ORIGINAL RESEARCH

Antibacterial Efficacy of Irrigants with Varying Osmolarity on *E. faecalis* Biofilm: An *In Vitro* Study

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Abstract

Aim: To evaluate the role of the addition of different concentrations of sodium chloride salt to conventional intracanal irrigants to vary their osmotic values and thereby compare their antibacterial efficacy.

Materials and methods: In an active attachment biofilm model, *Enterococcus faecalis* (ATCC 29212) biofilms were grown. Sodium chloride salts were added to 100 mL of distilled water to make 6M (hyperosmotic), 0.5M, and 0.25M (hypoosmotic) sodium chloride solutions, respectively. The experimental groups were divided into three groups: Group I: 5.25% sodium hypochlorite, group II: 2% chlorhexidine, and group III: 2% povidone iodine, and four subgroups within these three groups, such as subgroup A (without salt solution), subgroup B (with 6M of hyperosmotic salt solution), subgroup C (with 0.5M of hypoosmotic salt solution), and subgroup D (with 0.25M of hypoosmotic salt solution), respectively. Biofilms were treated with all the subgroups for a contact time of 15 min. A crystal violet assay was done to estimate the bacterial cell biomass.

Results: The results revealed that subgroups IIIB, IB, and IID, ID had a statistical reduction in bacterial biomass at $p < 0.05$. There were no significant differences between subgroups IC, IIC, and IIIC and subgroups IA, IIA, and IIIA.

Conclusion: The antibacterial efficacy of all three irrigants was significantly affected by varying the osmolarities.

Clinical significance: The results prove that the hyperosmotic and hypoosmotic salt solutions, along with irrigants, have enhanced antibacterial efficacy on *E. faecalis* biofilm due to its ability to vary the turgor pressure of cell wall, as well as the inherent properties of the irrigants such as hypochlorous acid formation, ionic interaction, and free radical interactions.

Keywords: Chlorhexidine, Hyperosmotic salt solution, Hypoosmotic salt solution, Irrigants, Osmolarity, Povidone iodine, Root canal disinfection, Sodium hypochlorite.

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Introduction

The presence of persisting microorganisms following endodontic therapy has been the main etiology for post-treatment disease and endodontic failure. Primary endodontic infections are mainly caused by obligate anaerobic species, while the foremost causative agent for endodontic failure is *E. faecalis*.[1] Its ability to invade human dentinal tubules,[2] stick to dentine,[3] high resistance to alkaline stress,[4] and proton pump function[5] may also be responsible for its survival in extreme conditions like high alkalinity. This bacterium has the ability to resist many intracanal medications and irrigants, and hence represents itself as a crucial microorganism within the endodontic biofilm population. Root canal irrigants can be adjuncts to mechanical debridement by flushing out debris, dissolving tissue, and disinfecting the root canal system.

Sodium hypochlorite (NaOCl) has an exclusive history and is a popular irrigant. Zand et al. evaluated the efficacy of varied concentrations of NaOCl and concluded that 5.25% NaOCl acts effectively on *E. faecalis* biofilm.[6] Chlorhexidine digluconate is widely used because of its excellent antimicrobial activity. Many studies have concluded that 2% chlorhexidine has an antimicrobial effect against *E. faecalis* bacteria.[7–9] Povidone iodine has the ability to kill bacteria, fungi, viruses, and spores. The 2% povidone iodine preparations are shown to be less cytotoxic and reduce the bacterial load.

Osmolarity is the number of milliosmoles of solutes per liter of solution. When a solution is divided into two parts by a membrane through which water molecules are permeable but not impermeable to solutes, the membrane is referred to as selective or semi-permeable. If the solute concentration is not equal on the two sides of a semipermeable membrane, the water molecules will migrate to the more concentrated side. Osmotic pressure is one of the most important physical parameters with which living cells must contend.

