

Endodontics

Comparative analysis of two experimental root-end filling cements about apical sealing, bioactivity and biocompatibility

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Aim: The aim of this study was to compare two innovative root end filling materials: an experimental penta-aggregate calcium silicate cement and a light-curing penta-aggregate calcium silicate cement doped with di-calcium-phosphate; the apical sealing ability, bioactivity and biocompatibility were the parameters analyzed for this study.

Methods: A penta-aggregate calcium silicate cement doped with di-calcium-phosphate (ENDOPASS LC), an experimental penta-aggregate calcium silicate (Exp. ENDOPASS) and a calcium silicate Portland-based (Control, PC) were assessed for their biocompatibility and alkalinizing activity (pH). Single-root canal teeth were endodontically treated, filled with gutta-percha and finally submitted to apicoectomy. Root end fillings were performed using all tested cements, and their apical sealing ability was evaluated up to 4 weeks of immersion in simulated body fluid (SBF). The mineral precipitation ability at the apical region and the cement adaptation to root dentine were also evaluated through non-destructive optical microscopy both at 24h and prolonged water storage (4 week).

Results: ENDOPASS LC had neutral pH, and it showed the greatest sealing ability after 24h. ENDOPASS also showed excellent fibroblasts proliferation. ENDOPASS presented excellent sealing ability after two and four weeks, as well as biocompatibility after 4 and 7 days similar to ENDOPASS LC. The control PC cement showed

the lowest sealing ability, the greatest alkalinization properties and greatest cytotoxicity compared to the tested experimental cements. Mineral precipitation, as well as optimal adaptation to the root dentine were observed with the use of the two experimental materials.

Conclusions: ENDOPASS LC and ENDOPASS may be promising materials for root end obturation as they present appropriate (in vitro) biocompatibility, sealing ability and aptitude to induce mineral precipitation.

Efficacy of photon-initiated photoacoustic streaming technique on root canal disinfection deep into dentinal tubules

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Aim: Photon-induced photoacoustic streaming (PIPS) technique is a root canal disinfection system that induces a profound photoacoustic shock wave, facilitating a three-dimensional movement of the irrigants. It is based on the use of a Er:YAG laser with both a radial and stripped tip at subablative power settings (0.3 W), placed only into the coronal portion of the root canal. Using low-energy levels and short microsecond pulse rates (50 µsec) peak power spikes are generated. The primary objective of the study was to compare antimicrobial efficacy deep into dentinal tubules of PIPS irrigation system compared with a traditional irrigation protocol with endodontic needles.

Methods: Thirty human single-root teeth were selected. Specimens were instrumented with ProGlider and ProTaper Next X1, X2 and X3 (Dentsply, Maillefer) at working length (WL) used at 300 rpm and torque 4.0 Ncm, alternating irrigation with NaOCl and EDTA after



each instrument. After ethylene oxide sterilization for six hours, the root canals were infected with a culture of *E. Faecalis* ATCC 29212 (3×10^7 CFU/ml) in Brain Heart Infusion (BHI; Oxoid, Milan, Italy) medium broth and further incubated aerobically at 37°C for 2 weeks. Specimens were randomly assigned to 2 experimental groups (n=11), plus positive and negative controls (n=2+2). Each group has been irrigated with 2mL of 2,5% NaOCl for 20". In the NaOCl group (control group) the irrigation has been carried by a 30 gauge endodontic needle at 2 mm from the WL. In the PIPS group (test group) the tip has been positioned in the pulp chamber to activate the irrigants, ensuring in the meantime a continuous replacement of the solution. The laser was set to 50 µsec pulse duration at a 15 Hz pulse rate and 20mJ of energy, thereby delivering a total of 0.3W of power. Specimens have been analyzed using the colony forming unit method (CFUs) and confocal laser scanning microscopy (CLSM). The CFUs method admitted to evaluate the bacterial load after treatment and the dead ratio in each study group. Then, each specimen has been fractured longitudinally and subjected to Live/Dead Backlight viability test to evaluate vital and non vital bacteria with the dyes Syto 9 and PI. A LEICA confocal microscopy with Krypton-Argon laser illumination has been used with 505-550 nm wave length (green) to observe the Syto 9 and 650-750 nm wave length (red) to observe the PI. CLSM was able to evaluate the disinfection depth inside dentinal tubules and the percent of red fluorescence (non vital bacteria) compared with the red + green (vital bacteria) fluorescence. Statistical analysis has been performed using Kolmogorov-Smirnov test and T-test for unpaired data.

Results: The PIPS group showed a more effective bactericidal effect than the NaOCl group. CLSM analysis showed a high efficacy in both groups in terms of deep disinfection inside dentinal tubules, without statistically significant differences.

Conclusion: Within the limits of this study, PIPS technology can be considered an effective aid in a simplified and faster treatment protocol.

Apical extrusion of debris after reciprocating WaveOne Gold® and WaveOne™ systems compared with rotary instrumentation: an *in vitro* study

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Aim: The purpose of this study was to compare the amount of apically extruded debris of NiTi

reciprocating instrumentation systems WaveOne Gold® and WaveOne™ with NiTi rotary instrumentation system Protaper Next™. As known, reciprocating system WaveOne™ produces a greater amount of apically extruded debris than full-sequence rotary instrumentation, however this study aims to assess whether WaveOne Gold®, characterized by new offset parallelogram shaped cross section and Gold-wire technology, produces less apical extrusion of debris.

