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Case Series

Dentistry Section

# Radiographic Assessment of Bone Formation Using rhBMP2 at Maxillary Periapical Surgical Defects: A Case Series

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## ABSTRACT

Periapical cysts are the most common inflammatory odontogenic cysts arising from untreated dental caries with pulp necrosis and periapical infection. The choice of treatment is often influenced by various factors like size, extension of the lesion, proximity to vital structures, systemic condition and compliance of the patient too. The treatment protocol for management of periapical cysts is still under discussion and options vary from conservative treatment by means of endodontic technique to surgical treatment like decompression or a marsupialisation or even to enucleation. Large bony defect secondary to periapical surgery compromising the tooth integrity often requires bone graft to enhance bone formation and thus restoring function at the earliest.

The present case series included 10 patients who had established periapical pathology secondary to history of trauma on upper anterior teeth as well patients with history of carious teeth with an apparent failure in root canal therapy. All ten patients were treated with cyst enucleation and apiceotomy along with 1.4cc Recombinant Human Bone Morphogenetic Protein-2 soaked Absorbable Collagen Sponge implantation at surgical defect. Radiographs and clinical examinations were done upto 3 months to evaluate healing. Radiographic and clinical assessments revealed bone regeneration and restoration of the maxillary surgical defects in all 10 patients. No evidence of graft failure was noted. The Recombinant Human Bone Morphogenetic Protein-2 soaked Absorbable Collagen Sponge carrier is thus proved to be a viable option for the treatment of maxillary periapical surgical defects.

**Keywords:** Bone morphogenetic proteins, Panoramic radiography, Radicular cyst, Type I Collagen

## CASE SERIES

The present case series included 10 patients with established periapical pathology with the need for periapical surgery. All the 10 patients underwent a detailed clinical examination, routine haematological investigations, preoperative Digital pantomogram. The clinical details are presented in [Table/Fig-1].

All these patients had a history of trauma in the upper anterior teeth as well as with the history of carious teeth with an apparent failure in root canal therapy. They had radiographic evidence of a periapical lesion. None of them had any history of known systemic illness. The surgical procedure constituted of a full thickness mucoperiosteal flap being raised depending upon the size and location of the lesion. The incision lines of the flap did not overlie any bony defect and the base of the flap was the widest point with no sharp corner. Thorough periapical curettage and apiceotomy was performed to remove the pathological tissue surrounding the apices and the root of the tooth. The surgical defect was grafted with INFUSE® BONE GRAFT KIT {1.4 CC OF rhBMP2+ACS preparation}. The methodology in preparation of reconstituting lyophilised rhbmp2 with its collagen carrier has been described below

INFUSE® Bone Graft kit {1.4 cc (X Small Kit) Preparation} contains a sterile Absorbable Collagen Sponge (ACS) with a dimension of Sponge 2.5cm × 5.08cm and with a graft volume 1.4 cc. It also contains two number 5 ml vials Sterile Water for Injection and two number 1.05 mg vials of Sterile rhBMP2.

In a sterile surgical field, the outer packaging of ACS was opened and the inner package containing one 1" × 2" collagen sponge was placed. Two sterile 3 ml syringes/needles were opened and placed into the sterile field.

By using one of the two remaining 3 ml syringes/needles 0.9 ml of sterile water for injection was aspirated. One of the 1.05 mg vials of the rhBMP-2 was reconstituted with 0.9 ml of sterile water. The reconstituted rhBMP-2 was gently swirled (not to be shaken) to ensure adequate mixing. Similarly the second 1.05 mg vial of the rhBMP-2 was reconstituted with 0.9 ml of sterile water in the same method as mentioned earlier.

