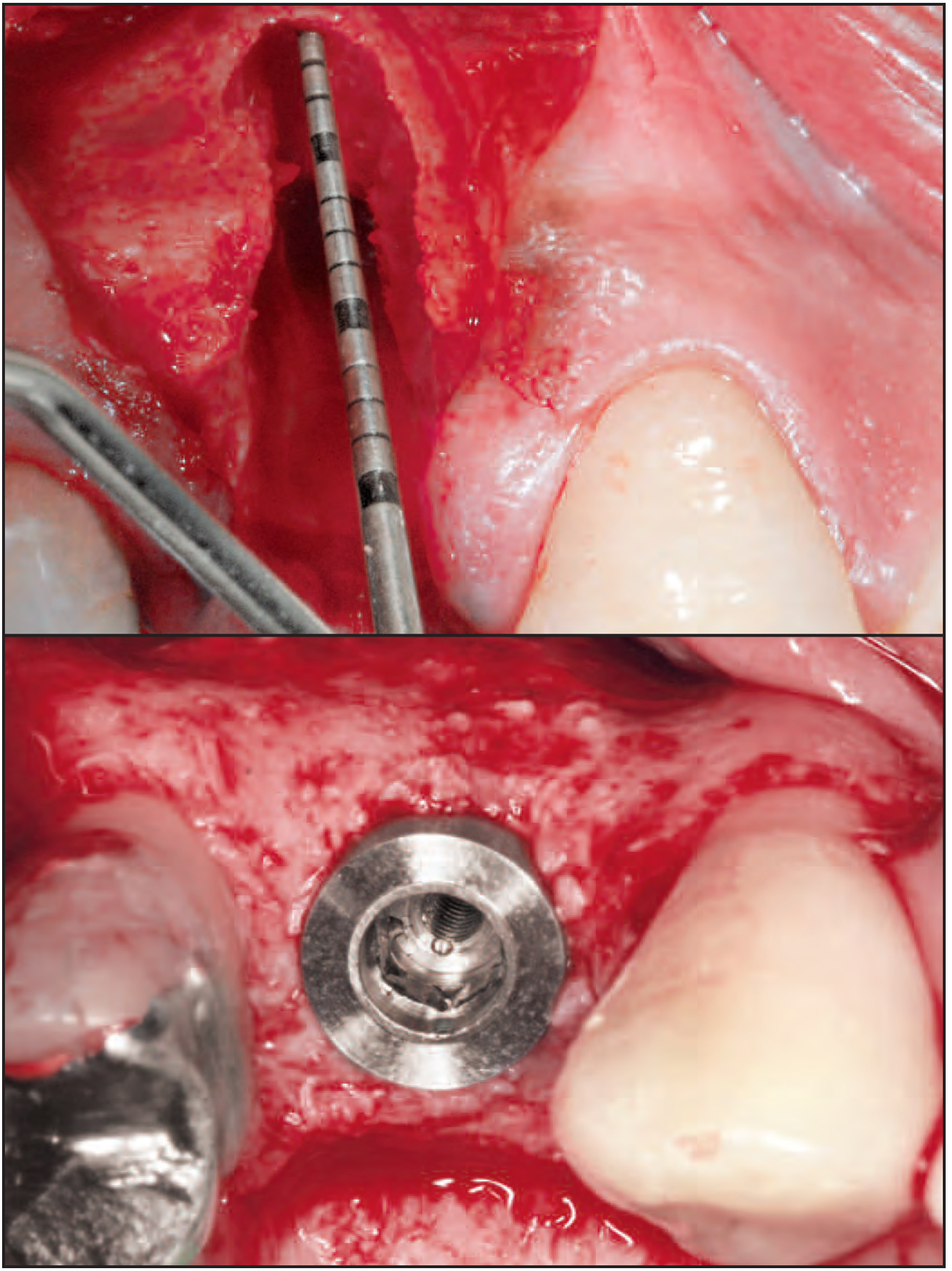

# Human Histologic Evaluation of Mineralized Collagen Bone Substitute and Recombinant Platelet-Derived Growth Factor-BB to Create Bone for Implant Placement in Extraction Socket Defects at 4 and 6 Months: A Case Series

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*The objective of this pilot study was to assess whether mineralized collagen bone substitute (MCBS) combined with recombinant human platelet-derived growth factor-BB (0.3 mg/mL) would generate adequate viable bone in buccal wall extraction defects to accommodate implant placement. The primary outcome variable was bone quality, as measured by microcomputed tomography and histologic evaluation. This was successfully accomplished in all eight specimens obtained from seven patients. The secondary outcome variables were bone quality and quantity as observed clinically, radiographically, and by the primary stability of implants at the time of placement. Soft tissue healing was excellent, and there were no unanticipated adverse events. Robust bone formation accompanied by MCBS resorption was evident in all 4- and 6-month specimens. This was accomplished without barrier membranes.* (Int J Periodontics Restorative Dent 2009;29:129–139.)

