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# In Vivo Efficacy of Advanced Drug Delivery System Loaded with Different Medicaments For Regenerative Endodontics

**Research Article**

**Menda Akkulu Naidu[1*]**

1. Faculty of Pharmacy, Mandsaur University, Rewas Dewda Road, SH - 31, Mandsaur, Madhya Pradesh- 458001, India.

## Abstract

Aim: The aim of this study is to evaluate the efficacy of an advanced drug delivery system loaded with calcium hydroxide and calcium silicate for regenerative endodontic procedures. Materials & Methods: Preparation of Drug Delivery System: A biocompatible carrier material hydrogel is prepared which is capable of encapsulating calcium hydroxide and calcium silicate particles. Characterisation: The drug delivery system is characterised for its morphology and release kinetics. Animal Study: In vivo studies are conducted using animal models of pulpal injury or infection. The drug delivery system is applied in root canal procedures, and the regeneration of pulp tissue and formation of hard tissue barriers are assessed using histological and radiographic analyses. Results & Discussion: Sustained Release Property: The drug delivery system exhibits potent endodontics regeneration property with sustained release of calcium hydroxide contributing to effective bacterial elimination. Tissue Regeneration: The incorporation of calcium silicate promotes dentinogenesis and facilitates the formation of hard tissue barriers, leading to enhanced tissue regeneration within the root canal space. Conclusion: In conclusion, the advanced drug delivery system loaded with calcium hydroxide and calcium silicate shows great promise for enhancing regenerative endodontic procedures. Its ability to provide sustained release of medicaments, promote tissue regeneration, and improve treatment outcomes makes it a valuable tool in modern endodontic practice.

**Keywords:** Regenerative endodontics, calcium hydroxide, calcium silicate, encapsulation technique.

## Introduction

Regenerative endodontics has emerged as a transformative approach in the field of endodontics, aiming not only to treat infected root canals but also to restore the vitality and function of dental pulp tissue. Traditional endodontic procedures focus primarily on the elimination of microbial pathogens within the root canal system, followed by the obturation of the canal space to prevent reinfection.(1) However, these approaches often result in the loss of vital pulp tissue and compromise the structural integrity of the tooth, leading to long-term complications such as tooth discoloration and susceptibility to fracture. Regenerative endodontic therapies seek to address these limitations by harnessing the innate regenerative capacity of dental pulp tissue to promote tissue repair and regeneration.(2)

Maturation and remodeling processes ensure the development of functional pulp tissue. Overall, the orchestrated interplay of cellular activities, growth factors, and signaling pathways drives the regeneration of endodontic tissues, aiming to restore structure and function within the root canal system.(3)

One of the key challenges in regenerative endodontics is the development of effective strategies for disinfection and tissue regeneration within the root canal space. Microbial infection is a major obstacle to successful regenerative outcomes, as residual bacteria can impede tissue healing and compromise the viability of regenerating pulp tissue. (4)

Additionally, the presence of necrotic tissue and inflammatory byproducts further exacerbates the inflammatory response and inhibits tissue regeneration. Therefore, an ideal regenerative endodontic protocol should not only eliminate microbial pathogens but also provide a conducive environment for tissue regeneration and repair. (5)

In recent years, there has been growing interest in the use of advanced drug delivery systems for enhancing the efficacy of regenerative endodontic procedures. These systems offer a promising platform for the controlled and sustained delivery of medicaments within the root canal space, thereby optimizing antimicrobial activity and promoting tissue regeneration. Among the various medicaments investigated for use in regenerative endodontics, calcium hydroxide and calcium silicate have garnered considerable attention due to their antimicrobial properties and ability to stimulate dentinogenesis. (6)

Calcium hydroxide has long been recognized for its potent antimicrobial activity against a wide spectrum of bacteria commonly associated with endodontic

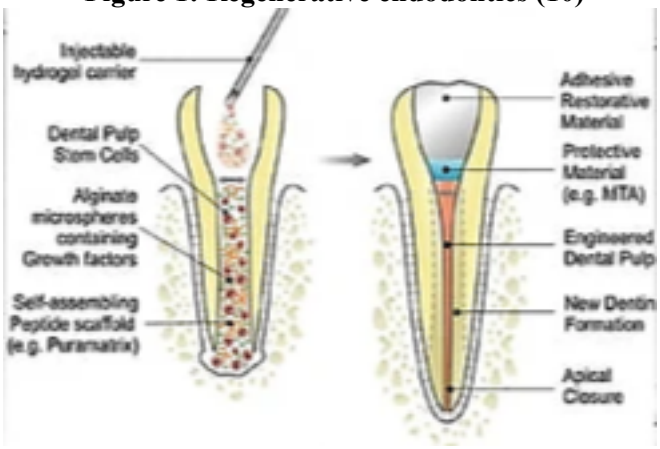
*** Corresponding Author:**
**Menda Akkulu Naidu**
Faculty of Pharmacy,
Mandsaur University,
Rewas Dewda Road, SH - 31, Mandsaur,
Madhya Pradesh- 458001, India..
Email Id: ma.naidu@meu.edu.in

