ORIGINAL RESEARCH

# Comparative Evaluation of Amniotic Membrane Derivative, Chitosan with Mineral Trioxide Aggregate, Diode Laser, and Ferric Sulfate as Pulpotomy Agents in Human Primary Molars: An *In Vivo* Study

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## Abstract

**Aim:** The aim of the present study was to compare the clinical and radiographic success of amniotic membrane derivative (AMD), chitosan with mineral trioxide aggregate (C-MTA), diode laser (DL), and ferric sulfate (FS) as pulpotomy agents in human primary molars.

**Materials and methods:** In this present study, pulpotomies were performed on 48 primary molars in 30 children aged between 4 and 8 years (12 teeth in each group). Following the pulpotomy procedure, teeth were evaluated clinically and radiographically at 1st, 3rd, 6th, and 9 monthly intervals.

**Results:** After 9 months of follow-up, the clinical success was 91.6% for AMD and C-MTA and 83.3% for DL and FS. Radiographic success was 91.6, 91.6, 75, and 83.3% for AMD, C-MTA, DL, and FS groups, respectively. There is no statistically significant difference between the four groups ($p > 0.05$).

**Interpretation and conclusion:** Results of our study showed that both AMD and C-MTA were equally successful compared to traditional agents like laser and ferric sulfate as pulpotomy agents.

**Clinical significance:** Amniotic membrane derivative (AMD) and C–MTA are alternative biomimetic pulpotomy agents that can be used in pediatric primary tooth pulpotomies.

**Keywords:** Amniotic membrane derivative, Chitosan with mineral trioxide aggregate, Diode laser, Ferric sulfate, Pulpotomy.

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## Introduction

Pulpotomy is one of the most common vital pulp therapies performed for carious, symptom-free primary molars.[1] This technique overcomes the complexities involved in pulpectomy, like accessing the tortuous and complicated root canal anatomy of primary teeth and the cumbersome method of filling the root canal with ideal material that can resorb at the same rate as physiological root resorption. The ultimate objective of this procedure is to save the uninfected and minimally inflamed radicular pulp vitality so that the primary tooth can functionally fit until its natural time of exfoliation, saving the arch integrity at par.[2] Pulpotomy in primary molars is classified as (1) devitalization, (2) preservation, and (3) regeneration,[3] based on the type of medicament and technique used. The success of pulpotomy usually depends upon the diagnosis,[4] techniques of isolation,[4] and the pulpotomy agent being used.[1] Formocresol has been a widely used pulpotomy medicament in pediatric dentistry for decades due to its ease of application and well-documented success rate,[5] but it has its own disadvantages like systemic absorption, inducing a chronic inflammatory response in pulp, cytotoxic, and also having carcinogenic potential.[6] Research concentrated on identifying a better substitute that can be biocompatible and can overcome the limitations of the previous materials being used; though some have been successful in doing so, the search for a more pulp-friendly material is still necessary.

With the advent of new techniques like gene amplification, gel chromatography, invitro protein synthesis, and other biochemical assays, pulp therapy has progressed to new heights. Many naturally occurring, inherent (homologous) proteins and signaling factors aid in wound healing through cellular proliferation, migration, and extracellular matrix production. Bone morphogenetic proteins and other growth factors like platelet-derived growth factors and insulin-like growth factors are some of them that regulate this process.[7] Hence, these could prove to be feasible alternatives to the materials currently used for pulpotomy.

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Various materials with the presence of inherent growth factors have been tried. Amniotic membrane derivative (AMD) is one material that is being extensively researched and applied in various medical fields[8] and also in dentistry[9] because of its germicidal and characteristic wound-healing properties.[10] It is extensively used in the management of burn injuries, skin abrasions and ocular surface disorders as a valuable surgical scaffold with good regenerative inductive properties.[11,12] Applications of this material so far in dentistry have been limited to the filling of surgical defects for early healing and in the field of periodontology, where the membrane form of this material is used for guided tissue regeneration.[9,13,14] So, in the present study, AMD was evaluated as a pulpotomy agent for its clinical and radiographic success.

Other bioactive agents like chitosan are also being extensively studied.[15] Chitosan is a natural carbohydrate (polysaccharide) that is extracted from the protective external skeleton-like structure seen in many crustaceans, which is simply a deacetylated version of chitin with excellent biocompatibility. It is biodegradable and nontoxic, with good antibacterial properties. It is mucoadhesive in nature and has the ability to improve wound healing. Chitosan, which has an improved capacity for chelating calcium ions, has been used along with mineral trioxide aggregate (MTA) for its activity as a potential bioactive scaffold *in vitro*.[16]

In the present study, clinical and radiographic evaluation was done between the following pulpotomy medicaments, that is, AMD, chitosan with MTA (C-MTA), diode laser (DL), and ferric sulfate (FS), in human primary molars. This study aimed to test the null hypothesis that there is no significant difference in clinical outcome between the different pulpotomy medicaments used in this study.