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The resorption of the alveolar ridge following tooth extraction is a physiologically undesirable and probably avoidable phenomenon.[1] The preservation of the morphology of the extraction socket becomes critical when one desires to place an implant for prosthetic restoration.[2] The healing of extraction wounds in human and animal trials has been studied extensively by evaluating the cascade of events histologically, radiographically, and clinically.[3–7] The findings indicate that alveolar ridge resorption is significantly greater on the buccal than the lingual aspect and results in a diminished buccal bone height.[8–10] The range of loss of bone contour averages 3 to 5 mm at 6 months and 50% at 1 year.[11-15] Ridge resorption is more likely in postextraction sites for teeth with prominent roots, which may be dehisced or fenestrated from the time of eruption.[2]

Contemporary therapeutics for extraction wounds employ a variety of osteoconductive materials with and without barrier membranes.[16–19] Reports suggest a range of clinical successes allowing implant placement, but histologic evaluation routinely demonstrates significant retention of

the graft material.[2,20–26] The use of bone allograft, mineralized or demineralized, with a barrier membrane is well documented, but the use of barrier membrane is technically difficult. Complications such as wound dehiscences or membrane exposures can compromise the outcome.[27–30] This provides the challenge of achieving and maintaining primary closure, covering the membrane to prevent exposure and infection. It is important to recognize that some animal research provides an opportunity to see different healing patterns compared to the human model in clinical results.[31]

Recent advances in recombinant gene technology have made cytokines (ie, growth factors) commercially available. The results of proof-of-principle and randomized controlled trials have demonstrated the efficacy of recombinant human platelet-derived growth factor-BB (rhPDGF-BB, Gem21S, Osteohealth) for periodontal regeneration.[32] The clinical community has tested it extensively in combination with bone replacement scaffolds to reconstruct lost periodontium as well as damaged localized ridges.[33–40]

Anorganic bovine bone graft (Bio-Oss, Osteohealth) has been used successfully to achieve periodontal regeneration to treat localized edentulous areas as well as to reconstruct bone in the maxillary sinus.[41–45] The histologic results demonstrate direct contact between graft materials and newly formed mineralized bone.[42–51]

Mineralized collagen bone substitute (MCBS; Bio-Oss Collagen, Osteohealth), a xenogeneic bone graft material composed of 90% bovine bone mineral and 10% porcine collagen, has been used as a bone replacement graft to induce periodontal regeneration.[52–56] The efficacy of this procedure has been demonstrated histologically in a human model.[52–54] The placement of MCBS in fresh extraction wounds in a canine model preserved the dimensions of the hard tissue socket walls and maintained the profile of the ridge.[31]

It would be beneficial to have a ridge preservation technique that predictably provides regeneration of the damaged alveolar process by stimulating new bone formation without the use of barrier membranes. MCBS has also been shown to bind rhPDGF-BB and to be a suitable carrier vehicle with potential for clinical applications.[57,58]

The present study evaluated the efficacy of a combination of rhPDGF-BB and MCBS to provide ridge preservation when delivered to extraction socket buccal wall dehiscence defects. The hypothesis was that extraction socket defects treated with rhPDGF-BB and MCBS would heal with adequate bone for implant placement.

## Method and materials

This investigation was designed and implemented as a single-center prospective open-label clinical study. Subjects were randomized through blinded selection to have surgical reentry at either 4 or 6 months. Subjects provided informed consent according to the Declaration of Helsinki.

Subjects were selected for enrollment from the population of patients presenting between January 15 and February 15, 2007. To be eligible, prospective subjects had to be between the ages of 18 and 70 years and be willing and able to follow the study procedures. Subjects provided signed informed consent and had to have at least one tooth diagnosed and prescribed for extraction with > 4 mm of bone from the apex of the tooth to the alveolar crest and > 2 mm of bone from the apex of the tooth to the floor of the maxillary sinus. In addition, subjects were excluded from entry into the study if (*1*) they had acute sinusitis or congenital or metabolic bone disorders or (*2*) were current smokers (within 6 months of entry into the study). Also, pregnant women were not eligible for the study.

