RESEARCH PAPER

# Cytotoxicity, Biocompatibility and Growth Factor Analysis of a Novel Pulp Capping Material - An invitro study

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**Abstract**

**Objectives**:

To evaluate the cytotoxicity, biocompatibility, and growth factor modulation of a novel pulp capping material using in vitro assays.

**Materials and Methods**:

3T3-L1 cells were cultured and treated with two concentrations of the experimental material. The MTT assay was used to measure cell viability at 24 and 48 hours. Gene expression of angiogenic and odontogenic markers (VEGF, TGF-β1, FGF-b, BMP2) was quantified using RT-qPCR and normalized by the ΔΔCt method. One-way ANOVA with Tukey's post hoc test was used to examine the data, and $p < 0.05$ was deemed significant.

**Results:**

The MTT assay revealed cell viability >95% across all experimental groups, with no significant difference from untreated controls. Gene expression analysis demonstrated mild downregulation of VEGF (0.92–0.89 fold), TGF-β1 (0.96–0.91 fold), FGF-b (0.95–0.92 fold), and BMP2 (0.95–0.90 fold) relative to untreated controls. The expression changes were consistent but remained within a narrow range, indicating preserved biological compatibility.

**Conclusions:**

The novel pulp capping material showed favorable cytocompatibility and biocompatibility, with minimal impact on growth factor signaling. While less stimulatory than conventional calcium silicate–based cements, its biological neutrality and safety profile suggest potential for clinical use in vital pulp therapy. Further in vivo and translational studies are recommended.

**Keywords:** pulp capping, cytotoxicity, biocompatibility, growth factors, Oral health, disease, healthcare material, medicine

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## Introduction

Preserving pulp vitality is an important aspect of modern restorative and endodontic dentistry. The most conservative approach of vital pulp therapy (VPT), which consists of conservative methods meant to maintain the health of the pulp–dentin complex, is direct pulp capping [1]. The therapeutic efficacy of pulp capping is largely dependent on the biological performance of the capping material, namely its ability to preserve pulp cell viability, encourage the formation of mineralized tissue, and modify signaling pathways involved in regeneration [2,4].

Since calcium hydroxide ($Ca(OH)_2$) has antibacterial properties and can cause dentin bridge development, it has long been regarded as the gold standard pulp capping agent [5,6]. However, its limitations restrict its predictability and long-term results, such as dissolving over time, poor sealing ability, and the creation of porous dentin bridges with tunnel faults[7].

A study by Priyadharshini et al depicted Resin-based ReCal LC exhibits encouraging bioactivity while remaining affordable, making it a promising pulp-capping material for further clinical evaluation.[33]

The invention of calcium silicate-based cements, especially mineral trioxide aggregate (MTA), which provided improved sealing ability, biocompatibility, and bioactivity, further advanced VPT [8,12]. It has been shown that MTA stimulates

odontoblast-like growth, encourages the formation of dentin bridges, and releases bioactive molecules from the dentin extracellular matrix (dECM), all of which activate pathways related to pulp repair [13,15]. Despite these advantages, MTA has disadvantages, including as difficult handling, long setting periods, and the potential for tooth discoloration [16,17].

Alternative materials, such as biodentine and resin-modified calcium silicates (TheraCal LC), have been developed to overcome these limitations1 [8–20]. Materials including resin are concerning due to the potential cytotoxic effects of unpolymerized monomers [24,25], even though biodentine is just as bioactive as MTA [21,22,23]. Clinical and histological research indicates that calcium silicate-based cements often produce superior results than $Ca(OH)_2$ [26,27,28]

Growth factors that are sequestered in dentin and necessary for pulp regeneration include fibroblast growth factor (FGF-b), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β1), and bone morphogenetic proteins (BMPs). Pulp capping agents [2,13] can encourage the release of these compounds, which coordinate pulp cell recruitment, angiogenesis, and differentiation. Therefore, the ideal material should be cytocompatible and able to preserve or enhance growth factor-mediated regeneration signals.

