

# Effect of Nisin on Root Dentine Microhardness - An Invitro Study

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**Abstract:** Successful treatment of apical periodontitis depends on elimination of the infective microflora from the necrotic root canal system and favors the healing of the periapex. Although chemo mechanical preparation of root canal is able to reduce the number of bacteria, an intracanal medicament with antibacterial action is required to maximize the disinfection of the root canal system in infected cases. The need for intracanal medication increases, especially in those cases where the infection is resistant to regular treatment and the therapy cannot be successfully completed due to the presence of pain or continuing exudates. Nisin, a naturally occurring antimicrobial peptide (discovered in 1928) is found to be effective against the *E. faecalis*. Nisin is produced by *Lactococcus lactis* and which is a class I bacteriocin. It is used as food preservative and found to be safe in human beings. It has been reported that the use of intracanal medicament are capable of causing alterations in the chemical composition of dentin. Micro-hardness determination helps to provide an indirect evidence of losing or gaining any mineral substance in the dental hard tissues. Hence this study is aimed at the evaluation of effect of nisin on root dentine microhardness.

**Keywords:** calcium hydroxide, intracanal medicament, microhardness, Nisin

## 1. Introduction

Success in endodontic treatment was originally based on the triad of debridement, thorough disinfection, and obturation of root canal system, with each and every aspects equally important.<sup>1</sup> Numerous measures have been introduced to reduce the number of microorganisms from the root canal system, including various mechanical instrumentation techniques, irrigation regimes, and intracanal medicaments. It is difficult to eliminate all microorganisms from an infected root canal system by mechanical instrumentation alone.<sup>2</sup> Hence the need for intracanal medication increases, especially in those cases where the infection is resistant to regular treatment and the therapy cannot be successfully completed due to the presence of pain or continuing exudates.<sup>3</sup> Since its introduction in 1920, calcium hydroxide has been widely used in endodontics as an inter-appointment intracanal medicament. It is a strong alkaline substance, which has a pH of approximately 12.5 and has various biological properties such as antimicrobial activity, tissue dissolving ability, inhibition of tooth resorption, and induction of repair by hard tissue formation. Because of such effects, calcium hydroxide has been recommended for use in several clinical situations.<sup>4</sup>

Its antibacterial effect is achieved through the ionic dissociation of Ca(2+) and OH(-) ions and their effect on vital tissues, the induction of hard-tissue deposition and the antibacterial properties. The lethal effects of calcium hydroxide on bacterial cells are probably due to protein denaturation and damage to DNA and cytoplasmic membranes.<sup>5</sup> In spite of various advantages and indications

of calcium hydroxide, it does have some limitations. There are some concerns regarding its effectiveness against several endodontic pathogens, including *Enterococcus faecalis* and *Candida* species, leading to various incidence of reinfection or flare up.<sup>6</sup> Furthermore, it has been observed that long term use of calcium hydroxide treated teeth showed a high failure rate because of an unusual preponderance of root fracture and it has been suggested that changes in the physical properties of dentin might be caused by the calcium hydroxide medicament.<sup>4</sup> Exposure of root dentin to the bioactive effects of calcium hydroxide may affect its physical characteristics and could have important clinical implications for the treatment of traumatized teeth and immature teeth with nonvital pulps.<sup>4</sup> A newer intracanal medicament Nisin, which is a naturally occurring antimicrobial peptide (discovered in 1928) is found to be effective against the *E. faecalis*. Nisin is produced by *Lactococcus lactis* and a class I bacteriocin. It is used as food preservative and found to be safe in human beings.<sup>3</sup> Hence this study was aimed to evaluate the effect of NISIN on root dentine microhardness in comparison with Ca(OH)<sub>2</sub>.

## 2. Materials and Methodology

This in vitro study was conducted in the department of conservative dentistry and endodontics at Krishnadevaraya college of Dental Sciences, Bangalore .

