RESEARCH ARTICLE

Chitosan–*Aloe vera* Combination Enhances STRO-1, DSPP, and Reparative Dentin Formation in a Rat Model of Reversible Pulpitis

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Abstract

BACKGROUND: Direct pulp capping (DPC) aims to preserve pulp vitality but requires agents that are both bioactive and biocompatible. Calcium hydroxide ($CaOH_2$) is widely used, however its long-term success is limited, and it may cause adverse effects. Natural biomaterials such as chitosan and *Aloe vera* have shown potential, but their combined regenerative effects are still not well understood. This study was conducted to evaluate the efficacy of chitosan–*Aloe vera* composites in promoting stem cell activation, odontoblast differentiation, and reparative dentinogenesis in a rat model of reversible pulpitis.

METHODS: Twenty-four Wistar rats with mechanically induced reversible pulpitis were divided into six groups: normal control, reversible pulpitis, $CaOH_2$, and chitosan–*Aloe vera* pastes at 20%, 30%, and 40% (CA20, CA30, CA40). Pulp capping was performed following standardized pulp exposure. After 28 days, reparative dentin thickness and dentin bridge formation were assessed histologically, and STRO-1 and dentine sialophosphoprotein (DSPP) expression were analyzed immunohistochemically.

RESULTS: Dentin bridge was observed in the CA40 group, presenting the thickest dentin formation (113.5±13.5 μm). STRO-1 and DSPP were significantly higher in all chitosan–*Aloe vera* combination groups compared with reversible pulpitis group (p<0.01), with DSPP in CA30 and CA40 also higher than $CaOH_2$ group. Both biomarkers demonstrated a positive correlation, and reparative dentin thickness showed a strong positive correlation with DSPP level (r=0.786, p<0.001).

CONCLUSION: Chitosan–*Aloe vera* combination showed encouraging biological activity in this 28-day preclinical model of reversible pulpitis. Although higher concentrations enhanced stem cell activation, odontoblast differentiation, and reparative dentin formation, these results should be interpreted cautiously due to the small sample size and study design limitations.

KEYWORDS: pulp capping, STRO-1, DSPP, dentin bridge, $CaOH_2$, natural biomaterial, endodontics

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Introduction

Inflammation of the pulp or periapical tissue is typically triggered by microbial invasion or exposure to mechanical, thermal, or chemical stimuli.(1) Vital pulp therapy represents a key minimally invasive approach designed to maintain the vitality of pulp tissue.(2) The dental pulp contains dental pulp stem cells (DPSCs), which function to preserve pulp vitality due to their regenerative ability to form new dentin, thereby protecting the tooth from further damage caused by caries and trauma.(3) DPSCs are stem cells derived from

the dental pulp tissue that have the potential to differentiate into various cell types, including odontoblasts. In response to pulp injury, odontoblasts migrate to the damaged area and initiate the formation of reactionary dentin. In cases of more severe damage, DPSCs may further differentiate into odontoblast-like cells.(4)

STRO-1 was selected as one of the evaluated markers because it is a well-established surface antigen used to identify mesenchymal stem cells, including DPSCs. Its expression reflects the presence and activation of undifferentiated progenitor cells that participate in early pulp healing and reparative dentinogenesis. Therefore, STRO-1 serves as an essential indicator of the regenerative potential of pulp tissue following injury.(5,6)

Exposure of the dental pulp caused by trauma, carious lesions, or iatrogenic procedures requires the implementation of direct pulp capping to preserve pulp vitality.(7) Ideal pulp capping materials should possess bioactive properties to promote hard tissue regeneration through reparative dentin formation, exhibit antimicrobial activity, and have sufficient mechanical strength to endure the forces exerted during restoration placement collectively contributing to the preservation of pulp vitality.(8)

Calcium hydroxide ($CaOH_2$) has traditionally been the preferred pulp capping agent due to its favorable biocompatibility and its capacity to induce the formation of a dentinal bridge.(9) However, this material has very low adhesion to dentin and is easily soluble. Additionally, $CaOH_2$ is a strong alkali, which can lead to necrosis of the superficial layer of the pulp tissue.(10) In addition, the failure rate increases significantly, with a success rate of only 37% at 5 years of treatment and 13% at 10 years.(11)

