Apatite-coated Collagen Sponge for the Delivery of Bone Morphogenic Protein-2 in Rabbit Posterolateral Lumbar Fusion

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Disclosures:
G. Im: None. J. Lee: None. J. Ahn: None. E. Kim: None.

Introduction: Bone morphogenetic proteins (BMPs) are potent osteo-inductive growth factors that have shown excellent fusion rate in many animal and clinical studies. Recent advances in genetic engineering enabled the production of recombinant human bone morphogenetic protein-2 (rhBMP-2), which has been put in commercial use. However, there are unsolved problems concerning the dose of BMPs and delivery systems. The short half-life and structural instability of BMP-2 require a high-dose administration of the protein. Implanted BMPs are rapidly released from ineffective carriers, leading to side effects. In the authors’ previous studies, the modification of type I collagen scaffold with apatite coating significantly increases the BMP-2 release period and osteogenic efficacy of BMP-2 in in vitro experiments and in vivo mouse calvarial defect models. Thus, the purpose of this study was to evaluate the effectiveness of apatite-coated collagen sponge as a long-term delivery system for BMP-2 versus conventional short-term delivery system in lumbar posterolateral fusion using a rabbit model.

Methods: Posterolateral lumbar fusion model
Group 1_Control group: uncoated collagen sponge without BMP-2: The first group (group 1, five rabbits; control group) was treated with uncoated collagen sponges without BMP-2 on both sides.
Group 2_Uncoated group: uncoated collagen sponge loaded with BMP-2: The second group (group 2, five rabbits) was treated with uncoated collagen sponge loaded with the BMP-2 in the dose of 40 μg on each side.
Group 3_Apatite-coated group: apatite-coated collagen sponge loaded with BMP-2: The third group (group 3, five rabbits) was treated with apatite-coated collagen sponge that was loaded with BMP-2 in the dose of 40 μg each side.

Operative procedures
Each rabbit underwent posterolateral intertransverse process fusion at L4-5 or L5-6 as previously described. Anesthesia was established using xylazine hydrochloride (Bayer, Seoul, Korea) at 5 mg/kg intramuscularly, followed by ketamine 35 mg/kg administered intramuscularly 5 min after the first injection. Anesthesia was performed by an independent animal handler in accordance with the standard protocol. After shaving and preparing the lower lumbar area, a longitudinal skin incision was made. Separate fascial incisions were followed on each side, and bilateral subperiosteal dissections were performed laterally to the transverse processes. The soft tissue dorsal to the intertransverse membrane was stripped laterally, and the transverse processes were decorticated with a high-speed burr. A collagen sponge was then placed on each side of vertebrae, and the fascia and skin were sutured.

Fusion assessment
All rabbits were euthanized 6 weeks after operation, and the fusion status were assessed by radiographic study, micro-CT, biomechanical study.

Results: Radiographic study: In the control group, there was no case that had achieved fusion in radiographic study (0/5). In the uncoated group, 3 cases had radiographic fusion at 6 weeks after surgery (3/5). In the apatite-coated group, 4 case achieved fusion by radiographic analysis (4/5). Apatite-coated group had significantly higher rate of fusion than the control group (p=0.024) while the uncoated group did not (p=0.083).
CT scan: Reconstructed CT images were used in the analysis of fusion masses from the operated segment. There was no evidence of ectopic ossification beyond inter-transverse spaces. Using 3-D reconstructed images, fusion rate was 0% (0/5) in the control group and 60% (3/5) in the uncoated group, and 80% (4/5) the apatite-coated group. Accordingly, the apatite-coated group had significantly higher rate of fusion than the control group (p=0.024) while the uncoated group did not.
Biomechanical test: There was no specimen that failed at sites other than the fusion mass. BMP-only group (202.3N, P=0.0016) and apatite-coated group (265.1N, P<0.0001) had significantly higher tensile strength than that of the control group (93.6 N). The difference between uncoated group and apatite-coated group was also significant (P=0.028).

Discussion: BMP-2 delivery system using apatite-coated collagen sponge showed efficient bone regeneration and more solid fusion compared with uncoated collagen sponge in posterolateral lumbar fusion model. Apatite-coated collagen sponge can be an effective carrier for the delivery of BMP-2 to promote spine fusion.

Significance: The short half-life and structural instability of BMP-2 require a high-dose administration of the protein, which can cause some adverse effects including the bony overgrowth, osteolysis, and various immune reactions as well as the increased
cost of application. Therefore, it is essential to develop an efficient delivery system to lower the required dose of BMP-2. Moreover, the bone formation efficacy of BMPs can be different according to the delivery system.

**Acknowledgments:** This study was supported by Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A100443).

**References:**
TABLE 1. The results of biomechanical testing

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<tr>
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<th>Tensile strength (SD)</th>
<th>P value (vs. control)</th>
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<tbody>
<tr>
<td>Control</td>
<td>93.6 (37.1)</td>
<td></td>
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<tr>
<td>BMP-only</td>
<td>202.3 (36.4)</td>
<td>p = 0.01514</td>
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<tr>
<td>BMP/HA</td>
<td>265.1 (38.1)</td>
<td>&lt;0.001</td>
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ORS 2014 Annual Meeting
Poster No: 1661