Methods: A total of 30 extracted human maxillary incisors with mature apices and straight root canal (<5°) according to the Schneider classification were randomly assigned to 3 groups (n = 10 teeth for each group of reciprocating instrumentation; n = 10 teeth for rotary instrumentation control group). Only single-rooted teeth with a single canal and a single apical foramen were included. This was verified by viewing their buccal and proximal radiographs. The external surface of all teeth was cleaned with a periodontal curette. Coronal access was performed with diamond burs. Glidepath was performed with Proglider™ (Dentsply Maillefer, Ballaigues, Switzerland) in all groups. The root canals were instrumented according to the manufacture's instructions using the reciprocating single-file systems WaveOne Gold® or WaveOne™ Primary File (Dentsply Maillefer, Ballaigues, Switzerland) or the rotary system Protaper Next™ X1-X2 (Dentsply Maillefer, Ballaigues, Switzerland) up to working length (WL). Bidistilled water was used as irrigant. The apically extruded debris during instrumentation was collected in preweighted glass vials using the Myers-Montgomery method. After drying, the average weight of debris of each group was assessed using a microbalance and statistically analyzed using ANOVA and post hoc Bonferroni test. Furthermore, the time required to prepare the canals with different instruments was recorded.

Results: Rotary instrumentation with Protaper Next™ (PTN) X1, X2 produces significantly less debris than both reciprocating single-file groups WaveOne™ (p <.01) and WaveOne Gold® (p <.05). WaveOne Gold® produces less debris than WaveOne™.

Conclusion: Under the condition of this study, all systems caused apical debris extrusion. Full-sequence rotary instrumentation was associated with less debris extrusion compared with the use of reciprocating single-file systems. New features of WaveOne Gold® could explain the lower production of debris than WaveOne™, despite the obtained differences were not statistically significant.

Histochemical evaluation of activated sodium hypochlorite into human dentin

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Aim: The aim of this study was to evaluate ex vivo the penetration depth of sodium hypochlorite in human dentin, with manual and ultrasonic activation, using a histochemical staining technique under vacuum and the bleaching ability of NaOCl.

Methods: The root canals of 36 extracted single canal teeth were shaped to a size 30. .07 taper. Dentinal tubules were stained with 8% copper sulphate solution and 5% ammonium hydroxide followed by a 1% rubeanic acid alcohol solution under vacuum. The samples were irrigated for 1 min with 5mL of 5.25% sodium hypochlorite solution, then the irrigant was activated for 1 minute by Manual Dynamic Agitation (MAD) with K-file (Kerr, Italy) or Passive Ultrasonic Activation (PUI) with Irrisafe insert (Satelec Acteon group, Merignac, France), according to the corresponding treatment group. A cross-section was obtained in the central portion of each root third. Under microscopic examination, sodium hypochlorite penetration into dentin was evaluated by measuring the following parameters: bleached circumference of the root canal with respect to the stained circumference; bleached areas, mean and maximum penetration depth. For statistical analysis, Pearson's chi-squared test and one-way ANOVA were used.

Results: Only 58% of the apical sections showed stained dentinal tubules; therefore, these were excluded from further comparisons. The percentage of copper sulphate-stained circumference of the root canals increased from the middle to coronal level in both groups, with a statistically significant difference ($P < 0.001$). In the middle and coronal third, the average root canal bleached circumference was 98-100% of the stained circumference, with no difference within and between groups, and the bleached area was 125490-298290 μm^2 , respectively. The bleached circumference values (also those related to root canal) and bleaching areas of the coronal third were significantly higher than those recorded in the middle third in each group, while no differences between groups at the same level were observed. The mean penetration depth ranged from 63 to 69 μm and the maximum was from 96 to 106 μm , without inter- and intra-group differences ($P = 1$).

Conclusion: No differences in dentinal tubules penetration of 5.25% sodium hypochlorite solution were observed with manual and ultrasonic activation in the middle and coronal root thirds of single canal teeth.

Endodontic treatment of patient with cleidocranial dysostosis: a case report

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Aim: Cleidocranial dysostosis (CCD) is a rare congenital bone disorder with an autosomal dominant hereditary inheritance pattern. This disorder can be caused by mutation in the transcription factor CBFA1 (RUNX2). The CBFA1 gene is essential for membranous as well as endochondral bone formation. The estimated prevalence of CCD is one per million, but it is most likely underdiagnosed because of the relative lack of medical complications in comparison to other skeletal dysplasias. CCD is reported in all ethnic groups, and there is no sex predilection. Clinically, CCD is characterized by hypoplastic/aplastic clavicles, brachycephalic skull, midface hypoplasia, delayed closure of fontanelles and moderately short stature. The most common dentoalveolar characteristics of CCD are: over-retained deciduous teeth with unresorbed roots, supernumerary teeth, retarded eruption, reduced height of the lower third of the face and a skeletal Class III tendency due to an underdevelopment of the maxilla and to an upward and forward mandibular rotation, seriously delayed root development of the permanent teeth. The multidisciplinary treatment procedure consists of a surgical phase followed by an orthodontic treatment, in which extrusion forces are applied.

Methods: A 18-years-old male student presented at Gemelli Hospital in June 2016 because of pain in the upper right back-molar region. Clinical and radiographic examinations revealed an extensive cavity in the upper right first molar and impaction of numerous teeth. In addition, supernumerary teeth were evident at orthopantomography. During treatment, an accidental pulp exposure occurred, so an interim medication with calcium hydroxide was performed. After 1 week, the patient keep on being symptomatic and returned for a recall evaluation, reporting pain and sensitivity.

