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An In-Vitro Comparative Evaluation of the Effect of Passive Sonic and Ultrasonic Agitation on the Dissolution of Pulp Tissue in Simulated Grooves in Root Canals Using Sodium Hypochlorite With or Without Surfactant.

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ABSTRACT:**Purpose**
Purpose of this study was to evaluate the effect of passive sonic and ultrasonic agitation on pulp tissue dissolution from simulated grooves in root canals using sodium hypochlorite with or without surfactant.

Material and methods
The root canals of 75 extracted human premolars were chemomechanically prepared and then the teeth split. A standardized groove was prepared in the root halves. Human dental pulp tissue samples were collected, adapted in order to fit into the grooves and weighed. The reassembled samples were divided into five groups based on the irrigation technique - Distilled water group, plain NaOCl (sodium hypochlorite) and passive sonic agitation, NaOCl with surfactant (cetrimide) and passive sonic agitation, plain NaOCl and passive ultrasonic agitation and NaOCl with surfactant and passive ultrasonic agitation. After irrigation the samples were again weighed and difference in the weight calculated.

Results
There was a statistically significant difference ($p<0.05$) seen for the values of distilled water group when compared to each of the test groups and the values of difference in weight did not differ significantly among the test groups. The highest mean percentage weight change value (77.18 %) was observed with ultrasonic agitation with added surfactant.

Conclusion
Combined use of surfactant and ultrasonic agitation enhanced the tissue dissolution ability of sodium hypochlorite.

KEYWORDS: Agitation, Grooves, Sonic, Surfactant, Ultrasonic

I. INTRODUCTION

One of the many challenges faced during endodontic therapy is eradication of infected pulp tissue from the complex configurations and intricate shapes of root canal system. (1) Geometrically symmetrical shaping instruments, cannot reach the intricacies of the root canal such as fins, isthmuses, lateral and accessory canals, multiple foramina, cul-de-sacs and deltas. Thus, pulp tissue removal from these areas completely relies on the action of irrigating solutions. Two critical factors that should be considered during the process of irrigation are effective delivery of the irrigant to the whole extent of the root canal system, particularly to the apical third, and the capacity of the irrigant to debride canal intricacies.(2) Previous studies reported in literature have compared the effect of positive pressure irrigation on organic tissue dissolution with either sonic or ultrasonic agitation using NaOCl (sodium hypochlorite) with or without surfactant.(3.4.5) Also, the previous studies have used palatal mucosa to study the effect on tissue dissolution.(4) Fewer studies have assessed the combined effect of sonic, ultrasonic agitation and use of surfactants the direct solubilizing effect of irrigants on pulp tissue obtained from human teeth. Considering the potential synergism of agitation and surface tension reduction on irrigant penetration and dissolution of human pulp tissue, this in vitro study was designed.

II. MATERIAL AND METHODS

Teeth selection and preparation –

Recently extracted 75 single rooted premolars with sound crowns and roots were assessed to exclude teeth with open apices, severe curvatures, resorptive defects, fracture lines and root caries.

All included teeth were stored in 10% formalin until further required. Specimen lengths were standardized to 16 mm by decoronating the teeth using a diamond disc (MDT Micro Diamond Technologies Ltd., Israel) at slow speed. Working length determination was accomplished by insertion of a #10 stainless steel K type hand file (Mani Inc., Tochgi, Japan) until it was just visible at the apical foramen and then teeth were mounted in alginate impression material. Actual working length was obtained by subtracting 0.5 mm from this length. Shaping and cleaning was performed for all the samples using a 'Step Back technique' using K-type hand files (Mani Inc., Tochgi, Japan) and during preparation, each canal was irrigated passively with freshly prepared 2.5% NaOCl using a 27-gauge needle (Ramsons Tools, Faridabad, India). Apical preparation was performed till size 30 K file. Specimens were removed from matrix and a longitudinal groove was created on the proximal aspects using a cutting disc. Roots were cleaved in two fragments using a scalpel blade (**Fig 1a**). Two lines perpendicular to the canal and long axis of the tooth at 2 mm and 6 mm from the apex were marked to standardize groove length (**Fig 1b**). 2 mm diameter round diamond point - No. 6 (Mani Inc., Tochgi, Japan) mounted in micromotor handpiece was used to create 2 mm wide, 2 mm deep, 4 mm long groove in all the specimens(**Fig 1c**).