In sterile field the inner ACS package was opened, leaving the collagen sponge in the plastic tray given. Using 3 ml syringe/needle 0.7 ml of reconstituted rhBMP-2 was aspirated by the surgeon from the vial held by the assistant. Later 0.7 ml of reconstituted rhBMP-2 was uniformly distributed on half of the 1" × 2" collagen sponge. Similarly the second 0.7 ml of reconstituted rhBMP-2 was aspirated and uniformly distributed on half of the 1" × 2" collagen sponge in the same method as mentioned earlier. The

| Description | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 | Case 8 | Case 9 | Case 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | 24 | 35 | 34 | 45 | 22 | 24 | 24 | 26 | 20 | 22 |
| Sex | Female | Male | Male | Female | Male | Male | Female | Female | Female | Male |
| Systemic Disease | None | None | None | None | None | None | None | None | None | None |
| Diagnosis | Radicular Cyst In 21, 22 | Radicular Cyst In 11, 21 | Radicular Cyst In 11, 12 | Radicular Cyst In 21, 22 | Radicular Cyst In 21 | Radicular Cyst In 21 | Radicular Cyst In 21 | Radicular Cyst In 21, 22 | Radicular Cyst In 11 | Radicular Cyst In 21, 22 |
| Treatment Done | Cyst Enucleation, Apiceotomy And Placement Of Graft [ rhbmp2+ACS] –Volume 1.4.Cc | | | | | | | | | |
| Follow Up Period | 3 Months In All 10 Cases | | | | | | | | | |
| Complication | Postoperative Oedema In All 10 Cases | | | | | | | | | |

**[Table/Fig-1]:** Clinical details.

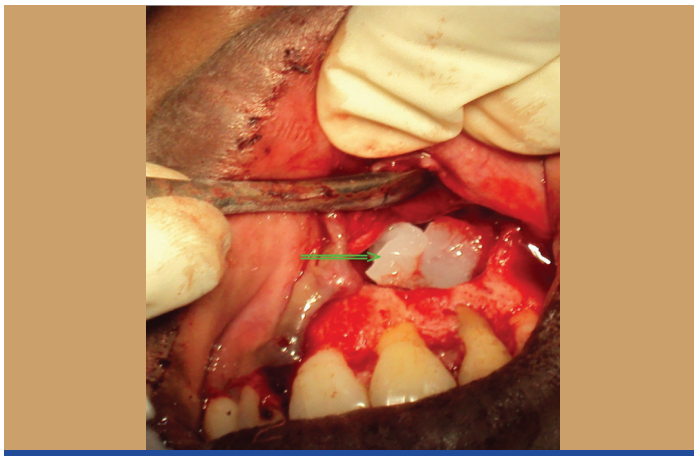
collagen soaked with reconstituted rhBMP-2 was allowed to wait for a minimum of 15 minutes to uniformly distribute reconstituted rhBMP-2 on the collagen sponge carrier.

The soaked collagen was placed in the periapical osseous defect followed cyst enucleation [Table/Fig-2]. Precaution was carried not to use irrigation or suction near the collagen implanted site.

The flap was replaced to its original position and sutured with 3-0-black braided silk (HISIL*) to ensure complete soft tissue coverage of the graft. Patients were given antibiotics and analgesics for five days. Sutures were removed on the 7th post operative day. The patients were called on the 1st day after surgery for evaluating immediate postoperative complication and recalled after three months postoperatively to assess the bone formation at periapical surgical defect sites.

Preoperative and postoperative measurement of radiolucency of the periapical lesion was done at 0 and 3 months with digital orthopantomogram [Table/Fig-3,4]. PLANMECA- DIMAXIS SOFTWARE with version Pro 4.16 (2006) was used for this purpose for assessing periapical lesion on the basis of morphometric analysis for measuring height and width of radiolucency to resolution level up to 0.00mm. The distance from the crest of the bone, corresponding the apical 3rd of resected root to the basal bone, corresponding palatal aspect in digital orthopantomogram was measured as the length of radiolucency in the cyst. The maximum mesio-distal distance of radiolucency was measured as the width of radiolucency in the cyst. Magnification factor was calculated using the standard formula.

Radiological assessment of preoperative and postoperative radiolucency have been shown in the [Table/Fig-5]. The comparison of mean values and standard error of mean of pre and post operative length and width is presented in [Table/Fig-6].

