This is the peer reviewed version of the following article:

Johnsen GF, Sunde PT, Haugen HJ. Validation of contralateral premolars as the substrate for endodontic comparison studies. International Endodontic Journal.

which has been published in final form at <https://doi.org/10.1111/iej.12902>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

INTRODUCTION

The main confounding factor in many *ex vivo* endodontic comparisons studies has been the anatomy of the root canal as they have not been screened and matched for symmetry prior to executing the experiments (De-Deus 2012, Xu *et al.* 2016).

Xu *et al.* (2016) suggest using strictly screened and matched premolars in order to create well-balanced experimental groups in endodontic comparative studies. Jorgensen (1975) was one of the first to suggest using contralateral teeth for dental comparative studies when he showed similar etching patterns in the enamel of contralateral canines, but his findings did not affect the study design of future comparative studies. A handful of comparative endodontic studies have, indeed, used contralateral teeth (Albrecht *et al.* 2004, Zehnder *et al.* 2006). The methodology for matching the teeth in these previous studies has unfortunately been incomplete. Furthermore, the supposition of their anatomical similarity has been based on a study using ipsilateral comparisons of mandibular second premolars crowns for the determination of monozygosity of twins (Wood & Green 1969, Zehnder *et al.* 2006). A recent paper report (Guimaraes *et al.* 2017) studied the ability of a continuous rotary versus a reciprocating (Reciproc®, VDW GmbH, Munich, Germany) file system to prepare oval-shaped canals in thirteen pairs of contralateral premolars matched for volume and surface area prior to instrumentation. They only describe visual validation for morphological similarity from the microcomputed tomography (micro-CT) scans. Even though they only used two quantifiable parameters their findings support the proposal that contralateral premolars matched with high-resolution micro-CT may serve as an ideal substrate for endodontic comparison studies (De-Deus 2012, Johnsen *et al.* 2016, 2017).

Shaping of the root canal system has historically been achieved by various modes of manual (Roane *et al.* 1985) and rotary instrumentation (Hülsmann *et al.* 2005). It is unknown if any given file system used in mirror-image premolars will produce equivalent outcomes. Identical outcomes from the same file system would serve as validation of higher certainty for using contralateral premolars in comparison studies. No studies exist where root canal systems contralateral premolars have been evaluated and compared with micro-CT before and after instrumentation using the same endodontic file system. This study presents a comparative evaluation of the root canals of contralateral premolars before and instrumentation.

The primary aim of this *ex vivo* study was to use micro-CT technology and a metrology (morphometry) software toolset to validate contralateral premolars as the ideal substrate for endodontic comparison studies. The secondary aim was to determine if the previously matched contralateral premolar roots from the same donors are identical enough to yield non-significantly different outcomes regarding dentine removal (volume), degree of twisting (*torsion*), three-dimensional shape (shape deviation analysis), and root canal diameter in the apical third after instrumentation with an endodontic file system. . The root canal lengths of the contralateral pairs were also measured and compared using three endometric methods.

The null-hypothesis (H_0) of this study is that there are no differences between the contralateral premolar root canals pre- or post-instrumentation regarding any of the examined outcomes. If the null-hypothesis is upheld (true) the data will have implications for the design of future comparative studies on root canal preparation and filling.

MATERIALS AND METHODS

SAMPLE SELECTION AND PREPARATION

This study was evaluated and found not to necessitate disclosure by The National Committee for Medical and Health Research Ethics and Health Research Ethics of Norway

(reference number 2012/2092-Remit Assessment). A total of 76 mandibular and maxillary contralateral premolar roots (CPR) were extracted from 20 volunteer patients on the basis of orthodontic treatment indications (7 female, 13 male; mean age: 15 years, SD 5 years; age span: 11-31 years) at the Department of Orthodontics, Institute of Clinical Dentistry, Faculty of Dentistry University of Oslo, Norway. Premolars from patients with known congenital dental disorders or conditions predisposing to dental developmental disorders were excluded. Further grounds for exclusion in the study included teeth with restorations and/or deep caries, resorptive defects, immature apices, CEJ-apex length < 10 mm, visible cracks or fractures, and contralateral pairs having different root canal classifications (Vertucci 1984). A total of 44 CPR (28 premolars (10 single-rooted and 18 double-rooted) from 12 donors (8 male and 4 female with age range 12-26 years (mean (SD): 16 (6)) passed the selection criteria. Premolars were decoronated perpendicular to their vertical axis using a high precision diamond edge band saw (Exakt, Otto Hermann, Denmark) under continuous water-cooling. The premolars were stored prior to investigation as previously described (Johnsen *et al.* 2016).