After local anesthesia (3% mepivacaine with noradrenaline 1:100.000) and rubber dam isolation, the pulp chamber was accessed using a spherical diamond bur and further refined with Endo Z bur. The working length was established with a Root ZX apex locator. The root canals were instrumented with NiTi rotary files, using copious irrigation with 2.5 % sodium hypochlorite. The MB2 canal was deeply calcified, so an interim root canal medication was performed with a pellet embedded with 17% EDTA and calcium oxide cement. The next appointment, under 2,5x loop magnification, the MB2 canal was rinsed by copious irrigation with 17% EDTA and instrumented

until working length. The root canal was dried with sterilized paper points and then filled with Thermafil gutta-percha cones and AH Plus sealer. After 5 days, coronal restoration was performed with composite resin.

Results: Our follow up plan consisted in a clinical visit and a control radiography of the tooth. After 9 months of follow up, the patient reports the complete remission of symptomatology attributable to the tooth treated.

Conclusions: As seen, there are many dental implications of this pathology. Therefore, it is very important to approach it with a correct multidisciplinary protocol. Since these patients have a great number of impacted teeth, it is fundamental to preserve their erupted teeth. In fact, keeping permanent teeth as much healthy as possible, enables to choose among different treatment possibilities in order to improve everyday life of our patients. In our case, an erupted tooth can be very important for the orthodontic treatment, since it may help during the orthodontic traction of impacted teeth after surgical exposure. In conclusion, the dental condition of these patients has to be analyzed carefully, because even a single tooth can give us the opportunity to improve oral condition of our patients.

Cytotoxic evaluation of three epoxy resin-based endodontic sealers

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Aim: The objective of this study was to assess the cytotoxicity of three epoxy resin-based endodontic sealer: AH Plus (Dentsply, Konstanz, Germany), Sicura Seal (Dentalica, Milano, Italia) and Top Seal (Dentsply, Konstanz, Germany).

Methods: The sealers were prepared according to the manufacturer's instructions under aseptic conditions. Direct and indirect cytotoxicity tests and fluorescence evaluation were performed. Cytotoxicity was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay to check the osteoblast-like cells (MG63 cells) viability at 1-2-3-7 days. Data were statistically analyzed by analysis of variance and the Kolmogorov-Smirnoff test with a significance level of 5%. Fluorescence evaluation was performed using the LIVE/DEAD® Viability/Cytotoxicity Assay kit (Invitrogen, Waltham, MA, USA). After incubation time of 3 hours, the images

were acquired using a Fluorescence Microscopy (EVOS FL, Thermo Fisher Scientific).

Results: The direct cytotoxicity, in all three groups, tended to decrease over time, and after 7 days all the sealers were not cytotoxic compared to controls. In addition, Sicura Seal showed the best pattern of biocompatibility at 2 days, while AH PLUS resulted more cytotoxic till the third day. Results related to the indirect cytotoxicity test revealed that elutes derived from all the sealers were significantly more cytotoxic than the negative controls up to 7 days from 24 hours ($p < 0.05$). No differences were found among the three tested sealers at 1, 2 and 7 day, however Sicura Seal showed a worst rate of cytotoxicity at the 2nd day compared both to AH PLUS and Top Seal.

The fluorescence images obtained after 3 hours of incubation with tested materials confirmed the cytotoxicity results, a higher number of dead cells was visible in cultures exposed to AH Plus, Top Seal and Sicura Seal, compared to the negative control.

Conclusion: Analysis of the cytotoxicity of AH Plus, Top Seal and Sicura Seal revealed that all the three epoxy resin-based sealers possess a moderate grade of cytotoxicity on human osteoblast-like cells. A direct cytotoxicity is present in the short term when sealers come directly in contact with cells, but it tends to strongly decrease after a week. While, cytotoxicity due to the release of exudates is present also after a week of culture. Although AH Plus has more cytotoxicity rate than Sicura Seal and Top Seal, all materials can be considered biocompatible.

Cytokine profile analysis of dental pulp stem cells

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Aim: Bone tissue is capable of self-repairing, but inborn malformations, impaired healing and critical-size bone defects make it the second most manipulated tissue after blood. As many issues hinder the traditional treatments, researchers have focused on bone regeneration. Adipose-derived stem cells (ASCs) are relatively abundant and easy to access; they may become a convenient source of mesenchymal stem cells for bone regenerative protocols. Surprisingly, very little is known about the most significant biomolecules that these cells produce and release after being osteoinducted. Therefore, the aim of the present study was to dose 13 candidates chosen among the most representative chemokines, cytokines

and growth factors within the conditioned media of osteodifferentiated and undifferentiated ASCs. Comparisons were made with two acknowledged osteoblastic cell models, i.e. MG-63 and SaOs-2 cells.

Methods: ASCs, isolated from fat tissue obtained from three different donors, were maintained in Dulbecco's Minimum Essential Medium, enriched with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS, Gibco Life Technologies), 100 mg/ml streptomycin, 100 U/ml penicillin. Saos-2 (ATCC number: HTB-85) and MG-63 (ATCC number: CRL-1427) cells were cultured following ATCC instructions. Conditioned media was characterized to analyze the profile of the biomolecules of all cells. The concentration of the following biomolecules was quantified: granulocyte-colony stimulating factor (G-CSF), interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), basic-fibroblastic growth factor (bFGF), inter-feron-gamma (INF- γ), monocyte chemoattractant protein-1 (MCP-1) (CCL-2), CXCL10 chemokine (IP-10), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF). The flexible Bio-Plex system (Bio-Rad Laboratories, Hercules, CA, USA) was employed. Alkaline Phosphatase (ALP) activity was evaluated using a colorimetric end-point assay, which measures the conversion of p-nitrophenol phosphate (PNPP), a colourless substrate, by the enzyme ALP to the yellow product p-nitrophenol.

