# The use of resorbable collagen membrane in conjunction with autogenous bone graft and inorganic bovine mineral for buccal/labial alveolar ridge augmentation: A pilot study

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**Statement of problem.** No study provides human histologic evidence regarding the use of resorbable collagen membrane for a 2-stage localized alveolar augmentation procedure.

**Purpose.** The purpose of this pilot study was to evaluate the potential of use of a resorbable collagen membrane in conjunction with an autogenous bone graft and inorganic bovine mineral (IBM) for labial/buccal alveolar ridge augmentation prior to placing dental implants.

**Material and methods.** Seven consecutively treated human patients participated in the study. All patients received labial/buccal alveolar ridge augmentation. An autogenous block graft was secured at the recipient site with fixation screws and a mixture of autogenous particulate with IBM was placed at the periphery. Resorbable collagen membrane was used as a barrier. Radiographic and laboratory measurements were made to quantify ridge augmentation and resorption rate. Preoperative and postoperative stone casts were used to quantify alveolar ridge augmentation. Volumetric evaluation was measured in mL whereas linear laboratory evaluation was measured in millimeters. Measurements were made 1 and 6 months after bone grafting. Histologic and histomorphometric analysis from the grafted area evaluated new bone formation, and osteoconductivity of IBM.

**Results.** For all patients Type II to III bone quality was achieved at the augmented sites. The implant survival rate was 100% at second-stage surgery. No complication was observed at the recipient sites. Radiographic evaluation revealed 4.65 mm labial/lingual augmentation, whereas laboratory analysis revealed 4.57 mm. Volumetric laboratory analysis demonstrated 1.00 (± 0.29) mL alveolar ridge augmentation 6 months after bone grafting and 13.79% resorption between months 1 and 6. Histomorphometric analysis revealed that on average, the area occupied by bone was 34.28% (range 24 to 50; ±9.05),] soft tissue 46.00% (±9.20%; range 30% to 55%), and IBM particles 19.71% (±11.74%, range 3% to 42%). The proportion of the surface of the IBM particles in contact with bone was 47.14% (range 15% to 64%; SD 17.21%).

**Conclusions.** Resorbable collagen membranes may be used as barriers for labial/buccal alveolar ridge augmentation procedures. (J Prosthet Dent 2003;90:530-8.)

**CLINICAL IMPLICATIONS**

*This pilot study demonstrated that resorbable collagen membranes may be used as barriers for labial/buccal alveolar ridge augmentation procedures rather than nonresorbable barriers that require a separate surgical procedure for removal.*

After the acceptance of dental implants as a valid treatment modality for the completely[1,2] or partially[3,4] edentulous patient, bone grafting has been proposed before[5-10] or simultaneously[8,11-13] with the placement of dental implants to obtain adequate bone volume.

Several methods, materials, and techniques have been used for bone grafting. The autogenous bone graft harvested either extraorally[11,12] or intraorally[5-9] appears to represent the gold standard whereas xenografts,[9,14-19] alloplastic bone grafts,[20,21] and allografts[22,23] have also been proposed alone or in combination with autogenous bone graft for localized alveolar ridge augmentation. Various techniques have been applied to secure the graft material at the recipient site. Nonresorbable membranes,[5,8,12,13,23-28] fixation screws,[6,7,9] dental implants,[11,12] or titanium mesh[29-31] are the most common securing devices.

Resorbable collagen membranes have also been described as barriers for 2-stage alveolar augmentation procedures.[32-34] Parodi et al[32] performed lateral alveolar ridge augmentation in 16 patients using a collagen membrane in conjunction with a collagen sponge with no histologic evidence of bone formation. Benque et al,[33] in a clinical report, demonstrated bone formation under light microscopy when a collagen membrane was

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**Table I.** Patient distribution

| Patient no. | Recipient site | Donor site | Age | Gender | Healing period (mos) | Number of implants | Type of provisional restorations |
|---|---|---|---|---|---|---|---|
| 1 | Maxillary right lateral incisor | Tori | 26 | M | 7 | 1 | RPD |
| 2 | Premaxilla | Chin | 77 | F | 6 | 3 | None |
| 3 | Premaxilla | Chin | 44 | F | 6 | 4 | RPD |
| 4 | Mandibular right posterior area | Chin | 38 | F | 6 | 3 | None |
| 5 | Maxillary right posterior area | Chin | 67 | M | 8 | 2 | None |
| 6 | Maxillary right posterior area | Ramus | 67 | M | 14 | 3 | None |
| 7 | Maxillary right lateral and central incisor | Chin | 27 | F | 8 | 2 | RPD |
| Average | | | 49.4 | | 7.86 | | |
| SD | N/A | N/A | 20.77 | N/A | 2.85 | N/A | N/A |
| Range | | | 26-77 | | 6-14 | | |

