

Platelet rich fibrin used as a pulpotomy medicament in a permanent molar with pulpitis: two case reports

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Abstract- Platelet-rich plasma (PRP) is a platelet concentrate that has been used widely to accelerate soft tissue and hard-tissue healing. Platelet-rich fibrin (PRF) was first described by Choukroun et al. in France. It has been referred to as a second-generation platelet concentrate, which has been shown to have several advantages over traditionally prepared PRP. Two patients reported to the Department of Conservative Dentistry and Endodontics in North Bengal Dental College & Hospital with established pulpitis in molar. Teeth had carious pulp exposure, with a history of lingering pain. After isolation, caries removal and pulp exposure, pulpotomy with PRF was performed and a permanent restoration was placed immediately. At the first recall (+1 day), no postoperative pain was reported. At 3, 6 and 12 months recall, the tooth responded positively to pulp sensibility tests, and radiographic examination revealed a normal periodontal ligament space. Positive results of these cases imply the need for more studies with larger sample sizes and a longer recall period to justify the use of this novel material for the treatment of pulpitis in human permanent molar teeth.

Index Terms- growth factors, platelet-rich fibrin, pulpitis, pulpotomy, regeneration.

I. INTRODUCTION

The term tissue engineering was originally coined to denote the construction in the laboratory of a device containing viable cells and biologic mediators (e.g., growth factors and adhesins) in a synthetic or biologic matrix, which could be implanted in patients to facilitate regeneration of particular tissues. In general, tissue engineering combines three key elements, namely scaffolds (collagen, bone mineral), signaling molecules (growth factors), and cells (osteoblasts, fibroblasts).

Pulpitis associated pain contributes a substantial financial and societal burden and affects quality of life. In addition to pain and suffering, pulp infection also causes severe and at times even fatal systemic infection¹. Appropriate management of dental pulp

infection and inflammation has tremendous significance in maintaining general health. Although immediate treatment is indispensable for a better prognosis, current endodontic therapies typically lead to destroying or compromising pulp vitality. In adult teeth, even when the lesion affects only part of the pulp, the entire pulp tissue is often removed (root canal therapy). The resulting nonvital tooth often requires extensive restorations, which in turn increase the cost and the risk of complications. In primary teeth, pulpotomy treatment involves application of formaldehyde, a toxic, mutagenic chemical that can be absorbed into the body^(2,3). Therefore it is important to develop biocompatible treatments directed at maintaining pulp vitality and increasing tooth vitality. Pulpotomy is a vital pulp therapy in which a portion of coronal pulp tissue is removed surgically, and the remaining radicular tissue is covered with a suitable material that protects the pulp from further injury and permits and promotes healing (Bakland 2002)⁴.

Several materials have been advocated to induce dentine bridge formation via the dentinogenic potential of pulpal cells (Schroeder 1985)⁵. In 1929, Hess reported a technique of pulpotomy using calcium hydroxide (CH; Hess 1929)⁶. Stanley (1989)⁷ strongly advocated CH for vital pulp therapy, and this material has been used for the protection of exposed dental pulps up to the present time.

Several case series have suggested pulpotomy as a viable treatment for pulp exposures with pulpitis; the rationale being the healing potential of the remaining radicular tissue and the biocompatibility of pulpotomy agents, especially mineral trioxide aggregate (MTA; Asgary & Eghbal 2010)⁸. To increase the success rate, a critical need exists to develop new biologically based therapeutics that reduce pulp inflammation and promote the formation of dentine pulp tissues.

Platelet-rich fibrin (PRF) was first described by Choukroun et al. (2006)⁹. It has been referred to as a second-generation platelet concentrate, which has been shown to have several advantages over traditionally prepared platelet-rich plasma. Its chief advantages include ease of preparation and lack of biochemical handling of blood, which makes this preparation

strictly autologous (Choukroun et al. 2006). Two case reports are presented where PRF was used as a pulpotomy material in treating pulpitis in human permanent molars.

