



Original Article

Amniotic Membrane versus Formocresol as Pulpotomy Agents in Human Primary Molars: An in vivo Study

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Abstract

Objective: To determine and compare the efficacy of Amniotic Membrane (AM) as a pulpotomy agent with Formocresol (FC) clinically and radiographically. **Material and Methods:** 30 deciduous molars warranted for pulpotomy in 24 children (4-9 years) were divided equally into two groups of 15 each. Group 1: Amniotic membrane pulpotomy and Group 2: Formocresol pulpotomy, which was followed by placement of glass ionomer cement and stainless steel crown restoration. The patients were recalled after 1, 3, 6 and 9 months for clinical and radiographic evaluation. Fisher's exact test and McNemar test were used for statistical analysis. **Results:** Results indicated both clinically and radiographically amniotic membrane performed at par with formocresol. **Conclusion:** Amniotic membrane with its regenerative, antibacterial properties and the ability to deliver growth factors has shown promising results comparable to gold standard formocresol when used as a pulpotomy agent and hence can be recommended as an alternative pulpotomy agent.

Keywords: Amnion; Formocresols; Tooth, Deciduous; Pulpotomy.

Introduction

The primary goal of any pulp therapy is to maintain the integrity and health of the teeth and their supporting tissues. One such pulp therapy technique used for preserving decayed primary molars is pulpotomy, which is done in a primary tooth with extensive caries but without evidence of radicular pathology. This technique involves removal of the coronal infected pulp and the remaining radicular pulp is opined to be vital and free of any pathological alterations [1,2].

Formocresol, introduced by Buckley, has been the drug of choice in pulpotomy for primary teeth due to ease of use and high clinical success rate [3]. In spite of its wide usage, it possesses known toxic, mutagenic and carcinogenic potential risk in humans as it is systemically absorbed, and increases the prevalence of hypoplastic and/or hypomineralization defects, and is known to cause necrosis and sloughing of the gingival tissue [4].

Recently, the fetal-derived mesenchymal stem cells (MSC) from the placenta or other gestational tissues like the amniotic fluid, umbilical cord are novel materials with rich stem cell reserves [5]. The matrix of Human Amniotic Membrane (HAM) contains abundant growth factors like keratinocyte growth factor (KGF), basic-fibroblast growth factor (b-FGF), transforming growth factor-beta (TGF- β), nidogen growth factor (NGF) and epidermal derived growth factor (EDGF) which promote tissue regeneration [6]. These growth factors provide a natural healing environment and mimic the stem cell niche for ex vivo growth.

Amniotic Membrane (AM) has a proven rate of success in the field of dentistry as guided tissue regeneration, root conditioning, haemostatic and wound dressing agent. It has inherent properties like low immune response and toxicity, ability to promote cellular growth and attachment [7]. Hence, the present study was aimed at comparing the success of pulpotomy outcomes using amniotic membrane and formocresol by evaluating them both clinically as well as radiographically.

Material and Methods

Sample

The present study was conducted on a group of 24 children (4-9 Year-old) who had attended the outpatient Department of Pedodontics and Preventive Dentistry with good general health and no history of systemic illness or hospitalization.

The selection of teeth were done according to the criteria proposed by previous authors [8] which includes a restorable tooth with large carious lesion, with no spontaneous pain, presence of at least 2/3rd of root length, with no sign of internal/external root resorption and hemorrhage from amputated sites that are easy to control.

A total of 30 deciduous molars from 24 children, which met the inclusion criteria [8] were randomly divided into two groups of 15 teeth each: Group 1: Amniotic Membrane Pulpotomy and Group 2: Formocresol Pulpotomy.

Clinical Procedure

After local anesthesia administration and rubber dam isolation, the involved teeth indicated for pulpotomy were treated by complete removal of residual caries by using handpiece with a round bur and access to the coronal pulp was obtained. The inflamed coronal pulp was removed with a sharp spoon excavator followed by gentle debridement of the coronal pulp chamber with saline.

Amniotic Membrane Pulpotomy Procedure

After removal of the coronal pulp a sterile saline wet cotton pellet was placed for 1 min on the pulp stumps and once bleeding was controlled, Dry Amniotic Membrane (Amnio-care, Biocover Laboratories, Model Town Karnal, India) wetted in saline for a minute was placed with the help of tweezers, in such a way that it completely covered the exposed pulp stumps followed by zinc oxide eugenol restoration over this amniotic membrane.

Formocresol Pulpotomy Procedure

After removal of coronal pulp, Formocresol (Pharmadent Remedies, Maharashtra, India) wet cotton pellet was placed for 1 min over the pulp stumps with tweezers and once hemorrhage was controlled, zinc oxide eugenol restoration was placed over the pulp stumps.

Following the pulpotomy procedure in both Group 1 and Group 2, Glass ionomer cement restoration (Fuji type II gold label) was placed coronally over the zinc oxide eugenol cement (prime dental product) followed by stainless steel crown after 1 week.