Natural biomaterials such as chitosan and *Aloe vera* have begun to attract attention.(12) The combination of chitosan and *Aloe vera* has demonstrated beneficial combined effects in wound healing. This combination is effective in increasing the number of odontoblast cells, accelerating wound healing, and enhancing the biological response to injury in cases of reversible pulpitis.(12) A study using chitosan as a pulpotomy treatment material showed that chitosan can increase the formation of reparative dentin and hard tissue.(13) Chitosan exhibits properties favorable for tissue engineering applications, including its biodegradable and biocompatible nature, abundance in natural sources, and structural resemblance to the extracellular matrix.(14) Chitosan is a product of the partial deacetylation of chitin.(15) Chitosan is a bioactive material that has been widely used in the field of medical materials due to its excellent biological properties, especially in serving as a carrier for drug delivery systems.(16–18) In a study it was also found that *Aloe vera* can prevent erythrocyte membrane lysis caused by hypotonicity and heat. The results of this study indicate that *Aloe vera* exhibits a protective effect on erythrocyte membranes that is nearly comparable to the standard anti-inflammatory drugs commonly used (NSAIDs). This suggests that the extract has potential as a natural anti-inflammatory agent.(19) One of the main components found in *Aloe vera* is acemannan, a polysaccharide that can stimulate collagen production and fibroblast proliferation, thereby supporting osteoinductive and osteogenetic processes.(20,21)

Several *in vitro* studies have further supported the regenerative potential of chitosan and *Aloe vera* in odontoblastic differentiation and dentinogenesis. For example, *in vitro* application of chitosan-based scaffolds has been shown to upregulate odontogenic markers such as dentin sialophosphoprotein (DSPP) and bone morphogenetic protein-2 (BMP-2) in human dental pulp stem cells, indicating enhanced differentiation toward odontoblast-like cells.(22,23) *Aloe vera* extracts have been reported to stimulate DPSC proliferation, increase transforming growth factor-beta1 (TGF-β1) expression, and promote the mineralization process in cultured pulp cells.(24,25) Moreover, *in vitro* co-incubation of chitosan and *Aloe vera* matrices demonstrated combined effects on cell viability, collagen synthesis, and alkaline phosphatase activity as a key early indicators of odontoblastic differentiation.(15,26) These findings collectively provide a strong biological basis for evaluating the combined effects of chitosan and *Aloe vera* in *in vivo* settings, particularly in the context of reversible pulpitis.

However, few *in vivo* studies have explored the combined effects of chitosan and *Aloe vera* in stimulating odontoblastic differentiation and dentin bridge formation, particularly in models of reversible pulpitis. Therefore, this study was conducted to evaluate the effectiveness of pulp capping materials made from combination of chitosan and *Aloe vera* in promoting odontoblast differentiation and reparative dentin formation in a rat model of reversible pulpitis. The assessment evaluated dentin formation and expression of STRO-1 and DSPP as cellular markers.

Methods

Preparation of Chitosan–*Aloe vera* Pastes

Chitosan powder (Chimultiguna, Indramayu, Indonesia; medium molecular weight, degree of deacetylation ~75–

85%) was dissolved in 1% acetic acid to obtain a 2% w/v stock solution. *Aloe vera* powder (Herbal Materia Medica Laboratory, Batu, Indonesia) was dissolved in deionized water to obtain a 2% w/v stock solution. The two stock solutions were mixed at a 1:1 ratio (v/v) and adjusted to pH 7 using sodium hydroxide. The mixture was centrifuged at 2000 rpm for 1 hour, filtered, and stored at 4°C. Final paste concentrations of 20%, 30%, and 40% (w/v) were prepared by adjusting the total solids content relative to the total solution volume. All pastes were prepared under aseptic conditions and sterilized using 0.22-µm membrane filtration. Resulting formulations had a smooth, gel-like consistency suitable for clinical handling during pulp capping.