*RPD,* Removable partial denture.

used in conjunction with hydroxyapatite for localized alveolar ridge augmentation. Kirkland et al[34] achieved alveolar ridge augmentation using a resorbable polylactide membrane in conjunction with allograft and bioactive glass.

This pilot study provided a clinical, laboratory, and histologic/histomorphometric analysis of the use of a resorbable collagen membrane for localized alveolar ridge augmentation in conjunction with intraorally harvested autogenous bone graft and inorganic bovine mineral.

## MATERIAL AND METHODS

### Patient selection

Seven consecutively treated patients (mean age 49.4 years old; range 38 to 77) participated in this study (Table I). The patients required bone grafting procedure before the placement of dental implants. For all patients, a resorbable collagen membrane (Bioguide; Osteohealth Co, Shirley, NY) was used during the bone grafting procedure in conjunction with intraorally harvested intramembranous bone graft and inorganic bovine mineral (Bio-Oss; Osteohealth Co, Shirley, NY). The bone grafting procedures were performed during the period between April 1998 and February 2001. Treatment was performed by postdoctoral students at the Center for Prosthodontics and Implant Dentistry at Loma Linda University (LLU). Patients signed the corresponding informed consent approved by the Institutional Review Board at LLU to have a biopsy specimen taken during implant surgery.

Patients were included in the study if they demonstrated the need for labial/buccal bone grafting procedure before placement of dental implants, had good oral hygiene, and no evidence of periodontal disease. Exclusion criteria included smoking, the need for vertical alveolar ridge augmentation in addition to labial/buccal augmentation, and systemic disease, precluding bone grafting or implant surgery (for example, uncontrolled diabetes or cancer).

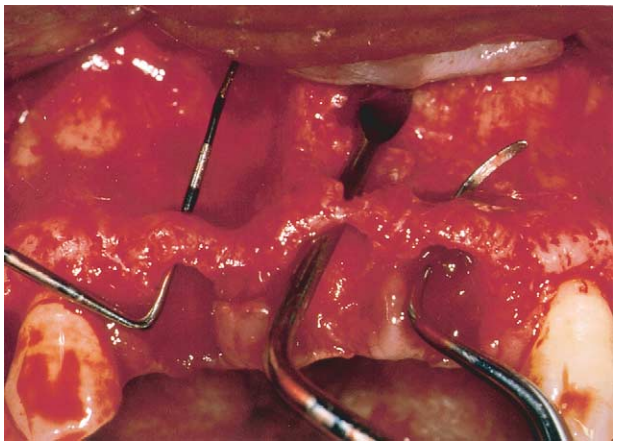

**Fig. 1.** Extraction of maxillary anterior teeth. Bone volume is inadequate for placement of implants.

### Surgical protocol

At the time of the bone grafting procedure or implant placement, the patients were given a choice of local anesthesia (LA) only, LA with oral sedation (Halcion 0.25mg), or LA with intravenous sedation (midazolam in conjunction with fentanyl). Full-thickness labial/buccal and lingual/palatal flaps were reflected at the recipient site (Fig. 1). The donor site was either the chin area (5 patients), the ascending ramus area (1 patient), or a mandibular tori (1 patient) (Table I). Harvesting of the bone graft was performed according to the previously described standard procedure.[6,35] The autogenous bone graft was removed in the form of 1 or 2 blocks (Fig. 2). Additional bone marrow was then removed in particulate form from the recipient area with a curette (ACE Surgical Supply Co, Brockton, Mich).

