In vitro evaluation of a new endodontic device for assessment of canal cleanliness

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Abstract

A new testing device (Endocator, Endocator Inc, Aptos, CA, USA) has been recently developed to assess presence of remaining organic debris inside canals , In the present study the reliability of the device was assessed in vitro by checking the risk of possible cross-contamination during the sampling procedure. Five new syringes were used for sampling procedure in five artificial canals which had been previously filled with organic and inorganic debris (contaminated canals). The recorded Endocator measurement were noted as Group 0. Other five new syringes were used for sampling procedures in artificial canals in which no instrumentation, debridement or filling with debris had been performed (untouched canals). The recorded Endocator measurement were noted as Group A (sterile syringes). In Group B (non-sterile syringes) the sampling procedure in the untouched canals was repeated by using the same 5 syringes previously used for sampling in the contaminated canals. In all cases procedure precisely followed the instruction of use (IFU) provided by the manufacturer. . Descriptive analysis was performed to determine mean and standard deviation (SD) of the findings for the 3 groups. Paired T-test with Bonferroni correction was executed to find out significant differences ($p<0.05$) between the 3 groups. Results showed that ,even if the canals were same and all well cleaned, Group B provided significantly higher mean values than Group A ($p<0.05$). In Group O canals (non-cleaned, contaminated canals) mean values were significantly higher than the other two groups ($p<0.05$) . Results showed a potential risk that even a minimal cross-contamination can significantly affect Endocator measurements due to the high sensitivity of the system. Therefore, authors suggested to use a new sterile syringe for each sampling procedure and proposed manufacturer should write this recommendation in the IFU (Instruction of Use) of the product.
Key words: canal cleanliness, testing device, endodontic

Introduction

In the last decades there has been a lot of progress in materials and techniques for the endodontic treatment, aiming at improving performance and clinical outcomes (1-4). The major innovation in the shaping and cleaning procedures, has been the widespread and consolidated clinical use of nickel-titanium (Niti) rotary instrumentation as the golden standard for canal preparation, and, more recently, the introduction of innovative proprietary heat treatments in the manufacturing process. Such innovations result in more flexible and resistant NiTi instruments (5-6), which allow to simplify canal shaping by making it easier and faster to perform. Moreover, they minimize iatrogenic errors and overall improve safety and efficiency of the shaping procedure also in complex canals (7-8).
Besides these improvements, innovations have been proposed also in the root canal cleaning procedures, mainly related to the development of new irrigating solutions and devices to activate irrigants inside canals (9-12). However, such improvements so far had a smaller impact on the quality of the procedure and on the treatment outcome. In recently published reviews there is no evidence about superiority of these new cleaning

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procedures and devices when compared to traditional syringe irrigation with sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) (13). Proper canal cleaning and disinfection is influenced by many factors including canal complexities, presence of biofilm, differences between composition of pulp remnants, iatrogenic errors which may lead to incomplete or unsatisfactory debridement and disinfection of the endodontic space (14-18). Even if some antibacterial effect can be provided by proper canal obturation by entombing bacteria (19-20), poor cleaning is likely the main factor which negatively affects both the short and long-term outcome of the treatment.

In clinical practice endodontists have to face a big challenge. While it is relatively easy to decide when to end shaping procedures (usually when working length is reached by a Niti rotary instrument with adequate tip size and taper), clinicians have little or no clue when to end the final cleaning procedure. Besides visualization under magnification, which may reveal only huge amount of left debris, and visualization of debris, blood or exudates on paper points used to dry the canal, clinicians have no devices to verify quality of cleaning (21). in the last decade new devices and techniques like fluorescence-based imaging or spectroscopy have been developed to provide clinical hints and help in detecting residual bacteria and/or debris after cleaning and shaping procedure are completed (22-24), but none of them so far has been successfully commercialized or gained popularity amongst practitioners. To become effective and widely used in clinical practice such devices should be simple, rapid to use, reliable and cost effective. A new testing device (Endocator, Endocator Inc, Aptos, CA, USA) has been recently developed to fulfill these requirements (fig.1) and assess presence of remaining organic debris inside canals (25). Ideally, the new device should provide a strong clinical recommendation through objective measurements, checking when root canal cleaning is satisfactory completed. It consists of a dedicated swab (Endotester) and a luminometer (Endocator) as shown by figs 1-2. Endotester is containing the swab and the reagent for testing the amount of debris inside a root canal (fig.2) , by using a methodology which utilizes an enzyme cycling method, based on a combination of luminescent reactions from firefly luciferase, pyruvate, orthophosphate dikinase (PPDK) and pyruvate kinase (PK). The luminescence is measured by Endocator and provides a quantitative analysis, or the above-mentioned reactions induced by organic and inorganic debris. This new device has been evaluated by the authors in previous in vitro research (25) with promising results, even if the procedure must be properly performed to avoid errors which could affect performance, including false positive or negative results. Therefore, in the present study the reliability of the device was assessed in vitro by checking the risk of possible cross-contamination during the sampling procedure.

