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long-distance prognosis, because of biomechanical problems (unfavourable coronal-radicular rate) After adequate clinical managment, it is fundamental that patients be followed up during a certain period of time for clinical treatment success. Our retrospective analysis evaluates conservative therapy success and fractured teeth survival according to the treatment given.

**Methods.** Through clinical and radiological investigations, the Authors have analyzed several cases of middle-root fractures in anterior teeth, particularly, 1.1-2.1-4.1-1.4 dental elements. 21 teeth have been studied in a population of 6 – 62 years old patients. Considered parameters were: possible related fracture, fragments delocation, mobility, tooth sensitivity and pulp complications, diastasis, periodontal inflammation. Observation was made after 6 months, 1 year and 3 years from traumatic event. Data have bene the statistically analyzed.

**Results.** In our statistic analysis, immediately after traumatic event (time 0), multiple fractures were releaved in 38.1% of cases. Mobility was observed in 28.57% of cases. From a clinical point of view, most frequent symptoms were: hypersensitivity in 66.7%, pulp lesion in 42.85%, periapical pathology in 19% (1 on 21). In time 0, in 47.61% of cases, delocation of fracture coronal fragment was observed. In that case, splinting with adjacent teeth, immediately followed by endodontic treatment of involved teeth in 52% of cases was planned. Endodontic treatment was necessary because of pulp complications onset in one of cases after 6 months and in two one-year follow-ups. In one of cases, after 3 years from the therapy, tooth extraction beacause of diastasis, mobility and flogosis was required.

**Conclusion.** Clinical and statistical analysis shows a high index of survival and success rate in restoration therapy of horizontal middle-root fractured teeth. Just in one case, tooth extraction was mandatory after 3 years. In most cases, an immediate endodontic treatment was necessary. In a restricted number of the cases a late endodontic treatment, beacause of complications onset, was needed.

## Evaluation of debris and smear layer removed by different irrigation protocols performed with and without the use of the passive ultrasonic irrigation: a SEM analysis.

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**Aim.** The purpose of this study is to evaluate by SEM the efficacy of different irrigation protocols in the removal of debris and smear layer from the root canal walls.

**Methods.** Fifty-six extracted human single teeth were selected for this study. Radiographs were taken in buccolingual and proximal directions to confirm the presence of a single canal and absence of calcifications or resorptions. Crowns were trimmed to a uniform length of 15 mm with a carborundum disk.Chemomechanical preparation was performed by a single operator with Reciproc system and irrigation was carried out with 3 mL of NaOCl 5,25% for 20 seconds. The groups differed in the final washing.

Samples were randomly divided into ten test groups (n=5) and three control groups (n=2): control 1, no irrigation; control 2, distilled water for 1 min; control 3, distilled water activated with PUI for 20 sec; test 1, 17%EDTA for 1 min; test 2, 17% EDTA activated with PUI for 20 sec; test 3, 5.25% NaOCl for 1 min; test 4, 5.25% NaOCl activated with PUI for 20 sec; test 5, 17% EDTA for 1 min+ 5.25% NaOCl for 1 min; test 6, 17% EDTA activated with PUI for 20 sec + 5.25% NaOCl for 1 min; test 7, 17%EDTA activated with PUI for 20 sec + 5.25% NaOCl activated with PUI for 20 sec; test 8, 17%EDTA with PUI for 20 sec + three cycles of 5.25% NaOCl with PUI for 20 sec; test 9, three cycles of 17% EDTA with PUI for 20 sec + 5.25% NaOCl with PUI for 20 sec; test 10, three cycles of 17% EDTA with PUI for 20 sec + three cycles of 5.25%NaOCl with PUI for 20 sec.

Finally, two longitudinal grooves were prepared on both buccal and lingual surfaces by using a diamond disc without penetrating the canal. The roots were then split into two halves with a hammer and chisel. 12 SEM photomicrographs were taken at X200 and X400 magnification at the coronal, middle, and apical thirds of each specimen. The canal walls were evaluated for the amount of debris and smear layer by using the 5-score system introduced by Hulsmann et al. by two blinded operators.

**Results.** Root canal walls absolutely free of smear layer were not observed with any irrigation solution. The Kruskal-Wallis test revealed statistically significant differences in all the canal sections between the groups that used chelation (EDTA17%)+PUI and other groups; the EDTA and EDTA/PUI groups presented similar smear layer removal, but EDTA/PUI removed significantly more debris as compared to EDTA alone. The groups with NaOCl+EDTA showed statistically significant differences in smear layer scores compared to NaOCl and EDTA alone. There were also statistically significant differences between the control group and all the experimental groups in three canal sections.

**Conclusion.** The best conclusion in this study were obtained when a final irrigation with a chelating agent was used. Within the limitations of this in vitro study it can be concluded that PUI did not improve smear layer removal by EDTA. Moreover, the smear layer scores were similar regardless of the root canal third after the final irrigation protocol with EDTA. On the other hand EDTA/PUI removed significantly more debris as compared with EDTA alone.

