Development of a novel osteophilic BMP-2 carrier using polyphosphate diester

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INTRODUCTION: Bone morphogenetic protein 2 (BMP-2) is a cytokine with strong osteoinductive ability and have been clinically applied for nonunions and spinal fusion surgeries combined with collagen carrier. However, collagen carriers have poor retention of BMP-2, and side effects such as dose-dependent inflammation and osteolysis caused by burst release of BMP-2 prevent their widespread use (Ref.1). To mitigate these adverse events, the use of various carriers such as polymers, inorganic materials and hydroxyapatite (HAp) have been investigated, but there have been few reports as for carriers having osteophilic properties. Polyethylene sodium phosphate is a hydrophilic polymer that mimics the main chain structure of DNA and has recently been reported to show high cytocompatibility and bone affinity (Ref.2). The purpose of this study is to develop a novel osteophilic BMP-2 carrier using a type of polyethylene sodium phosphate (CHPEP·Na).

METHODS: CHPEP·Na was synthesized by a previously described method (Ref.3). BMP-2 (400 nM) and CHPEP·Na (600 μM) in PBS was mixed at room temperature or 90 °C with stirring for 10 min to form CHPEP·Na/BMP-2 complex. The free polymers were removed by centrifugal filtration and the complexes were then redispersed in PBS. The size and PDI of complexes were analyzed by dynamic light scattering (DLS) and transmittance electron microscopy. All animal experiments were approved by the Animal Experimental Committee of our institution. We investigated *in vivo* release kinetics from grafted bone with subcutaneous allogeneic bone grafting model of rat, and bone formation analysis with subfascial allogeneic bone grafting model of rat. Firstly, we prepared the allogeneic graft bone: After harvesting allogenic iliac bone of ten rats, the bone was pulverized into small pieces and packed into one hundred and eighteen plastic tubes for bone graft (50mg per each). Secondly, we formed a fluorescently labeled BMP-2 with an amine-reactive (NHS ester) near-infrared fluorochrome, and the complex with fluorescently labeled BMP-2 was formed in the procedure previously described above. 1μg of labeled BMP-2 were mixed with an allogenic graft bone and implanted in the left upper leg, and the complex containing 1μg of labeled BMP-2 were mixed with an allogenic graft bone and implanted in the right upper leg of four 8-weeks-old male SD rats (n = 4). Fluorescence imaging was performed with an in vivo imaging system (IVIS) until 21 days after implantation. Thirdly, 10μg of BMP-2 were mixed with fifty-four allogenic graft bones and implanted the graft bones underneath the left fascia of the dorsal muscle (3 for one rat), and the complex containing 10μg of BMP-2 were mixed with fifty-four allogenic graft bones and implanted the graft bones underneath the right fascia the dorsal muscle of SD rats (3 for one rat, total 18 rats). Six rats each were euthanized at 0 day, 10 days and 21 days after implantation (n=6). The excised bone graft samples were used for microcomputed tomography (micro-CT) and histology analyses. An unpaired t test was used for statistical analysis to compare bone volume (BV) of grafted bone.

RESULTS: DLS measurements confirmed the formation of a complex with a diameter of 73 nm (polydispersity: 0.26) upon mixing with BMP-2. The IVIS analysis showed that in the BMP-2 group, BMP-2 was released early after implantation (half-life: 3.2 hours), whereas in the complex group, it was confirmed that it remained in the grafted bone for a long time after implantation (half-life: 6.9 days) (Fig.1). The micro-CT analysis showed that the total BV of grafted bone samples was significantly higher with complex group than BMP-2 group at day 10 (BV: BMP-2 group, 24.0 mm^3; complex group, 40.8 mm^3; $p<0.05$ by unpaired t test.) (Fig.2A, B). The histological analysis revealed that newly formed bone volume was higher with complex group than BMP-2 groups at day10 (Fig.2C). The bone volume of BMP-2 groups at day 21 was significantly decreased than that at day 10, whereas the bone volume loss was suppressed in complex group (BV: BMP-2 group, 3.27 mm^3; complex group, 28.4 mm^3; $p<0.001$ by unpaired t test.).

DISCUSSION: We successfully developed CHPEP·Na/BMP-2 complex and demonstrated the *in vivo* long-term BMP activity of the complex. The characteristics of the complex led to the enhanced new bone formation. The osteophilic properties of the complex are also expected to inhibit the diffusion of BMP into surrounding tissues and reduce complications associated with BMP use. Thus, the CHPEP·Na/BMP-2 complex can be an alternative to the current BMP/collagen products for efficient and safe bone regeneration by BMP.

SIGNIFICANCE: We Developed a novel bone tropic carrier for BMP-2 using polyethylene sodium phosphate. The complex of BMP-2 with CHPEP-Na, a bone tropic carrier, can be an option for efficient bone regeneration with BMP-2.

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IMAGES AND TABLES:

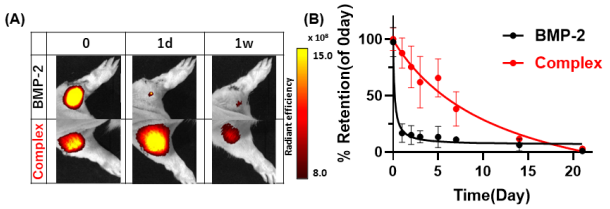

Figure 1. *In vivo* release kinetics of fluorescently labeled BMP-2
In the BMP-2 alone group, BMP-2 was released early after implantation, whereas in the complex group, it was confirmed that it remained in the grafted bone for a long time after implantation.
(A) Images of IVIS at day 0, 1, 7. (B) In vivo release kinetics based on fluorescence quantification at the implantation sites.

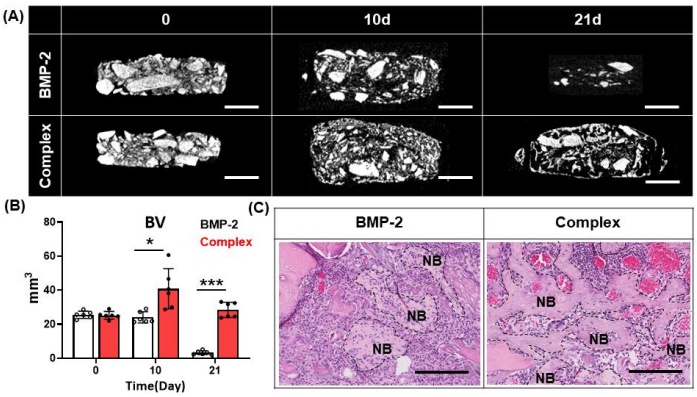

Figure 2. Bone formation analysis with subfascial bone graft model
(A) The total BV of grafted bone samples was significantly higher with complex group than BMP-2 group at day 10 and the total BV of BMP-2 groups at day 21 was significantly decreased than that at day 10, whereas the bone volume loss was suppressed in complex group. (Scale bar = 1mm) (B) Total bone volume of grafted bone (*p<0.05, **p<0.01, ***p<0.001). (C) The histological analysis revealed that newly formed bone volume was higher with complex group than BMP-2 groups at day10. (Scale bar = 200μm, NB = new bone)