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An Invitro Evaluation of Antimicrobial and Anti-Adhesive Properties of Nano-Chitosan, Biodentine and Theracal Against Dental Biofilms- Sem Analysis

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KEYWORDS

Nanochitosan Theracal, antimicrobial, biodentin

ABSTRACT:

Introduction: Vital pulp therapy has gained importance with the advent of bioactive pulp capping materials. The success of these materials depends largely on their antimicrobial properties and ability to prevent biofilm formation. Nano-chitosan, Biodentine, and TheraCal LC are widely used materials with varying biological and physicochemical characteristics.

Objectives:To evaluate and compare the antimicrobial and anti-adhesive properties of nano-chitosan, Biodentine, and TheraCal against dental biofilms using scanning electron microscopy (SEM)

Methods: Thirty non-carious extracted molars were collected and sectioned to obtain dentin discs. The specimens were demineralized and randomly divided into three groups (n=10): nano-chitosan, Biodentine, and TheraCal. Materials were applied in a standardized thickness and incubated in artificial saliva. The samples were then inoculated with Streptococcus mutans and incubated for 24 and 48 hours. Biofilm formation was assessed using crystal violet assay, colony-forming unit (CFU) count, and SEM analysis. Statistical analysis was performed using one-way ANOVA and post hoc tests with significance set at $p<0.05$.

Results: Nano-chitosan demonstrated significantly reduced bacterial adhesion and disrupted biofilm architecture compared to other groups. Biodentine showed moderate antimicrobial activity with reduced but evident biofilm formation. TheraCal exhibited comparatively higher bacterial adhesion and denser biofilm structure. SEM analysis confirmed smoother surfaces and minimal bacterial colonization in the nano-chitosan group. The differences among groups were statistically significant.

Conclusions: Nano-chitosan exhibited superior antimicrobial and anti-adhesive properties against dental biofilms compared to Biodentine and TheraCal. Its dual action in inhibiting bacterial growth and promoting remineralization highlights its potential as an effective pulp capping material. Further clinical studies are recommended to validate these findings.

1. Introduction

Emphasis has shifted from the “Doomed” organ concept of an exposed pulp to one of the hope and recovery. The era of vital-pulp therapy has been greatly enhanced with introduction of various pulp capping agents.[1]

Pulp capping material should have the ideal properties like stimulating reparative dentin formation, maintain pulpal vitality, release fluoride to prevent secondary caries, adhere to dentin, adhere to restorative material and provide bacterial seal.[2]

Chitosan is a polysaccharide produced from chitin.[3]chitin is a polymeric compound in most shelled marine animals, such as shrimps, crabs, fish scales.

Chitosan has bioactive, anti-inflammatory, and non-toxic properties and plays an active role in wound healing, bone repair and hemostasis. Therefore, the use of

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chitosan is now being developed as alternative medicament in dentistry.[3]

Nano-chitosan is a nanostructured derivative of chitosan. It has high surface area and enhances interaction with pulp tissue and growth factors. [4]

Biodentine is a calcium silicate- based bioactive material introduced as a "bio-dentin" substitute due to its physicochemical and biological properties closely resembling natural dentin.[5]

Clinically, biodentine offers advantages such shorter setting time, good handling charecteristics and mechanical properties similar to dentin making it suitable for pulp capping agent.[6]

TheraCal LC is a light-curable resin-modified calcium silicate material developed as a pulp capping agent .it combines calcium silicate with a resin matrix allowing immediate light activation, easy handling.[7]

TheraCal LC releases calcium and hydroxide ions, creating an alkaline environment that is believed to stimulate apatite formation and reparative dentinogenesis and provides protective seal to the exposed pulp tissue.[8]

when it comes to detailed visualization of biofilm formation and material interaction at the microstructural level SEM & EDS gives valuable insights.[9]

2. Objectives

The purpose of the study is to Compare and Evaluate:

- Antimicrobial properties of Nanochitosan, Biodentine and Theracal
- Anti-adhesive properties of Nanochitosan, Biodentine and **theracal** against dental biofilms using SEM analysis.

3. Methods

A total of 30 non-carious multi rooted teeth extracted for periodontal or orthodontic purpose were collected for the study. Teeth with carious lesion, severe attrition, fractures, cracks & developmental defects, open apices were excluded.

Thirty molars was collected for the study and extracted teeth were cleaned of debris and placed in chloramine T solution till use.

Teeth were sectioned vertically to obtain dentin discs and immersed in demineralizing solution lactic acid buffer 0f 4.5 Ph for 48hrs to induce demineralization.

Demineralized discs were treated with different pulp capping agents.