The Hurdle effect was first highlighted by Leistner. It is most commonly used in the food industry. In the food industry, hurdles like thermal, osmolarity, and pH are applied. When exposed to hurdles, the microorganism tries to adapt to the environmental changes and becomes exhausted once there are hurdles to beat. Environmental stress is an external factor that has an adverse effect on the physiological welfare of microbial cells, resulting

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in a reduction in their rate of growth and, in extreme cases, necrobiosis.[10]

It has been concluded by Rossi-Fedele and Guastalli that hyperosmotic preparations have an additional antimicrobial effect with irrigants.[11] Van der Waal et al.[12] concluded that hyperosmotic media treated with 2% sodium hypochlorite effectively reduced the dual-species biofilm of *E. faecalis* and *Pseudomonas aeruginosa*.

There is limited evidence in the literature for the antibacterial efficacy of various other root canal irrigants with differing osmolarities. The aim of this *in vitro* study was to evaluate the role of the addition of varied osmotic salt solutions of sodium chloride salt to conventional intracanal irrigants to vary their osmotic values and thereby compare their antibacterial efficacy.

Materials and Methods

From August 2020 to September 2020, this study was conducted at the Department of Biotechnology, Sri Ramachandra Institute of Higher Education and Research, Chennai.

Bacterial Strain and Growth Conditions

E. faecalis (ATCC 29212) was obtained from the Department of Microbiology, Sri Ramachandra Institute of Higher Education and Research, Chennai. It was incubated in a fresh brain heart infusion (BHI) broth in an aerobic atmosphere at 37°C for 24 hours. During this experiment, *E. faecalis* culture was grown on the medium which was composed of 0.2% sucrose, 0.3% wt./vol yeast extract, sodium chloride 5.0 gm/L, and 1 L of distilled water.

Sodium Chloride Hyperosmotic, Hypoosmotic, and Isosmotic Solutions

To make 6M (hyperosmotic), 0.5M (hypoosmotic), and 0.25M (hypoosmotic) sodium chloride solutions, 5.4 gm, 0.45 gm, and 0.22 gm of sodium chloride salts were added to 100 mL of distilled water, respectively.

Preoperative Colony-forming Unit (CFU) Counts

The broth culture was adjusted to a level of 1.0106 CFU per milliliter (CFUs/mL) at an optical density (OD) of 600 nm. The results were verified by spectrophotometry and colony counting.

Biofilm Formation

Biofilms were grown in an active attachment biofilm model. This model consisted of a sterile flat-bottom 96-well polystyrene plate into which 200 µL of the free-floating planktonic culture (*E. faecalis* ATCC 29122) was carefully pipetted into the wells so that the upper fringe of the aliquot just reached the center of the bottom well and therefore the nutrient-rich medium was added to the wells to grow the cells.[13] The microplates were incubated at 37°C for 3 days to permit biofilm formation.

Reagent Addition

The reagents were divided into 3 groups and 4 subgroups according to Table 1. Four wells were left without adding reagents for negative. For every subgroup in group I (5.25% sodium hypochlorite), group II (2% chlorhexidine), and group III (2% povidone iodine), 0.1 mL of the respective irrigants without sodium chloride solution, 0.1 mL of the respective irrigants with 0.1 mL of 6M sodium chloride solution, 0.1 mL of the respective irrigant with 0.1 mL of 0.5M of sodium chloride solution, and 0.1 mL of the respective irrigant with 0.1 mL of 0.25M of sodium chloride solution were added to the wells in quadruplicates and incubated for 15 minutes. The experiment for each subgroup was done in quadruplicates to eliminate errors during recording the results.

