Bone-to-Implant Contact and New Bone Formation Within Human Freeze-Dried Bone Blocks Grafted Over Rabbit Calvaria

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Purpose: To assess the extent of osseointegration with rough-surface implants and new bone formation (NBF) within human freeze-dried bone blocks (h-FDB) grafted over rabbit calvaria. Materials and Methods: A total of 18 rectangular h-FDB blocks were stabilized bilaterally to the calvaria of nine New Zealand rabbits by two mini titanium screws each. A total of 18 rough-surface implants (5.0 × 6.0-mm) were placed, 9 simultaneously (immediate placement [IP]) on one side and 9 at 3 months after block grafting (delayed placement [DP]) on the contralateral side. At 12 weeks after the second surgical procedure, block biopsies were harvested and processed for histologic analysis. Morphometric measurements consisted of bone-to-implant contact (BIC) and the extent of NBF from the calvarial surface and outward into the block. A paired t test was applied for statistical analysis. Results: All h-FDB blocks were integrated, and the implants showed clinical stability. Histologically, the BIC was primarily between the apical end of the implants and the host rabbit calvaria. Bone growth between the implant threads was minimal and inconsistent among all animals. Morphometric measurements showed that the mean BIC of the IP and DP implants with the blocks was 10.50% ± 5.99% and 23.06% ± 9.58%, respectively (P < .001). NBF was observed primarily in the cancellous compartment of the block adjacent to the recipient calvarial bed. The extent of NBF into the block around the IP and DP implants was 9.95% ± 8.41% and 12.90% ± 11.07%, respectively (P = 0.2). Conclusion: In this model, a significantly lower BIC was demonstrated when implants were placed simultaneously with h-FDB block grafting compared to those placed in a two-stage mode. However, both techniques showed limited osseointegration. INT J ORAL MAXillofac Impl Ants 2017;32:768–773. doi: 10.11607/jomi.5366

Keywords: bone-implant contact, FDB bone blocks, freeze-dried bone, graft vascularization, new bone formation, osseointegration

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lveolar bone deficiency is one of the major obsta-
cles in implant reconstructions, often requiring restoration of the alveolar process via bone augmentation procedures. The application of autogenous bone block transplantation has a predictable outcome and well-established long-term success1–5; however, graft resorption,6–9 donor site limitations, and morbidity considerations10–14 call for alternative approaches. Consequently, other sources of allogeneic and xenogeneic origins have been proposed.15–29 Clinical reports on the use of onlay human freeze-dried bone (h-FDB) blocks with subsequent implant placement have shown promising clinical results.15,30–36 However, no controlled trials, comparative studies, or histologic analyses have been conducted to validate the efficacy of these grafted biomaterials.

In their systematic review,37 Waasdorp and Reynolds gathered literature on h-FDB onlay grafts for alveolar ridge augmentation from 1950 to 2008. Only nine publications met their inclusion criteria: two case
reports and seven case series with short-term follow-up. No randomized controlled clinical trials (RCTs) were identified in the search. Those authors concluded that there is insufficient evidence to establish treatment efficacy of graft incorporation, alveolar ridge augmentation, and long-term dental implant survival.

A recent systematic review of the literature provided updated histomorphometric and histologic characteristics of h-FDB blocks. Of the 15 articles that met the inclusion criteria (361 blocks), there was not a single report on the quality/quantity of the osseointegration. Only two studies that evaluated fresh-frozen osseous blocks also included a control group, and both reported that the majority of slides demonstrated large numbers of empty osteocyte lacunae in non-vital segments of necrotic bone and no direct contact between remodeled and grafted bone.

