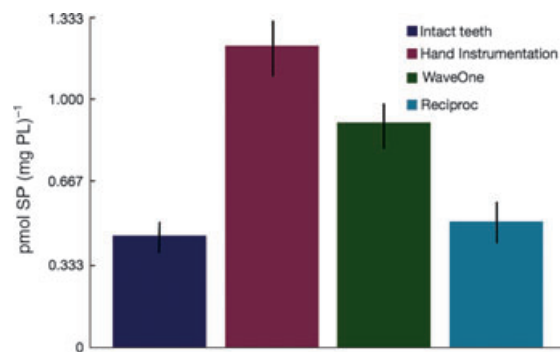
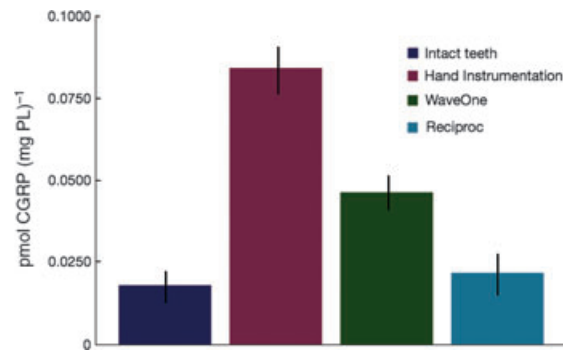
SP per mg of PDL (Fig. 1). Kruskal–Wallis test revealed significant differences between groups ($P < 0.001$). Tukey HSD *post hoc* tests showed significant statistical differences between the intact-teeth control group and the WaveOne and Hand instrumentation groups ($P < 0.001$). Differences between the Reciproc and the WaveOne groups were also statistically significant ($P < 0.001$). There was no statistically significant difference between the intact-teeth and the Reciproc groups ($P = 0.165$). Differences between hand instrumentation and all the other groups were also significant ($P < 0.001$).

Highest CGRP levels were observed in the Hand instrumentation group, with a mean value of 0.084 ± 0.006 pmol CGRP per mg of PDL, followed by the WaveOne group with a mean value of 0.046 ± 0.006 pmol CGRP per mg of PDL. The mean value for the Reciproc group was 0.022 ± 0.006 pmol CGRP per mg of PDL. Lowest CGRP levels were observed in the intact-teeth control group samples with a mean value of 0.018 ± 0.004 pmol CGRP per

mg of PDL (Fig. 2). Kruskal–Wallis test revealed significant differences between groups ($P < 0.001$). Tukey HSD *post hoc* tests revealed significant differences between the intact-teeth control group and the WaveOne and Hand instrumentation groups ($P < 0.001$). Differences between the Reciproc and the WaveOne groups were also statistically significant ($P < 0.001$). There was no statistically significant difference between the intact-teeth and the Reciproc groups ($P = 0.42$). Differences between hand instrumentation and all the other groups were also statistically significant ($P < 0.001$).

Discussion

A previous study demonstrated that hand instrumentation, as well as some continuous rotation systems, increased the expression of SP and CGRP in the PDL of teeth after root canal preparation (Caviedes-Bucheli et al. 2010). Therefore, this study was conducted under the same methodology to quantify and compare

**Figure 1** Substance P expression in periodontal ligament from healthy human premolars after the root canal preparation with single-file reciprocating systems.**Figure 2** Calcitonin gene-related peptide (CGRP) expression in periodontal ligament from healthy human premolars after the root canal preparation with single-file reciprocating systems.

the effect of two single-file reciprocating root canal preparation systems on SP and CGRP expression in PDL tissue.

Substance P and CGRP values were obtained from healthy premolars where extraction was indicated for orthodontic reasons. Neuropeptide values in the intact-teeth control group were similar to the ones previously reported (Caviedes-Bucheli *et al.* 2010). These baseline values are necessary under physiological conditions to maintain and regulate tissue homeostasis and vascular tone (Caviedes-Bucheli *et al.* 2008a).