Table 1: Order of grouping reagents

Group I	5.25% Sodium hypochlorite
Subgroup IA	5.25% Sodium hypochlorite without adding sodium chloride solution
Subgroup IB	5.25% Sodium hypochlorite + 6M of sodium chloride solution
Subgroup IC	5.25% Sodium hypochlorite + 0.5M of sodium chloride solution
Subgroup ID	5.25% Sodium hypochlorite + 0.25M of sodium chloride solution
Group II	2% Chlorhexidine
Subgroup IIA	2% Chlorhexidine without adding sodium chloride solution
Subgroup IIB	2% Chlorhexidine + 6M of sodium chloride solution
Subgroup IIC	2% Chlorhexidine + 0.5M of sodium chloride solution
Subgroup IID	2% Chlorhexidine + 0.25M of sodium chloride solution
Group III	2% Povidone iodine
Subgroup IIIA	2% Povidone iodine solution without adding sodium chloride solution
Subgroup IIIB	2% Povidone iodine solution + 6M of sodium chloride solution
Subgroup IIIC	2% Povidone iodine solution + 0.5M of sodium chloride solution
Subgroup IIID	2% Povidone iodine solution + 0.25M of sodium chloride solution

Crystal Violet Assay

After treating the wells with reagents, the contents of the microplates were extracted, and the remaining suspension within the wells containing free-floating planktonic bacteria was removed by washing twice with phosphate-buffered saline (PBS). The quantification of the biofilm was performed based on a previous study by Pourhajibagher et al.[14] About 200 µl of 0.1% (wt./vol) crystal violet was added to stain biofilm bacteria at room temperature for 15 minutes. After washing the wells with PBS thrice, the wells were treated with 95% ethanol for 10 minutes at room temperature and left to dry. The wells were then filled with 150 µl of 33.0% (vol/vol) acetic acid, and therefore the absorbance was read by a microplate reader at a wavelength of 570 nm.

Phase Contrast Microscope

To confirm the biofilm formation, the microtiter plate was placed flat on the stage of the phase contrast microscopy (Nikon Microscopy) and viewed under 20× magnification. Therefore, the digital images were obtained.

Statistical Analysis

The results of the study were analyzed using SPSS software version 16.0. The mean and standard deviation were used to describe the results of the experimental groups and subgroups.

Table 2: Comparison of 96% CI of group I with all other subgroups of group I, group II, and group III

	Subgroup IA		*Subgroup IB*		*Subgroup IC*		*Subgroup ID*	
	95% CI of mean value	*p-value*	*95% CI mean value*	*p-value*	*95% CI mean value*	*p-value*	*95% CI mean value*	*p-value*
Subgroup IA	–	–	0.00749–0.07951	**0.015**	–0.02241–0.04961	0.77	0.00949–0.08151	**0.011**
Subgroup IB	–0.07956–0.00752	**0.018**	–	–	–0.06591–0.00611	0.128	–0.03401–0.03801	1.00
Subgroup IC	–0.04973–0.02249	0.95	–0.00606–0.06484	0.136	–	–	–0.00411–0.06791	0.095
Subgroup ID	–0.08093–0.00934	**0.016**	–0.03891–0.03482	1.008	–0.06571–0.00201	0.08	–	–
Subgroup IIA	–0.04961–0.02241	0.77	0.00736–0.07864	**0.019**	–0.02173–0.04854	0.74	0.00927–0.08092	**0.013**
Subgroup IIB	–0.07938–0.00739	**0.025**	–0.00619–0.06576	0.119	0.00445–0.08905	**0.027**	–0.03201–0.03604	1.12
Subgroup IIC	–0.04895–0.02176	0.75	–0.08609–0.00404	0.022	–0.02275–0.04989	0.82	–0.10005–0.01545	**0.006**
Subgroup IID	–0.08151–0.00949	**0.011**	–0.03801–0.03401	1.000	0.01475–0.10375	**0.008**	–0.00428–0.06778	0.090
Subgroup IIIA	–0.04748–0.02269	0.727	0.00840–0.07850	**0.011**	–0.02361–0.04860	0.753	0.00843–0.08550	**0.014**
Subgroup IIIB	–0.07863–0.00758	**0.019**	–0.00720–0.06694	0.110	0.01737–0.10394	**0.008**	–0.03304–0.03508	1.053
Subgroup IIIC	–0.04961–0.02241	0.740	0.01636–0.10484	**0.009**	–0.02241–0.04961	0.767	–0.09844–0.01200	**0.021**
Subgroup IIID	–0.08256–0.00957	**0.013**	–0.03905–0.03703	1.040	0.01204–0.09987	**0.018**	–0.00514–0.06899	0.093