At the screening visit, eligibility was assessed, patient histories were taken, and extraoral and intraoral exams were performed. Periapical radiographs and photographs were taken at the time of the surgical procedure. Dental prophylaxis was performed prior to the surgery.

At the baseline (day 0) visit, periapical radiographs and photographs were taken. Local anesthesia was administered (2% lidocaine with 1:100,000 epinephrine), and full-thickness mucoperiosteal flaps were elevated. Intracrevicular incisions, with vertical incisions as necessary, were used for access to and closure of the treatment site. Study teeth were extracted, and the sockets were debrided of granulation tissues. Three measurements were made of each extraction socket defect (Fig 1a): defect depth (vertically from the apex of the defect to the most coronal bone crest); buccal plate loss (most coronal bone crest vertical to the coronal

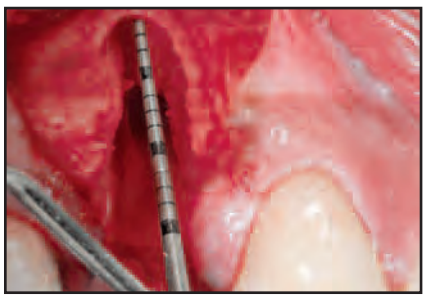


**Fig 1a** *Maxillary right first premolar extraction socket with a buccal wall defect measuring vertically 14 mm and 10 mm wide and buccal bone plate loss of 10 mm.*

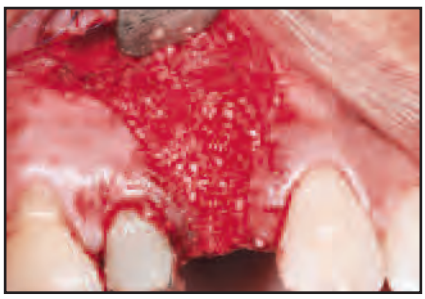


**Fig 1b** *The defect was treated with a combination of MCBS and rhPDGF-BB.*

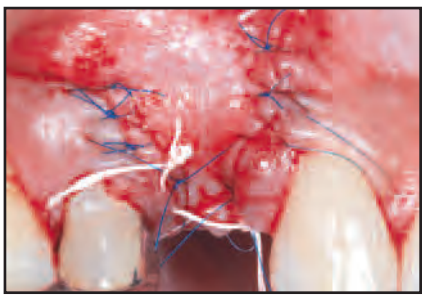


**Fig 1c** *A buccal flap was advanced for primary closure over the treated defect.*

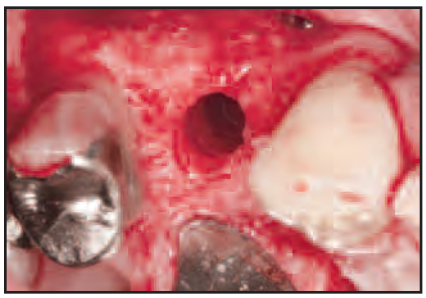
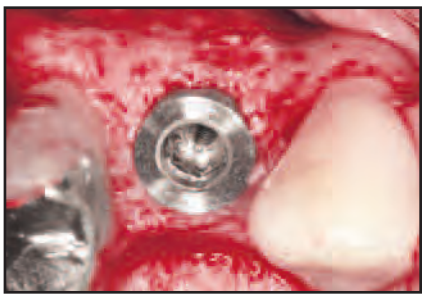


**Fig 1d** (left) *Clinical view after obtaining a trephine core biopsy sample.*

**Fig 1e** (right) *A dental implant is placed in the healed defect.*



extent of buccal plate and horizontal at crest); and socket width (at the widest point). Intramarrow bone penetration was performed as needed to stimulate bleeding into the defect to allow access for progenitor cells and angiogenesis.

The extraction socket defect was filled with MCBS (Bio-Oss Collagen) hydrated with rhPDGF-BB (Gem 21S), which was placed incrementally into the defect (ratio: 250 mg MCBS/0.5 mL rhPDGF-BB) and condensed to achieve stability of the particulate material (Fig 1b). Primary wound approximation was achieved with releasing incisions, as necessary, and a combination of vertical mattress and interrupted sutures (Fig 1c).

