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Case Series
Pre-Implant Surgery

Bone augmentation of the atrophic anterior maxilla for dental implants using rhBMP-2 and titanium mesh: histological and tomographic analysis

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Abstract. The loss of multiple teeth or trauma to the anterior maxilla often results in a deficient ridge width for prosthetic tooth rehabilitation. This study evaluated the use of titanium mesh and recombinant human bone morphogenetic protein 2 (rhBMP-2) for the repair of major bone defects in the alveolar bone. Five patients were enrolled in the study; these patients required implant replacements for two contiguous missing teeth in the anterior maxilla, which lacked sufficient bone. Residual ridges were augmented with rhBMP-2 and titanium mesh to direct the geometry of the newly formed bone. Seven months later, a bone biopsy specimen was removed from the implantation site before osteotomy and insertion of dental implants. Cone beam computed tomography (CBCT) scans were obtained preoperatively, postoperatively (baseline), and 48 months after implantation to evaluate implant healing. All dental implants were placed in the grafted sites without the need for further bone augmentation. The most frequent adverse effects were facial oedema and oral erythema. Biopsy specimens were used to evaluate bone quality. CBCT scans provided a prediction of alveolar restoration and long-term success. The combination of rhBMP-2 and titanium mesh provided effective augmentation of the atrophic anterior maxilla prior to implant placement.

Key words: dental implant; atrophic ridge; follow-up.

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In 1965, Urist et al. demonstrated that bone morphogenetic proteins (BMPs) extracted from bovine bone are able to induce ectopic bone formation subcutaneously in rats.[1] These results suggested the potential use of BMPs to induce bone regeneration and dental implant osseointegration. A subfamily of the transforming growth factor beta (TGF-β) superfamily, BMPs mediate signalling pathways that affect cell proliferation, cell differentiation, and extracellular matrix formation. In particular,

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recombinant human BMP-2 (rhBMP-2) has been shown to stimulate adult mesenchymal stem cells to induce clinically relevant bone formation.[2]

Since 2002, the US Food and Drug Administration (FDA) has approved the use of absorbable collagen sponge (ACS) as a carrier for rhBMP-2 in lumbar fusion and long bone fracture repairs. In 2007, rhBMP-2 was used for alveolar ridge and maxillary sinus augmentation.[3] Relevant bone formation for various skeletal defects, including those of the craniofacial complex, has been observed in the presence of rhBMP-2 in clinical studies.[2,4] However, only a few reports have evaluated the use of rhBMP-2 or other BMP family members in conjunction with dental implants in the aesthetic zone.[4,5] Therefore, the objective of this prospective case series study was to evaluate the clinical and histological effects of rhBMP-2 alveolar bone augmentation in the rehabilitation of the severely resorbed anterior maxilla with osseointegration implants.

Materials and methods

Selection of patients

This prospective case series study was conducted in accordance with the STROBE guidelines for observational studies and the Declaration of Helsinki. The study was approved by the local ethics committee.

Five consecutive patients (one male, four females; mean age 49.4 ± 20.8 years) who were referred to the authors' institution in 2009 for implant therapy in the anterior region were considered for inclusion in the study. Patients were included if they had trauma- or pathology-induced alveolar bone defects in all three dimensions in the anterior region (Fig. 1), an absence of two contiguous teeth, and were in good general health at the time of surgery. Patients were excluded if they met any of the following criteria: (1) endodontic treatment required for a tooth adjacent to the evaluated site, (2) smoking habit of >10 cigarettes a day, (3) parafunctional habits, (4) pregnancy, (5) known hypersensitivity to rhBMP-2, bovine type I collagen, or any other component of the Infuse bone graft kit, (6) active or suspected malignancy, and (7) undergoing treatment for malignancy.

Reconstructive surgery

Patients received 2 g of amoxicillin 1 h before surgery. They were instructed to wash their mouths with a 0.12% chlorhexidine solution for 30 s immediately before surgery. All reconstructive surgeries, implant placements, and prosthetic restorations were completed by the same professional (SR).

Briefly, local anaesthesia consisting of 2% lidocaine (1:50,000 epinephrine) was administered. An incision was made along the ridge through the keratinized gingiva in the alveolar crest, and a lateral releasing incision was made at the base on both sides. A mucoperiosteal flap was reflected to expose the atrophic ridge completely. Future implant sites were planned. The site for insertion of a 0.2-mm-thick titanium mesh for graft coverage was assessed. The lateral borders of the mesh were to be extended slightly beyond the desired area of augmentation to contact the residual ridge. The concave region below the mesh would be the site for the rhBMP-2-containing ACS. The cortex of the crest was perforated multiple times to produce bleeding at various sites (Fig. 2), while pilot holes for fixation screws were prepared for the titanium mesh.