Preparation of PRF- The patients were informed about the treatment modality – coronal pulpotomy using **PRF** as an alternative treatment to root canal treatment. After obtaining written consent from the patient, PRF was prepared by drawing the required amount of blood(fig-1) into a 10-mL test tube without an anticoagulant and centrifuged immediately using a table top centrifuge (REMI Laboratories, Mumbai, Maharashtra, India) for 10 min at 2500 rpm(fig-2). The resultant product consisted of the following three layers(fig-3,4):

- Acellular platelet poor plasma at the top of the tube;
- Fibrin clot (PRF) in the middle of the tube; and
- Red blood corpuscles at the bottom of the tube.

Because of the absence of an anticoagulant, blood begins to coagulate as soon as it comes in contact with the glass surface. Therefore, for successful preparation of PRF, rapid blood collection and immediate centrifugation, before the clotting cascade is initiated, are absolutely essential. PRF was obtained in the form of a membrane by squeezing out the fluids in the fibrin clot.

II. CASE REPORT-1

A 17-year-old male patient (fig-5) reported to the Department of Conservative Dentistry and Endodontics with pain in the lower right posterior region. On clinical examination, occlusal caries was seen on the right mandibular first molar (fig-6). An intra-oral periapical radiograph revealed deep occlusal caries invading the pulp with slight periapical rarefaction (fig-7). The diagnosis of pulpitis was determined on the basis of clinical assessment, including history of spontaneous pain and intense, lingering pain to cold stimulus. Tooth 46 was first anaesthetized with Lidocaine 2% and adrenaline 1/80 000 (Astra-Zeneca Pharma India Ltd, Bangalore, India) and isolated with a rubber dam (Hygenic, Coltene whaledent). Pulpotomy was performed with a round bur (Mani, Japan) in a high-speed handpiece (NSK, Japan) with copious irrigation (fig-8); coronal pulp tissue was removed to the level of pulp chamber floor. Haemostasis was achieved by irrigating the cavity with sterile saline (Baxter, India, Pvt.limited) and cotton pellets. The blood clot-free pulpal wound was covered with a small piece of PRF (fig-9). An approximately 2 mm thick layer of MTA (ProRoot; Dentsply Tulsa Dental Specialty, Tulsa, OK, USA) was placed over the PRF (fig-10) and a final restoration of glass-ionomer cement type-II (GC Corporation, Japan) was placed(fig-11). The patient was recalled after 1 day for radiographic examination and evaluation of postoperative pain. The patient had no pain or discomfort and at 3, 6, 12 and 24 months recall (fig-12,13,14,15), the tooth responded positively to pulp tests, and radiographic examination revealed normal periodontal ligament space.

III. CASE REPORT-2

A 20-year-old male patient (fig-16) reported to our department with pain in the lower left posterior region. On clinical examination, occlusal caries was seen on the left mandibular second molar. An intra-oral periapical radiograph revealed deep occlusal caries invading the pulp(fig-17). The diagnosis of pulpitis was determined on the basis of clinical assessment, including history of spontaneous pain and intense, lingering pain to cold stimulus. The treatment (18) was same for that patient as the former. The patient was recalled after 1 day for radiographic examination and evaluation of postoperative pain. The patient had no pain or discomfort and at 3 and 6 months recall (fig-19,20), the tooth responded positively to pulp tests, and radiographic examination revealed normal periodontal ligament space.

IV. DISCUSSION

Pulpotomy is a universally accepted treatment for teeth with incompletely formed roots involving pulpal exposure (Camp & Fuks 2006, Witherspoon et al. 2006)¹⁰. In permanent teeth, it has been postulated that extirpating pulpal tissue and undertaking root canal treatment in many cases is not cost-effective as it is time-consuming and difficult for both patient and clinician. In addition, failure of a vital pulp therapy would not reduce the outcome of future root canal treatment for the tooth (Camp & Fuks 2006)¹⁰. However, more studies are required to evaluate this procedure in mature permanent teeth.

