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Case Report

A novel regenerative endodontic procedure in a traumatized immature tooth using amniotic membrane

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ABSTRACT

Tooth nonvitality is one of the frequently seen consequences of dental trauma that causes the arrest of root development. Amniotic membrane has received a lot of attention for its use in transplantation and regeneration procedures. This article reports a unique and novel case of successful regenerative endodontic procedure done using amniotic membrane in a traumatized immature right maxillary central incisor of an 8-year-old girl. The clinical and radiographic evaluation done during the recall visits at 1, 3, 6, 9 and 12 months showed a progressive root growth with apical closure.

Key Words: Amnion, dental pulp necrosis, regenerative endodontics, tooth fractures

INTRODUCTION

Regenerative endodontic procedures (REP) are biologically based procedures, designed to replace damaged structures, including dentine and root structures, along with the cells of the pulp-dentine complex.[1] The three essential elements in any regenerative procedure include stem cells, scaffold, and growth factors.[2]

Human amniotic membrane is an avascular tissue of fetal origin. It is the innermost lining of the human placenta, which is disposed after the baby is born.[3] A thick basement membrane together with an avascular stromal matrix constitutes the structural aspect of amniotic membrane. It contains two types of cells – embryonic ectodermal-derived human amnion epithelial cells and embryonic mesodermal-derived human amnion mesenchymal stromal cells. Both the cell types share similar immune phenotype and are capable of differentiation into major mesodermal lineages.[4] The human amniotic membrane also acts like a scaffold for cell proliferation and differentiation.[5]

Thus, lot of attention has been paid toward the use of amniotic membrane for transplantation and regeneration procedures. The preserved human amniotic membrane has been already tried in various areas of medicine and dentistry to regenerate the lost tissues and to accelerate repair.[6] However, there is not much documented literature, where amniotic membrane has been used for REP. This case report describes the clinical and radiographic findings of a

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successful REP of a nonvital immature permanent tooth with apical periodontitis, using amniotic membrane.

CASE REPORT

An 8-year-old female patient reported with the chief complaint of trauma to the upper front teeth 1 day ago due to a fall from the bed while playing at home. No significant findings were present on extraoral examination.

Intraoral examination revealed mixed dentition status with an Elli's Class I fracture of tooth 11 and Elli's Class II fracture of tooth 21. Both the teeth were tender on vertical percussion. Radiographic examination revealed an open apex in relation to teeth 11 and 21 without any signs of periapical pathology [Figure 1a-c]. Thus, Type III glass ionomer cement (GC Corporation, Tokyo, Japan) was placed on the fractured incisal edges of 11 and 21.

During the initial three recall visits, the patient claimed mild tenderness on vertical percussion in relation to both the teeth. However, no definitive signs of nonvitality were seen. On the fourth recall visit, the patient complained of pain on biting with respect to 11, and it was noticeably tender on vertical percussion. On radiographic examination, widening of the apical periodontal ligament of 11 was noted, exhibiting a periapical index score[6] of 2, thereby suggesting a diagnosis of pulpal necrosis and symptomatic apical periodontitis [Figure 2a]. The crown root ratio of 11 as calculated in the preoperative radiograph using Image J software was 1.12 (National Institutes of Health, University of Wisconsin, Madison, United States). REP in relation to tooth 11 using amniotic membrane was planned, after obtaining an informed consent. Ethical clearance was also obtained from the institutional ethics committee for the use of amniotic membrane in a child patient for endodontic procedures (Reference No.: 18087).

Access opening was done under local anesthesia, 2% lidocaine with 1:200,000 adrenaline (Xylocaine 2%, AstraZeneca Pharma India Ltd., India), and rubber dam isolation. Necrotic pulp tissue was extirpated; the root canal was then irrigated with 20 mL of 1% sodium hypochlorite (NaOCl) (Septodont, France) and saline using Navitip® (Ultradent Products, USA).[7] Following working length determination, coronal dentine was sealed using a bonding agent (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA), and an intracanal medicament was placed in the form of triple antibiotic mixture (ciprofloxacin [ciplox Tablet 250 mg, Cipla, India], metronidazole [Flagyl Tablet 400 mg, Nicholas Piramal India Ltd.], and minocycline [Divaine tablet 100 mg, Cipla, India]). Care was taken to fill the triple antibiotic mixture only till the cementoenamel junction, and cotton pellets soaked in absolute alcohol were used to wipe off any residual paste on the pulp chamber to avoid possible tooth discoloration due to the use of minocycline. A closed dressing using Type II glass ionomer cement was given. The patient was recalled after 2 weeks, where the access was regained, and final irrigation of the canal was done using 20 mL of 1% NaOCl and 20 mL of 17% ethylenediaminetetraacetic acid (EDTA)(Dent Wash, Prime Dental products, India).[4] After drying the canals, a 2 cm × 2 cm sheet of amniotic membrane (Tata Memorial Hospital, Mumbai, India) was cut into approximately 1 mm × 1 mm size, moistened with saline, folded with the help of a tweezer, and placed in the canal till 3–4 mm short of the orifice [Figure 2b]. Mineral trioxide aggregate (MTA-Angelus) was placed over the amniotic membrane till the orifice and glass ionomer cement restoration was done and postoperative radiograph was taken [Figure 2c].

