

# Analysis of root canal organic tissue dissolution capacity according to the type of irrigation solution and agitation technique

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## Abstract

**Aim:** To analyze the root canal organic tissue dissolution capacity promoted by irrigating solutions, with or without the use of different agitation techniques. **Methods:** Bovine pulp tissue fragments were initially weighed. The following irrigating solutions were tested: 2.5% sodium hypochlorite, 2% chlorhexidine digluconate solution, and distilled water. The irrigating protocols were: immersion, mechanical agitation with endodontic files, and ultrasonic or sonic systems (Endoactivator® and Easy Clean®). At the end of the protocols, the pulps were weighed to determine their final weight. For comparison, the average percentage of tissue dissolution in relation to the groups was analyzed using the Kruskal-Wallis nonparametric test complemented by multiple comparisons test. The significance level was set at 5%. **Results:** Among the irrigation solutions, 2.5% sodium hypochlorite showed a higher dissolving power than 2% chlorhexidine digluconate and distilled water. Furthermore, ultrasonic and sonic systems were more effective irrigating protocols than immersion and mechanical agitation with endodontic files. **Conclusions:** The combination of sodium hypochlorite with an agitation system promotes a greater degree of tissue degradation.

**Keywords:** Endodontics. Dissolution. Sodium Hypochlorite. Chlorhexidine. Vibration.

## Introduction

Because of the anatomical complexity of the root canal system, approximately 50% of the root canal walls remain untouched by instruments during preparation, which results in insufficient cleaning<sup>1</sup>. Necrotic tissue debris may provide a nutrient source for the surviving bacteria<sup>2</sup>.

Sodium hypochlorite is widely used to irrigate the root canal during endodontic procedures; its properties are low surface tension, antimicrobial capacity<sup>3</sup>, and ability to dissolve organic tissue<sup>4</sup>. However, because of its harmful action on periapical tissues and its cytotoxicity<sup>5</sup>, other substances have been used as alternatives, such as chlorhexidine digluconate. Chlorhexidine has antimicrobial properties, power of substantivity, and is less harmful to vital tissues compared with sodium hypochlorite<sup>6</sup>.

Many studies have reported the significant influence of a variety of factors such as concentration, time, temperature, contact area of tissue irrigation, and mechanical action of the irrigation process on tissue dissolution<sup>7,8</sup>. Currently, there have several resources to assist in the agitation process of an irrigating solution in the root canal. Sonic and ultrasonic systems with different tips and inserts and with different protocols have been tested. Unlike ultrasonic systems, which function at a high frequency and with a range of inserts for different areas of dentistry and with very well-defined

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purposes, the sonic systems such as the Endoactivator® (Dentsply/ Maillefer, Ballaigues, Switzerland) and Easy Clean® (Easy Equipamento Odontológicos, Belo Horizonte, MG, Brazil) provide a specific mechanism of action and applicability for its use in the endodontic irrigation process. The tips of these two devices are flexible and have a small caliber in all extensions, which helps improve the action of the irrigation solution in areas of difficult access inside the root canal.

The aim of this study was to analyze the dissolution capacity of pulp tissue according to the type of the irrigating solution and the agitation technique employed in the chemical protocol of endodontic instrumentation.

## Material and methods

Overall, 150 fragments of bovine pulp tissue, obtained from extracted mandibular central incisors, with an initial mean weight of  $3.5 \pm 0.5$  mg, were selected and divided into 15 experimental groups (Table 1).

**Table 1** - Experimental groups.

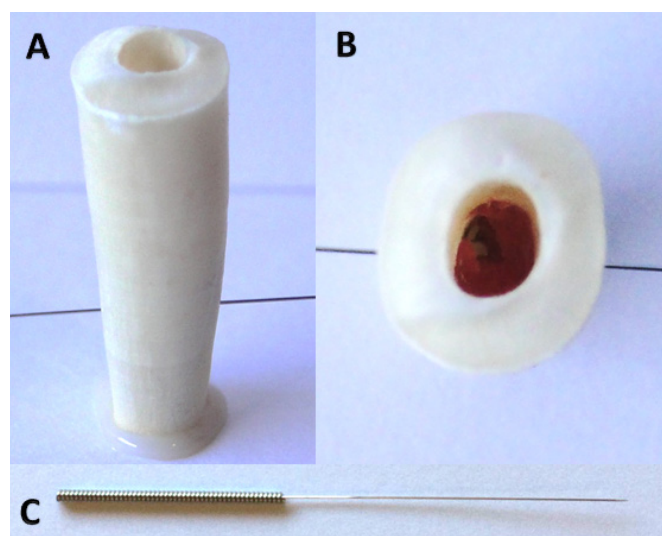
Experimental Group	n	Irrigation Solution	Agitation Protocol
G1	10	Distilled Water	Immersion
G2	10	Distilled Water	Mechanical Agitation
G3	10	Distilled Water	Sonic System (Easy Endo®)
G4	10	Distilled Water	Sonic System (Endoactivator®)
G5	10	Distilled Water	Ultrasonic System
G6	10	2.5% Sodium Hypochlorite	Immersion
G7	10	2.5% Sodium Hypochlorite	Mechanical Agitation
G8	10	2.5% Sodium Hypochlorite	Sonic System (Easy Endo®)
G9	10	2.5% Sodium Hypochlorite	Sonic System (Endoactivator®)
G10	10	2.5% Sodium Hypochlorite	Ultrasonic System
G11	10	2% Chlorhexidine Digluconate	Immersion
G12	10	2% Chlorhexidine Digluconate	Mechanical Agitation
G13	10	2% Chlorhexidine Digluconate	Sonic System (Easy Endo®)
G14	10	2% Chlorhexidine Digluconate	Sonic System (Endoactivator®)
G15	10	2% Chlorhexidine Digluconate	Ultrasonic System

