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Experimental Evaluation of a Novel Device to Quantify Canal Cleanliness: An In Vitro Study

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Abstract: Endodontic treatments are performed to avoid extractions and maintain the natural dentition. Root canal treatments are undertaken to eliminate or prevent an infection within the root canal system. Chemical and mechanical root canal debridement are the main methods used in endodontics to remove necrotic tissue, microorganisms, and microbial byproducts from the canal. However, to date there is no objective method to clinically determine the proper root canal disinfection level and thus proceed with the obturation. Clinicians just rely on their experience and habits or can trust in empirical methods such as the insertion of paper cones inside the canal and then check their appearance after the removal. Even in the in vitro and ex vivo scientific studies there is no objective method to analyze and compare the efficacy of different endodontic chemo-mechanical techniques and materials. The most frequently used method is to visually analyze some areas with a scanning electron microscope (SEM), even if the resulting images are hardly quantifiable and could greatly vary according to the analyzed area. A new device to clinically test the cleanliness of a root canal and display the result in an objective score was recently developed. The device analyzes the luminescence generated by an enzyme cycling method that process the adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) present in organic residues. The aim of the present in vitro study was to test the efficacy and reliability of this novel device (Endocator) in a controlled in vitro environment, before using it in clinical practice. The device sensitivity was tested on 5 single canal resin blocks. Three consecutive sampling were executed by one operator for each block to test the device repeatability. Results were recorded according to Endoscore (ES) and relative light unit (RLU) scales. Descriptive analysis and comparison between the 5 resin blocks and the 3 consecutive sampling were performed. Only the comparison between the first and third measurements both for ES (p = 0.00115999) and RLU (p = 0.00532749) resulted significant. Endocator was able to determine small variations of canal contamination in a controlled laboratory environment, showing high sensitivity and repeatability.

Keywords: root canal; cleaning; debridement; irrigating solution

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1. Introduction

Ensuring adequate canal cleanliness and debridement is essential for successful endodontic treatment, allowing infection control, promoting tissue healing, and increasing long-term preservation of the tooth by reducing the potential risks of reinfection [1]. Even if root canal obturation can help entombing bacteria and some filling materials exhibit short-term antibacterial properties, tissue remnants inside root canal can be a potential source of food for remaining bacteria or more likely, for bacteria re-infecting the endodontic

space [2]. Moreover, removing organic and inorganic debris allows for better adaptation of the filling material to the canal walls, which promotes a more effective seal, thus preventing reinfection of the obturated canals [3]. Overall, proper canal debridement directly impacts on both short-term and long-term clinical outcomes.

Clinicians are aware that the success of endodontic treatment heavily relies on the cleanliness and debridement of the root canal system, which is not easy due to complex canal anatomy and limitations related to the use of instruments mainly designed to shape round canals [4,5]. Therefore, they look for improvements in the shaping and cleaning procedures with new material and techniques, aiming at improving removal of pulp tissue, bacteria and infected debris [6–8].

However, despite its crucial clinical relevance, assessing canal cleanliness in vivo is not a simple, well-defined, and objective procedure [9]. It typically involves methods like visual inspection using magnification and the detection of debris or dirt on instruments or paper points, which are based mainly on individual judgement. Despite progress in digital imaging techniques, currently radiographs or cone-beam computed tomography (CBCT) cannot detect and evaluate debris and tissue inside canals and canal morphology. Microbiological sampling techniques to detect bacterial presence have also been proposed as an alternative means to decide when shaping and cleaning procedures in vivo are completed, suggesting when they can obturate properly disinfected canals. However, these microbial culturing techniques that quantify bacterial presence (and not residual debris) are not simple to use in a clinical environment, and are currently used mostly for in vitro studies [10–12].

Assessing canal cleanliness in vitro often involves techniques such as scanning electron microscopy (SEM) to visualize the surface of the canal walls for debris and biofilm, and chemical analyses to detect residual organic or inorganic materials [13]. These methods can provide valuable images of the cleanliness of root canal systems in laboratory settings, but there is a limitation related to the fact that it is not easy and/or reliable to evaluate and count debris, taking into consideration all the canal walls' surfaces. Using digital imaging analysis software may help to quantify debris removal more precisely and consistently, but still it is a procedure usually limited to small portions of the canal space. The same problem can also be a limit of histological studies, which usually show only small part of the canals [14].