## Comparison on sealer penetration into dentinal tubules using self-adjusting file cleaning-shaping-irrigation system and conventional endodontic needle irrigation

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**Aim.** The aim of the study was to compare the effect of the self-adjusting file cleaning-shaping-irrigation system and conventional needle root canal irrigation on sealer penetration into dentinal tubules using confocal laser scanning microscopy.

**Methods.** Twenty-two upper incisors were selected from a pool of extracted teeth. The teeth had a single round canal with a long-short cross-section diameter ratio ≤2,5 at 8 mm from the apex and similar root canal anatomy. The teeth were randomly divided into two groups in according to the irrigation technique used: self-adjusting file system (SAF) (group 1) and conventional endodontic needle irrigation (group 2). The groups resulted homogeneous according to the canal width. The same operator performed all experimental procedures. The crowns were sectioned at the cementoenamel junction and the root length was standardized to 13 mm from the apex. The coronal thirds was enlarged with size 1, 2, 3 and 4 Gates-Glidden drill. The working length (WL) was established by the insertion of a 21-mm #10 K-File until its tip appeared at the apical foramen under microscopic vision at 10x. A glide path was performed using size 13, 16 and 19/.02 PathFile at 300 rpm. In the group 1 the 1,5-mm-diameter SAF was operated for four minutes and continuous irrigation with 5,25% NaOCl was performed by a VATEA peristaltic pump at a rate of 4 mL/min. In the group 2 the canals was instrumented using Protaper Universal to a size of the F2 instruments at the working length and irrigated with 1 ml 5% NaOCl at every instrument changes. In both groups a final irrigation of 2.0 mL 1% EDTA for 30s was performed. Roots filling was performed with Guttacore Obturators with TopSeal labeled with 0,1wt% Rhodamin B. Transverse sections at 2, 5 and 7 mm from apical foramen were observed using a confocal laser scanning microscopy. Total percentage and maximum depth of sealer penetration were measured and registered using ImageJ software. Statistical analysis was performed by ANOVA and Turkey test.

**Results.** The 7-mm and 5-mm sections of group 1 showed a significantly higher percentage and maximum depth of sealer penetration respect the group 2. Not significant difference was found at 2-mm sections between the two groups.

**Conclusion.** The self-adjusting file cleaning-shaping-irrigation system improved the sealer penetration at coronal and middle sections respect the conventional endodontic needle irrigation. At apical sections not differences were found.

## HyFlex EDM rotary Ni-Ti prototypes: the effect of an innovative machining technology on Ni-Ti wear

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**Aim.** HyFlex EDM files were recently introduced presenting an innovative electro discharge machining (EDM) process of fabrication. The aim of this study was to evaluate the surface and microstructural alterations of new and in vitro used HyFlex EDM Ni-Ti rotary prototypes.

**Methods.** The surface and microstructural characteristics of 15 new HyFlex EDM instruments were analyzed by ESEM equipped with energy dispersive x-ray spectrophotometry (EDS) and optical metallographic imaging. Instruments were subjected to instrumentation tests on severely curved root canals (ranging between 50° and 70°) of extracted multi-rooted teeth. Once that canal patency was verified with a #10 K-file, the working length was determined by subtracting 1mm. HyFlex EDM files were used with a 16:1 reduction handpiece X-Smart (Dentsply Maillefer, Baillagues, Switzerland) following the manufacture's direction, at 500 rpm and 2.5Ncm, with slightly apical pressure and pecking motion. The operative sequence was: 25/12 at 2/3 of the WL, 10/05 and 25/08 at WL. Irrigation was performed at every change of instrument, with a total amount of 3 ml of 5% NaOCl and 3 ml of 10% EDTA (Ogna, Muggiò, Italy). Each instrument was used in 10 curved canals, washed in an ultrasonic bath containing detergent for 10 min and then autoclaved at 134°C. Surface and microstructural characterizations were repeated on used instruments at same points and with same angulations to compare the pre- and postoperative micrographs, in order to verify the appearance of fractures, unwinding, microcracks, blade disruption and tip deformation.

**Results.** Surface and microstructural characterization of new instruments revealed the typical features of a NiTi ED-Machined alloy with an irregular and "craters-like" surface. High magnification micrographs disclosed a non-uniform structure were pits, pores and voids caused the peculiar aspect of a "rough-spark-machined" surface. No fractures were registered during instrumentation of curved canals. Surface and microstructural characterization of used files revealed no wear and no degradation of the 25.12 and 25.08 files. The tip segment was confirmed as the most mechanically stressed portion of 10.05 prototypes. All the instruments, after several uses, well-preserved the "craters-like" irregular surface without cutting edge alterations. The metallographic inspection on the cross section of brand new HyFlex EDM files showed an homogeneous martensitic phase. The microstructure appeared uniform from the surface to the bulk, and no microcracks or defect were identified, even at high optical magnification (1000X).

**Conclusion.** Unaltered spark-machined surface and low microstructural degradation are the main features of recently introduced HyFlex EDM. Caution would be recommended regarding reuse of small HyFlex EDM files. Instruments exhibited a safe in vitro use in presence of severely curved canals.

## Vascular endothelia growth factor (VEGF) expression in human tooth germs early and later stage development

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**Aim.** The angiogenesis plays a crucial role in many human physiological and pathological processes during