Experimental Animals

This *in vivo* experimental study employed a post-test only control group design. All procedures were conducted at the Laboratory of Experimental Animals, Inter-University Center (PAU), Universitas Gadjah Mada; the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada; and the Biochemistry and Biomolecular Laboratory, Faculty of Medicine, Universitas Brawijaya. Ethical approval was obtained from the Health Research Ethics Committee of Dr. Moewardi General Hospital, Surakarta, Indonesia (Approval No. 818/IV/HREC/2025, April 26, 2025).

Twenty-four healthy male Wistar rats (*Rattus norvegicus*), aged approximately three months and weighing 150–180 g, were used. The animals were housed in standard cages under controlled environmental conditions (22±2°C, 55±10% relative humidity, 12-h light/dark cycle) with *ad libitum* access to standard pellet diet and water. A total of 24 rats (n=4 per group) were allocated to six experimental groups: NC (normal control), RP (reversible pulpitis without treatment), CH (reversible pulpitis treated with $CaOH_2$), and CA20, CA30, and CA40 (reversible pulpitis treated with chitosan–*Aloe vera* paste at concentrations of 20%, 30%, and 40%, respectively).

Induction of Reversible Pulpitis and Pulp Capping Procedure

Animals were anesthetized by intramuscular injection of ketamine hydrochloride (OGBdexa, Tangerang, Indonesia). Reversible pulpitis was induced through controlled mechanical exposure. Standardized Class I cavities were prepared on the maxillary first molars using a high-speed handpiece (PANA-MAX, Tochigi, Japan) with a 0.8-mm (Edenta, Au, Swiss) round bur under continuous sterile saline irrigation. The remaining dentin was thinned until a pinpoint pulp exposure was achieved, after which the pulp chamber roof was gently perforated using a sterile probe to produce controlled, mild inflammation consistent with reversible pulpitis.

Following induction, the assigned pulp-capping materials were applied according to the group allocation. $CaOH_2$ paste (Calcipex II, Nippon Shika Yakuhin Co., Yamaguchi, Japan) was used as the standard pulp-capping material in the CH group. In the experimental groups, combination of chitosan and *Aloe vera* were applied at concentrations of 20%, 30%, and 40% (CA20, CA30, and CA40, respectively). All cavities were sealed with temporary restorative material Cavit™ (3M ESPE, St. Paul, MN, USA) to prevent microleakage and bacterial contamination.

Tissue Processing and Histological Analysis

On day 28, all animals were euthanized and the maxillary molars were dissected for histological and immunohistochemical evaluation. The maxillary molars were harvested and fixed in 10% neutral-buffered formalin (Sigma-Aldrich, St. Louis, MO, USA) for 24 hours, then decalcified in formic acid–HCl (Sigma-Aldrich) for seven days. Samples were dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, and sectioned longitudinally at 4–6 µm using rotary microtome (Sakura, Tokyo, Japan).

Animals were randomly allocated to the six groups using simple randomization. Each animal contributed one maxillary first molar as the experimental unit, and analyses were performed per tooth (n=4 teeth per group). Histological scoring was performed by two independent blinded examiners. Histological evaluation for dentin thickness measurements was performed under a light microscope (Olympus CX23®, Olympus Corporation, Tokyo, Japan) at 1000× magnification. Reparative dentin formation was assessed using a semi-quantitative scoring system.(27) The scoring was performed by two blinded and calibrated examiners under a light microscope (Olympus CX23, Olympus Corporation) at 400× magnification. The reparative dentin bridge was assessed based on its presence, continuity, and thickness at the site of pulp exposure and categorized as follows score 0 no evidence of dentin bridge formation, score 1 partial dentin bridge formation with discontinuous or thin reparative dentin at the exposure site, score 2 complete and continuous dentin bridge formation with uniform thickness sealing the pulp exposure.

