During the past two decades, augmentation of the bone at dental implant sites, sometimes done simultaneously with dental implant placement, has been reported in the literature. In general, clinicians have placed various bone graft materials on top of the resorbed alveolar ridge (ie, onlay grafting), although interpositional (inlay) grafting is sometimes done. The potential advantage of onlay augmentation is development of height, width, and alveolar form in three-dimensional space. However, this has been a difficult task to accomplish in the clinical setting because of collapse of the scaffold, matrix, or membrane applied.

One innovative clinical report followed 11 patients treated with titanium shells, which created space for bone growth without added graft material. A titanium membrane, which was not perforated, was fixed to the maxillary bone and left to heal for 12 to 18 months. Bone slowly formed, apparently from the endosteal...
surface, in a volume sufficient to host implants. A follow-up study was carried out to use tissue engineering to accelerate the bone formation.

To further define this unexpected phenomenon, an animal experiment was proposed using titanium chambers placed over so-called “high-profile” dental implants (ie, implants placed supracrestally) inserted into rabbit tibia with and without bone morphogenetic protein-2 (BMP-2) in an absorbable collagen sponge carrier (ACS) to test the hypothesis that signal-enhanced tissue engineering might accelerate and enhance bone formation in this setting. The present study sought to answer two questions: (1) What is the optimal grafting material to place beneath an imperforate titanium membrane? and (2) Could BMP-2 be useful in forming bone in this setting? The aim of the study, therefore, was to determine the osteogenic potential of recombinant human BMP-2 (rhBMP-2) used with collagen sponge around high-profile dental implants occluded from the periosteum by an imperforate titanium shell.

MATERIALS AND METHODS

The study was approved by the Animal Use Committee of Hebrew University, Jerusalem, Israel. Eight male New Zealand white rabbits, weighing 3 to 4 kg each, were divided into four groups of two rabbits each (Table 1).

All groups had free access to water and laboratory food. Prior to the surgical procedure, the animals were anesthetized with a mixture of 1.5 mL ketamine (100 mg/mL) + 0.5 mL xylazine (20 mg/mL) at a dosage of 0.11 mL/100 g, delivered via a subcutaneous injection. Cephazoline (90 mg intramuscular) prophylactic antibiotics were administered in addition to preoperative analgesics in the form of Rimadyl (Pfizer) 0.4 mL (200 mg/mL). The animals were intubated, and general anesthesia was maintained with isoflurane. The heart rate, respiratory rate, pulse oximetry, and temperature of the animals were monitored. Povidone iodine was used as an antiseptic agent. Surgery was performed under local anesthesia (1.8 mL lidocaine hydrochloride 2% + epinephrine 1:100,000). Tramadol (0.3 mL) was given as an analgesic agent, and antibiotic coverage was achieved with Penstrep (5 mg/kg, Norbrook Laboratories) for 3 days postoperative.

An incision was made in the area of the right anterolateral tibia to elevate the skin and periosteum (Fig 1a). Two implant sockets were prepared based on a standard implant protocol using a succession of 2-mm, 2.8-mm, and 3.2-mm drills (Fig 1b). The medullary content was left in place and 3.7-mm-wide × 10-mm-long acid-etched implants (MIS Implant Technologies) were inserted 6 mm into the prepared socket until they contacted the opposing cortical bone. This resulted in 4 mm of protrusion (ie, 4-mm high-profile implants). Implants were inserted at a distance of 8 mm from one another. Group A (control) implants were left as is. The implants in groups B, C, and D were covered by an occlusive chamber made of imperforate titanium leaves, which had been hand cut from titanium sheeting so that when folded over each other they fitted together intimately. The spacing around each implant was similar. Next, two separate square chambers were fixed into place around the implants by cover screws, with good visual fit observed at the bone site. The chambers were both very secure and immobile but were not directly fixed to the bone except by the cover screw. The margin appeared to have a gap of 1 mm or less in most cases. Group B chambers were left untreated. The chambers

