A STUDY ON IMPLANTATION OF A NOVEL BIOMATERIAL, HYDROXYAPATITE/COLLAGEN COMPOSITE INTO THE WEIGHT-BEARING SITE

Relevance to Musculoskeletal Condition: Hydroxyapatite (HAp) has been popular as a substitute for autogenous bone implantation. However, HAp ceramic is not bioabsorbable, and because the rate of substitution in the case of artificially made HAp is as slow as 1 mm/year, loosening or breakage of HAp implants may occur after implantation. Furthermore, the brittleness and weakness of the metallic prostheses have restricted its medical application to only bone filler and film coating for metal materials. The development of a new material which is bioabsorbable and has osteoconductive activity is needed.

Introduction: A novel biomaterial, hydroxyapatite/collagen (HAp/Col) composite in which the c-axes of HAp nanocrystals were aligned along the collagen molecules was prepared under a cold isostatic pressure of 200 MPa. The 3-point bending strength of the composite was found to be 39.5±0.88 MPa, and Young’s modulus was 2.5±0.38 GPa. To increase its mechanical strength, the surface of the composite was cross-linked by gultaraldehyde. Osteoconductive activity of these materials with and without rhBMP-2 treatment implanted in the weight-bearing site were examined.

Materials and methods:

1. Implantation into the bone defect of tibia: The composite materials with surface cross-linking were trimmed into implants 15 mm in diameter and 20 mm in length, which had 1 central and 8 satellite vertical drill holes (each 3 and 1mm in diameter) and 16 transverse drill hole (1mm in diameter). They were implanted into bone defect of 20 mm of tibiae in 3 beagles and fixed with Ilizarov method. The composites containing rhBMP-2 (200 µg/ml) were also implanted in 3 beagles. In both the BMP-treated and non-BMP-treated groups, the implant was harvested from 1 dog after 12 weeks. The Ilizarov fixators of 2 dogs in the BMP-treated group were removed after 12 weeks, and full weight bearing began. The implants were harvested at 18 and 24 weeks after surgery. In the non-BMP-treated group, full weight bearing was continued with the Ilizarov fixator, and the implants were harvested at 18 and 24 weeks. Soft x-ray images were prepared each week. The bone mineral density (BMD) of the implant was measured at the time of implantation and when specimens were collected. The specimens were divided for 2 types of histological observation: 1) decalcified and undecalcified sections stained with hematoxylin and eosin (HE) and toluidine blue, and 2) undecalcified sections ground to thickness of 100 µm and stained with Villanueva bone stain.

2. Anterior fusion of the cervical spine: The composite materials without surface cross-linking were trimmed into cylindrical implants of 5x5x10 mm. With the implants containing rhBMP-2 (400 µg/ml) or its vehicle only anterior fusion between C3 and C4 vertebrae was operated on 5 beagles. In both the BMP-treated and non-BMP-treated groups, the implants were harvested from each 2 dogs at 12 weeks and 3 dogs at 24 weeks after surgery. Soft x-ray images were prepared each week. The specimens were prepared for light microscopic observation with HE, toluidine blue and Villanueva stain.

Results:

1. Implantation into the bone defect of tibia: Although, in the non-BMP-treated group callus formation was poor, the implant united with bone on the x-ray images until 12 weeks after operation. The composite had contacted tightly with the newly formed bone without interposed connective tissue with Villanueva bone stain. The Haversian system, and the non-calcified soft tissue (osteon) laid scattered in the boundary between the composite and the newly formed bone. A Howship's lacunae-like structure was formed on the composite, and multinucleated giant cells were observed attached to the surface of the composite with HE stain (Fig. 1). Spindle-shaped cells were observed arranged along the newly formed bone. Chondrocyte column was also observed. While, in the BMP-treated group, callus formation covering the whole implant was observed in the early stage after implantation. Medullary structure was formed through the implant at week 12, and maturation of the newly formed bone was observed at week 24. Histologically, too, hypertrophy of the cortical and trabecular bone were observed in the rhBMP-treated groups. Although maturation of the newly formed bone was not necessarily enhanced, chondrocyte column was scarcely observed. The composite materials with surface cross-linking remained as a block even after 24 weeks. The BMD of the intact bone of tibiae was 2.596±0.097 g/cm². The BMD was 2.806±0.155 g/cm² before implantation. The BMD in the non-BMP-treated group was 2.515 g/cm² and in the BMP-treated group 2.622 g/cm² after 12 weeks. The BMD in the non-BMP-treated group was 3.301 g/cm² and in the BMP-treated group 3.327 g/cm² after 24 weeks.

2. Anterior fusion of the cervical spine: In the non-BMP-treated group, callus formation was poor on the x-ray images, but the outline of the implants became faint by 12 weeks after surgery. Histological findings revealed that the greater parts of the composite materials without surface cross-linking were absorbed and permuted to bone as early as 12 weeks, and that the implant united with recipient bone without interposition of the soft tissue. In the BMP-treated group, callus formation appeared as early as after 2 weeks, and thick new bone coverage in front of the implant was observed after 12 weeks.

Discussion: The period until bone union after implantation of the HAp/Col implant on the x-ray image was approximately the same as that of autogenous bone graft. The transient of the BMD of the HAp/Col implants suggests that the material is once absorbed and implanted and the newly formed bone is added to the interface of the implant and recipient bone. Histological findings suggest that each implants grafted in the tibiae and the cervical spine could induce the development of osteogenic cells and bone remodeling units. After surface cross-linking by gultaraldehyde to increase mechanical strength of the composite the speed of permutation to bone was delayed. And chondrocyte column formation was observed especially in the non-BMP-treated group, which is thought due to micromotion between the implant and recipient bone. These findings suggest it may be effective to use the implants containing rhBMP-2 in the weight-bearing site, because it facilitates early callus formation and fixes the implant tightly.

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