This study presents and assesses a new pulp capping material. The MTT assay was used to evaluate its (i) cytotoxicity, (ii) biocompatibility in preserving pulp cell viability, and (iii) expression of VEGF, TGF-β1, FGF-b, and BMP2. The material would exhibit similar biological safety and regenerative capacity to current pulp capping agents, according to the null hypothesis.

## Materials and Methods

### Reagents

The culture media includes fetal bovine serum (FBS; HiMedia), Dulbecco's Modified Eagle's Medium (DMEM, HiMedia, India), and an antibiotic mixture of penicillin and streptomycin (HiMedia).RNase, propidium iodide (PI) dye, and dimethyl sulfoxide (DMSO) were purchased from HiMedia. Abcam (Cambridge, UK) provided the MTT cell viability kit. The active ingredient in the new pulp capping substance being studied, calotropin, was acquired from Sigma Aldrich (India). mRNA was extracted using the total RNA isolation kit (Invitrogen, USA). Eurofins Genomics India Pvt. Ltd. created unique forward and reverse primers for the target genes (VEGF, TGF-β1, FGF-b, and BMP2).

### Cell Culture

The 3T3-L1 cell line (NCCS, Pune, India) was used for cytotoxicity testing. The cells were grown in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. The cultures were maintained in a humidified incubator at 37 °C with 5% CO2.Cells between passages 3 and 6 were used in all tests, and sub-culturing was done when the cells achieved 70–80% confluency.

### Cell Viability Assay (MTT Assay)

In 96-well plates, the cells were planted at a density of $1 \times 10^2$ cells per well and incubated for the whole night. The experimental groups included an untreated control, a positive control (20% DMSO), and two test concentrations of the novel drug (low and high dose, produced in serum-free media). Each well received 100 µL of MTT solution (5 mg/mL) after exposure for 24 and 48 hours, and the supernatant was discarded. The formazan crystals were incubated at 37 °C for four hours before being dissolved in 100 µL of DMSO. The absorbance at 590 nm was measured using a microplate reader. A comparison was made between the percentage of cells that survived treatment and the untreated control.

### Gene Expression Analysis

For 24 and 48 hours, the cells were exposed to the novel chemical at an IC•¹ concentration after being seeded at a density of $5 \times 10^2$ cells/well in 6-well plates. The Invitrogen RNA isolation kit was used to extract total RNA, and spectrophotometry was used to confirm its purity. cDNA was created using a reverse transcription kit. Quantitative RT-PCR was performed on VEGF, TGF-β1, FGF-b, and BMP2, with β-actin acting as the housekeeping control. The ΔΔCt technique was used to determine relative gene expression.

### Statistical Analysis

Every experiment was carried out three times independently and in triplicate. The outcomes are shown as mean ± SD. One-way ANOVA was used to compare the groups, followed by Tukey's post hoc test. For pairwise comparisons, the student t-test was utilized. P-values below 0.05 were considered statistically significant. GraphPad Prism v8.0 was used for the statistical analysis.

## Results

### Cell Viability (MTT Assay)

After being exposed to the new pulp capping material for 48 hours, cell viability was evaluated using the MTT assay. With relative viabilities of 100.0%, 99.3%, 96.3%, and 94.9%, the mean absorbance values were 0.864 ± 0.010 for the untreated group, 0.858 ± 0.032 for the control, 0.833 ± 0.028 for the low-dose group, and 0.821 ± 0.040 for the high-dose group (Table 1).

A one-way ANOVA revealed no statistically significant differences between the groups ($F(3,8) = 1.46$, $p = 0.296$). Tukey's post hoc test revealed that the viability of both the high-dose and low-dose treatments was comparable to that of the untreated and control groups ($p > 0.05$). These results demonstrate that, at

the levels tested, the innovative pulp capping material has no discernible cytotoxic effects (Figure 1). The MTT assay findings at 48 hours are shown in Figure 1. In comparison to the untreated control, cell viability is expressed as mean ± SD. There were no discernible group differences (ANOVA, $p = 0.296$). ns stands for not significant.

**Gene Expression Analysis**

Table 2 and Figure 2 describe the novel material's effects on angiogenic and odontogenic markers.

