Evaluation of an automated system for root canal irrigation: a scanning electron microscopy study

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This study evaluated a new automated system using alternative irrigants for root canal cleaning treatments. This method relies on a system inserting an enzymatic solution based on Trypsin flowing inside the pulp chambers and root canals, completely avoiding traditional endodontic instrumentation. Sixty freshly extracted human molar teeth were randomly divided into 4 groups to assess 3 regimens (R1–3) differing in 0.25% Trypsin/EDTA and 5% Sodium Hypochlorite (NaOCl) solutions administration. Scanning electron microscopy observations and scores taking into account changes in dentin tubules were used to assess treatment effects in pulp chambers and roots. Significant changes in root cleaning ability relative to administration timing were observed, with the best results found in R3, with scheduled alternated cycles of Trypsin/EDTA and NaOCl inside the tooth. The non-invasive root canal method demonstrates good teeth cleaning ability independent of root morphology. This equipment may provide lower discomfort levels for patients undergoing endodontic treatment.

Keywords: Automated system, Root canal cleaning, Irrigants, SEM

INTRODUCTION

Meticulous cleaning and shaping of the root canal space, adequate obturation and coronal tight seal are the goals for successful endodontic therapy. In order to create a fluid tight seal, it is imperative that the endodontic filling material closely adapts to the tooth’s structure. This, however, is impaired by the presence of an amorphous mass known as smear layer, which is formed and deposited onto the root canal wall during biomechanical instrumentation. The smear layer is composed of inorganic material (dentin chips containing hydroxyapatite) and organic material (necrotic or vital pulp tissue, odontoblastic remnants, coagulated proteins, blood cells, nerve fibres, collagen, saliva, bacteria and their products)9. In endodontics its removal is considered to be advantageous and highly desirable. For this purpose, cleaning of the root canal by means of endodontic instrumentation is performed. However, irregularities of the root canal wall, including oval extensions, isthmuses and apical deltas2, create barriers by effectively allowing instruments to reach only 40% of the canal wall area. As a consequence, irrigation forms an essential process of root canal treatment.

Irrigants such as sodium hypochlorite (NaOCl), hydrogen peroxide (H2O2), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine gluconate have been widely used8,9, particularly due to their antibacterial properties and the ability to dissolve necrotic tissues3,5,7,9. These irrigants have been found to be less efficient in narrow portions of the canal. Long application time is required for optimum results as the canal anatomy becomes more complex, a feature often observed at the apical third of the roots. Yamada et al.7 found that a final rinse with 10 mL of 17% EDTA followed by 10 mL of 5.25% NaOCl was an effective method. Given that the use of these irrigants at such concentration levels may also reduce dentin micro-hardness, a more diluted concentration of these chemicals in order to reduce their side-effects was recommended10.

This study evaluates, by means of a Scanning Electron Microscope (SEM), the efficacy of a new automated non-invasive method for root canal treatment and irrigation. Avoiding the use of classic endodontic instrumentation, this system instead relies on the ability of the enzymatic solutions (i.e. Trypsin/EDTA and NaOCl), to flow simultaneously inside the tooth to clean the root canal, effectively dissolving both the organic and inorganic components. This process relies on the differences between input and output tooth pressure to determine the intensity of the turbulence and amount of contact of the solution with root canal tissues. The aim is to clean the pulp chamber and root canal surfaces with a less invasive endodontic treatment, effectively creating less discomfort for the patient.

MATERIALS AND METHODS

The equipment is schematically illustrated in Fig. 1. It consists of 5 reservoirs. The first 3 (LQ1–LQ3) are filled with the solutions reagents to be employed, another reservoir is empty (LQ4) and the 5th (LQ5) contains the residual discharging material (Fig.1). Each reservoir is connected to a peristaltic volumetric pump (P1–P4), which delivers the precise amount of reagents during the endodontic treatment (Fig.1). An additional pump
P5) is connected through an air filter to the external environment allowing for circuit and internal tooth drying. P6 determines the solution administration time.

Time control of the solution’s reaction against pulp chamber and root tissues is determined by a micro-controller. Constant chemical reaction speed is maintained by thermo-regulating the collector block throughout the chemical solutions flow. The reagents that reach the patient’s teeth have a physiologic temperature. These monitors allow for more than 80% of the solution to be released from each reservoir in order to infuse in the pulp chamber once it comes into contact with the organic material with a high turbulence.