Nanochitsan - powder is mixed with 1% Acetic acid until homogenous mass is obtained.

Biodentine- is mixed with distilled water until homogenous mass is obtained.

Theracal- is applied and light cured for 20secs

Thin layer of 2mm thickness of pulp capping agents applied on tooth surface. Discs are incubated in artificial saliva at 37c for 14days. Mineral content are assessed to determine remineralization efficacy by SEM. Dentin discs then incubated with S.mutans cultures. After 24 and 48hrs, biofilm formation is evaluated using crystal violet assay to quantify biofilm biomass. Biofilm- covered discs are stained with crystal violet, and absorbance is measured at 590nm to quantify biofilm mass.

The specimens was randomly divided into 3 groups.

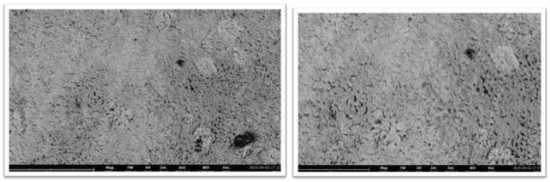
GROUP 1: Demineralized dentin treated with Nanochitosan

GROUP 2: Demineralized dentin treated with Biodentin

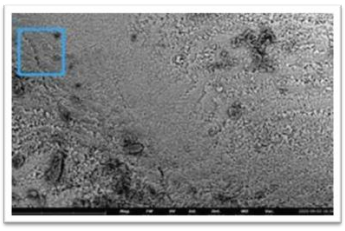
GROUP 3: Demineralized dentin treated with Theracal

4. Results

SEM IMAGES OF DEMINERALIZED SAMPLES



SEM IMAGES OF REMINERALIZED SAMPLES



NANOCHITOSAN

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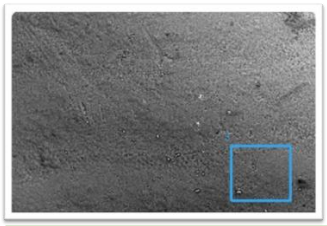
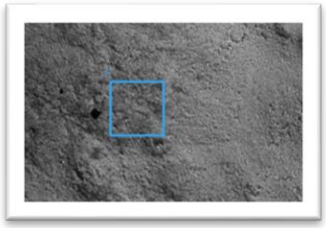


BIODENTINE

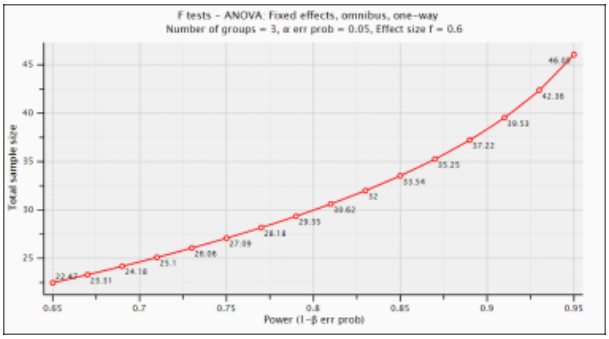


THERACAL

STATISTICAL ANALYSIS

The sample size estimation was performed at 5% alpha error (α = 0.05), with an effect size of 0.60 [Based on the Cohen classification of effect size for more than 2 groups, a larger effect size of 0.60 will be considered b/w 3 groups] & the power of the study at 80%, demonstrated that a minimum of 30 samples was needed for the present study. So, each study group was consisted of 10 samples [10 samples x 3 groups = 30 samples].

POWER ANALYSIS CURVE:



Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0 Released 2013 Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis includes expression of remineralization potential & absorbance of biofilm mass in terms of mean & SD for each group.

Inferential Statistics:

One-way ANOVA Test followed by Bonferroni's post hoc Test / Kruskal Wallis Test followed by Dunn's post hoc test [Based on the Data Distribution] was used to compare the mean remineralization potential & absorbance of biofilm mass between 3 groups.

The level of significance set at P<0.05.

	Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
	8	O	Oxygen	66.948	47.141
	15	P	Phosphorus	13.389	18.255
	17	Cl	Chlorine	0.386	0.602
	20	Ca	Calcium	19.277	34.002

	Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
	6	C	Carbon	10.007	5.717
	8	O	Oxygen	63.256	48.144
	15	P	Phosphorus	11.026	16.249
	17	Cl	Chlorine	0.297	0.502
	20	Ca	Calcium	15.414	29.388

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	14.028	8.350
8	O	Oxygen	62.419	49.497
14	Si	Silicon	0.434	0.604
15	P	Phosphorus	9.697	14.889
20	Ca	Calcium	13.421	26.660

EPS Extraction and Quantification (Phenol–Sulfuric Acid Method)

Sl. No	Group/Samples	EPS at 490 nm			EPS conc µg/mL
		1	2	Avg	
1	Control	0.988	1.082	1.035	527.14
2	Demineralized	1.345	1.438	1.3915	709.68
3	Theracal	0.726	0.655	0.6905	350.74
4	Biodentine	0.872	0.862	0.867	441.12
5	Nano chitosan	0.529	0.477	0.503	254.74

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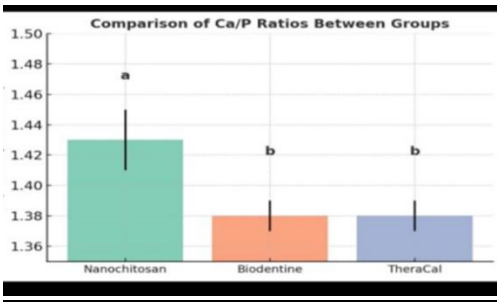
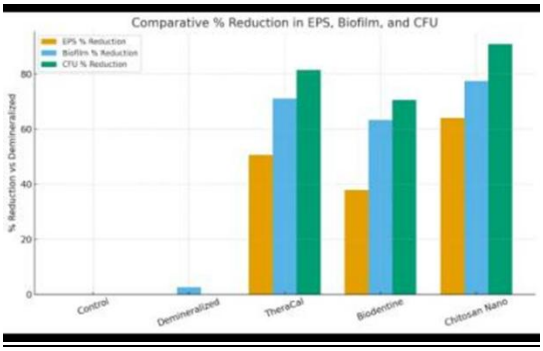
CRYSTAL VOILET ASSAY(ANTI-ADHESION/ BIOFILM BIOMASS)

Sl. No	Group/Samples	Crystal violet at 590 nm			% reduction
		1	2	Avg	
1	Control	1.678	1.867	1.7725	
2	Demineralized	1.726	1.729	1.7275	2.53
3	Theracal	0.552	0.472	0.512	71.11
4	Biodentine	0.657	0.644	0.6505	63.30
5	Nano chitosan	0.412	0.390	0.401	77.37

COLONY FORMING UNIT ENUMERATION

SL No	Group/Samples	CFU/0.1mL			CFU/mL
		1	2	Avg	
1	Control	300	300	300	3.00×10^4
2	Demineralized	450	450	450	4.50×10^4
3	Theracal	78	89	83.5	8.35×10^3
4	Biodentine	112	153	132.5	1.33×10^4
5	Nano chitasan	52	30	41	4.10×10^3





5. Discussion

The main purpose of pulp capping agents is to protect the dental pulp and promote its healing when it is exposed or nearly exposed due to caries removal, trauma or cavity preparation.

They act as a barrier between the pulp and external irritants while encouraging dentin regeneration. **The main goal of pulp capping agents are:**

PULP PROTECTION: shields the pulp from bacterial contamination, thermal, chemical or mechanical irritation.[10]

PROMOTE HEALING AND REPARATIVE DENTIN FORMATION: Stimulates the pulp cells to form tertiary dentin and helps in maintaining pulp vitality[11]

PROVIDE A SEALING EFFECT: Minimizes microleakage and prevents further progress of caries.[12]

Nano-chitosan refers to chitosan in nanoparticle form, which improves its solubility, bioactivity and penetration into dentinal tubules. It has cationic property disrupt bacterial cell membranes and reduces infection risk at exposure site**.** Enhances odontoblast differentiation and stimulates deposition of tertiary dentin. Enhances odontoblast differentiation and stimulates deposition of tertiary dentin.[13]

Biodentin is a tricalcium silicate based bioactive material. It is considered as next generation alternative to calcium hydroxide.[14]

Biodentin has antimicrobial activity due to calcium hydroxide release but because of its higher porosity it allows microleakage and biofilm attachment more easy. Over time, loss of material integrity it will compromise its antibiofilm action**.**[14]

- Theracal LC contains calcium silicate particles in a resin matrix and provides sustained calcium ions over time.[15]

Calcium release stimulates hydroxyapatite crystal formation and secondary dentinal bridge formation. Calcium ions are required for differentiation and proliferation to odontoblast-like cells.[16]The resin component helps in maintaining material stability and prevents rapid washout allows longer and continuous ion release.Its high alkaline environment makes condition unfavourable for bacterial adhesion and biofilm maturation. The resin matrix makes the surface less

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porous and smoother reducing sites for bacterial colonization.[17]

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