ROOT CANAL LENGTH DETERMINATION AND COMPARISON

The root canal length (RCL) was measured after decoronation both visually, with an electronic apex locator (Raypex 6 EAL, VDW), and using micro-CT scans.

VISUAL MEASUREMENT

The visual measurements were acquired by advancing the tip of a size 10 K-file (Ready-Steel™, Dentsply Sirona, Ballaigues, Switzerland) apically until it was detected exiting the apical foramen using a dental-operating microscope (DOM) (Global Surgical, St. Louis, MO, USA) at 12.8x magnification. The silicone-rubber stopper was fixed at the flat coronal reference point using a flowable light-cured composite (Filtek Supreme XTE Flow, 3M ESPE, St. Paul, Mn, USA) and this measurement was recorded as the RCL using a digital calliper (Cocraft, Insjon, Sweden) with an accuracy of ± 0.03 mm (Christofzik *et al.*

2015, Piasecki *et al.* 2016). The composite fixation of the stopper was subsequently broken and measurements were repeated on two further overcomings.

ELECTRONIC APEX LOCATOR

The samples were placed in 0.9% saline-filled well in a Plexiglas electro-conductive system (Figure 1) and secured with a plastic vice in (ElAyouti *et al.* 2002). A size 10 K-file was advanced apically and the silicone rubber stopper was again secured with flowable composite at the red visual indicator bar on the Raypex 6 EAL (VDW) (Piasecki *et al.* 2016). The measurements were repeated on two further overcomings and only recorded if the red bar remained stable for at least 5 s (Lucena *et al.* 2014).

INSTRUMENTATION

The apices were sealed and roots wrapped with ParaFilm (Bemis, Neenah, WI, USA) to simulate the effect from vapour lock and apical gas entrapment in a closed canal system (Tay *et al.* 2010, Zuolo *et al.* 2017). A glide path was created by instrumenting canals up to a stainless steel size 20 K-file (Ready-Steel™, Dentsply Maillefer, Ballaigues, Switzerland) up to the working length (WL).

The WL for instrumentation of the root canals was set at the mean value of RCL acquired with the micro-CT minus 1.00 mm. All canals were instrumented with Reciproc® Blue (VDW) files in a Silver Reciproc® Endomotor (VDW) set to the RECIPROC ALL setting. Instrument selection was done in accordance with manufacturer's recommendations, and performed by a single experienced operator. All canals were either instrumented with R40 (Size 40, .06 taper) or R50 (Size 50, .05 taper). The R50 file was used to instrument 2 CPR pairs while the remaining 20 CPR pairs were instrumented with R40. The instrumentation protocol was in compliance with both a recent study and manufacturer's recommendations (Guimaraes *et al.* 2017). The roots were irrigated during instrumentation with 1% NaOCl (10 ml total) with closed-end, side-port Vista-Probe™ delivery syringes (Inter-Med, Racine, WI, USA). A final rinse of 17% EDTA (5 ml) was completed after instrumentation followed by drying of the canals with absorbent paper points (Reciproc) (Endal *et al.* 2011). Patency was checked using a size 10 K-file. The roots were then wrapped in Parafilm "M" Laboratory Film

(Bemis, Neenah, WI, USA) to prevent contamination with foreign particles and debris before micro-CT scanning. A new file was used for every root canal preparation.

MICRO-CT SCANNING AND QUANTITATIVE EVALUTION

Micro-CT-scans were done in the desktop SkyScan 1172 (Bruker microCT, Kontich, Belgium) both pre- and post root canal instrumentation of the. A flat field correction was performed prior to scanning to correct for variations in the pixel sensitivity of the camera (Robinson *et al.* 2012). The specimens were placed in a customized plastic tube allowing rotation around the CPR's vertical axis and scanned at high resolution (isotropic resolution of 9.91 μm) at 180° rotation with a rotation step of 0.4°. The camera exposure time was 1475 milliseconds from an X-ray source with 100 kV and 100 mA, using a 500- μm Al and 38- μm Cu filter.

Images of each specimen were reconstructed (NRecon v.1.6.10; Bruker microCT) using previously described parameters (Johnsen *et al.* 2016). Furthermore, a customized algorithm in CTAn SkyScan software (CTAn and CTvol; Bruker microCT) was used to segment the root canal space for both visualization and analyzation.

