Histomorphometric Evaluation of Onlay Freeze-Dried Block Bone and Deproteinized Bovine Bone with Collagen in Rat

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The aim of this study was to evaluate the effect of human freeze-dried bone block (FDBB) and deproteinized bovine bone with collagen (DBBC) on bone formation when applied as an onlay graft in rat calvariums. Thirty male Sprague-Dawley rats received collagen sponge (control), FDBB, or DBBC onlay grafts trimmed into 8-mm disks measuring 4-mm height. Each graft was secured onto the calvarium surface using horizontal mattress sutures. Rats in each group were killed at 2 (n=5) or 8 (n=5) weeks postoperatively for histologic and histomorphometric analysis. The total augmented area (mm²), new bone area (mm²), and bone density (%) were measured. The FDBB and DBBC groups showed significantly more new bone formation and bone density than the control group at 2 and 8 weeks. The increased new bone area was significantly greater in the FDBB group than in the DBBC group (p<0.05). The total augmented area was significantly higher in the FDBB and DBBC groups at 2 and 8 weeks than in the control group (p<0.05), and at 8 weeks, the area was significantly decreased in the DBBC group compared to that in the FDBB group and the area at 2 weeks (p<0.05). Within the limitations of the present study, we concluded that onlay FDBB and DBBC grafts caused new bone formation through an osteoconductive mechanism. In addition, compared to FDBB, DBBC had less capacity to form new bone and maintain the space.

Key Words: Human freeze dried corticocancellous bone block; Deproteinized bovine bone with collagen; Onlay graft; Bone formation; Space maintenance

INTRODUCTION

Augmentation of the alveolar bone has gained attention in the field of dental surgery, and many studies have investigated methods for restoration of resorbed alveolar bone following tooth extraction, especially as dental implants have become increasingly common. One frequently used alveolar bone augmentation technique is bone grafting with autogenous bone or bone substitute [1-3]. Among the multiple factors considered in bone grafting procedures, the particular type of bone graft material is one of the important elements determining clinical success. Autogenous bone is the gold standard and first choice clinically and has long been considered the most stable graft material with superior ability to trigger bone formation [4]. However, there are several concerns in autogenous bone grafting, it requires a second donor site, carries an increased surgical time and cost, and may be limited by the volume of obtainable bone [5-7]. Therefore, development and advancements in artificial graft materials lacking these limitations as a replacement of autogenous bone are being actively pursued.

Several studies and clinical reports of allogenic bone graft materials, such as human freeze-dried bone allograft and human demineralized freeze-dried bone allograft (DFBA), and xenogenic bone graft materials, such as deproteinized bovine bone (DBB) and deproteinized bovine bone with collagen (DBBC), have found that these as graft materials can replace autogenous bone [8-10]. Recently, allogenic graft materials that were fabricated into block form have been evaluated for the restoration of vertical height in patients with severe resorption of the alveolar ridge. These studies were intended to address the limitations of particle-type graft materials, which are especially susceptible to deformation caused by stress that de-
velops within the tissue during postoperative healing [11,12]. There is a need to prevent collapse of the graft material and maintain the space for bone regeneration by using additional materials such as titanium mesh or a titanium-reinforced barrier membrane [13].

Several clinical studies confirmed the predictability of block-type allografts, and found that a tight bone fusion could be established at the onlay bone-recipient interface [13-15]. In addition to these benefits, allogenic graft materials also carry the risk of infection and may cause non-uniform bone formation depending on the supplying bone bank [16]; as a result, xenograft and alloplast are being actively investigated as alternatives to overcome these disadvantages. Among them, deproteinized bovine graft material (Bio-Oss® collagen, Geistlich Pharma AG, Wolhusen, Switzerland), which has a bony trabecula that closely resembles the bone in humans, is one of the most widely used bone substitutes compared with other xenogenic bones. It reportedly has effective osteoconductivity, and many recent studies of DBBC have added 10% collagen to the grafted bovine bone to provide shape [17,18].

Nevins et al. [19] reported that owing to its superior ability to maintain the alveolar space, DBBC can be used without a barrier membrane for intrabony defects in relatively good shape; they also found that its particle adhesion to the damaged area was outstanding. Sculean et al. [20] demonstrated how easily DBBC can be applied clinically as the collagen fibers maintain the shape of the tissue by holding the particles together. Most of those studies were performed in periodontal defects, extraction sockets, and bony dehiscence, in which structural integrity was preserved and space was maintained for bone regeneration [21,22]. However, there are no reports describing DBBC grafting in onlay form, and histological and histomorphometric analysis studies are lacking.

Therefore, in this report, the bone formation and space maintenance in human freeze-dried bone and DBBC grafted in block form onto rat calvarium were comparatively analyzed using histological and histomorphometric analyses.

**MATERIALS AND METHODS**

**Experimental animals**
Thirty male Sprague-Dawley rats (body weight, 200–300 g) were used. They were maintained in cages in a room with 7-h day/night cycles, an ambient temperature of 21°C, and a standard laboratory pellet diet. Animal selection and management, surgical protocols, and preparation procedures were approved by the Institutional Animal Care and Use committee, Ewha Medical Center, Seoul, Korea (confirmation number: ESM 12-0188).

**Materials**
Absorbable atelo CS (TERUPLUG®; Olympus Terumo Bio-materials, Tokyo, Japan) consists of 85–95% collagen type I and 5–15% collagen type III, and is cross-linked by heat treatment for biocompatibility. Human freeze-dried bone block (FDBB) (AlloBone; Osteo.in, Seoul, Korea), which is sterilised by low dose gamma irradiation, was used. It was composed of outer cortical and inner cancellous bone. DBBC (Bio-Oss® Collagen; Geistlich Pharma AG, Wolhusen, Switzerland) consists of cancellous bovine bone granules with the addition of 10% purified porcine collagen, and is sterilised by gamma irradiation. It is served as a matrix consisting of interconnection with macro and micropores (250 to 450 µm). The calcium components were varied in 38~42% and the phosphorus were in 12.5~17.5%. All the materials were trimmed into disk shape with 8 mm in diameter and 4 mm in height (Fig. 1).

**Surgical procedures**
The animals were anaesthetised by an intramuscular injection [0.1 mL/10 g of a 3:2 solution of Zoletil® (Virbac, Carros, France): xylazine (Rompun, Bayer Korea, Seoul, Korea)]. Full-thickness flap was reflected, thus exposing the calvarial bone. With the use of round burs, the recipient bed was subjected to six 1-mm wide monocortical perforations. Then the material was implanted on the parietal bone and stabilised with horizontal mattress suture, and for control experiments, the CS was im-

**Figure 1.** Preparation of graft materials. (A and B) Freeze-dried bone block group. (C and D) Deproteinized bovine bone with collagen group.