Comparative Evaluation of the Antimicrobial Efficacy of 3% Nisin-Modified Endoflas F.S and Conventional Endoflas F.SAgainst *Escherichia coli*: An In Vitro Study.

Abstract:

Background

Successful pulpectomy in primary teeth relies on effective elimination of microorganisms from the root canal system and prevention of reinfection. Endoflas F.S. is a widely used obturating material in pediatric endodontics owing to its broad-spectrum antimicrobial activity, favorable resorption pattern, and biocompatibility. However, the emergence of antimicrobial resistance has prompted the search for novel modifications capable of enhancing the antibacterial properties of existing obturating materials. Nisin, a naturally occurring antimicrobial peptide (lantibiotic), has demonstrated potent activity against several oral pathogens and has recently gained attention as a promising additive in dental biomaterials.

Aim

To compare the antimicrobial efficacy of 3% (w/w) Nisin-modified Endoflas F.S. and conventional Endoflas F.S. against *Escherichia coli* using agar disc diffusion and direct contact tests.

Materials and Methods

An in vitro experimental study was conducted using two experimental groups: conventional Endoflas F.S. and 3% (w/w) Nisin-modified Endoflas F.S. Antibacterial activity was evaluated against *Escherichia coli*. Agar disc diffusion was performed to determine the zone of inhibition after 24 hours of incubation on Mueller–Hinton agar. Direct antibacterial activity was assessed using the direct contact test by measuring optical density spectrophotometrically at 650 nm after 3, 6, and 24 hours.

Results

The 3% Nisin-modified Endoflas F.S. demonstrated a larger zone of inhibition than conventional Endoflas F.S. The direct contact test also revealed lower optical density values for the modified material at all observation periods, indicating enhanced antibacterial activity and greater inhibition of bacterial growth.

Conclusion

Incorporation of 3% (w/w) Nisin significantly improved the antimicrobial efficacy of Endoflas F.S. against *Escherichia coli*. Nisin-modified Endoflas may represent a promising bioactive obturating material capable of enhancing disinfection during pulpectomy procedures in primary teeth. Further in vivo and clinical studies are recommended to validate these findings.

Keywords: Endoflas F.S; Nisin; Pediatric Endodontics; Obturating Material; Antimicrobial Activity; *Escherichia coli*; Direct Contact Test; Agar Disc Diffusion Test.

Introduction

Pulpectomy remains one of the most effective treatment modalities for retaining primary teeth affected by irreversible pulpitis or pulpal necrosis until their normal exfoliation. Preservation of primary teeth is essential for maintaining arch integrity, mastication, phonetics, esthetics, and proper eruption of the permanent dentition.[1] The success of pulpectomy largely depends on thorough chemomechanical debridement of the root canal system followed by complete obturation using a material capable of eliminating residual microorganisms while exhibiting favorable biological properties.[2,3]

Despite advances in instrumentation and irrigation protocols, complete eradication of microorganisms from the complex root canal anatomy of primary teeth remains challenging because of accessory canals, ribbon-shaped canals, physiological root resorption, and numerous anatomical irregularities.[4] Residual microorganisms within the canal system are considered one of the major causes of endodontic failure, emphasizing the need for obturating materials possessing sustained antimicrobial activity.[5]

An ideal obturating material for primary teeth should exhibit excellent antimicrobial efficacy, biocompatibility, resorbability in harmony with physiological root resorption, ease of manipulation, radiopacity, dimensional stability, and minimal irritation to the surrounding tissues.[6] Although several obturating materials have been introduced for pediatric endodontics, none fulfills all the characteristics of an ideal material. Consequently, continuous modifications are being explored to improve their biological and antimicrobial performance.[7]

Endoflas F.S. is a zinc oxide eugenol-based obturating material containing iodoform, calcium hydroxide, and other constituents that provide broad-spectrum antimicrobial activity and favorable clinical outcomes. Owing to its ability to resorb extra-radicularly while remaining relatively stable within the canal, Endoflas has gained widespread acceptance in pediatric endodontics.[8,9] However, the increasing prevalence of antimicrobial resistance and the persistence of resistant microorganisms have encouraged researchers to investigate naturally derived antimicrobial agents capable of enhancing the antibacterial efficacy of conventional dental materials.[10]

Nisin is a naturally occurring antimicrobial peptide (lantibiotic) produced by *Lactococcus lactis* and has been safely used as a food preservative for several decades. It is recognized as safe by both the United States Food and Drug Administration (FDA) and the World Health Organization (WHO).[11] Nisin exerts its antibacterial action by binding to lipid II, an essential precursor in bacterial cell wall synthesis, leading to pore formation, membrane disruption, and rapid bacterial cell death.[12] Unlike conventional antibiotics, Nisin demonstrates a low propensity for inducing bacterial resistance and has shown excellent activity against several oral pathogens, making it an attractive candidate for incorporation into dental biomaterials.[13]

Escherichia coli was selected as a standardized laboratory organism because of its predictable growth and reproducible response to antimicrobial agents, making it suitable for comparative in vitro evaluation of dental materials.