Collection of pulp tissue samples-

Freshly extracted sound teeth extracted for orthodontic or disimpaction purpose, were selected and placed in normal saline. A groove was prepared on the proximal aspects using a diamond disc without entering the pulp chamber or the root canals and the teeth were carefully split using a surgical blade. Pulp tissue was carefully removed using an excavator (**Fig 1d**), placed in normal saline and was refrigerated at four degree Celsius temperature as soon as possible to assist in sectioning. Thereafter, the pulp tissue was blotted and were sectioned with a surgical blade into fragments that would approximately adapt to the size of the prepared groove.(Estevez et al., 2017)

Weighing procedure 1-

The pulp tissue samples were inserted in the groove created in root canals of both the halves of each sample (**Fig 1e**). Both the halves of each tooth sample were weighed together using an analytical balance (S.R. Lab Instruments Pvt. Ltd., Mumbai, India) and the readings were recorded.

Reapproximation of teeth samples-

The halves of the teeth samples were reapproximated carefully using an adhesive tape (Swadesh Enterprises, Delhi, India) and embedded in alginate impression material (**Fig 1f**). The reapproximated samples were then randomly divided into five groups and subjected to irrigation procedure as follows-

Irrigation procedure-

GROUP 1: The control group -7 ml distilled water was used to irrigate the canals for 120 secs (n=15)

GROUP 2: Irrigation using 3 ml NaOCl- Plain delivery for 45 secs, passive sonic activation using Endoactivator (DentsplyMallifer, Ballaigues, Switzerland) for 15 secs, 1 ml 17% EDTA for 30 secs and 3 ml NaOCl for 30 secs (n=15)

GROUP 3: Irrigation using 3 ml NaOCl- with surfactant delivery for 45 secs, passive sonic activation using Endoactivator (DentsplyMallifer, Ballaigues, Switzerland) for 15 secs, 1 ml 17% EDTA for 30 secs and 3 ml NaOCl for 30 secs. (n=15)

GROUP 4: Irrigation using 3 ml NaOCl- Plain delivery for 45 secs, passive ultrasonic activation using Acteon ultrasonic tips for 15 secs, 1 ml 17% EDTA for 30 secs and 3 ml NaOCl for 30 secs. (n=15)

GROUP 5: Irrigation using 3 ml NaOCl- with surfactant delivery for 45 secs, passive ultrasonic activation using Acteon ultrasonic tips for 15 secs, 1 ml 17% EDTA for 30 secs and 3 ml NaOCl for 30 secs. (n=15)

Solutions were delivered with the Luer- Lok syringe (Becton Dickinson, India, Pvt. Ltd.) and a side vented needle (Ramsons Tools, Faridabad, India) in all irrigation assays.

Finally, canals were dried using paper points (DiaDent, British Columbia, Canada) and the sections were dissembled.

Weighing procedure 2-

The dissembled halves of each tooth sample were weighed using an analytical balance and the readings were recorded. The results obtained for all the groups were correlated and statistically evaluated to draw a reliable conclusion. Data obtained was compiled and then subjected to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM).

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III. RESULTS

Table1shows pairwise comparison in difference in weight using Tukey's Post Hoc Tests. There was a statistically significant difference **(**p<0.05**)** seen for the values of control group i.e. Group 1 when compared to each of the test groups- Group 2, Group 3, Group 4 and Group 5. However, the values of difference in weight did not differ significantly among the test Groups 2, 3, 4 and 5.

Table 2 shows mean percentage weight change among the five groups. The highest mean percentage weight change value (77.18 %) was observed for Group 5- ultrasonic activation with added surfactant, followed by Group 3- sonic activation with added surfactant (73.63%), Group 2- sonic activation without surfactant (71.24%) and Group 4- ultrasonic activation without surfactant (68.40%).

