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Evaluation of Recombinant Human Platelet Derived Growth Factor (rhPDGF-BB) Along with Tricalcium Phosphate (β-TCP) In Impacted Mandibular 3rd Molar Sockets

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Abstract

Aim: The aim of this study was to evaluate efficacy of Recombinant Human Platelet derived Growth Factor (rhPDGF-BB) mixed with Tricalcium Phosphate (β-TCP) particles in mandibular 3rd molar extraction socket for bone regeneration.

Setting And Design: A comparative clinical trial consisting of 10 patients who wanted to undergo the surgical extraction of mandibular 3rd molar bilaterally, were undertaken in the institutional settings, where in each patient one side of lower third molar extraction site was kept as control and in the other side graft material was used, after the approval from the institutional ethical committee.

Materials And Methods: In this study 10 patients were included with mean age of 28.3 years (Ranging from (20-35 years). These were 4 male and 6 female patients. In each patient one side of lower third molar extraction site was kept as control site and in the other side graft material was used kept as study site. Hence 10 control sites were compared with 10 study sites. Selection of site for placement of graft was done randomly. In 6 patients study site was on right side and in 4 patients study site was on left side. Clinical parameters like pain, facial swelling, total number of analgesic tablets required evaluated at 48hrs and at 7th day. While, radiographically bone density was evaluated after 1st and 3rd month postoperatively. Other parameters like infection, sinus discharge, wound dehiscence, dry socket or graft leakage were also observed.

Results: rh PDGF with β – TCP when used in mandibular third molar extraction socket results in rapid bone regeneration seen via increased bone density. It also results in less post-operative discomfort to the patient like pain and swelling. Complications like infection, sinus discharge, wound dehiscence, dry socket or graft leakage were not encountered and the tissue showed good acceptance with no graft rejection.

Conclusions: The rh PDGF/β – TCP combination is a novel bone grafting material with excellent results and should be included in the armamentarium when bone defect is encountered.

Keywords: 3rd Molar, Bone Defect, Rh PDGF, β-TCP.

Introduction

Growth factors are proteins that have the ability to stimulate growth, proliferation and differentiation of cells. Platelet Derived Growth Factor (PDGF) is stored in the alpha granules of platelets and are also produced by osteoblasts, macrophages and monocytes. Three main isomers of PDGF exist: PDGF-AA (alpha/alpha), PDGF-BB (beta/beta), and PDGF-AB (alpha/beta)[1]. PDGF-BB has chemotactic and mitogenic effects on periodontal mesenchymal stem cells, cementoblasts, periodontal ligament fibroblasts and osteoblasts[2]. Because of these properties PDGF-BB has been investigated as a potential biologic material to aid in soft and hard tissue regeneration[2].

Centrifugation of the whole blood of the patient provides platelet rich plasma (PRP) which is a source of PDGF[3].However procuring PDGF in this manner requires phlebotomy which is psychologically uncomfortable for certain patients. Moreover, the number of platelets (and consequently PDGF-BB levels) in PRP varies depending on the procurement method[1], it is time consuming and additional equipment increases expense. Because of this non-dependability of PRP, a new commercial concentrate of growth factor is expected to be very useful in dealing with bone defects.

Recombinant human PDGF-BB (rhPDGF-BB) is now commercially available which contains pure and consistent concentrations of the protein. Hence it also reduces the need for additional equipment and does not require a phlebotomy. The application of it is many folds. Like used in implant procedures, sinus lift, ridge augmentation, periodontal tissue regeneration procedures[2,4], distraction osteogenesis,[5] diabetic and geriatric conditions,[6] fracture healing[7], etc.

The purpose of this study was to prove the efficacy of rhPDGF-BB in bone regeneration after removal of third molar, which if proved can be used for various purposes in maxillofacial surgical procedures. Hence it would become a novel bone grafting material.

Subjects And Methods

A comparative clinical trial of rhPDGF-BB mixed with Tricalcium Phosphate (β-TCP) particles in bone regeneration was undertaken in the Department of Oral and Maxillofacial Surgery, College of Dental Science And Research Centre, Bopal, Ahmedabad. after the approval from the institutional ethical committee.

