

A Comparative Evaluation of Tissue Dissolution Capacity of Sodium Hypochlorite Stored in Dark and White Coloured Bottles: An In Vitro Study

Meghana Velagaleti¹ R.V.S.C. Raju² G. Deepthi Sarvani³ CH. Nanda Sai Krishna⁴ M. Vishnu Vardhan⁵ H.V.S. Sasi Kiran⁶

¹PG Student, Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Moinabad, Telangana, India,

²Professor and Head, Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Moinabad, Telangana, India.

³Associate Professor, Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Moinabad, Telangana, India.

⁴PG Student, Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Moinabad, Telangana, India,

⁵PG Student, Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Moinabad, Telangana, India.

⁶PG Student, Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Moinabad, Telangana, India.

ABSTRACT

Background: Achievement of a successful endodontic treatment depends upon thorough chemical and mechanical debridement of root canals. Though instrumentation achieves most of this specified task, a proper irrigation protocol accesses all the parts of root canal system including those inaccessible to the instrumentation and ensures much efficient debridement. The main qualities of irrigants that make them play the pivotal role in cleaning of the canals are their flushing action, antimicrobial effect, tissue dissolution capacity and low clinical toxicity.

Keywords: Tissue dissolution capacity, Sodium Hypochlorite, coloured bottles.

INTRODUCTION

Achievement of a successful endodontic treatment depends upon thorough chemical and mechanical debridement of root canals. Though instrumentation achieves most of this specified task, a proper irrigation protocol accesses all the parts of root canal system including those inaccessible to the instrumentation and ensures much efficient debridement. The main qualities of irrigants that make them play the pivotal role in cleaning of the canals are their flushing action, antimicrobial effect, tissue dissolution capacity and low clinical toxicity. (1)

Many irrigating solutions have found their use in endodontic treatment like EDTA, normal saline, citric acid, hydrogen peroxide and sodium hypochlorite.(2-4)(5)(6) Sodium hypochlorite has been popularly used as an endodontic irrigant ever since it was introduced by Walker in 1936. It has

the superior combination of the three crucial properties of an ideal irrigating solution – the tissue dissolving capacity, antimicrobial activity and absence of clinical toxicity when used in intended manner.(2,7) The oxidizing properties and reactivity of sodium hypochlorite gives it an antimicrobial and tissue dissolving effect, thus making it an anti-biofilm root canal irrigant. (8) The postoperative complications like pain, accident etc. have also been reported by various authors in their trials and case reports.(9-16) Substantial evidence on the effect of solution on rotary instruments is also available.(17) Though all the properties of sodium hypochlorite are well known, the effective shelf life of the prepared solutions are still under research. It has been proved that the stability of sodium hypochlorite is affected by exposure to high temperature, light, air and presence of any

Received: Apr. 3, 2024; Accepted: May. 18, 2024

*Correspondence Dr. Meghana Velagaleti.

Department of Conservative Dentistry and Endodontics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

Email: meghanavelagaleti@gmail.com

contaminants, either organic or inorganic. (18–27) It has also been stated that the antibacterial property of the solution decreases over time and the effective storage shelf life maybe 3 months. (28)(26)

The purpose of the present study was to analyse and evaluate the effect of storage condition by storing them differently in white (clear) and dark coloured bottles on the efficacy of sodium hypochlorite in terms of tissue dissolution capacity.

MATERIALS AND METHODS

Materials used

1. Sodium hypochlorite - white bottle - VISHAL DENTOCARE 5%
2. Sodium hypochlorite - dark bottle - CHLORAXID 5.25% CHLORAXID XTRA 5.25%
3. Human umbilical cord tissue
4. Petri dishes
5. Microweight scale

Methodology

Fresh human umbilical cord tissue was collected, frozen and preserved until study. Tissue was allowed to defreeze till room temperature and sliced into thin sections, 50mg for each section by observer 1. The petri dishes were labelled A, B and C. The solutions were poured into the dishes by observer 2.

Group A: Sodium hypochlorite - white bottle - VISHAL DENTOCARE 5%.

Group B: dark bottle - CHLORAXID 5.25%

Group C: dark bottle - CHLORAXID XTRA 5.25%

The sectioned tissues were immersed in these solutions. The samples were removed from the dishes and washed in distilled water and blotted dry before weighing for every 5, 10, 15 minute intervals. The time taken for complete dissolution of tissue was also noted. This was done by a third observer. 20 samples were observed for each solution, making a total sample size of 60 for the study intended. The same methodology of the study was repeated after 30 days.