Recent studies have demonstrated successful incorporation of Nisin into restorative materials such as glass ionomer cements, pit and fissure sealants, and endodontic sealers without adversely affecting their physical properties.[14,15] Modification of obturating materials with Nisin may therefore provide enhanced antibacterial activity while maintaining the desirable clinical characteristics of the parent material.

Although previous investigations have explored the antimicrobial potential of Nisin in restorative and endodontic materials, limited evidence exists regarding its incorporation into Endoflas F.S. for pediatric endodontic applications. Therefore, the present in vitro study aimed to compare the antimicrobial efficacy of 3% (w/w) Nisin-modified Endoflas F.S. and conventional Endoflas F.S. against *Escherichia coli* using agar disc diffusion and direct contact tests. It was hypothesized that incorporation of Nisin would significantly enhance the antibacterial efficacy of Endoflas F.S.

MATERIALS AND METHODS

The present investigation was designed as an **in vitro experimental study** to evaluate and compare the antimicrobial efficacy of **3% (w/w) Nisin-modified Endoflas F.S.** with conventional **Endoflas F.S.** against *Escherichia coli*. The antimicrobial activity of the test materials was assessed using the **agar disc diffusion method** and the **direct contact test**, two established laboratory techniques for evaluating the antibacterial properties of dental materials.

Materials

The materials used in the study included Endoflas F.S. (Sanlor Laboratories, Sanlor& Cia. S. en C.S., Colombia) and Nisin powder procured from CK's Ingredients Pvt. Ltd., Tripunithura, Kochi, Kerala, India. Mueller-Hinton Agar (MHA) served as the culture medium for bacterial growth, and *Escherichia coli* was used as the test microorganism.

Preparation of 3% Nisin-Modified Endoflas

Nisin was incorporated into Endoflas F.S. at a concentration of **3% (w/w)**. The required amount of Nisin powder was accurately weighed using a precision analytical balance and thoroughly blended with the Endoflas powder to obtain a homogeneous mixture. The modified powder was subsequently mixed with the manufacturer's recommended liquid immediately before testing. Conventional Endoflas F.S. prepared according to the manufacturer's instructions served as the control material.

Experimental Groups

Group I: Conventional Endoflas F.S.

Group II: 3% (w/w) Nisin-modified Endoflas F.S.

Microbiological Procedure

A fresh culture of *Escherichia coli* was prepared under aseptic laboratory conditions. The bacterial suspension was uniformly inoculated onto Mueller-Hinton Agar plates using sterile cotton swabs to produce a confluent bacterial lawn.

Agar Disc Diffusion Test

Sterile paper discs impregnated with freshly mixed conventional Endoflas F.S. and 3% Nisin-modified Endoflas were placed on the inoculated agar surface. The plates were incubated aerobically at **37°C for 24 hours**. Following incubation, the diameter of the **zone of inhibition** surrounding each disc was measured in millimetres using a digital Vernier caliper. Greater inhibition zones indicated superior antibacterial activity.

Direct Contact Test

The direct contact test was performed to evaluate antibacterial activity following direct interaction between the material and bacterial suspension. Equal quantities of each test material were exposed to *E. coli*, and bacterial growth was assessed spectrophotometrically by measuring optical density (OD) at **650 nm** after **3, 6, and 24 hours**. Lower optical density values indicated greater inhibition of bacterial proliferation.

Outcome Measured

The primary outcome measured included:

- Diameter of the zone of inhibition (mm)
- Optical density values at 3, 6, and 24 hours

RESULTS:

The results of the present in vitro study are presented using figure, tables and graphical representations to illustrate the comparative antimicrobial efficacy of conventional EndoflasF.S. and 3% (w/w) Nisin-modified Endoflas F.S. The zone of inhibition and optical

density values obtained from the agar disc diffusion and direct contact tests are summarized below.

