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## Riboflavin Activated Chitosan Scaffold: A Novel Approach for Pulp Revascularization of Immature Necrotic Teeth: A Case Report

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### KEYWORDS

Regenerative endodontics; immature permanent teeth; pulp necrosis; chitosan scaffolds; riboflavin cross-linking

### ABSTRACT:

Background:

Regenerative endodontic procedures (REPs) have redefined the management of immature permanent teeth with necrotic pulp by promoting biological regeneration rather than simple apical barrier formation. However, predictable root maturation remains inconsistent, prompting the exploration of advanced scaffold materials to achieve more predictable regenerative treatment outcomes.

Aim:

To report the clinical and radiographic criteria of a successful regenerative endodontic procedure using a riboflavin-activated chitosan scaffold in an immature permanent tooth with pulpal necrosis.

Method:

A 17-year-old patient with a previously traumatized immature right maxillary central incisor presented with pulpal necrosis, periapical pathology associated with an open apex. After minimal instrumentation, the canal was disinfected using 1.5% sodium hypochlorite and 17% EDTA, followed by placement of calcium hydroxide as an intracanal medicament. At the subsequent visit, intracanal bleeding was induced to provide endogenous stem cells and growth factors. A chitosan scaffold was prepared, incorporated with riboflavin, photo-crosslinked, and injected into the canal, where it was mixed with blood in situ. The canal was coronally sealed with Biodentine and restored with composite resin. Clinical and radiographic follow-up was performed to evaluate success of the procedure.

Conclusion:

Within the limitations of this case report, the riboflavin-activated chitosan scaffold supported periapical healing and continued root development in an immature necrotic tooth. This photo-cross linked scaffold might have enhance the biological predictability of regenerative endodontic therapy. Further long-term clinical and histological studies with larger sample size are required to confirm its regenerative potential.



## INTRODUCTION

The management of immature permanent teeth with necrotic pulp has undergone a paradigm shift with the advent of regenerative endodontics, moving beyond the traditional objective of apical barrier formation toward true biological restoration of the pulp–dentin complex.<sup>1</sup> Unlike conventional approaches that primarily aim to achieve apical closure, regenerative endodontic procedures seek to re-establish a functional tissue within the root canal system, thereby enabling continued root development, restoration of protective mechanism pulp–dentin complex which lead to strengthening of the tooth structure.<sup>2</sup>

Pulp revascularization represents a biologically driven treatment strategy that capitalizes on the regenerative potential of stem cells derived from the apical papilla, residual pulp tissue, and periodontal ligament.<sup>2,3</sup> When appropriately stimulated, these cells migrate into the disinfected canal space, proliferate, and differentiate into odontoblast-like cells capable of depositing hard tissue along the canal walls. This process facilitates dentin formation, increases root length, and promotes thickening of dentinal walls, significantly enhancing the fracture resistance and long-term prognosis of immature teeth.<sup>4</sup>

In contrast, conventional apexification approaches using calcium hydroxide or mineral trioxide aggregate (MTA), although reliable in achieving apical barrier formation, are inherently limited by their inability to facilitate continued root maturation, often resulting in thin dentinal walls and an increased risk of tooth fracture.<sup>5</sup> Regenerative endodontic therapy overcomes these limitations by promoting both apical healing as well as progressive root development, thereby offering a biologically based and structurally more favorable therapeutic outcome.<sup>6</sup>

Recent advances in regenerative endodontic materials and clinical protocols have further improved the predictability and success of revascularization procedures.<sup>7</sup> The introduction of bioactive materials with enhanced biocompatibility and signaling potential has optimized the intracanal microenvironment, supporting stem cell survival, differentiation, and tissue regeneration.<sup>8</sup> These innovations have expanded the clinical applicability of regenerative endodontics and

reinforced its role as a viable treatment option for immature necrotic permanent teeth.

This case report describes the successful management of an immature permanent tooth with necrotic pulp using a regenerative endodontic approach incorporating a combination of advanced bioactive materials. The treatment outcome highlights the potential of regenerative therapy to promote healing, facilitate continued root maturation, and restore structural integrity, underscoring its value in contemporary endodontic practice.