Immunohistochemical Examination

Slides were stained with hematoxylin–eosin for histological evaluation and processed for immunohistochemistry using

STRO-1 and DSPP antibodies. The immunohistochemical staining was performed on 4–6 µm paraffin sections using a standardized protocol from the Biochemistry and Biomolecular Laboratory, Faculty of Medicine, Universitas Brawijaya. Antigen retrieval was conducted using heat-induced epitope retrieval (HIER) in citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 minutes, followed by non-specific protein blocking using 1% bovine serum albumin (BSA).

Sections were incubated overnight at 4 °C with primary antibodies against STRO-1 and DSPP (commercially available validated antibodies routinely used in the facility). After washing, slides were treated with HRP-linked secondary antibodies using a ready-to-use detection kit (3,3'-Diaminobenzidine (DAB) chromogen). Hematoxylin was used as counterstain. Negative controls were processed by omitting the primary antibody, while positive controls consisted of tissue sections previously validated by the laboratory for each marker. Quantification was performed using ImageJ by calculating the percentage of DAB-positive area within the region of interest. Two blinded examiners performed the evaluation, and inter-rater agreement was acceptable (κ>0.75).

Statistical Analysis

All data were expressed as mean±standard deviation (SD). The Shapiro–Wilk test was used to evaluate normality, and Levene's test was used to assess homogeneity of variances. Parametric data, including reparative dentin thickness and immunohistochemical expression, were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Non-parametric data, including dentin bridge scores, were analyzed using the Kruskal–Wallis test followed by pairwise comparisons. The Kruskal–Wallis test was selected because dentin bridge scores represent ordinal categorical data and did not meet the assumptions of normality or homogeneity required for parametric analysis. Given the small sample size (n=4 per group), a nonparametric approach provided a more appropriate and robust method for comparing intergroup differences. Pearson correlation analysis was conducted to determine the association between reparative dentin thickness, STRO-1, and DSPP expression. A p-value<0.05 was considered statistically significant. All statistical analyses were performed using IBM® SPSS® Statistics version 25 (64-bit, IBM Corporation, Armonk, NY, USA) and GraphPad Prism version 10.1.1 (270) for macOS (GraphPad Software, Boston, Massachusetts, USA; www.graphpad.com).

Results

STRO-1 Expression Increases with Higher Chitosan–*Aloe vera* Concentrations

Immunohistochemical analysis revealed significant differences in STRO-1 among the treatment groups (p<0.001, Table 1, Figure 1). The RP group showed weak staining with only scattered brown-positive cells, whereas CH displayed a moderate signal. In contrast, CA20, CA30, and CA40 demonstrated progressively stronger STRO-1 staining, with CA40 exhibiting the greatest intensity. Quantitative analysis confirmed that STRO-1 levels in CA40 were significantly higher than in RP and CH (p<0.05).

DSPP Expression Expression Increases with Higher Chitosan–*Aloe vera* Concentrations

DSPP immunostaining showed a pattern consistent with odontoblastic differentiation (p<0.001, Table 1, Figure 2). RP demonstrated only faint DSPP labeling, while CH and CA20 presented intermediate signal intensity. CA30

Table 1. Thickness of reparative dentin and expression of STRO-1 and DSPP in each experimental group.

Group	STRO-1 (REU)	Thickness (µm)	DSPP (REU)
NC	8.3±1.7[a]	0.0±0.0	5.5±1.3
RP	2.5±1.3	0.0±0.0	2.8±1.7
CH	4.3±1.5[c]	4.8±6.2	5.5±1.3
CA20	7.3±1.7[a]	82.3±33.4[abc]	8.8±2.5[a]
CA30	8.5±2.4ab	84.3±14.4[abc]	10.5±1.7[abc]
CA40	10.5±1.3[ab]	113.5±13.5[abc]	12.0±1.8[abc]
p-value	0.001***	0.001***	0.001***

Statistical analysis was performed using one-way ANOVA with Tukey post hoc test; ***Statistically significant compared to all groups (p<0.001); [a]Significant *vs.* RP (p<0.05); [b]Significant *vs.* CH (p<0.05), [c]Significant *vs.* NC (p<0.05). REU: Relative expression unit.