An Infuse bone graft kit (Medtronic) was used to repair each defect. The collagen sponge included in the kit was saturated evenly with 0.7 ml of 1.5 mg/ml rhBMP-2 for 15 min, in accordance with the manufacturer's instructions. The titanium mesh was then applied to cover the rhBMP-2-containing collagen sponge (Figs. 3 and 4). The periosteum of the buccal flap was released to allow tension-free coronal advancement of the flap,

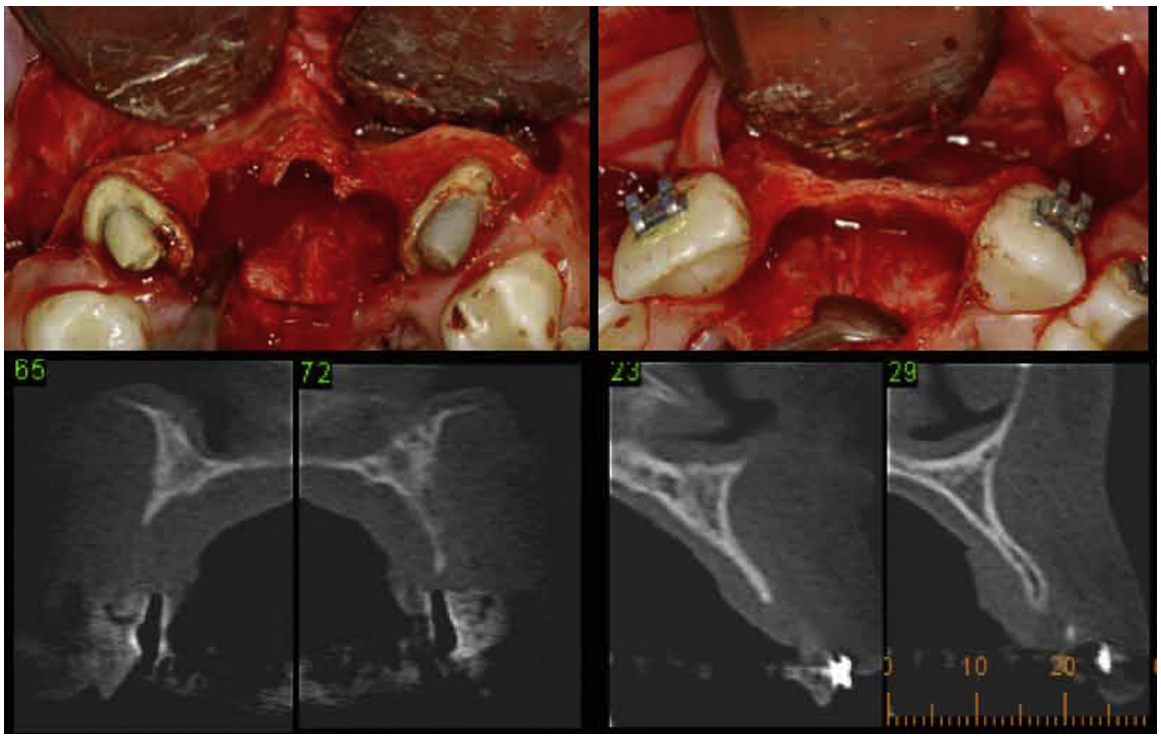

Fig. 1. Clinical and CBCT preoperative views of a representative atrophic maxilla that underwent implantation as part of this study.

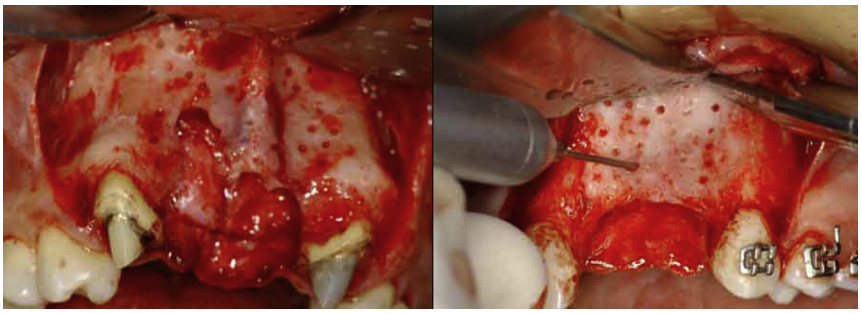

Fig. 2. The cortex of the maxilla was perforated with a round bur to gain access to the marrow.