## CASE REPORT

A 17-year-old patient reported to the Department of Conservative Dentistry and Endodontics with the chief complaint of intermittent pain in the maxillary anterior region for the past three weeks. The pain was gradual in onset and associated with the previously traumatized tooth. The patient provided a history of dental trauma resulting from a fall at the age of seven years, for which no definitive dental treatment was undertaken at that time. Since then, the tooth remained asymptomatic until the onset of the present complaint.

Clinical examination revealed slight discoloration of the involved tooth, which was tender on percussion. Radiographic evaluation demonstrated the presence of a periapical radiolucency (fig 1). Pulp sensibility testing, including the cold test and electric pulp test, elicited no response. The diagnosis was established as pulp necrosis associated with periapical pathosis and open apex with respect to 11.

Following informed consent, local anesthesia was administered using 2% lidocaine with 1:100,000 epinephrine. The tooth was isolated with a rubber dam. After access cavity preparation, working length was determined using an electronic apex locator and confirmed radiographically (Fig. 2).

Chemo-mechanical preparation was carried out using a minimally invasive approach. Irrigation was performed with 1.5% sodium hypochlorite, followed by saline and 17% EDTA, and a final rinse with distilled water. The canal was dried with sterile paper points, and calcium hydroxide was placed as an intracanal medicament.

During the second visit, a chitosan scaffold was prepared by dissolving chitosan powder in 1% acetic acid under



continuous stirring to obtain a clear, homogeneous solution, with the pH adjusted to near neutral using sodium hydroxide (fig 3). Riboflavin was incorporated as a 0.1% solution in deionized water and thoroughly mixed with the chitosan scaffold, followed by light activation for 120 seconds in accordance with established protocols to achieve effective collagen

crosslinking (fig 4, 5). Subsequently, bleeding was induced within the root canal to provide a source of stem cells and growth factors. The riboflavin-treated chitosan scaffold was then injected into the canal and mixed with the blood in situ (fig 6, 7). An intra-orifice barrier was placed using Biodentine, followed by definitive restoration with composite resin.



Figure 1 : Pre Operative Radiograph

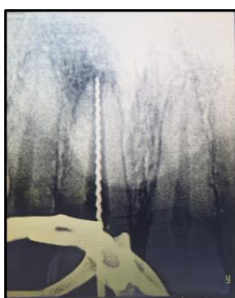


Figure 2 : Working Length Determined



Figure 3 : Chitosan Scaffold

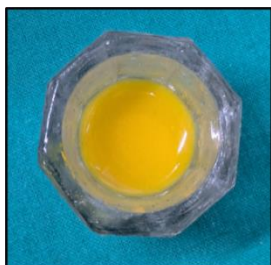


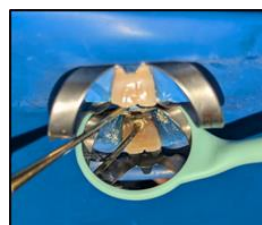
Figure 4 : Riboflavin Prepared,



Figure 5 : Mixed with Scaffold and Light activated



Figure 6 & 7 : Placement of Injectable Scaffold done after Induction of Bleeding followed by Coronal Plug formation by BIODENTIN



Post Operative Radiograph



6 Months follow up



9 Months follow up



## DISCUSSION

Regenerative endodontic procedures (REPs) have demonstrated consistently high clinical success rates in the management of immature permanent teeth with necrotic pulp. Evidence from systematic reviews and meta-analyses reports success rates ranging from 83.3% to nearly 100%, largely based on favorable clinical outcomes such as resolution of symptoms, healing of apical periodontitis, and absence of treatment-related complications including postoperative pain, persistent infection, pathological root resorption, and crown discoloration.<sup>9</sup> Despite these encouraging findings, radiographic evidence of true biological regeneration - characterized by continued root elongation, dentinal wall thickening, and complete apical closure (Cvek stage V) is achieved in a comparatively limited number of cases. Previous studies indicated that only approximately 45% of clinically successful REP cases demonstrate complete radiographic root maturation, highlighting a significant discrepancy between clinical success and biological tissue regeneration.<sup>10</sup>