In the present study, h-FDB blocks were used as onlay grafts over rabbit calvaria. The rabbit skull has been used extensively as an appropriate site to observe and analyze grafted biomaterials, including xenografts, and animal models are well accepted for the analyses of these blocks both histologically and morphometrically. Minimal morbidity of the animal, ease of access to the site, and predictability of soft tissue management over the augmented site are just a few advantages of choosing this animal model. Therefore, the aim of this study was to evaluate the amount of direct bone-to-implant contact (BIC) between the h-FDB blocks and the implants, which were placed either simultaneously or in a delayed two-stage fashion. The different placement methods were to allow testing of whether the timing of implant placement has any impact in a non-integrated block vs a 3-month integrated block. The extent of new bone formation (NBF) in these blocks and the nature of the osseous connection between the grafted blocks and the recipient calvarial beds were also assessed.

**MATERIALS AND METHODS**

The institutional committee of animal care of Tel Aviv University approved the study. The study comprised 10 New Zealand female rabbits aged from 4 to 6 months weighing from 2.5 to 3.0 kg. They were kept in a calm secluded room in separate cages, fed Teklad Global Rabbit Diet (Envigo) daily, and given tap water ad libitum.

The surgical procedures were performed under general anesthesia following pre-sedation with 1.5 cc (20 mg) 2% xylazine base IM (Sedaxylan Veterinary, Eurovet Animal Health BV), followed by an IV combination of ketamine (Clorketam, Vetoquinol) 5 mg/kg + xylazine base (XYL – M 2, Veterinary) 1 mg/kg. In addition, a transdermal slow release (50 µg/h) sticky patch of fentanyl 8.25 mg (Novosis AG) was adhered to the rabbit’s shaved upper back for 3 days.

Local infiltration of 2% lidocaine hydrochloride with norepinephrine (1:100,000) was administered for hemostasis and reduction of postoperative pain.

Once anesthetized, the rabbit calvarium was exposed via a midsagittal longitudinal incision. Full-thickness dermal flaps were reflected, exposing the calvarial cortex. Cortical perforations were established by a 1-mm rounded diamond burr to increase vascular flow at the relevant site. In each calvarium, a pair of 10 mm (W) × 10 mm (L) × 5 mm (H) rectangular corticocancellous h-FDB blocks (LifeNet Health, Inc) were adjusted and placed on the rabbit’s parietal calvarial surface bilaterally. Blocks were stabilized by two 1.2-mm titanium screws (Osteomed) and an implant (5.0 mm × 6.0 mm; ATID, Alpha-Bio Tec Ltd) placed. This was referred to as the immediately placed (IP) implant (Fig 1).

The surgical wounds were closed by suturing in layers. The periosteal margins were approximated by
simple interrupted suture after releasing the flaps by periosteal incisions parallel to the main surgical one. A primary non-tensional soft-tissue closure was obtained using interrupted horizontal internal mattress suture followed by a continuous interlock suture. All sutures were made using a 5-0 resorbable Vicryl suture. At 3 months following this first phase of the surgery, a second implant, which was referred to as the delayed placed (DP) implant, was placed on the contralateral block (Fig 2).

An effort was made to place the implants at the block level while 1 mm of the most apical portion of the implant would be placed in the native calvarial bone. However, with this method, the implant platform might be left slightly higher than the level of the block.

Postoperative antibiotics were given for 3 consecutive days after each surgical intervention (0.5 cc of durabiotic 5% IM, Baytril, Bayer AG). During follow-up, one rabbit showed signs of distress and started to lose weight. Consequently, this animal was euthanized and dropped from the study, leaving nine rabbits available for study.

At 6 months following the first surgical procedure, the animals were injected with ketamine (1 cc) + xylazine (1.5 cc) followed by a lethal dose (30 mg/kg) of pentobarbitone sodium 200 mg/ml IV (CTS, Pharmaceutical Industries Inc). The animals were decapitated, and the surgical sites were retrieved en bloc. The specimens were put in 10% buffered formalin for 1 week and then transferred to a 70% ethanol solution. Radiographs of the specimen blocks were taken before further histologic processing.