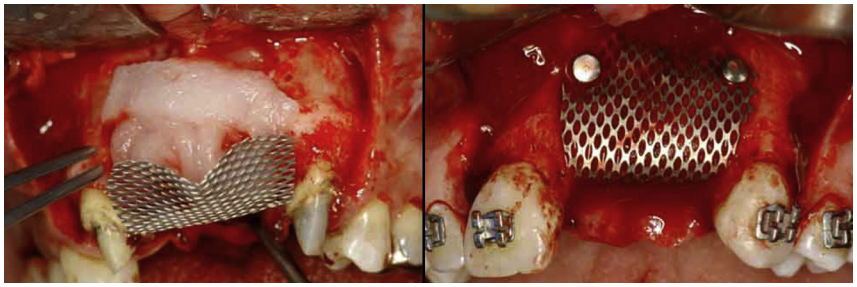

Fig. 3. ACS containing rhBMP-2 that was placed in the concave area of the titanium mesh, which was secured with titanium screws for subsequent implantation.

wound closure, and suturing. The flaps were closed with 4–0 Vicryl (Ethicon) interrupted and horizontal mattress sutures.

All patients were prescribed oral antibiotics, including 500 mg of amoxicillin (or 1 g clindamycin in the case of allergy), and 100 mg of the non-steroidal anti-inflammatory agent nimesulide for seven consecutive days after the reconstruction procedure. Postoperative instructions included the maintenance of a liquid/soft diet and use of 0.12% chlorhexidine mouthwash until the sutures were removed between 10 and 15 days after the reconstruction.

Implant placement

Seven months after the reconstruction, implants were placed (without the need for additional augmentation procedures) and the insertion torque was evaluated. Briefly, after the administration of local anaesthesia, a full mucoperiosteal flap was raised to expose the titanium mesh, which was cut with a diamond disc. The buccal portion was maintained to protect the newly formed bone (Fig. 5). A bone biopsy specimen (2.2 mm in diameter) was harvested from the previously augmented area using a bone trephine drill. This site was also the location of the implant osteotomy. Osteotomies were prepared according to the manufacturer's drilling sequence and dental implant placement guidelines (Nobel Biocare Replace) (Fig. 6).

The bone density was determined on the basis of the Lekholm and Zarb index[6] (D1 to D4), and the bone density at each site was recorded clinically for the surgeon. The Lekholm and Zarb index consists of a density scale that ranges from 1 (densest bone) to 4 (least dense bone). Soft tissue augmentation procedures were performed

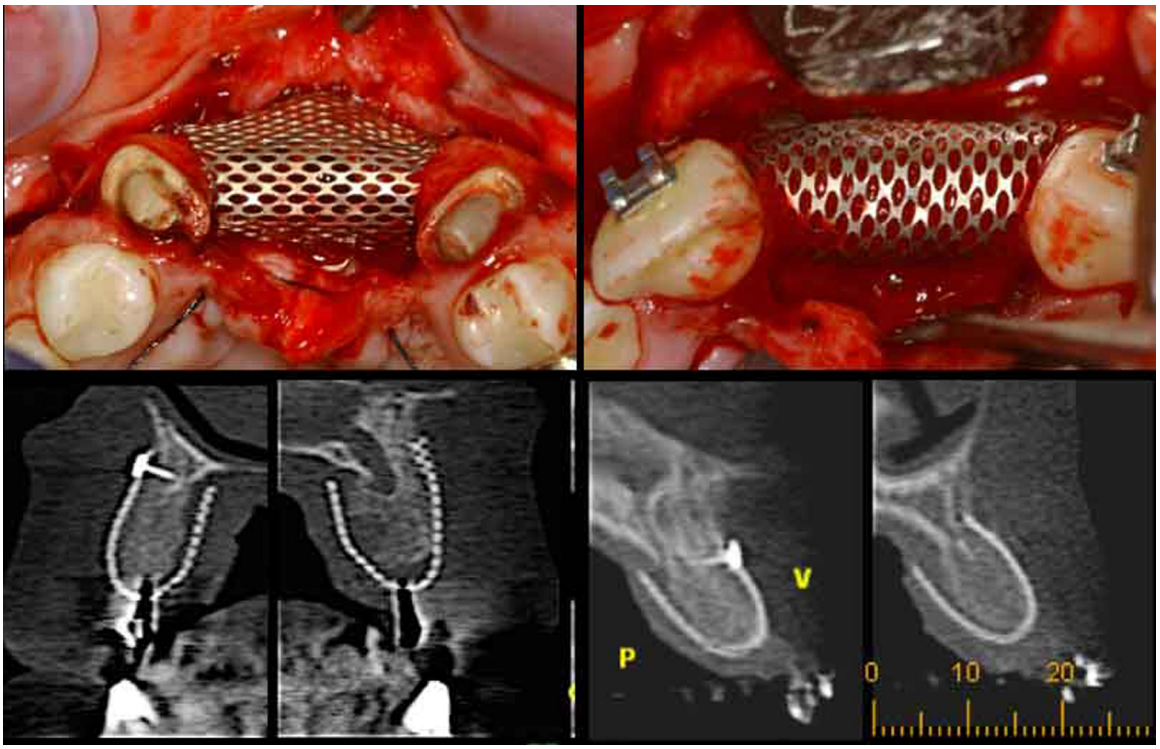

Fig. 4. Clinical and CBCT views of the rhBMP-2/ACS protected by the titanium mesh.