The recipient site was perforated with a surgical fissure bur under copious irrigation to induce bleeding and promote the incorporation of the graft.[36] One or 2 autogenous block grafts were placed at the recipient site on

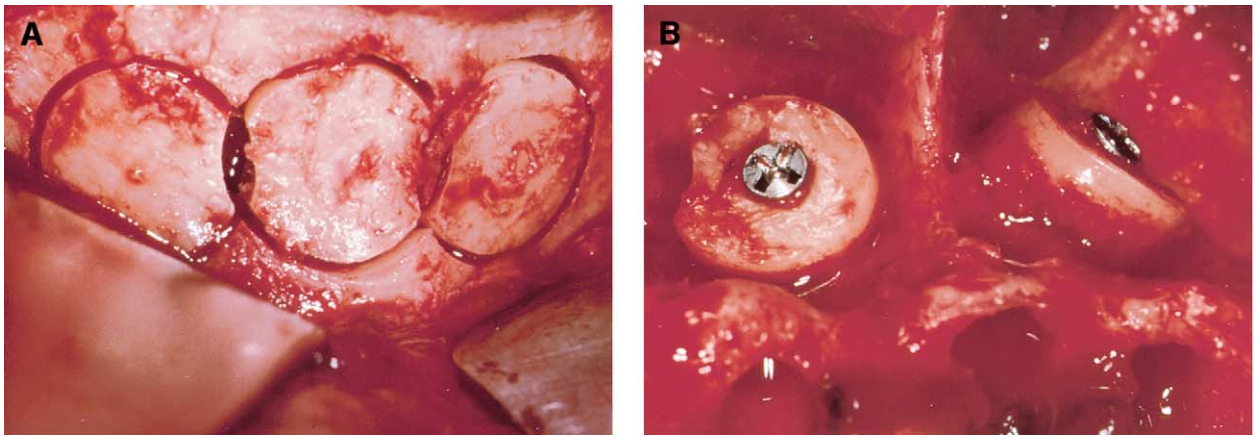


**Fig. 2. A,** Autogenous bone graft harvested from chin area. **B,** Autogenous bone used as block at recipient site.

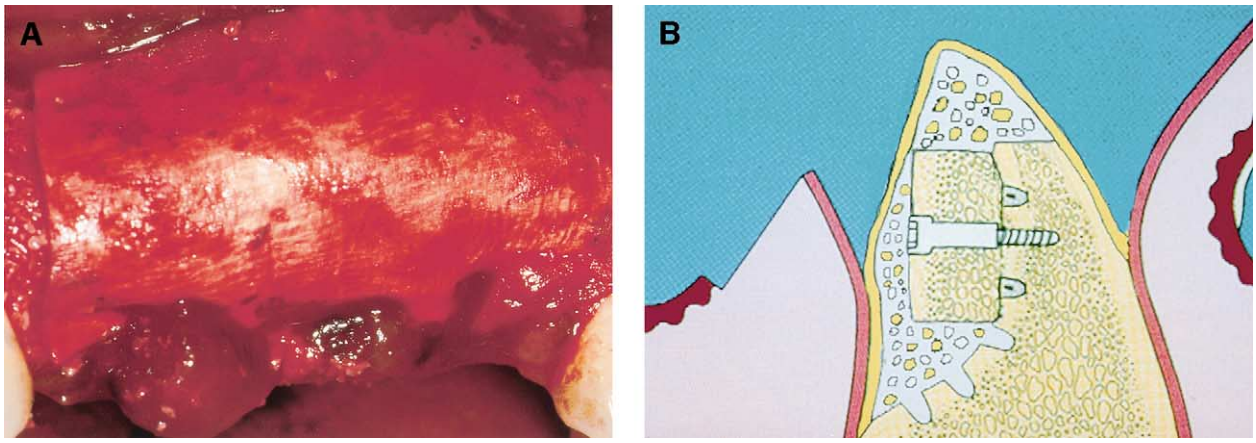


**Fig. 3. A,** Resorbable collagen barrier placed to cover grafted area. **B,** Autogenous block graft secured in place with fixation screw. Particulate autogenous bone graft mixed with Bio-Oss and collagen membrane covers grafted area.