infections. By releasing hydroxyl ions into the surrounding environment, calcium hydroxide disrupts bacterial cell membranes and denatures intracellular proteins, effectively eliminating microbial pathogens. Furthermore, calcium hydroxide has been shown to promote the formation of hard tissue barriers at the apex of the root canal, thereby facilitating the regeneration of pulp tissue and preventing apical microleakage. (7)

Regeneration of endodontic tissues involves an inflammatory response triggering stem cell mobilization. Dental pulp stem cells proliferate and differentiate into odontoblasts, fibroblasts, and endothelial cells. These cells synthesize extracellular matrix components, promote angiogenesis, and facilitate innervation. (8)

In addition to calcium hydroxide, calcium silicate-based materials have emerged as promising agents for use in regenerative endodontics. These materials, such as mineral trioxide aggregate (MTA), exhibit bioactive properties that promote dentinogenesis and facilitate the formation of a dentin-like barrier within the root canal space (figure 1). By releasing calcium and silicate ions into the surrounding tissue, MTA induces the differentiation of stem cells into odontoblast-like cells, leading to the deposition of mineralized tissue and the formation of a hard tissue barrier.(9)

**Figure 1: Regenerative endodontics (10)**




Despite their individual benefits, the use of calcium hydroxide and calcium silicate in regenerative endodontics is often limited by their delivery and retention within the root canal space. Conventional delivery methods, such as pastes or powders, may result in inadequate distribution of the medicament and premature washout from the canal system, leading to suboptimal antimicrobial activity and tissue regeneration. Moreover, the lack of sustained release kinetics may necessitate multiple treatment sessions and compromise patient compliance. (11)

To address these challenges, researchers have explored the development of advanced drug delivery systems capable of encapsulating calcium hydroxide and calcium silicate particles for controlled and sustained release within the root canal space. These systems offer several advantages over conventional delivery methods, including improved drug retention, enhanced antimicrobial activity, and promotion of tissue regeneration. By encapsulating the medicaments within a biocompatible carrier material, such as a hydrogel or porous scaffold, these delivery systems can provide sustained release of the active ingredients while supporting tissue ingrowth and repair. (12)

The design and optimization of advanced drug delivery systems for regenerative endodontics represent a multidisciplinary endeavor, drawing upon principles from materials science, pharmacology, and tissue engineering. Key considerations in the development of these systems include the selection of appropriate carrier materials, optimization of drug loading and release kinetics, and evaluation of biocompatibility and tissue response. Additionally, the clinical translation of these technologies requires rigorous testing in preclinical models and validation through well-designed clinical trials.(13)

In this context, this study aims to provide an overview of the current state-of-the-art in advanced drug delivery systems loaded with calcium hydroxide and calcium silicate for regenerative endodontics. We will discuss the underlying principles of drug delivery and tissue regeneration, highlight recent advances in material design and formulation strategies, and evaluate the preclinical and clinical evidence supporting the efficacy and safety of these systems.

Furthermore, we will identify key challenges and opportunities for future research and development in this rapidly evolving field, with the ultimate goal of improving treatment outcomes and patient care in regenerative endodontics.

## Materials and Methods

### Selection of Carrier Material

Hydrogels have been selected as a carrier material for encapsulating calcium hydroxide and calcium silicate particles.

### Preparation of Carrier Material

The preparation of a hydrogel carrier material via polymerization of hydrophilic monomers involves a series of steps to synthesize a crosslinked network structure capable of encapsulating calcium hydroxide and calcium silicate particles for use in advanced drug delivery systems for regenerative endodontics. Here's a detailed procedure:

### Materials

Hydrophilic monomers (e.g., acrylamide) - 2 g**,** Crosslinking agent (e.g., N,N'-methylene bisacrylamide) - 0.2 g**,** Initiator (e.g., ammonium persulfate) - 0.1 g**,** Catalyst (e.g., N,N,N',N'-tetramethylethylenediamine, TEMED) - 0.1 g**,** Solvent (e.g., water) - Sufficient amount**,** Stirring apparatus**,** Glassware (e.g., beakers, flasks)**,** Analytical balance**,** Sonicator (optional, for particle dispersion)**,** Vacuum desiccator (optional, for drying).