VEGF: From control (0.99 ± 0.035) to low dosage (0.92 ± 0.045) and high dose (0.89 ± 0.032), the expression progressively decreased. ANOVA demonstrated significance ($p = 0.008$), and post-hoc analysis confirmed that the expression of the high-dose group was considerably lower than that of the untreated group ($p < 0.05$).

TGF-β1: In high-dose group, expression dropped to 0.91 ± 0.054. ANOVA was significant ($p = 0.048$), and Tukey's test showed a significant decline in the high-dose group compared to the untreated group ($p < 0.05$).

FGF-b: There were no appreciable variations across groups ($p = 0.103$), and expression levels stayed mostly constant (0.96-0.92 fold).

BMP2: The expression decreased to 0.90 ± 0.042 in the high-dose group. ANOVA showed significance ($p = 0.049$), and Tukey's test confirmed considerably decreased expression in the high-dose group compared to the untreated group ($p < 0.05$).

The relative expression of VEGF, TGF-β1, FGF-b, and BMP2 is shown in Figure 2. Fold change (mean ± SD) of the data in relation to the untreated control. FGF-b did not significantly change in the high-dose group, whereas VEGF, TGF-β1, and BMP2 were considerably downregulated ($p < 0.05$).

**Discussion**

In comparison to untreated or control cells, the novel pulp capping material investigated in this work demonstrated exceptional cytocompatibility, with cell viability surpassing 94% in all groups and no statistically significant cytotoxicity. According to earlier studies, materials based on calcium silicate are better and more biocompatible than $Ca(OH)_2$, which has been connected to cytotoxicity and insufficient dentin bridges[5,6,26]

Our results corroborate earlier findings that Biodentine and MTA preserve good pulp cell viability and cause little cytotoxic reactions [8,18,22]. In contrast, pulp irritation and leachable monomers have been linked to resin-modified materials like TheraCal LC [24,25]. Our innovative material's lack of cytotoxicity suggests that it might be a safer option for direct pulp contact applications.

This study's evaluation of growth factor expression was a novel feature. At large doses of the novel substance, VEGF, TGF-β1, and BMP2 were significantly downregulated ($p < 0.05$), while FGF-b was unaffected. TGF-β1 and BMP2 control pulp cell differentiation and matrix mineralization[13,21]. VEGF is essential for angiogenesis during reparative dentinogenesis [2,29]. Although fold changes stayed within a small range (0.89–0.91), their downregulation indicates a dose-dependent inhibitory impact. The results show that nHALS@DXC may be a more effective pulp capping agent than nHALS in terms of lowering inflammation and stimulating dental pup cells to improve pulp healing following damage.

These findings are in contrast to research on MTA and Biodentine, which have been demonstrated to increase pro-regenerative signaling and induce the release of dentin matrix-bound growth factors[2,13,22]. Within the pulp microenvironment, biodentine has the ability to promote the development of human dental pulp stem cells[32]. The novel material's biological neutrality could be an indication of a cautious cellular reaction that is less stimulating of regeneration yet safe for pulp survival. According to recent research, because carbonated hydroxy apatite closely resembles the mineral phase of real bone tissue, it provides a more biomimetic environment for tissue regeneration and repair. The purpose of this study is to ascertain whether employing CHA as a pulp capping material in VPT31 is practically feasible.

A pulp capping substance must first guarantee pulp survival in a clinical setting. Strong regeneration stimulation is ideal, but biocompatibility and lack of cytotoxicity come first. The innovative material is a potential candidate for biocompatible vital pulp therapy because of its neutral biological profile, which may lower the risk of pulp inflammation. However, its capacity to actively promote dentin bridge development may be restricted when compared to materials like MTA and Biodentine [2,18,22].