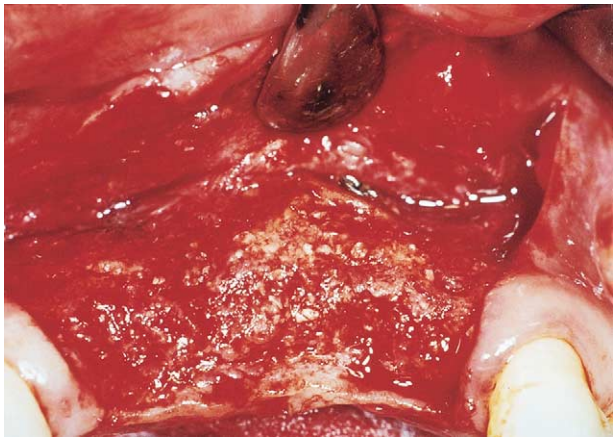

**Fig. 4.** Graft incorporated at recipient site 6 months after augmentation.

the buccal/labial surface and secured in place using 1 fixation screw (Osteromed Inc, Addison, Tex) for each block graft (Fig. 2, *B*). The particulate autogenous bone marrow was then mixed at a 50/50 ratio with inorganic bovine mineral particles (Bio-Oss; Osteohealth Co, Shirley, NJ) and placed at the recipient site. A resorbable collagen membrane was then trimmed and placed above on the bone graft (Fig. 3). Periosteal fenestration[37] was performed along the labial/buccal flap to enable primary closure. The flap was then sutured.

Two weeks after bone graft surgery, the sutures were removed. The bone graft was allowed to heal for 6 to 8 months before the placement of the implants (Table I). One patient (number 5) was unable to return earlier and received the implants 14 months after the bone grafting procedure.

Full-thickness labial/buccal and palatal/lingual flaps were reflected during the placement of the implants (Fig. 4). Hydroxyapatite-coated root form implants

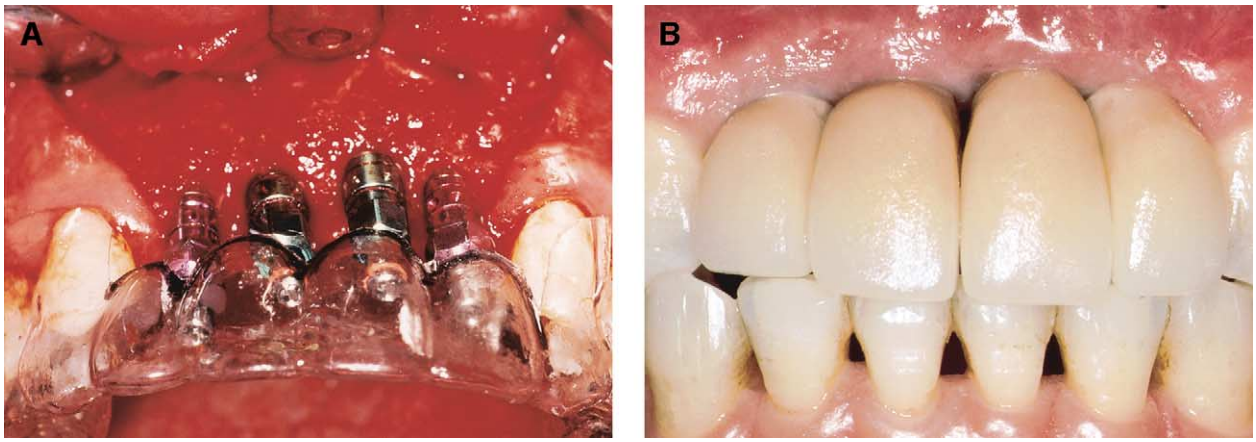


**Fig. 5. A,** Adequate bone volume obtained for placement of 4 root form implants. No dehiscence or fenestration of implants was noticed. **B,** Final result, facial view.

(Steri-Oss; Nobel Biocare, Yorba Linda, Calif) were placed (Fig. 5, *A*), and the bone quality (type I to IV)[38] as assessed by the clinician was recorded. A calibration process for assessment of bone quality was performed before implant surgeries to enhance reproducibility of data collection. The treatment plan was for all patients to receive an implant-supported, screw-retained fixed partial denture (Fig. 5, *B*).

Second-stage surgery was performed 6 to 8 months after implant placement. Implant mobility was evaluated (bidigitally, with the handles of 2 instruments). Periapical radiographs were made. Patients were asked to return 2 weeks after the bone grafting procedure for suture removal and in 1, 3, and 6 months for clinical re-evaluation.