Root canal preparation procedures were standardized for both single-file systems. Only one instrument was used for the entire preparation procedure strictly following manufacturer's recommendations. The apical size of file was 0.40 mm, and preparation time did not exceed 1 min. The total number of instruments used for the hand instrumentation technique was 6, being the main apical file with a 0.40-mm tip diameter and preparation time did not exceed 5 min. Irrigant solution concentration and volume were also standardized as it has been shown that irrigant used during root canal preparation can be extruded causing chemical irritation that directly affects the PDL (Kustarci *et al.* 2008). EDTA-gel was used during root canal preparation to reduce the heat produced by instrument friction against dentinal walls, as well as for smear-layer removal. It has been reported that EDTA toxicity is directly related to its concentration, and that EDTA extrusion to periapical tissue would generate an inflammatory process and could compromise tissue reparation (Scelza *et al.* 2010). Therefore, the amount and concentration of EDTA were standardized for all groups.

Taking in consideration the results of a previous study (Caviedes-Bucheli *et al.* 2010), the hand instrumentation group was included in this study as a positive control, as it was expected that this group would generate a greater expression of both neuropeptides in comparison with both reciprocating systems. These results could be attributed to the fact that hand preparation techniques require more time, therefore, generating increased mechanical stress, more debris extrusion due to the filing movement, in which the instrument pushes debris and irrigant into the periapical area. Furthermore, K-type files have a constant 40° helicoidal angle, giving them a reduced ability to remove debris coronally and a greater tendency of accumulating dentine in the spaces between flutes (Al-Omari & Dummer 1995, Reddy & Hicks 1998, Ferraz *et al.* 2001).

Reciproc was associated with a neuropeptide expression similar to the intact-teeth control group. This could be explained by the file S-shaped cross-section, which gives the instrument a smaller core diameter with only two cutting blades and deep flutes, providing enough space to remove debris coronally (Yared 2011). Moreover, Reciproc files have noncutting guiding tips that safely maintain the shape and position of the apical foramen, and, therefore, reduce the amount of debris extruded to the periapical tissue (Bürklein *et al.* 2012).

On the other hand, WaveOne was associated with a significantly higher neuropeptide expression when compared with the intact-teeth control group. This could be explained by the concave triangular cross-section at the tip end of the files and the convex triangular cross-section at their coronal end. This cross-section design provides greater mass to the instrument core reducing the depth of flutes, therefore limiting its ability to allow coronal removal of debris and making the instrument more rigid (Webber *et al.* 2011).

It is also important to point out that differences between WaveOne and Reciproc could be influenced by the different speeds and angles of alternating rotation (150° CCW and 30° clockwise at 300 rpm for Reciproc; 170° CCW and 50° clockwise at 350 rpm for WaveOne). Reciproc's lower speed and shorter angles of rotation allow a better control of the instrument and lower flexural stiffness than WaveOne, which in turn is a more aggressive instrument with higher torsional stiffness, favouring debris extrusion (Kim *et al.* 2012).

It is interesting to compare the results of this study with the ones obtained in a previous study (Caviedes-Bucheli *et al.* 2010) for Mtwo and ProTaper Universal rotary files, as both studies were carried out under the same conditions. The SP and CGRP values for Reciproc and Mtwo are very similar, despite the differences between techniques, such as the type of movement (continuous versus alternating rotation), the number of instruments and the time required to complete canal preparation. Therefore, based on the results of this study, these differences did not influence neuropeptide expression with these systems, suggesting that instrument design is more important than type of movement and the number of instruments.

On the other hand, SP and CGRP values for ProTaper Universal in the previous study (Caviedes-Bucheli *et al.* 2010) were significantly higher than

the ones obtained in this study for WaveOne, suggesting that the differences between both techniques, such as the type of movement, the number of instruments, the time required to complete canal preparation and the tip design (semicutting for WaveOne and noncutting for ProTaper Universal) (Bürklein et al. 2012), influenced the PDL neuropeptide expression in this study. The higher values for ProTaper Universal could also be explained due to the continuous debris extrusion between each file of the system, implying in this case that instrument design is more important than type of movement and the number of instruments.