Statistical analysis:

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
day 1 time	1	20	10.7725	2.34170	.52362	9.6766	11.8684	6.50	13.40
	2	20	22.6200	2.91829	.65255	21.2542	23.9858	18.50	27.60
	3	20	10.2250	2.41549	.54012	9.0945	11.3555	6.50	14.00
	Total	60	14.5392	6.29615	.81283	12.9127	16.1656	6.50	27.60
day 30 time	1	20	21.8100	4.22485	.94471	19.8327	23.7873	8.00	26.50
	2	20	5.7225	1.81460	.40576	4.8732	6.5718	2.30	8.30
	3	20	5.9375	1.58537	.35450	5.1955	6.6795	4.00	8.50
	Total	60	11.1567	8.08296	1.04351	9.0686	13.2447	2.30	26.50

The results after the first day and the 30th day were statistically analysed using the ANOVA test of significance (Analysis of Variance) and the results

were tabulated as below showing the p value to be statistically significant ($p < 0.005$).

		Sum of Squares	df	Mean Square	F	Sig.
day 1 time	Between Groups	1961.994	2	980.997	148.377	.000
	Within Groups	376.857	57	6.612		
	Total	2338.850	59			
day 30 time	Between Groups	3405.268	2	1702.634	215.929	.000

	Within Groups	449.455	57	7.885		
	Total	3854.722	59			

Table 3: Tests of Between-Subjects Effects						
Dependent Variable: day 1 time						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	1962.191 ^a	3	654.064	97.243	.000	
Day 30 time group	1575.404	2	787.702	117.112	.000	
Error	376.659	56	6.726			
Total	15022.092	60				
a. R Squared = .839 (Adjusted R Squared = .830)						

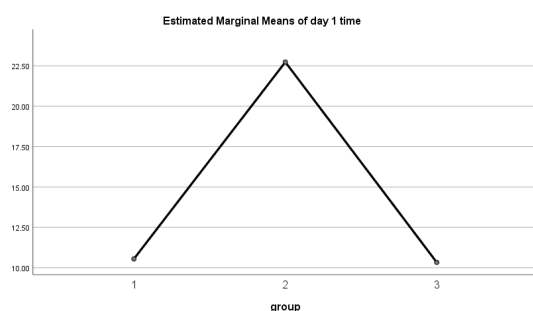


Fig 1: Graph depicting the total time taken for the three sample groups for total dissolution of the tissue, where numbers 1,2 and 3 represented as Group A,B and C respectively.

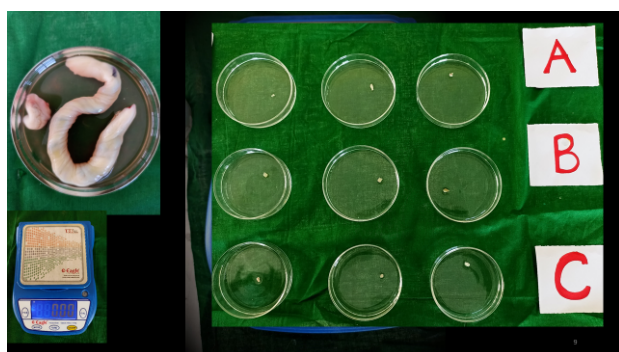


Fig 2: The apparatus used for testing tissue dissolution i.e., of freshly collected umbilical cord,

Petri dishes containing tissue samples and weighing device that corrects up to 0.5mg.

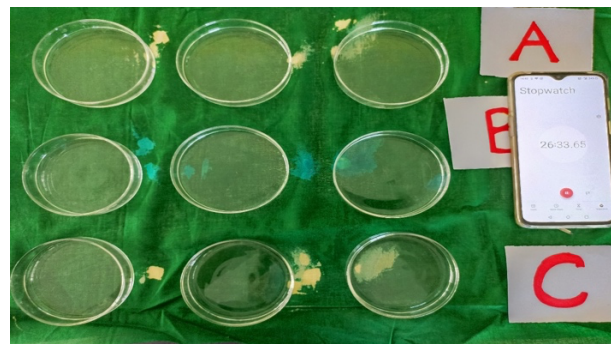


Fig 3: Depicts complete dissolution of tissue in all sample Petri dishes containing sodium hypochlorite 5.25%

RESULTS

Significant differences in tissue dissolution capacity between sodium hypochlorite stored in white and dark bottles.

Not much difference noted in both the groups after a shelf life of 1 month.

However, after 30 days, the variation in efficacy of sodium hypochlorite stored in dark and white colored bottles was not statistically significant.

