Extraction socket grafting using Recombinant human bone morphogenetic protein-2 -Clinical implications and histological observations.

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Research note

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Abstract

Objectives – The main indication for preserving osseous structures following tooth extraction has been the rehabilitation of edentulous ridges to facilitate the placement of dental implants. Recombinant Bone Morphogenetic Protein-2(rhBMP-2) is exploited for bone augmentation due to its osteoinductive capacity. This study aimed to determine the effectiveness of bone induction for implant placement by rhBMP-2 delivered on beta-tricalcium phosphate graft (β -TCP) and PRF following tooth extraction.

Results- At three months following socket grafting, minimal changes in the crestal bone width compared to baseline values were seen. A bone loss of 0.6 ± 0.13 mm and 0.5 ± 0.13 mm at the mesiodistal and buccolingual aspects, respectively, was seen. While drilling before the implant placement, the bone's clinical hardness evaluated through tactile was analogous to drilling into spruce or white pine wood. Complete radiographic bone fill was seen in 3 months, and there was no need for any additional augmentation during implant placement. Besides, histology revealed no residual bone particulate graft. Therefore, the data from this study demonstrated that the novel combination of rhBMP-2 + β -TCP mixed with PRF has an effect on de novo bone formation and can be recommended for socket grafting before implant placement.

Introduction

At present, endosseous implants have become the choice of therapy for the management of an edentulous site (1) (2) (3). Following extraction, however, the alveolar process undergoes resorption due to osteoclastic activity leading to diminished alveolar ridge dimensions in both the vertical and horizontal planes. (4) Alveolar ridge alterations ranging from 40–60% tend to occur as early as three months after extraction. (5) The altered height and width present with functional as well as aesthetic problems resulting in compromised rehabilitation. Non-grafted edentulous sites may lose up to fifty percent of ridge width within 12 months following the tooth's removal. (6) An average reduction reported in the width and height of grafted edentulous sites' is minimal compared to non-grafted sites, with most cases requiring no additional augmentation to have the implants placed. (7) Therefore, surgical approaches aiming to maintain ridge volume by preservation before implant placement are deemed useful. Platelet-rich fibrin (PRF) comprises of fibrin matrix enriched with platelets and growth factors. Hence it is a powerful bioscaffold for tissue regeneration. (8) Alloplastic materials like tri-calcium phosphate (TCP) are viable substitutes for autogenous bones or other bone substitutes. (9) When blended with bone substitutes, the ability of PRF for regeneration is enhanced. The Recombinant human bone morphogenetic protein-2 (rhBMP-2) is promising in various bone augmentation procedures. (10) An animal study has reported unique bone formation escorted with rhBMP-2 impregnated β -TCP scaffold in the cranial bone defect model. (11) The utility of rhBMP-2 and Acellular Collagen Sponge (ACS) in extraction defects helps to reconstruct the buccal plate. The alveolar ridge dimensions are crucial for implant placement, and rhBMP-2 aids in maintaining ridge configuration favoring implant placement. (12) Similarly, the inherent amount of vital bone formation in the healing socket is also essential. Therefore, the effectiveness of ridge preservation using rh-BMP-2 in combination with β -TCP and PRF were evaluated clinically, radiographically, and histologically.

Materials And Method

Materials and Method

Following informed consent, two systemically healthy patients (M:F1:1, aged 23 years and 30 years, respectively) who required the extraction of teeth for reasons other than periodontitis were recruited. After proper examination and diagnosis, initial therapy consisting of oral hygiene instructions, supragingival and subgingival scaling was performed. The full mouth plaque index (FMPI)(13) and Full mouth papillary bleeding index (FMPBI) scores were low at the baseline and remained low (< 1) during the treatment period.(Table 1) (14). We included four sites in two patients. Following this, clinical and radiographic examination of the selected sites was carried out. (Table 2).