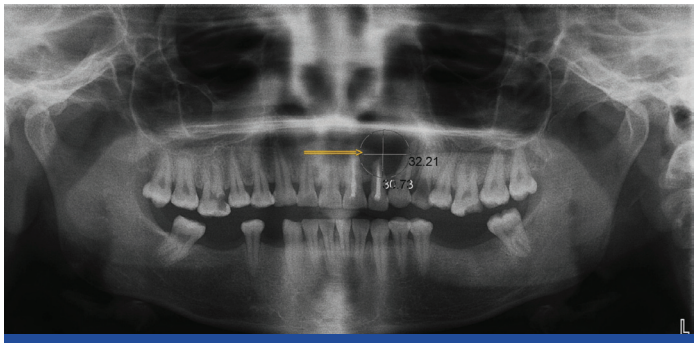


**[Table/Fig-2]:** Perioperative image with green arrow shows placement of rhBMP2 + ACS in bony defect.

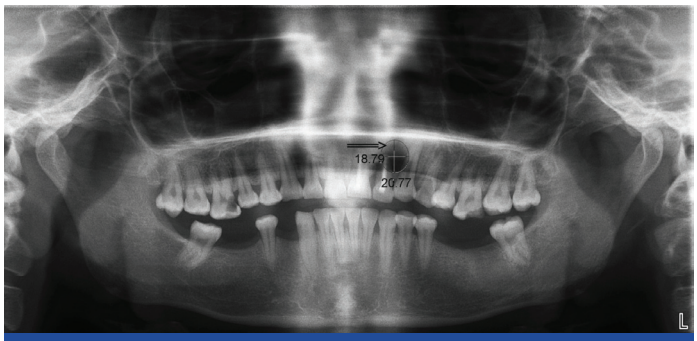
On statistical analysis, significant difference in reduction in post -operative mean length of radiolucency ($p < 0.05$) and mean width of radiolucency ($p < 0.05$) were observed in [Table/Fig-5,6] which is indicative of bone formation radiographically at a periapical surgical defect site at the end of the third month after placement of rhBMP-2.

It can be inferred from the analysis that:

1. Mean Difference in Length of preoperative and postoperative radiolucency was 4.65mm.
2. Mean Difference in Width of preoperative and postoperative radiolucency was 5.57mm.
3. Statistically significant p-value<0.05 is indicative of significant difference in length as well as in width between preoperative and 3 month postoperative radiolucency.




**[Table/Fig-3]:** Pre operative orthopantomogram with yellow arrow shows large radiolucent lesion along left upper anterior teeth region.




**[Table/Fig-4]:** Third month postoperative orthopantomogram with black arrow shows reduction in radiolucency with mixed radiopacity suggestive of bone formation at surgical defect.

| Patient's Serial No. | Pre-op Radiolucency in mm | Post op Radiolucency in mm |
|---|---|---|
| 1 | Length-28.75<br>Width-31.22 | Length-20.77<br>Width-18.79 |
| 2 | Length-15.53<br>Width-19.60 | Length-12.52<br>Width-17.85 |
| 3 | Length-27.75<br>Width-26.27 | Length-19.35<br>Width-19.33 |
| 4 | Length-12.50<br>Width-11.85 | Length-11.82<br>Width-9.52 |
| 5 | Length-18.13<br>Width-17.16 | Length-11.53<br>Width- 10.20 |
| 6 | Length-19.21<br>Width- 14.96 | Length- 13.13<br>Width-12.48 |
| 7 | Length- 19.12<br>Width- 15.84 | Length-12.87<br>Width-11.87 |
| 8 | Length-18.42<br>Width- 19.08 | Length-14.54<br>Width-11.84 |
| 9 | Length- 11.24<br>Width- 15.53 | Length-7.98<br>Width-9.32 |
| 10 | Length- 10.29<br>Width - 20.30 | Length- 9.92<br>Width- 14.87 |

**[Table/Fig-5]:** Radiological assessment of preoperative and postoperative radiolucency

| Details of Pre and Postoperative Length and width | Mean ± Standard deviation | Mean Difference | Standard Deviation Difference | t-value | p-value |
|---|---|---|---|---|---|
| Pre-Op Length | 18.09 ± 6.28mm | 4.65mm | 2.85mm | 5.16mm | < 0.05 |
| Post–Op Length | 13.44 ± 3.94mm | | | | |
| Pre-Op Width | 19.18 ± 5.74 mm | 5.57mm | 3.18mm | 5.53mm | < 0.05 |
| Post-Op Width | 13.61 ± 3.85mm | | | | |

**[Table/Fig-6]:** Comparison of pre and Postoperative length and width of radiolucency
TEST OF SIGNIFICANCE USED-> Paired Student's t-Test
< 0.05 – Significant at 5% level

## DISCUSSION

Bone has a unique ability to repair and regenerate itself throughout life upon fracture and surgical removal for various reasons like a

cyst, tumour and various other maxillofacial pathologies. Bone regeneration is a well-harmonized series of biological events involving a number of cell types and intracellular and extracellular molecular signalling pathways, with a definable temporal and spatial sequence, in an effort to optimize skeletal repair and restore skeletal function [1].