Double-rooted premolars were opened in DataViewer (Bruker microCT) for selection of individual roots as separate volumes-of-interest. These were distinguished by starting at the first slice where each root canal space was completely surrounded by dentine to the last slice with visible dentine present apically. Next, the root canal was divided in thirds for evaluation of the apical third. The *Average Object Equivalent Circle Diameter per Slice* (AED) was evaluated in the apical third for all contralaterals pre- and post-instrumentation. AED is defined as the diameter of a circle having the same area as the measured object, and was calculated for every slice in the apical third. The average diameter of all the slices in the apical third from pre- to post-instrumentation provided insight on the instruments effect on the apical third.

An increase in root canal volume was calculated by subtracting the pre-instrumented binary from the post-instrumented binary, which equals the removed dentineal tissue.

Degree of twisting was evaluated as changes in *torsion* of the centreline of each root canal by placing source and target landmarks on the pre- and post-instrumentation models (Figure 2) in the Vascular Modelling ToolKit Lab (VMTKLab; Bergamo, Italy) Software (VMTKLab), which allows for classic differential geometry analysis to compute point-wise torsion (Bulant *et al.* 2017). VMTKLab allows for quantification of the degree twisting of the centreline, *i.e.* change in torsion, from the occlusal to the apical landmarks.

Instrumented root canals were evaluated and compared to pre-instrumented canals in terms of a geometric and morphometric shape deviation analysis (SDA) through an iterative closest point algorithm with the metrology software Geomagic® Control X™ (Geomagic, Morrisville, NC, USA) according to a previously described method (Johnsen *et al.* 2016). Standard tessellation language (STL) datasets from the pre-instrumented were superimposed with the STL dataset from the post-instrumented canals to evaluate the three-dimensional (3D) deviations. Deviations were calculated as root-mean-square (RMS) error, which is a global value of the entire root canal model. The RMS error was evaluated and compared for contralateral pairs pre-instrumentation, post-instrumentation, and pre- vs. post-instrumentation.

The RCL was measured from the centreline of the root canal surface models on 3D surface models created on CTan using Marching Cube 33 algorithm after micro-CT scanning with the SkyScan 1172 (Bruker microCT). The placement of landmarks and length computation in VMTKLab of the models' RCL was triplicated, and the mean was used as the RCL for each models. These lengths were verified by statistical comparison of lengths from both pre- and post-instrumentation models.

STATISTICAL ANALYSIS

All datasets were examined for parametric or non-parametric distributions (Shapiro-Wilk test). The Wilcoxon Signed Rank Test was used to compare the geometric and morphometric descriptors for pre- and post-instrumented root canals, pre-instrumented CPRs, and post-instrumented CPRs. One-Sample Signed Rank Test was used on the intra-

pair differences pre- and post-instrumentation for volume (hypothesized population median: 0.25mm³).

The Friedman test compared the three methods for acquiring working length. The Wilcoxon Signed Rank Test was used for comparison of the WL intra-contralateral pairs acquired by the three individual methods. The software package SigmaPlot 13.0 (San Jose, CA, USA) was utilized for all statistical analyses. Significance level was set to $p < 0.05$. All results are presented as mean with standard deviations.

RESULTS

There were no significant differences between the contralateral premolar roots before or after instrumentation with respect to volume of dentine removed, three-dimensional morphology or the average root canal diameter (AED). Furthermore, the majority of contralateral premolars that did not have any significant difference in degree of twisting prior to instrumentation remained this way post-instrumentation.

Table 1 shows the results from the analysis and comparison of volumes pre- and post-instrumentation for the total root canal length and the apical third. Instrumentation led to root canal volume enlargement in all of the root canals (0.38 – 3.25 mm³). The mean volume for the total length of the root canals increased from 7.26 (6.44) to 8.41 (6.93) mm³, and this increase was significantly different ($p < 0.05$). The mean volume of the apical third increased significantly ($p < 0.001$). There was no significant difference in volume between pairs prior to instrumentation, no significant difference between pairs post instrumentation or between pre- and post-instrumented roots for the entire root length. The apical third had a significant difference ($p = 0.040$) between pairs prior to instrumentation. The 3D-models increased 20.95 (13.45)% in CTan and 20.20 (12.34)% in Geomagic® Control X™, and the difference between software programs was not significant.

The mean difference of the difference in volume from pre- to post-instrumentation between left versus right contralaterals for the entire root canal length was 0.38 (0.35) mm³, and the One-Sample Signed Rank Test was non-significant ($p=0.24$).

The diameter of the apical thirds (Table 2) increased significantly ($p<0.001$) from pre- to post-instrumentation (0.46 (0.16) to 0.57 (0.15) mm).

