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Comparative Evaluation of Anti - Bacterial Activity of Nisin Polypeptide Coated Gutta Percha and Conventional Gutta Percha against Enterococcus Faecalis Using Agar Diffusion Test- An in Vitro Study

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Abstract

Background: E.faecalis has been a constantly recovered from persistent periapical infections. It is one of the primary causes of retreatment. Nisin, a polypeptide has shown to be effective against this organism when used as intracanal medicament, thus the present study assessed antimicrobial efficacy of nisin coated gutta percha against E.faecalis.

Materials and Method: Forty 6% taper GP cones were used for the study. Twenty cones were coated by nisin polypeptide using 5g of powder in 100ml solution of ethanol and 100ml of distilled water. E.faecalis (ATCC 29212) was cultured using blood agar and transferred in BHI media. After that it was inoculated in Mueller hinton agar media. The petri dishes were divided in two halves and each received one test and one control GP cones. They were incubated for 24 hours. The zone of inhibition was measured using vernier calliper. They were incubated again for next 24 hours and final zone of inhibition was measured at 48 hours post inoculation.

Statistical analysis was done using students t test with significance set at $p<0.05$.

Results: Nisin coated GP had greater zone of inhibition (1.63) than non-coated GP (0.31). The difference was statistically significant.

Conclusion: Nisin polypeptide coating notably enhances the antibacterial activity of gutta percha against E. faecalis under in vitro conditions, suggesting potential clinical benefits in reducing endodontic failure rates.

Keywords: Nisin polypeptide, Gutta percha cones, Enterococcus faecalis, direct contact test

Introduction

The endodontic infections are caused by complex clusters of microorganisms. Persistent microbes such as Enterococcus faecalis (E. faecalis) have been isolated from the infected canals with a prevalence as high as 24%–77%.[1] The aim for the endodontic therapy remains to completely eradicate the root canal of this microbial flora. The complete cleaning and shaping of the canal remove the bacteria present within the direct reach of instrumentation while the chemical disinfection used eliminate the persistent pathogens from the difficult to reach areas. However, a 100% sterilization of the canal is difficult to achieve and hence placing material with sustained antimicrobial efficacy will prevent the chances of reinfection.[2]

To achieve hermetic seal is a fundamental requirement for a successful endodontic prognosis. It cuts off infiltration of the canal with external flora as well as maintains the sanctity of the disinfected canal. Gutta percha (GP) has been an ideal material for obturation. The viscoelastic and thermoplastic properties of GP along with an endodontic sealer, allows efficient sealing of the canal. It has been a gold standard for obturation however, there has been certain shortcomings in the form its antibacterial efficacy hence, various methods have been employed to overcome this.[3] In the past, the antimicrobial efficacy has of GP have been enhanced using incorporation or surface coating with materials such as tetracycline, iodoform and chlorhexidine. These agents have been effective against a broad spectrum of microorganisms but E.faecalis have been eluding most of them.[4,5] Thus, need of such a material which can be efficiently target E.faecalis have been pursued in various researches. This search directed us towards Nisin, a polypeptide.

Nisin is a lantibiotics, a term used for antimicrobial peptide produced by lactic acid producing bacteria. It is isolated from several strains of Lactococcus lactis. It has an antibacterial property against both gram positive as well as gram negative bacteria. The mechanisms include ion leakage, inhibition of cell wall synthesis and pH disbalance causing cell death.[6] It have been used with various vehicles for canal medicament and produced positive results. It was effective against E.faecalis.[7] Thus, the present study used the nisin coated GP and compared it against the non-coated GP against E.faecalis strains in order to assess the antimicrobial efficiency of nisin. The null hypothesis proposed no difference between coated and non-coated GP.

Materials and Method

The study was conducted in department of Conservative dentistry and Endodontics and department of microbiology (Pt. JNM Medical College). This in-vitro study included 40 samples in total based on the effect size calculated as 0.42[8] at 80% power keeping 95% confidence interval using A priori analysis of t test in G power software. GP cones used were of Dentsply sirona, with 6% taper. The samples were divided in two groups as:

- Test group: GP cones coated with bioactive nisin

- Control group: GP without any coating or conventional GP

Coating of Nisin: Nisin solution was prepared using 5g of powder in a solution of 100ml ethanol as well as100ml of distilled water. GP cones were sterilized and placed in these/each solution for 48 hours. After that they were removed and air dried in a controlled environment. (Sarvamangal Healthcare Pvt Ltd)

Bacterial strain preparation: A standard strain of Enterococcus faecalis (ATCC 29212) was employed in this study. E.faecalis was carried on to blood agar plate using sterile loop and then incubated at37°C for 24 hours. After that, a single E.faecalis colony was isolated from the same cultured plate and Gram's staining was performed to confirm the purity of microbial growth, it was checked under oil immersion microscope under 1000X magnification (Labomed, California, united states). Brain heart infusion broth was prepared using 7.5 grams of powder in 200ml distilled water and sterilized in autoclave. Then bacteria were inoculated to brain-heart infusion (BHI) broth (Hi Media, Mumbai, India) using a sterile loop. The BHI-broth was incubated at 37°C for 24 hours period and checked for bacterial growth by changes in turbidity of the media.

