INTRODUCTION

Alveolar regeneration of the jaw bone is essential for implant-prosthetic oral rehabilitation, and adequate bone quality and quantity are required to ensure stability upon implant insertion. Several bone-grafting materials such as autogenous bone, allogeneic and xenogeneic bone, and alloplastic bone substitutes have been used in mandibular and maxillary alveolar bone augmentation. However, each graft material has various shortcomings, such as donor site morbidity and bone resorption after autogenous bone transplantation, infection risk and possible immune rejection reaction in allogeneic and xenogeneic material, and reduced bone forming capacity in allogeneic, xenogeneic and alloplastic graft materials [1,2]. To overcome these disadvantages, various approaches using osseous growth factors with or without stem cells have been tested [3-5].

Among the many growth factors, bone morphogenetic protein-2 (BMP-2) has been recognized as one of the most potent osteoinducers, as it can trigger the differentiation of mesenchymal stem cells (MSCs) to osteogenic cells for accelerated new bone formation, and it is now regarded as an important modulator in the formation and remodelling of bone tissue [6-9]. The application of BMP-2 has demonstrated that bone regeneration increases new bone formation in animal models [10,11] and in clinical applications for alveolar bone regeneration and maxillary sinus floor augmentation [12,13]. However, a higher dose of BMP-2 is required for enhanced bone formation in humans compared to animals [12,13], which can cause undesirable complications such as extensive swelling, seroma formation, and cystic bone lesion [14,15]. To avoid these complications, it is necessary to reduce the dose of BMP-2, which can decrease its bone forming capacity.

Comparative Study of BMP-2 Alone and Combined with VEGF Carried by Hydrogel for Maxillary Alveolar Bone Regeneration

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The effect of vascular endothelial growth factor (VEGF) combined with bone morphogenetic protein-2 (BMP-2) for bone regeneration is still controversial as to whether or not VEGF has a synergistic or additive effect. This study attempted to evaluate the synergistic effect of VEGF and BMP-2 compared to BMP-2 alone for maxillary alveolar bone regeneration using collagen sponge/hydrogel complex sheets in a canine model. After mixing BMP-2 and VEGF with a hyaluronic acid-based hydrogel (HAH), the collagen sponge/hydrogel complex was transplanted into maxillary alveolar bone defects (n=14) after the extraction of canine upper first molars on both sides. Bone regeneration was evaluated in three groups (control group without growth factors, experimental groups I and II with BMP-2 alone and BMP-2 and VEGF, respectively) using micro-computed tomography and histological staining. The total amount of new bone formations and bone mineral density were significantly higher in the group with BMP-2 only and the group with BMP-2 combined with VEGF than it in the control group. The area with positive staining of von Willebrand factor bone defect was significantly greater in the group with BMP-2 only and with dual growth factors than the control. BMP-2 released from the HAH promoted new bone formation. However, the combination of BMP-2 and VEGF did not show a synergistic or additive effect on bone regeneration at canine maxillary alveolar bone defects.

Key Words: Maxillary alveolar bone defect; Bone regeneration; Bone morphogenetic protein-2; Vascular endothelial growth factor; Synergistic effect

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Vascular endothelial growth factor (VEGF) can enhance osteogenesis through osteoblast differentiation and the transport of precursor mesenchymal cells to the mineralized region via newly formed vessels [16,17]. Therefore, an additional growth factor such as VEGF may provide helpful compensatory action in bone regeneration with low-dose BMP-2. Moreover, a synergistic effect of VEGF in combination with BMP-2 may be beneficial for enhanced bone regeneration, especially for early functional loading of dental implants after bone grafting. However, there is still controversy in animal studies whether the effect on osteogenesis of VEGF in combination with BMP-2 is synergistic or additive. Patel et al. [18] reported that combined delivery of VEGF and BMP-2 significantly enhanced osteogenesis in a rat critical-size defect model compared with BMP-2 alone. Zhang et al. [19] reported that biomaterial involving the growth factors BMP-2 and VEGF increased angiogenesis and new bone formation for maxillary sinus augmentation in experimental animals. However, a study by Young et al. [20] and Kempen et al. [21] showed that VEGF combined with BMP-2 did not have a synergistic effect on bone regeneration in rat bone calvarial or femoral defects. In a study by Kim et al. [22], bone regeneration at supra-alveolar peri-implant defects in the canine mandible was not significantly different between BMP-2 alone and BMP-2 combined with VEGF.