More recently, techniques like fluorescence-based imaging or spectroscopy have been proposed as an aid in detecting bacteria and residual organic material [15]. These technologies can help assessing the cleanliness during endodontic procedures, and could be used both in vitro and in vivo.

The aim of the present study was to evaluate the in vitro sensitivity and precision of a new device to quantify the presence of organic debris inside an artificial root canal.

2. Materials and Methods

2.1. Specimen Selection

Five single canal endodontic transparent resin training blocks were selected. The artificial canals (SystemB blocks, Kerr, Glendora, CA, USA) were designed for evaluation of root canal filling techniques and consequently their dimensions were approximately .06 tapered with apical size 25. Such transparent blocks were also chosen to visually check the cleanliness and dirt of the specimens. A 60° curvature was present in the apical third with a radius of 5 mm, thus allowing proper insertion of needles and other irrigating devices (Figure 1). Moreover, the artificially prepared canals were selected to avoid variation in canal shaper and in debris production generated by mechanical instrumentation. Therefore, there was no need to instrument or prepare the artificial canals, and the only variables were related to artificial contaminations and subsequent debridement. The blocks were not instrumented to avoid the creation of debris, revealed from a preliminary analysis. Sample size was determined using Power Analysis and calculated based on preliminary data obtained after 4 initial measurements with a power of 80% and a 0.05 alpha type error

(G*Power, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). With an effect size of 1.48, sample calculation was 3, and consequently a total number of 5 artificial blocks was considered sufficient to provide significant data.

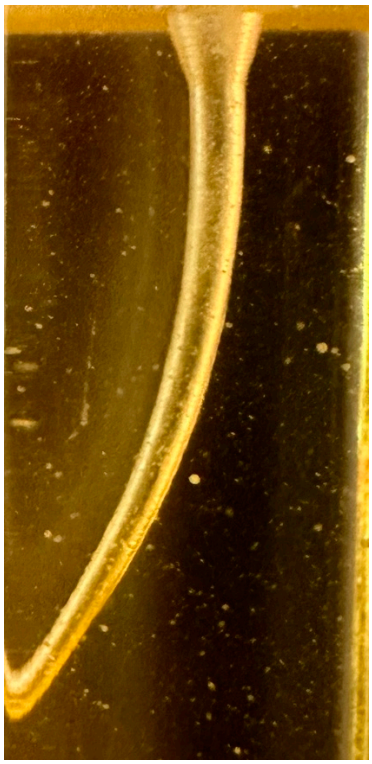

Figure 1. Artificial resin canal, as provided by the manufacturer.

2.2. Device

The analyzed procedure is based on the use of 1 device consisting of a dedicated swab (Endotester, Endocator Inc., Aptos, CA, USA) and a luminometer (Endocator, Endocator Inc., Aptos, CA, USA) (Figures 2 and 3). Endotester is containing the swab and the reagent for testing of a root canal (Figure 2). Endotester uses an enzyme cycling method based on a combination of luminescent reactions from firefly luciferase, pyruvate, orthophosphate dikinase (PPDK), and pyruvate kinase (PK). This method produces a given amount of luminescence that is proportional to the amounts of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) present. ATP is a source of energy necessary for various forms of life that are present in organic residues, such as microorganisms and biological substances that originate from other living organisms. This ATP monitoring system allows you to measure and detect organic residues at high speed and high sensitivity by detecting ATP using luciferase, which is why it is widely used in determining cleanliness levels. However, conventional ATP monitoring system is insufficient because ADP and AMP generated from ATP degradation are completely overlooked. A new ATP + ADP + AMP monitoring system is shown in Figure 4. This method enables highly sensitive analyses of a wider range of organic residues. This kit is a simple integrated testing instrument that contains both the test reagent and the swab device required for testing cleanliness levels (Figure 2). The luminescence is measured by the Endocator (Figure 3).

