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Combination of hydroxyapatite, platelet rich fibrin and amnion membrane as a novel therapeutic option in regenerative periapical endodontic surgery: Case series

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ABSTRACT

INTRODUCTION: Periapical surgery is the last resort in the arsenal of an endodontist to effectively deal with periapical lesions that result from necrosis of the pulp. Bone grafts, growth factors and membranes form an array of regenerative materials that influence the healing outcome of periapical surgery.
PRESENTATION OF CASE: The main purpose of the two cases reported here was to assess the potential benefits of a combination of bone graft, platelet-rich fibrin (PRF) and amnion membrane in terms of reduced post-operative discomfort, radiographic evidence of accelerated periapical bone healing and present a novel therapeutic option in the management of large periapical lesions. Two cases of radicular cysts were treated through a combined regenerative approachof Bio-Gen mix®, PRF and amnion membrane. The patients were assessed for discomfort immediate post-operatively and after a week. The patients were recalled every month for the next 6 months for radiographic assessment of the periapical healing.
DISCUSSION: Literature is replete with articles that have substantiated the role of demineralized bone matrix comprising a mixture of cancellous and cortical bone graft particles in enhancing regeneration. To the best of our knowledge, there has been no evidence related to the application of a human placental membrane in periapical surgery. Hence, the rationale of using a combined approach of Bio-Gen mix®, PRF and amnion membrane was to combine the individual advantages of these materials to enhance clinical and radiographic healing outcomes. Our present case reports provide an insight into this novel therapeutic option.
CONCLUSION: The results of this case seriessubstantiatesthe credibility of using a combination ofamnion membrane with a bone graft and PRF to enhance radiographic healing outcome with decreased post-operative discomfort and present a viable regenerative treatment modality in periapical surgery.

1. Introduction

Long standing nonvital tooth usually results in a periapical pathology leading to bone destruction. The main objective of periapical surgery is to create an optimal environment for periradicular tissue healing. Though this can be accomplished by eliminating unhealthy tissues through the use of conventional surgical procedures, it eventually results in connective tissue repair; a less desired outcome from the endodontist's point of view [1,2].

With the dawn of the new century, dentistry has made progress in leaps and bounds with introduction of new bone graft materials, which have, to a certain extentproved their caliber in periodontal regeneration with varied success in periapical surgery [3,4]. However, the final desired outcome and the key to true regeneration is the restoration or reconstitution of the lost periapical tissues which has been made possible with the introduction of regenerative therapies that utilized growth factors and barrier membranes [5,6].

Incorporation of biologically active molecules, particularly growth factors with platelets as the source has yielded promising

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results. This evinced clinical interest in platelet derived products and lead to the evolution of platelet concentrates; first generation platelet concentrates such as platelet – rich plasma (PRP) and second generation such as platelet- rich fibrin (PRF) [7].

Regeneration with new attachment became a reality with the introduction of guided tissue regeneration (GTR) technique andcreated interest in membranes, as these maintained space and formed a key to regeneration. Placement of a mechanical barrier such as a membrane, over an osseous defect can prevent proliferating oral epithelium and gingival connective tissue from growing into the defect. Proliferating cells with osteogenic potential can then repopulate the defect resulting in more predictable bone repair [8,9].

Literature is replete with articles which put forth the use of numerous forms of bone graft materials, but there is dearth of studies that documented the use of bone graft along with platelet-rich fibrin and barrier membranes in periapical surgeries [10–12]. Survey of the existing literature revealed that there was no documented evidence of the use of an amnion membrane along with PRF and bone graft as a combination in endodontic surgery to the best of our knowledge.

Hence, the aim of our present case series is to evaluate the post-operative discomfort and assess the regenerative potential in terms of radiographic evidence of accelerated periapical bone healing process by a combination of bone graft and PRF to fill the periapical osseous defects coupled with the use of an amnion membrane as a mechanical barrier. Institutional ethical approval has been obtained as well as informed consent has been taken from the participants prior to the study. Ref. No. for Ethical clearance from the institute 323/SSCDS/IRB-E/OS. This project is compliant with the PROCESS guidelines. [13]

Case report 1: A 38-year-old male patient presented to a private dental hospital with the complaint of pus discharge associated with a sinus tract in relation to 11. The patient revealed a history of trauma 3 years ago. Clinical examination revealed presence of an Ellis class 2 fracture without any discoloration. Patient's medical history was noncontributory.

Radiographic examination revealed the presence of large well-defined periapical radiolucency in relation to the apices of 11,12 and 13. The teeth were non responsive to thermal and electric tests. Teeth11 and 13 exhibited tenderness on percussion test.

Case management was discussed with the patient with the primary treatment that comprised of a root canal treatment, but the necessity of a periapical surgery was explained and an informed consent was obtained.