### 2.1 Preparation of Teeth

Fifteen extracted human canines were collected and stored in distilled water until required. The teeth were decoronated at the cemento-enamel junction using a diamond saw mounted on a slow speed micromotor under water coolant. A fifteen-sized k-file was introduced in the canal and the stopper was set at the point where the file was seen at the apex. Instrumentation with Protaper was done using the following sequence (S1 until 2/3rds of the WL- Sx till the middle third- S1 until the working length- then F1, followed by F2 and F3 until the working length). Between each step the canals were thoroughly irrigated with 5 ml of 5% sodium hypochlorite and saline. The roots were split longitudinally into two parts using diamond disc at slow speed, making a total of 30 segments and the root halves were horizontally embedded in auto polymerizing acrylic resin so that their dentin was exposed. The dentin surfaces of the mounted specimens were grounded flat and smooth on a digital polishing machine with a series of ascending grades of silicon carbide abrasive papers (600-grit, 1,200-grit, and 2,000-grit) under distilled water to remove any surface scratch and finally polished with 0.1-mm alumina suspension on a rotary felt disk.

### Determination of Microhardness

Three separate indentations were made at the cervical, middle, and apical levels of the root dentin in each sample with a Vickers diamond indenter microhardness tester at X 10 parallel to the edge of the root canal lumen at a depth of 0.5 mm from the pulp-dentin interface, each using a 200-g load and a 10-s dwell time and the pretreatment microhardness values were calculated

### Grouping of Samples

Specimens were randomly divided into two groups (n = 15) and were prepared as follows:

G1 — the Nisin group, in which the specimens were treated with Nisin application for 1 week. This was applied on the flattened smooth dentinal surface;

G2 — the CaOH group, in which the specimens were similarly treated with CaOH for 1 week.

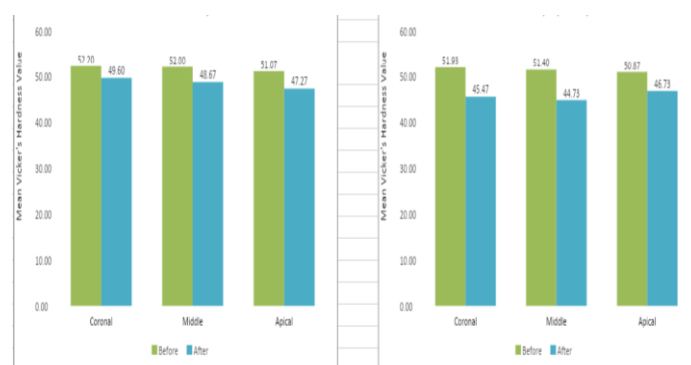
All the specimens were applied with intracanal medicament by using a microbrush. After 1 week the specimens were rinsed in distilled water, and blotted dry. Three indentations were made 0.5mm away from the pulp dentine interface and the microhardness values were calculated using the following formula [  $VHN = P \times 1.8544 / d^2$  (P=load applied in kg and d=arithmetic mean of the diagonals d1 & d2 caused by the indentation)].

## 3. Statistical Analysis

Independent Student t Test was used to compare the mean Vicker's Hardness values between two study groups at different thirds of the root specimens. Repeated measures of ANOVA followed by Bonferroni's post hoc analysis was used to compare the mean Vicker's Hardness values among different thirds in each study group. The level of significance will be set at  $P < 0.05$ .

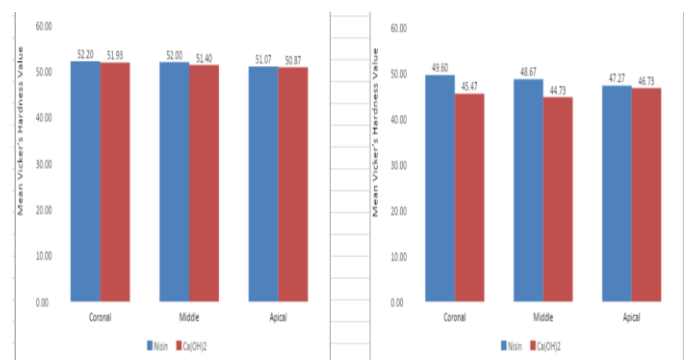
## 4. Results

The pre treatment microhardness values (mean  $\pm$  standard deviation) for all tested specimens for Nisin group at the cervical, middle, and apical levels were (52.20 $\pm$ 4.06, 52.00 $\pm$ 5.82, 51.07 $\pm$ 3.77) and CaOH group at the cervical, middle and apical levels were (51.93 $\pm$ 6.10, 51.40 $\pm$ 6.25, 50.87 $\pm$ 5.04) respectively. The intracanal medicament on application reduced the dentin microhardness at all the levels for both Nisin and CaOH groups. In the Nisin group, the percentage reduction in microhardness was less at the coronal level than the middle and apical level where as in CaOH group, less reduction in the microhardness was observed at the apical level than the coronal and middle level. Nisin group showed less reduction in microhardness compared to CaOH group and the difference were statistically significant.