Inclusion Criteria

- Indication for surgical removal of bilateral mandibular 3rd molar.
- Similar angulation and difficulty level of 3rd molars of opposite quadrants for non-biased comparison.
- Subject willingness for study and follow up visits.

Exclusion Criteria

- Pre-existing infection in the region of lower 3rd molars.
- Carious or compromised 1st and 2nd molar whose treatment may alter the radiographic parameters during follow up comparison.

- Medically compromised patients or systemic diseases like uncontrolled diabetes, prolonged steroids therapy, compromised immunity, and associated bone pathology.
- Pregnant or lactating mothers.
- Patients who are not willing for being a part of study.

Method

Preoperative Evaluation

- A standard proforma was used to collect necessary information and history of all cases. Informed consent about the study and the procedure was taken from the patients.
- Photographic records were taken.
- Preoperative radiography included OPG on which difficulty tracing of both lower 3rd molars was done using Pederson's scale.
- Preoperative measurement of face were noted – The horizontal distances between the corner of mouth and the lobe of the ear and the angle of the mandible were measured with a tape[10].
- Preoperative investigation included routine blood profile, BT, CT, HIV, HBsAg

Operative Technique

- All patients were prescribed prophylactic amoxycillin 500mg 1 day prior the procedure for three times a day.
- Painting with beatdine and draping was done in standard manner.
- Inferior alveolar nerve block with long buccal nerve block was given on one side, using 2% lignocaine HCL with 1:80,000 Adrenaline.
- Triangular incision was places to expose the 3rd molar. Full mucoperiosteal flap was raised.
- Space was created around the tooth using straight fissure (No.702 carbide bur) or round bur (No.6 carbide bur) as required and a surgical handpiece under constant cool normal saline irrigation.
- Tooth sectioning was done if required.
- After the removal of 3rd molar the socket was curetted, sharp bony margins were filed and the flap edges were freshened.
- Opposite side lower 3rd molar was removed in the same manner.
- After that immediate postoperative OPG was obtained.
- Graft material was prepared. 2 cc rhPDGF was mixed with 0.5 cc β-TCP particles in a sterile vessel and allowed to soak for 10 minutes.
- The grafting material was placed into any one of the sockets chosen randomly.
- Mucoperiosteal flap was sutured with 4-0 silk on both sides.
- Patients were advised to continue amoxicillin 500mg. TDS and a combination of diclofenac sodium 50mg. + paracetamol 500mg. for pain relief.

Postoperative Assessment

- Facial swelling with same parameters were measurd on 2nd and 7th postoperative day.
- Percentage facial swelling (%)

$$= \text{Postoperative measure} - \text{Postoperative measure} \times 100^{10} \div \text{Preoperative measure}$$

- Subjective pain assessment was done by visual analogue scale on 2nd and 7th postoperative day.
- No. of analgesic tablets required for pain on study and control site was noted.

- OPG was obgtained after 1 month and 3month using the same exposure parameters as preoperative and immediate postoperative OPGs.
- Both groups were observed intra-orally for complications like infection, sinus formation, wound dehiscence, and graft leakage.
- Bone density measurements were performed on 1 month and 3rd month radiographs.
  Bone Density Measurement
- A box of size 1.5 × 1.0 inches was cropped from the OPG on both sides which included 2nd molar and the socket of 3rd molar.
- To standardize the images for comparison the cropping box was placed with the mesial and occlusal contours of 2nd molar in contact with its lines.
- Such box of fixed size and pixels were cropped from immediatge post-operative and post-operative 1 month, 3month OPGs from control and study site.
- The subsequent images were opened in Adobe Photoshop CS6 software and their mean grey level histogram values are noted
- Grey level histogram: The grey level histogram of an image represents the distribution of the pixels in the image over the gray-level scale from 0 to 255 where 0 represents black colour and 255 represents white. It can be visualised as if each pixel is placed in a bin corresponding to the colour intensity of that pixel. All of the pixels in each bin are then added up and displayed on a graph. Hence more radio-opaque an image, more the graph will shift towards right (255-white).
- Mean grey level histogram values of images at 1 month and 3 months were compared to the immediate post-operative images and the difference i.e. increase in the histogram values for study and control site were noted.