Bold *p*-value < 0.05 statistically significant

Table 3: Comparison of 96% CI of group II with all other subgroups of group I, group II, and group III

	Subgroup IIA		*Subgroup IIB*		*Subgroup IIC*		*Subgroup IID*	
	95% CI mean value	*p-value*	*95% CI mean value*	*p-value*	*95% CI mean value*	*p-value*	*95% CI mean value*	*p-value*
Subgroup IA	–0.01478–0.03409	0.560	–0.07831–0.00758	**0.013**	–0.04665–0.03706	0.996	0.00940–0.09470	**0.025**
Subgroup IB	0.00801–0.07963	**0.025**	–0.00150–0.08190	0.074	–0.08806–0.00454	**0.035**	–0.03185–0.05340	0.980
Subgroup IC	–0.08278–0.00193	0.061	0.00454–0.08854	**0.021**	–0.04867–0.03689	0.998	0.01564–0.10016	**0.004**
Subgroup ID	–0.09376–0.00950	**0.011**	–0.05323–0.03179	0.956	–0.10019–0.01536	**0.009**	–0.03169–0.05348	0.996
Subgroup IIA	–	–	–0.00168–0.08269	0.068	–0.04876–0.03619	0.994	0.00895–0.09360	**0.019**
Subgroup IIB	–0.08267–0.00139	0.061	–	–	–0.08918–0.00456	**0.031**	–0.03152–0.05348	0.965
Subgroup IIC	–0.03624–0.04876	0.994	0.00452–0.08914	**0.024**	–	–	0.01532–0.10016	**0.008**
Subgroup IID	–0.09375–0.00919	**0.019**	–0.05345–0.03124	0.921	–0.10013–0.01587	**0.002**	–	–
Subgroup IIIA	–0.01464 –0.03314	0.532	–0.09063–0.00242	**0.034**	–0.04862–0.03618	0.996	0.00934–0.09363	**0.024**
Subgroup IIIB	0.00238 –0.09079	**0.034**	–0.00159–0.08276	0.063	–0.08914–0.00465	**0.037**	–0.03148–0.05328	0.936
Subgroup IIIC	–0.08279 –0.00175	0.064	0.00445–0.08905	**0.027**	–0.04855–0.03605	0.990	0.01545–0.10005	**0.006**
Subgroup IIID	–0.09376 –0.00931	**0.018**	–0.05389–0.03143	0.932	–0.10018–0.01587	**0.008**	–0.03153–0.05345	0.931

Bold *p*-value < 0.05 statistically significant

One-way analysis of variance (ANOVA) test was used for intergroup comparison and *post-hoc* Tukey honestly significant difference (HSD) test for multiple subgroup comparison. The mean difference is significant at the 0.05 level.

Results

The results are presented as a 96% confidence interval comparison of the mean absorbance value of biofilm and their *p* values in reagent treated and untreated wells (Tables 2 to 4). On analysis of results, between the subgroups without salt solution (subgroup IA, IIA, IIIA); with hyperosmotic salt solution (subgroup IB, IIB, IIIB); with 0.5M hypoosmotic salt solution (subgroup IC, IIC, IIIC); and 0.25M hypoosmotic salt solution (subgroup ID IID, IIID) of 5.25% sodium hypochlorite, 2% chlorhexidine, and 2% povidone iodine, the mean absorbance value of subgroup IIIB (2% povidone iodine with 6M hyperosmotic salt solution), subgroup IID (2% chlorhexidine with 0.25M hypoosmotic salt solution) and subgroup IB (5.25% sodium hypochlorite with 6M hyperosmotic salt solution) and subgroup ID (5.25% sodium hypochlorite with 0.25M hypoosmotic salt solution) were statistically significantly low at $p < 0.05$. This result implies complete statistically significant bacterial inhibition in the *E. faecalis* biofilm. The intergroup comparison between group I (5.25% sodium hypochlorite), group II (2% chlorhexidine), and group III (2% povidone iodine) was statistically not significant at ($p < 0.05$).