Subjects were instructed to use a mouth rinse (0.12% chlorhexidine) twice daily and to avoid brushing of the treated site for 2 weeks. In addition, oral antibiotics (amoxicillin 500 mg or clindamycin 300 mg) were prescribed for 7 days (three times daily), and ibuprofen (800 mg three times a day for 3 days) was taken for anti-inflammatory and pain relief. Patients were also provided instructions on when to return for follow-up visits, as indicated in the protocol schedule (Table 1).

At follow-up visits 3, 4, and 5 (days 7, 14, and 28), photographs were taken of the treatment sites. The treatment sites were inspected and cleaned with saline-soaked gauze, and soft tissue healing was assessed according to a previously reported wound healing index.[59] Descriptions of the three possible scores are as follows: 1 = uneventful wound healing with no gingival edema, erythema, suppuration, patient discomfort, or flap dehiscence; 2 = uneventful wound healing with slight gingival edema, erythema, or patient discomfort, but slight flap dehiscence and no suppuration; 3 = poor wound healing with significant gingival edema, erythema, patient discomfort, significant flap dehiscence, or any suppuration. Oral hygiene instruction was reviewed at each visit, and the 3-month visit included the same evaluations, as well as periapical radiographs and dental prophylaxis.

**Table 1 Study protocol**

| | Visit no. | | | | | | |
|---|---|---|---|---|---|---|---|
| **Event** | **1 (screening)** | **2 (day 0)** | **3 (day 7)** | **4 (day 14)** | **5 (day 28)** | **6 (mo 3)** | **7 (mo 4 or 6)** |
| Medical history (eligibility criteria) | X | | | | | | |
| Extraction and treatment* | | X | | | | | |
| Periapical radiographs | X | X | | | | X | X |
| Photographs | X | X | X | X | X | X | X |
| Defect measurements | | X | | | | | X |
| Postoperative instructions and medications | | X | | | | | |
| Oral exam–WHI** | | X | X | X | X | X | X |
| Dental prophylaxis | X | | | | | X | X† |
| Re-entry and biopsy | | | | | | | X |
| Implant placement | | | | | | | X |

*Treatment = MCBS and rhPDGF-BB.
**Wound healing was assessed by a wound healing index (WHI).
†Prophylaxis was performed at 3 and 6 months for all subjects in the study.

**Table 2 Subject demographics and surgical measurements (baseline)***

| | | | | | | Defect dimensions (mm) | | |
|---|---|---|---|---|---|---|---|---|
| **Subject** | **Age** | **Gender** | **Treatment site** | **MCBS (mg)** | **rhPDGF (mL)** | **Vertical** | **Width** | **Buccal loss** |
| 1 | 59 | M | 13 | 250 | 0.5 | 10 | 11 | 5 |
| 2 | 49 | F | 6 | 375 | 0.75 | 20 | 6 | 16 |
| 3 | 31 | F | 5 | 500 | 1.0 | 14 | 9 | 12 |
| 4 | 49 | F | 5 | 250 | 0.5 | 14 | 10 | 10 |
| 5 | 36 | F | 7 | 375 | 0.75 | 12 | 6 | 9 |
| 6 | 35 | F | 8 | 500 | 1.0 | 16 | 6 | 14 |
| 7 | 36 | F | 9 | 375 | 0.75 | 15 | 6 | 11 |
| 8 | 42 | M | 10 | 375 | 0.75 | 12 | 8 | 6 |

*Subjects 1 to 4: re-entry after 4 months of healing; subjects 5 to 8: re-entry after 6 months.

Surgical re-entry of the treatment site was performed at either 4 or 6 months (per randomization) under local anesthesia with the elevation of full-thickness mucoperiosteal flaps. Evidence of any graft particulate was noted along with observations of the exposed bone. Photographs of the treatment sites were taken prior to and after re-entry. Trephine drills were used to obtain core biopsies (minimum 2 mm wide by 7 mm deep) from the center of the treated area (Fig 1d). The biopsy cores were preserved intact with the trephines in formalin. These nondecalcified bone cores were scanned, and the data were quantified using three-dimensional microcomputed tomography (micro-CT) (mCT 40, Scanco Medical).

Dental implants (SIHS Dental Implants) were then placed into the regenerated sites if primary stability could be achieved (Fig 1e). Photographs were taken at the time of implant placement and periapical radiographs were taken after implant placement. All subjects in the study received dental prophylaxis at 3 and 6 months.