The irrigating solutions used were 2.5% sodium hypochlorite (Bellafarma Farmácia de Manipulação, Caxias do Sul, RS, Brazil), 2% digluconate chlorhexidine (Bellafarma Farmácia de Manipulação, Caxias do Sul, RS, Brazil), and distilled water (Iodontosul - Industrial Odontológica do Sul Ltda., Porto Alegre, RS, Brazil) as control. The type of agitation protocols included mechanical with endodontic files, ultrasonic system, and sonic systems (Endoactivator® and Easy Clean®). The immersion of pulp tissue only in distilled water solution was used as the control group.

For the experiment, 10 bovine incisor roots were prepared to simulate the conditions of the pulp tissue within the root canal during the execution of the protocols, as reported in a recent

study<sup>9</sup>. Roots were standardized at 16 mm in length and the root canal was dilated to an equivalent of a number 4137 bur tip (KG Sorensen Indústria e Comércio Ltda., Barueri, SP, Brazil). The dental models were fixed on a metal tray with cyanoacrylate (Loctite, Hartford, CT, USA) to enable the solution agitation protocols.

In order to firm the pulp fragment in the apical third of the root canal, a surgical steel needle 0.20 mm in diameter was transfixed in the mesiodistal direction (Moxom Acupuncture, Budapest, Hungary) approximately 4 mm short of the apex (Figure 1). Initially, the pulp fragment was weighed on an electronic scale (Exacta Série Basic/ BL-1200AS, Curitiba, PR, Brazil). Afterwards, the bovine tissue was fixed within the canal and the canal was filled with 3 mL of irrigation solution.



**Fig.1.** Image of pulp tissue fragment fixed inside the root canal: A (coronal view of the tooth), B (axial view of the tooth), and C (surgical steel needle).

Before the solution agitation protocol inside the root canal, the endodontic instrument for the manual-mechanical agitation and the sonic and ultrasonic tips were calibrated on 8 mm from the root edge.

For the immersion (control) group, there was no agitation protocol. The pulp tissue fragment remained immersed in the solution (distilled water) for 2 min.

For the mechanical agitation with endodontic files, sonic (Endoactivator® and Easy Clean®), and ultrasonic groups, the proposed protocol was to intersperse three immersing cycles of the pulp fragment inside the root canal containing irrigating solution (20 s) followed by agitation (20 s), totaling 2 min, according to the protocol suggested by the systems manufacturers.

For mechanical agitation with endodontic files, an endodontic instrument type K file number 25 was used (Dentsply/ Maillefer). For the ultrasonic system, an insert GT40 was used (Gnatus Equipamentos Médico-Odontológicos, Ribeirão Preto, SP, Brazil) and activated with an ultrasonic vibration of 50% potency of the Jet Sonic device 30,000 Hz (Dabi Atlante®, São Paulo, SP, Brazil), without irrigation.

For the sonic systems Endoactivator® and Easy Clean®, their respective flexible tips with diameters of 0.25 and 0.04

taper were used. The Easy Clean® system was activated with the aid of a handpiece (KaVo in Brazil Industria e Comercio Ltda, Joinville, SP, Brazil) linked to a micromotor (KaVo in Brazil Industria e Comercio Ltda).

After completion of the agitation protocols associated with irrigating solutions, the pulp fragments were removed, dried with absorbent paper, and reweighed on a precision scale.

The T test was used to obtain the mean and standard deviation of the experimental groups. To compare the tissue dissolution capacity regarding the association of irrigating solutions and agitation protocols, the nonparametric Kruskal-Wallis test was used, complemented by a multiple comparisons test. The significance level used in the study was set at 5%.

