Effects of Block Bone Substitutes Loaded with *Escherichia Coli*-Produced Recombinant Human Bone Morphogenetic Protein-2 on Space Maintenance and Bone Formation in Rat Calvarial Onlay Model

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We aimed to evaluate the effects of onlay-type grafted human freeze-dried corticocancellous bone block (FDBB) and deproteinized bovine bone with collagen (DBBC) loaded with *Escherichia coli*-produced recombinant human bone morphogenetic protein-2 (ErhBMP-2) on space maintenance and new bone formation in rat calvaria. Collagen sponge (CS), FDBB, or DBBC disks (8×4 mm) with ErhBMP-2 (2.5 μg) were implanted onto the calvaria of male Sprague-Dawley rats, whereas CS with buffer was implanted onto the calvaria as controls (n=20/carrier). Rats were killed at 2 or 8 weeks post-surgery for histologic and histomorphometric analyses; total augmented area, new bone area, and bone density were evaluated. At both time-points, all ErhBMP-2 groups showed significantly higher new bone area and bone density than the control group (p<0.05). ErhBMP-2/FDBB and ErhBMP-2/DBBC groups showed significantly higher total augmented area than ErhBMP-2/CS group (8 weeks), and ErhBMP-2/FDBB group showed significantly higher new bone area and bone density than ErhBMP-2/DBBC group (p<0.05). ErhBMP-2/CS group showed the highest bone density (p<0.05). Combining ErhBMP-2 with FDBB or DBBC could significantly improve onlay graft outcomes, by new bone formation and bone density increase. Moreover, onlay-grafted FDBB and DBBC with ErhBMP-2 could be an alternative to autogenous block onlay bone graft.

Key Words: Freeze-dried corticocancellous bone block; Deproteinized bovine bone with collagen; *Escherichia coli*-produced recombinant human bone morphogenetic protein-2; Onlay graft; Space maintenance; New bone formation

INTRODUCTION

Sufficient bone width and height are the prerequisite for functional and esthetic implant. Horizontal and vertical bone augmentation of severely destroyed alveolar ridge would be necessary, and various biomaterials and surgical techniques have been developed for bone augmentation [1].

Autogenous bone grafts have been considered the gold standard in reconstructive surgery because of their bone regeneration mechanisms through osteogenesis, osteoinduction, and osteoconduction [2]. However, reconstruction with autogenous block onlay bone graft has shown clinical limitations such as donor site morbidity, limited volume of obtainable bone, increased time, the potential for complications, and pronounced resorption, especially in sites receiving mechanical load and soft tissue tension [3-5].

For these reasons, recent researches have been concentrating on the development and evaluation of allogenic or synthetic block type bone substitutes for onlay graft. Allografts obtained from donors of the same species have biologic activity containing human mineralized component and collagen. The most common used form of allografts are frozen, freeze-dried, demineralizes freeze-dried, and irradiated. Freezing or freeze-drying the bone significantly reduced the antigenicity [6]. The freeze-dried bone allografts (FDBA) have been frequently applied for the periodontal and perimplant defects especially in particulated form. FDBA effectively enhanced bone formation through long graft resorption time and induce induc-
tive potential through growth factors stored in matrix [7].

Freeze-dried corticocancellous bone block (FDBB) has better mechanical stability compared to the particulated FDBA as well as osteoconductivity. Despite the successful results with FDBB in orthopedic surgery for decades, there have been only limited studies including case reports in alveolar ridge augmentation [8-11]. In our previous study, onlay grafts of human FDBB showed new bone formation in the rat calvarium through an osteoconduction. However, it appeared that de novo bone formation was limited [12].

Deproteinized bovine bone mineral (Bio-Oss®, Geistlich AG, Wollhusen, Switzerland) is a widely utilized commercial product in the form of cortical granules and well documented in periodontal and implant dentistry [13]. It is a natural matrix which is identical to the mineral phase of human bone. It has been reported to be highly osteoconductive and to have a very low resorption rate [14]. It has the macro- and micro-pore structure and the pores are of optimal size and configuration to facilitate vascular ingrowth, which is essential for new bone formation [15]. Thus, it was used as an efficient and affordable drug delivery systems [7]. However, despite the many advantages of deproteinized bovine bone mineral, it has also a shortcoming of particulated graft materials, i.e., difficulty in space maintenance without additional membrane.