Agar Disc Diffusion Test

The agar disc diffusion test demonstrated superior antibacterial activity for the 3% Nisin-modified Endoflas F.S. compared with conventional Endoflas F.S. The modified material exhibited a **zone of inhibition of 2.3 cm**, whereas conventional Endoflas F.S. produced a zone of **2.1 cm(Figure 1)**. The graphical representation of the zone of inhibition is shown in graph 1, and the percentage zone of inhibition is presented in graph 2. The enhanced inhibition observed with the modified material suggests improved diffusion of antibacterial components and greater inhibitory activity against *E. coli*.

Table 1. Zone of inhibition

Group	**Zone of inhibition (cm)**
Conventional Endoflas F.S.	2.1
3% Nisin-modified Endoflas F.S.	2.3

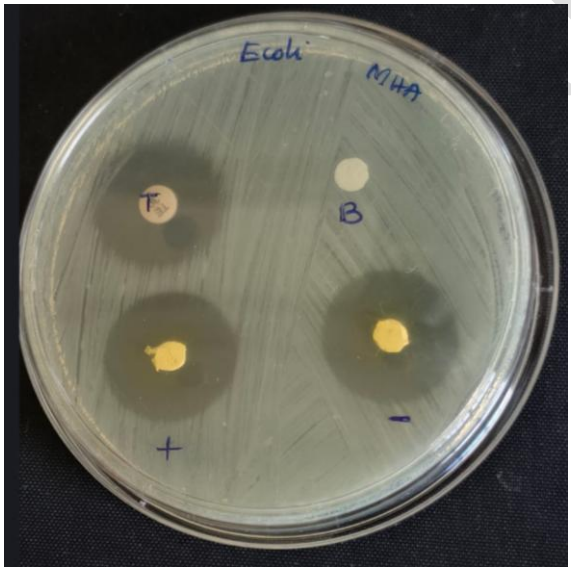
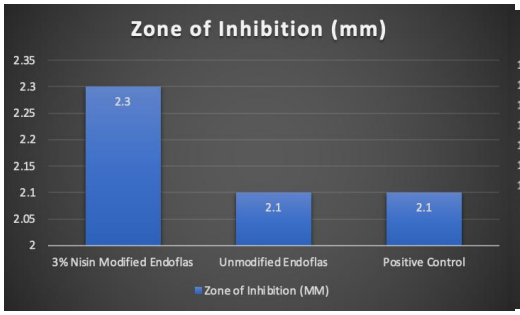
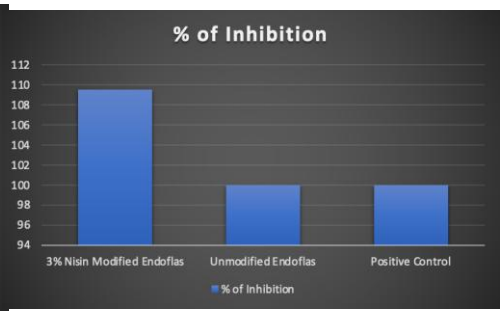


FIGURE 1





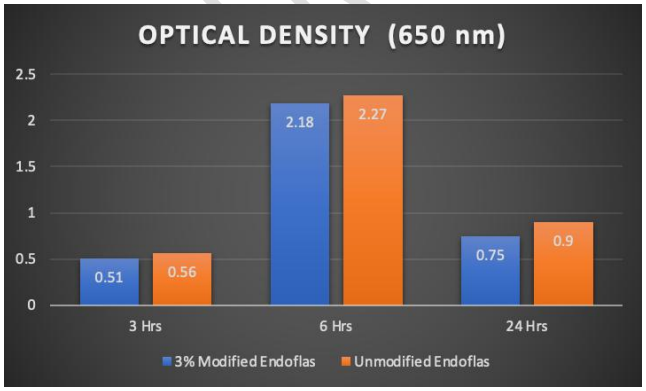
GRAPH 1 GRAPH 2

Direct Contact Test

Optical density values recorded at 3, 6, and 24 hours consistently demonstrated lower bacterial growth in the Nisin-modified group than in the conventional Endoflas group. (Table 2 and Graph 3)

Table 2. Optical density values

Time	Conventional Endoflas	3% Nisin-modified Endoflas
3 hours	0.56	0.51
6 hours	2.27	2.18
24 hours	0.90	0.75



Graph 3

These findings indicate that incorporation of Nisin enhanced the antibacterial efficacy of Endoflas throughout the experimental period.