Surgical procedure

A pre-surgical rinse with chlorhexidine gluconate 0.12% mouthwash (Rexidine®, Indoco Remedies Ltd) for 2 minutes was advocated. After administering local anesthesia (Xicaine®, ICPA Health Products Limited), flapless minimally invasive periostomy was carried out with a Bard-Parker Surgical blade number #15 (Glassvan®, Niraj Industries Pvt Ltd) to facilitate atraumatic extraction without causing damage to the buccal plate. Consecutively, the walls of the socket were verified for integrity. Twenty milliliters of venous blood was withdrawn in two test tubes from the antecubital vein. Centrifugation of the blood was carried out at 3000 rpm for 12 minutes to obtain PRF. (15) One part of PRF was mixed with β -TCP (OSTEON TM II Genoss Co., Ltd, Korea) and 1.5 mg/mL concentration of rhBMP-2 (GibcoR, Recombinant Human Bone Morphogenetic protein-2, Life Technologies, Van Allen Way, Carlsbad, California) (16) to enhance wound healing. The socket was filled up to the level of the alveolar crest. The remaining clot was compressed to a membrane of high tensile strength. The membrane was then carefully placed, covering the graft material. The approximation of the surgical site was made using a 3-0 silk suture. (Centisilk Non- Absorbable Surgical Suture U.S.P)

The patients were reviewed after three months for implant placement. Following administration of local anesthesia (Xicaine®, ICPA Health Products Limited), an incision was given from the line angle of adjacent teeth to elevate the mucoperiosteal flap and expose the underlying bone. Alveolar ridge measurements were taken intrasurgically and were compared with the pre-operative alveolar dimensions (Table 2). A 2 mm trephine drill was initially used to harvest bone core for histological examination. The bone tissue was moved to a mixture containing 10% neutral buffered formalin. A decalcification protocol was used to remove the bone mineral component to ease the fine section and better study of new bone-forming cells. The tissues were stained with hematoxylin & eosin (H&E) (Fig.1) (17).

Sequential drills were used for osteotomy preparation, based on the size of the implant selected. An implant (Equinox, Myriad Plus TM implant system) of the largest possible dimension was placed according to the manufacturer's recommendations, and further follow-up was carried out. Four implants were placed in two patients.

Statistical Analysis - The Wilcoxon sign ranked test was used to compare the means of the parameters used before and after socket grafting. A $p < 0.05$ was considered to be statistically significant. (18)

Discussion

Healthy osseous contour provides a skeleton for peri-implant soft tissues. Atraumatic extraction aims to minimize bone resorption and maintain the alveolar ridge dimensions during healing. In the present study, three months following socket preservation, minimal changes in the alveolar ridge dimensions were seen (mesiodistal bone loss 0.6 ± 0.13 mm, and buccolingual bone loss 0.5 ± 1.13 mm), and the results were not significant. The bone's clinical hardness evaluated during drilling before implant placement provided a tactile sensation analogous to drilling into spruce or white pine wood at all the four sites. (19) Although bone density evaluation using Hounsfield units was not done, dense to the thick porous cortical bone on the crest and coarse trabecular bone underneath was seen on radiographic examination, suggesting D2 bone quality. (20) Histologically, the presence of vital mineralized osteoid trabeculae and soft tissue containing spindle nuclear cells indicated new bone formation. All four implants achieved good primary stability. As soft tissue grows six times faster than the bone tissue (21), in the present study, a saddle of PRF membrane covering the socket helps prevent the gingival connective tissue down-growth. It also held the osseous graft in place. (22) Additionally, the membrane also aided in clot stabilization. PRF principally contains a fibrin matrix rich in platelets and leukocytes. Cytokines such as IL-1 β , -4, and -6, and growth factors such as PDGF, TGF-beta, IGF, EGF, and VEGF are other vital components present. These are the critical elements in bone regeneration. (23) Fibrin gel in coagulation cascade aids in fibrinogen molecules collaboration and formation of a highly biocompatible three-dimensional fiber network. (24) Therefore, PRF has resulted in improved wound healing and enhanced bone graft density and maturation. (19) β -TCP is (purified, microcrystalline porous) a form of calcium phosphate. Remarkable resorption of β -TCP particulate is estimated at around 3 to 6 months after placement.

Besides, particles become well-incorporated into new bone formation, creating a dense cancellous matrix. Biodegradation of graft takes place both by osteoclastic activity as well as chemical dissolution by tissue fluids. A solid new bone formation was evident during implant placement, which favored the placement of ideal implant size. The use of rhBMP-2 necessitates the use of a carrier for its osteoinductivity (25). Misch emphasized the advantages of rhBMP-2 in new bone growth in patients requiring extraction. In the present study, rhBMP-2 was used as an agent to promote socket repair and aid in ridge augmentation when combined with a part of β -TCP. Fiorellini et al. (16) in their study showed significantly greater bone augmentation with 1.50 mg/mL/ rhBMP-2 compared to a concentration of 0.75 mg/mL in socket defects. Therefore, in the present study, a concentration of 1.50 mg/mL/ rhBMP-2 was used. Various studies (26) (27) have supported using a mixture of β -TCP and rh-BMP- 2 to facilitate the increased bone formation; hence rh-BMP-2 was used in combination with β -TCP to promote bone growth.