The mean distance between root canal surfaces before and after preparation was 0.057 (0.019) mm. Representative differences between 3D-models are visualized as colour maps in Figure 3. It was evident that the pre- and post-instrumentation models deviated the most in the apical third and in the most curved portions of the root canals. This observation was supported by a significant difference in the volume change between the entire root lengths versus the apical thirds ($p<0.001$). The average distance from the root canal surfaces in pre-instrumented contralateral roots was 0.084 (0.018) mm and 0.082 (0.017) mm in instrumented contralateral roots.

The degree of twisting in a total of 18 out of 22 pairs (82%) was not significantly different from each other prior to instrumentation., and 15 of these 18 pairs (83%) remained this way post-instrumentation (Figure 2). Only one of these pairs had a SIGNIFICANT DIFFERENT change in degree of twisting for both roots from pre- to post-instrumentation. Overall, only 14 of the 44 (32%) roots had a significantly different ($p\leq 0.05$) degree of twisting between pre- to post-instrumentation. The roots with an overt s-shape demonstrated significant changes in degree of twisting from pre- to post-instrumentation (Figure 2)

There was no significant different in the length between the contralaterals for any of three endometric methods (Table 3).

DISCUSSION

The present study provides proof to the supposition that contralateral premolars are ideal as the substrate for root canal comparison studies based on the similarity between the pre- and post-instrumentation geometric and morphometric descriptors. This is the first study

to compare the results of instrumentation of contralateral premolar roots using a single instrumentation system.

Comparative endodontic studies should use anatomically matched contralateral premolars (Guimaraes *et al.* 2017) where the volume and area of the pre-instrumented oval-shaped contralateral premolar was matched. The present study explored even more morphometric geometric parameters, provides validity to their endodontic comparative study. It matched the centreline and overall 3D-shape in future endodontic studies in addition to volume and area as recommended.

The mean percentage change of circumferential pulpal dentine volume removed in other studies using the original Reciproc® system, or ProTaper F2 (size 25 with fixed 8% taper over the first 3 mm) with reciprocating kinematics have ranged from 23.03 – 83.00% (Paque *et al.* 2011, Versiani *et al.* 2013, Guimaraes *et al.* 2017). In the present study, the volume change in the total length of the root canals was 5.6- 58.2%. The difference in dentine removal between these different studies may be explained by the type of teeth used, the length of roots instrumented, differences in file design and or not brushing motion (Versiani *et al.* 2013), or by the age span of donors. The mean apical third volume increase in the present study was approximately three times greater than the apical 4 mm of premolars instrumented by Reciproc® evaluated in the study by Guimaraes *et. al* (2017), where the final apical size of instrumentation was R40 (Size 40, .06 taper) in oval canals. The present results may be greater than theirs due to the fact that all the roots in the aforementioned study were oval shaped (2:1 ratio between the buccolingual and mesiodistal dimensions throughout its coronal two thirds) making it more difficult for the instrument to touch all aspects of the root canal system. The mean (SD) apical third 4.50 (0.51) mm in the present study was 0.5 mm longer than reported by Guimaraes *et. al* (2017), but this would not fully explain the difference in volume increase.

Landmark based three-dimensional metrological software for evaluation of distance between root canal surfaces before and after preparation has previously demonstrate the spatial distribution of deviations with a pixel size of 30.7 µm (Markvart *et al.* 2012).

Geomagic Control performs an automatic alignment with an iterative closest point algorithm for co-registration followed by three-dimensional deviation analysis, which is why dental research applies it for evaluation of accuracy with superimposition of reference and target models (Goodacre *et al.* 2016, Rudolph *et al.* 2016, Renne *et al.* 2017). This method creates readily available quantitative data as well as visual qualitative data for the evaluation of morphometric geometric changes induced by the file system. Overall, the similarity in both data and visualization of RMS error for the contralateral pairs demonstrate how contralateral roots may serve as excellent substrates for root canal comparison studies. The creation of STL datasets for each premolar root canal volume allows for non-invasive and efficient evaluation of several 3D-parameters *ex vivo*. Markvart *et al.* (2012) defined therapeutic mean deviation as beyond 200 μm . This definition is based on studies evaluating how far microorganisms can penetrate dentineal tubules (Markvart *et al.* 2012), and the mean RMS error of $\sim 60\ \mu\text{m}$ in the present study was comparable with their value of $\sim 65\ \mu\text{m}$ for the entire root canal in what was by them subjectively defined as *large roots*.