DISCUSSION

Successful pulpectomy in primary teeth depends on effective elimination of microorganisms and prevention of reinfection. The complex anatomy of primary root canals often limits complete microbial removal through instrumentation alone, highlighting the importance of obturating materials with intrinsic antimicrobial activity.[1–3]

Endoflas F.S. has gained widespread acceptance in pediatric endodontics because of its antimicrobial properties, favorable resorption pattern, and biocompatibility. Nevertheless, persistent microorganisms and increasing antimicrobial resistance have encouraged the incorporation of naturally derived antimicrobial agents into conventional dental materials.[4,5]

Nisin is a polycyclic antibacterial peptide (lantibiotic) produced by *Lactococcus lactis*. It exerts its antibacterial effect by binding to lipid II, thereby inhibiting cell wall synthesis and creating pores in the bacterial membrane, resulting in rapid cell death.[6] Unlike conventional antibiotics, Nisin demonstrates a low tendency for bacterial resistance and has been recognized as safe for human use by both the WHO and the FDA.[7]

In the present study, incorporation of 3% (w/w) Nisin into Endoflas F.S. resulted in enhanced antibacterial activity compared with conventional Endoflas F.S., as evidenced by both the agar disc diffusion and direct contact tests. The larger inhibition zone observed for the modified material indicates improved antibacterial diffusion and inhibitory capacity. Similarly, consistently lower optical density values at 3, 6, and 24 hours indicate sustained suppression of bacterial growth following direct contact.

These findings are consistent with those of Hegde et al., who reported that incorporation of Nisin into high-viscosity glass ionomer cement significantly enhanced antibacterial activity without adversely affecting its physical properties.[14] These findings support the concept that Nisin can be successfully incorporated into dental materials while preserving their functional properties. Likewise, Chan et al. highlighted the broad potential of Nisin in oral healthcare because of its potent antimicrobial activity and excellent safety profile.[11] Niaz et al. also

demonstrated that Nisin exhibits a low rate of bacterial resistance, making it an attractive antimicrobial adjunct for biomedical applications.[13]

The antimicrobial activity demonstrated by the Nisin-modified Endoflas observed in the present study can be attributed to the unique mechanism of action of Nisin. Unlike conventional antibiotics, Nisin binds specifically to lipid II, an essential precursor involved in bacterial cell wall synthesis, resulting in membrane pore formation, disruption of membrane integrity, and rapid bacterial cell death. This dual mechanism contributes to its potent antibacterial activity while minimizing the development of bacterial resistance.[16]

Recent advances in pediatric dental biomaterials have increasingly focused on incorporating naturally derived antimicrobial agents to improve the biological performance of restorative and endodontic materials. Antimicrobial peptides such as Nisin offer several advantages over conventional antibiotics, including broad-spectrum antibacterial activity, excellent biocompatibility, biodegradability, and a reduced likelihood of inducing antimicrobial resistance.[17]

Although *Enterococcus faecalis* is the microorganism most frequently associated with persistent endodontic infections, *Escherichia coli* was selected in the present investigation as the test organism because it provides a standardized laboratory model for evaluating antimicrobial activity under controlled in vitro conditions. Future studies should investigate the efficacy of Nisin-modified Endoflas against polymicrobial biofilms and clinically relevant endodontic pathogens to better simulate the microbiological complexity of infected primary root canals. The present findings suggest that incorporation of Nisin enhances the antibacterial potential of Endoflas F.S. without altering the manipulation protocol. Such modification may improve microbial control during pulpectomy procedures and contribute to greater long-term clinical success. Nevertheless, further investigations evaluating physicochemical properties, cytocompatibility, sealing ability, solubility, and long-term clinical performance are required before routine clinical application.

CLINICAL SIGNIFICANCE

The incorporation of **3% (w/w) Nisin** into Endoflas F.S. enhanced its antibacterial activity against *Escherichia coli* under laboratory conditions. Nisin-modified Endoflas has the potential to improve root canal disinfection during pulpectomy procedures in primary teeth and may serve as a promising bioactive obturating material in pediatric endodontics.

LIMITATIONS

- In vitro design may not replicate the clinical environment.
- Only *Escherichia coli* was evaluated.
- Biofilm models were not investigated.
- Cytocompatibility using fibroblast and stem cell cultures.
- Micro-computed tomography (micro-CT) evaluation of obturation quality.
- Long-term physicochemical properties were not assessed.

CONCLUSION

Within the limitations of this in vitro study, incorporation of **3% (w/w) Nisin** into Endoflas F.S. enhanced its antibacterial efficacy against *Escherichia coli*. The modified material demonstrated a larger zone of inhibition and lower optical density values compared with conventional Endoflas F.S., indicating superior antimicrobial performance. Nisin-modified Endoflas may represent a promising bioactive obturating material for pediatric endodontics. Further laboratory investigations and well-designed clinical studies are required to confirm its biological safety, physicochemical properties, and long-term clinical effectiveness.

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