TABLE 1 Pairwise comparison using Tukey's post hoc test

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p value
1	2	.0021467*	.0003673	.000**
1	3	.0022467*	.0003673	.000**
1	4	.0022333*	.0003673	.000**
1	5	.0022000*	.0003673	.000**
2	3	.0001000	.0003673	.999#
2	4	.0000867	.0003673	.999#
2	5	.0000533	.0003673	1.000#
3	4	.0000133	.0003673	1.000#
3	5	.0000467	.0003673	1.000#
4	5	.0000333	.0003673	1.000#

TABLE 2 Mean difference percentage weight change

Groups	N	Mean	%Weight change	Std. Deviation	Std. Error
1	15	.184559	18.45	.4961664	.1281096
2	15	.712457	71.24	.1697371	.0438259
3	15	.736361	73.64	.2060182	.0531937
4	15	.684087	68.40	.1117192	.0288458
5	15	.771861	77.19	.3171406	.0818853
Total	75	.617865	61.79	.3603596	.0416107

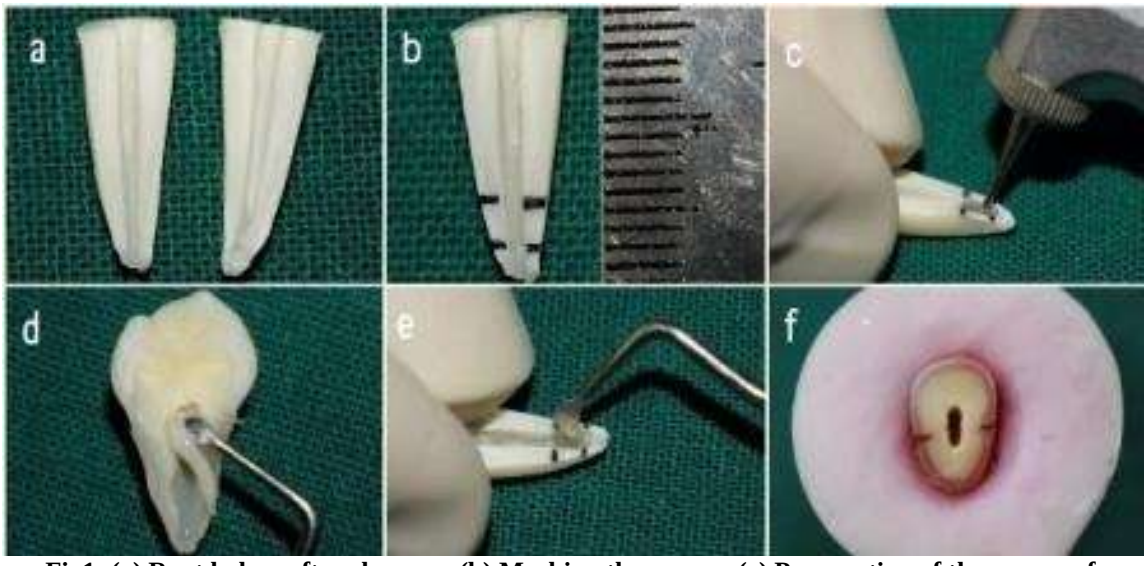

Fig1: (a) Root halves after cleavage, (b) Marking the groove, (c) Preparation of the groove of standardized dimensions, (d) Retrieval of pulp tissue from recently extracted teeth, (e) Placement of pulp tissue inside the groove, (f) Reassembled sample

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IV. DISCUSSION

A large portion of available endodontic literature focuses on different types of endodontic instruments used for cleaning and shaping. However, it has been clearly stated that instruments only shape the root canals(5,6) Whereas, it is the irrigant which actually cleans them. Multiple studies on effect of sonic and ultrasonic activation on organic tissue dissolution are available in the literature.(7,8,9)Fewer studies have assessed the combined effect of sonic, ultrasonic agitation and use of surfactants the direct solubilizing effect of irrigants on pulp tissue obtained from human teeth.

Considering the difficulties such as lack of standardization due to anatomical variations and complexities of the root canal system in in vivo and ex vivo study designs, this in vitro study was planned.

In the present study, the values of difference in weight did not differ significantly among the groups 2, 3, 4 and 5. Similar results were observed in study conducted by **Plotino et al.**(9)In their study, efficacy of different sonic and ultrasonic devices in elimination of debris from canal irregularities in artificial root canals was assessed. However, this study used Eddy system having the frequency of 10000 Hz for 20 sec , while the present study used Endoactivator with the frequency of 6000 Hz for 15 sec.