Group 1 and Group 2 were clinically assessed for pain, swelling, the presence of sinus tract, mobility and radiographically assessed for periodontal ligament (PDL) widening, furcal/periradicular radiolucency (FR/PR), internal/external resorption (IR/ER), premature exfoliation and all cases were evaluated both clinically and radiographically at a period of 1 month, 3 months, 6 months and 9 months interval.

Statistical Analysis

Data were subjected to Fishers exact test and the Mc Nemar test to evaluate the efficacy of each material between 1 month, 3 months, 6 months and 9 months and significance was set at $p < 0.05$.

Ethical Aspects

Written informed consent was obtained from the parents/guardians of children who required pulpotomy treatment and ethical clearance was obtained from the institutional ethical committee.

Results

Comparison of clinical criteria, i.e., status of pain, sinus tract, swelling and mobility, at 1 month, 3 months, 6 months and 9 months interval showed a non-significant difference ($p>0.05$). Clinically, both Group 1 and Group 2 showed 100 % clinical success rate (Table 1).

Table 1. Comparison of amniotic membrane and formocresol clinically at different time points.

Clinical Findings	Preoperative		1 month		3 months		6 months		9 months		Overall success rate (%)	
	AM	FC	AM	FC	AM	FC	AM	FC	AM	FC	AM	FC
Pain	15	15	0	0	0	0	0	0	0	0	100.0	100.0
Mobility	0	0	0	0	0	0	0	0	0	0	100.0	100.0
Swelling	0	0	0	0	0	0	0	0	0	0	100.0	100.0
Sinus tract	0	0	0	0	0	0	0	0	0	0	100.0	100.0

AM = Amniotic Membrane; FC = Formocresol; Fishers exact test.

Observations from radiographic findings in Group 1 revealed one tooth with IR at 1-month follow-up and this IR further increased in size at 3rd and 6th-month follow-up intervals without clinical symptoms, but the same tooth showed a reduction in the size of IR at the 9th-month follow-up. Another two teeth showed development of FR at 3rd-month follow-up in which one tooth showed reduced FR whereas in the other tooth same size FR was observed at 6th-month follow-up and FR had further increased in size with non-involvement of permanent tooth bud at 9th-month follow-up. In Group 1, overall radiographic success rate stood at 80 % at 6 months, which improved to 93.3% at 9 months follow-up (Table 2).

In Group 2, two teeth developed IR in which one tooth showed IR at 3rd month follow-up and the other tooth at 6th month follow-up, thus the overall radiographic success rate for Group 2 stood at 93.3% at the end of 3 months follow-up, which deteriorated to 86.6% at 6th month thus in Group 2 at the end of 9 months overall radiographic success rate was 86.6% (Table 2). In both the Groups, teeth with radiologic findings of either IR/ER or FR/PR were clinically asymptomatic and hence no clinical intervention was undertaken for these teeth.

Table 2. Comparison of amniotic membrane and formocresol radiographically at different time intervals.

Radiographic Findings	Preoperative		1 month		3 months		6 months		9 months		Overall success rate (%)	
	AM	FC	AM	FC	AM	FC	AM	FC	AM	FC	AM	FC
Internal/external resorption (IR/ER)	15	15	1	0	1	1	1	2	0	2	100.0	86.6
Furcation/ periapical radiolucency (FR/PR)	Permanent tooth bud not involved		0	0	0	0	2	0	2	0	93.3	100.0
	Involving Permanent tooth bud		0	0	0	0	0	0	0	0	100.0	100.0
	Premature exfoliation		0	0	0	0	0	0	0	0	100.0	100.0
Periodontal ligament widening	0	0	0	0	0	0	0	0	0	0	100.0	100.0

AM = Amniotic Membrane; FC = Formocresol.

Furthermore, with respect to radiographic criteria, i.e., premature exfoliation, periodontal ligament (PDL) widening, IR/ER and PR/FR, we observed a non-significant difference ($p>0.05$) for both Group 1 and Group 2 after 1, 3, 6 and 9 months follow-up (Table 3).

Table 3. Comparison of p values for amniotic membrane and formocresol at different time intervals.

Radiographic Findings for Groups 1 and 2	P- value									
	Pre-operative		1 month		3 months		6 months		9 months	
	AM	FC	AM	FC	AM	FC	AM	FC	AM	FC
Internal/external resorption (IR/ER)	1.0000	1.0000	0.9999	0.9999	0.9999	0.9999	0.9999	0.9999	1.0000	0.9999
Furcation/periapical radiolucency (FR/PR)	1.0000	1.0000	0.9999	1.0000	0.9999	1.0000	0.9999	0.9999	0.9999	1.0000
Premature exfoliation	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Periodontal ligament widening	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

AM = Amniotic Membrane; FC = Formocresol; p value determined by McNemar test where $P=0.0001^*$; $p<0.05$ determined as significant.

Discussion

In search of ideal pulpotomy medicament, materials like formocresol, ferric sulfate, glutaraldehyde, calcium hydroxide, adhesive liners, enamel matrix derivative, MTA, bioactive glass, bone morphogenic protein, growth factors, pulpotech, collagen and other techniques like electrosurgery and lasers and antibiotic based pulpotomy [9] have been tried out with variable clinical, radiological and histological success for pulpotomy procedure in both primary and permanent dentitions [10].