In (Fig. 1A) the phase contrast microscopy image shows a 3-day biofilm with interspersed bacterial cells which acts as a control. In (Fig. 1B), the phase contrast microscopy images of wells treated with 2% povidone iodine with 6M hypertonic salt solution showed the absence of bacterial cells. These were in accordance with the colorimetric analysis of biofilm's biomass.

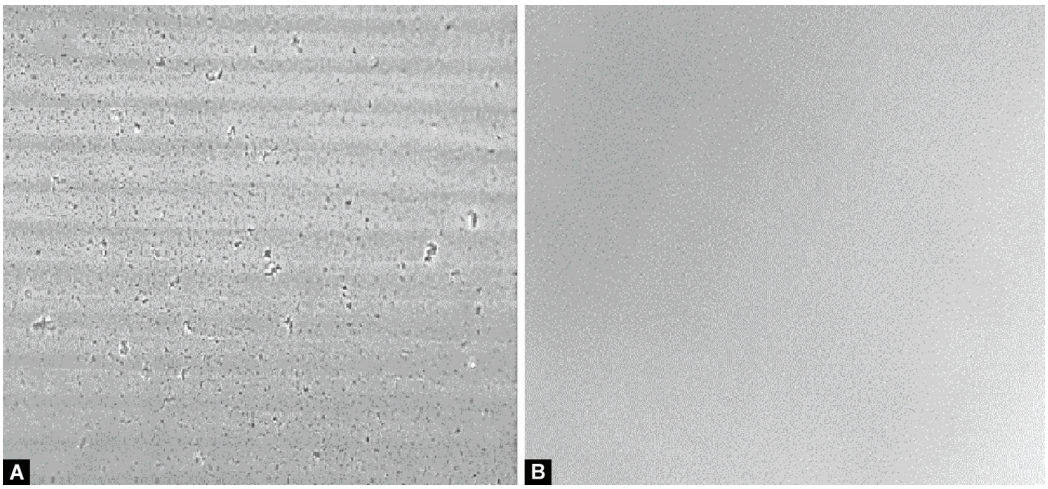
Discussion

In this study, the hyperosmotic and hypoosmotic environments significantly reduced the bacterial biomass. Subgroup IIIB (2% povidone iodine with 6M hypertonic salt solution), subgroup 2D (2% chlorhexidine with 0.25M hypotonic salt solution), and subgroup 1D (5.25% sodium hypochlorite with 0.25M hypotonic salt solution) (Tables 2 to 4) had the lowest mean absorbance value at 95% confidence interval, signifying a greater reduction in bacterial biomass compared to all the other groups. They showed statistical

Table 4: Comparison of 96% CI of group III with all other subgroups of group I, group II and group III

	Subgroup IIIA		*Subgroup IIIB*		*Subgroup IIIC*		*Subgroup IIID*	
	95% CI mean value	*p-value*	*95% CI mean value*	*p-value*	*95% CI mean value*	*p-value*	*95% CI mean value*	*p-value*
Subgroup IA	−0.00854–0.03963	0.226	0.00232–0.09084	**0.028**	−0.05858–0.02987	0.864	−0.08161–0.00938	**0.011**
Subgroup IB	−0.09067–0.00224	**0.041**	−0.03892–0.04964	0.999	0.01642–0.10479	**0.005**	−0.00329–0.08532	0.081
Subgroup IC	−0.02984–0.05847	0.858	0.01636–0.10484	**0.008**	−0.05853–0.02987	0.856	0.01113–0.09952	**0.013**
Subgroup ID	0.00954–0.08149	**0.012**	−0.03885–0.04962	0.997	−0.09953–0.01107	**0.013**	−0.04968–0.03893	0.998
Subgroup IIA	−0.00847–0.03945	0.226	0.00205–0.09052	**0.037**	−0.05853–0.02987	0.852	−0.09377–0.00917	**0.018**
Subgroup IIB	−0.09063–0.00216	**0.038**	−0.03884–0.04957	0.989	0.01643–0.10492	**0.005**	−0.00331–0.08537	0.079
Subgroup IIC	−0.02993–0.05851	0.859	0.01648–0.10489	**0.008**	−0.05835–0.02987	0.857	0.01104–0.09953	**0.015**
Subgroup IID	0.00929–0.09374	**0.018**	−0.03881–0.04953	0.989	−0.09952–0.01117	**0.016**	−0.04964–0.03894	0.998
Subgroup IIIA	–	–	0.00217–0.09063	**0.036**	−0.05832–0.02987	0.859	−0.00332–0.08529	0.078
Subgroup IIIB	−0.09063–0.00207	**0.035**	–	–	−0.10472–0.01642	**0.002**	−0.04964–0.03893	0.998
Subgroup IIIC	−0.02987–0.05845	0.863	0.01642–0.10493	**0.004**	–	–	0.01113–0.09951	**0.014**
Subgroup IIID	−0.08537–0.00331	0.081	−0.03883–0.04962	0.992	−0.09951–0.01118	**0.013**	–	–