Antimicrobial activity by agar diffusion assay: Petri dishes containing Mueller hinton agar (Hi Media, Mumbai, India) were inoculated with bacteria by pouring the BHI broth enriched with E.faecalis. Each petri dish was marked and divided into two equal portions. A flame sterilized tweezer was used for placement of conventional GP (n=20) on the left side and nisin coated GP (n=20) on the right side. Mueller hinton agar plates were then incubated for 24 hours at 37 degree celsius. After 24 hours the largest zone of inhibition is measured using digital vernier caliper. It was again incubated for next 24 hours and again measured using digital vernier caliper. Thus, two measurements were taken 24 hours apart. The largest zone of inhibition was measured in terms of millimeters. (figure 1)

Statistical analysis: The data obtained by measuring zone of inhibition was statistically analysed by using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). A comparison of the antibacterial activity of nisin coated gutta percha and conventional gutta percha was evaluated using student's t-test. Statistical significance was obtained when probability value was less than 0.05.

Results

A zone of inhibition was seen in both test and control groups. The zone of inhibition observed in test control was significantly more pronounced than the control group. On comparison of means of both test and control groups there was statistically significant difference at 24 hrs (1.04) as well as 48 hrs (1.32). (Table 1 and 2)The results suggested greater antimicrobial activity of the nisin coated GP cones as compared to conventional GP cones.

Discussion

The infected pulp and biofilm created within an apical infection is removed using the chemo-mechanical preparation in the endodontic therapy. Even with meticulous preparation, there is still a chance of resistant bacteria which may evade removal. The statistics of the previous literature suggests that E.faecalis has been stubbornly present in the persistent periapical lesions as well as the secondary infections after a primary therapy.[9] It has been attributed to mutation of the antibiotic-resistant genes through pheromone prompted dissemination within its own species.[10] It possesses lytic enzymes, aggregation substances and lipoteichoic acid and has ability to adhere onto and within the dentinal tubules. It can penetrate upto 500 to 1000 μm.[11] Since it

has been routinely isolated from reinfections lesions, it was used as the bacteria of choice to perform this study. Although there have been studies which used an antimicrobial infused or coated GP, E.faecalis eradication is still a hitch for reducing the chances of reinfection.

Nisin, a product obtained from plants, is colorless and odourless product which has been widely used as preservative and is safe for humans. It had demonstrated antibacterial activity against E. faecalis when used in a different manner thus, it was used as a coating on the obturation material. It has the capacity to integrate with bacterial plasma membrane. This causes lysis of peptidoglycans and disruption of cell.[12] It was assumed that a coating on GP can produce the direct contact within the canal and thus the agar diffusion method was chosen for the study.[13] The results have shown effective inhibition of E. faecalis strain during the 24 and 48 hours of incubation period.

Nisin has been used in earlier studies[10,14,15] where it has demonstrated antibacterial activity against E. faecalis. In these studies, nisin has been used as an intracanal medicament, thus a direct comparison to our study was not applicable however, this was the basis of using this component on to the coating of GP. Nisin has shown that the mechanism of its bactericidal activity is independent of pH and it can function both in acidic and alkaline state making it a suitable candidate for use in infectious lesions.[16] Moreover, as the direct contact with the root canal when used as a coating of obturating material, it may penetrate to depth of dentinal tubules reaching that tissue bound E.faecalis. Chinni et al[17], in their study have exhibited that nisin does not significantly affects the microhardness of dentin which can be added advantage in clinical applications of nisin coated GPs. In a study done by Haryanti et al, the authors have found that efficacy of nisin against E.faecalis is dose dependent and is achieved porous formation due to lipid II molecule. It had shown reduced activity as compared to other test materials.[18]

In case of direct contact method, GP cones were efficacious but clinical protocol dictates placement of sealer within the root canal, thus the antibacterial activity in presence of a sealer coating was not determined in the present in vitro study which could be a limitation of the study. Another drawback is that the zone of inhibition was almost similar within 24 hours apart, which does suggest that antibacterial activity has reached its maximum, though a similar distance imply continuous action during those 48 hours. Thus, in order to apply this clinically in vivo, standardization of the nisin coated GP cones and its commercial preparation is proposed by the authors based on the results of the study.

Conclusion

The present employed nisin as a coating on the GP cones in order to assess it activity in condition of direct contact within the canal. The result of 24 and 48 hours have shown effective antibacterial activity against E. faecalis establishing its novelty. Although other medicated products as coating or incorporating in GP have been used in the past, the efficacy against E.faecalis was diminished. Due to coating on the GP, antibacterial activity of nisin will not be confined to one particular organism but will combat wide range of micro-organisms post obturation, reducing the chancers of reinfection and flare-ups.

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Legend Tables

Table 1: Mean and standard deviation of test and control group

Group Descriptives						
		N	Mean	Median	SD	SE
24 hours	Control	20	0.316	0.00	0.557	0.125
	Test	20	1.36	1.52	0.719	0.161
48 hours	Control	20	0.316	0.00	0.557	0.125
	Test	20	1.63	1.80	0.762	0.170

SD: Standard deviation, SE: Standard error, Group A: Conventional gutta percha, Group B: Conventional gutta percha

Table 2: T-test comparison of test and control group

Students ’s t-test						
		Statistic	df	p	Mean difference	SE difference
24 hours	Student's t –test	5.12	38.0	<.001*	1.04	0.203
48 hours	Student's t –test	6.23	38.0	<.001*	1.32	0.211

df: Degree of freedom, P: probability value

Figure 1: Measurement of antibacterial activity against E.faecalis