**Histologic Processing**

Nine calvaria, providing 18 specimens total, were available for non-decalcified histologic processing, which was performed according to a standardized procedure. In brief, tissue biopsies were dehydrated using ascending grades of alcohol and xylene, infiltrated, and embedded in methylmethacrylate (Technovit 9100 NEU, Heraeus Kulzer). Three sections approximately 300 µm in thickness were obtained per block at the most central aspect of the titanium implant, and both osteosynthesis screws were fixed in place using a diamond band saw (Exakt, Apparatebau). The sections were ground to a final thickness of approximately 40 µm and stained with toluidine blue.

All measurements were jointly taken by two investigators (Z.A. and K.A.L.) while the identification of the site was masked. Histomorphometry was conducted on a screen monitor attached to the microscope (magnification ×35) using the Bioquant Nova Prime System (Bioquant Image Analysis Corp) software.

Direct BIC was compared between the IP and DP implants within the surrounding new bone out of the total block housing and particularly at its cancellous area (or portion). The BIC was also measured at the calvarial implant zone to be used as a reference. New bone formation (NBF) was calculated as the percentage of distance penetration of newly formed osseous tissue out of the total vertical dimension of the h-FDB. This was achieved by the mean measurements taken from peripheral mineralized stained areas proximal to the implants and proximal to the fixation screws close to the observed BIC. Also, a demarcation between the outer calvarial bony envelope and pale staining of osteoid formation served as accessory tools to distinguish between the host and the block mineralized zones. Apart from the IP and DP implants, the fixation screws, which represent machined-surface titanium, were also evaluated and recorded.
Statistical analysis was performed using the paired t test.

RESULTS

Histology and Histomorphometry

NBF was observed primarily in the cancellous compartment of the block adjacent to the recipient calvarial bed. Bone growth between the implant threads was limited and inconsistent in all specimens. The BIC was observed primarily between the apical end of the implant and the calvarium. The BIC within the h-FDB block was established mainly at the implant threads proximal to the host bone. Neither BIC nor bone growth into the threads was evident at the cortical zone of the block. The graft matrix showed no signs of osteoclastic or other resorption, and the osteocyte lacunae were, in general, empty (Figs 3 and 4).

The BIC in the IP and DP implant groups was 10.50% ± 5.99% and 23.06% ± 9.58%, respectively (Table 1). The differences were statistically significant (P < .001). Excluding the cortical portion, the BIC at the cancellous portion was 14.61% ± 7.51% and 35.67% ± 16.14% for the IP and DP implants, respectively (P < .002).

The BIC at the calvarial zone was significantly higher than the BIC within the block, with the mean grade varying from 68.11% ± 12.00% for the IP implants to 74.73% ± 19.36% for the DP implants. However, there was no significant difference between the groups. The NBF, indicating the vertical extent of bone formation into the block scaffold from the calvarial surface, was 9.95% ± 8.41% and 12.90% ± 11.07%, respectively (P = .2). After excluding the cortical non-vital zone of the blocks, the NBF was 15.30% ± 13.81% and 19.71% ± 16.59%, respectively (P = .216). There was no significant correlation between the BIC and the NBF in any of the groups.

It is noteworthy that the BIC between the machined surface titanium fixation screws and the bone growth was 10.8% ± 5.23% within the h-FDB and 15.0% ± 16.18% at its cancellous portion (Fig 5). The BIC to the fixation screws at the calvarial zone was remarkably higher (79.6% ± 27.3%; P < .0001).

<table>
<thead>
<tr>
<th>Implant/screw</th>
<th>BIC/calvaria (%)</th>
<th>BIC/blocks (%)</th>
<th>BIC/cancellous (%)</th>
<th>NBF/blocks (%)</th>
<th>NBF/cancellous (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP implants (n = 9)</td>
<td>70.7</td>
<td>10.5</td>
<td>14.6</td>
<td>9.9</td>
<td>15.3</td>
</tr>
<tr>
<td>DP implants (n = 9)</td>
<td>72.9</td>
<td>23.1</td>
<td>35.7</td>
<td>12.9</td>
<td>19.7</td>
</tr>
<tr>
<td>Fixation screws (n = 35)</td>
<td>79.6</td>
<td>10.8</td>
<td>15.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Calvaria = the host bed; block = trabecular + corticalis; cancellous = only the trabecular zone; N/A = not applicable.