Results: Interestingly, there is a big difference in the expression pattern of interleukins, chemokines and growth factors among different cells. ASCs produce considerable levels of MCP-1, IL-6, IL-8 and VEGF without particular variations between control and osteodifferentiated condition, with the exception of IL-8. MG-63 show high levels of expression of MCP-1, IL-12, IL-10 and VEGF. Importantly, the expression of IL-12, IL-10 and VEGF decreases in osteodifferentiating conditions. SaOs-2 shows low expression levels of the considered molecules, except for VEGF. Similarly to MG-63 cells, the osteodifferentiating medium inhibits the expression of IL-12 and VEGF in SaOs-2. Cells osteogenic potential has been assessed at the early stage by quantifying the ALP activity. Interestingly, the osteodifferentiating condition significantly increased the ALP activity for each cell type.

Conclusion: Once more this study supports the viability of ASCs in bone tissue engineering based on the levels of cytokines, chemokines and growth factors detected.

Confocal laser scanning evaluation of bactericidal effect of photon-initiated photoacoustic streaming disinfecting protocol

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Aim: Photon-induced photoacoustic streaming (PIPS) technique is a disinfection system that induces a profound photoacoustic shock wave, facilitating irrigants' three-dimensional movement. It's based on the use of a Er:YAG laser with both a radial and stripped tip of new design at subablative power settings (0.3 W), placed only into pulp chamber. Bactericidal effect of Diode laser is based on the generation of a dose-dependent increase of temperature. This method is triggered by a probe, positioned deep into the root canal lumen, activated with light energy at two different wavelengths. Both systems have been proposed to improve disinfection in minimally invasive endodontics.

Methods: Forty-two human single-root teeth were stored in 4% thymol solution after extraction. The teeth were sectioned 1 mm below the cemento-enamel junction, then instrumented with ProTaper Next system X1, X2 e X3 (Dentsply, Maillefer) to WL. Irrigation was performed with 2,5% NaOCl throughout instrumentation and EDTA 10% for 5 min. After ethylene oxide sterilization, the canals (n=42) were infected with *Enterococcus faecalis* ATCC 29212 (3x10⁷ CFU/ml) in Brain Heart Infusion medium broth, afterward incubated aerobically at 37°C for 3 weeks to allow penetration of *E. faecalis* into dentin tubules. After three weeks of infection, specimens were subdivided into different groups with the objective to compare antimicrobial efficacy of PIPS system with Diode laser and traditional irrigation protocol. Specimens were randomly assigned to 3 groups (n=10) plus positive (n=2) and negative (n=2) controls. In PIPS's group irrigation was performed with 2mL NaOCl 2,5% and activated with PIPS system. In Diode group the laser had a double wavelength with combined emission (980 nm and 645 nm) and was used with apical-coronal movement into the canal filled with NaOCl 2,5%. In NaOCl group irrigation was performed with 2,5% NaOCl for 20 seconds using an endodontic needle at 2 mm from WL. After the different irrigation protocols all the teeth were washed with saline solution for 30 seconds and data about the bacterial charge in the root canal lumen were collected through sterile paper points. The paper points were centrifuged into tubes containing 1 mL of 0.85% saline for 1 minute. After 10 serial dilutions, 0.1 mL of solution were inoculated on the culture medium and incubated at 37 ° C in anaerobiosis for 24 hours. The resulting bacterial colonies (CFUs) were counted and multiplied by the dilution factor. The samples were analysed by confocal laser scanning microscope (IX70, Olympus Optical) and coloration Viability Staining (coloration: LIVE/DEAD BacLight Bacterial Viability stain) (Molecular Probes, Eugene, OR)). Images have



been reworked with ImageJ (NIH, Bethesda, MD). Differences between groups were analysed with the Kruskal-Wallis test and Dunn's post-hoc ($P < 0.05$). The level of significance was set at $P < 0.05$.

Results: Dead Ratio: NaOCl vs Diode (KW=17,28, $P < 0.01$) - NaOCl vs PIPS (KW=17,28, $P < 0.001$) - Diode vs PIPS (KW=17,28, $P > 0.05$).

Conclusions: The results show a significant reduction of the counts of CFU for all the techniques, compared to the positive control group. Both methods based on laser technology (PIPS and diode lasers) shown increased antibacterial efficacy than conventional endodontic irrigation. The PIPS method proved more performing cleaning than the one performed with diode laser, difference was not statistically significant. CLSM analysis of the penetration within the dentinal tubules showed that all systems have a high efficiency in terms of deep cleaning of dentin structure, with no differences between groups.

Influence of continuous rotation or reciprocation of optimum torque reverse motion on cyclic fatigue resistance of five nickel-titanium rotary instruments

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Aim: To evaluate the resistance to cyclic fatigue of ProTaper Next (PTN), Revo-S, Mtwo, Twisted Files (TF) and EndoWave used in continuous rotation or in reciprocation of Optimum Torque Reverse motion (OTR).