## Radiographic evaluation

All patients received preoperative and immediate postoperative panoramic radiographs. In addition, linear tomographs were made before the bone grafting procedure and before the placement of the implants. Measurements for the labial/buccal bone augmentation were made by evaluation of the preoperative and postoperative linear tomographs. All measurements were made by 1 investigator (P.P.). For the linear tomographs, the distortion rate (1.7) provided by the manufacturer of the tomographic unit ("Scanora" Type SBR 1C; Orion Co, Helsinki, Finland) was considered when the measurements were made. Measurements were made under original magnification ×10.[39] Tomographs were standardized using 2 external light beams that were incorporated in the tomographic unit for standardization purposes.

## Laboratory evaluation

Impressions were made around the grafted area with a custom tray with irreversible hydrocolloid (Coe Alginate; GC America Inc, Alsip, Ill). The impressions were made before operation, 1 month after the bone grafting procedure, and 6 months after the bone grafting. The impressions were poured with Type III dental stone (Microstone; Whip Mix Co, Louisville, Ky).

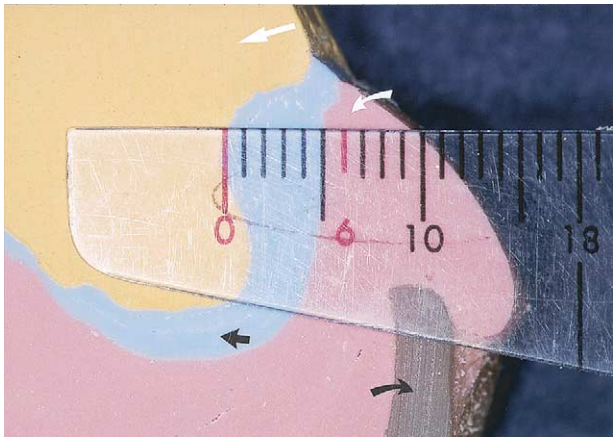


**Fig. 6.** Laboratory measurements allowed linear and volumetric measurements of grafted area. In illustrated cross-section, interocclusal registration material (*blue area, black arrow*) represents augmented alveolar ridge. *Curved black arrow* represents custom tray, pink material (*curved white arrow*) represents silicone matrix used when making impression from postoperative stone cast, and yellow material (*white straight arrow*) represents preoperative stone cast.

The postoperative stone casts were used to quantitatively assess the volume of the alveolar ridge augmentation by using the following technique. An impression was made with a custom tray from the postoperative stone cast using silicone (Lab-putty; Coltene/Whaledent Inc, Mahawan, NJ). The custom tray was removed and separating medium (Al-Cote Dentsply International Inc, York, Pa) was internally applied. Polyvinylsi-

**Table II.** Clinical assessment

| Patient no. | Bone quality | Complications—donor site | Complications—recipient site | Bio-Oss particles |
|---|---|---|---|---|
| 1 | III | None | None | The Bio-Oss particles appeared well attached to surrounding grafted area and incorporated to regenerated alveolar ridge. |
| 2 | II | Temporary hypoesthesia | None | |
| 3 | III | None | None | |
| 4 | II | None | None | |
| 5 | III | Temporary hypoesthesia | None | |
| 6 | II | None | None | |
| 7 | III | None | None | |

**Table III.** Increases in alveolar ridge volume (mL)

| Patient no. | 1 month after bone grafting | 6 months after bone grafting |
|---|---|---|
| 1 | 0.53 | 0.47 |
| 2 | 1.34 | 1.12 |
| 3 | 1.42 | 1.17 |
| 4 | 1.64 | 1.35 |
| 5 | 1.10 | 0.96 |
| 6 | 0.84 | 0.79 |
| 7 | 1.29 | 1.17 |
| Average | 1.16 | 1.00 |
| SD | 0.38 | 0.29 |
| Range | 0.53–1.64 | 0.47–1.35 |

**Table IV.** Linear laboratory measurements of buccal/labial ridge augmentation (mm)

| Patient | 1 month after bone grafting | 6 months after bone grafting |
|---|---|---|
| 1 | 4 | 3 |
| 2 | 7 | 6 |
| 3 | 5 | 5 |
| 4 | 6 | 5 |
| 5 | 4 | 4 |
| 6 | 5 | 4 |
| 7 | 6 | 5 |
| Average | 5.28 | 4.57 |
| SD | 1.11 | 0.98 |
| Range | 4–7 | 3–6 |