In recent years, MTA has been introduced for pulpotomy in primary molars (Messer 2008)¹¹ and has demonstrated good biocompatibility (Asgary et al. 2008)¹², excellent sealing ability (Aqrawabi 2000)¹³ and stimulation of healing in the pulpal tissue (Asgary et al. 2008)¹⁴. In the first report of MTA pulpotomy in human mature permanent teeth, a case series of 14 human mature permanent molars with so-called irreversible pulpitis, a histological examination revealed complete dentinal bridge formation, pulp vitality and absence of inflammation in all the cases (Eghbal et al. 2009)⁸. However, the exact per-operative status of the pulp was never determined and it is likely the pulps were not actually irreversibly inflamed.

A number of laboratory studies have been conducted to evaluate the biocompatibility of MTA by measuring various parameters such as proliferation and viability using different types of cells in direct and/or indirect contact with MTA. MTA in its freshly mixed state shows a higher cytotoxicity (Haglund et al. 2003, Balto 2004)¹⁵⁻¹⁶, which could be due to its high pH (Camilleri 2008)¹⁷. Therefore, it is important to develop biocompatible treatments directed at maintaining pulp vitality and increasing tooth longevity (Wang et al. 2010)¹⁸.

One such biologically based therapeutic is PRF. PRF is a second-generation platelet concentrate widely used to accelerate soft and hard-tissue healing. Its advantages over the better known platelet-rich plasma (PRP) include ease of preparation/application, minimal expense and lack of biochemical modification (no bovine thrombin or anticoagulant is required).

Platelet-rich fibrin is a strictly autologous fibrin matrix containing a large quantity of platelet and leucocyte cytokines (Sunitha Raja & Munirathnam Naidu 2008)¹⁹. Growth factors play a pivotal role in signalling the events of tissue formation and repair

in the dentine-pulp complex. They are responsible for signalling many of the key events in tooth morphogenesis and differentiation, and recapitulation of these processes after dental injury allows tissue regeneration (Smith 2003)²⁰.

Platelet-derived growth factor stimulates DNA and protein synthesis in osseous tissues, mitogenic effects on mesenchymal cells and angiogenic effect on endothelial cells (Sunitha. et al.)¹⁹. Transforming growth factor β stimulates angiogenesis, enhanced woven bone formation, stimulate matrix synthesis in most culture systems, chemotactic effect on osteoblastic cells, stimulates endothelial chemotaxis; stimulates bone formation by inhibitory effect on osteoclasts.

A number of reports of the in vivo (Hu et al. 1998)²¹ or in vitro (Sloan & Smith 1999)²² placement of exogenous growth factors, particularly TGF- β s and Bone Morphogenetic Proteins, on exposed pulps have demonstrated the potential of these molecules to signal reparative dentinogenic events. Transdental or direct application of TGF-1 and BMP-7 to the odontoblasts of unexposed pulps in cultured tooth slices has also shown the ability of these growth factors to signal reactionary dentinogenesis (Sloan & Smith 1999)²². In an experimental trial, the growth factor content in PRP and PRF aliquots was measured using Elisa kits. The results suggest that the growth factor content (PDGF and TGF- β) was comparable in both (Sanchez et al. 2003)²³.

In the current cases, an effort was made to use such growth factors to help in repair of a tooth with pulpitis. As discussed earlier, PRF was prepared with the patients own blood and was placed in the pulp chamber after a pulpotomy procedure. A layer of MTA was placed over PRF and the final restoration of glass-ionomer cement was placed immediately. MTA was chosen as it is hydrophilic and requires moisture to set, which is a favourable property when there is potential for moisture contamination in the clinical setting (Gancedo-Caravia & Garcia-Barbero 2006)²⁴. Also a double coronal seal was created to eliminate microleakage. At 3, 6 and 12 months, the teeth were asymptomatic and responded positively to sensibility tests. Follow-up radiographs revealed normal peri-apical and periodontal region.

