

# Effects of rhBMP-2 loaded hydroxyapatite granules/ $\beta$ -tricalcium phosphate - hydrogel (HA/ $\beta$ -TCP/hydrogel) on a new rat model of nonunion

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**INTRODUCTION:** Nonunion is one of the most challenging conditions to treat in orthopedics. The current standard treatment for nonunion is bone grafting (autologous or artificial bone), but bone fusion rates are not satisfactory. One promising treatment is bone regeneration therapy using bone morphogenetic protein 2 (BMP-2). BMP-2 has strong osteoinductive properties and has attracted attention in the treatment of nonunion. However, insufficient bone-forming effects and adverse events related to inflammatory reactions have prevented its clinical application. We have developed a novel composite material, NOVOSIS putty (NP), which combines hydroxyapatite(HA),  $\beta$ -tricalcium phosphate microsphere( $\beta$ -TCP)/poloxamer 407-based hydrogel, and recombinant human (rh) BMP-2. Hydrogel in NP enables sustained release of BMP-2 for more than 2 weeks, and  $\beta$ -TCP and HA provide a scaffold for bone formation. NP demonstrated excellent bone regeneration potential in a spinal fusion model. (Ref.1,2). In this study, we investigated the effects of NP on nonunion using a novel rat model of femoral nonunion.

**METHODS:** The Animal Experimental Committee of our institution approved all animal studies (approval number: 04-078-001). Forty 8-week-old male Sprague–Dawley (SD) rats (Charles River Laboratories, Japan Inc.) were used in this study. Nonunion was created by intervening silicone discs at the femoral fracture site, followed by intramedullary nail fixation for three weeks. The acquisition of bone fusion in this nonunion model was compared between the three grafting materials for nonunion sites: NP + 10 $\mu$ g of BMP 10  $\mu$ g (NP group, n=13), collagen sponge (CS) + 10 $\mu$ g of BMP (CS group, n=14), iliac bone (IB group, n=13). The rats were sacrificed six weeks after the grafting surgery and evaluated by X-ray,  $\mu$ CT, and histology (H&E and Safranin-O staining) for bone fusion rate and new bone quality.

**RESULTS:** By inserting a silicon disc at the fracture sites, the cortical bone in the nonunion area was atrophied and formed a soft callus. And, these pathological conditions were similar to nonunion in clinical practice. After the grafting surgeries, the fusion rate of the nonunion was 0% in the IB group, 69.2% in the NP group, and 50% in the CS group. The fusion rate in the NP group tended to be higher than that in the CS group, but the difference was not statistically significant (p=0.44). The  $\mu$ CT analysis showed that the BV/TV and BMD of the new bone in the NP group were significantly higher than those in the CS group (BMD: NP group, 0.73g/cm<sup>3</sup>; CS group, 0.23 g/cm<sup>3</sup>; p<0.0001 by Student's t-test. BV/TV: NP group, 64.5%; CS group, 54.7%; p<0.05 by Student's t-test.). The histological analysis revealed that the fracture gap was filled with new bone with abundant trabecular bone (TB) and a small amount of remnant HA granules (Fig.3 yellow arrows). In contrast, the number of trabecular bones was scarce in the CS group, and fatty bone marrow made up the majority (Fig.3).

**DISCUSSION:** Bone fusion in nonunion is more challenging than spinal fusion due to a lack of blood flow and osteogenic cells. Although NP did not show significant differences in bone fusion, it did allow regeneration with high-quality new bone. These results suggest that NOVOSIS Putty is expected to replace preexisting BMP-2 products (collagen sponge carrier) as a nonunion treatment option.

**SIGNIFICANCE:** In this study, a new clinically relevant rat nonunion model was created to test the therapeutic efficacy of NP as a new BMP carrier, demonstrating that BMP treatment is helpful for nonunion treatment.

## REFERENCES:

1. S. Nakagawa, T. Kaito, et al. Scientific Reports 2022 Vol. 12 Issue 1
2. D. Tateiwa, T. Kaito, et al. Scientific Reports 2021 Vol. 11 Issue 1

## IMAGES AND TABLES:

Figure 1. A novel rat model of nonunion in the femur

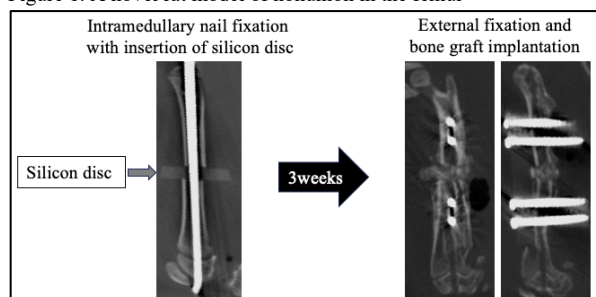


Figure 3. Histological analysis of newly formed bone at nonunion sites

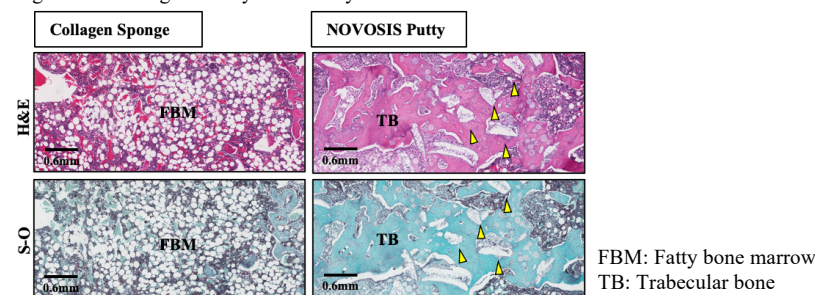


Figure 2.  $\mu$  CT result of nonunion