At the 3rd month recall visit, the patient was asymptomatic, and the radiographic evaluation revealed an increase in the root length with apex heading

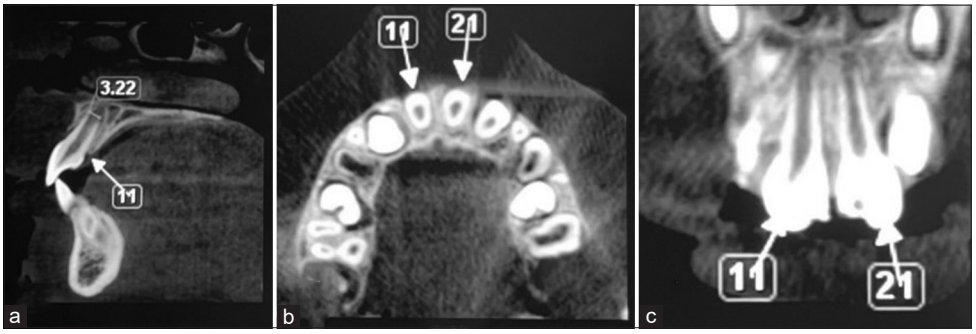

Figure 1: Preoperative cone-beam computed tomography (a) Sagittal view of tooth 11 showing a wide-open apex of diameter 3.22 mm. (b) Axial view of teeth 11 and 21. (c) Coronal view of teeth 11 and 21.

toward closure [Figure 3a] with a crown root ratio of 0.98 (Image J software). At the 6th month radiographic evaluation, root showed continued increase in the length with apex closed [Figure 3b]. However, there were no gross changes in the thickness of the root canal wall. At the 9th month follow-up, intraoral periapical radiograph taken showed complete closure of root apex [Figure 3c] with a crown root ratio of 0.86 (Image J software). The regenerated tooth showed positive response similar to the adjacent teeth (teeth 12 and 22) to electrical pulp testing. The 12th month intraoral periapical radiograph showed a closed apex without any periapical abnormality in relation to 11; however, the tooth 21 showed an immature root apex [Figure 3d].

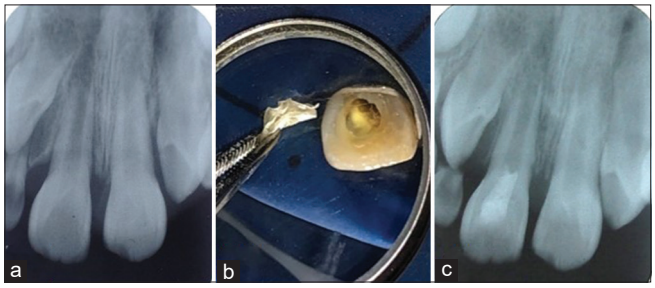

Figure 2: (a) Preoperative radiograph showing teeth 11 and 21 with immature root apex. (b) Amniotic membrane being placed in the root canal of tooth 11 with the help of a tweezer. (c) Posttreatment Intra oral peri-apical Radiograph with amniotic membrane in the root canal space, mineral trioxide aggregate placed till the cementoenamel junction and resin-modified glass-ionomer cements restoration done on tooth 11.

Clinically, the teeth 11 and 21 were asymptomatic and were not tender on percussion [Figure 4a]. Cone-beam computed tomography report revealed closed apex and no periapical pathology in tooth 11, whereas tooth 21 showed an open apex with a well-defined periapical radiolucency suggestive of a periapical cyst [Figure 4b]. Based on this incidental radiographic finding, REP has been initiated even in relation to tooth 21.