<table>
<thead>
<tr>
<th>Group</th>
<th>Implantation description</th>
<th>Rabbit no.</th>
<th>Time of sacrifice</th>
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<tbody>
<tr>
<td>Group A</td>
<td>Two high-profile titanium implants</td>
<td>6583</td>
<td>3 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6612</td>
<td>6 wk</td>
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<tr>
<td>Group B</td>
<td>Two high-profile titanium implants covered with titanium chamber, which were fitted and placed during implant insertion</td>
<td>6584</td>
<td>3 wk</td>
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<td></td>
<td></td>
<td>6623</td>
<td>6 wk</td>
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<tr>
<td>Group C</td>
<td>Two high-profile titanium implants covered with titanium chambers that were fitted and placed during implant insertion, with the void between the cage and the bone filled with a buffer-soaked collagen sponge</td>
<td>6585</td>
<td>3 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6732</td>
<td>6 wk</td>
</tr>
<tr>
<td>Group D</td>
<td>Two high-profile titanium implants covered with titanium chambers that were fitted and placed during implant insertion, with the void between the cage and the bone filled with an rhBMP-2– enriched collagen sponge</td>
<td>6733</td>
<td>3 wk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6586</td>
<td>6 wk</td>
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in group C were filled with collagen sponge soaked with buffer, while the chambers in group D were filled with an rhBMP-2–enriched collagen sponge (Medtronic). The chambers were then covered by custom-fit titanium shells. The muscle and periosteum were returned to their places and closed with Vicryl 3/0 sutures (Fig 2). One animal from each group was sacrificed after 3 weeks, and the second animal was killed after an additional 3 weeks (total of 6 weeks).

Following this protocol, the right tibia was harvested from each animal, radiographed, and prepared for histologic examination. From each tibia, one implant was embedded in methyl methacrylate and sectioned using a Leica 1600 saw microtome (Leica Microsystems) into 100-μm slices. Slices were ground down to a thickness of 40 to 50 μm using an Exakt cutting/grinding system (Exakt Apparatebau). The second implant was decalcified, embedded in paraffin, and sectioned into 10-μm-thick specimens.

The ground sections were stained with Goldner trichrome and the paraffin sections were stained with hematoxylin-eosin for histomorphometric analysis.
RESULTS

All animals survived the placement of the implants and chambers. All animals resumed normal dietary habits during the first 24 hours after the operation, and none of the animals lost more than 10% of their body weight during the study.

Several complications were observed during the experiment. Rabbit no. 6585 (group C) was excluded from the experiment as a result of a fracture in the right tibia and dislocation of the implants in a manner that did not permit histologic evaluation. An additional two rabbits (no. 6612 [group A] and no. 6623 [group B]) showed fractures in the tibia, but histologic examination was nevertheless carried out (Fig 3). No fractures occurred in rabbits that received rhBMP-2 (group D).

Sections from each specimen were produced—one implant ground section, and one implant decalcified and embedded in paraffin. Specimens retrieved after 3 weeks (Fig 3) and 6 weeks (Fig 4) were examined and compared.

Figs 3a to 3c  Radiographs demonstrating pathologic fractures. (a) Rabbit no. 6585: The leg was not fully functioning, and the animal was sacrificed after 3 weeks. (b) Rabbit no. 6612, sacrificed after 6 weeks. (c) Rabbit no. 6623, sacrificed after 6 weeks.

Figs 4a to 4f  Histologic specimens stained with Goldner trichrome. (Top row) rabbits sacrificed after 3 weeks (left to right): Rabbit no. 6583, implant without chamber; rabbit no. 6584, implant covered with empty chamber; rabbit no. 6733, implant covered with chamber filled with rhBMP-2. (Bottom row) rabbits sacrificed after 6 weeks (left to right): Rabbit no. 6586, implant covered with chamber filled with rhBMP-2.
THREE-WEEK SPECIMENS

Rabbit no. 6583, with two implants and no shell covering (group A), was sacrificed after 3 weeks. Histologic examination showed that the implant was “floating” in soft tissue and had not osseointegrated (Fig 4a).

Specimen no. 6584 (group B) had two implants covered with a titanium chamber without filler material. There was little evidence of bone growth inside the chamber or adjacent to the cortical bone. Most of the chamber was filled with noncalcified granular tissue (Fig 4b).

Specimen no. 6585 (group C) was sacrificed at 3 weeks minus 1 day as the result of fracture of the right tibia, which restricted the rabbit’s ability to move. The specimen was not sent for histologic examination because of implant mobility and displacement (Fig 3a).