This gap has prompted ongoing research focused on optimizing the biological components of REPs, particularly through advancements in scaffold design, bioactive molecules, and stem cell-based strategies. Among these components, the scaffold plays a pivotal role by providing a three-dimensional framework that supports stem cell migration, their attachment, proliferation, and differentiation. It also facilitates angiogenesis, spatial organization of the newly formed tissue, and the diffusion of nutrients and metabolic by-products essential for stem cell survival and function.<sup>11</sup> In the present case, a photo-crosslinked Riboflavin-Chitosan Scaffold was employed to enhance the predictability and quality of regenerative outcomes.

Chitosan, a naturally derived polysaccharide, possesses favorable properties including excellent biocompatibility, biodegradability, and bio-adhesive characteristics, making it well suited for intracanal regenerative applications. Chitosan interacts favorably with growth factors through electrostatic binding between its positively charged amino groups and the negatively charged domains of growth factors. This interaction enables adsorption and retention of endogenous growth factors released from the blood clot, protecting them from rapid degradation and allowing

their sustained release. Its inherent antimicrobial activity is particularly advantageous in the previously infected environment of immature necrotic teeth, where residual microbial contamination may adversely affect regenerative outcomes. Furthermore, the porous architecture of chitosan supports stem cell adhesion and proliferation collectively creating a favorable environment for tissue regeneration.<sup>12</sup>

The regenerative potential of the chitosan scaffold was further refined by the incorporation of riboflavin and subsequent photoactivation, a process that enhances crosslinking within the scaffold matrix. This photo-induced stabilization enhances mechanical strength and preserves structural integrity, allowing the scaffold to endure the biologically dynamic environment of the root canal. Concurrent inhibition of matrix metalloproteinase (MMP) activity limits enzymatic degradation of collagen, safeguarding the extracellular matrix during the early and critical phases of healing. By preserving scaffold stability throughout healing, this strategy creates a biologically ordered microenvironment that directs cell adhesion, migration, and proliferation, while simultaneously promoting angiogenesis through endothelial ingrowth, collectively driving sustained regenerative activity and predictable, long-term functional regeneration within the root canal space.<sup>13</sup>

Induction of intracanal bleeding complemented the scaffold by delivering endogenous stem cells particularly stem cells of the apical papilla along with a rich reservoir of growth factors essential for tissue regeneration.<sup>14</sup> The resulting scaffold-blood construct functioned as a bioactive regenerative unit capable of orchestrating cell homing, vascular ingrowth, and odontogenic differentiation, ultimately promoting regeneration of the pulp-dentin complex.

In the present case, clinical and radiographic follow-up demonstrated resolution of periapical pathology accompanied by evidence of continued root development, suggesting successful stem cell-mediated regeneration facilitated by the riboflavin-activated chitosan scaffold. These findings underscore the potential of photo-crosslinked chitosan-based scaffolds as promising next-generation biomaterials in regenerative endodontics, with the capacity to bridge the gap between clinical success and true biological root maturation. Nevertheless, long-term clinical follow-up



and histological evaluation are necessary to validate the reproducibility, durability, and functional outcomes of riboflavin-activated chitosan scaffolds in pulp revascularization protocols.

## CONCLUSION

This case highlights the ability of a riboflavin-activated chitosan scaffold to create a biologically favorable milieu for regenerative endodontic therapy by facilitating cell homing, neovascularization, and ongoing root maturation in an immature necrotic tooth. The observed clinical resolution and radiographic evidence of healing in the present case indicate that photo-activated cross-linked scaffolds may contribute to improved predictability and outcomes of regenerative endodontic procedures. Nevertheless, larger clinical studies with long-term follow-up and histological evaluation are necessary to confirm their efficacy, reliability, and long-term success of the treatment.

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