The potential theory behind the success of the presented case could be attributed to a study conducted by Wang et al. (2010)¹⁸ that the pulp cells residing in pulp clinically diagnosed with pulpitis might still have stem cell potential similar to healthy pulp cells and therefore might be a resource for autologous pulp regeneration. These findings suggest exciting opportunities for biologically based therapeutic approaches to dental tissue repair as well as providing valuable insights into how natural regenerative processes may be operating in the tooth. Further research on this topic is required with regard to the histological assessment of such treated teeth on a larger sample size.

V. CONCLUSION

The slow polymerizing potential of PRF and the fibrin technology accounts for a favourable physiologic structure to support healing. Growth factors can help in providing a blue print for tissue regeneration within tooth, thus creating new opportunities for biological approaches to dental tissue repair.

It can be concluded that there is a reasonable biological argument to carry out pulpotomy as a possible alternative treatment in mature permanent teeth with pulpitis. Further studies

(histological and clinical) can add significant weight to this argument.

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Figures-



Fig-1 ,Blood drawn from patient

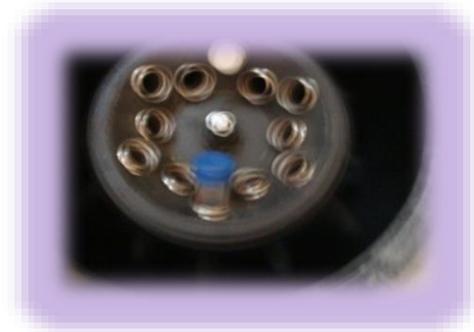


Fig-2, blood centrifuge without anticoagulant



Fig-3, three layers after centrifugation

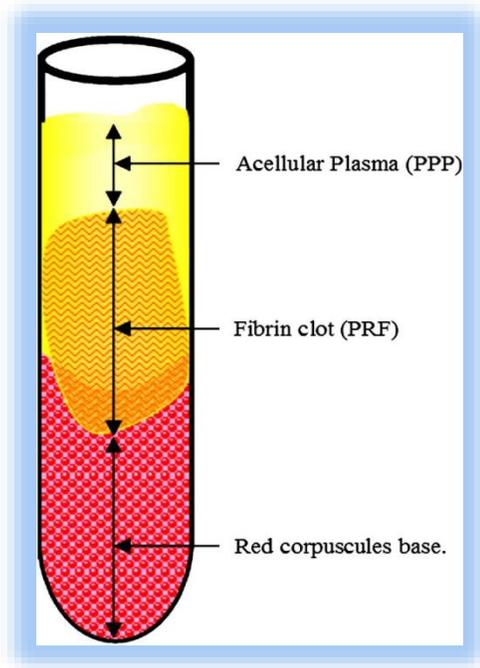


Fig-4, three layers after centrifugation



Fig-5



Fig-6, clinically occlusal

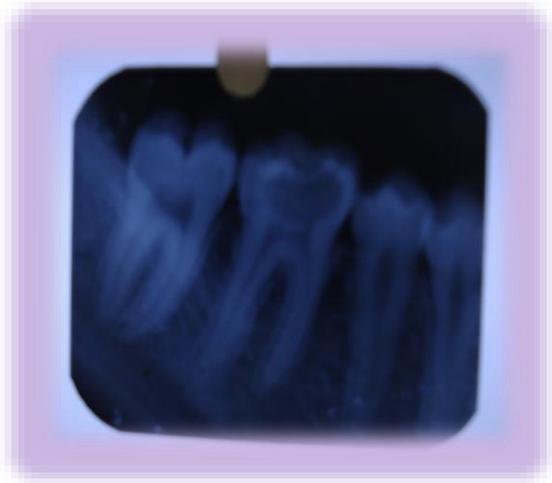


Fig-7, IOPA showing deep occlusal caries



Fig-8, pulpotomy done



Fig-9, PRF given



Fig-10, MTA applied over PRF



Fig-11, final restoration with GIC



Fig-12, 3 months after pulpotomy



Fig-13, 6 months after pulpotomy



Fig-14, 12 months after pulpotomy



Fig-15 , 24 months after pulpotomy



Fig-16, case report
2



Fig-17, IOPA showing deep occlusal caries



Fig-18, PRF given to pulp chamber



Fig-19, after 3 months