**Table V.** Histomorphometric analysis

| | Histomorphometric evaluation (%) | | | |
|---|---|---|---|---|
| Patient no. | Bone | Fibrous tissue | Bio-Oss residual particles | Bone-residual particles contact |
| 1 | 36 | 48 | 16 | 37 |
| 2 | 28 | 30 | 42 | 62 |
| 3 | 50 | 47 | 3 | 50 |
| 4 | 30 | 55 | 15 | 64 |
| 5 | 24 | 53 | 23 | 15 |
| 6 | 30 | 52 | 18 | 44 |
| 7 | 42 | 37 | 21 | 58 |
| Average | 34.28 | 46.00 | 19.71 | 47.14 |
| SD | 9.05 | 9.20 | 11.74 | 17.21 |
| Range | 24–50 | 30–55 | 3–42 | 15–64 |

loxane interocclusal record registration material (IRRM) (Exabite II NDS; GC America Inc) was placed in the tray which was then placed on the preoperative stone cast and the IRRM was allowed to polymerize (Fig. 6). The IRRM representing the augmented portion of the postoperative alveolar ridge was then removed from the tray. The excess material was trimmed. The weight of the material was assessed and, by considering the weight per volume of the material provided by the manufacturer, it was possible to calculate the volume of the alveolar ridge augmentation.

In addition, linear measurements were made by evaluating the buccal/labial thickness and vertical height of the IRRM. Linear measurements were made with a caliper (Darby Dental Supply Inc, Rockville, NY). The accuracy and reproducibility of this method has been previously published.[40] This method has been used in other clinical studies involving localized alveolar ridge augmentation.[9,31]

## Specimen harvesting

During implant surgery, a biopsy specimen was taken from the grafted area using a 2-mm internal diameter trephine bur (ACE Surgical Supply Co) as the first drill during the osteotomy preparation for implant placement. The specimens were fixed in 10% buffered formalin.

## Histologic processing

The specimens were dehydrated in alcohol and embedded in resin (Technovit 7200 VLC; Kulzer, Wehrheim, Germany). Initial midaxial sections of 200 $\mu$m were made by means of the cutting-grinding system (Exact Medical Instruments, Oklahoma City, Okla). The sections were then ground to 40 to 50 $\mu$m and stained with Stevenel's blue and Van Gieson's picrofuchsin for histomorphometric and light fluorescent microscopy analyses.[41,42]

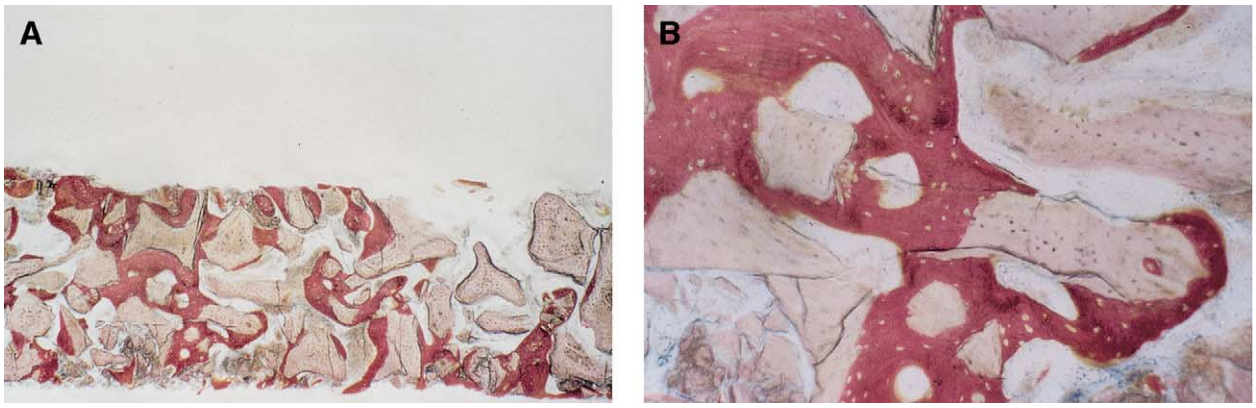


**Fig. 7. A,** Histologic overview of specimen. (Original magnification ×3.) **B,** Residual bovine mineral (Bio-Oss) particles in tight contact with surrounding bone. Particles appear interconnected with bony tissue. No sign of inflammation or resorption is observed. (Original magnification ×10.)

