Case Report

Analysis of the rate of maturogenesis of a traumatized Cvek's stage 3 anterior tooth treated with platelet-rich fibrin as a regenerative tool using three-dimensional cone-beam computed tomography: An original case report

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ABSTRACT

Regenerative endodontic procedures are biologically based procedures which deal with the regeneration of pulp-like tissue, more idealistically the pulp-dentin complex. The regeneration of this pulp-dentin complex in an infected necrotic tooth with an open apex is possible only when the canal is effectively disinfected. Though there are various procedures for treating open apex ranging from $Ca(OH)_2$ apexification, mineral trioxide aggregate apexification and surgical approach, regeneration of tissues has always taken superior hand over the repair of tissues. The mechanics behind the regenerative endodontic procedures is that despite the tooth being necrotic, some pulp tissue can survive apically which under favorable conditions proliferate to aid in the process of regeneration. In the past 2 decades, an increased understanding of the physiological roles of platelets in wound healing and after tissue injury has led to the idea of using platelets as therapeutic tools in the field regenerative endodontics. In the present case report with an open apex, high sterilization protocol is followed using triple antibiotic paste as intra-canal medicament, followed which platelet rich fibrin is used as the regenerative material of choice. Over an 18-month follow-up period, clinically patient is asymptomatic and radiographically there is complete regression of the periapical lesion and initiation of the root end closure.

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Trauma whether major or a minor one, always leaves an impact. Dental trauma has always been one of the most common reasons for seeking dental care and which requires immediate treatment both pathologically and psychologically. Trauma to the anterior teeth accounts for one-third of all traumatic injuries in boys and one-fourth of all injuries in girls. Most dental injuries occur between 8 and 12 years of age.[1] This is the period during which the root development will be taking place and any trauma during this period would hamper the root development, resulting in immature open apex with thin dentinal walls.

There are various treatment options for treating immature nonvital teeth, which includes: Surgical endodontics, apexification, and regenerative endodontics. Though surgical approach can be used, it has many disadvantages like: It is an invasive procedure, which results in an altered crown root ratio, leading to psychological distress, with possible surgical complications and increased cost of treatment.[1]

The next and the most common treatment option for open apex is apexification, which is a method to induce development of the root apex of an immature pulpless tooth by formation of osteocementum/bone-like tissue. $Ca(OH)_2$ apexification is the oldest method which was first introduced by Kaiser in 1964. The main drawbacks with this procedure are, it is time-consuming (6–24 months), makes tooth brittle, and its high pH is known to be toxic to vital cells and prevents migration of multipotent undifferentiated mesenchymal cells into the canal.[2,3]

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In order to overcome disadvantages of calcium hydroxide, mineral trioxide aggregate (MTA) is used for this procedure which is a biocompatible material that sets in the presence of moisture, has a good sealing ability and the procedure can be completed in single visit.[4] Regardless of the material used, apexification procedure only forms apical bridge and does not strengthen the remaining tooth structure. Alternatively, regenerative treatment procedures are potential regimes which help in the natural development of the tooth rather than the repair.

Earlier attempts were made in this direction by use of graft materials for healing of periapical region. But, now regeneration of tissues rather than a replacement with an artificial substitute is an emerging and exciting field in the health sciences. Regenerative endodontics has been defined as biologically based procedures designed to replace damaged structures such as dentin, root structures, and cells of the pulp-dentin complex. This paper describes a case of tissue regeneration with platelet-rich fibrin (PRF) in an immature nonvital tooth after thorough disinfection protocol.

CASE REPORT

A 15-year-old male patient reported to the department with the chief complaint of broken upper front tooth. His medical history is noncontributory, with a history of trauma 5 years back. On clinical examination, there was a discolored Ellis Class IV fracture of the upper front toot [Figure 1], which was tender on palpation. Radiographic examination revealed open apex which was 3 mm wide with thin dentinal walls and large periapical radiolucency with an impacted mesiodens between two central incisors [Figure 2]. Pulp sensitivity tests gave a negative response of 12, 11, and 21. The various treatment options for this particular scenario include – surgical approach, apexification, and regenerative endodontics. All the procedures were explained to the patient, and informed consent was obtained for use of regenerative procedures.

Figure 1: Pre-operative clinical photograph of 11 with Ellis Class III fracture

Initially, access cavity preparation was done under rubber dam isolation followed by working length determination using ingles method which was about 21 mm and a minimal biomechanical preparation was done using hand K-files (Mani, Japan). Then, after thorough irrigation with saline, 0.5% NaoCl and 2% chlorhexidine, canal was packed with triple antibiotic paste (TAP) mixed with propylene glycol and macrogol [Figures 3 and 4]. Individually, these tablets are crushed after removing the sugar coatings and then they are taken in 1:1:1 ratio which is mixed with 1:1 ratio of propylene glycol and macrogol till the creamy consistency is obtained. This was packed using lentulospirals below the cementoenamel junction (CEJ) and coronal seal done with cavit. After a period of 21 days, patient was recalled and under rubber dam isolation TAP was flushed out with saline and thoroughly irrigated.