The primary outcome variable was bone quality according to histology and micro-CT. Secondary outcome variables were bone quality (assessed by clinical photographs at re-entry, periapical radiographs, and primary stability of implants at placement), soft tissue healing according to the wound healing index, and incidence of any unanticipated adverse healing events.

## Results

### *Clinical findings*

Baseline information for all eight subjects (with a total of eight treatment sites) is presented in Table 2. Healing was uneventful in all subjects, with no unanticipated adverse events. All eight treatment sites achieved adequate bone for the placement of standard size implants and trephine core biopsy at the time of re-entry. Clinical observation demonstrated a firm, bonelike substance at all sites, whether entered after 4 or 6 months, with minimal graft particulate observed after flap reflection.

### *Micro-CT findings*

Core specimens at 4 and 6 months revealed robust new bone, with some MCBS particulate persisting (Fig 2). The MCBS and new bone were visualized directly in intimate contact in the three-dimensional cross sections. The bone trabeculae were continuous and abundant throughout the biopsies (Fig 3).

**Fig 2** *Micro-CT scan of a trephine core biopsy showing bone in red and MCBS in white.*

**Fig 3** *Longitudinal section showing bone only.*

### *Histologic findings*

Core specimens at 4 and 6 months revealed robust new bone formation throughout the extraction sockets, with intimate contact between the new bone and remnants of the MCBS (Figs 4 to 7). Osteocytes were present with extensive osteoid lines with osteoblasts. The remaining MCBS particles demonstrated demineralization lines on the surface in intimate contact with multinucleated giant cells (Fig 8). Much of the MCBS surface was surrounded by new bone, with cutting cones and Haversian systems evident. Eosinophils were noted in isolated areas of the marrow spaces.

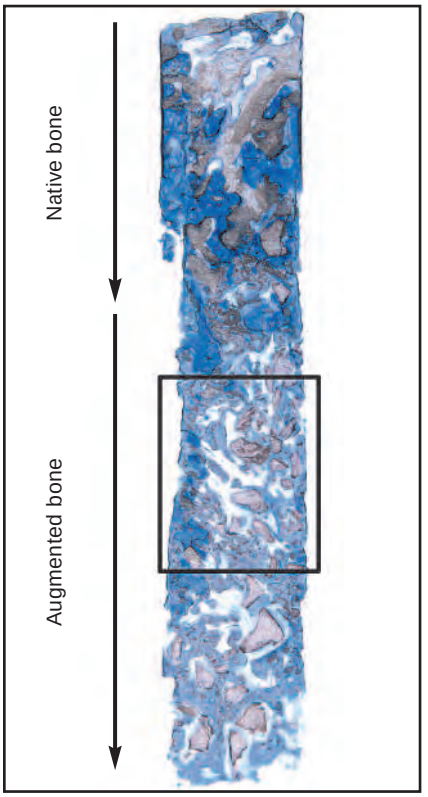



**Fig 4** (left) *Trephine biopsy demonstrates augmented and native bone.*

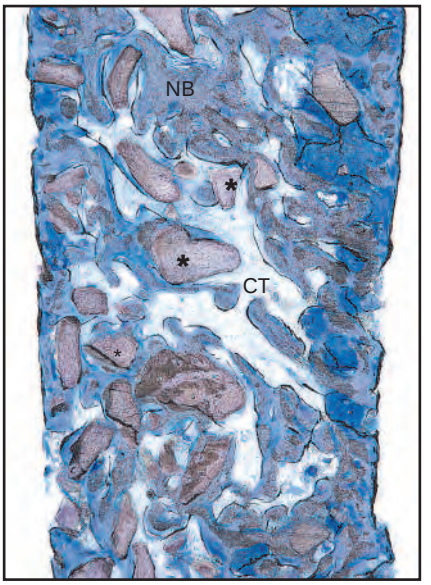



**Fig 5** *Higher magnification of area in Fig 4. Note the MCBS particles* (asterisks)*, new bone (NB), and connective tissue (CT).*