Methods: Files from five NiTi rotary brands (PTN X2 size 25, .06 taper, Revo-S SU size 25, .06 taper, M-two size 25, .06 taper, TF size 25, .06 taper and EndoWave size 25, .06 taper) were used. A total of one-hundred and twenty instruments (25-mm long), twenty-four for each brand, were divided into 2 groups ($n=12$) on the basis of the motion tested: Group 1 in continuous rotation and Group 2 in reciprocation of OTR motion. Resistance to cyclic fatigue was determined by recording time to fracture (TtF) in a stainless steel artificial canal with a 60° angle of curvature and 5 mm radius of curvature. The length of the fractured file tip was measured by using a digital microcaliper. The TtF data were analysed by using the two-way analysis of variance (ANOVA) and Bonferroni post-hoc tests at 0.05.

Results: Reciprocating OTR motion improved TtF of

all of the tested instruments ($P < 0.0001$). Mtwo and TF had significantly higher TtF when compared with all other instruments, both in continuous rotation and reciprocation of OTR motions ($P < 0.0001$ and $P < 0.05$, respectively). No difference was observed between Mtwo and TF ($P > 0.05$), in both motions. PTN was associated with higher cyclic fatigue resistance than Revo-S and EndoWave, both in continuous rotation and reciprocation of OTR motions ($P < 0.0001$). No difference was observed between Revo-S and EndoWave, in both motions ($P > 0.05$). The mean length of the fractured fragment (5.0 mm) was not significantly different for all of the tested instruments ($P > 0.05$). SEM images of the fracture surface revealed mechanical damage due to cyclic fatigue failure in all of the groups tested both in OTR reciprocating motion and continuous rotation with dimpling and cone formations from the ductile rupturing.

Conclusion: Reciprocation of OTR motion improved significantly cyclic fatigue resistance of all instruments tested compared with continuous rotation. Moreover, Mtwo and TF showed significantly higher cyclic fatigue resistance than the other tested instruments, both in continuous rotation and reciprocation of OTR motion. Because reciprocation of OTR motion increases cyclic fatigue resistance of rotary instruments, clinicians should consider the possibility to use OTR motion and its reciprocating motion (with instruments designed for CW continuous rotation) in the clinical situations that produce high cyclic fatigue as in shaping of curved canals.

Two fibre post systems luted with or without post space preparation: cement thickness analysis

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Aim: To appraise the cement thickness around two adhesively luted fibre posts in straight single-rooted teeth: a double-tapered post placed after traditional post space preparation and a single-tapered post adapted to the canal without further removal of root dentine.

Methods: Twenty straight single-rooted permanent teeth were cross-sectioned to obtain 14 mm-long roots, whose canal was shaped with Mtwo rotary files up to size 40/.04 and then filled with the continuous wave of condensation technique. The coronal portion of the canal was left empty for 9 mm. The roots were randomly allocated to two experimental groups of 10

elements each. In group 1 (G1), the post space was drilled by means of dedicated burs corresponding to the relative double tapered DT light post. In group 2 (G2), the canal was not further enlarged prior to receiving a single taper TechES post, which underwent standardised trimming to fit into the prepared canal. In both groups, posts were luted with self-adhesive cement (RelyX Unicem). The specimens were longitudinally cut with a microtome in mesiodistal direction, sputter-coated with gold and observed at the scanning electron microscope. For each side of the post, a technician acquired three microphotographs at 250× for every third of the post length (coronal, middle and apical third). An independent calibrated examiner measured the cement thickness 20 times per image (120 readings per post third, 360 readings per specimen). The mean cement thickness was compared with parametric statistical tests between the two post systems and among different post thirds ($p < 0.05$).

Results: The fit of the post at the coronal level was excellent irrespective of the experimental group. In G1, the cement thickness did not significantly vary among the post thirds; the impression left on canal walls by the tip of the bur was observed at the apical third of the post. In G2, the post fit decreased in the apical third ($p < 0.05$), reaching maximum thickness at the post tip (200–250 μm).

Conclusion: In straight single-rooted teeth, the post fit at the apical third of the post was superior when the post space was prepared by drilling, at the cost of removing sound dentine. Single taper posts allowed for good fit, which slightly decreased along with the canal depth, but required no further removal of tooth structure. The relatively greater cement thickness detected at this level was ascribable to the methodological standardization required in the research setting.

Torsional and cyclic fatigue resistance of a new nickel-titanium instrument manufactured by electrical discharge machining

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Aim: The purpose of this study was to evaluate the torsional and cyclic fatigue resistance of the new Hyflex EDM OneFile manufactured by electrical discharge machining and compare the findings with

the ones of Reciproc R25 and WaveOne Primary.

Methods: One hundred-twenty new Hyflex EDM One-File (#25/0.08), Reciproc R25, and WaveOne Primary files were used. Torque and angle of rotation at failure of new instruments ($n = 20$) were measured according to ISO 3630-1 for each brand. Every instrument was inspected for defects or deformities before the experiment under a stereomicroscope; none were discarded. Cyclic fatigue resistance was tested measuring the number of cycles to failure in an artificial stainless steel canal with a 60° angle and a 3-mm radius of curvature. The length of the fractured file tip was measured by using a digital microcaliper. The fracture surfaces of all fragments were examined under a scanning electron microscope to look for topographic features of the fractured instruments. Data were analyzed using the analysis of variance test and the Student-Newman-Keuls test for multiple comparisons.

Results: The cyclic fatigue of Hyflex EDM was significantly higher than the one of Reciproc R25 and WaveOne Primary ($P < .05$ and $P < .001$, respectively). Hyflex EDM showed a lower maximum torque load ($P < .05$) but a significantly higher angular rotation ($P < .0001$) to fracture than Reciproc R25 and WaveOne Primary. No significant difference was found comparing the maximum torque load, angular rotation, and cyclic fatigue of Reciproc R25 and WaveOne Primary ($P > .05$). The mean length of the fractured fragment (3.0 mm) was not significantly different for all of the instruments tested ($P > .05$). Scanning electron microscopy of the fracture surface showed similar and typical features of cyclic fatigue and torsional failure for the 3 brands.