### Procedure

**Monomer Solution Preparation:** Dissolve 2 g of hydrophilic monomers in a sufficient amount of solvent

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(e.g., water) to prepare the monomer solution. Stir the solution until the monomers are completely dissolved.

**Crosslinking Agent Addition:** Add 0.2 g of the crosslinking agent (e.g., N,N'-methylenebisacrylamide) to the monomer solution. Stir the mixture to ensure homogeneity.

**Initiator and Catalyst Addition:** Dissolve 0.1 g of the initiator (e.g., ammonium persulfate) and 0.1 g of the catalyst (e.g., TEMED) in the monomer solution. Stir the mixture gently to avoid premature initiation of the polymerization reaction.

**Polymerization Initiation:** Initiate the polymerization reaction by adding the initiator-catalyst mixture to the monomer-crosslinker solution. Stir the mixture gently to ensure uniform distribution of the components.

**Gel Formation:** Allow the polymerization reaction to proceed until the gel forms. The gel should have a uniform consistency and appearance throughout the solution.

**Curing and Drying:** Cure the hydrogel in an oven or under suitable conditions to promote crosslinking and enhance mechanical strength. Dry the hydrogel under vacuum or in a desiccator to remove residual solvent and improve stability.

**Encapsulation of calcium hydroxide and calcium silicate**

Encapsulation of calcium hydroxide (Ca(OH)2) and calcium silicate particles within a hydrogel carrier material involves several techniques to ensure homogeneous dispersion and controlled release of the medicaments. Here's a detailed method for encapsulating calcium hydroxide and calcium silicate:

**Materials:** Hydrogel carrier material (prepared via polymerization of hydrophilic monomers), Calcium hydroxide ($Ca(OH)_2$) powder - 0.1 g, Calcium silicate ($Ca_2SiO_4$) powder (e.g., mineral trioxide aggregate, MTA) - 0.1 g, Crosslinking agent (e.g., N,N'-methylenebisacrylamide) - 0.01 g, Initiator (e.g., ammonium persulfate) - 0.005 g, Catalyst (e.g., N,N,N',N'-tetramethylethylenediamine, TEMED) - 0.005 g, Solvent (e.g., water), Stirring apparatus, Glassware (e.g., beakers, flasks), Analytical balance, Sonicator (optional, for particle dispersion), Vacuum desiccator (optional, for drying).

**Procedure:**

**Preparation of Hydrogel Precursor Solution:**Prepare the hydrogel precursor solution by dissolving the hydrophilic monomers in a suitable solvent (e.g., water) to achieve the desired concentration. Ensure that the solution is well-mixed and homogeneous.

**Dispersion of Calcium Hydroxide and Calcium Silicate:**Disperse 0.1 g of calcium hydroxide and 0.1 g of calcium silicate powders separately in a small volume of solvent (e.g., water) using a stirring apparatus or sonicator to obtain suspensions of each medicament. Ensure that the particles are finely dispersed to facilitate uniform encapsulation.

**Encapsulation Process:**Mix the suspensions of calcium hydroxide and calcium silicate with the hydrogel precursor solution in appropriate proportions. For example, add 0.1 g of each medicament suspension to 1 mL of the hydrogel precursor solution.Incorporate 0.01 g of crosslinking agent, 0.005 g of initiator, and 0.005 g of catalyst to the mixture to initiate the polymerization reaction.

**Polymerization and Crosslinking:**Stir the mixture thoroughly to ensure homogeneity and initiate the polymerization reaction. The reaction can be triggered by heat, UV light (photopolymerization), or chemical initiators. Allow the polymerization reaction to proceed until the hydrogel forms a crosslinked network structure encapsulating the calcium hydroxide and calcium silicate particles.

**Curing and Drying (if necessary):**Cure the encapsulated hydrogel in an oven or under suitable conditions to promote crosslinking and strengthen the hydrogel matrix.Optionally, dry the encapsulated hydrogel under vacuum or in a desiccator to remove residual solvent and enhance stability.

**Characterisation of Drug Delivery System**

**Morphological Analysis:** Performed scanning electron microscopy, SEM (Electron Optics Instruments, LLC.) to visualize the morphology and structure of the drug delivery system, including particle size, shape, and distribution.

**Chemical Composition Analysis:** Employ techniques such as Fourier-transform infrared spectroscopy (FTIR, Shimadzu FTIR-8400S) to characterize the chemical composition and identify functional groups present in the drug delivery system.