**Limitations and Future Directions**

This study's in vitro design, which is unable to fully capture the intricacy of immune and vascular responses in vivo, is one of its limitations. Additionally, a wider network of signaling molecules is involved in pulp repair, however the evaluation was limited to four growth factors [3,27,28]. In vivo animal models, comparative trials with MTA and Biodentine, and broader gene expression analysis, including indicators of inflammation and mineralization, should all be part of future research. The importance of natural

products in endodontic applications is shown by their improved efficacy when compared to modern pulp capping agents and the discovery of synergistic effects in combined treatments.[30]

**Conclusion**

Overall, the new pulp capping material showed good biocompatibility and no negative effects, although at higher dosages, it inhibited VEGF, TGF-β1, and BMP2 in a mild, dose-dependent manner. These results confirm that it is a biocompatible alternative to critical pulp therapy. However, more research is needed to demonstrate its potential for long-term therapeutic effectiveness and regeneration.

**References**


1. Hwas M, Sandrik JL. Acid and water solubility and strength of calcium hydroxide bases. J Am Dent Assoc. 1984;108(1):46-48.
2. Gandolfi MG, Siboni F, Prati C. Chemical–physical properties of TheraCal, a novel light-curable MTA-like material for pulp capping. Int Endod J. 2012;45(6):571-579.
3. Schmitt D, Lee J, Bogen G. Multifaceted use of ProRoot MTA root canal repair material. Pediatr Dent. 2001;23(4):326-330.
4. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A. 2000;97(25):13625-13630.
5. Sun Q, Gustin JW, Tian F cong, et al. Effects of pre-mixed hydraulic calcium silicate putties on osteogenic differentiation of human dental pulp stem cells in vitro. J Dent. 2021;108(March):103653.
6. Rodríguez-Lozano FJ, Lozano A, López-García S, et al. Biomineralization potential and biological properties of a new tantalum oxide (Ta(2)O(5))-containing calcium silicate cement. Clin Oral Investig. 2022;26(2):1427-1441.
7. Jeanneau C, Laurent P, Rombouts C, Giraud T, About I. Light-cured Tricalcium Silicate Toxicity to the Dental Pulp. J Endod. 2017;43(12):2074-2080.
8. Bakhtiar H, Nekoofar MH, Aminishakib P, et al. Human Pulp Responses to Partial Pulpotomy Treatment with TheraCal as Compared with Biodentine and ProRoot MTA: A Clinical Trial. J Endod. 2017;43(11):1786-1791.
9. Poggio C, Arciola CR, Beltrami R, et al. Cytocompatibility and antibacterial properties of capping materials. ScientificWorldJournal. 2014;2014:181945.
10. Tomson PL, Lumley PJ, Smith AJ, Cooper PR. Growth factor release from dentine matrix by pulp-capping agents promotes pulp tissue repair-associated events. Int Endod J. 2017;50(3):281-292.
11. Cooper PR, Takahashi Y, Graham LW, Simon S, Imazato S, Smith AJ. Inflammation-regeneration interplay in the dentine-pulp complex. J Dent. 2010;38(9):687-697.
12. Tran X V, Gorin C, Willig C, et al. Effect of a calcium-silicate-based restorative cement on pulp repair. J Dent Res. 2012;91(12):1166-1171.
13. Goldberg M, Farges JC, Lacerda-Pinheiro S, et al. Inflammatory and immunological aspects of dental pulp repair. Pharmacol Res. 2008;58(2):137-147.
14. Nilsen BW, Jensen E, Örtengren U, Michelsen VB. Analysis of organic components in resin-modified pulp capping materials: critical considerations. Eur J Oral Sci. 2017;125(3):183-194.
15. Arandi NZ, Rabi T. TheraCal LC: From Biochemical and Bioactive Properties to Clinical Applications. Int J Dent. 2018;2018(1):3484653.
16. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod. 1993;19(12):591-595.
17. Parirokh M, Torabinejad M. Mineral Trioxide Aggregate: A Comprehensive Literature Review-Part I: Chemical, Physical, and Antibacterial Properties. J Endod. 2010;36(1):16-27.
18. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review--part II: leakage and biocompatibility investigations. J Endod. 2010;36(2):190-202.
19. Camilleri J. Hydration mechanisms of mineral trioxide aggregate. Int Endod J. 2007;40(6):462-470.
20. Camilleri J. Evaluation of the effect of intrinsic material properties and ambient conditions on the dimensional stability of white mineral trioxide aggregate and Portland cement. J Endod. 2011;37(2):239-245.
21. Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. Dent Mater. 2013;29(5):580-593.
22. Laurent P, Camps J, About I. BiodentineTM induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012;45(5):439-448.
23. Han L, Okiji T. Uptake of calcium and silicon released from calcium silicate-based endodontic materials into root canal dentine.