Ten milliliter of blood was withdrawn from the medial cubital vein of the patient [Figure 5] and was immediately centrifuged without the addition of anticoagulants at the speed of 2800 rpm for 10 min [Figure 6]. After the centrifugation, blood was segregated into three layers, the bottom layer red blood cells (RBCs), the middle layer PRF and the top layer being acellular plasma [Figures 7 and 8]. Plasma was discarded; PRF was removed and placed into the canal with the help of hand pluggers [Figure 9]. Over PRF, 3 mm of white MTA plug was placed and closed with moist cotton and cavit. Patient was recalled after 3 days and coronally double sealed with glass ionomer cement (GIC) and composite [Figure 10].

Patient was clinically and radiographically evaluated every 1 week, 1 month, 3 months, 6 months, 1 year, and 18 months [Figures 11 and 12]. As regular radiovisuographs (RVG) is only two-dimensional (2D) imaging, in order to confirm the results cone-beam computed tomography (CBCT) was taken after 18 months [Figures 13 and 14]. When the results were analyzed, there was complete regression of

Figure 2: Pre-operative radiovisuography of 11 with wide open apex and periapical pathology

Figure 3: Dispensing of ciprofloxacin 500 mg, minocycline 50 mg and metronidazole 400 mg on a glass slab

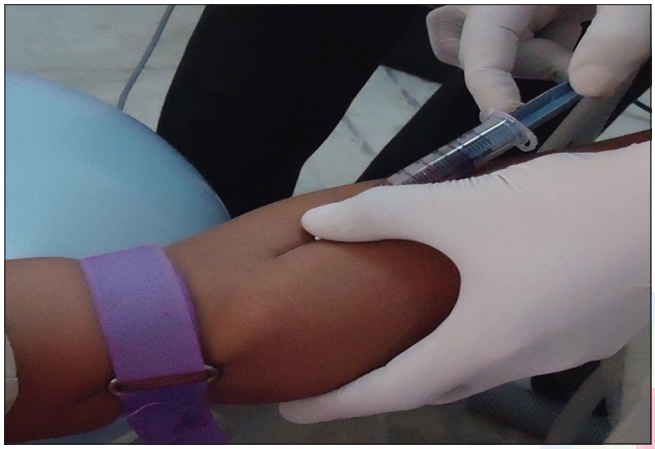

Figure 5: Withdrawal of 10 ml of blood from left median cubital vein

Figure 7: After centrifugation, blood segregated into three layers. Bottom being red blood corpuscules, middle platelet-rich fibrin, and top acellular plasma

Figure 4: Digital radiovisuography image at the end of 21 day period of disinfection of 11 with triple antibiotic paste

Figure 6: Remi Mini centrifugal machine (C - 852), Remi Elektrotechnik Ltd., Vasai, Maharashtra, India

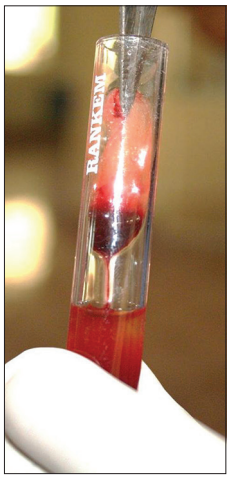

Figure 8: Removal of platelet rich fibrin gel with straight tweezer

periapical lesion and initiation of the root end closure indicating the success of the procedure.

DISCUSSION

Open apex cases pose a challenge to dentists, because of the presence of large apical foramen, thin dentinal walls and occasionally associated with periapical lesion. In this scenario, there is a paradigm shift from repair of tissues to