Conclusions: Within the limitations of this study, our results showed higher flexibility and angular rotation to fracture but a lower maximum torque load to failure of HEDM (CM-wire) compared with reciprocating instruments (M-wire for both files), and they highlight the potential of EDM for use in the manufacturing of endodontic mechanical instruments.

Diagnosis and treatment of dens invaginatus with open apex in adult patient, by using cone-beam computed tomography and operative microscope

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Aim: To present a clinical case of dens invaginatus, where the invagination gave rise to a structure radiologically similar to a tooth inside of a maxillary



lateral incisor, that became necrotic before the complete formation of the apex.

Methods: A 35-year old male patient referred a history of recurring abscesses with a vestibular fistula corresponding to the left maxillary lateral incisor. The intraoral periapical radiograph showed the presence of a periapical radiolucency of the 2.2 which had an open apex, and dentine tissue similar to a tooth within the pulp chamber. Cone Beam Computed Tomography (CBCT) confirmed the diagnosis of "dens invaginatus class II Ohler". The concerned tooth did not respond to both electric and heat test. Provisional diagnosis was concluded as non-vital tooth with periapical lesion in relation to 2.2. After proper isolation with rubber dam an appropriate access cavity was prepared to allow the debridement of the necrotic pulp. A barbed broach was used for debridement. The glide path was performed with a manual K-file n. 10, which crossing the invaginated canal reached the immature apex at a working length of 22 mm measured by Root ZX apex locator. Thanks to the operating microscope it was possible to find access to the "real" endodontic space and to clean it circumferentially to the invaginated hard tissues. Gentle circumferential filing had been performed with minimal dentin removal using #80 H file. The canal was then irrigated with 5.25% NaOCl. Paper point had been introduced inside canal to dry it. Trying to follow and respect the C-shaped, already highlighted by CBCT. At first the whole endodontic space, both the real and the invaginated one, was filled with calcium hydroxide for 3 months. After 3 months the calcium hydroxide was removed by irrigation with 5.25% NaOCl, and the endodontic space was dried. The intraoral radiograph and CBCT showed that the internal hard structure was almost completely detached from the "real" canal walls; so, it was mobilized and removed with ultrasonic tip (StartX #3) through the access cavity, and the wide endodontic space was filled with MTA, condensed for approximately 3 mm by using a Schilder's plugger and an ultrasonic tip for 10 seconds; it was covered with a moist cotton pellet, and the access was sealed with Cavit. One week later, a dual composite sealer (Relyx Unicem 2) was placed over the MTA cement, and the tooth was restored with an universal nano-hybrid composite (Tetric Evoceram).

Results: After the first sessions of root canal shaping and medication with calcium hydroxide, the clinical symptoms and the fistula, which to date (two years) have not presented, disappeared. The radiographs, 12 months after the end of endodontic treatment, showed that the periapical lesion was gradually reduced both in size and intensity of gray.

Conclusion: The cases of dens invaginatus may differ and consequently different is the treatment approach. In the case described, through the use of CBCT and the operating microscope, it was possible to locate

and remove the hard tissue invaginated and then to proceed as a classic endodontic treatment of a tooth with open apex. The patient was included in a follow-up program to check and verify the complete periapical bone healing of the affected tooth.

Activation of two irrigating solutions with the XP-Endo finisher: canal cleanliness analysis

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Aim: To evaluate the formation of smear layer and debris on canal walls of prepared root canals after activating the irrigating solution by using the XP-endo Finisher either with sodium hypochlorite or ethylenediaminetetraacetic acid (EDTA).

Methods: The crowns of 24 single-rooted similarly-sized extracted teeth were removed with a cutting disc. After working length determination and glide path creation, root canals were shaped with BT Race files up to size 40/04. During the enlargement phase, the canals were rinsed with 2 ml of 5% sodium hypochlorite solution after each instrument. The prepared canals were rinsed with 3 ml of distilled water and dried with sterile paper points. At this stage, the roots were randomly allocated to a control and two test groups (n=8). The control group received three minutes-long rinses with 17% EDTA followed by 5% sodium hypochlorite. The canals of the remaining roots were rinsed with the same irrigants, activating for one minute with the XP-endo Finisher (800 rpm, 1 Ncm) the EDTA solution in the first test group and the sodium hypochlorite solution in the second. Two longitudinal grooves were created on the external surface of the roots, which were split, sputter coated with gold and observed at the scanning electron microscope. Five microphotographs per canal third were acquired at 2000 magnifications and scored according to the Hülsmann scale for smear layer and debris formation. The median value of the scores attributed to each third was regarded as statistical unit. Non-parametric statistical analysis was performed to assess the differences among groups and canal thirds ($p < 0.05$).

Results: The coronal third was found to be satisfactorily cleaned irrespective of the experimental group; on the contrary, all the considered irrigation protocols were unable to avoid smear layer and debris accumulation in the apical third. In the group where the sodium hypochlorite was activated, significantly worse scores were attributed to the middle third in terms of both smear layer and debris, in comparison to the other two

groups ($p < 0.05$).