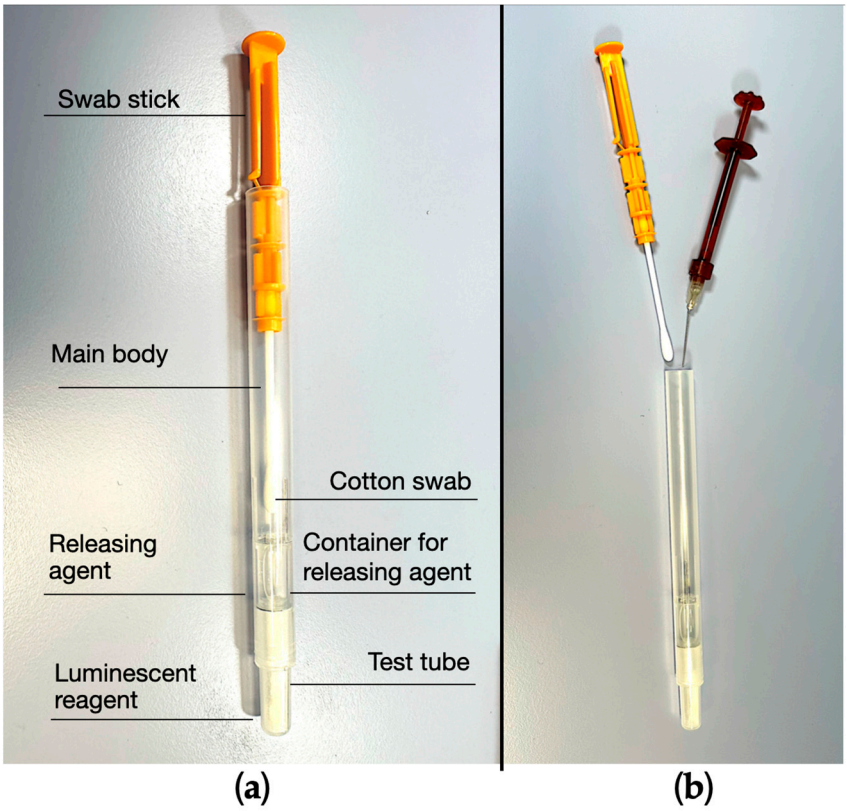

Figure 2. (**a**) Endotester specifics; (**b**) Endotester swab outside the main body and sampling syringe.

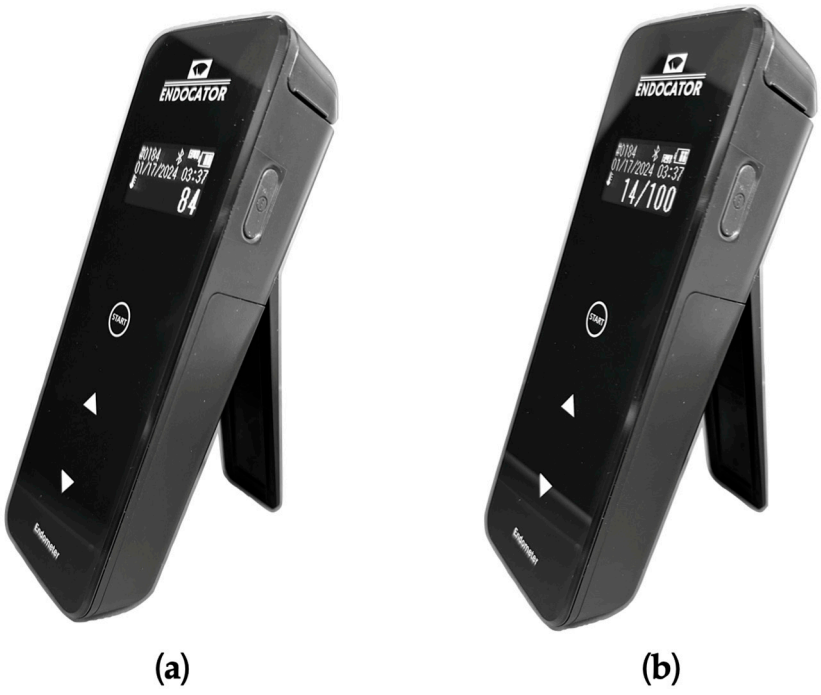

Figure 3. (**a**) Endocator with RLU display (RLU = 84); (**b**) Endocator with ES display (ES = 14/100). RLU: Relative light unit. ES: Endoscore.