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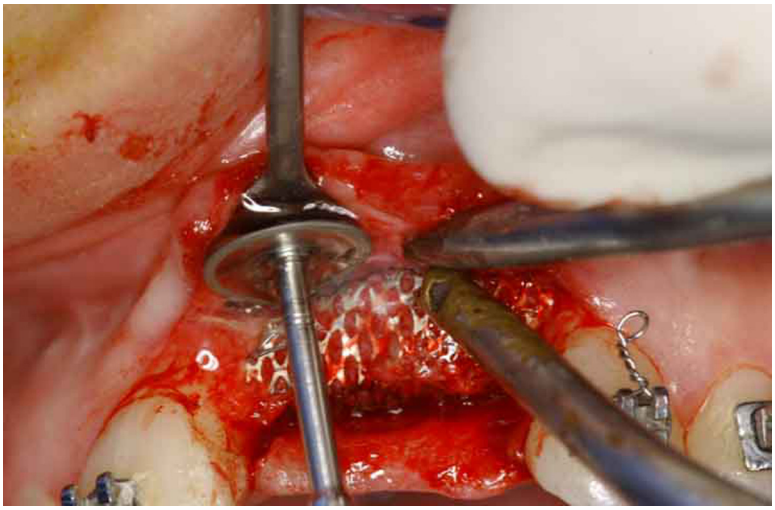

Fig. 5. The mesh was cut with a diamond disc, and the buccal portion was maintained to protect the newly formed bone.

at each surgery stage. Cone beam computed tomography (CBCT) scans, study plaster models, and clinical photographs were used for the planning of rehabilitation of all selected patients. After a 6-month healing period, the implants were uncovered for placement of an unsplit cemented prosthetic restoration on a custom zirconia abutment. A single experienced prosthodontist performed all of the prosthetic procedures.

All patients were evaluated every 6 months during a 48-month follow-up period. Every follow-up visit included a clinical examination of implant mobility, occlusion, and hygiene conditions. Periapical radiographs were obtained at every visit. CBCT and clinical photographs were also obtained at the last follow-up visit.

Aesthetic evaluation

One previously calibrated researcher (BSSM), who was not involved in the treatment steps, performed all evaluations of the soft tissue and implant crown aesthetics. The state of soft tissue aesthetics was evaluated with the Jemt index. Two clinical standardized oral photographs were obtained 24 h after definitive crown delivery and at the last follow-up visit (48 months). These photographs included at least one adjacent tooth on each side, and were obtained under the same lighting conditions with similar framing.

CBCT evaluation

All CBCT scans were acquired with the same system (i-CAT 3D Imaging System, i-CAT Vision Software; Imaging Sciences International) through the soft tissue CBCT technique.[7] The maxilla was scanned preoperatively and at 48 months postoperatively. Sagittal sections (1.0 mm in thickness) were obtained as CBCT reconstructions.

A previously trained examiner (BSSM) assessed all CBCT images independently. For the measurements, the most central sagittal sections of the titanium mesh (preoperative CBCT) and of each implant (postoperative CBCT) were selected. The examiner measured the distance from the lingual border to the buccal bone ridge border using the Image Tool software package. Measurements were performed at a minimum of 2-week intervals. Means and standard deviations (SDs) were calculated for each reference measurement. The examiner recorded the second set of