Marshall R Urtist in 1965 made the key discovery of the proteins, namely "Bone Morphogenetic Proteins (BMP)", which were extracted from the Demineralised Bone Matrix (DBM) of mammals being capable of inducing new bone when reconstituted with insoluble collagen and implanted in ectopic sites [2]. The major component of DBM is type I collagen (95%) and the remaining as non-collagenous proteins, including small amounts of growth factors like bone morphogenetic proteins (BMPs) which are responsible for the natural bone healing process [3].

The Bone Morphogenetic Proteins (BMPs) are growth and differentiation factors and form a large family of proteins structurally related to Transforming growth factor-beta (TGF-β) and activin [4]. Clinically useful BMP is a cloned Recombinant Human Bone Morphogenetic Proteins (rhBMP-2) that can regenerate bone *de novo* [5].

Commercially available Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2) namely INFUSE® (*Medtronic Sofamor Danek USA*) have been approved by the Regulatory agencies in US, Europe, Canada for selective oral & maxillofacial and dental-osseous regenerative purposes like sinus augmentations, and localized alveolar ridge augmentations, defects associated with extraction sockets [6] .

Numerous studies on the biological role of carrier for rhBMP-2, state that the carrier along with rhBMP-2 plays a vital role in new bone formation [6,7]. Till date type I collagens has been the preferred carrier for rhBMP-2 [8]. In the present study, we have assessed the clinical efficacy of rhBMP2+ACS in bone formation by performing radiographic assessment of bone formation at surgical defect, within three months of its placement.

The most commonly encountered cyst in the oral and maxillofacial region are periapical cyst. It occurs at any age. The distribution of the patient's gender and their mean age in our study revealed that there is no age or sex predilection for this lesion to occur as stated by Robert E. Marx and Diane Stern [9]. The periapical cyst is a sequel of untreated dental caries with pulp necrosis and periapical infection [10]. Clinically often these cysts are associated with a carious tooth, which have undergone improper restorative or endodontic therapy or tooth that have sustained trauma due to sports and assault, road traffic accident [10,11]. In our study 9 out of 10 patients revealed a history of trauma to the upper anterior tooth, thereafter immediate restorative or conservative treatment was carried out. One patient revealed a history of carious tooth with an apparent failure in root canal therapy.

Radiologically periapical cyst appears as an apical radiolucent region ranging from 0.5cm to 5cm or exceeding 5cm involving entire quadrant as observed by Shafer [12]. Periapical cyst may cause a regular smooth resorption of adjacent tooth roots, or there may be irregular resorption of their tooth root of origin, Presumably because of infection or osteoclastic factors elaborated by the cyst [9].

The treatment of pulpal pathosis following trauma or caries consists of Root Canal Therapy (RCT) [13]. However, periapical surgery is the ultimate treatment option to save a tooth with apical pathology that cannot be managed by conventional non-surgical endodontics [14].

After curettage or enucleation of the periapical cyst, spontaneous healing is noted in small periapical lesion up to 0.5cm in diameter. However, in the larger periapical lesion (>1cm) only a periapical scar is noted in the follow up radiographs. In such cases, complete bone healing may not be achieved leading to a compromised tooth integrity as observed by Lingaraj et al., in their study [15]. Hence, it is often essential to perform bone grafting in order to enhance bone formation at surgical defect and thus restoring tooth integrity at the earliest.

In such a consideration, acceleration of the bone-healing process, for example by implantation of a bioactive substance, would be of obvious benefit. Study based on freeze dried bone allograft in periapical osseous defect have shown satisfactory bone formation [15]. The present study attempted to evaluate the efficacy of Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2) for managing periapical surgical defects.