DISCUSSION

Sodium hypochlorite is hypertonic (2800 mOsmol/kg) and highly alkaline (pH = 11.5 to 11.7). Solvent activity and antimicrobial properties are due to sodium hypochlorite's ability to oxidize and hydrolyze cell proteins, (b) the release of chlorine to form hypochlorous acid, and (c), to some extent, its ability to osmotically draw fluids out of cells. Sodium hypochlorite is nonspecific in action and is cytotoxic to most of the cells. (28)

When sodium hypochlorite contacts tissue proteins, nitrogen, formaldehyde, and acetaldehyde are formed. Fragmented Peptide links and disintegrated proteins permit hydrogen in the amino groups (-NH-) to be replaced by chlorine (-NCl-) forming chloramines; which plays a pivotal role for antimicrobial efficacy. (29)

It acts as an organic and fat solvent that degrades fatty acids and transforms them into fatty acid salts (soap) and glycerol (alcohol), reducing the surface tension of the remaining solution. Sodium hypochlorite neutralizes amino acids by forming water and salt. When chlorine dissolves in water and it is in contact with organic matter, it forms hypochlorous acid. It is a weak acid with the chemical formula HClO that acts as an oxidizer. Hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) lead to amino acid degradation and hydrolysis. It also acts as a solvent, releasing chlorine that combines with protein amino groups (NH) to form chloramines (chloramination reaction). Chloramines impede cell metabolism; chlorine is a strong oxidant and inhibits essential bacterial enzymes by irreversible oxidation of SH groups (sulfhydryl group). The antimicrobial effectiveness of sodium hypochlorite is based on its high pH (hydroxyl ions action). The high pH interferes in cytoplasmic membrane integrity due to irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism, and phospholipid degradation observed in lipid peroxidation. (29)

The fact that addition of a surfactant improves the efficacy of sodium hypochlorite as an irrigant is well documented. (19,30,31) Hence, taking this aspect into consideration, the efficacy of sodium hypochlorite stored in dark coloured bottles with and without surfactant were also compared. However, there was not much significant difference in the time taken by these solutions for tissue dissolution.

Human umbilical cord was selected for use as a pulp substitute owing to the uniformity of obtaining samples and its similarity to pulp tissue in a histological aspect. It primarily contains a gelatinous ground substance (Wharton's jelly) that is rich in proteoglycans and collagen fibers. Umbilical cord is of fetal mesodermal origin and the principle cell type is a specialized form of fibroblast. Blood vessels and fetal autonomic nerve fibers are also present. Using umbilical cord as the tissue sample, the collection and preservation problems inherent in working with other tissue types as well as the lack of histological uniformity found in collections of human pulp tissue were avoided. The general gross similarity in appearance of umbilical cord sections and human pulp and the manner in

which umbilical cord dissolves further supports its use as a pulp substitute. (28)

However, there were certain limitations in the present study that open up multiple dimensions furtherly for research. Firstly, umbilical cord tissue was uniformly sectioned into samples and dissolved in petri dishes. This might not simulate the original clinical scenario of root canals, their morphology and the pulp tissue. However, the exact clinical scenario cannot be simulated as the organic content of the pulp tissue might be different in different cases varying with the stage of pulpal inflammation and diagnostic term. Standardization of sample was achieved this way which would support the use of a substitute tissue. Secondly, duration of 1 month might not be enough to evaluate the effective shelf life of the solution; however further research is being continued for different time intervals. Thirdly, the cost effectiveness of using dark coloured bottles has to be analysed in terms of volume of solution required, number of cases efficiently worked upon and other clinical factors.

CLINICAL SIGNIFICANCE

The efficacy of sodium hypochlorite as an irrigant for pulp tissue is already well documented. However, this study aimed at establishing the efficacy of solutions when stored differently. It addresses the cost effectiveness and efficacy of sodium hypochlorite.

CONCLUSION

The efficacy of sodium hypochlorite as an irrigating solution, its pulp dissolution ability, toxic and allergic effects, effect of external factors on stability have been well documented and reported so far. However, the influence of storage containers over the efficacy has not been substantially evaluated, which the present study did. The results concluded that there is clinical and statistical significance in the efficacy of sodium hypochlorite stored in white and dark bottles.

REFERENCES

1. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. *J Endod*. 2010 Sep;36(9):1558-62.