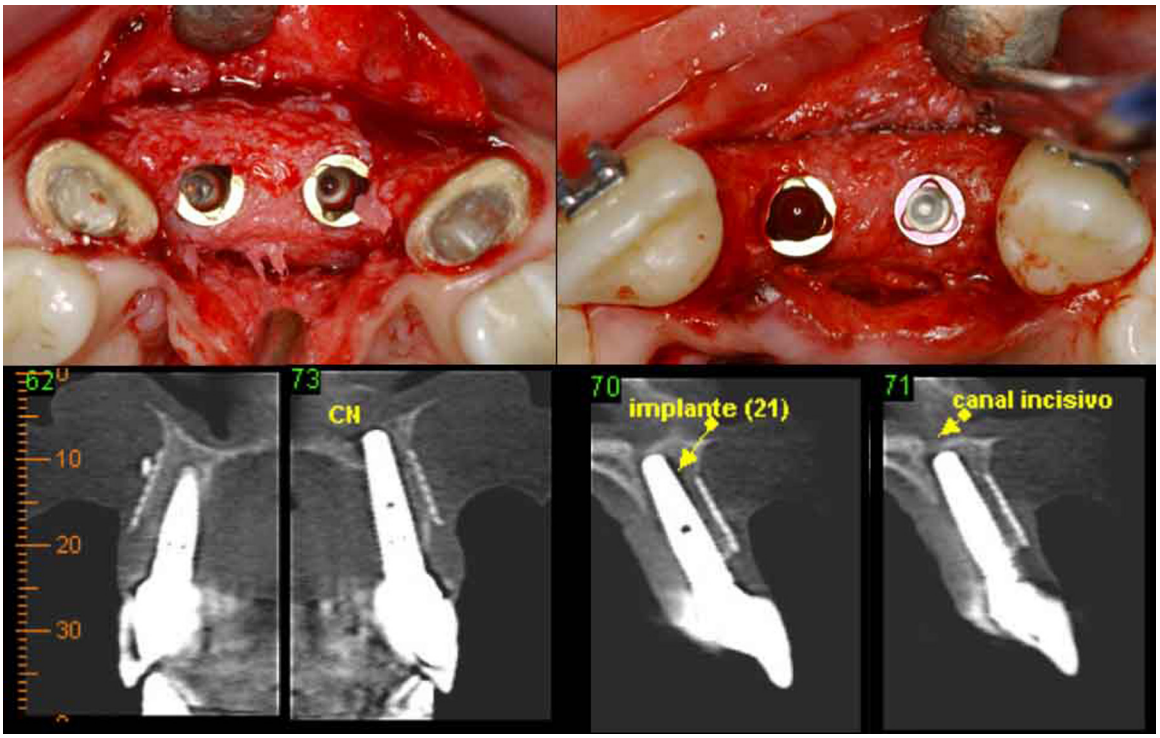

Fig. 6. Clinical and CBCT views of implant placement, showing the presence of the titanium mesh on the buccal side.

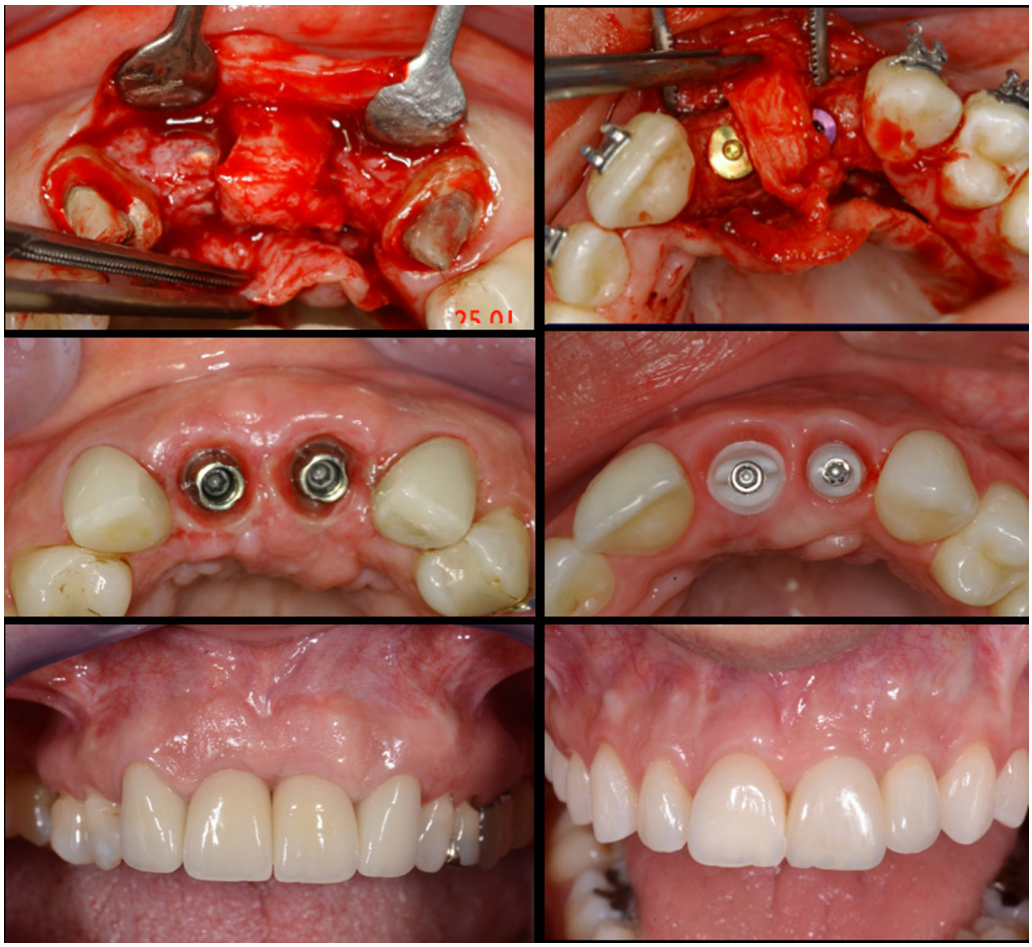

Fig. 7. Soft tissue augmentation procedures were performed, and the final clinical view was obtained at 48 months after implantation.

measurements while blinded to the first set, in order to evaluate the reliability of the recordings.