Figure 1: Pre-operative intra oral picture

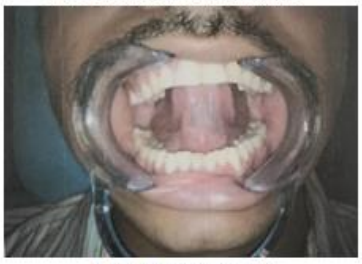


Figure 2: Pre-operative OPG

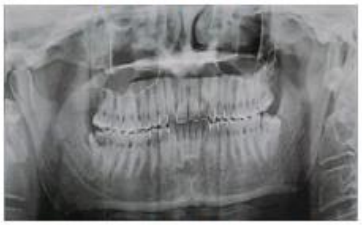
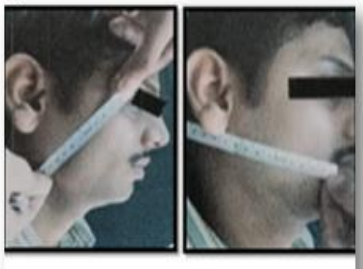


Figure 3: Facial measurements – horizontal & vertical



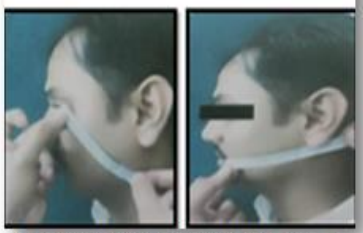


Figure 4: Grafting material was prepared

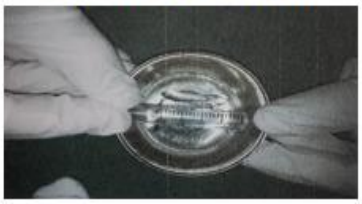


Figure 5: Grafting material was placed into socket

Figure 6: Intra oral study and control sites were observed

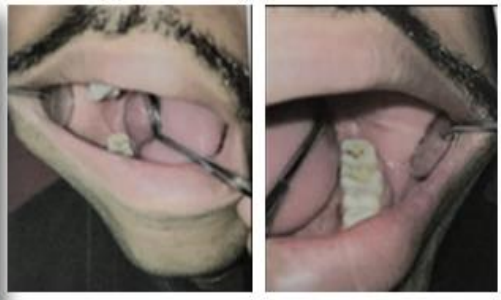
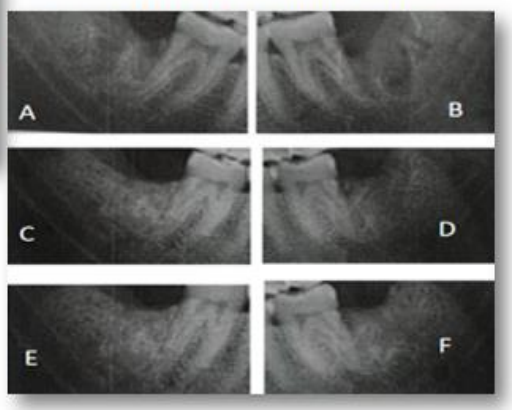


Figure 7: A,B-immediate post operative; C,D-1 month postoperative; E,F- 3 month postoperative placement of graft in 3rd quadrant



Observation And Result

In this study, 10 patients were included with mean age of 28.3year (Ranging from 20-35 years). These were 4 male and 6 female patients. In each patient one side of lower third molar extraction site was kept as control and in the other graft material was used. Hence 10 control site and 10 study sites were compared. The selection of site for placement of graft was done randomly. In 6 patients the graft was placed on right side and in 4 patients graft was placed on left. Pederson difficulty tracing was done and the difficulty of the impacted teeth on both control and study site were confirmed to be the same so that no bias existed between both groups.

(1) Facial swelling - Facial swelling measurements were recorded pre-operatively and were compared with that measured after 48 hours post-operatively and 7 days post-operatively. In this study the average percentage increase in facial swelling was 3.21% on study site as compared to 6.36% on control site. At 7 days post-operatively the percentage increase in facial swelling was 0.84% on study site and 1.91% on control site. Hence the swelling on control site was 98.13% more than study site at 48 hours post-operative and 127.4% more than study site at 7 days post-operatively.