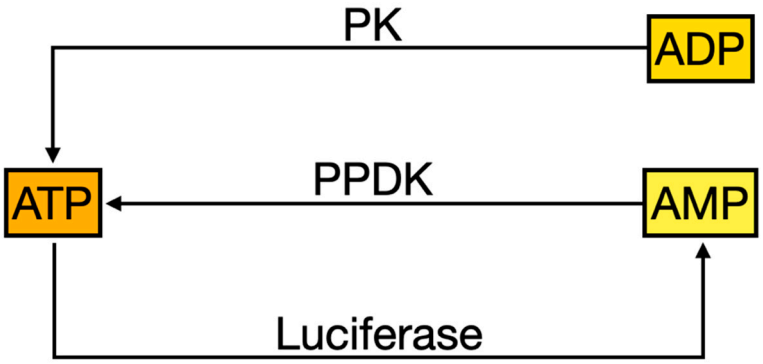

Figure 4. Principle of luminescence method using the enzyme cycling. PK: Pyruvate kinase. PPDK: Pyruvate orthophosphate dikinase. ATP: adenosine triphosphate. ADP: adenosine diphosphate. AMP: adenosine monophosphate.

2.3. Samples and Sampling Procedure

All the samples were collected from blocks that were not instrumented and/or cleaned because, from a preliminary analysis, we found that the blocks provided by the manufacturer inside their plastic packaging already contained some organic contamination, even if there was no visual sign of any debris or manufacturing remnants inside the canal (Figure 1).

To test the efficacy and reliability of this novel device in vitro, for each canal, 3 consecutive measurements were taken and compared. The null hypothesis was that there should be no significant difference amongst the 5 samples and there should be no significant difference between the 3 consecutive measurements on the same samples.

The sampling procedure strictly followed the instruction of use (IFU) provided by the manufacturers. An endodontic needle mounted on a syringe was inserted into the canal to rinse with 1 mL distilled water. A delicate up and down movement of the needle was performed to agitate the irrigating solution inside the artificial canal. Then, the needle tip was positioned in the apical third (3 mm from the apex), and the irrigating solution was collected and transferred to the Endotester. For each procedure, a single sterile needle and syringe were used to avoid any type of cross contamination.

The swab stick was then removed from the main body and 1–2 drops of the sample liquid were released by the needle inside the upper part of the tube main body (Figure 2b. The swab stick was reinserted in the tube and moved to ensure proper absorption of the sample liquid. Then, the swab stick was completely inserted inside the casing to mix the sample solution with the releasing reagent surfactant (Benzalkonium chloride) and the luminescent reagent (Luciferin, Luciferase, Magnesium acetate, Phosphoenolpyruvic acid, Pyrophosphoric acid, PPDK, PK). Correct mixing of the two components was ensured by shaking the Endotester casing for at least 10 s and allow visual assessment of dissolution of the luminescent reagent with the sample solution. Finally, the Endotester was inserted into the Endocator to measure the generated luminescence. The outcomes were displayed according to 2 different measuring scale, each of them chosen by the examiner (Figure 3): Endoscore (ES), and Relative Light Unit (RLU). The measurements were displayed after 10 s, and the overall procedure was completed in less than 1 min. All the measurements were performed by one trained operator to eliminate variables amongst examiners.

2.4. Outcomes

ES is a 0 to 100 analogic scale, where 0 corresponds to the absence of organic material and 100 to dirty canal. RLU is a continuous scale, the higher the score the higher is the amount of organic material collected, with values ranging from 0 to more than 600,000. No information about the correlation between the two scales was provided by the manufacturer. In the IFU, the following values were suggested only for the ES: 0–30 is clean, 31–60 is contaminated, and 61–100 is dirty.

2.5. Statistical Analysis

All the data were recorded using both scales (ES and RLU) and were than divided in 6 groups: A, B, and C representing the first, second, and third sampling for each block, according to ES, and D, E, and F representing the first, second, and third sampling for each block, according to RLU.

Descriptive analysis was performed to determine mean and standard deviation (SD) of the findings for the 6 groups. A paired T-test with Bonferroni correction was executed to find out significant differences ($p < 0.05$) between the 6 groups per score type. Statistical analysis was undertaken using SPSS (SPSS, v25.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results

Descriptive results are shown in Table 1. Mean and SD of ES/RLU for first, second, and third sampling resulted to be 31 ± 6.32/263.8 ± 88.23, 25.8 ± 8.87/210.8 ± 139.74, and 17.8 ± 4.81/110.8 ± 37.51 (A/D, B/E, and C/F).