Currently, novel bone biomaterials incorporated with various osteogenic growth factors are being studied to replace bone-grafting materials. It has been shown that various biomaterials from the gelatin hydrogel complex [23], injectable hydrogel [19] or collagen sponge [12] incorporate with growth factors enhance bone regeneration in human and animal experiments. However, these osteogenic growth factors were difficult to maintain at defect sites for treatment in vivo [24]. Of the scaffolds for new bone formation, hydrogels plays a key role in new bone formation applications, such as osteogenic cell proliferation and the repair of bone defects. In particular, biomimetic hydrogels are a smart material, controlling degradability and growth factor release [3,19,25]. In addition, osteogenic effects of BMP-2 with collagen sponge have been extensively investigated, especially new bone formation and dental restoration [1,12,26]. Thus, the combination of hydrogel and collagen gel for bone regeneration could be more effective in bone regeneration than only a single osteoconductive scaffold.

Based on this knowledge, we used scaffolds of collagen sponge/hydrogel incorporated with BMP-2 and VEGF and compared the effects of BMP-2 alone and the combination of BMP-2 and VEGF growth factors on an alveolar bone defect dog model. Furthermore, based on the controversial results in relation to the effect of dual delivery of BMP-2 and VEGF, especially at the jaw bone with relatively abundant vasculature, we have attempted to evaluate the synergistic or additive effect of VEGF combined with BMP-2 delivered with collagen sponge/hyaluronic acid-based hydrogel (HAH) on maxillary alveolar bone regeneration in large animals in comparison with BMP-2 alone.

**MATERIALS AND METHODS**

**Materials**

**Preparation of the hydrogel**

MMP-sensitive hyaluronic acid (HA)-based hydrogel was prepared using acrylated HA, as previously described [27,28]. Briefly, acrylated HA (4wt%, 230 kDa) was dissolved in 0.3 M triethanolamine (TEA) buffered solution (pH 8). MMP-sensitive peptide (GCRDGQPGIWGQDRCG) was dissolved in 0.3 M TEA buffer and then added to acrylated HA solution with the same molar ratio of acryl and thiol groups. The reaction mixture was incubated at 37°C for gelation. The HA-based hydrogel was formed via Michael-type addition reaction. VEGF (recombinant human VEGF165, R&D Systems, Minneapolis, MN, USA) and recombinant human BMP-2 (Novosis®-Dent, CGBio Inc., Seongnam, Korea) were incorporated into the HA-based hydrogels. These HA-based hydrogels were used for the in vivo experiments.

**Preparation of the collagen sponge**

Collagen matrices were prepared as described elsewhere with slight modifications [29]. Briefly, homogenized 1.5% collagen solution (w/v, pH 7.4) was poured into the mould and lyophilized. Freeze-dried collagen matrices were cross-linked with 20 mM EDC for 24 h. Residual EDC was washed out with autoclaved distilled water for 5 times. Rinsed collagen matrices were relyophilized and kept at 4°C until further use.

**Animals**

Alveolar bone defects at the upper first molar on both sides (n=14) in seven adult beagle dogs (1 year old, 10 kg) were used in this study. All the animals were treated and handled in accordance with the “Recommendations for Handling of Laboratory Animals for Biomedical Research” compiled by the Committee on the Safety and Ethical Handling Regulation for Laboratory Experiments at the School of Dentistry at Seoul National University. The animals were maintained in the animal facility, where a constant room temperature of 22°C was maintained. The animals were fed a soft diet during the first two postoperative weeks, otherwise, there was no restriction of food.

**Surgery**

The surgical design of this study involved the creation of an...