## Histomorphometric evaluation

Histomorphometric evaluation was performed by 1 investigator (P.P.) using a computer-assisted linear analysis program, Ribbon, developed at Loma Linda University.[43] For each histologic specimen, 1 to 4 images were analyzed, depending on the size of the specimen. All histomorphometric analysis was performed by capturing an image under original magnifications ×2 (Olympus Microscope, Model BH-2; McBain Instruments, Chattworth, Calif). The percentage of bone formation, soft tissue, and residual Bio-Oss particles (RBP) were calculated. In addition, the portion (%) of the RBP in direct contact with bone was measured.

# RESULTS

## Clinical evaluation

Patients reported no pain or discomfort at the grafted area (Table I). In 2 patients (1 and 4) the area adjacent to the grafted area tooth developed a periodontal abscess of an endodontic-periodontic nature 3 months after the bone grafting procedure (patient 1) and caries (patient 4). Temporary hypesthesia developed at the donor side (chin area) on 2 occasions (patients 2 and 5). No exposure of the membrane or any clinical sign of inflammation or infection was observed at the recipient site in any of the 7 patients.

During implant surgery, the RBP appeared well incorporated into the grafted area (Fig. 4). During implant placement, the grafted area had Type II-III bone quality (Table II). Primary stability was achieved during the placement of all implants. During second-stage surgery, none of the implants appeared to be mobile or have any symptom or clinical sign of pathosis (pain, discomfort, deep periodontal probing depths, or bone loss). This resulted in a 100% survival rate at second-stage surgery.

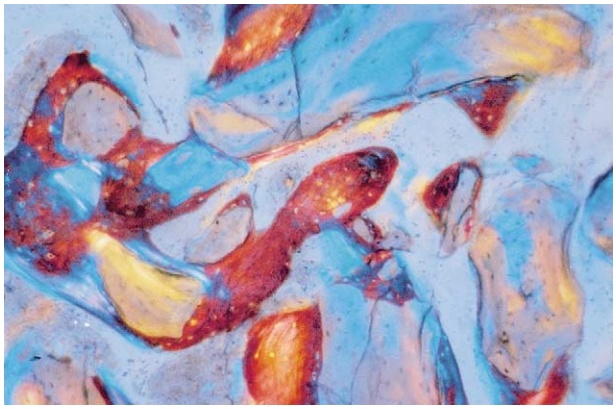

**Fig. 8.** Polarized microscopy emphasizes remodeling activity of bone surrounding Bio-Oss particles. (Original magnification × 10.)

## Radiographic evaluation

Radiographic analysis with tomographs revealed 4.65 mm labial/buccal augmentation (range 4 to 6 mm). For all patients adequate bone volume was clinically observed for the placement of root form implants at a prosthetically ideal position.

## Laboratory evaluation

Laboratory volumetric measurements revealed that 1.16 mL (+0.38, range 0.53 to 1.64 mL) of ridge augmentation was achieved 1 month, and 1.00 mL was achieved 6 months after operation (±0.29, range 0.47 to 1.35 mL) (Table III). These measurements indicated that 13.79% resorption occurred 6 months after the bone grafting procedure.

Linear measurements revealed that 1 month after the bone grafting procedure, 5.28 mm (±1.11, range 4 to 7 mm) of labial/buccal ridge augmentation was obtained

(Table IV). The corresponding number for the 6-month postgrafting measurements was 4.57 mm (±0.98, range 3 to 6 mm).

### Histologic evaluation

In all specimens, a mixture of bone, connective tissue, and RBP was observed (Fig. 7, *A*). In most patients the RBP appeared to be in tight contact with bone (Fig. 7, *B*). Polarized microscopy emphasized the active remodeling pattern of the bone around the RBP (Fig. 8). No sign of resorption or active inflammatory process was identified in any of the specimens.

### Histomorphometric evaluation

The average area of all 7 core sections occupied by bone was 34.28% (±9.05%; range 24% to 50%) (Table V). The analogous value for soft tissue was 46.00% (±9.20; range 30 to 55) and for RBP 19.71% (±11.74%; range 3% to 42%). The proportion of the surface of the RBP in contact with bone was 47.14% (±17.21; range 15% to 64%).