Fig 5 A nondecalcification section of the titanium fixation screw in the h-FDB. The BIC is evident only at the calvarial host bed (H) and in the proximal area of the cancellous portion (Can) of the block. There is no sign of osseointegration at the cortical layer (Cor) of the block (nondecalcified toluidine blue staining, ×17.5 magnification).

DISCUSSION

This study was undertaken to investigate the potential use of h-FDB onlay grafts for alveolar ridge augmentation. Block stabilization was achieved by two fixation screws to a cortical perforated intramembranous osseous bed, which allowed vascular passage into the cancellous part of the block. Implants were placed either simultaneously (IP) or after 3 months of block integration (DP); thus, the establishment of osseointegration was assessed in two different non-vital/viable tissue surroundings. Clinically, all h-FDB blocks had been well integrated. At 6 months, the NBF as well as some sparse BIC around the implants’ titanium surfaces were evident. Histologically, the amount of NBF in the blocks was quite limited. Both NBF and BIC were observed primarily near the calvarial bed. Since there was only an average of 10% to 13% of NBF ingrowth in the h-FDB, most of the block became a non-vital scaffold. The middle and coronal parts of the implant's
osseous housing remained non-vital, and no osseointegration process could be identified. A similar outcome was recently reported in humans who received fresh-frozen allogeneic bone. 28,29

An appropriate biomaterial scaffold should allow vascular development. The fact that there is no evidence of vascularization in the block margins (ie, the compact cortex) would clinically eventually prevent vitalization, followed by inability to establish NBF in this particular area. Apparently, the perfusion of vessels within this region is obstructed. This might explain in part the difference between the cortical and the trabecular zones in terms of tissue replacement. Currently, the present authors are investigating tracing the vascular formation rate and angiogenesis in these grafted blocks in a similar animal model by tetracycline and calcein labeling.

Implants were placed in their apical zones at the native/host bone. This was to assure initial stability and to initiate an immediate osseointegration process.

In addition, the calvarial cortical perforations enabled enhancement of vascular pathway to the stabilized attached block.

Implants were placed at two points in time: simultaneously with the blocks and at 3 months following block grafting. The outcomes of both BIC and NBF showed that the two-stage approach had an advantage over the combined technique—there was a significant difference in favor of the DP implants over the IP implants. This could be attributed to the fact that the DP implants were installed in a 3-month partly vital h-FDB, which provides better vascularization and better mechanical stability at the portion in which there is direct contact between the pristine calvarial bone and the implant surface.

In general, the greater BIC over NBF could be related to the fact that the rough surface of the implant serves as an osteoconductive vehicle, which is not proven as related to the blocks themselves. Apparently, the combination of placing an implant surface that has been proven by evidence to be osteoconductive in an already remodeled and viable grafted block would be the timing of the ideal treatment approach.

Previous studies with autogenous blocks 47,48 and/or particulate biomaterials 49 have also shown a discernible difference in the amount of BIC and NBF in favor of the DP approach. It would appear that augmented bone could serve as appropriate osseous housing for an osseointegrated implant, provided that the grafted biomaterial (h-FDB) had first been revascularized and repopulated by new bone growth, thus making it comparable to a pristine alveolar ridge.

The lack of long-term evidence-based data, as reflected in current reviews, 50,51 warrants in-depth methodical future research to determine the long-term efficacy of h-FDB in terms of stable osseointegration and the capability of total new bone growth replacement.

CONCLUSIONS

The experimental animal model used in the current study demonstrated limited and inconsistent new bone growth into human freeze-dried corticocancellous bone blocks. This indicates that success of osseointegration of implants placed in these block grafts in augmentation procedures is probably uncertain.

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REFERENCES