(2) Pain - VAS score was noted subjectively by the patient and in this study at 48 hours post-operative average VAS score was 4.8 for study site and 6.7 for control site. Whereas at 7days post-operatively the VAS scored neared zero for both groups with average score on study site 0.6 and on control site 1.4.

(3) Number of analgesics required - The patients were also asked to note the number of analgesic tablets required for both side the average analgesics required for study site were 10.8 and for control site 13.4. Hence, in this study the pain score was slightly higher for the control site as compared to study site. Correspondingly the number of analgesics required by the patients were also higher for the control site.

(4) Bone density - The pre-operative mean grey level histogram values were compared with that at 1 month and 3 months of interval. In this study the increase in mean grey level values at 1 month was 14.21 for study site and 8.43 for control site. The increase in grey level values at 3 months was 34.67 for study site and 18.80 for control site Hence for study site there was 18.71% increase in bone density at 1 month and 45.65% increase at 3 months. As compared to that the increase in bone density for control site at 1 month was 11.11% and at 3 months 24.77% proving the efficacy of rhPDGF for bone regeneration.

In this study, no complications like infection, sinus discharge, wound dehiscence, dry socket or graft leakage were observed post-operatively in either group. Hence the tissue showed good acceptance to the graft material and no adverse reactions were seen.

Discussion

The reconstruction of large tissue defects is one of the main challenges to face the modern oral and maxillofacial surgeon. Being either iatrogenic in origin after serious operations for head and neck cancer or caused by traumatic injury or congenital deformity, the need to reconstruct multi-layered defects is growing as surgical techniques advance. While the transfer of autologous tissue such as bone grafts or tissue free flaps are well described, they are not without complications. With this in mind, the prospect of using principles of tissue engineering to reconstruct defects in tissues of the head and neck continues to gain the attention of the reconstructive surgeon[21].

Various synthetic bone grafts are available which only act as a scaffold for new bone formation. With the use of autologous bone graft the added surgical and anesthetic time, cost, and overall morbidity morbidity in patients per year who undergo autologous bone graft harvest is a significant cost to the health care system. Given that the rate of complications with autologous bone graft can approach 25%, room for improvement exists. Theses cost and patient complication issues have driven the medical community to search for bioactive alternatives like rhPDGF.

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma and it too is a source of PDGF. However, the centrifugation process for developing PRP must be sterile and precisely suited to platelet separation from red blood cells and their sequestration in high concentrations without lysing the platelets or damaging them so that they no longer can actively secrete their growth factors. Marx found that of all of the devices tested, only two FDA-cleared PRP devices produced the greatest platelet concentrations and, most important, release of a therapeutic level of bioactive growth factors[1]. This non dependability of PRP, equipment cost and equipment hassles show that the use of commercial substitute for PDGF is more feasible and dependable. When comparing to autogenous bone, in a study for ankle or hindfoot fusion the rate of radiographic union, time to full weightbearing, and outcomes scores between the rhPDGF/β-TCP and autologous bone graft subjects were similar[26]. The two treatment groups had highly similar radiographic, clinical, functional, and quality-of-life outcomes, but the patients in the rhPDGF-BB/ β-TCP group had fewer adverse events and complications and no donor site pain when compared with patients in the autogenous bone graft group[6]. The rhPDGF/ β-TCP group also demonstrated a reduction in surgical time of 22% compared to those patients with autogenous bone graft, and were spared of any potential donor site morbidity or pain associated with graft harvest[22].

The β-TCP serves as a matrix that physically fills bone defects providing a biocompatible, osteoconductive scaffold that allows bone formation and cellular ingrowth into the osseous defects[7,8]. The calcium and phosphate content of β-TCP is similar to bone mineral (Ca:P ratio of 1.5) and provides a substratum for bone-forming cells to adhere[21]. The porous β-TCP matrix allows for absorption of the protein solution and acts as a temporary reservoir of rhPDGF. Moreover, rhPDGF remains biochemically unaltered following release. Over the time, β-TCP is resorbed through dissolution, phagocytic, and osteoclastic activity and is replaced by new bone. Hence, the β-TCP is an effective delivery system for rhPDGF-BB[21].