**Graph 1A:** Mean vicker's microhardness values between before and after Nisin application.

**Graph 1B:** Mean vicker's microhardness values between before and after CaOH application.



**Graph 2A:** Mean vicker's microhardness values between Nisin and CaOH group before application.

**Graph 2B:** Mean vicker's microhardness values between Nisin and CaOH group after application.

## 5. Discussion

The present study compared the microhardness of the root dentine between Nisin and the calcium hydroxide group. Microhardness was used as a parameter to assess the strength of the teeth. Microhardness of a material is not a measure of

a single property as it is influenced substantially by other fundamental properties of the material such as yield strength, tensile strength, modulus of elasticity and crystal structure stability. Thus, it can be used as an indicator of the overall strength or resistance to deformation when compared with baseline information. Vicker's microhardness testing was employed as it is more sensitive to measurement errors, less sensitive to surface conditions and small specimens can be tested with good accuracy. For evaluation of a reduction in hardness, standardization was done by estimating the pretreatment hardness of every sample and then comparing with the posttreatment hardness.<sup>4</sup>

CaOH is the most commonly used dressing for treatment of the vital pulp. It also plays a major role as an inter-visit dressing in the disinfection of the root canal system.<sup>5</sup> The action of calcium hydroxide in the root canal space mainly depends on its dissociation into calcium and hydroxyl ions. CaOH (pH 12.5) molecules have a highly alkaline inorganic structure, and because of their small size, they can penetrate the intrafibrillar structure of the mineralized collagen fibrils and alter the 3-dimensional conformation of tropocollagen. As a result, it reduces the elastic modulus and microhardness of dentin. Consequently, because of the alkaline properties of CH, it has a strong denaturing effect on the organic matrix of dentin.<sup>7</sup>

Nisin (Mol. Wt 3500) is a naturally occurring antimicrobial peptide and was discovered in 1928. It is a cationic peptide antibiotic produced by streptococcus lactis sub sp lactis. Nisin is safe to humans and is used extensively as a food preservative in over 40 countries, mainly in preservation of meat and dairy products.<sup>8</sup> The effect of Nisin as an antibacterial agent is recorded in several studies. An in vitro investigation of the antibacterial effect of Nisin in root canals and canal wall radicular dentin found that Nisin is effective against *E. faecalis*. From previous studies, the mode of action of nisin have shown that this basic polypeptide acts by inserting into the plasma membrane and triggering the activity of bacterial murein hydrolases resulting in damage or degradation of the peptidoglycan and lysis of the cells.<sup>3</sup> Hemadri et al, found *E. faecalis* showed increased resistance to Calcium hydroxide than to Nisin. The mode of action of Nisin differs to that of calcium hydroxide, and is not reliant on a highly alkaline environment for effective killing.<sup>8</sup>

In the current study, dentin treated with Nisin groups showed no significant reduction in microhardness at the cervical, middle, and apical levels. The ability of nisin to effectively kill *E. faecalis* by a mechanism that is not reliant on achieving a high pH may provide a means to eliminate this species by a method to which it has no defense mechanism. In the present study, investigation of Nisin as an Intracanal medicament on root dentine microhardness has provided some encouraging results. However, the systemic effect of this medicament, its biocompatibility, allergic potential, and bacterial resistance needs further investigation.

## 6. Conclusion

Within the limitations of this study, Nisin has substantial antibacterial activity with no effect on microhardness of the radicular dentin layer at all the levels. Hence Nisin has a potential to be used as intracanal medicament if its antibacterial activity could be enhanced.

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