For specimen no. 6733 (group D), a small amount of bone outside the chamber was evident clinically. Histologic specimens showed that granular tissue filled the chamber and necrotic tissue was present next to the cortical bone. There was little evidence of vertical bone formation inside the chamber. Osseointegration was developing around the implant inside the tibia (Fig 4c).

SIX-WEEK SPECIMENS

During harvesting of the tibia of specimen no. 6612 (control group A), a fracture was discovered. The fracture deformed the specimen, preventing histologic analysis (Fig 3b).

Clinically, a fracture distal to the chamber was discovered during harvesting of the tibia from specimen no. 6623 (group B) (Fig 3c). There was almost no evidence of bone formation outside or inside the chamber in this specimen. Histologic examination showed that a small amount of vertical bone migration had occurred (~0.5 mm) inside the chamber around the implant. There was no bone growth outside the chamber (Fig 4d).

Clinically, there was no evidence of bone formation in specimen no. 6732 (group C). Histologic examination showed vertical bone formation of approximately 2 mm. This bone formed around the implant threads but appeared to be restricted by granular tissue and collagen matrix (Fig 4e).

In the group D rabbit (specimen no. 6586), large amounts of bone had formed outside the chamber, covering it completely (Fig 3c). Inside the chamber, approximately 3 mm of bone (measured vertically) had formed around the implant. Further bone growth appeared to be occurring, but a large portion of the collagen sponge remained and had not fully absorbed; it was being replaced by nonmineralized granular tissue. Necrosis was present within the titanium chamber as well as within the cortical bone (Fig 4f).

DISCUSSION

The two rabbits in the rhBMP-2 group finished this study with intact tibiae, in contrast to the other three groups, in each of which one rabbit developed a fracture at the operative site. This implies that rhBMP-2 encouraged osseous repair following bone trauma or bone strain, despite its confinement to the titanium chamber.17

Control specimens with empty chambers as well as chambers filled with only collagen sponge and buffer showed negligible bone growth. It is possible that the margins of the chambers moved under function, although the chambers were quite rigid and well scarred into place at the time of specimen harvest. Mobility of the chambers could help explain the lack of bone formation but bone formed outside the chamber indicates the chamber was indeed immobile.

rhBMP-2 induced greater bone growth than empty chambers. However, most of the induced bone formed at the periphery; this was especially apparent in the 6-week rhBMP-2 specimen (Fig 5). This
specimen did show 3 mm of bone growth (vertically) around the implant, but the response was relatively weak compared to the peripheral bone formation. In addition, this bone covering the chamber appeared to arise from the soft tissues in continuity with the basal bone, where the chamber did not fit intimately and the BMP-2 likely extruded. The modest 3 mm of bone growth inside the chamber of the 6-week rhBMP-2 specimens, therefore, could have arisen from outside of the chamber, at least in part. Therefore, the lack of bone formed within the chamber is likely to have been caused by isolation from the surrounding soft tissues and a lack of adequate vascularization.18,19

In general, the majority of the space within the chambers was not filled with bone, as the titanium barrier appeared to block the migration of bone-forming cells from surrounding soft tissue as well as stifle angiogenesis.18,19 This occurred despite multiple bone perforations into the marrow space of the basal bone enclosed beneath the chamber.

Several preclinical studies have been undertaken to demonstrate augmentation using BMP-2 in various space-making strategies. Since Cochran et al first showed that bone could form in a critical-size defect and that osseointegration would develop,20 there have been various attempts to establish combined bone augmentation and osseointegration in implants with exposed threads, usually placed supracrestally.21–24 Although bone forms in this setting, the bone appears to taper and become thin over the titanium threads as it grows crestally, such that the width of the alveolar process is not recovered. That is to say, an adequately shaped alveolar process could not be obtained without some type of architectural bone-grafting strategy. In the present study, not unlike the study by Caplanis et al that used expanded polytetrafluoroethylene (e-PTFE) membranes, minimal amounts of bone formed and only at the base, not filling the confined space when a barrier was used.18