Methodology

Ten transparent resin training blocks (fig.3) for root canal obturation (SystemB blocks, Kerr, Glendora, CA, USA) were selected and randomly divided into two groups of 5 each. The use of transparent blocks allowed to visually check the procedure, including proper insertion of the sampling needle and visual (macroscopic) check of presence of debris which could interfere with results. Each block consisted in one artificial curved canal with dimensions already designed (taper .06 and apical size 25) for root canal filling (Fig 3), thus reducing any bias related to production of additional debris or any artificial contamination generated by NiTi rotary instrumentation. Sample size was selected by using Power Analysis and

Figure 1.

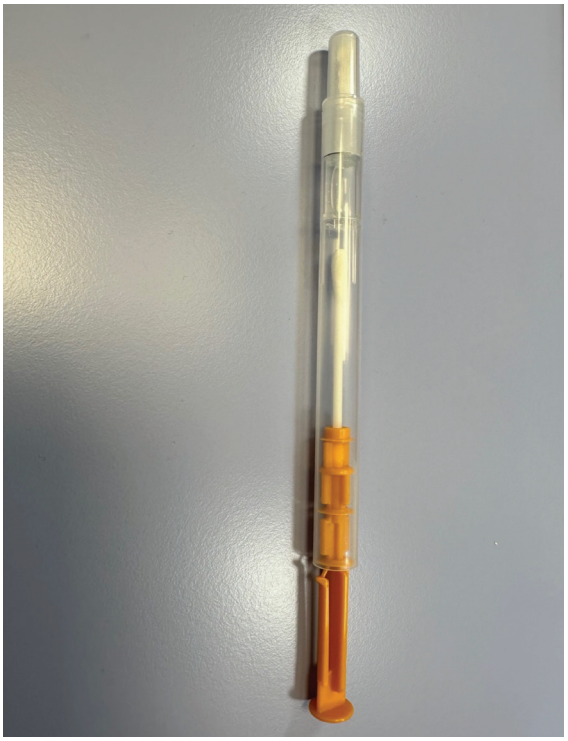

Figure 2.

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calculated from results obtained after 4 preliminary measurements, by choosing a power of 80% and a 0.05 alpha type error (G*Power, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Since sample calculation showed that 3 specimens could be sufficient to provide significant data (effect size = 1.48), a total number of 5 artificial transparent block was chosen for each group.

Five new syringes were used for sampling procedure in five transparent training blocks which had been previously filled with organic and inorganic debris (artificially contaminated canals). The recorded Endocator measurement were noted as Group 0. Other five new syringes were used for sampling procedures in artificial canals in which no instrumentation, debridement or filling with debris had been performed (untouched canals). The recorded Endocator measurement were noted as Group A (sterile syringes). In Group B (non-sterile syringes) the sampling procedure in the untouched canals was repeated by using the same 5 syringes previously used in the contaminated canals. In all cases the sampling procedure precisely followed the instruction of use (IFU) provided by the manufacturer. A sterile irrigation syringe with sterile 27-gauge needle containing 1 ml of distilled water was inserted into the artificial canal, injecting the solution and then agitating it with a slight up and down motion of the needle. Then, after inserting the needle 3-4 mm from the apex, the irrigating solution was collected and transferred to the Endotester.

After removing the swab stick from the main body of Endotester and the collected sample liquid (one/two drops) was released by the needle inside the upper part of the tube main body (fig 4). Injection was easy to perform but attention was paid since a too small amount or a too big amount of liquid may interfere with results, by diluting or concentrating the amount of debris. Then swab stick was inserted inside the casing, initially moved in the upper part to collect the liquid inside the tube and then inserted to the full length to mix the sample solution with the releasing surfactant reagent. A correct mixing of the two components was then provided by shaking the Endotester casing for at least 10 seconds and checked by visualizing the dissolution of the luminescent reagent within the sample solution. As the last step, the Endotester was inserted into the Endocator to measure the generated luminescence, and results where quickly displayed after 10 seconds. Overall, each sampling and measuring procedure was completed in less than 1 minute and was performed by one trained operator to eliminate intra-examiner variables. Three consecutive samples were taken for each block to assess consistency of results