Figure 3 shows that most of the morphometric and geometric changes took place in the apical portions of the root canal, most likely making the apical part of the root canal system accessible for flushing by antibacterial irrigating solutions. Several studies have shown that apical enlargement facilitates removal of bacteria (Rollison *et al.* 2002, Rodrigues *et al.* 2017). Within the limitations of the present study, Reciproc® Blue files seem to fulfil the desire for increased apical preparation. The canals were instrumented with R40 or R50 and the mean (SD) apical third was 4.50 (0.51) mm. The diameter for R40 and R50 increase from 0.40 and 0.50 mm to 0.58 and 0.65 mm over the first 3 mm, respectively. The mean Average Object Equivalent Circle Diameter per Slice for the apical third was approximately 0.55 mm post-instrumentation and this clearly demonstrates that the instrument enlarges the canal apically as expected.

The mean absolute torsion (degree of twisting of curve out of the osculating plane for each centreline point (Bulant *et al.* 2017, Gallo *et al.* 2017)) of the entire length of the root canal was compared pre- and post-instrumentation to evaluate the influence of the file and

instrumentation mechanics (Bergmans *et al.* 2001). Torsion is expressed as a negative or positive value depending on whether the twisting is counter clockwise or clockwise, and this study considered the absolute mean of the point-wise torsion along the entire length of the root canal models. In other words, the present methodology enabled a quantitative appreciation of the degree of twisting of the centreline, *i.e.* change in torsion. Again, the contralateral roots demonstrate a high degree of matching symmetry both pre- and post-instrumentation. However, almost a third (32%) of the torsion changed significantly from pre- to post-instrumentation and the greatest changes were most evident in roots with greater and/or multiple curvatures (Figure 2). The present preliminary findings are not supported by previous studies on the original Reciproc® in severely curved canals (Bürklein *et al.* 2012, Capar *et al.* 2014, Saber *et al.* 2015). The evaluation of Reciproc® in curved canals has previously been done after instrumentation with R25 (Size 25, .06 taper), while the roots in the present were instrumented with either R40 (Size 40, .06 taper) or R50 (Size 50, .05 taper). Furthermore, the two studies found increased flexibility and decreased cyclic fatigue resistance when considered the R25 files (De-Deus *et al.* 2017, Keskin *et al.* 2017). In a recent paper, (Topcuoglu 2017) compared Reciproc® with RB (R40 and R50) in S-shaped canals in stainless steel blocks and found increased cyclic fatigue resistance in the RB files. In any case, the tendency in the present paper for RB files (R40) to disrespect tortuous root canal anatomy demands further scrutiny in future studies.

The manual placement of terminal landmarks in VMTKLab for construction of the centreline of the root canal is operator dependent (semi-automated), and this could be a possible source of errors. However, since the software calculates the point-wise torsion from the centreline placed in the centre of the STL model between the source and target landmarks, discrepancies in placement of terminal landmarks between CPRs will not affect the validity of the results. Other studies have considered centring ability and canal transportation (da Silva Limoeiro *et al.* 2016, Venino *et al.* 2017), and it is recommended that future studies on contralateral premolars include these parameters.

The use of micro-CT in order to study the complex internal anatomy of teeth is the most reliable method available (Ordinola-Zapata *et al.* 2017), however, as with all types of radiographic imaging, introduction of artefacts and noise can cloud interpretation and analysis (Boas & Fleischmann 2012). Sources of error were reduced using previously described precautionary and post-scanning procedures (Johnsen *et al.* 2016).

Contralateral premolars have been used previously to compare endodontic file systems (Guimaraes *et al.* 2017), and the results from the present study provide validity and power to their results.

Both the EAL and micro-CT RCL differed by approximately 0.5 mm from the visual length, which is in agreement with the difference (0.59mm) found by Lucena *et al.* (2014) when comparing visual, Raypex 6, and cone-beam computed tomography methods for determination of root canal length. Nevertheless, in order to expedite *ex vivo* endodontic experiments involving micro-CT using the root canal length acquired from the datasets is suggested. From a clinical standpoint, the underestimation of the RCL with EAL compared with visual endometrics means that the Raypex 6 is safe to use for estimation of working length (stable red bar $\geq$ 5s minus 1.00mm) as overestimation is known to have a detrimental effect on root treatment prognosis (Sjögren *et al.* 1990).

CONCLUSIONS

Canal instrumentation of contralateral premolars produced non-significantly different geometric and morphometric changes for the studied parameters. The present study provides further evidence for inherent matching symmetry in contralateral premolars in terms of volume, length, three-dimensional shape and degree of twisting both before and after instrumentation. The H_0 could not be rejected, and within the limits of the study, the findings herein gives impetus for using matched contralateral premolars for valid and reliable root canal comparison studies.

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