The use of bone mixtures, such as hydroxyapatite or xenograft with BMP-2, were at first advocated to help define and maintain space but were later shown to form bone of inconsistent quality and lacking volumetric stability.25,26 The use of some type of scaffold or bone-shaping device remains justified given the present non–space-maintaining BMP-2 carrier. A rigid shell concept will potentially be important in the clinical edentulous setting when considering reconstruction of alveolar vertical height into orthoalveolar form. A study by Molly et al15 used customized perforate titanium shells placed without grafting material and observed that alveolar bone developed in the maxilla after 1 to 1.5 years in 11 patients. However, even after this amount of time, bone was still incompletely mineralized at the crest, and the implant failure rate was about the same as that associated with iliac augmentation grafting.15

The titanium shell idea was studied using titanium caps placed onto rat calvaria.27 Bone formed in this setting, but very slowly. Further studies showed that occlusive titanium caps performed better than occlusive e-PTFE membranes. Yamada et al28 using a rabbit calvaria model, found that totally occlusive titanium caps resulted in less bone formation than perforated titanium caps but that soft tissue entered the chamber through the pores and prevented the entire chamber from filling with bone. They concluded that, given more time, a nonperforated titanium shell would likely fill completely with bone—and this in a setting of no added graft material over a perforated osseous bed.28

Subsequent efforts have attempted to stimulate or accelerate this slow bone formation process by adding stem cells to the blood clot or altering the titanium membrane with a nanohydroxyapatite/polyamide coating to promote osteoconduction.16,29 Bone formation using these approaches was nevertheless slow, and BMP-2 was not tried.

Barboza et al developed the use of a nonocclusive, well-perforated space-maintaining device made from e-PTFE, which allowed for osseointegration as well as shaped the bone in a high-profile implant dog model using BMP-2/ACS.30 This approach had been studied previously in the dog using canine allograft and autograft but under an occlusive e-PTFE membrane; bone formed to fill the space and osseointegration of exposed screw threads occurred by osteoconduction, although an admixture of nonvital bone remained.31 Wikesjö found that with the use of BMP-2/ACS only, completely vital bone formed, which resulted in osseointegration of the implant and filled the entire space though with highly trabecular bone.32 The question then arose: Could a relatively sparse bone architecture mature under functional loading from a primarily stable implant and give rise to a well-mineralized, well-formed alveolus? The answer to the question of whether both alveolar form and osseointegration would persist is uncertain.

This present titanium shell pilot study sought to examine this question from the standpoint of establishing shaped bone morphology through the use of a more rigid space-making device, ie, one made of titanium. Imperforation of the shell was suggested by a study done by Lundgren in rat calvaria, which showed that perforate titanium barriers resulted in predictable bone augmentation.33 This present pilot study did not show this. Also, unlike the clinical report of occlusive titanium membranes, here, the imperforate rigid titanium device demonstrated very little early bone formation, similar to an occlusive e-PTFE barrier membrane in the presence of a morphogen.18
Wikesjö also studied BMP-2–coated implants preclinically, finding that BMP-2–impregnated implants appeared to form denser augmented bone at a much lower overall BMP-2 dose.32 This suggests that the carrier function of the collagen sponge (and the BMP-2 dosage) may not be ideal against an implant surface for creating the appropriate bone quantity/quality, despite promoting osseointegration of the exposed titanium surface; however, this technique does not establish a specified alveolar form.

CONCLUSION

This study shows potential for further elucidating the role of recombinant human bone morphogenetic protein-2 (rhBMP-2) in encouraging morphologically specific alveolar ridge expansion under confining devices. Further investigation and study will be needed to explore the most effective bone augmentation strategies. It appears conclusive that soft tissue must not be fully excluded for optimal bone formation to occur in a timely manner and that a completely immobile and optimally fitted titanium shell must be used. This study also demonstrated a minimal effect of cortical bone perforations on bone formation in the setting of an excluding titanium barrier and that necrotic processes present within the titanium shell suggest an absence of blood supply and scavenger properties. The rhBMP-2 signal appeared to be unable to function within the chamber. However, where the signaling protein leaked out from the chamber margin, exuberant healthy bone formed peripherally, without evidence of necrosis.

Further studies should explore a shaping "barrier" function that allows for cell migration, proliferation, and differentiation at the site of interest, as well as BMP-2 delivery function, with an adequate number of specimens to achieve statistical relevance.

REFERENCES