The device allows to display results using to 2 different measuring scales, in which the higher the score the higher is the amount of organic material collected. RLU is a continuous scale, with values ranging from 0 to more than 600000, while ES shows values from 0 to 100. In the IFU, the following clinical correlation was provided only for ESE values : 0-30 is clean, 31-60 is contaminated, and 61-100 is dirty. On the contrary,no information about the correlation between the two scales was provided by the manufacturer. The Endoscore (ES) is suggested only to briefly simplify clinical evaluation of canal cleanliness by proposing a division in the three above mentioned categories , which are not being precisely or directly correlated to RLU results. RLU results are more precise and accurate, and consequently in the present study statistical analysis was performed only using them. Descriptive analysis was performed to determine mean and standard deviation (SD) of the findings for the 3 groups. Paired T-test with Bonferroni correction was executed to find out significant differences (p<0.05) between the 3 groups . Statistical analysis was undertaken using SPSS (SPSS, v25.0 for Windows, SPSS Inc Chicago, IL, USA

Figure 3.

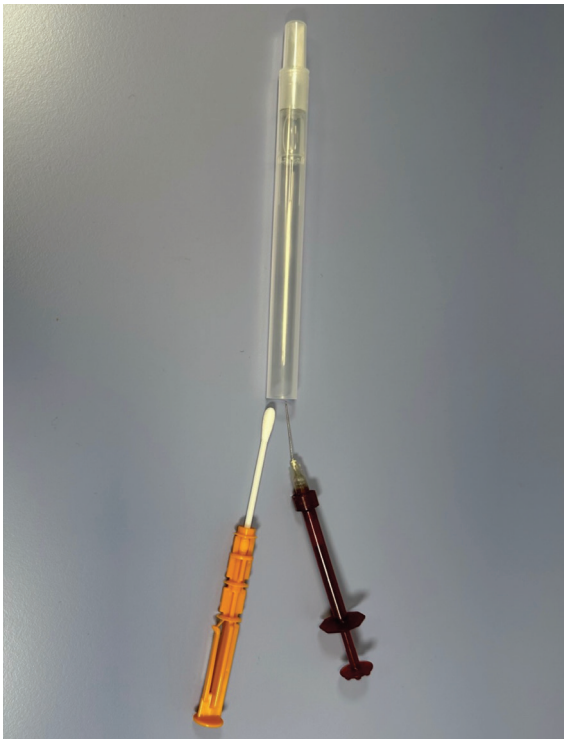

Figure 4.

Table 1. Descriptive analysis. ES (Endoscore). RLU (Relative Luminescence Unit). Group 0 (dirty blocks, clean syringe),

Measurement unit					
Group		**0**			
Sample		1st	2nd	3rd	
Resin block	1	100/192932	100/113568	100/88764	
	2	100/198885	100/102934	100/95411	
	3	100/679199	100/213908	100/110980	
	4	100/488916	100/113515	100/70267	
	5	100/310117	100/98766	100/90457	
Sample Mean		100/374009.8	100/128538.2	100/91175.8	
Sample SD		0/208497.0	0/48165.8	0/14608.7	
Overall group Mean		100/197907.9			
Overall group SD		0/173224.9			

Results

All Measurements, mean and standard deviation (SD) values for groups 0 (dirty blocks, clean syringe), A (clean blocks, clean syringe) and B (clean blocks, dirty syringe), consecutive samples (1st, 2nd, 3rd) and resin blocks (1, 2, 3, 4, 5) are reported in Table 1. A significant difference was noted between Group A (sterile syringes) and Group B (non-sterile syringes) . Even if the canals were same and well cleaned, Group B provided significantly higher mean values than Group A (p<0.05). Group O canals (non-cleaned, contaminated canals) mean values were significantly higher than the other two groups (p<0.05). Group 0 showed highest values 100/197907.9±0/173224.9, followed by Group B 84.3/32352.4±21.4/39095.1 showing that not only dirty blocks, but also contaminated syringes used on clean blocks could lead to ES/RLU values corresponding to dirty canal. On the other hand, Group A expressed the lowest values 24.9/201.1±8.5/123.5, showing that collecting a sample from a clean resin block with a clean syringe could lead to values corresponding to clean channel even if a small amount of organic residue was always detected. Considering the 3 consecutive samples, each group expressed a decreasing trend, which is related to the fact that each sampling procedure adds some new irrigant and provides some agitation of the solution.