Int Endod J. 2011;44(12):1081-1087.
24. Huang GT-J, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod. 2008;34(6):645-651.
25. Simon S, Cooper P, Smith A, Picard B, Ifi CN, Berdal A. Evaluation of a new laboratory model for pulp healing: preliminary study. Int Endod J. 2008;41(9):781-790.
26. Tziafas D, Alvanou A, Papadimitriou S, Gasic J, Komnenou A. Effects of recombinant basic fibroblast growth factor, insulin-like growth factor-II and transforming growth factor-β1 on dog dental pulp cells in vivo. Arch Oral Biol. 1998;43(6):431-444.
27. Sloan AJ, Smith AJ. Stem cells and the dental pulp: potential roles in dentine regeneration and repair. Oral Dis. 2007;13(2):151-157.
28. Nasri Z, Jahromi MZ, Aminzadeh A. Clinical and histological response of human pulp tissue to direct pulp capping with mineral trioxide aggregate, Biodentine and propolis. Dent Res J (Isfahan). 2022;19:40.
29. Aguilar P, Linsuwanont P. Vital pulp therapy in vital permanent teeth with cariously exposed pulp: A systematic review. J Endod. 2011;37(5):581-587.
30. Turbatmath K, Sharma CS. Evaluating The Synergistic Antibacterial Efficacy Of Nanoparticle-Enhanced Propolis And Contemporary Pulp Capping Agents On Streptococcus Mutans: A Comparative Study.
31. Priyadharshini SS, Ragavendran C, Sherwood A, Ramya JR, Krithikadatta J. Evaluation of mineral induction ability and cytotoxicity of carbonated hydroxyapatite for pulp tissue regeneration: an in vitro study. Restorative dentistry & endodontics. 2024 Oct 29;49(4):e40.
32. Priyadharshini SS, Ragavendran C, Unnikrishnan M, Sherwood IA, Amaechi BT. Physicochemical and biological properties of pulp capping agents: a systematic review of in vitro studies. Journal of International Oral Health. 2024 Jul 1;16(4):274-82.
33. Priyadharshini S, Ragavendran C, Unnikrishnan M, Ganapathy S, Jeevan MB. Comparative Analysis of the Mineral Induction Potential of Resin-Containing and Conventional Pulp-capping Materials: An In Vitro Study. Journal of International Oral Health. 2025 Mar 1;17(2):154-60.

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**TABLES**

**Table 1. MTT assay results (48 h, mean ± SD)**

| Group | Absorbance (Mean ± SD) | Viability (%) |
|---|---|---|
| Untreated | 0.864 ± 0.010 | 100.0 |
| Control | 0.858 ± 0.032 | 99.3 |
| Sample B (Low dose) | 0.833 ± 0.028 | 96.3 (ns) |

**Table 2. Relative gene expression (fold change, mean ± SD)**

| Group | VEGF (Mean ± SD) | TGF-β1 (Mean ± SD) | FGF-b (Mean ± SD) | BMP2 (Mean ± SD) |
|---|---|---|---|---|
| Untreated | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.032 |
| Control | 0.99 ± 0.035 | 0.99 ± 0.065 | 0.96 ± 0.035 | 0.97 ± 0.037 |
| Sample B (Low dose) | 0.92 ± 0.045 | 0.96 ± 0.035 | 0.95 ± 0.052 | 0.95 ± 0.057 |
| Sample B (High dose) | 0.89 ± 0.032* | 0.91 ± 0.054* | 0.92 ± 0.014 (ns) | 0.90 ± 0.042* |

($p < 0.05$ vs untreated; ns = not significant

**FIGURES**

* Fig 1- MTT Assay – Cell Viability (48 h)

** Fig 2 – Relative gene expression (Fold change vs Untreated)