Histological evaluation

Biopsy specimens were fixed in 10% neutral buffered formalin, dehydrated in ethanol, embedded in paraffin, and sectioned (4 μm). These sections were stained with haematoxylin and eosin. A descriptive histological analysis was performed with a standard light microscope by an experienced examiner. This analysis included observations of new bone formation and resorption, woven and lamellar bone, cortex formation, seroma formation, fibrovascular tissue and marrow, and inflammatory responses. The cell and tissue morphologies were also identified.

Results

Five patients were treated with rhBMP-2 and titanium mesh. All patients achieved successful regeneration of their alveolar defects. All regeneration sites exhibited swelling and mild erythema of the healing soft tissue. However, the recovery period for each patient was largely uneventful. All patients progressed to implant placement and final prosthetic reconstruction. No exposure of the mesh was observed during the healing process, and none of the implant sites required further bone augmentation (Fig. 7). Table 1 shows the bone gain, bone quality, insertion torque, and implant size for all patients. After 6 months of healing, all of the implants achieved primary stability, although the bone quality of the regenerated tissue was rated as soft (D4) for all of the sites.

Aesthetics, evaluated with the Jemt score, showed an improvement in all cases. All patients, except patient 3, had a score of 1 at the first evaluation and 2 at the follow-up. For patient 3, the score remained at 1 at the follow-up evaluation. Bone biopsies revealed large amounts of new bone and bone marrow/connective tissue. Moderate to large numbers of osteoblasts and capillaries were observed in the bone marrow of the newly induced bone. Leukocytes were observed in the connective tissue and interspersed among the trabecular bone (Fig. 8).

Discussion

The goal of modern dentistry is to achieve the most inconspicuous reconstruction or replacement of missing teeth and periimplant hard and soft tissue components. However, endodontic failure, advanced periodontal disease, trauma, root fracture, and other conditions requiring tooth extraction are frequently associated with severe alveolar bone resorption and, sometimes, soft tissue loss.[8–11] This study sought to evaluate the long-term reliability of using rhBMP-2 and titanium mesh for regenerating the alveolar bone, and to determine the success rate of implants in the resulting anterior maxilla. All 10 implants in all five patients were successfully integrated in the tissue at the 4-year follow-up visit, as documented by clinical, CBCT, and histological results.

Postsurgical swelling and a bluish complexion of the alveolar mucosa were observed locally for all osseointegrated

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Table 1. Bone and implant characteristics for five patients undergoing bone augmentation of the atrophic anterior maxilla using rhBMP-2 and titanium mesh.

Patient no.	Gender	Tooth site	Insertion torque (N cm)	Bone quality	Implant size (mm^2)	Preop. ridge thickness (mm)	Postop. ridge thickness (mm)
1	M	21	30	D4	4.3 × 13	2.34	6.52
		22	20	D4	3.5 × 13	1.96	5.34
2	F	22	25	D4	3.5 × 11.5	1.85	4.89
		23	20	D4	4.3 × 13	2.14	5.67
3	F	11	30	D4	4.3 × 13	2.23	6.01
		21	20	D3	4.3 × 13	3.10	6.13
4	F	11	15	D4	3.5 × 13	2.13	5.69
		21	15	D4	3.5 × 13	2.22	6.04
5	F	21	20	D4	4.3 × 13	1.42	6.55
		22	15	D4	3.5 × 13	1.22	6.02

rhBMP-2, recombinant human bone morphogenetic protein 2; M, male; F, female; Preop., preoperative; Postop., postoperative.

implants. These results are consistent with those of several previous studies that have included the use of rhBMP-2/ACS.[4,5,12] CBCT scans demonstrated that alveolar bone regeneration was achieved during the period evaluated.[13,14]

The successful maintenance of bone gain may have been due to the use of the titanium mesh, with only partial removal of this mesh at the time of implant placement, leaving it *in situ* on the buccal side. Titanium mesh acts as a protective matrix to maintain the space and facilitate bone ingrowth. The use of titanium mesh has been shown to provide both space for rhBMP-2/ACS-induced bone formation and geometric direction for the newly formed bone.[5] Used in other membranes, titanium mesh has been shown not to occlude cells that may contribute to the bone-forming process or the vascularity derived from the soft tissue flap.[15] In the present study, the thickness of the titanium mesh (0.2 mm) was adequate to resist flexing and micromovements during the healing process, while being thin enough to mould easily.