## Materials and Methods

This study was conducted on patients attending the outpatient Department of Pediatric Dentistry at SVS Dental College, Telangana. Children with good general health and no history of any systemic illness, previous hospitalization, or allergic manifestations were included. Parents and guardians were asked to read the study protocol, and written informed consent was obtained. The methodology was reviewed, approved, and documented by the institute's Institutional Human Ethical Committee.

A total of 100 children were screened and examined for the study. A total of 78 patients who met the inclusion criteria were selected. Parents of 33 children denied their consent for the study. Three of the patients dropped out of the study during the follow-up due to accessibility issues. Finally, 30 children were present for the study. A total of 48 primary molar teeth requiring pulpotomy in these children aged between 4 and 8 years were selected. The sample was randomly divided into four groups, with 12 teeth allocated to each group. Teeth were selected irrespective of upper or lower and first or second primary molars. This was a prospective, randomized clinical trial.

The inclusion criteria for teeth indicated for pulpotomy are[17]:

- Teeth with carious lesion approximating pulp.
- Teeth with more than two-thirds root intact without physiologic root resorption.
- Absence of any pain history suggestive of irreversible pulpitis-like spontaneous pain.
- Absence of any chronic periapical inflammation like swelling or medullary abscess formation and abnormal tooth mobility.
- Hemorrhage from the pulpal chamber is controlled with a wet cotton pellet for <5 minutes.

Teeth with evidence of degenerating pulp, presence of pathologic resorption, and calcific degeneration were excluded from the study.

After adequate infiltration or block anesthesia and isolation using a rubber dam and access cavity was prepared using a high-speed airotor with a no. 5 or no. 6 round bur, the coronal pulp chamber was exposed and amputated either by a sharp sterile spoon excavator or the same bur under slow speed. Bleeding from the amputation site was controlled by using a moist cotton pellet dipped in saline. If the bleeding was beyond 5 minutes, then the tooth was indicated for pulpectomy and was excluded. All the cases were allocated randomly to four groups.

Amniotic membrane derivative (AMD) (cryopreserved, freeze-dried amnion, Tata Memorial Tissue Bank, Mumbai) was used to cut the membrane as per the requirement with a sterile scissor, and the remaining was discarded. It was folded and placed in the pulp chamber and pushed so that it soaked in the blood. Chitosan (medical grade, obtained from Kerala Seafoods) was mixed with MTA (ProRoot, Dentsply) powder in 1:1 proportion and then mixed with distilled water and placed over the floor of the pulp. DL (Picasso, AMD) was used at 1.5 W power in continuous, noncontact mode. Pulp stumps at each orifice were lased for 5 seconds. FL (Viscostat, Ultradent) was applied to the pulp orifices with a brush applicator provided.

The pulpotomy procedure was followed by placing intermediate restorative material on the pulpal orifices (Dentsply) over which glass ionomer cement (Fuji II, GC corporation) was restored. After 1 day, the tooth was prepared for a stainless steel crown (Unitek, 3M ESPE) and restored. Follow-up evaluation was scheduled at intervals of 1, 3, 6, and 9 months. Clinical and radiographical evaluation was done at all intervals based on the following criteria.

An asymptomatic tooth without any adverse clinical signs or symptoms suggestive of progress in pulpal inflammation such as pain, abnormal swelling, draining sinus opening in the attached gingiva, pain on vertical percussion, or pathological mobility. The tooth should be devoid of any radiographic evidence of pathologic external or internal root resorption, periodontal ligament widening, furcation radiolucency, or periapical abscess.[4,17]

## Results

The Chi-squared test was used to evaluate the difference in clinical and radiographic success between the pulpotomy medicaments used. There were no statistically significant differences between the different materials used in the study. The success rates in terms of clinical and radiographic outcomes at each follow-up period were similar in all the groups.

Other confounding variables, such as age, teeth in concern, first or second molars, and teeth with no preoperative root resorption, were also statistically analyzed. Analysis revealed that the parameters mentioned did not have a profound effect on the success of treatment ($p > 0.05$).

The pulpotomized teeth were clinically assessed at 1, 3, 6, and 9 months. None of the teeth showed clinical failure in 1- and 3-month recall in all four groups. At 6 months, recall one tooth each showed pain in group II (CMTA), two teeth in group III (DL), and group IV (FS). At 9 months, recall one tooth showed pain in group I (AMD) and one tooth in group IV (FS). There was no statistically significant difference in the treatment outcomes between the four groups evaluated (Table 1).