2. Rosenfeld EF, James GA, Burch BS. Vital pulp tissue response to sodium hypochlorite. *J Endod.* 1978 May;4(5):140-6.
3. Georgopoulou M, Kontakiotis E, Nakou M. Evaluation of the antimicrobial effectiveness of citric acid and sodium hypochlorite on the anaerobic flora of the infected root canal. *Int Endod J.* 1994 May;27(3):139-43.
4. Svec TA, Harrison JW. Chemomechanical removal of pulpal and dentinal debris with sodium hypochlorite and hydrogen peroxide vs normal saline solution. *J Endod.* 1977 Feb;3(2):49-53.
5. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. *J Endod.* 2004 Nov;30(11):785-7.
6. Baumgartner JC, Brown CM, Mader CL, Peters DD, Shulman JD. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. *J Endod.* 1984 Nov;10(11):525-31.
7. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. *Int Endod J.* 1982 Oct;15(4):187-96.
8. Petridis X, Busanello FH, So MVR, Dijkstra RJB, Sharma PK, van der Sluis LWM. Factors affecting the chemical efficacy of 2% sodium hypochlorite against oral steady-state dual-species biofilms: Exposure time and volume application. *Int Endod J.* 2019 Aug;52(8):1182-1195.
9. Pontes F, Pontes H, Adachi P, Rodini C, Almeida D, Pinto D Jr. Gingival and bone necrosis caused by accidental sodium hypochlorite injection instead of anaesthetic solution. *Int Endod J.* 2008 Mar;41(3):267-70.
10. Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. *Int Endod J.* 2004;37(4):272-80.
11. Mostafa MEHAA, El-Shrief YAI, Anous WIO, Hassan MW, Salamah FTA, El Boghdadi RM, El-Bayoumi MAA, Seyam RM, Abd-El-Kader KG, Amin SAW. Postoperative pain following endodontic irrigation using 1.3% versus 5.25% sodium hypochlorite in mandibular molars with necrotic pulps: a randomized double-blind clinical trial. *Int Endod J.* 2020 Feb;53(2):154-166.
12. Witton R, Henthorn K, Ethunandan M, Harmer S, Brennan PA. Neurological complications following extrusion of sodium hypochlorite solution during root canal treatment. *Int Endod J.* 2005 Nov;38(11):843-8.
13. Witton R, Henthorn K, Ethunandan M, Harmer S, Brennan PA. Neurological complications following extrusion of sodium hypochlorite solution during root canal treatment. *Int Endod J.* 2005 Nov;38(11):843-8.
14. Behrents KT, Speer ML, Noujeim M. Sodium hypochlorite accident with evaluation by cone beam computed tomography. *Int Endod J.* 2012 May;45(5):492-8.
15. Ballal NV, Das S, Rao BSS, Zehnder M, Mohn D. Chemical, cytotoxic and genotoxic analysis of etidronate in sodium hypochlorite solution. *Int Endod J.* 2019 Aug;52(8):1228-1234.
16. Çalışkan MK, Türkün M, Alper S. Allergy to sodium hypochlorite during root canal therapy: a case report. *Int Endod J.* 1994 May;27(3):163-7.
17. Nóvoa XR, Martin-Biedma B, Varela-Patiño P, Collazo A, Macías-Luaces A, Cantatore G, Pérez MC, Magán-Muñoz F. The corrosion of nickel-titanium rotary endodontic instruments in sodium hypochlorite. *Int Endod J.* 2007 Jan;40(1):36-44.
18. Berutti E, Marini R. A scanning electron microscopic evaluation of the debridement capability of sodium hypochlorite at different temperatures. *J Endod.* 1996 Sep;22(9):467-70.
19. Palazzi F, Morra M, Mohammadi Z, Grandini S, Giardino L. Comparison of the surface tension of 5.25% sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. *Int Endod J.* 2012 Feb;45(2):129-35.
20. Gambarini G, De Luca M, Gerosa R. Chemical stability of heated sodium hypochlorite endodontic irrigants. *J Endod.* 1998 Jun;24(
21. Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, Diogenes A. Concentration-dependent effect of sodium hypochlorite on stem

cells of apical papilla survival and differentiation. *J Endod.* 2014 Jan;40(1):51-5.

22. Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. *J Endod.* 2005 Sep;31(9):669-71.

23. Tang HM, Nordbö H, Bakland LK. Pulpal response to prolonged dentinal exposure to sodium hypochlorite. *Int Endod J.* 2000 Nov;33(6):505-8.

24. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, in vitro. *Int Endod J.* 1997 Jul;30(4):279-82.

25. Jungbluth H, Marending M, De-Deus G, Sener B, Zehnder M. Stabilizing sodium hypochlorite at high pH: effects on soft tissue and dentin. *J Endod.* 2011 May;37(5):693-6.

26. Frai S, Ng YL, Gulabivala K. Some factors affecting the concentration of available chlorine in

commercial sources of sodium hypochlorite. *Int Endod J.* 2001 Apr;34(3):206-15.

27. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J.* 2001 Sep;34(6):424-8.

28. Johnson BR, Remeikis NA. Effective shelf-life of prepared sodium hypochlorite solution. *J Endod.* 1993 Jan;19(1):40-3.

29. Olujide S. Cohen's Pathways of the Pulp. Vol. 48, Dental Update. 2021. 248-248 p.

30. Guerreiro MYR, Belladonna FG, Monteiro LPB, Lima CO, Silva EJNL, Brandão JMS. The influence of the addition of surfactants to sodium hypochlorite on the removal of hard tissue debris. *Int Endod J.* 2020 Aug;53(8):1131-1139.

31. De-Deus G, de Berredo Pinho MA, Reis C, Fidel S, Souza E, Zehnder M. Sodium hypochlorite with reduced surface tension does not improve in situ pulp tissue dissolution. *J Endod.* 2013 Aug;39(8):1039-43.