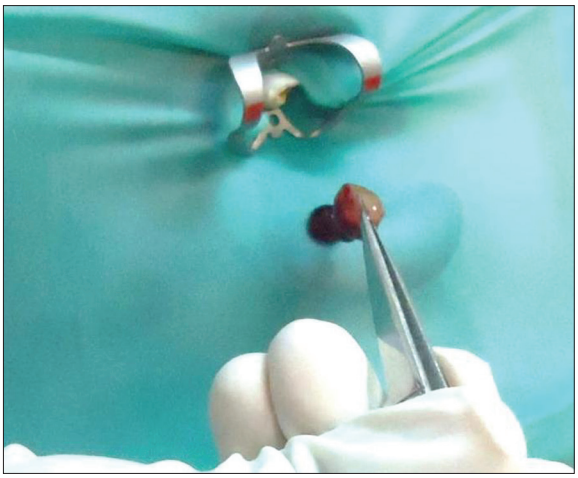

Figure 9: Carrying of platelet rich fibrin gel for placement into the canal

Figure 10: Digital radiovisuography verification after double coronal seal in 11

Figure 11: Digital radiovisuography image at 1 month period follow-up

Figure 12: Digital radiovisuography image at 18 month follow-up period

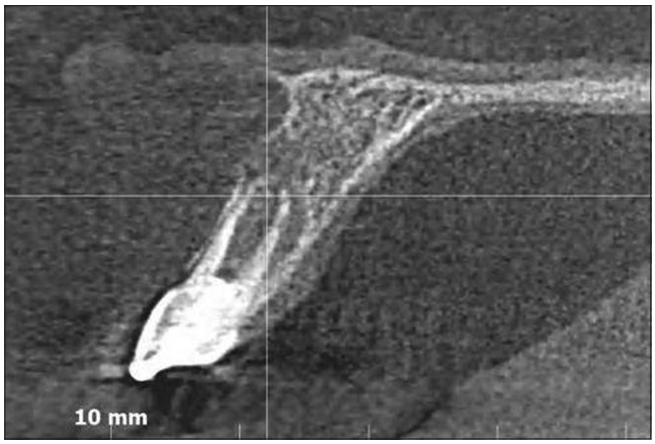

Figure 13: Sagittal section evaluation of cone-beam computed tomography

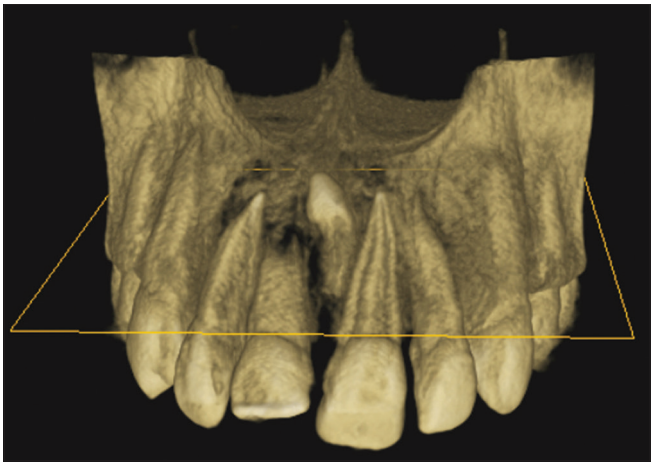

Figure 14: Three-dimensional view of cone-beam computed tomography

regeneration. The three key elements for the regeneration of any tissue are scaffolds, cells, and signaling molecules. The term used to describe root development mainly because of the regeneration of tissue in the pulp canal space is maturogenesis.

Revascularization is one of the earliest procedures, first introduced by Ostby in 1961. Where, intentionally bleeding is induced into the canal space by instrumenting the canal

beyond the working length. But, the major limitations are that it causes discomfort for the patient while mechanically irritating the periapical tissues and in certain situations where bleeding cannot be induced this procedure cannot be used.

Platelet concentrates are another type of regenerative therapy which is used. They are basically two types: Platelet-rich plasma (PRP) which is a first-generation platelet concentrate and PRF, which is a second generation platelet concentrate. The significance of platelets is that they have a fundamental role in hemostasis and are a natural source of growth factors. It is a simple strategy to concentrate platelets or enrich natural blood clot, which forms in normal surgical wounds, to initiate a more rapid and complete healing process. Natural blood clot has about 95% RBC, 5% platelets, <1% white blood cell (WBC) whereas platelet concentrate has 4% RBC, 95% platelets, 1% WBC.[5]

The present case had a wide open apex which can be classified to be at Cvek's stage 3.[6] The reason why we have chosen PRF over PRP is that, PRF preparation is a very simple and an easy procedure which does not require biochemical handling of blood, it consists of many growth factors like platelet-derived growth factor, transforming growth factor β, platelet-derived angiogenesis factor, insulin-like growth factor. It causes a slow release of growth factors with peak release at 14 days, this slow polymerization procedure results in a trimolecular/equilateral fibrin matrix which is similar to natural collagen matrix and most important is that it is an autologous material as there is no addition of external anticoagulants, which is required in PRP preparation.[7] PRF does not dissolve quickly after application; it causes proliferation of human dental pulp cells and increases the protein expression of osteoprotegrin and alkaline phosphatase.[8]

In recent years, the concept of lesion sterilization and tissue repair therapy has been developed, that employed a mixture of antibacterial drugs for disinfection.[9] Disinfection is one of the key steps in any regenerative procedures undertaken.