BMP's constitute a subfamily of the transforming growth factor-ß super family. Following their discovery and extensive purification [2], identification [2,3], molecular cloning, recombinant BMPs have been made available for evaluation and treatment of osseous defects in the appendicle, axial, and craniofacial skeleton [16-20].

Although the usage of rhBMP-2 in craniofacial osseous defect has been extensively studied in dogs and nonhuman primates and shown to be effective for several indications in the craniofacial osseous defect [16-20] only a few exclusive studies like Bergenholtz et al., and Jong-Bum Lee et al., have considered its usefulness in the surgical management of periapical surgical defects [21,22].

Bony repair of periapical lesions after endodontic or surgical therapy is usually monitored in a subjective manner using conventional radiographs, which does not assure the precise identification of periapical lesion changes or extension [23], hence it is necessary to evaluate and standardize the radiographic assessment by using new and promising diagnostic methods such as USG, CT scans Digital radiographic analysis as stated by Ingle JI [24].

FB Carvalho et al., have used morphometric analysis and outlined the radiographic images of periapical lesion and the radiolucent areas were measured by image analysis software [25]. Our study used Digital orthopantomogram with Planmeca-Dimaxis Software along with morphometric analysis for assessing the healing of periapical lesion which is more superior and accurate in comparison to conventional radiographs.

Numerous animal as well as human studies have been done in determining the optimal dosage of rhBMP-2 needed for bone formation. Cochran et al., experimented with 0.43mg/ml and Howell et al., evaluated with 0.83mg/ml of rhBMP-2 for ridge augmentation [18,26]. Both authors noticed only a minimal clinically evident ridge augmentation which is suggestive of a conclusion that rhBMP-2 induces bone in a concentration–dependent manner.

However, Alexandre Valentin Opran et al., from his multicentre study concluded that 1.5mg/ml was the most effective rhBMP-2 dosage for inducing bone of higher mineral density [27]. Hence the rhBMP-2 concentration of 1.5 mg/ml has been used in the present study.

Kuber T. Sampath stated that the carrier plays an important biological role as a component of the BMP device to affect bone formation [8]. As of now type I collagen is the preferred carrier for BMP as it is a natural component of bone and considered as the gold standard for comparison as it undergoes resorption comparable to that of extra-cellular matrix component of bone and gets degraded within 2 to 4 week period of time. Preclinical research has found that many carriers with fast degradation rates, such as calcium sulphate, tricalcium phosphate, and demineralised bone which are poor carrier for rhBMP-2 and should be avoided [28].

The safety profile of rhBMP-2 and type I collagen carrier in ACS has been extensively studied by numerous authors [16,26,29]. T Howard Howell et al., conducted first human study focused

predominately on the safety of rhBMP-2 and its carrier, where patients were evaluated for potential antibody formation for anti-rhBMP-2 or anti-bovine collagen type I or anti-human collagen type I protein. It was found that all the results of blood samples to measure serum chemistry, haematology and urine analysis were found within normal limit [26].

Triplett et al., had observed a greater degree of clinical oedema in their human studies with rhBMP-2. He stated that the clinical oedema was presumably a biological effect of cell recruitment and neovascularisation at rhbmp2 implanted surgical site [30]. In our study all the 10 patients had gross postoperative oedema which was persistent for 3 days and subsided gradually.

Minimal bone formation has been observed radiographically within 4 weeks and healed by 12 weeks in an experimental bone defect of animals implanted with rhBMP-2/ACS [31]. In the present study, we observed the healing of periapical surgical defects by reduction of radiolucency in length and width of the lesion along with the increase of radiopacity in the deficit by the end of 12 weeks. Thus, our present study is comparable and similar to the above mentioned author's study [31]. Thus, this small sampled short term study proved the clinical efficacy of rhBMP-2 as biologically useful alternate bone graft material in bone formation at periapical surgical defect.

## CONCLUSION

It has been proved by ample literature that rhBMP-2 has good osteoinductive potential, eliminating the need for further surgical graft procedure, and reducing hospitalization time, and thus can be even done on an outpatient basis. As our study was on the technical feasibility of rhBMP-2 in periapical surgical defects with a limited number of selected cases; to substantiate, prove and apply these findings and results to a generalization for the public, it is necessary to do more research on this issue with more and definite sample size.

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