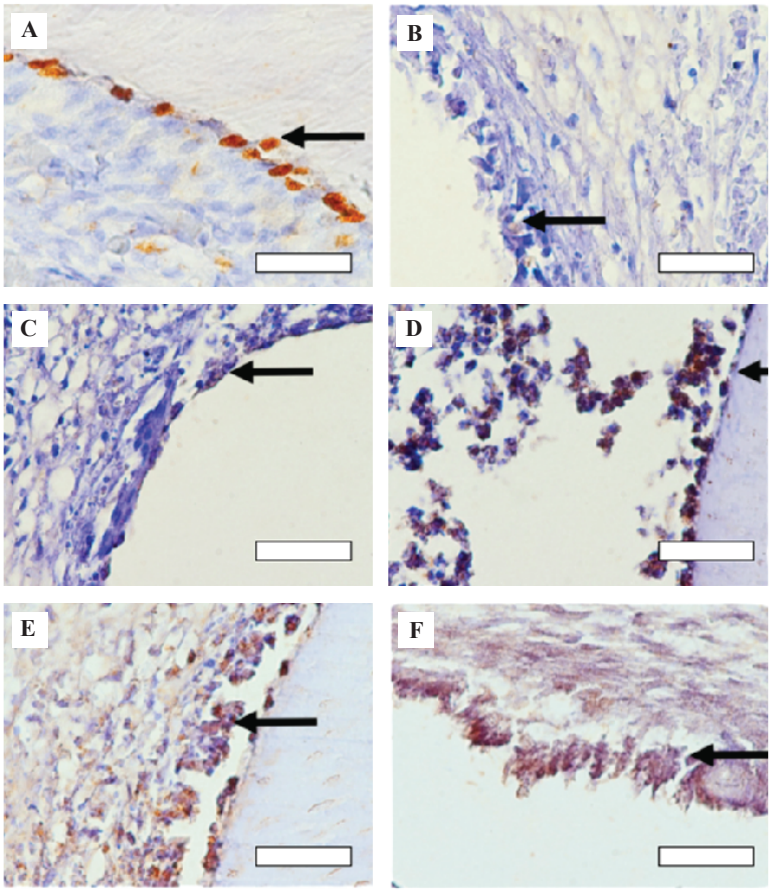

Figure 1. Immunohistochemical expression of STRO-1 in all experimental groups. A: NC group. B: RP group. C: CH group. D: CA20 group. E: CA30 group. F: CA40 group. Brown-stained STRO-1–positive cells (black arrows) are observed adjacent to the odontoblastic layer. STRO-1 expression is weak in RP, moderate in CH and CA20, and markedly increased in CA30 and CA40. White bar: 20 µm.