## DISCUSSION

This study provided histologic evidence in humans regarding the potential of using a resorbable collagen membrane in conjunction with autogenous bone graft and inorganic bovine mineral for buccal/labial alveolar ridge augmentation. Nonresorbable polytetrafluoroethylene membranes have been used for alveolar ridge augmentation[5,8,12,13,23-27] because of their rigidity providing stability and protection of the graft material from external mechanical forces. However, polytetrafluoroethylene membranes have been associated with infection on exposure,[5,8,24,27] compromising the amount of alveolar ridge augmentation.[8,27] Zitzmann et al,[27] in a comparative study, demonstrated that the use of a collagen membrane does not compromise the final result when membrane exposure occurs, as opposed to polytetrafluoroethylene membranes that results in incomplete bone healing when exposed.

In addition, a layer of connective tissue has been observed between polytetrafluoroethylene membranes and the regenerated bone.[13,23,25,26-28] This is similar to what Boyne et al[29] described as "pseudoperiosteum" underneath the titanium mesh. It seems that nonresorbable barriers tend to induce the formation of a thick connective tissue layer underneath the barrier and above the regenerated bone. It is unknown what stimulates this connective tissue formation; it may be hypothesized that the micromovement of the barrier could induce the formation of this layer of connective tissue. Simion et al[25] demonstrated incomplete bone regeneration because of the consistent presence of this layer of connective tissue underneath a nonresorbable barrier. In this study, this thick connective tissue layer was not observed, and bone regeneration appeared to be complete. It appeared that the placement of the block graft at the mid-buccal or mid-facial area provided the necessary rigidity for the support of the membrane and the particulate graft. It could be speculated that in spite of the lack of rigidity, collagen membranes posses some biologic advantages over nonresorbable barriers for alveolar ridge augmentation procedures. Nevertheless, this study demonstrated lateral alveolar ridge augmentation, which is a relatively predictable treatment modality.[5] Further research is needed to evaluate the potential of the technique presented to achieve vertical alveolar ridge augmentation.

The particles of Bio-Oss identified in the histologic analysis of this study appeared to be in tight contact with bone along the 47% of their external surface. The biocompatibility of the Bio-Oss particles has been observed in both humans[9,16,19,31] and animals.[15,17] The particles of the inorganic bovine mineral appear to act as a scaffold for new bone formation.[15-18] However, it is difficult to explain the tight contact between these particles and the newly formed bone. It has been reported that the structural compatibility of Bio-Oss to cancellous bone enhances the osteogenic cell in growth and bone apposition.[15] On the other hand, other studies have demonstrated the inability of the Bio-Oss to induce new bone formation when used as an onlay graft alone with no addition of an osteoinductive material.[14,19] It seems that the addition of cancellous autogenous bone graft in this study offered the necessary osteogenic cells that induced new bone formation around the Bio-Oss particles, an observation made in other human studies as well.[9,31,44]

Regardless of the selected technique and methodology, it should be emphasized that bone grafting is essential to provide not only adequate bone volume for implant placement, but in addition, better implant angulation and position,[8] improved implant-to-crown ratio,[24,25,39] and esthetics.[31] Adequate treatment planning and communication between the surgeon, prosthodontist, laboratory technician, and patient are essential to achieve the desired results.

In this study, a newly developed method was used to measure alveolar ridge augmentation in the laboratory. This method has been reported to be reproducible and accurate.[39] However, a limitation of this study is that the impressions were made intraorally before and after bone augmentation procedures; it is unknown whether the alveolar ridge augmentation represents bone or soft-tissue augmentation. Impressions could be made during surgery and after full-thickness flap reflection to provide information regarding hard tissue augmentation only.

In addition, only 7 patients were evaluated in this study. Even though histologic evaluation demonstrated good results, further studies with a larger sample followed for a longer period of time are needed to establish the usage of a resorbable barrier as a predictable treat-

ment modality for alveolar ridge augmentation procedures.

## CONCLUSIONS

This study demonstrated 34.28% bone formation under light microscopy when a resorbable collagen membrane was used in conjunction with autogenous bone graft and Bio-Oss for buccal or labial alveolar ridge augmentation. The augmented alveolar ridges had a solid consistency, and no sign of inflammation or resorption was seen under light microscopy. A total of 1.00 mL of ridge augmentation was achieved 6 months after bone grafting whereas 13.79% resorption occurred between months 1 and 6. Radiographic evaluation revealed 4.65 mm alveolar ridge augmentation whereas linear laboratory measurements revealed 4.57 mm.

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