In this study the average percentage increase in facial swelling was 3.21% on study site as compared to 6.36% on control site. VAS score was noted subjectively by the patient and in this study at 48 hours post-operative average VAS score was 4.8 for study site and 6.7 for control site. Hence, in this study the pain score was slightly higher for the control site as compared to study site. Hence for study site there was 18.71% increase in bone density at 1 month and 45.65% increase at 3 months. As compared to that the increase in bone density for control site at 1 month was 11.11% and at 3 months 24.77% proving the efficacy of rhPDGF for bone regeneration. No complications like infection, sinus discharge, wound dehiscence, dry socket or graft leakage were observed post-operatively in either group. Hence the tissue showed good acceptance to the graft material and no adverse reactions were seen. Patient-reported outcome measures were reported by only 1 study. This study showed that when rhPDGF and TCP were used with the single flap surgical approach, less patient morbidity in terms of self-reported pain and consumption of analgesics was encountered as compared with the double-flap surgical approach Schincaglia et al. 2015. Kaigler et al. 2012 in their review study stated that there was a significantly greater

clinical attachment level gain at three months for the 0.3 mg/ml rhPDGF-BB (Group I), as compared to the β-TCP controls (Group III), indicating an early benefit of rhPDGF-BB treatment[26].

Because of these shortcomings of PRP and autologous bone graft, rhPDGF/ β-TCP combination acts as a boon for reconstruction by performing equally and without any complications and cost. Due to its excellent efficacy in enhancing rapid bone regeneration, the rhPDGF can be used in a variety of procedures in oral and maxillofacial surgery. It will act as an exemplary grafting material for ridge augmentation procedures, implant procedures, sinus lift, socket preservation, etc. It will also be ideal material for filling bone defects created due to removal of any pathology. It also has a role in periodontal regenerative procedures and guided tissue regeneration procedures. Moreover, in diabetic and geriatric conditions (with compromised vascular supply) where other bone grafts are contraindicated, rhPDGF is not only a miraculous alternative but is above par for rapid bone regeneration.

Conclusion

Recombinant human PDGF with β-TCP when used in mandibular third molar extraction socket results in rapid bone regeneration seen via increased bone density. It also results in less post-operative discomfort to the patient like pain and swelling.Complications like infection, sinus discharge, wound dehiscence, dry socket or graft leakage were not encountered and the tissue showed good acceptance with no graft rejection.Hence rhPDGF/β-TCP combination is a novel bone grafting material with excellent results and should be included in the armamentarium when bone defect is encountered.

References

1. Marx RE., “Clinical controversies in Oral and Maxillofacial Surgery,: Part two. Platelet- Rich Plasma: Evidence to Support Its Use”, J Oral Maxillofac Surg 2004, 62(4),89-96.
2. Ton V., “Recombinant Human Platelet-Derived Growth Fact or-BB (rhPDGF-BB) Clinical Update”,2012,34(1).
3. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR., “Platelet-rich plasma Growth factor enhancement for bone grafts”, Oral Surg Oral Med Oral Pathol Oral Radiol Endod,1998,85(6),638-46.
4. Schliephake H., “Bone growth factors in maxillofacial skeletal reconstruction’’, Int J Oral Maxillofac Surg,2002,31(4),69-84.
5. Moore DC, et al., “Recombinant human platelet-derived growth factor-BB augmentation of new-bone formation in a rat model of distraction osteogenesis”, J Bone Joint Surg Am, 2009,91(8),73-84.
6. DiGiovanni CW, et al., “North American Orthopedic Foot and Ankle Study Group. Recombinant human platelet-derived growth factor-BB and beta-tricalcium phosphate (rhPDGF-BB/B-TCP): an alternative to autogenous bone graft J”, Bone Joint Surg Am. 2013,95(13),84-92.
7. Al-Zube L, et al., “Recombinant human platelet-derived growth factor BB (rhPDGF-BB) and beta-tricalcium phosphate/collagen matrix enhance fracture healing in a diabetic rat model”, Orthop Res, 2009,27(8),74-81.
8. Rocchietta Dellavia C, Nevins M, Simion M, “Bone regenerated via rhPDGF-6B and a deproteinized bovine bone matrix: backscattered electron microscopic element analysis”, Int J Periodontics Restorative Dent, 2007,27(6),39-45.
9. McGuire M, Nevins M., “rhPDGF-BB promotes healing of periodontal defects: 24 month clinical and radiographic observations”, Int J Periodontics Restorative Dent, 2006,26(2),23-31.