Discussion

The new testing device is based on a method producing a given amount of luminescence, which is directly related to the amounts of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) present inside canals. In the past ATP monitoring technology has been used in determining cleanliness levels, allowing rapid and accurate measurements of organic residues by detecting ATP using luciferase, even if with some risks of bias (25). Therefore, Endocator technology uses an improved new ATP + ADP + AMP monitoring system which can analyze a greater range of organic residues with increased sensitivity and precision. However, such higher sensitivity may more easily produce variations and errors in the measurements due to inadvertent contamination or errors in the sampling technique, which is the most critical step in the procedure.

The monitoring technology is a simple, easy to perform step which allows objective measuremenst and is minimally influenced by operators' skills. On the contrary the sampling procedure is more subjective and can be influenced by many factors, including the risk of cross-contamination. For example the dentists could use a syringe to collect the liquid for evaluation of the initial presence of debris (first measurement after starting of the procedure) and then use the same syringe to collect the liquid for final evaluation of the cleaning results (second measurement). A similar situation could also happen when dentists check only final canal debridement, but results provided by Endocator are not good, thus suggesting further cleaning procedures. After performing such improved cleaning, dentists may check again canal cleanliness (second measurement) by using the same syringe used for the first measurement. All these clinical situation may lead to some contamination and reduce reliability of the system. It is intuitive that initially the syringe should be a sterile one, while

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A (clean blocks, clean syringe) and B (clean blocks, dirty syringe). SD (Standard Deviation).

	ES/RLU					
	A			B		
	1st	2nd	3rd	1st	2nd	3rd
	38/456	41/457	26/176	100/82652	97/16461	78/4707
	37/333	25/175	18/108	100/113421	100/24409	70/2798
	30/270	24/168	16/96	96/15131	64/1916	25/157
	24/168	20/140	14/84	100/98756	100/32277	73/3080
	26/182	19/114	15/90	100/72432	94/14733	68/2356
	31/281.8	25.8/210.8	17.8/110.8	99.2/76478.4	91/17959.2	62.8/2619.6
	6.3/118.3	8.8/139.7	4.8/37.5	1.8/37679.7	15.3/11364.8	21.5/1638.2
	24.9/201.1			84.3/32352.4		
	8.5/123.5			21.4/39095.1		

same concept may not be so intuitive when applied to a second measurement in the same clinical case. Moreover, no recommendation is provided by manufacturer about syringes. Hence, the purpose of this study was to assess the risk of cross-contamination with syringe, and to assess whether this risk could significantly affect measurements and promote the use of a new syringe for each sampling procedure, even if in the same tooth in the same clinical procedure

Results of the present study showed that the risk of cross contamination is significant. Clean, non-instrumented canals were properly assessed by the methodology showing only a minimal quantity of residual debris (probably derived from manufacturing) in a very precise and reliable way when a new sterile syringe was used. On the contrary second measurements of the same sample using a syringe who had been previously slightly contamined (being used for sampling in a canal full of debris) provided significantly different results and canals were evaluated as partially or poorly cleaned. This is obviously related to the high sensitivity of the Endocator, and to the presence of residual debris in the needle or in the syringe previously used. In fact in a previous study performed by authors to determine precision and reliability of measurements using Endocator, successive measurements on the same sampling provided better results than the first ones (25), because the sampling method provides more irrigant and some agitation which slightly improve debridement amongst consecutive measurements. The present study also confirmed these findings by performing three subsequent measurements,

Measurements are displayed by Endocator in two different ways: ES and RLU. ES is a 0 to 100 analogic scale, where 0 corresponds to a properly cleaned canal without any organic material and 100 to a non-cleaned canal. The ES scale is meant to be used in clinical practice with a simplified range of values as described in IFU: 0-30 clean, 31-60 contaminated, and 61-100 dirty. In the present study, even if number of specimens was low, variations in the measurements in a few cases due to inadvertent contamination were able to wrongly differentiate a cleaned canal into a contaminated canal, according to the proposed ES scale. RLU is a continuous scale, with wider values ranging from 0 to more than 600.000, which is probably more precise and useful for vitro or clinical studies. However, manufacturer provided no information about the correlation between RLU values and canal cleanliness in vivo.

Hence, we may conclude that there is a significant risk that even a minimal cross-contamination can significantly affect Endocator measurements due to the high sensitivity of the system. Therefore, authors recommend clinicians to use a new sterile syringe for each sampling procedure and suggest manufacturer to write this recommendation in the IFU (Instruction of Use) of the product.

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