Root canal treatment was performed in relation to all the effected teeth. Calcium hydroxide was used for frequent dressings as the intra canal medicament throughout the visits. Though an intra-canal medicament was used, weeping canals were evident at every subsequent visit. Hence, endodontic surgery was incorporated into the treatment plan apart from the root canal treatment to effectively treat the periapical lesion.Under proper isolation, the root canals were obturated using gutta percha (Dentsply Maillefer, Ballaigues, Switzerland) and AH plus sealer (Dentsply DeTreyGmbH,Philadelphia, USA) prior to the surgery. A radiograph was taken to assess the root canal treatment (Fig. 1).

Surgical intervention was facilitated using a crevicular incision aided with a unilateral posterior release after thorough anesthesia was attained using 2% lignocaine. Following the reflection of the full-thickness mucoperiosteal flap, a bony window was created through the cortical bone (Fig. 2). This facilitated access to the cystic lining and the granulation tissue, which was carefully curetted and sent for a biopsy. Residual inflammatory connective tissue was curetted, the root ends were resected. Root end cavities were prepared with low speed bur and filled with Glass-ionomer cement (GIC) as a retrograde filling material. The surgical area was thoroughly irrigated and isolated.

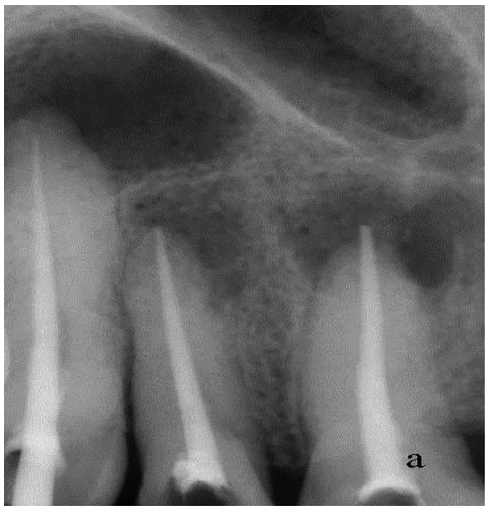

Fig. 1. Pre-surgical radiograph representing the root canal treatmentin the effected teeth.

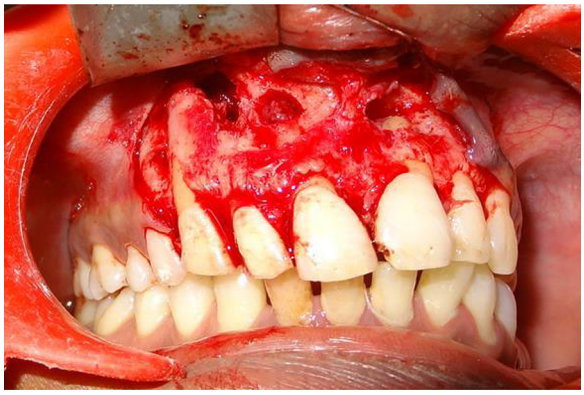

Fig. 2. Intra-operative picture of the lesion curetted and root end resection completed.

Fig. 3. Bio-Gen mix® bone graft material.

A xenograft bone graft material (derived from Horse femur bone) Bio-GenMix® (Bioteck Company, Italy) consisting of a mix of cancellous and cortical bone particles was used (Fig. 3). Platelet-rich fibrin (PRF) preparation protocol described by Choukrounet al.

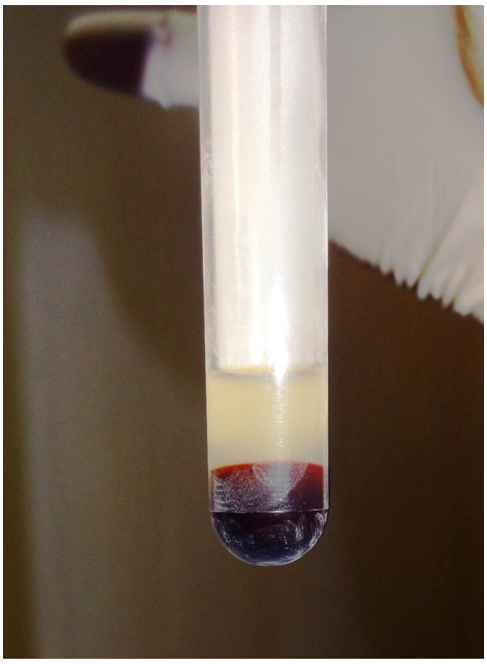

Fig. 4. Three layers obtained after the centrifugation process with PRF in the middle.