Results from this study showed that SP and CGRP have the same release tendency, which indicates that both neuropeptides potentiate the inflammatory process. Whilst SP mediates the initial component of the vasodilator response, the continued long-lasting rise in blood flow is dependent on CGRP. Moreover, it has been demonstrated that CGRP prolongs the SP effect by inhibiting peptidase degradation (Caviedes-Bucheli et al. 2008a). Therefore, it is suggested that neuropeptides contribute to the pathophysiology of peripheral inflammation, and that root canal preparation generates an inflammatory process in the periapical tissues that could be related to symptomatic apical periodontitis (Närhi et al. 1992).

It is important to note that this research was carried out on teeth with vital pulps, where the neuropeptide release in the PDL is attributed to a mechanical and chemical irritation to the periapical tissue; which could be significantly different in teeth with infected canals, where even a slight extrusion of debris contaminated with microorganisms would magnify the inflammatory process (Er et al. 2005). Finally, it should be pointed out that PDL inflammation is multifactorial, and may be influenced by several features of root canal preparation, such as mechanical stress, debris and/or irrigant extrusion and apical patency verification (Georgopoulou et al. 1986, Reddy & Hicks 1998, Stashenko et al. 1998, Siqueira et al. 2002, Tanalp et al. 2006, Caviedes-Bucheli et al. 2010).

Conclusion

Hand instrumentation and WaveOne single-file root canal preparation technique increased SP and CGRP expression in human PDL. Reciproc did not show significant differences with the baseline levels of the control group.

Acknowledgements

The authors deny any conflicts of interest.

References

- Al-Omari MA, Dummer PM (1995) Canal blockage and debris extrusion with eight preparation techniques. *Journal of Endodontics* **21**, 154–8.
- Bürklein S, Hinschitzka K, Dammaschke T, Schäfer E (2012) Shaping ability and cleaning effectiveness of two single-file systems in severely curved root canals of extracted teeth: Reciproc and WaveOne versus Mtwo and ProTaper. *International Endodontic Journal* **45**, 449–61.
- Caviedes-Bucheli J, Correa-Ortiz JA, Garcia LV, Lopez-Torres R, Lombana N, Munoz HR (2005) The effect of cavity preparation on Substance P expression in human dental pulp. *Journal of Endodontics* **31**, 857–9.
- Caviedes-Bucheli J, Munoz HR, Azuero-Holguin MM, Ulate E (2008a) Neuropeptides in dental pulp: the silent protagonists. *Journal of Endodontics* **34**, 773–88.
- Caviedes-Bucheli J, Ariza-Garcia G, Restrepo-Mendez S, Rios-Osorio N, Lombana N, Munoz HR (2008b) The effect of tooth bleaching on substance P expression in human dental pulp. *Journal of Endodontics* **34**, 1462–5.
- Caviedes-Bucheli J, Rojas P, Escalona M et al. (2009) The effect of different vasoconstrictors and local anesthetic solutions on substance P expression in human dental pulp. *Journal of Endodontics* **35**, 631–3.
- Caviedes-Bucheli J, Azuero-Holguin MM, Gutierrez-Sanchez L et al. (2010) The effect of three different rotary instrumentation systems on Substance P and Calcitonin gene-related peptide expression in human periodontal ligament. *Journal of Endodontics* **36**, 1938–42.
- Caviedes-Bucheli J, Azuero-Holguin MM, Correa-Ortiz JA et al. (2011a) Effect of experimentally induced occlusal trauma on Substance P expression in human dental pulp and periodontal ligament. *Journal of Endodontics* **37**, 627–30.
- Caviedes-Bucheli J, Moreno JO, Ardila-Pinto J et al. (2011b) The effect of orthodontic forces on Calcitonin gene-related peptide expression in human dental pulp. *Journal of Endodontics* **37**, 934–7.
- DiRenzo A, Gresla T, Johnson BR, Rogers M, Tucker D, Begole EA (2002) Postoperative pain after 1- and 2-visits root canal therapy. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **93**, 605–10.
- Er K, Sumer Z, Akpınar KE (2005) Apical extrusion of intracanal bacteria following use of two engine-driven instrumentation techniques. *International Endodontic Journal* **38**, 871–2.
- Ferraz CC, Gomes NV, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ (2001) Apical extrusion of debris and irrigants using two hand and three engine-driven instrumentation techniques. *International Endodontic Journal* **34**, 354–8.