In a matured tooth disinfection is carried out by instrumentation, irrigation, and intra-canal medicament. In case of immature teeth with open apex major disinfection is carried out by intra-canal medicament. The infection of the root canal system is considered to be a polymicrobial infection, consisting of both aerobic and anaerobic bacteria. More likely a combination would be needed to address the diverse flora encountered. A combination of antibiotics would also decrease the likelihood of the development of resistant bacterial strains.[10]

Hence, we have chosen TAP as the intra-canal medicament introduced by Hashimo, which is a combination of ciprofloxacin 500 mg, metronidazole 400 mg, and minocycline 50 mg. Ciprofloxacin, a synthetic fluoroquinolone, has a bactericidal action, potent against Gram-positive pathogens.[11] Metronidazole is a nitroimidazole compound that exhibits broad spectrum activity against protozoa and anaerobic bacteria, which is bactericidal. In addition, metronidazole and ciprofloxacin can generate fibroblasts. Minocycline is bacteriostatic, has a broad spectrum of activity against Gram-positive and Gram-negative microorganisms. Tetracycline is biocompatible, inhibits collagenases and matrix metalloproteinase.

As minocycline has the potential of staining, the pulp chamber is coated with the bonding agent and also the TAP is placed below the CEJ, which rendered the tooth free of staining. The liquid component propylene glycol and macrogol were used for diffusion of the medicament.[12] It is been shown in a study that TAP rendered 70% of the root canals bacteria free after 2 weeks.[10]

We have chosen local application of drugs over systemic administration because, for systemic use of drugs proper blood circulation is very important, which is not the case for the teeth with necrotic pulps where the blood circulation is compromised. Hence, local administration of drugs is preferred.[13]

It is important to create a bacteria tight seal coronally to inhibit bacterial invasion into the pulp space before regeneration of tissues take place.[14] In this particular case, we have double sealed with Type II GIC and Composite in order to provide an impervious seal for bacteria and any other contaminants of the oral cavity.

There is always a debate whether a mesiodens has to be retained or removed. There are certain situations where the mesiodens are deliberately retained: In cases where patients are asymptomatic and when the root development is incomplete.[15] Here, in this case, mesiodens is left behind as the patient is asymptomatic, and the root development of 11 is not yet finished. Patient is kept under observation till the root development is completed and periodically evaluated.

According to investigators, the kind of tissue which is expected in this particular procedure can be: (1) Revascularization of the pulp with accelerated dentin formation leading to pulp canal obliteration, (2) Ingrowth of cementum and periodontal ligament (PDL), (3) Ingrowth of cementum, PDL, and bone, (4) Ingrowth of bone and bone marrow.[16]

According to another set of authors, the kind of tissue anticipated is intra-canal cementum, intra-canal bone, connective tissue similar to PDL and in one case, there is survival of pulp tissue.[6] The research is still ongoing in this area to standardize the type of tissue formed.

The success of the procedure is gauged both clinically and radiographically. Clinically, patient was asymptomatic at

all the periodic evaluations. Radiographs showed continued thickening of the dentinal walls and regression of the periapical lesion.

As the radiographs are the 2D imaging of a three-dimensional (3D) object, in order to confirm and standardize the results, we have taken CBCT. In the CBCT, the 3D reconstruction of the image showed complete healing of the periapical lesion, where the score of 0 can be given according to the CBCT periapical index scoring.[17] In the sagittal section, when each slice of 11 is observed, there is perfect initiation of the root apex, indicating the successful execution of the procedure.

CONCLUSION

In lieu of the advances in science and technology, it is important to adapt to newer exciting possibilities of regeneration of tissues within the pulp space and further induction of root development in immature traumatized teeth. Regenerative modalities with PRF definitely stand as an alternative to routine conventional root canal procedures. Taking into consideration the age, oral hygiene status, patient motivation, and compliance, we have attempted regenerative therapy over conventional procedures for the management of an immature necrotic tooth. Furthermore, the sacred tenets for successful regenerative procedures which include - proper case selection, strict sterilization protocol, appropriate choice of regenerative material, and a bacterial tight seal have been judiciously performed to achieve a predictable outcome. The case under observation was systematically followed-up at timely intervals of 1, 3, 6, 9, 12, and 18 month period where, the patient was subjected to clinical and radiological evaluation. In the current case, due to the thin radicular dentinal walls the success of the procedure could not be accurately analyzed using 2D imaging, hence, a CBCT imaging technique was performed to better analyze and confirm the results of the treatment. The CBCT scan revealed a dense bone fill in the periapical region with a 3D closure evident which was further confirmed using reconstructive 3D CBCT technology. This provided upper hand information on lesion resolution and root end closure which was not evident in the 2D imaging techniques like intraoral peripaical RVG. However, further evidence-based clinical trials with longer follow-up periods are required to establish the success of clinical cases treated with regenerative techniques.

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