Bold *p*-value < 0.05 statistically significant



Figs 1A and B: Phase contrast microscopy images of the 96 well plates after crystal violet assay: (A) Three day biofilm phase contrast microscopy images; (B) Reduction of biofilm in 2% Povidone iodine with 6M hypertonic salt solution

significance with subgroup 3A ($p = 0.038$ at pc0.05); subgroup IC ($p = 0.006$ at $p < 0.05$); subgroup IIA ($p = 0.038$ at $p < 0.05$); subgroup IIC ($p = 0.027$ at p < 0.05); subgroup IIIC ($p = 0.006$ at $p < 0.05$); subgroup IA ($p = 0.015$ at $p < 0.05$). No statistical significance was observed with subgroup IIID ($p = 0.995$ at $p < 0.05$); subgroup IIB ($p = 0.064$ at $p < 0.05$); subgroup 1B ($p = 0.128$ at $p < 0.05$).

This could be due to two factors: the irrigant's inherent antibacterial activity and the antibacterial effect of varying osmolarities.

The hyperosmotic preparations of povidone iodine, chlorhexidine, and hypoosmotic preparations of sodium hypochlorite did not have a statistically significant reduction in bacterial biomass (Tables 2 to 4) compared to the hypoosmotic preparation of povidone iodine and hyperosmotic preparation of sodium hypochlorite, which can be attributed to their equally effective inherent antibacterial activity. Because of varying osmolarity, the 0.25M hypoosmotic preparations of sodium hypochlorite, chlorhexidine, and povidone iodine groups showed statistical significance in reduction of bacterial biomass compared to the 0.5M hypoosmotic preparations of sodium hypochlorite, chlorhexidine, and povidone iodine groups.

Povidone iodine's antibacterial activity is due to the povidone molecule (polyvinyl pyrrolidone) and triiodide (I_3^-), which cause permanent pore formation on the cell wall. The free iodine released from povidone iodine in the solution can cause oxidation of double bonds in the phospholipid fraction of cell walls. Solid–liquid interfaces at lipid membrane level are also generated and this can lead to loss of cytosol contents in addition to enzymatic denaturation due to direct iodine reaction.[15]

Estrela et al. reported that sodium hypochlorite exhibits saponification action, neutralizing action, hypochlorous acid formation, solvent action, and high pH.[16] Gomes et al. found that 5.25% sodium hypochlorite was the most effective irrigant among other concentrations of sodium hypochlorite.[8] In a study by Van der Waal et al., they achieved significant antibiofilm activity of hyperosmotic sodium hypochlorite irrigant solution at 10 minute contact time. The results of present study were similar to those of Van der Waal et al.[12]