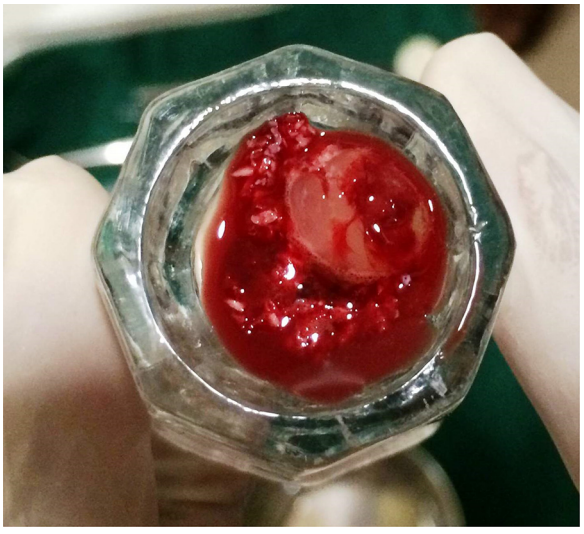

Fig. 5. Composite graft prepared with mixture of PRF and Bio-gen mix.

[14] was used in our present case reports. The PRF protocol comprised of a collection of a blood sample in 10 ml tubes without any anticoagulant and was immediately centrifuged at 3000 rpm for 10 min in a table top centrifuge (REMI laboratories, Bombay, India). The PRF clot that was obtained after the processing was retained, whereas platelet-poor plasma (PPP) and the exudates with RBCs were discarded (Fig. 4).

The bone graft material was mixed with freshly prepared PRF to mold it into a composite graft (Fig. 5). This was gently placed into the osseous defect. An amnion membrane (Tata memorial Hospital Tissue Bank, Mumbai, India)was used in our case reports. After the defects were grafted, the area was covered with an amnion membrane to act as a mechanical barrier (Fig. 6). Hemostasis was achieved and the mucoperiosteal flap was sutured back to its original anatomical position with 4-0 silk sutures.

Necessary post-operative instructions were advised and the patient was prescribed antibiotics and analgesics. Proper plaque

Fig. 6. Amnion membrane applied over the defect area.

Fig. 7. Post-operative radiographic image depicting periapical bone healing after 3 months.

control was emphasized with the use of 0.2% chlorhexidine gluconate mouthwash twice daily for at least 2 weeks. A post-surgical visit after one week revealed no adverse effects or any allergic reactions to the material. A VAS scale was used to score the discomfort immediate post-operatively and at the patient's recall visit after a week. The patient was recalled every month for the next 6 months. Radiographs taken after a six-monthfollow-up revealed adequate bone healing in the periapical region. (Fig. 7).

The curetted lesionthat was sent for histopathologic examination exhibited a nonkeratinized stratified squamous epithelium lined with mixed inflammatory infiltrate. These features support the diagnosis of anodontogenic radicular cyst (periapical cyst).

Case report 2: A 26-year-old female patient reported to a private dental hospital with the chief complaint of swelling in relation to the buccal aspect of 11 and 12. Dental history revealed that she had an incident of trauma to her front teeth 4 years ago and underwent root canal treatment. But she never went for a follow-up visit to her dentist. Clinical examination revealed grade 1 mobility with respect to 11 with discoloration. Radiographic examination revealed the presence of periapical radiolucency with respect to the apices of 11 and 12 (Fig. 8).

Case management comprised of a periapical surgery and was very much similar to case 1. The regenerative protocol adopted for case 1 was followed in this case too (Figs. 9–11). The patient was

Fig. 8. Pre-surgical radiograph revealing over obturated root canals of the effected teeth.

Fig. 9. Intra-operative picture of the lesion curetted and root end resection completed.

recalled every month and a final recall after 6 months revealed sufficient bone healingin the periapical area of both the teeth as observed by a radiographic examination (Fig. 12).

2. Discussion

Treatment of large periapical lesions through a conventional surgery was a challenging task to most of the endodontists,since healing post a conventional surgery resulted in a connective tissue repair, resulting in a poor prognostic outcome.

Fig. 10. Placement of PRF mixed with Bio-Gen mix® into the defect.

Fig. 11. Amnion membrane is placed over the defect site to enhance wound healing.

Technological advances in material science have brought forth new materials and membranes that have enhanced the regenerative capacity in such large periapical lesions, certainlya boon for the practitioners.

We have used Bio-Gen mix® as the bone graft material to fill the defects. Literature is replete with articles that have substantiated the role of demineralized bone matrix comprising a mixture of cancellous and cortical bone graft particles in enhancing regeneration [15,16]. But the use of bone graft; a hydroxyapatite, tricalcium phosphate or xenograft alone might lead to fibrous encapsulation of the graft material and does not permit an ideal periapical healing outcome [17].

A critical factor in wound healing is the presence of a blood clot that stabilizes the wound matrix; this is provided by our body's biological product, PRF. PRF is a second-generation autologous fibrin gel consisting of concentrated platelets that are pooled up during the centrifugation process [18]. Platelet alpha (α) granules provide a rich source of growth factors, platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF). These growth factors are the key to healing as they potentiate vital functions such as cellular proliferation and bone formation [19,20].