Conclusion: Under the conditions of the present study, the use of the XP-endo Finisher at the end of the root canal preparation procedure did not appear to contribute to the cleaning of the canal walls at a microscopic level. More specifically, no visible improvement was detected in the most clinically relevant canal portion, namely the apical third. Other studies are needed to understand which is the ideal role that the XP-endo Finisher can play in an irrigant activation protocol to maximise canal cleanliness.

Micro-CT evaluation of root filling quality with warm guttapercha vertical technique and guttacore obturation systems

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Aim: Successful endodontic therapy depends on effective chemomechanical instrumentation of the root canal system and a successive 3D filling which provides hermetic sealing of the available spaces. Therefore the aim of this study was to evaluate, by microCT analysis, the root filling quality of two different obturation systems: warm gutta-percha vertical technique and GuttaCore. A quantitative and qualitative evaluation of gutta-percha filled areas (GPFA) and voids and their specific location in root canal cross sections was made.

Methods: Thirty human extracted mandibular first molar were selected with fully formed apexes and independent mesial canals. The teeth were free of root caries, cracks and artificial alterations. After access cavity and pulp removal, a size- 10 K-file was inserted up to the apical foramen and then withdrawn 1 mm, determining the working length (WL). Mechanical glide path was performed with ProGlider (Dentsply Maillefer Ballaigues, Switzerland). Irrigation was performed with a solution of EDTA 10% and NaOCl 5%. Teeth were shaped with WaveOne Gold (Dentsply Maillefer Ballaigues, Switzerland). Then half of the samples were filled with warm gutta-percha vertical technique and half with GuttaCore. In both cases ThermaSeal Plus sealer was used to achieve an hermetic seal. Specimens were scanned after the canal obturation to perform the matching volumes and for post-treatment analysis (SkyScan, Bruker-microCT, Kontich, Belgium). Every tooth was scanned with the same parameters. The X-Ray tube was operating at 50 kV and 200 μ A with 15 μ m resolution and 360° rotation. An Al+Cu filter was used to suppress beam-hardening artifacts. Afterward the

images were reconstructed and analyzed by NRecon and CTAn softwares (Bruker-microCT) to evaluate the area of GPFA, interfacial gaps at the filling/dentine surface and voids surrounded by filling material. The bidimensional area occupied by voids in sections of the apical 5 mm was obtained with 1 mm of distance from each other.

Results: both obturation systems demonstrated the ability to obtain a three-dimensional hermetic seal of the root canal system. However GuttaCore system demonstrated a lower amount of cross sectional areas occupied by voids, especially in the apical 5 mm.

Conclusion: The reproducibility and accuracy of micro-CT for analysing root canal fillings have been well demonstrated in previous studies. This non-destructive technique provides rapid imaging of the gaps and voids present in the filled canal space and produces impressive 3-D visualisation of the orientation and continuity of these deficiencies within the filled canal. According to the literature, this study confirmed that warm vertical compaction technique is much more operator dependent than GuttaCore technique even if both ensures a good 3D filling. Due to the similar obturation quality between GuttaCore and warm vertical compaction, the GuttaCore core-carrier technique represents a valuable alternative for canal obturation.

Immediate and residual antimicrobial efficacy of irrigants activated by cordless sonic or ultrasonic devices: an ex-vivo study

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Aim: The improvement of irrigating protocols is essential to achieve better disinfection and ensure greater residual antimicrobial activity in the root canal system. Aim of this study was to evaluate immediate and residual antimicrobial activity of irrigants activated by two different cordless devices (sonic or ultrasonic) on root canals infected by *Enterococcus faecalis*.

Methods: One hundred and ten single-rooted extracted teeth were infected with biofilm of *E. faecalis*. After preparation, the roots were randomly divided into six groups ($n=15$): 3% sodium hypochlorite (NaOCl) with EndoUltra or EndoActivator or conventional syringe irrigation without activation; sterile bi-distilled water



with EndoUltra or EndoActivator or conventional syringe irrigation without activation. The conventional syringe irrigation protocol involved the use of a needle bended at 15 mm from the tip, introduced as close to canal terminus as possible without binding and deposition of 1 mL of irrigant solution every 20 seconds, for a total of 3 ml of irrigant. According to the manufacturer, the EndoUltra protocol was performed by a frequency of 40 KHz with a 15.02 tip, maintained at a distance of 2mm from WL. The EndoActivator treatment was performed with a 15/02 tip at 10,000 cycles per minute. Colony-forming units (CFUs) and turbidity were recorded from bacteriological samples taken before (S1), after treatment (S2) and after 24 hours of reincubation (S3). Data were analysed by statistical software using Kruskal-Wallis and analysis of variance with Dunn's multiple comparison post hoc tests, and a P value of < .05 was considered statistically significant.

Results: Before treatment, all samples of the six test groups showed a similar high bacterial growth and turbidity ($P > .05$), instead after treatment, there were a significant lower bacteria count and turbidity for groups irrigated with 3% NaOCl-EndoUltra activation compared with groups treated with 3% NaOCl-EndoActivator or no activation ($P < .01$ for each comparison). No statistically significant differences were found in comparison with groups treated with 3% NaOCl with or without sonic activation ($P > .05$). No bacteria reduction was found in groups irrigated with sterile bi-distilled water with or without activation ($P > .05$). After 24 hours re-growth of bacteria was significantly lower in 3% NaOCl-EndoUltra teeth than the ones where it was used EndoActivator or no activation ($P < .05$).

Conclusion: Within the limitations of the present study, EndoUltra- 3%NaOCl produces an immediate and residual lower bacterial count compared with the one obtained with Endoactivator. Clinical studies should be undertaken to assess the portability of the present in vitro data.