Deproteinized bovine bone with collagen (DBBC) (Bio-Oss Collagen®, Geistlich AG, Wollhusen, Switzerland) consists of 90% of cancellous bovine bone granules and 10% of porcine collagen. Combining deproteinized bovine bone mineral with collagen enhances its handling characteristics making DBBC formable and easy to handle, and this give DBBC mechanical integrities. However, despite the many advantages of DBB, xenogenic block grafts could be brittle and show low toughness when used in vertical bone augmentation [11]. It has shown that it served as a scaffold and preserved the ridge profile, but did not enhance the new bone formation [16]. Moreover, in our previous study the onlay grafted DBBC showed limited new bone formation, because the collagen of DBBC was absorbed after a few weeks and did not replace the barrier function of membrane [17]. Thus, it is necessary to combine an osteoinductive growth factor, such as bone morphogenetic protein (BMP) with FDBB and DBBC [18].

BMPs are a set of growth and differentiation factors acting on early osteoprogenitor cells so that they differentiate into mature osteoblasts. Several BMPs, including BMP-2, -4, -6 and -7, have been reported to have significant osteoinductive potential [19,20]. Among these, recombinant human BMP-2 (rhBMP-2) was found to have strong in vivo bone-inducing ability [20-22]. Previously, most rhBMPs have been produced in mammalian cells, such as the Chinese hamster ovary cells [23]. However, its low yield (ng/mL) and high cost produced in this eukaryotic protein expression system might be a limitation for clinical applications. Therefore various attempts have been made to evaluate biologically active rhBMPs in Escherichia coli (E. coli), as an alternative to mammalian cells. It has shown comparable biologic activity in comparison with rhBMP produced in a eukaryotic system [24-26], and would enable the high yield of rhBMPs at low cost.

However, despite plenty of BMP researches, the ideal carrier has not yet been found. Absorbable collagen sponge (CS) is one of the most frequently used carriers of BMPs, and its regenerative capacity has been identified in many researches [27]. However, the structural stability was uncertain in some animal and human studies [28,29], it becomes victim to compressive forces especially when used for non-space-providing onlay indications [25]. If the block type bone substitutes such as FDBB and DBBC are used for BMPs’ carrier, we might expect that the cancellous portion of FDBB or the porous structure of DBBC could entrap the BMPs and release them slowly and, in addition, the rigid structure of them could provide resistance against compressive force.

Therefore, in this study, we used three carriers—CS, FDBB, and DBBC—loaded with Escherichia coli-produced recombinant human bone morphogenetic protein-2 in rat calvarium. The aims of the current study were to evaluate the effects of onlay type grafted FDBB and DBBC loaded with ErhBMP-2 on the space maintenance and new bone formation in the rat calvarium and to elucidate the efficacy of onlay grafts with FDBB and DBBC in point of eliminating the need for autogenous block onlay bone graft.

MATERIALS AND METHODS

Animals
Eighty male Sprague-Dawley rats (200–300 g) were used and maintained in cages in a room with 7-h day/night cycles, an ambient temperature of 21°C, and a standard laboratory pellet diet. All procedures were approved by the Institutional Animal Care and Use committee, Ewha Medical Center, Seoul, Korea (confirmation number: ESM 12-0188).

Materials
The expression of rhBMP-2 in E. coli was performed at the Research Institute of Cowellmedi Co. Ltd., Busan, Korea. Absorbable atelo CS (TERUPLUG®, Olympus Terumo Biomaterials, Tokyo, Japan) is cross-linked by heat treatment for biocompatibility. FDBB (AlloBone, Osteo.in, Seoul, Korea), and DBBC, a xenograft blended of granules of deproteinized bovine bone (90%) and purified porcine collagen fibers (10%) (Bio-Oss® Collagen; Geistlich