The high biocompatibility of Human Amniotic Membrane (HAM), in addition to its low immunogenicity and the presence of several growth factors, recommend it to be used as a tissue engineering scaffold [11-13]. The structure of HAM is similar to dental pulp tissue in several aspects. First, both of them are soft connective tissues. Second, the presence of monolayer cells in the peripheral zone of both of them, i.e., epithelial cells in HAM and odontoblasts in pulp tissue, which gives a notable distinguished similarity [12].

There are no studies reported in the literature regarding amniotic membrane clinical efficiency as a pulpotomy agent in human primary molars. This formed the basis for our current study. The success rate of amniotic membrane pulpotomy in this study showed absence of adverse clinical signs such as pain, swelling, and sinus/fistula, which could be due to its antibacterial [14], anti-inflammatory and regenerative properties [15]. The 100% clinical success rates of formocresol pulpotomy in our study can be attributed to its germicidal action [16].

As reported in many studies, the most common finding after pulpotomy procedure is the occurrence of internal resorption (IR) [17-20]. Among the formocresol pulpotomy group, one tooth showed IR at 3rd month follow-up and another tooth at 6th month review, but without any signs of recovery at the 9th month follow-up, which may be due to the chronic inflammation of the residual pulp caused by formocresol [21] whereas in amniotic membrane group, one tooth displayed internal resorption in all follow-up intervals but at 9th month review we noted a decrease in internal resorption for this tooth.

Amniotic membrane is similar to platelet-rich fibrin scaffold [22] as it increases host stem cell populations in treated tissues and, in turn modulating stem cell responses in the wound bed by the growth factors stimulation and thus the cause for internal resorption might be due to stimulation of undifferentiated mesenchymal stem cells by the amniotic membrane which might have

differentiated into odontoclasts and caused internal resorption. Recovery of this tooth at a later stage which might be attributed to the effect of combination of stimulation of undifferentiated mesenchymal cells into odontoblasts, growth factors stimulation by the intact amniotic membrane and the anti-inflammatory properties of amniotic membrane [23]. Similarly, in Group 1 additional two teeth showed furcational radiolucency at 6 months follow-up in which one tooth showed signs of recovery with interradicular bone formation observed at the 9th-month review.

Pulp canal obliteration (calcific metamorphosis) was found in the one tooth at 9 months follow-up treated with amniotic membrane which was similar to results obtained with MTA [24] and this might be due to the influence of (TGF- β) from amniotic membrane [25] which may have caused increased odontoblastic activity and suggesting that the tooth has retained some degree of vitality and function over time.

The freeze-dried amniotic membrane can be readied for use by soaking in normal saline for 1 minute. Amniotic membrane returns to a layered structure similar to that of fresh amnion when it absorbs water [26] and similarly, in our study, dry amniotic membrane was placed in saline for 1min to rehydrate and activate clinically.

Amniotic membrane enhances gingival wound healing properties and reduces scarring. Excellent revascularization of the amniotic membrane is another favorable property [27]. This was evident in our study, as direct application of amniotic membrane on the radicular pulp tissue could have promoted faster healing and recovery of the remaining radicular pulp to a normal state.

Amniotic membrane can be a favorable graft material for vestibuloplasty, promoting healing and preventing relapse. It is easily available and preserved and is a cost-effective material [28].

The mechanisms involved in accelerated wound healing by amniotic membrane are as follows: • Immunomodulative and Immune privilege; • Anti-microbial (broad spectrum effect against bacteria, fungi, protozoa and viruses); • Reduction of pain; • Anti-scarring and anti-Inflammatory; • Tissue reparative activities with enhanced bone remodeling, osteogenesis and chondrogenesis; • Speed fibrogenesis and angiogenesis; • Increased extracellular matrix deposition and • Potent source of mesenchymal stem cells [29].

In our study, amniotic membrane was used as a naturally derived pulpotomy agent, which acted as a scaffold and might have stimulated growth factors and in turn pulp undifferentiated mesenchymal cells, which may have allowed for the rapid recovery of the radicular pulpal tissue. With the many advantages of amniotic membrane, we were able to obtain significant success rates with no significant statistical differences with formocresol.

The use of amniotic membranes requires skill; thus, operator's inexperience is a limitation. There is always an associated risk of infection transmission with transplantation of amniotic membranes. In this study, gamma irradiated dry amniotic membrane was used thus reducing any chances of cross infections. Adequate precautions should be taken and safety criteria should be included in the application of these biological membranes. Amniotic membranes are fragile

membranes, so they need to be dealt with very carefully and also the procedure associated with the use of these membranes is technique-sensitive [30].

Conclusion

Amniotic membrane with its growth factors and high biocompatibility is a natural source, which is easily available, applicable and hence can be suggested as an alternative pulpotomy agent owing to its clinical and radiographic success in the present study. Further long-term studies are required to assess other beneficial properties of amniotic membrane as a regenerative pulpotomy agent.

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