DISCUSSION

Existing literature on endodontic regeneration of immature permanent teeth, successfully documents the use of blood clot, platelet-rich plasma, and platelet-rich fibrin as different approaches toward the root development and apex closure. However, these methods have few disadvantages, namely invasiveness in the procedure, associated discomfort while inducing bleeding/withdrawal of the blood, time and difficulty involved in the preparation, and relatively high treatment cost.[8]

Suresh *et al*.[9] reported the first case, where REP was performed in a nonvital immature permanent central incisor using human amniotic membrane. At the end of 3 years, they found approximately 78%–86% reduction in the volume of periapical lesion size, increase in canal width, as well as positive response to pulp sensitivity tests. Even in the present case, we observed gradual and significant increase in the root

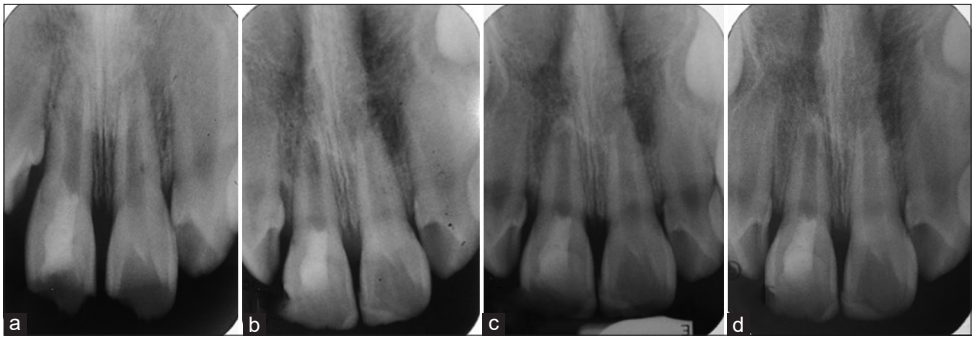

Figure 3: Progressive root closure with respect to tooth 11 seen at (a) 3-month follow-up. (b) 6-month follow-up. (c) 9-month follow-up. (d) 12-month follow-up.

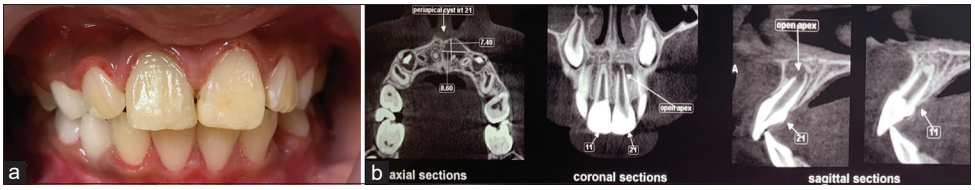

Figure 4: (a) Postoperative clinical photograph at 12 months. (b) Cone-beam computed tomography image showing complete apical closure of tooth 11 and a well-defined radiolucent lesion at the apex of tooth 21.

length and apex closure at 3rd, 6th, 9th, and 12th month follow-ups.

The regenerating ability of the amniotic membrane can be explained in manifold. The cells of amniotic membrane are pluripotent in nature. The membrane acts as an ideal scaffold to encourage migration, adhesion, and differentiation of epithelial cells, while preventing their apoptosis. The amniotic membrane cells are also capable of producing multiple growth factors including vascular endothelial growth factor (VEGF) and insulin-derived growth factor. These growth factors favor the formation of granulation tissue and the epithelialization leading to neovascularization.[10]

The criteria that we chose for case selection and the procedure followed were similar to that of any other regeneration case. We used triple antibiotic paste for root canal disinfection, due to its effectiveness in combating the existing diverse microflora present in the necrotic pulp, root canal dentine, and periapical lesions.[10] MTA was used to provide a tight coronal seal, as it also has an added advantage of promoting the growth of stem cells through the release of signaling molecules.[11]

Amniotic membrane is cost-effective and easy to handle. Disinfected amniotic membrane obtained from a consented donor following an elective cesarean delivery, is the ideal choice, and it can be even preserved for future use.[12] Although it is a human-derived tissue, it carries a negligible risk of infection or immunogenicity.[10] We used processed and stored membranes, which were available for research purpose. These membranes are ultrathin in thickness due to which it can be easily molded to any root canal irrespective of its shape and configuration. *In vitro,* it has shown unaltered strength and shape when soaked in sterilized physiologic saline solution for 1 month. However, the biodegradable rate after a month is unknown.[3]

CONCLUSION

The amniotic membrane is a promising material to be used for revascularization of immature permanent teeth. When used for the same, it results in continued root development, thereby indicating the successful revascularization.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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