Chlorhexidine is a powerful basic molecule belonging to the polybiguanide group, and its antibacterial activity is due to the ionic interaction. The positively charged chlorhexidine molecule binds to extracellular cell wall complexes of microbes and negatively charged microbial cell walls, thereby altering the cells' osmotic equilibrium. At high concentrations like 2% chlorhexidine, precipitation of cytoplasmic contents causes cell death.[17] A 2-minute rinse of 2% CHX liquid can be used to take away the *E. faecalis* from the superficial layers of dentinal tubules up to 100 µm.[18]

Hyperosmotic preparations of povidone iodine and sodium hypochlorite cause water loss from the cytoplasm, causing the cell to shrink (plasmolysis). This is detected by turbidity.[19] Microorganisms physiologically balance the emigration of free intracellular water due to the extracellular hyperosmotic value by replenishing the intracellular solute concentration by uptake or synthesis of small ions and molecules.[20] Extracellular high osmotic values are lethal since storing and synthesizing compatible solutes are energy-demanding processes. Once the stocks of compatible solutes or energy resources are depleted, microbes enter the stationary phase, and eventually.[12]

A hypertonic salt solution induces a hyperosmotic stress response due to which a loss of turgor was seen in the cell, causing loss of intracellular water. Water loss could cause membrane hardening and potential loss of structural integrity and eventual cell death. Solheim et al. in their study analyzed a high NaCl concentration induced expression of chaperons and cell envelope related traits like the enterococcal polysaccharide antigen (epa) locus, absolute repression of the gelE-sprE operon, and defective gelatinase activity.[21] This might be the reason why hypertonic salt solution gave a significant reduction in bacterial biomass.

In hypotonic preparations (0.25M) of 5.25% sodium hypochlorite and 2% chlorhexidine, a statistically significant antibacterial activity was achieved, compared to 5.25% sodium hypochlorite and 2% chlorhexidine without salt solutions (Tables 2 to 4). The rationale behind this is that after a decrease in osmolarity (hypoosmotic shock), there is a rapid influx of water into the cell, increasing turgor. This leads to expansion of the cell envelope, resulting in "cracks" in the membrane that may expand the existing pores (aquaporins) or activate stretch-activated channels. A downshift in osmolarity may result in the extrusion of osmolytes and ions in addition to the loss of amino acids, nucleotides, and other solutes from the cytoplasm. When adding a hypotonic solution, it leads to an influx of water into the cytoplasm, which causes the cell to swell (plasmoptysis) and may burst in a process called osmotic lysis, causing cell death.[19] There is limited to no evidence in the literature stating the effect of hypo osmosis in the field of root canal disinfection.

The hyperosmotic preparation of 2% chlorhexidine was not statistically significant with 2% chlorhexidine without salt solution at $p = 0.064$ at $p < 0.05$ (Table 3). When hyperosmotic salt solution is added to chlorhexidine, a white salt precipitate is obtained. This was similar to the findings of Prado et al.[22] They found that the precipitate formed between chlorhexidine and saline solution was attributed to the salting-out process, i.e., the introduction of the saline solution precipitated the chlorhexidine salts.

Hypoosmotic preparation (0.25M) of 2% povidone iodine did not have statistical significance compared with 2% povidone iodine within the group with $p = 0.076$ at $p < 0.05$ (Table 4). However, the mechanism of action remains unclear and needs to be further explored.

Limitations

Because this is an *in vitro* study, the clinical relevance is questionable, and further clinical trials are warranted. This study is being conducted in a 96-well microtitre plate where biofilm adhesion varies more than adhesion of biofilm to dentinal tubules. Despite not simulating the dentinal surfaces, the biofilms are grown on polystyrene wells because of the tissue culture treatment done by the manufacturer to promote cell attachment and growth.[23]

Conclusion

Within the limitations of the study, it was concluded that varying the osmolarities had a significant influence on the antibacterial efficacy of all the three irrigants. The addition of 6M hypertonic salt solution and 0.25M hypotonic salt solution to the irrigant resulted in a significant reduction in bacterial biomass. Irrigants without added salt solutions and irrigants to which 0.5M hypotonic salt solution was added had shown similar antibacterial efficacy.

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