Table 1. Descriptive analysis. A/D = first samples, B/C = second samples, D/E = third samples; ES = Endoscore; RLU = Relative Light Unit; SD = Standard Deviation.

Measurement Unit		**ES**			**RLU**		
Sample group		A	B	C	D	E	F
Resin block	1	38	41	26	366	457	176
	2	37	25	18	333	175	108
	3	30	24	16	270	168	96
	4	24	20	14	168	140	84
	5	26	19	15	182	114	90
Mean		31	25.8	17.8	263.8	210.8	110.8
SD		6.32	8.87	4.81	88.23	139.74	37.51

When comparing the first five measurements, no significant difference was noted amongst the five specimens. However, all specimens showed a debris contamination which demonstrated a high sensitivity of the device. Same non-significant differences were noted when comparing the second and third measurements. For each canal, the score comparison between the three consecutive samplings showed statistically significant difference only between the first and third measurements both for ES ($p = 0.00115999$) and RLU ($p = 0.00532749$).

4. Discussion

The cleaning ability of endodontic procedures is based on the evaluation of the percentage of debris, produced and/or left inside canals, and the percentage of tissue remnants in un-instrumented root canal walls in extracted teeth. In many studies using histological sections of human root canals (usually curved canals in maxillary and mandibular molars), it was noted that no system was able to perfectly clean the endodontic space, especially when complex anatomy was present [16,17]. These in vitro studies include histological preparation of specimens, obtainment of serial cross-sections (µm) and assessment of remaining pulp tissue using a morphometric approach, but are usually limited to a very small, specific portion of canals. Moreover, they are in vitro studies which can provide some general information but no real useful benefits for practitioners, because canal anatomy, especially in molars or in roots with more than one canal, can be very different and sometimes very complicated, with a relevant part of the canal space often untouched by the instruments and maybe not adequately washed out by irrigants [18,19]. Therefore, the quality of canal debridement cannot be properly determined in vivo, and the cleaning and shaping procedure are completed only by subjective decision of the clinician. The same problem

is related to studies which have investigated cleaning ability using the SEM evaluation, which is also a complex procedure which must be adopted only for extracted teeth [17]. It includes splitting the specimen into two halves and preparing the most visible section for SEM examination, with different parameters being evaluated: smear layer, pulpal debris, inorganic debris, surface profile. Without consideration the limitations inherent to only a partial visualization of the entire root canal system, as previously shown for histological studies, the SEM studies demonstrated that even with most sophisticated instrumentation and irrigation techniques, there might be some canal walls or inner endodontic spaces that cannot be reached, cleaned or instrumented by files and irrigants. Therefore, all these studies suggest that, currently, even the best techniques of root canal instrumentation are unable to completely remove pulpal debris from irregularities such as dentin grooves and depressions [17,20,21].

The present study was conducted in a controlled laboratory environment on prefabricated resin blocks, trying to reduce all possible variables related to cross contamination and sampling (no differences in canal anatomy and/or endodontic procedures, since all tests were performed in non-instrumented wide canal, which allowed proper needle insertion at the desired length). Measurements were performed by a skilled operator and a skilled assistant to minimize any procedural error.

The main limitation of the present study is that no data comparison is available in the scientific literature, as this is the first investigation of the present device and methodology. Furthermore, the choice of one operator could have hidden the operator effect, and the learning curve was not analyzed. As an in vitro study the reported outcomes should not be directly translated to clinical scenarios where other variables could possibly alter the methodology results. Furthermore, qualitative analysis of the organic remnants was not analyzed. Future studies combining a qualitative microbial analysis to the quantitative organic analysis are strongly recommended.

Results show that the Endocator device is rapid, simple to use, and provides precise measurements, detecting the organic components present or left inside an artificial canal. The device exhibits high sensitivity, because it can check minimal, non-visible, organic debris contamination, as shown in new canals that were not instrumented or contaminated by pulp or other organic tissues. The high sensitivity is demonstrated by the fact that a new, clean canal (not used or artificially contaminated) provides RLU values ranging from 168 to 366 (for the first measurements), which are very small values compared to the measuring scale, which allows a maximum RLU of 450,000. In the other modalities (ES), the highest values are defined as 100 Over, and values for the first measurements from the present study ranged from 24 to 38 for the first initial measurement.