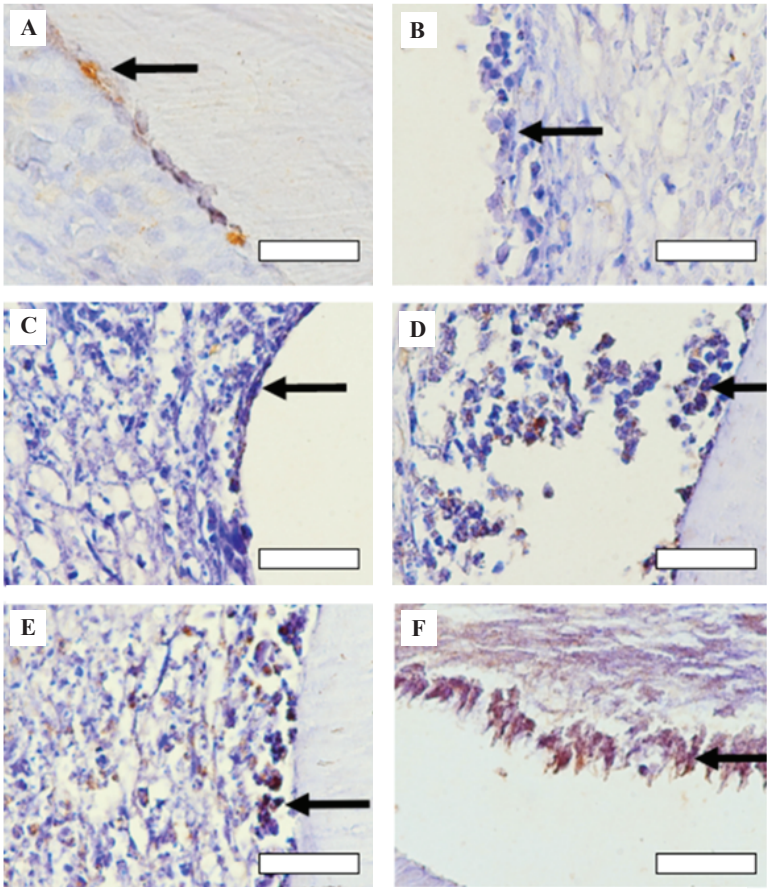

Figure 2. Immunohistochemical expression of DSPP across experimental groups. A: NC group. B: RP group. C: CH group. D: CA20 group. E: CA30 group. F: CA40 group. Brown cytoplasmic DSPP–positive cells (black arrows) are observed along the dentin–pulp interface. DSPP expression is weak in RP and CH, moderate in CA20, and markedly increased in CA30 and CA40. White bar: 20 µm.

and CA40, however, displayed dense brown cytoplasmic staining in odontoblast-like cells, reflecting enhanced secretory activity. Statistical analysis indicated that DSPP levels in CA40 were significantly higher than in RP and CH (p<0.05).

Reparative Dentin Thickness Increases with Higher Chitosan–*Aloe vera* Concentrations

Reparative dentin thickness increased progressively across the treatment groups (p<0.001, Table 1). RP showed only minimal hard tissue deposition, whereas CH and CA20 produced a modest dentinal layer. CA30 and CA40 generated substantially thicker reparative dentin, with CA40 achieving the greatest mean thickness (p<0.05). This pattern supports a dose-dependent effect of the chitosan–*Aloe vera* combination on reparative dentinogenesis.

Dentin Bridge Formation Score

Histological evaluation of dentin bridge formation using HE staining demonstrated significant differences among groups (Figure 3). The RP group primarily presented Score 0 (no bridge formation), while the CH group showed Score 1 (partial formation). The CA30 and CA40 groups predominantly achieved Score 2, indicating complete and continuous dentin bridge formation. Statistical comparison confirmed a significant increase in bridge formation scores in CA30 and CA40 compared with RP and CH (p<0.05) (Table 2).

Correlation Between DSPP Expression and Reparative Dentin Thickness

Pearson's correlation analysis revealed a strong positive correlation between DSPP expression and reparative dentin thickness (r=0.786, p<0.001) (Table 3). This indicates that enhanced odontoblast differentiation, as evidenced by DSPP expression, was closely associated with increased reparative dentin formation. Although the CA20–CA40 groups demonstrated a clear dose-response pattern in both STRO-1 and DSPP expression, as well as a strong correlation between DSPP levels and reparative dentin thickness, these findings should be interpreted as preliminary. The study was conducted in a small, short-term preclinical model with limited sample size, and the absence of single-component control groups prevents definitive conclusions regarding synergism or concentration-dependent efficacy.

Discussion

Direct pulp capping aims to preserve pulp vitality by providing an environment conducive to healing and reparative dentinogenesis.(2) In this study, a rat model of mechanically induced reversible pulpitis was used to evaluate the biological efficacy of a chitosan–Aloe vera combination. Rat molars provide a reproducible model with pulp dentin healing responses similar to those of human teeth, enabling systematic comparison of regenerative outcomes across treatment groups.(28,29)