None of the teeth in the four groups showed any positive radiographical findings at the 1- and 3-month recall. At 6 months

**Table 1:** Comparative evaluation of clinical success

| Follow-up period | AMD | | C- MTA | | DL | | FS | |
|---|---|---|---|---|---|---|---|---|
| | Success | Failure | Success | Failure | Success | Failure | Success | Failure |
| 1 month | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – | 12 (100%) | |
| 3 months | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – |
| 6 months | 12 (100%) | – | 11 (91.6%) | 1 (8.4%) | 10 (83.3%) | 2 (16.7%) | 11 (91.6%) | 1 (8.4%) |
| 9 months | 11 (91.6%) | 1 (8.4%) | 11 (91.6%) | – | 10 (83.3%) | – | 10 (83.3%) | 1 (16.7%) |

Chi-squared test (*p*: 0.919)

**Table 2:** Comparative evaluation of radiographic success

| Follow-up period | AMD | | C-MTA | | DL | | FS | |
|---|---|---|---|---|---|---|---|---|
| | Success | Failure | Success | Failure | Success | Failure | Success | Failure |
| 1 month | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – |
| 3 months | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – | 12 (100%) | – |
| 6 months | 12 (100%) | – | 11 (91.6%) | 1 (8.4%) | 9 (75%) | 3 (25%) | 11 (91.6%) | 1 (8.4%) |
| 9 months | 11 (91.6%) | 1 (8.4%) | 11 (91.6%) | – | 9 (75%) | – | 10 (83.3%) | 1 (16.7%) |

Chi-squared test (*p*: 0.753)

in group II (CMTA) and group IV (FS), one tooth and three teeth in group III (DL) showed pathological resorption. At 9 months of recall, one tooth in group I (AMD) and one tooth in group IV (FS) showed pathological resorption (Table 2).

In the present study, the amniotic membrane and chitosan with MTA showed 91.6% overall clinical success rates, whereas in the DL and FL groups, the success rate was 83.3%. According to the Chi-squared test, there was no statistically significant difference between the four groups (*p*: 0.919). The radiographic success rates of the amniotic membrane and chitosan with MTA were 91.6%, whereas, in the FL group, the success rate was 83.3%; the DL showed the least radiographic success of 75%. There was no statistically significant difference between the four groups evaluated (*p*: 0.753).

## Discussion

The present study evaluated the success of four different pulpotomy medicaments in human primary molars with minimal coronal inflammation. AMD and C-MTA are expected to be regenerative pulpotomy agents, whereas DL and FS are preservative pulpotomy agents. Devitalizing pulpotomy agents were not chosen in the present study as there are better agents to choose from. The current research is also inclined toward the development of biological agents, so current pulpotomy research is also oriented toward the development of regenerative agents.

There was no statistically significant difference among the four test groups for all the follow-up periods of 1, 3, 6, and 9 months. The results of the present study are in favor of the hypothesis that there are no differences in clinical and radiographic success among different pulpotomy materials.

Knowing AMD's antibacterial properties and the presence of angiogenic and growth factors,[10] it was selected to investigate the influence of its application in the repair of amputated pulp. It also serves as a means of studying the effect of amniotic membranes on pulp tissue. In the present study, AMD showed a 91.3% overall clinical and radiographic success rate. Even though the success based on percentage appears to be moderate, only one case has failed after 9 months of follow-up out of 12 teeth. This tooth was considered a failure because the patient complained of pain due to mobility, but there were no other clinical symptoms of failure except radiographically. There was complete resorption of the root, so the tooth was extracted. The patient's age at the time of extraction was 9 years, so the resorption of the root might be physiologic and expected to be the reason for the pain.

The results of the present study showed that AMD has good clinical success as a pulpotomy agent, which can be attributed to the presence of biological healing enhancers like growth factors. As it is relatively inexpensive and easy to handle, it can be considered an alternative to MTA for pulpotomy, but further studies are required to confirm this.

A study done by Chevrier et al. showed that chitosan has the property of enhancing neoangiogenesis and stimulating the healing of budding tissue.[18] Klokkevold and Newman[19] reported that chitosan enhances osteoblastic activity and aids in bone formation. Kim et al.[20] extensively studied chitosan and its derivatives for their applications in tissue engineering of various human tissues like skin, cartilage, bone, nerve fibers and blood vessels and reported its virtuous healing capacity. According to Budiraharjo et al.,[16] the bioactivity of chitosan was enhanced by MTA, and chitosan was incorporated with MTA in this study.

Mineral trioxide aggregate (MTA) has demonstrated clinically, radiographically and histologically a successful pulpotomy medicament by many studies where the clinical success ranged between 97 and 100% and radiographic success ranged between 85 and 100%.[21–25] Agamy et al.,[21] Naik and Hegde,[22] and Farsi et al.[23] reported 100% success rates clinically and radiographically for MTA pulpotomy. Maroto et al.[24] reported a 98.5% success rate, and Holan et al.[25] reported 97% radiographic success after MTA pulpotomy. In the present study, the results of chitosan with MTA are similar to those of other studies where MTA was used. There was one failure after 6 months; thereby, the success rate is 91.3%. So, based on this study, chitosan can be incorporated with MTA for its beneficial effects, and further studies are required to assess various mixing proportions of chitosan with MTA with a larger sample size and longer follow-up. Chitosan can also be tested as an individual pulpotomy agent.