A pressure transducer, which is connected to the collector block, measures the internal pressure. A micro-processor is used to determine the pump speed (1 cc/s) and direction of rotation. A gravity valve removes air and gas bubbles from the capillary tubes of the annular circuit, minimizing the solution segmentations and guarantying baric equilibrium between the pulp chamber (0 kPa) and external environment (100 kPa). This means that the endodontic treatment is performed at environmental pressure, assuring that reagents do not escape from the dental apex. The latter process is also counteracted by the individual diastolic pressure. Differences between input (130 kPa) and output (70 kPa) pressures inside the tubes (1.2 mm²) determine turbulence levels, thus affecting solution contact with root canal tissues. The pressure applied during the treatment was of 2.5 kPa.

Trypsin/EDTA (T4049 Sigma-Aldrich, Milan, Italy) used in this study is a 0.25%, sterile-filtered, BioReagent, suitable for cell culture. 2.5 g porcine trypsin and 0.2 g EDTA•4Na per liter of Hanks’ Balanced Salt Solution with phenol red (Sigma). NaOCl solution (Sigma) was used at 5%. After each cycle of reagent flowing, rinsing with saline solution (NaCl 8%) was performed.

The experimentation involved a random selection of 60 extracted human posterior teeth (30 mandibular third molars, 30 maxillary third molars). The teeth were immediately fixed using buffered formalin at 4% and stored at 4°C. The roof of the pulp chamber was opened and a hole was created using a diamond bur (#206, Komet-Brasseler, GmbH, Lemgo, Germany) mounted in a high-speed hand-piece. The head of the endodontic device was inserted and fastened to the hole using an epoxisid resin, ensuring a much closed fixation to secure the endodontic treatment.

Teeth were randomly divided into 4 groups to assess 3 regimens (R 1–3), each differing in the timing of Trypsin/EDTA and NaOCl solution administration and contact length. An additional regimen (R4) using only saline solution for 35 min was used as the control group. The regimen sequences are summarised in Table 1.

Fifteen teeth were treated within each regimen and processed for SEM analysis. The teeth were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 4 h, rinsed in 0.2 M cacodylate buffer pH 7.4 and dehydrated in ascending ethanol series. They were then longitudinally (10 teeth for each treatment) or transversally (5 teeth for each treatment) sectioned using a low speed diamond saw (Buehler Isomet Low Speed Saw) under water irrigation. Each section was polished by increasing grid SiC paper under constant deionised water irrigation (Buehler Metaserv Grinder-Polisher). The polished specimens were then coated using a gold sputter coater (Emitech K 550), air-dried and mounted on aluminium stubs for observation under a SEM Philips XL20 (FEI Italia SRL, Milan, Italy).

![Fig. 1 Device schematic design.](image)

Table 1 Scheme of the different regimens (R) tested

<table>
<thead>
<tr>
<th>R1</th>
<th>Trypsin/EDTA 0.25%</th>
<th>NaOCl 5%</th>
<th>Trypsin/EDTA 0.25% sol</th>
<th>NaOCl 5%</th>
<th>NaCl 8%</th>
<th>Tooth internal pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>10 min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5 kPa</td>
</tr>
<tr>
<td>15 min</td>
<td>15 min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5 kPa</td>
</tr>
<tr>
<td>10 min</td>
<td>10 min</td>
<td>10 min</td>
<td>5 min</td>
<td>-</td>
<td>-</td>
<td>2.5 kPa</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>35 min</td>
<td>2.5 kPa</td>
</tr>
</tbody>
</table>
images were performed using ×1000 magnification. Contrast was manually adjusted to produce optimal conditions for the evaluation; 4 random image fields for each tooth were analysed.

The representative areas (A, pulp chamber; B, coronal; C, middle; and D, apical root thirds) were evaluated by two independent assessors, unaware of the regimens to which each of the samples belonged. The score was assigned as follows: 0- all dentin tubules opened; 1- more than 50% dentin tubules opened; 2- less than 50% dentin tubules opened; 3- obliterated dentin tubules. The data were subjected to non-parametric Wilcoxon rank-sum test.