- Georgopoulou M, Anastassiadis P, Sykaras S (1986) Pain after chemomechanical preparation. *International Endodontic Journal* **19**, 309–14.
- Glennon JP, Y-L NG, Setchell DJ, Gulabivala K (2004) Prevalence of and factors affecting postpreparation pain in patients undergoing two-visit root canal treatment. *International Endodontic Journal* **37**, 29–37.
- Kim H-C, Kwak S-W, Cheung GS, Ko D-H, Chung S-M, Lee W (2012) Cyclic fatigue and torsional resistance of two new nickel-titanium instruments used in reciprocation motion: Reciproc versus WaveOne. *Journal of Endodontics* **38**, 541–4.
- Kuhn WG, Carnes DL Jr, Clement DJ, Walker WA III (1997) Effect of tip design of nickel-titanium and stainless steel files on root canal preparation. *Journal of Endodontics* **23**, 735–8.
- Kustarci A, Akpınar KE, Er K (2008) Apical extrusion of intracanal debris and irrigant following use of various instrumentation techniques. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **105**, 257–62.
- Leonardi LE, Atlas DM, Raiden G (2007) Apical extrusion of debris by manual and mechanical instrumentation. *Brazilian Dental Journal* **18**, 16–9.
- Närhi M, Jyväsjärvi E, Virtanen A, Huopaniemi T, Ngassapa D, Hirvonen T (1992) Role of intradental A- and C-type nerve fibres in dental pain mechanisms. *Proceedings of the Finnish Dental Society* **88**(Suppl 1), 507–16.
- Peters O (2004) Current challenges and concepts in the preparation of root canal systems: a review. *Journal of Endodontics* **30**, 559–67.
- Pruett JP, Clement DJ, Carnes DL Jr (1997) Cyclic fatigue testing of nickel-titanium endodontic instruments. *Journal of Endodontics* **23**, 77–85.
- Reddy SA, Hicks ML (1998) Apical extrusion of debris using two hand and two rotary instrumentation techniques. *Journal of Endodontics* **24**, 180–3.
- Scelza Z, Santos Da Silva V, Alves M, Esmeraldo L, Scelza P (2010) Evaluation of inflammatory response of EDTA, EDTA-T, and citric acid in animal model. *Journal of Endodontics* **36**, 515–9.
- Schäfer E, Vlassis M (2004) Comparative investigation of two rotary nickel-titanium instruments: ProTaper versus RaCe. Part 2. Cleaning effectiveness and shaping ability in severely curved root canals of extracted teeth. *International Endodontic Journal* **37**, 239–48.
- Siqueira JF Jr, Rôcas IN, Favieri A et al. (2002) Incidence of postoperative pain after intracanal procedures based on an antimicrobial strategy. *Journal of Endodontics* **28**, 457–60.
- Stashenko P, Teles R, D'Souza R (1998) Periapical inflammatory responses and their modulation. *Critical Reviews in Oral Biology and Medicine* **9**, 498–521.
- Tanalp J, Kaptan F, Sert S, Kayahan B, Bayırlı G (2006) Quantitative evaluation of the amount of apically extruded debris using 3 different rotary instrumentation systems. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **101**, 250–7.
- Veltri M, Mollo A, Mantovani L, Pini P, Balleri P, Grandini S (2005) A comparative study of Endoflare-Hero Shaper and Mtwo NiTi instruments in the preparation of curved root canals. *International Endodontic Journal* **38**, 610–6.
- Webber J, Machtou P, Pertot W, Kuttler S, Ruddle C, West J (2011) The WaveOne single-file reciprocating system. *Roots International Magazine of Endodontology* **7**, 28–33.
- Yared G (2011) Canal preparation with only one reciprocating instrument without prior hand filing: a new concept. Available at: http://www.vdw-reciproc.de/images/stories/pdf/GY_Artikel_en_WEB.pdf. Accessed 23 October 2011.