Lasers have been found to have wide applications in oral surgical procedures involving soft tissues.[26] The DL, with its high wavelength absorbance, is most suited for the pulpotomy technique and has the advantage of having a nill effect on other hard tissues. Other studies done using carbon dioxide and erbium

lasers have demonstrated moderate degrees of success in vital pulp therapy.[27,28] Carbon dioxide laser has a minor disadvantage of collateral thermal damage to the surrounding pulp tissue.[29] Many histopathological studies of various other DLs have shown reduced thermal damage and accelerated wound healing in the pulp.[30,31] Based on these characteristics, the DLs are reported to have promising results as an alternative to other pulpotomy medicaments.

Saltzman et al.[32] reported the possibility that pulp ablation might be used in hyperemic pulps as well. Pulpal ablation through repeated laser application results in pulp stumps free of hemorrhage, which might raise hyperemia of the residual pulp tissue, in turn influencing the success of treatment outcomes. In light of current evidence, in this study, the lasing of pulp tissue at the orifice was done after achieving hemostasis.

In the DL group, two teeth failed clinically. Thereby, the clinical success rate was 83.3%, three teeth showed internal resorption, and the radiographic success rate was 75%. It might be because of a rise in temperature or because of an operator's mistake, like placing the laser tip in contact with tissues for a longer time. Studies with lasers have shown variable results, with clinical success ranging from 77 to 100% and radiographic success ranging between 60 and 90%. Golpayegani et al.,[33] after 12 months of follow-up, showed 67% radiographic success, which is significantly less than the formocresol group, which showed 89% success.

Studies with lower success rates have attributed the failure to technical difficulties associated with laser and also to microleakage from the final restoration.[32] Generally, authors are skeptical about the use of laser for pulpotomy, and that's why literature is scanty, and there are no proper guidelines on the use of DL as a pulpotomy agent. Further research and studies are required to validate the use of DLs as pulpotomy agents.

It has been suggested that pulp capping materials should not be placed against a bleeding pulp or a clinically observed blood clot.[34] One of the most commonly used hemostatic agents in medicine and dentistry is ferric sulfate, which has also been showing good results as a medicament for primary teeth pulpotomies.[35] The vascular proteins get agglutinated from the reaction of blood constituents with ferric and sulfate ions. This ferric ion-protein complex seals the cut vasculature and produces immediate hemostasis mechanically. Hemostatic plugs occlude the capillaries and also prevent the formation of blood clots, thereby minimizing chances for inflammation and internal resorption in pulp therapy. With the current evidence, FL is considered a good alternative to formocresol.

Ferric sulfate (FL) has shown 91.6% success clinically and radiographically after 6 months and 83.3% after 9 months of follow-up. This is in accordance with previous studies where clinical success ranged between 78 and 100%, and radiographic success ranged between 70 and 90% (Fuks et al.[36]; Smith et al.[37]; Neamatollahi and Tajik[38]). FL is relatively inexpensive, easy to use, and reliable as a pulpotomy agent. However, in this era of regenerative techniques, when more biological agents can be used, a search for more biological agents is required.

All the failed teeth in our study were first primary molars with proximal caries. The probable reason for the failures can be attributed to the presence of irreversibly affected radicular pulp without any clinical signs and symptoms, diagnosing which is beyond the scope of current techniques employed generally in day-to-day practice.

The limitations of our study are a shorter follow-up duration of 9 months and a small sample size. To have a better radiographic picture, it is better to select only mandibular second primary molars for the study, which was not possible in the present study. To have a better understanding of the biological interaction of the materials used in the study, histological evaluation is mandatory, which is theoretically impossible, so the interaction of the material at the cellular and tissue level was studied.

## Conclusion

The following conclusions were drawn from the present study:

- The four medicaments showed promising results clinically and radiographically.
- Amniotic membrane derivative (AMD) and C-MTA have shown superior results over other agents.
- Amniotic membrane derivative appears to be a promising agent when factors like sterility, cost, isolation, and ease of manipulation are taken into consideration. As the agent has to be placed on the pulp directly, a sterile material like an amniotic membrane, which requires lesser manipulation, prevents contamination and thus promotes the success rate.
- Results of our study showed that both AMD and C-MTA were successful and can be considered alternative pulpotomy medicaments.
- Further studies using larger sample sizes with longer evaluation periods are recommended to get to a scientifically sound conclusion.

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