RESULTS
The flux of irrigants proceeded regularly in all treated teeth without escaping from the apex and providing a turbulence which assures root canal cleaning. Indeed, SEM observations (Fig. 2) and score analysis (Tables 2 and 3) showed significant changes in root cleaning ability in relation to both length and timing of solution administration. Morphological analysis of the teeth treated using Regimens 1 and 2 (herein R1 and R2) demonstrated that the cleaning of dentin tubules decreased from the pulp chamber to the tooth apex, though in some cases pulp remnants were found (Fig.
Cleaning procedure using R1 and R2 performed better in teeth with one, two or fused roots than in those presenting multiple root canals. The best results were found in R3, with scheduled alternated cycles of Trypsin/EDTA and NaOCl inside the tooth. In fact, both pulp chamber and root canals did not present debris and dentin tubules were opened right through to the tooth apex (Fig. 2e–h). No differences were detected between teeth with one, two or fused roots and those with multiple root canals. The control group, using saline solution, revealed the presence of organic debris. It also yielded very poor results in relation to cleaning both in the pulp chamber and in the roots (Fig. 2i–k).

A statistical analysis of the different scores, summarised in Table 3, showed that:

- As expected all regimens revealed a significantly better result ($p<0.05$) than the saline solution (R4).
- No differences between R1 and R2 were observed.
- There was a significant difference between R1 and R3 ($p<0.05$) in all of the analysed regions, while the difference was significant between Regimens 1 and 4 only for regions A ($0.75 \pm 0.11$ vs $1.50 \pm 0.23; p<0.05$) and B ($1.75 \pm 0.26$ vs $2.50 \pm 0.83; p<0.05$).

### DISCUSSION

Cleaning and shaping of the root canal is the key stage in root canal treatment, since it eliminates all tissue debris from the root canal space while removing the inner layers of root canal dentin\(^{13}\). Mechanical instrumentation alone is ineffective at completely removing residual bacteria and necrotic debris\(^{14}\).

Various materials and techniques have been reported with wide variations in their efficacy regarding the removal of the intra canal smear layer\(^{3,5,15,16}\). Alternative methods facilitating clinical work with effective root canal cleaning include laser technology\(^{17,18}\) and passive ultrasonic irrigation (PUI)\(^2\). Treatment of the radicular dentinal walls with the laser has been shown to promote cleaner surfaces when compared with a combination of sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA), which might result in better adaptation of the filling material to the root canal walls\(^{19}\). PUI is based on a noncutting methodology. This procedure reduces the potential to create aberrant shapes within the root canal and allow efficient cleaning\(^2\). In 1993 Lussi et al.\(^{20}\) proposed a method which does not involve mechanical instrumentation, thus limiting smear layer formation. This proposed treatment with a new device resulted in similar cleanliness and obturation quality when compared with the control group. Total treatment time using the new non-instrumental technique, however, was

### Table 2 Score analysis

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
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<tbody>
<tr>
<td>R 1</td>
<td>0.75±0.11</td>
<td>1.75±0.26</td>
<td>2.25±0.52</td>
<td>2.75±0.50</td>
</tr>
<tr>
<td>R 2</td>
<td>0.67±0.52</td>
<td>1.50±0.55</td>
<td>1.83±0.58</td>
<td>2.50±0.55</td>
</tr>
<tr>
<td>R 3</td>
<td>0±0</td>
<td>0±0</td>
<td>0.72±0.70</td>
<td>1.36±0.91</td>
</tr>
<tr>
<td>R 4</td>
<td>1.50±0.23</td>
<td>2.50±0.83</td>
<td>2.50±0.73</td>
<td>3.00±0.00</td>
</tr>
</tbody>
</table>

A, pulp chamber; B, coronal root third; C, middle root third and D, apical root third; 0- all dentin tubules opened; 1- more than 50% dentin tubules opened; 2- less than 50% dentin tubules opened; 3- obliterated dentin tubules

### Table 3 Statistical analysis of the score

<table>
<thead>
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<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
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<tbody>
<tr>
<td>R 1</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>R 1 vs R2</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
</tr>
<tr>
<td>R 1 vs R3</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
</tr>
<tr>
<td>R 1 vs R4</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>R 2 vs R3</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
</tr>
<tr>
<td>R 2 vs R4</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>R 3 vs R4</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
<td>$p&lt;0.05$</td>
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A, pulp chamber; B, coronal root third; C, middle root third and D, apical root third; differences of the scores across different regions were analysed using the non-parametric Wilcoxon rank-sum test.
less than half of that using hand instrumentation\textsuperscript{21,22}. Our novel and non-invasive method is in fact a modified and improved version of Lussi’s method. This study has shown that it effectively cleans and irrigates root canals. Indeed, it has also shown to reach the apical part of the canals, while preserving the tooth structure and keeping the anatomy of the canal intact.