Gadaalay et al(8)assessed the effectiveness of different irrigation activation devices including Endoactivtor, passive ultrasonic activation and manual dynamic agitation in removing debris from the isthmus area of mandibular molars was assessed. No significant differences were found between Endoactivator and passive ultrasonic agitation groups.

The reason for these observations, can be attributed to the fact that ultrasonic activation acts at a higher frequency and creates acoustic microstreaming producing sufficient stresses to dislodge debris from instrumented canals.(10)These characteristics of ultrasonic devices are suggestive of a better agitation efficacy compared to sonic devices.

However, an ultrasonic device also creates an unwanted dampening effect especially when it touches the lateral walls of root canal.(10)

The results of a study conducted by **Nunes et al** (7)are contrasting with the results of the present study. In this study, the tissue dissolution capacity of NaOCl and chlorhexidinedigluconate agitated using sonic and ultrasonic devices was compared. The tissue dissolution capacity of NaOCl with ultrasonic agitation was significantly better than the sonic agitation. However, this study used a lower concentration (2.5 %) of NaOCl agitated for 20 sec.

The possibility of reducing the surface tension by addition of surfactants to improve penetration of NaOCl solutions in root canal complexities has been given little attention in the literature until recently. This has probably been due to the difficulty in identifying a suitable surfactant, because NaOCl destroys most organic materials.(Clarkson and Moule, 1998)In this study, 0.2 % cetrimide was chosen as the surfactant added in NaOCl. The concentration chosen was based on previous studies.(11,12) It has been suggested that it can dissolve the tissue in canal complexities when it comes in contact with the tissue. In the present study on intergroup comparison, the tissue dissolving capacity of sodium hypochlorite for Group 5 was found to be the highest.

This finding is in agreement with the studies conducted by **Cameron**(13)which assessed human pulp tissue dissolution ability of a modified household bleach with added surfactant (Fluorad 99). When looking at complete tissue dissolution, no difference was found between the solutions. Even though the method of assessment of pulp tissue dissolution (weighing method) was similar, the chemical nature of the surfactant used was different.

The results of the present study are also in agreement with a study conducted by **Clarkson et al**.(14) The four NaOCl solutions tested were Hypochlor 1% and Hypochlor 4% forte, which contained surfactant, and two identical solutions without surfactant. Twenty pulp specimens were immersed in each of the four NaOCl solutions and the time to dissolution of each sample was determined by stopwatch.

It was observed that reduced surface tension resulted in greater soft tissue dissolution by NaOCl. The reason behind this finding could be the fact that diffusion of the active chlorine to target areas rather than wettability seemed to be deciding factor in the ability to dissolve pulp tissue.(15)

A study conducted by **Stojicic et al**(16)showed contrasting results. Three NaOCl solutions with concentrations of 1%, 2%, 4%, and 5.8% were tested at various temperatures with and without agitation by ultrasonic and sonic energy. However, some methodological differences were observed such as use of porcine muscle tissue to assess the tissue dissolution and quantity of surface- active agents. Use of warm irrigant, use of test tubes for containing the solutions may be the reasons for disagreements in the results.

Along with use of surfactants, active chlorine content of the NaOCl irrigant is considered as the deciding factor for its tissue dissolution ability.(17) In the present study, active chlorine content was not determined but the same concentration of NaOCl was used for all the irrigation procedures. This has to be considered as a limitation of this study as it could be a potential source of bias. Also, pH of the solution was not considered even though it has been shown to have an effect on the dissolution capacity.(16)

V. CONCLUSION

Thus, within the limitations of the present study, following conclusions were drawn-

1. Intracanal agitation of NaOCl increased its tissue dissolution ability.
2. Out of the two agitation techniques, ultrasonic agitation was found to be more effective than sonic agitation.
3. Surfactant (cetrimide) added to NaOClenhanced tissue dissolution ability of NaOCl.
4. Ultrasonic vibration along with surfactant has the highest tissue dissolution ability.

Considering the limitations of this study, further basic research is necessary to understand the effect of lowering the surface tension and sonic and ultrasonic agitation of NaOClwhen used during root canal shaping or as the final rinse protocol. Subsequently, its potential translation into patient-based outcomes needs to be evaluated.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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