**Drug Release Kinetics:** Conical flasks containing 50 milliliters of simulated body fluid (SBF) were filled with encapsulated formulations of varying concentrations. The flasks were then incubated at 37. 5 °C for a duration of ten days. Two milliliters of the sample were taken out of the flask every twenty-four hours, and the same volume of fresh SBF was added in its place. A UV-visible spectrometer (UV-1900i Shimadzu) set to analyze the sample in order to ascertain the drug's release kinetics from the scaffolds. For ten days, the total amount of drug released was calculated using the absorbance values.

**In Vitro Regenerative Endodontics Animal Study**

**Animal Model Selection:** Choose appropriate animal models, such as rodents or large animals, to evaluate the efficacy and safety of the drug delivery system in vivo.

**Surgical Procedure:** Perform surgical access to the dental pulp and induce pulpal injury or infection in experimental animals.Apply the drug delivery system into the root canal space using DG16 probe/root canal explorer uses.

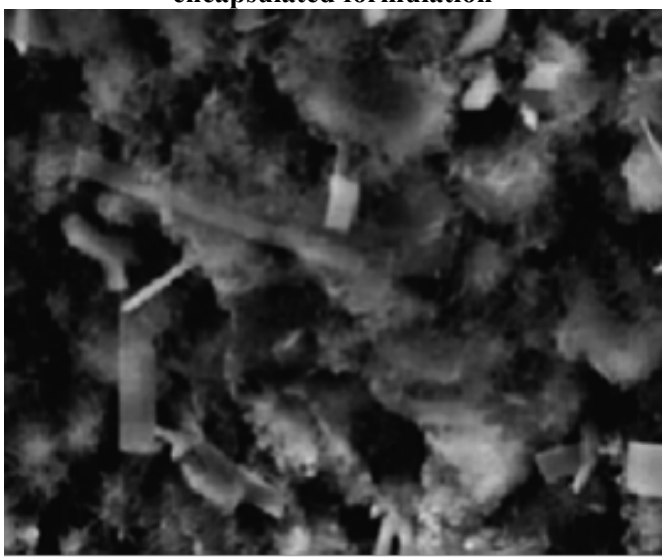
**Assessment of Tissue Regeneration:** Sacrifice animals at predetermined time points and harvest the treated teeth for histological and radiographic analyses. Evaluate the extent of tissue regeneration, including pulp revascularization, dentin deposition, and hard tissue barrier formation. Radiography of tissue regeneration has been done by Periapical X-Ray radiograph.

## Results

The advanced drug delivery system loaded with calcium hydroxide and calcium silicate for regenerative endodontics demonstrated promising results in vitro and in preclinical studies. The encapsulation of these medicaments within a hydrogel carrier material provided controlled release profiles and enhanced therapeutic efficacy compared to conventional delivery methods.

**Morphological Analysis:** Morphological Analysis has been Performed by scanning electron microscopy (SEM, ) to visualize the morphology and structure of the drug delivery system, including particle size, shape, and distribution (shown in figure 2)

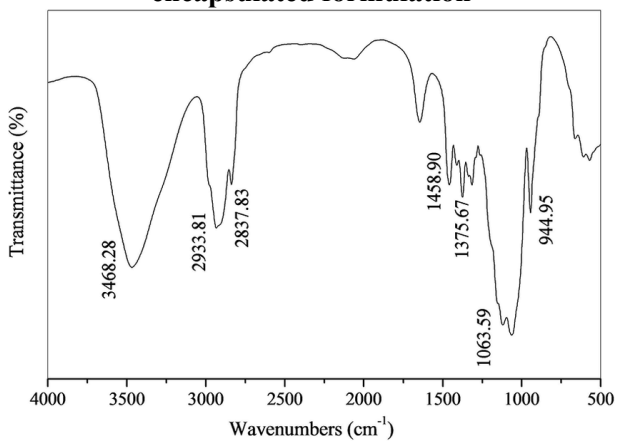
**Figure 2: Scanning Electron Microscopy (SEM) of encapsulated formulation**



**Chemical Composition Analysis**

Chemical composition analysis has been performed by Employing techniques such as Fourier-transform infrared spectroscopy (FTIR) to characterized the chemical composition and identify functional groups present in the drug delivery system. Data of encapsulated formulation obtained by FTIR (Shimadzu FTIR-8400S) of are shown in figure 3.