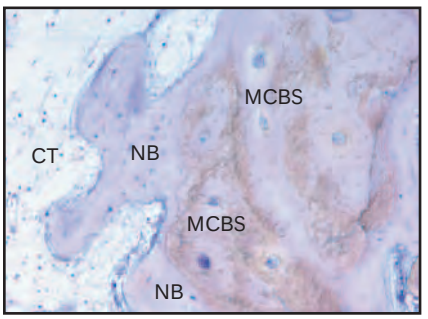



**Fig 6** *MCBS particle (Bio-Oss) with adjacent newly formed bone. NB = new bone; CT = connective tissue.*

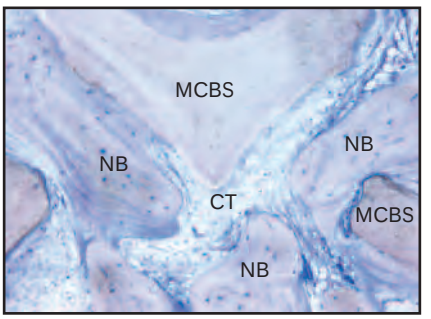



**Fig 7** *Histologic section demonstrates robust bone formation and remodeling. The MCBS particles are surrounded by new bone (NB). CT = connective tissue.*

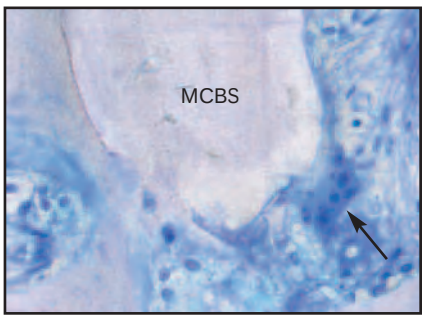



**Fig 8** *Demineralization of a MCBS particle by a multinucleated giant cell* (arrow)*.*

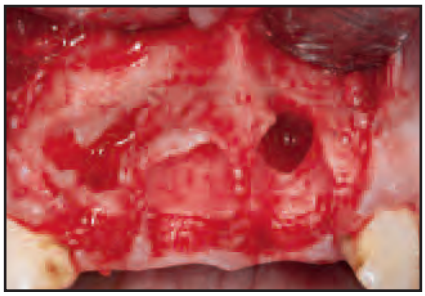


**Fig 9a** *The extraction sockets have been degranulated and debrided, revealing buccal wall defects.*

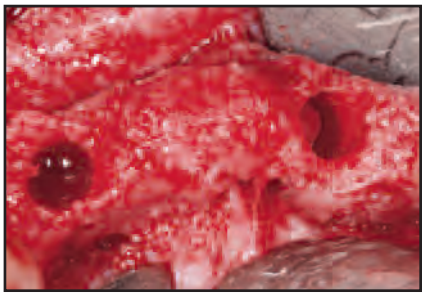


**Fig 9b** *Adequate bone volume existed for a trephine core biopsy sample to be obtained.*

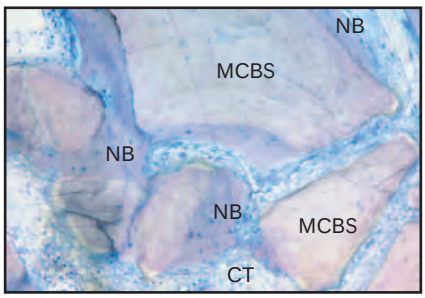



**Fig 9c** *Higher magnification reveals new bone formation (NB) around MCBS particles and remodeling of MCBS particles with resorption and replacement with new bone.*

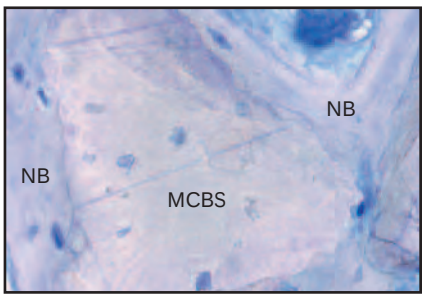



**Fig 9d** *Additional section reveals intimate contact between MCBS particle and new bone (NB).*

### *Histomorphometric findings*

Based on micro-CT evaluation, the mean percentages of new bone (23.2% ± 3.2%), residual MCBS (9.5% ± 9.1%), and soft tissue (67.3% ± 11.6%) in the 4-month group were comparable to the mean percentages of new bone (18.2% ± 2.1%), residual MCBS (17.1% ± 7.0%), and soft tissue (64.7% ± 7.1%) seen in the 6-month group.

Figure 9 demonstrates the described technique and findings in a subject whose biopsy sample was obtained after 6 months of healing.