Consistent with previous studies, $CaOH_2$ demonstrated the ability to induce dentin bridge formation; however, its limitations such as tunnel defects, superficial necrosis, and reduced long-term predictability, remain well documented. (11,26,30–32) These drawbacks have motivated the exploration of alternative natural biomaterials with better biocompatibility and regenerative properties. Direct comparisons with established pulp-capping materials further contextualize our findings. Numerous studies demonstrate that mineral trioxide aggregate (MTA) has been regarded as the gold standard for direct pulp capping, showing success rates of 80–100 % and superior dentin bridge quality compared with $CaOH_2$.(33) In recent years, Biodentine has emerged as a promising alternative to MTA, offering similar

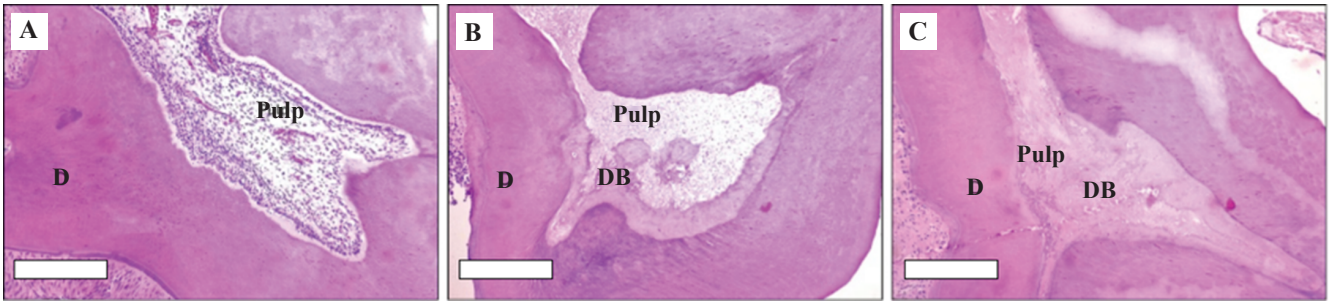

Figure 3. Histological features of dentin bridge formation. A: No dentin bridge (score 0). B: Partial dentin bridge (score 1). C: Complete dentin bridge (score 2). D = Dentin; DB = Dentin Bridge; Staining by Hematoxylin and Eosin. White bar: 100 µm.

Table 2. Histological scoring of reparative dentin formation in all experimental groups.

Group	Score 0	Score 1	Score 2	Total	*p*-value
NC	4 (100%)	0 (0%)	0 (0%)	4	0.001**
RP	4 (100%)	0 (0%)	0 (0%)	4	
CH	2 (50%)	2 (50%)	0 (0%)	4	
CA20	0 (0%)	3 (75%)	1 (25%)	4	
CA30	0 (0%)	1 (25%)	3 (75%)	4	
CA40[ab]	0 (0%)	0 (0%)	4 (100%)	4	
Total	10	6	8	24	

Statistical analysis was performed using the Kruskal–Wallis test with post hoc pairwise comparisons; ***Statistically significant compared to all groups ($p<0.01$); [a]Significant *vs.* NC ($p<0.05$); [b]Significant *vs.* RP ($p<0.05$).

clinical success, improved handling properties, and a shorter setting time. For example, there were no significant difference reported in outcomes between MTA and Biodentine in a comprehensive review of literature.(34) In our study, the chitosan–*Aloe vera* combination at higher concentrations (CA30/CA40) achieved reparative dentin thickness and marker expression levels that approach those reported for MTA and Biodentine in the literature; however, direct head-to-head comparison was not performed. Therefore, while our data are encouraging, we cannot conclude that the novel biomaterial surpasses or matches the performance of MTA or Biodentine without further comparative trials.