## Results

Table 2 shows the comparison between the initial and final weight for pulp tissue dissolution according to the type of the irrigant and agitation protocol. Regardless of the irrigating solution, the ultrasonic system and the sonic systems Endoactivator® and Easy Clean® differed significantly from immersion and mechanical agitation with endodontic files. In addition, there was a significant difference for mechanical agitation with endodontic files combined with 2.5% sodium hypochlorite.

Table 3 shows the comparison of the agitation protocols with each of the irrigating solutions. There was no significant difference in the tissue dissolution capacity between the irrigants in the immersion ( $p = 0.418$ ) and mechanical agitation with endodontic files ( $p = 0.665$ ). However, the use of ultrasonic system ( $p = 0.041$ ) and Endoactivator® ( $p = 0.013$ ) and Easy Clean® systems ( $p = 0.040$ ) resulted in a statistically significant difference. The combination of these devices with 2.5% sodium hypochlorite promoted greater dissolution capacity compared with the other two tested irrigators.

When comparing the solutions in relation to the agitation protocols (Table 4), it was observed that regardless of the irrigant, the use of sonic and ultrasonic systems promoted a greater capacity of tissue dissolution.

## Discussion

Several studies<sup>10-12</sup> have tested the outcomes of tissue dissolution during the process of endodontic irrigation. However, it is known that the capacity and efficiency of organic tissue degradation processes are related to the type, concentration, and quantity of irrigating, and how the solution is agitated<sup>7,8</sup>. Thus, this study aimed to analyze the capacity for tissue dissolution according to the combination of different types of irrigating solutions and agitation methods.

**Table 2** - Comparison of irrigating solutions and agitation protocols as to the tissue dissolution capacity.

Agitation protocol	Irrigation Solution								
	Distilled Water			2% Chlorhexidine Digluconate			2.5% Sodium Hypochlorite		
	Initial Weight	Final Weight	p	Initial Weight	Final Weight	p	Initial Weight	Final Weight	p
Immersion	0.033±0.015	0.033±0.015	1.000	0.029±0.011	0.029±0.011	1.000	0.039±0.017	0.036±0.016	0.081
Mechanical Agitation	0.040±0.009	0.037±0.011	0.081	0.029±0.011	0.026±0.009	0.081	0.036±0.014	0.031±0.011	0.015
Ultrasonic System	0.040±0.014	0.035±0.014	0.015	0.032±0.010	0.027±0.011	0.015	0.032±0.009	0.018±0.009	0.001
Endoactivator® System	0.040±0.010	0.030±0.009	0.008	0.039±0.011	0.029±0.011	0.008	0.039±0.015	0.012±0.004	0.001
Easy Clean® System	0.032±0.009	0.027±0.009	0.015	0.031±0.007	0.026±0.007	0.015	0.037±0.017	0.023±0.010	0.001

**Table 3** - Average and medium ranks of the differences between the values of initial and final weight of the pulp fragments after the irrigation protocols.

Agitation protocol	Irrigation Solution			p	
	Distilled Water	2% Chlorhexidine Digluconate	2.5% Sodium Hypochlorite		
Immersion	Average (P25;P75)	0.000 (0.000; 0.000)	0.000 (0.000; 0.000)	0.000 (0.000; 0.010)	0.418
	Rank Medium	14.05 <sup>A</sup>	14.10 <sup>A</sup>	18.50 <sup>A</sup>	
Mechanical Agitation	Average (P25;P75)	0.000 (0.000; 0.010)	0.000 (0.000; 0.010)	0.005 (0.000; 0.010)	0.665
	Rank Medium	14.45 <sup>A</sup>	14.50 <sup>A</sup>	17.50 <sup>A</sup>	
Ultrasonic System	Average (P25;P75)	0.005 (0.000; 0.010)	0.005 (0.000; 0.010)	0.015 (0.007; 0.020)	0.041
	Rank Medium	14.75 <sup>B</sup>	14.50 <sup>B</sup>	21.00 <sup>A</sup>	
Endoactivator® System	Average (P25;P75)	0.010 (0.000; 0.012)	0.010 (0.000; 0.012)	0.020 (0.010; 0.042)	0.013
	Rank Medium	12.30 <sup>B</sup>	12.40 <sup>B</sup>	21.90 <sup>A</sup>	
Easy Clean® System	Average (P25;P75)	0.005 (0.000; 0.011)	0.005 (0.000; 0.011)	0.015 (0.007; 0.020)	0.040
	Rank Medium	13.50 <sup>B</sup>	13.20 <sup>B</sup>	21.10 <sup>A</sup>	

Average followed by different capital letters in rows differ significantly by the nonparametric Kruskal-Wallis test, at a significance level of 5%.

