

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

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Abstract

Aim: To compare and evaluate the tissue dissolution capacity of sodium hypochlorite when stored in white and dark coloured bottles. **Materials:** 1) Sodium hypochlorite - white bottle - vishal dentocare 5%, 2) Sodium hypochlorite - dark bottle - chloraxid 5.25%, 3) chloraxid xtra 5.25%, 4) Human umbilical cord tissue, 5) Petri dishes, 6) 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 of 40mg each. The tissue samples were placed in petri dishes that were categorized into three groups A, B and C. 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 samples were washed in distilled water and blotted dry before weighing for every 5, 10, 15 minute intervals. The time taken for complete dissolution of tissue is also noted. The study is repeated after 30 days. **Results:** Sodium hypochlorite stored in dark containers showed quicker and efficient dissolution of tissue than that stored in white container. **Conclusion:** Significant difference was found in the time taken for complete dissolution of tissue when immersed in sodium hypochlorite stored in dark and white bottles. **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.

Keywords: Colored bottles, Sodium hypochlorite, Tissue dissolution capacity.

INTRODUCTION

Achievement of a successful endodontic treatment depends upon thorough chemical and mechanical debridement of root canals. Although 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 such as EDTA, normal saline, citric acid, hydrogen peroxide, and sodium hypochlorite.^[2-6] 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 such as pain and accident have also been reported by various authors in their trials and case reports.^[9-15] Substantial evidence on the effect of solution on rotary instruments is also available.^[16] Although 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 contaminants, either organic or inorganic.^[17-26] It has also been stated that the antibacterial property of the solution decreases over time and the effective storage shelf life maybe 3 months.^[25,27]

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The purpose of the present study was to analyze and evaluate the effect of storage condition by storing them differently in white (clear) and dark-colored 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, 50 mg for each section by observer 1. The petri dishes were labeled 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% (Figure 1).

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 min intervals. The time taken for complete dissolution of tissue was also noted (Figure 2). 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

The results after the first day and the 30th day were statistically analyzed using the ANOVA test of significance (Analysis of Variance) and the results were tabulated as below (Tables 1-3) showing the *P*-value to be statistically significant ($P < 0.005$).

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

RESULTS

Significant differences in tissue dissolution capacity between sodium hypochlorite stored in white and dark bottles (Figure 3).

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 = 11.5–11.7). Solvent activity and antimicrobial properties are due to sodium hypochlorite's ability to oxidize and hydrolyze cell proteins, the release of chlorine to form hypochlorous acid, and to some extent, its ability to osmotically draw fluids out of cells. Sodium hypochlorite is non-specific in action and is cytotoxic to most of the cells.^[27]

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 play a pivotal role for antimicrobial efficacy.^[28]

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

Table 1: Statistical analysis on the day 1 and 30 of sample testing

Comparison among the time periods	n	Mean	Standard Deviation	Standard Error	95% confidence interval for mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Day 1 time								
1	20	10.7725	2.34170	0.52362	9.6766	11.8684	6.50	13.40
2	20	22.6200	2.91829	0.65255	21.2542	23.9858	18.50	27.60
3	20	10.2250	2.41549	0.54012	9.0945	11.3555	6.50	14.00
Total	60	14.5392	6.29615	0.81283	12.9127	16.1656	6.50	27.60
Day 30 time								
1	20	21.8100	4.22485	0.94471	19.8327	23.7873	8.00	26.50
2	20	5.7225	1.81460	0.40576	4.8732	6.5718	2.30	8.30
3	20	5.9375	1.58537	0.35450	5.1955	6.6795	4.00	8.50
Total	60	11.1567	8.08296	1.04351	9.0686	13.2447	2.30	26.50

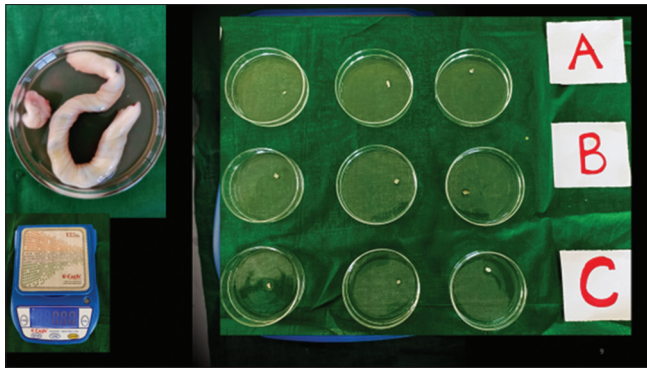


Figure 1: The apparatus used for testing tissue dissolution, i.e., of freshly collected umbilical cord

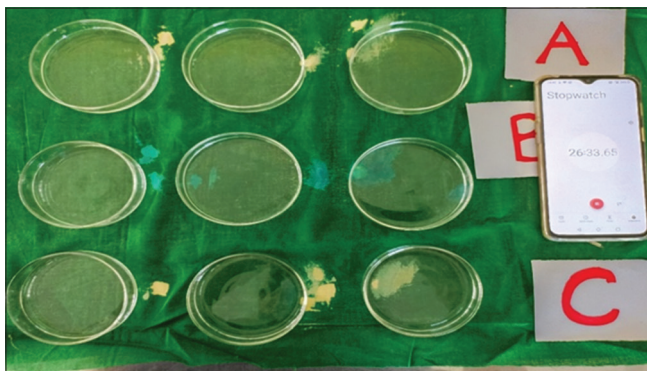


Figure 2: Depicts complete dissolution of tissue in all sample petri dishes containing sodium hypochlorite 5.25%

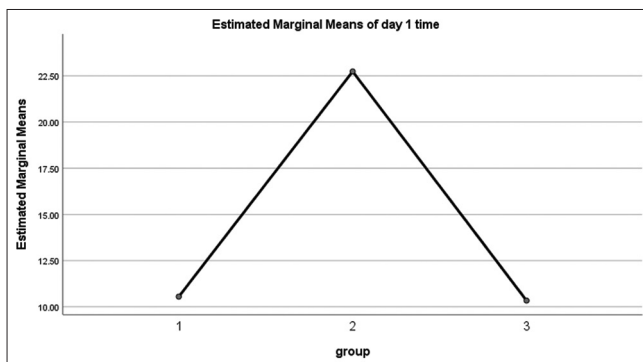


Figure 3: Graph depicting the total time taken for the three sample groups for total dissolution of the tissue, where numbers 1, 2, and 3 represented as Group A, B and C, respectively

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.^[28]

Table 2: ANOVA on the day 1 and 30 of testing for inter-group comparison

Comparison among the time periods	Sum of Squares	df	Mean square	F	Sig.
Day 1 time					
Between groups	1961.994	2	980.997	148.377	0.000
Within groups	376.857	57	6.612		
Total	2338.850	59			
Day 30 time					
Between groups	3405.268	2	1702.634	215.929	0.000
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	0.000
Day 30 time group	0.198	1	0.198	0.029	0.864
Error	1575.404	2	787.702	117.112	0.000
Total	376.659	56	6.726		
Total	15022.092	60			

^aR Squared=0.839 (Adjusted R Squared=0.830)

The fact that addition of a surfactant improves the efficacy of sodium hypochlorite as an irrigant is well documented.^[18,29,30] Hence, taking this aspect into consideration, the efficacy of sodium hypochlorite stored in dark-colored bottles with and without surfactant was 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.^[27]

However, there were certain limitations in the present study that open up multiple dimensions furtherly for research. First, 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. Second, 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. Third, the cost effectiveness of using dark-colored bottles has to be analyzed 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.

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