10. Amin MM, Laskin DM., "Prophylactic use of indomethacin for prevention of postsurgical complications after removal of impacted third molars", Oral Surg Oral Med Oral Pathol, 1983,55(5),448-51.
11. Rasubala L, Yoshikawa H., "Nagata K, lijima T, Ohishi M. Platelet-derived growth factor and bone morphogenetic protein in the healing of mandibular fractures in rats" Br J Oral Maxillofac Surg, 2003,41(3),173-8.
12. Lalani Z, Wong M, Brey EM, Mikos AG, Duke PJ., "Spatial and temporal localization of transforming growth factor-betal, bone morphogenetic protein-2, and platelet- derived growth factor-A in healing tooth extraction sockets in a rabbit model", J Oral Maxillofac Surg, 2003.
13. Fiedler J, Etzel N, Brenner RE., "To Go or Not to Go: Migration of Human Mesenchymal Progenitor Cells Stimulated by Isoforms of PDGF", Journal of Cellular Biochemistry, 2004,93,90-98.
14. Kilian O, et al., "New blood vessel formation and expression of VEGF receptors after implantation of platelet growth factor-enriched biodegradable nanocrystalline hydroxyapatite Growth Factors", 2005,23(2),125-33.
15. Sarment DP, et al., "Effect of rhPDGF-BB on bone turnover during periodontal repair", Clin Periodontol, 2006,33(2),135-40.
16. Giannoudis PV, Einhorn TA, Marsh D., "Fracture healing: the diamond concept", Injury ,2007,38(4),3-6.
17. Schindeler A, McDonald MM, Bokko P, Little DG., "Bone remodeling during fracture repair: The cellular picture", Semin Cell Dev Bio,2008,19(5),459-66.
18. Nedeau AE, Bauer RJ, Gallagher K, Chen H, Liu ZJ, Velazquez OC., "A CXCLS- and bFGF-dependent effect of PDGF-B-activated fibroblasts in promoting trafficking and differentiation of bone marrow-derived mesenchymal stem cells", Exp Cell Res, 2008,314(11-12),2176-86.
19. Gaissmaier C, Koh JL, Weise K., "Growth and differentiation factors for cartilage healing and repair" Injury, 2008,39(1),588-96.
20. Hollinger JO, Hart CE, Hirsch SN, Lynch S, Friedlaender GE.. " Recombinant human platelet-derived growth factor: biology and clinical applications", J Bone Joint Surg Am, 2008 ,90(1),48-54.
21. Young CS, et al., "Release, biological potency, and biochemical integrity of recombinant human platelet- derived growth factor-BB (rhPDGF-BB) combined with Augment™ Bone Graft or GEM 218 beta-tricalcium phosphate (beta-TCP). J Control Release", 2009,140(3),250-55.
22. Deschaseaux F, Sensébé L, Heymann D., "Mechanisms of bone repair and regeneration", Trends Mol Med, 2009,15(9),417-29.
23. Gawande PD, Halli R., "Efficacy of platelet rich plasma in bone regeneration after surgical removal of impacted bilateral mandibular third molars: pilot study", J Maxillofac Oral Surg, 2009,8(4),301-7.
24. Singh P, D.K. S, Kaushal S, Dabra S, Kaushik A., "Evaluation of recombinant human platelet derived growth factor-bb, beta-tricalcium phosphate with collagen membrane in the treatment of gingival recession", International Journal of Dental Clinics, North America,2011.
25. AI, Correa D., "PDGF in bone formation and regeneration: new insights into a novel mechanism involving MSCs", J Orthop Res, 2011,29(12),1795-803.
26. Kaigler D, Avila G, Wisner-Lynch L, et al., "Platelet-derived growth factor applications in periodontal and peri-implant bone regeneration", Expert Opin Biol Ther, 2011, 11(3),375-85.