Primary soft tissue closure over a grafted socket is thought to be necessary for proper incorporation of a graft. Tension-free closure of the soft tissue flaps over the grafted site is used to prevent wound dehiscence and early exposure of the mesh.[16] In the present study, none of the patients exhibited mesh exposure.

Histological samples collected 6 months after the first surgery was completed revealed remodelling of the immature woven bone into lamellar bone. The use of rhBMP-2 as a differentiation agent and the titanium mesh may have provided a growth factor/carrier combination that is conducive to progenitor cell ingrowth. BMPs differentiate into mesenchymal progenitor cells, chondroblasts, and osteoblast lineage cells. These proteins promote the expression of markers that are characteristic of chondroblast and osteoblast phenotypes. Moreover, BMPs have been shown to enhance the synthesis of extracellular matrix.[17,18] However, in preclinical studies, rhBMP-2 initially induced woven trabecular bone formation, and then remodelled this bone into lamellar bone, consistent with the anatomical location involved.[4,19] This finding is consistent with the bone quality noted in the present case series.

All five patients had developed D4 quality bone by the start of implant placement

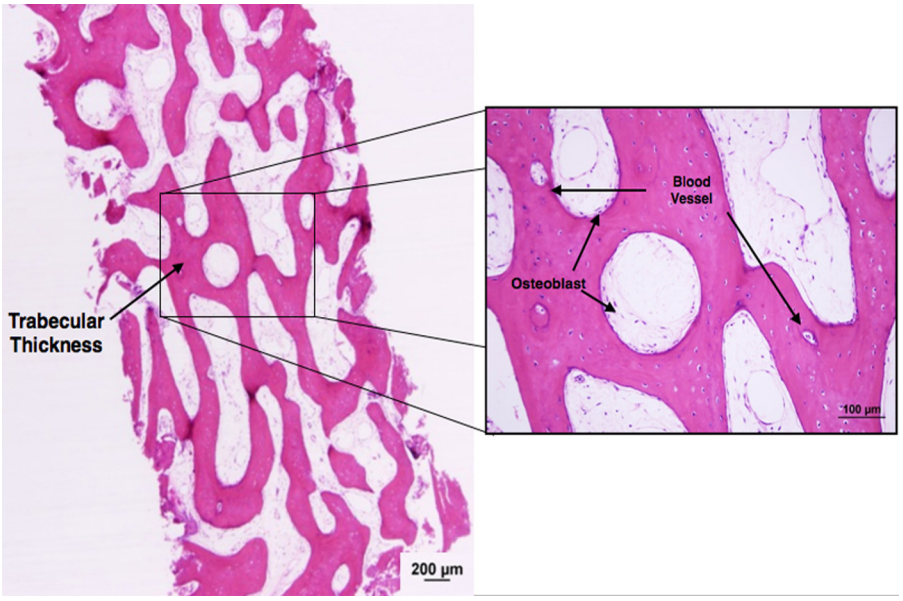

Fig. 8. Histological analysis showed a large amount of newly formed bone and bone marrow/connective tissue.

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surgery. Minimal resistance to drilling was observed. In a sinus bone graft study conducted by Boyne et al.,[20] rhBMP-2 grafts exhibited significantly less radiographic bone density compared to the autograft sites after 4 months of healing.[20] Similarly, Misch reported that *de novo* bone induction by rhBMP-2 requires a longer period of time for mineralization to occur.[16] Therefore, the use of an undersized osteotomy may help in attaining implant stability at softer bone sites.

In observational studies such as case series, the study investigators do not usually control which intervention(s) the research participants receive. This is the primary limitation of the present case series study. The lack of a control group is another limitation. However, the design of this study permitted the identification of hypotheses that will be useful in designing future studies, including randomized controlled trials.[21] Another limitation of this preliminary clinical case series is the small sample size. To confirm these results, a clinical trial is needed that includes a long observation period and a larger sample size.

Funding

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Competing interests

No conflict of interest.

Ethical approval

This research was approved by the Ethics Committee of the São Leopoldo Mandic Institute and Research Centre (reference number 134/2007).

Patient consent

Not applicable.