Such differences promote the use of RLU in laboratory testing because if provides a wider, more accurate range of values, and consequently more precise comparisons. The suggested clinical ES values (0–30 clean, 31–60 contaminated, and 61–100 dirty) should be re-evaluated with further studies. In the present study, when using the proposed "clinical score", two samples showed contamination values after the first measurements, one of them also after the second measurement, and none of them after the third one. Such differences, however, are based on an empirical scale, which need to be validated, considering it is not supported by the data of the present study.

Results from the present study showed that, when comparing differences amongst the five canals, there was no significant difference amongst the data when analyzing the same measuring step (first, second, and third), showing the precision and reliability of the test ($p < 0.05$). Such results show that the Endocator could be a valid and predictable device for objective evaluation of canal cleanliness using an easy, not expensive, non-distractive methodology.

The precision of Endocator was also confirmed by differences between first, second, and third measurements performed on the same canal. In such cases, to make the measurements, some irrigating solution (distilled water) and some activation (using the needle with up and down motion) were added, as a consequence of the sampling technique. In all tested

canals there was a reduction of the canal contamination in the following measurements, and the device was able to detect it: in fact, a statistically significant difference was noted in all specimens between the first and third measurements (p = 0.001 and 0.005 for the ES and RLU values, respectively), even if no visible sign of contamination could be visually detected. These results also show the future importance of preliminary testing of artificial canals to be used in in vitro studies before assessing quality of canal debridement provided by different techniques and materials.

The differences shown in each sample using consecutive measurements could be also very useful in performing in vitro studies about cleaning when the different methodologies are implemented during use [22]. Traditional microscopic or histological studies can only show the results of a methodology, while the Endocator can evaluate the different steps inside a procedure [23–26]. For example, it may allow to quantify how a technique can be improved by adding more steps or increasing time or volumes of irrigants.

The high sensitivity of RLU values is an extremely positive factor in providing accurate measurements in vitro, and we expect similar results in vivo, which makes the device very useful for experimental research and clinical cases. In clinical cases, the device can display the amount of contamination left inside the canal, and then the dentist can choose whether the final cleaning procedure should be implemented or not. On the other hand, such a high sensitivity requires to be very careful in the sampling procedure to avoid any cross contamination related to needles, syringes, gloves, etc. Further studies will be necessary to improve or standardize the sampling procedure in terms of quantity of solution to be collected, depth of needle insertion, and possible influence of relevant amounts of blood, exudate, or chemicals in the sample liquid.

These in vitro results enforce the concept of developing a system which could help clinicians in making a crucial decision about whether to stop or continue the cleaning and shaping procedure based on an objective evaluation of canal cleanliness. Such an improvement could lead also to a better knowledge between the amount of canal cleanliness and outcome, because we have many cases where incomplete canal and shaping procedure resulted in a successful treatment over many years. To date, we have no certainty about the correlation of these parameters. We only know that we should ideally clean canals, but we are never able to achieve these results. Since many cases fail and other may not be due to incomplete debridement, a proper quantitative evaluation would be extremely useful for two main reasons: assess clinical relevance of different levels of incomplete canal cleanliness and provide a more case-related evaluation of quality of cleaning procedure, which may suggest or not further cleaning before obturation.

5. Conclusions

Within the limitations of the present study, it is possible to conclude that Endocator was able to determine small variations of canal contamination in a controlled laboratory environment, showing precise and reliable measurements. These findings suggest the possible use of the Endocator for in vitro comparative studies amongst different irrigation techniques and materials with an objective, non-distractive methodology. This device is a significant improvement towards a more evidenced-based decision process on when ending the shaping and cleaning procedure, because it provides some objective data about canal cleanliness in vivo. Further research is needed to correlate such values with positive outcomes, trying to provide clinicians with more precise data about the clinical relevance of medium-poor canal cleanliness. Therefore, the new tested device can now offer an initial first recommendation, and will probably help in determining adequate level of canal cleanliness which will be consider sufficient to promote healing of the endodontic treated teeth. Further studies are, however, necessary to determine its use in clinical studies or clinical practice because the technique is very sensitive and there is a potential risk that differences in the sampling technique may affect results. So, the proposed ES scale should be also validated.

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