**Table 4** - Comparative analysis of the power to dissolve tissue of each irrigating solution for the different agitation protocols.

Stirring Agitation	Irrigation Solution		
	Distilled Water	2% Chlorhexidine Digluconate	2.5% Sodium Hypochlorite
Immersion	15.4 <sup>B</sup>	15.5 <sup>B</sup>	13.5 <sup>C</sup>
Mechanical Agitation	22.7 <sup>BC</sup>	22.8 <sup>BC</sup>	16.8 <sup>BC</sup>
Ultrasonic System	27.3 <sup>AB</sup>	27.5 <sup>AB</sup>	29.4 <sup>AB</sup>
Endoactivator® System	34.3 <sup>AC</sup>	34.6 <sup>AC</sup>	38.6 <sup>A</sup>
Easy Clean® System	27.5 <sup>AB</sup>	27.8 <sup>AB</sup>	30.2 <sup>AB</sup>
p	0.013	0.015	< 0.001

Average followed by different capital letters in columns differ significantly by the nonparametric Kruskal-Wallis test, at a significance level of 5%.

Bovine pulp tissue fragments were used according to methods reported in recent studies<sup>13,14</sup>. The ease of obtaining and standardizing the length and weight of the pulp bovine tissue is an advantage because variation in these measures may influence the dissolution ability<sup>15</sup>. Bovine pulp tissue has been shown to be similar to the human pulp tissue<sup>2</sup>.

The delineation of an approximate initial weight of 0.03 g bovine tissue fragment is in agreement with the mass of the pulp tissue in a human molar tooth ( $0.04 \text{ mL} \times 1 \text{ g/mL} = 0.04 \text{ g}$ )<sup>16</sup>.

In order to approximate this experiment to a more credible clinical condition, the dissolution capacity of the pulp tissue was measured within a tooth structure previously prepared for use, as previously reported in studies by Slutzky-Goldberg et al.<sup>17</sup> (2013) and Arslan et al.<sup>18</sup> (2015). Other studies<sup>13,14</sup> used the same methodology, but immersed on a culture plate.

There are a variety of experimental methods for measuring the dissolution capacity, such as measuring the time of dissolution<sup>19</sup> and visual examination<sup>12</sup>. In this study, the initial and final weights of the sample were compared, as reported in other studies<sup>4,14</sup>.

The definition and standardization of an experimental time of 2 min for each sample in all groups followed the manufacturers' recommendations for use of the Endoactivator® and Easy Clean® systems.

Among the tested solutions, 2.5% sodium hypochlorite showed a higher tissue dissolution capacity than 2% chlorhexidine digluconate and distilled water, as reported elsewhere<sup>20</sup> (1981). One of the known properties of sodium hypochlorite is its capacity of degradation organic tissue<sup>21</sup>.

On the other hand, chlorhexidine digluconate and distilled water showed no significant difference in their ability to dissolve tissue, and none of them was effective in the degradation of pulp tissue, which has been observed previously<sup>4,22</sup>. According to Marley et al.<sup>23</sup> (2001), chlorhexidine digluconate has no solvent action on organic tissues, and neither does distilled water.

Regarding the agitation protocols, the combined use of sonic (Endoactivator® and Easy Clean®) and ultrasonic systems with irrigating solution has been shown to increase the power to dissolve tissue, as seen in other studies<sup>24-26</sup>. In the present study, the power of tissue dissolution with the combination of these systems with chlorhexidine digluconate and distilled water was more efficient compared with the use of the immersion and mechanical agitation with endodontic files methods. It is believed that this result was

possibly due to the direct action afforded by the vibration equipment: Ultrasound (28.000 Hz), Endoactivator® (190 Hz), and Easy Clean® (approximately 4000 Hz). Although at different frequencies, the similarity in results between the sonic and ultrasonic devices may have been provided by the small diameter of the tips used for operating the sonic devices. Both the Endoactivator® and Easy Clean® systems have tips with a diameter of 0.25 and 0.04 taper. However, the ultrasonic insert used in this study has a pointed shape with initial diameter of 0.40, which is therefore greater.

The fact that none of the protocols tested was fully effective in pulp tissue degradation can be associated with and justified by the agitation time used in the study. In a way, this condition may be of concern if we take into account faster preparations. However, it is believed that the process of agitation associated with the appropriate irrigating solution and not just the action of irrigating leverage the power of tissue dissolution. The repetition of several cycles of agitating the irrigating solution during chemomechanical preparation can promote the complete dissolution of the pulp tissue.

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