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References

1. Urist MR. Bone: formation by autoinduction. *Clin Orthop Relat Res* 1965;**2002**(395):4–10.
2. Wikesjo UM, Polimeni G, Qahash M. Tissue engineering with recombinant human bone morphogenetic protein-2 for alveolar augmentation and oral implant osseointegration: experimental observations and clinical perspectives. *Clin Implant Dent Relat Res* 2005;**7**:112–9.
3. Spagnoli DB, Marx RE. Dental implants and the use of rhBMP-2. *Oral Maxillofac Surg Clin North Am* 2011;(23):347–61. vii.
4. de Freitas RM, Susin C, Spin-Neto R, Marcantonio C, Wikesjo UM, Pereira LA, et al. Horizontal ridge augmentation of the atrophic anterior maxilla using rhBMP-2/ACS or autogenous bone grafts: a proof-of-concept randomized clinical trial. *J Clin Periodontol* 2013;**40**:968–75.
5. Misch CM. The use of recombinant human bone morphogenetic protein-2 for the repair of extraction socket defects: a technical modification and case series report. *Int J Oral Maxillofac Implants* 2010;**25**:1246–52.
6. Lekholm U, Zarb G. Patient selection and preparation. In: Brånemark PI, Zarb GA, Albrektsson T, editors. *Tissue integrated prostheses: osseointegration in clinical dentistry*. Chicago: Quintessence; 1985. p. 199–209.
7. Januario AL, Barriviera M, Duarte WR. Soft tissue cone-beam computed tomography: a novel method for the measurement of gingival tissue and the dimensions of the dentogingival unit. *J Esthet Restor Dent* 2008;**20**:366–73. discussion 374.
8. Rosa JC, Rosa AC, Francischone CE, Sotto-Maior BS. Esthetic outcomes and tissue stability of implant placement in compromised sockets following immediate dentoalveolar restoration: results of a prospective case series at 58 months follow-up. *Int J Periodontics Restorative Dent* 2014;**34**:199–208.
9. Araujo MG, da Silva JC, de Mendonca AF, Lindhe J. Ridge alterations following grafting of fresh extraction sockets in man. A randomized clinical trial. *Clin Oral Implants Res* 2015;**26**:407–12.
10. Araujo MG, Wennstrom JL, Lindhe J. Modeling of the buccal and lingual bone walls of fresh extraction sites following implant installation. *Clin Oral Implants Res* 2006;**17**:606–14.
11. Covani U, Cornelini R, Calvo JL, Tonelli P, Barone A. Bone remodeling around implants placed in fresh extraction sockets. *Int J Periodontics Restorative Dent* 2010;**30**:601–7.
12. Butura CC, Galindo DF. Implant placement in alveolar composite defects regenerated with rhBMP-2, anorganic bovine bone, and titanium mesh: a report of eight reconstructed sites. *Int J Oral Maxillofac Implants* 2014;**29**:e139–46.
13. Anitua E, Alkhraisat MH, Miguel-Sanchez A, Orive G. Surgical correction of horizontal bone defect using the lateral maxillary wall: outcomes of a retrospective study. *J Oral Maxillofac Surg* 2014;**72**:683–93.
14. Mounir M, Beheiri G, El-Beialy W. Assessment of marginal bone loss using full thickness versus partial thickness flaps for alveolar ridge splitting and immediate implant placement in the anterior maxilla. *Int J Oral Maxillofac Surg* 2014;**43**:1373–80.
15. Wehrhan F, Amann K, Molenberg A, Lutz R, Neukam FW, Schlegel KA. Critical size defect regeneration using PEG-mediated BMP-2 gene delivery and the use of cell occlusive barrier membranes—the osteopromotive principle revisited. *Clin Oral Implants Res* 2013;**24**:910–20.
16. Misch CM. Bone augmentation of the atrophic posterior mandible for dental implants using rhBMP-2 and titanium mesh: clinical technique and early results. *Int J Periodontics Restorative Dent* 2011;**31**:581–9.
17. Salata LA, Franke-Stenport V, Rasmusson L. Recent outcomes and perspectives of the application of bone morphogenetic proteins in implant dentistry. *Clin Implant Dent Relat Res* 2002;**4**:27–32.
18. Casap N, Laviv A, Debecco M, Alterman M, Laster Z, Jensen OT. Imperforate titanium shell enclosing recombinant human bone morphogenetic protein-2-induced bone formation for high-profile dental implants in rabbit tibia. *J Oral Maxillofac Surg* 2015;**73**:245–52.
19. Kao DW, Kubota A, Nevins M, Fiorellini JP. The negative effect of combining rhBMP-2 and Bio-Oss on bone formation for maxillary sinus augmentation. *Int J Periodontics Restorative Dent* 2012;**32**:61–7.
20. Boyne PJ, Lilly LC, Marx RE, Moy PK, Nevins M, Spagnoli DB, et al. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 2005;**63**:1693–707.
21. Kooistra B, Dijkman B, Einhorn TA, Bhandari M. How to design a good case series. *J Bone Joint Surg Am* 2009;**91**(Suppl. 3):21–6.

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