## Discussion

This report demonstrates the potential of rhPDGF-BB combined with MCBS to provide regeneration for extraction socket wounds with buccal wall defects. Clinical results at 4 and 6 months revealed preservation of ridge form, which allowed for implant placement in all eight patients without adverse healing events.

Bone cores obtained from the study sites were assessed by micro-CT and processed for histologic analysis. This osteopromotive graft combination stimulated new bone formation and appeared to up-regulate bone metabolism. The micro-CT scans showed MCBS particles surrounded by host bone, proving that the bone cores were definitely taken from the grafted sites. All eight specimens were consistent, with no obvious differences between the 4- and 6-month groups.

The histologic analysis confirmed that new vital bone surrounded the MCBS particles with the surface presence of osteoid along with aligned osteoblasts. Bone modeling units were observed in both the 4- and 6-month specimens showing the expected

cascade of events in bone maturation. The presence of multinucleated giant cells on the MCBS particles was associated with reversal lines, indicative of active resorption of the particles. This allowed for host bone replacement, as evidenced by the osteoid and osteoblasts in the region of resorption. This is in contrast to multiple studies with xenograft or MCBS, which have demonstrated minimal remodeling or elimination of xenograft particles.[19,25,31,60] The utility of the MCBS scaffold combined with a potent mitogenic growth factor, rhPDGF-BB, to regenerate advanced buccal wall defects treated at the time of dental extraction appears to be excellent. This compares favorably with the use of recombinant human bone morphogenetic protein 2 (rhBMP-2) to treat similar defects.[16] The present study lacks a no-treatment control group because Fiorellini et al[16] and Nevins et al[2] have demonstrated that a significant portion of the buccal plate would be lost to resorption when teeth with prominent roots are extracted. For example, no treatment resulted in only 9 of 20 sites (45%) receiving an implant without additional bone grafting, compared to 18 of 21 sites (88%) that received rhBMP-2. Nevins et al reported ridge preservation with a xenograft alone with no membrane to treat extraction sockets without defects.[2] There is ample evidence supporting the use of bone replacement grafts with membranes, but the goal of the present study was to accomplish bone regeneration without membrane applications.

Simion et al demonstrated significant ridge augmentation with rhPDGF-BB combined with a xenograft block.[37] This study compared three treatment cohorts: Group A used a deproteinized bovine bone block in combination with a collagen membrane, group B infused the block with rhPDGF-BB, and group C covered the rhPDGF-BB–infused block with a collagen membrane. Histologic examination of group B samples revealed a significant quantity of newly formed bone replacing the resorbed xenograft block. Group A sites suffered dehiscence of the graft, and group C sites showed significantly less bone development.

A significant finding by Simion et al is the documentation of remodeling of the xenograft.[37] The particles had demineralization seams similar to those reported in the present study, along with evidence of intense physiologic remodeling. This is consistent with the results of the present study and supports the ability of rhPDGF-BB to up-regulate osteogenic wound healing. Both the 4- and 6-month groups in this human study demonstrated resorption and replacement of MCBS with vital bone. This is in contrast to previous reports that xenograft is osteoconductive but is not resorbed and replaced when used in periodontal defects, extraction defects, ridge augmentation, or sinus elevation.[41–45] The present histologic findings document multinuclear giant cells on the surface of the MCBS particles with resorptive lacunae and demineralization seams.

MCBS was chosen for the present investigation because of its biologic and mechanical qualities and its well-documented binding and release data with rhPDGF-BB.[57,58] This biomaterial

is adequate for natural bone spacing defects without the use of tenting screws or membranes. It will allow for the optimal environment for growth factor–mediated healing, obviating the need for a membrane that appears to block periosteal-based progenitor cells.

The limitations of the MCBS scaffold are related to the amount of three-dimensional structure that can be obtained because of its spongelike consistency. The clinical technique used here increased the volume of graft placed into the defect to account for compression and proved effective to produce bone volume sufficient for implant placement in prosthetically optimal positions. Further research is required to evaluate the graft's performance in less contained defects.

## Conclusion

Mineralized collagen bone substitute in combination with recombinant human platelet-derived growth factor-BB was used successfully to treat extraction wounds with buccal wall defects. The primary outcome of bone quality was supported by microcomputed tomography and histologic analysis. The bone quantity was visualized during surgery at the time of implant placement. Primary stability of implants was accomplished in all eight sites. The 4- and 6-month bone samples were identical. The final conclusion is that this treatment protocol can shorten the clinical treatment time to 4 months.

## Acknowledgment

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