Antibacterial activity of different root canal sealers by using two different methods

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Aim: Complete elimination of microorganism is the great goal of endodontic treatment, the use of endodontic sealers with antibacterial activity could improve the chances of achieving the aim. The purpose of this study was to evaluate the antibacterial

activity of eight different root-canal sealers against *Enterococcus faecalis*, both the agar-diffusion test (ADT) and direct-contact test (DCT) were performed.

Methods: The antibacterial activity was evaluated using a standard strain of *E. faecalis* cultivated in Brain Heart Infusion and prepared different sealers following manufacturer's instructions. In ADT Six replica plates were spread with 0.1 mL of the bacterial suspension, sterile paper discs were immersed in freshly mixed sealers and deposited onto agar plates, then incubated at 37° C for 48 h under aerobic conditions. The inhibition zones around each well was measured by the same operator in two perpendicular locations with a millimeter ruler with accuracy of 0.5 mm. In DCT the antibacterial properties were evaluated by counting the number of bacterial colonies; every sealer was placed in a sterile cylinder-shaped plastic blocks. The samples were placed in incubator at 37°C and the humidity of 100% for a period of 7 days. The obtained sealer blocks were powdered. A different suspension of every sealer was created using 1 ml of saline suspension and 50mg of powder and then it was added to bacterial suspension at equal volumes. Six, fifteen and sixty minutes after mixing the suspensions were diluted ten thousand times, 0.01 ml of diluted suspension was plated in triplicate on the already-provided BHI agar plates after incubation at 37°C for 24 h, the colonies formed on the agar plated were counted. Then, the number of colony-forming unit (CFU) was calculated. These experiments were repeated three times.

Results: The ADT was used to evaluate the antibacterial activity on freshly mixed sealers; BioRoot™ RCS, TotalFill BC Sealer, MTA Fillapex and Sealapex Root Canal Sealer revealed no activity; both Pulp Canal Sealer EWT and AH plus sealers showed a significant increase ($P < 0.05$) in antibacterial effect. The mean diameters of the bacterial inhibition zone by EasySeal and N2 sealers ($P < 0.01$). The direct contact test evaluated the activity of sealers on set; the results are express as percentage of antibacterial activity compare to the negative control. AH plus and Sealapex Root Canal Sealer revealed no antibacterial activity after 6 min of contact, but data increase after 15 and 60 min ($P < 0.05$ and $P < 0.01$ respectively). The effect of Sealapex Root Canal Sealer, compare to AH plus shown a higher effect ($P < 0.01$). BioRoot™RCS, MTA Fillapex, Pulp Canal Sealer EWT and N2 revealed at least means ($4 \pm 2 \times 10^7$ /ml) of the number of colonies after 6 min of contact. Except for N2, a significant increase in bactericidal effect ($P < 0.05$) after 15 and 60 min for the other compared sealers (BioRoot™RCS, MTA Fillapex and Pulp Canal Sealer EWT). TotalFill BC Sealer and EasySeal were bactericidal against *E. faecalis* and killed all bacteria for every contact times considered.

Conclusion: In the ADT N2 sealer showed maximum

antibacterial activity and in the DCT TotalFill BC Sealer and EasySeal demonstrated the highest bactericidal effects. Therefore, the technique and components of the tested materials affected the results of microbiological studies.

Influence of proper or optimum torque reverse motion on cyclic fatigue resistance of 3 single-file nickel-titanium rotary instruments

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Aim: The purpose of this study was to evaluate the resistance to cyclic fatigue of HyFlex EDM OneFile (HEDM), Reciproc R25 (Rec) and WaveOne Primary (WO) used in proper rotation or in Optimum Torque Reverse motion (OTR).

Methods: Three NiTi rotary systems (HyFlex EDM size #25, 0.08 variable taper, Reciproc R25 size #25, 0.08 variable taper and WaveOne Primary #25, 0.08 variable taper) were used in this study. All seventy-two files used were 25-mm long. Twenty-four instruments for each brand were divided into 2 groups (n=12) on the basis of the motion tested: group 1 in proper

rotation (continuous rotation at 500 rpm for HEDM, reciprocal motion with the "RECIPROC ALL" mode for Rec and reciprocal motion with the "WAVEONE ALL" mode for WO), and group 2 in OTR motion at 500 rpm. Resistance to cyclic fatigue was determined by the recording time to fracture (TtF) in a stainless steel artificial canal with a 60° angle of curvature and 5 mm radius of curvature. The length of the fractured file tip was measured by using a digital microcaliper. The broken fragments were evaluated under the scanning electron microscope (SEM) for topographic features of the fracture surfaces. Data were analyzed by two-way analysis of variance with significance level at 0.05.

Results: HEDM showed higher cyclic fatigue in OTR motion than proper rotation ($p < 0.0001$). No statistically significant difference was observed in OTR than proper motion for Rec and WO ($p > 0.05$). HEDM showed higher TtF when compared with other instruments, both in proper motion and OTR motion ($p < 0.0001$). Rec showed higher TtF than WO in both movements ($p < 0.0001$). The mean length of the fractured fragment (5.0 mm) was not significantly different for all of the instruments tested ($p > 0.05$). SEM of the fracture surface showed similar and typical features of cyclic fatigue failure for the 3 brands.

Conclusion: Within the limitations of this study, cyclic fatigue of HEDM in OTR motion was higher than in proper rotation. No difference was found in cyclic fatigue of Rec and WO activated in its own motion or OTR movement. HEDM showed higher cyclic fatigue resistance than all other instruments in both motions.