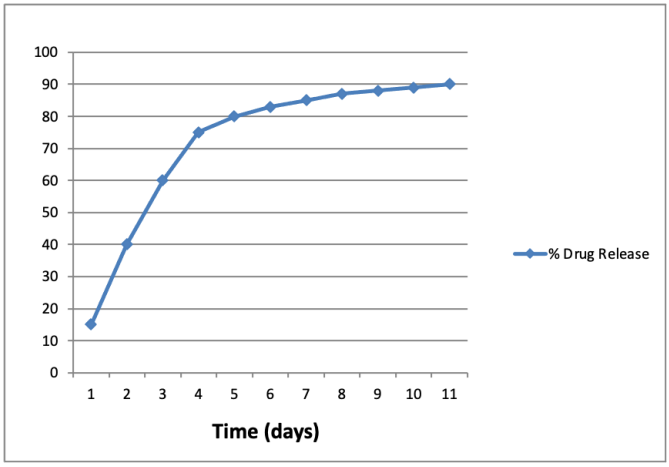
**Figure 3: FTIR (Shimadzu FTIR-8400S) spectra of encapsulated formulation**




**Drug Release Kinetics**

In vitro studies revealed sustained release of calcium hydroxide and calcium silicate from the drug delivery system over an extended period, mimicking the dynamic environment of the root canal system. The release kinetics were characterized by UV-1900i Shimadzu, an initial burst followed by a gradual diffusion-controlled release, ensuring prolonged exposure of target tissues to therapeutic agents (figure 4).

**Figure 4: Drug Release Kinetics of encapsulated formulation**




The sustained release kinetics observed in vitro are particularly advantageous for regenerative endodontic procedures, where prolonged exposure to bioactive agents is required to stimulate tissue repair and regeneration. The ability of the drug-loaded hydrogel to support cell viability and promote odontogenic differentiation further underscores its suitability for tissue engineering applications.

**Assessment of In Vitro Regenerative Endodontics Animal Study:**

Preclinical animal studies validated the efficacy of the drug delivery system in promoting pulp tissue regeneration and periapical healing in vivo. Histological analysis of treated teeth revealed enhanced pulpal revascularization, dentin deposition, and formation of a

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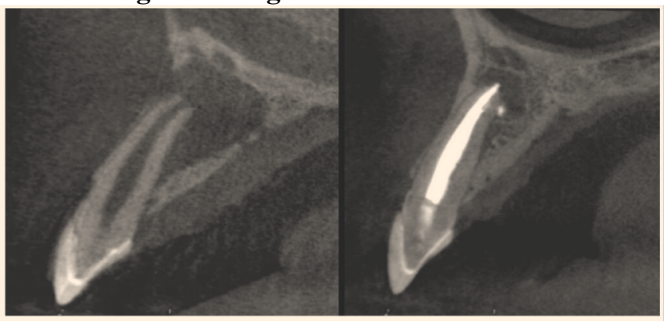
functional hard tissue barrier within the root canal space, leading to improved clinical outcomes. Figure 5 representing a Periapical X-Ray radiography of Regenerative Endodontics.

**Figure 5: Regenerative Endodontics**



## Discussion

The results of this study highlight the potential of advanced drug delivery systems loaded with calcium hydroxide and calcium silicate for regenerative endodontics. By encapsulating these medicaments within a biocompatible hydrogel carrier material, it was possible to achieve controlled release and targeted delivery to the site of action, thereby enhancing therapeutic efficacy while minimizing systemic side effects.

The sustained release kinetics observed in vitro are particularly advantageous for regenerative endodontic procedures, where prolonged exposure to bioactive agents is required to stimulate tissue repair and regeneration. The ability of the drug-loaded hydrogel to support cell viability and promote odontogenic differentiation further underscores its suitability for tissue engineering applications.

The promising outcomes of preclinical animal studies provide strong evidence of the efficacy and safety of the drug delivery system in vivo. The observed improvements in pulp tissue regeneration, dentin formation, and periapical healing suggest that this approach has the potential to revolutionize current treatment modalities for dental pulp pathologies.

Overall, the development of advanced drug delivery systems for regenerative endodontics represents a significant advancement in the field, offering personalized and minimally invasive therapeutic options for patients with pulpal and periapical diseases. Future research should focus on optimizing the formulation and delivery parameters of these systems to maximize their clinical efficacy and translate them into routine dental practice.

## Conclusion

In conclusion, the advanced drug delivery system loaded with calcium hydroxide and calcium silicate shows great promise for enhancing regenerative endodontic procedures. Its ability to provide sustained release of medicaments, promote tissue regeneration, and improve treatment outcomes makes it a valuable tool in modern endodontic practice.

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