The key difference between Lussi and co-workers and this new modified version is the different hydrodynamic techniques used to complete the chemical reaction in the cavities. Lussi \textit{et al.} used pressure pulses aimed at penetrating the root canals. However, residual pulpal tissue was always discovered in the apical zone\textsuperscript{23}. With this new method, on the other hand, a continuous circulation of the same reagents was maintained, with little ripple of pressure, very similar to the one of the ambient pressure.

Indeed, the machine used by Lussi \textit{et al.} generates a relative pressure of about $+5$ kPa and a vacuum pressure of $+95$ kPa\textsuperscript{26}. This compares with a pressure of less than $\pm3$ kPa under the new method. Indeed, the higher the pressure, the higher the risk of extruded reagents beyond the apex. Nevertheless, it is also worth nothing that \textit{in vivo}, the open apex and the surrounding tissues do not guarantee the maintenance of a closed system ---a critical factor to achieve the hydrodynamic turbulence necessary to clean the root canals.

Three regimens differing in Trypsin/EDTA and NaOCl administration timing of supply were tested. Irrigating solutions during root canal treatment may in fact play a part in influencing the physical and mechanical properties of dentin potentially changing the mineral content of root dentin\textsuperscript{24-27}. Ari and Erdemir\textsuperscript{28} found that the Calcium and Phosphate levels in dentin decreased after treatment with all irrigating solutions. Goldberg and Spielberg\textsuperscript{29} advised that the optimal cleaning effect is only achieved after a 15 min application. The scanning electron micrographs showed perfectly opened dentinal tubules without debris after the alternating use of Trypsin/EDTA and NaOCl. Therefore this regimen seems to be a promising endodontic tool, since the EDTA action supplemented in the Trypsin solution may remove calcium and magnesium ions with its chelating effects. The proteolytic enzyme Trypsin associated with EDTA is normally used for the decellularization of the heart valves with excellent results\textsuperscript{29}. Moreover, it is also commonly used to digest dentin noncollagenous proteins\textsuperscript{30} and to “recover” the pulp cells detaching from the matrix\textsuperscript{31}. Thus, in the present study we suggest to add Trypsin to the commonly used irrigants, in order to remove all “living” tissue, even though it has never been used in dentistry. In this respect, this study demonstrated that the administration of the combination of Trypsin and EDTA associated with NaOCl efficiently removed the organic and inorganic components, mainly from the middle third. However, this treatment in some cases was not so effective in the apical third. In particular, not so encouraging results were obtained with first regimen (R1), since dentinal canaliculi were opened only in the pulp chamber and pulpal residue remained in the coronal root third after treatment. Results from the second regimen (R2) were better, although only marginal. It is noteworthy that the treated root canals exhibited some variations of anatomical and morphological characteristics\textsuperscript{32,33}. Indeed, molars with one, two or fused roots and regular shaped canals obviously resulted in better cleaning than molars with multiple roots and c-shaped canals, due to the straight and direct path maintained by the circulating reagents. After R1 and R2, the dentinal canaliculi were cleaned only in “single root canal” teeth, while in teeth presenting “multiple root canals” they were not tidied or, at most, they were opened only in one root.

This decline in the efficiency of irrigating solutions along the apical part of the canals\textsuperscript{33} in the case of R1 and R2 could possibly be explained by the fact that dentin in the apical third is much more sclerosed and the number of dentinal tubules present is less. Further, the use of an application time of less than 15 min\textsuperscript{28} for Trypsin/EDTA and NaOCl solutions respectively may potentially have such a pronounced action at the narrow apical portion as in the apical root third. Therefore, only when alternating cycles of Trypsin/EDTA and NaOCl (R3) were used, optimal results were obtained. In fact, this alternating of cycles was able to remove the smear layer in the middle and in the apical third of the root canal, also when teeth exhibited multiple canals.

CONCLUSION

The root canal cleaning system tested in this study was innovative because of its non-instrumental approach. Indeed, root canals are cleaned without using any manual or rotary instrumentation and the best results were achieved using an alternative irrigant solution consisting on Trypsin combined with EDTA (0.25%) and NaOCl (alternating the irrigation cycles). With this new method, the device simply uses irrigant solutions that penetrate into the canals and create turbulence, thus resulting in a good cleaning of the dentinal tubules, demonstrated by the scanning electron micrographs.

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