In the present findings, the combination of chitosan and *Aloe vera* significantly enhanced reparative dentinogenesis, as evidenced by increased dentin bridge formation, greater reparative dentin thickness, and elevated expression of STRO-1 and DSPP. These outcomes are aligned with earlier research showing the regenerative potential of chitosan or *Aloe vera* based materials in stimulating odontoblast activity and dentin formation.(12–14,22,25,27)

The dose-dependent increase in STRO-1 and DSPP expression observed in CA20–CA40 suggests a progressive enhancement of stem cell activation and odontoblastic differentiation at higher concentrations. Specifically, CA40 produced the most substantial reparative response, including complete dentin bridging in all specimens and the greatest dentin thickness. This parallels reports that *Aloe vera*-based materials exhibit improved regenerative outcomes with increasing concentration.(27) The strong correlation between DSPP expression and dentin thickness further supports a biological link between odontoblast functional activity and reparative dentin formation.(24,25) The complementary properties of these biomaterials provide a plausible explanation for the enhanced reparative effects observed.

Additionally, the observed dose-response trend (CA20–CA40) and the correlation between DSPP and dentin thickness should be regarded as preliminary findings. The small sample size (n=4 per group), the short 28-day observation period, and the use of the Federer formula instead of a priori power analysis reduce statistical power and generalizability. The biological response was assessed at a single time point, and long-term biocompatibility or pulp vitality was not evaluated. Furthermore, the unit of analysis was the tooth, and systemic responses or inter-individual variability were not assessed.

However, the present study did not include single-component control groups (chitosan-only or *Aloe vera*-only), which limits conclusions regarding true synergism versus additive or independent effects. Therefore, although the combination was effective, the specific contribution of each component cannot be determined within this study design.

Table 3. Pearson correlation between dentin thickness and immunohistochemical markers.

Correlated Variables	Pearson *r*	*p*-value	95% CI of *r*	Strength of Correlation
DSPP vs Thickness	0.786	0.000	0.5605 to 0.9031	Strong
STRO-1 vs DSPP	0.636	0.001	0.3135 to 0.8274	Moderate-Strong
STRO-1 vs Thickness	0.599	0.002	0.2576 to 0.8072	Moderate

r<0.3 = weak; 0.3-0.5 = fair; 0.5-0.7 = moderate; >0.7 = strong correlation.

Despite these limitations, the study provides promising evidence supporting chitosan–*Aloe vera* as a potential natural alternative to traditional pulp-capping agents. Nonetheless, more comprehensive studies are required particularly those incorporating individual-component controls, longer follow-up periods, larger sample sizes, and advanced formulation optimization to validate the efficacy, mechanism, and safety of this combination for future clinical application.

The combination of Chitosan–*Aloe vera*, particularly at a 40% concentration, has been proven to accelerate pulp tissue regeneration by enhancing the activity of stem cells and odontoblasts. This supports its potential use as a promising pulp capping biomaterial in conservative dental practice. The small sample size (n=4 per group), determined using the Federer formula rather than a priori power analysis, may limit the statistical power and generalizability of the findings. Therefore, future studies with larger sample sizes and formal power calculations are needed to validate these results.

The unit of analysis in this study was the maxillary first molar from each animal, which may limit the extrapolation of findings to whole-organ or systemic responses. Furthermore, although no adverse effects or complications were observed during the 28-day period, the study did not evaluate long-term biocompatibility or systemic responses, which should be addressed in future research.

Conclusion

This study demonstrated that the chitosan–*Aloe vera* combination, particularly at the 40% concentration, resulted in increased STRO-1 expression, enhanced odontoblastic differentiation (DSPP), and more pronounced reparative dentin bridge formation compared with untreated and $Ca(OH)_2$ groups in a 28-day reversible pulpitis rat model. Overall, these findings indicate that chitosan–*Aloe vera* may serve as a promising regenerative pulp-capping material. Nevertheless, confirmation through studies with larger sample sizes, extended observation periods, and additional control groups is required to establish its long-term efficacy and clinical relevance.

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Authors Contribution

AST was responsible for the conceptualization and methodology of the study, carried out data collection, performed formal analysis, and prepared the original draft of the manuscript. RR provided supervision, project administration, and funding acquisition, and was actively involved in investigation, validation, and critical review and editing of the manuscript. YN contributed to supervision, project administration, and funding acquisition, and participated in investigation, validation, and review and editing. AFA managed data curation, conducted formal analysis and visualization, and contributed to the review and editing of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest or competing interests related to the content of this manuscript.

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