Fig. 12. Post-operative radiograph image depicting periapical bone healingafter 3 months.

The rationale for the use of PRF along with the bone graft in our case report can be explained from the following facts. PRF acts a matrix maintaining the integrity of the bone graft material and enhances revascularization between the bone graft particles through neo-angiogenesis [21,22]. The fibrin matrix of PRF gets slowly resorbed and releases the growth factors, such as PGDF, TGF and VEGF that maintain a viable and everlastingfield to enhance healing. Many studies have validated that the combination of bone graft along with PRFhad the potential to enhance bone formation [23,24].

PRP and PRF are platelet concentrates, which are a potential source of growth factors that play a crucial role in bone regeneration. In our present case reports, we have used PRF, owing to a number of advantages of PRF over PRP. PRF preparation is simple, considering the fact that the blood sample is centrifuged only once without any anticoagulant whereas in PRP, the collected blood sample has to be centrifuged twice and also involves the addition of an anticoagulant, thrombin and a platelet activator like calcium chloride [25,26]. Other major advantage of PRF over PRP is that PRF provides a stable matrix that remodels slowly unlike PRP which resorbs quickly [27].

Guided tissue regeneration technique with the use of various membranes has been a valuable tool for endodontists considering their significance in periapical surgeries performed in the esthetic zone. Literature is abundant with studies that have used GTR incorporating various types of membranes in endodontic surgery with varied results [28–30]. The rationale for the use of an amniotic membrane in our present case reports can be explained from the following facts.

First, amnion membrane, derived from the human placenta is absorbable and contains various growth factors exhibiting anti-inflammatory and antimicrobial properties with relative ease of processing and procurement [31]. In our reported cases, there were bony dehiscencesaround the teeth which were quite extensively seen after removal of the granulation tissue. These dehiscences can contribute to gingival recession in the post healing period, a matter of esthetic concern for the patient. Amnion membrane, with a diameter of 300 μm has an added advantage over other collagen membranes which have a 700 μm thickness. The use of this thin amnion membrane enables a mechanical protection as it has a self-adhering property ensuring a proper adaption to the underlying bone and to the contours of the roots, covering these dehiscences intimately. This enables the regenerative cells to proliferate and excludes the down growth of the long junctional epithelium, a crucial step in regeneration [32,33]. Hence the use of a membrane reduces the chances of recession in the anterior teeth and provides the much needed impetus for the surgeons to perform surgeries in the esthetic zone with confidence.

Most important, amnion membrane provides a rich source of factors which enhance angiogenesis; these include angiogenin, fibroblast growth factor and vascular endothelial growth factor, to name a few.These factors enhance proliferation of endothelial cells and boost the angiogenesis with new vessel formation [34]. Evidence substantiates that amnion membrane also plays a crucial role in recruitment of mesenchymal progenitor cells and help in accelerating wound healing. Finally, unlike the other membranes used in GTR that have reported adverse immune response, this membrane is completely safe [35].

To the best of our knowledge, there has been no evidence related to the application of a human placental membrane in periapical surgery. Our present case seriesprovides an insight into this novel therapeutic option. There were certain limitations; procurement of patient's own blood to prepare PRF is difficult with apprehensive patients and associated with certain morbidity. To overcome these limitations, over the last few years researchers have evinced interest in the introduction of new biological molecules such as the recombinant human platelet-derived growth factor (rhPDGF-BB), in commercial preparations such as Periogen [36]. These preparations in combination with bone graft materials retained the wound healing properties of platelet derived products without the need to draw the patient's blood and have exhibited excellent soft and hard tissue healing outcomes.

3. Conclusion

Bone graft materials along with growth factors derived from platelet derivatives have upped the ante in wound healing. To conclude within the limitations, the cases reported here demonstratethat amnion membrane combined with bone graft and PRF have enhanced the radiographic healing outcome and reduced the post-operative discomfort. This clinical result must be considered with caution and further research through histological studies and use of advanced radiographic techniques is necessary to validate the role of PRF and amnion membrane in regeneration on a long-term basis.

Conflict of interest

None

Funding

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Ethical approval

The case is reported in accordance with the institutional ethical guidelines. 323/SSCDS/IRB-E/OS 2016

Consent

Each patient on admission signs a consent to use his anonymous health data for research purposes.

Author contribution

UKU, BK, PK: Study concept, data collection, writing the paper, critical revision of the paper. NV, KP, VM, SP, SS: data collection, writing the paper. UKU, BK, PK, SP, SS, LAS: study concept, critical revision of the paper.

Registration of research studies

323/SSCDS/IRB-E/OS 2016

Guarantor

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