Limitations

The present study describes the results obtained in a small sample. Although there is evidence of good-quality bone formation during a short period following the use of a combination of rhBMP-2, β -TCP, and PRF, more extensive population studies may further improve the significance of the results obtained. PRF was used to cover and hold the graft in place. The increase in keratinized tissue thickness following the PRF membrane placement was not evaluated. Additionally, only six months follow-up results have been reported; further long term evaluation of the patients would be beneficial. Even though pre-operative CBCT was taken but due to the patient's cost concerns and unwillingness, the post-operative CBCT could not be accepted.

Abbreviations

rhBMP-2- recombinant bone morphogenetic protein, β -TCP- beta-tricalcium phosphate, PRF-platelet rich fibrin, PDGF platelet-derived growth factor, TGF-beta transforming growth factors, IGF-the insulin-like growth factor, EGF - epithelial growth factor, VEGF-vascular endothelial growth factor. Full Mouth Papillary Bleeding Index (FMPBI), Full Mouth Plaque Index (FMPI), MB-Mesio-buccal root, DB-Disto-buccal root, RBH-Residual bone height- measured from alveolar crest to anatomical structure, M-D Mesiodistal width, B-L Buccolingual width.

Declarations

Acknowledgements-None

Authors' contributions

Primary author KJD performed the surgical procedures and drafted the manuscript. Second author VSB conducted the examinations and reviewed the manuscript. The third author SBS, reviewed & revised the manuscript. The fourth author PVD changed the final writing of the document. Fifth author GCR assisted in all surgical procedures and helped in drafting the manuscript through a literature search. All authors have read and approved the manuscript.

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Ethics approval and consent to participate -The study protocol No- DMIMS(DU)/IEC/2016-17-5080 was first approved by the Ethical Committee of Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha, Maharashtra (India), and written informed consent was obtained from all the patients.

Consent for Publication-Taken

Competing interests– The authors declare that they have no competing interests.

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Tables

Table no 1 - Full Mouth Papillary Bleeding Index (FMPBI) and Full Mouth Plaque Index (FMPI)

Cases	FMPBI at Baseline	FMPBI at 3 months	FMPI at Baseline	FMPI at 3 months
Cases 1	0.5	0.4	0.4	0.4
Cases 1	0.5	0.3	0.4	0.3
Cases 2	0.5	0.5	0.5	0.5
Cases 2	0.5	0.5	0.5	0.5
Mean±SD	0.5± 0	0.45± 0.05	0.425± 0.08	0.425± 0.08
p-value	0.215 NS		0.391 NS	

NS- Not significant

Table no -2 Measurements at ridge prior tooth/root piece extraction (at baseline) and at 3 months (Post-operative)

Cases	Site	At Baseline				At 3 Months			Mean ± SD (Bone loss)				
		Mesio-distal width (mm)	Bucco lingual width (mm)	Measurement of root piece (mm)		Distance from root apex to anatomical structures (mm)	Mesiodistal width (mm)	Buccolingual width (mm)	Apico coronal length (RBH) (mm)	M-D (mm)		B-L (mm)	
										At Baseline	At 3 months	At Baseline	At 3 months
										9.6±1.67	9.0±1.54	8.4±1.13	7.9±1
Cases 1	11	8.5	7.5	12	3	8	6.5	14.5	Mean Difference (Bone Loss) (mm) = 0.6 ± 0.13		Mean Difference (Bone Loss) (mm) = 0.5 ± 0.13		
Cases 1	12	7.5	7	11	3	7	7	13.5					
Cases 2	46	11.5	9.5	MB-8	DB-6	4	10.5	9	12	p=0.015*		p=0.058 NS	
Cases 2	47	11	9.5	MB-7	DB-7	5	10.5	9	12.5				

NS- Not significant

* Statistically significant

Figures

Figure 1

Implant placement

Figure 1

Implant placement

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