ECTOPIC BONE FORMATION BY INJECTION OF BMP-2 CONTAINED IN HYDROXYAPATITE-CHONDROITIN SULFATE COMPOSITES

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Introduction
Although the use of bone morphogenetic protein (BMP) has advantage in the treatment of fracture or large bone defect, BMP are rapidly resorbed and redistributed. Injectable drug delivery system for BMP is expected to have many advantages, because they can be administered repeatedly and less invasive even after surgery. For this purpose, we have developed hydroxyapatite-chondroitin sulfate (HAp-ChS) composites for a time-controlled BMP release and reported characteristic of this material at past ORS.

In the present study, we prepared injectable HAp-ChS composites with incorporated recombinant human BMP-2 (rhBMP-2) and evaluated their efficiency for ectopic bone formation.

Materials and methods
20mg of the HAp-ChS microparticles was dispersed in 6ml of 1/10 diluted PBS solution including rhBMP-2 with different concentration at 21.5°C and pH was maintained at 6.85-7.34 for 4 hours.

Forty-eight male ICR mice (8weeks old) were anesthetized with diethyl ether. The test implants were injected into the back subcutaneous tissue of following seven treatment groups.

- **Group A**, implanted compound liquid of 0.25μg rhBMP-2 and 0.5ml saline solution
- **Group B**, implanted compound liquid of 1.0μg rhBMP-2 and 0.5ml saline solution
- **Group C**, implanted compound liquid of 4.0μg rhBMP-2 and 0.5ml saline solution
- **Group D**, implanted compound liquid of 50mg HAp-ChS and 0.5ml saline solution
- **Group E**, implanted compound liquid of 50mg HAp-ChS incorporated 0.25μg rhBMP-2 and 0.5ml saline solution
- **Group F**, implanted compound liquid of 50mg HAp-ChS incorporated 1.0μg rhBMP-2 and 0.5ml saline solution
- **Group G**, implanted compound liquid of 50mg HAp-ChS incorporated 4.0μg rhBMP-2 and 0.5ml saline solution

Three weeks after surgery, the implants were harvested together with surrounding tissue. The number of HAp-ChS remained samples was counted in each group. Samples were radiographed with a soft X-ray apparatus, and the areas of ectopic bone were calculated by Scion image.

Hematoxylin and eosin staining (H-E staining) was performed.

The number of implants were analyzed using chi-square for independence test. The areas of ectopic bone were analyzed using analysis of variance.

Results
Macroscopically, any ectopic bone was not recognized in group A, B, and C (Table 1). In group D, two soft HAp-ChS samples were remained (Fig. 1a). In group G, hardened samples were surrounded with intensive vascular invasion (Fig. 1b). The number of implants has relevance to each treatment significantly (p < 0.01).

In H-E staining, group D showed 10μm-20μm in diameter spherical particles were stained dark purple, and bone formation was not observed (Fig. 1b). Group E and F, some particles were stained pink which seem to be surrounded new bone, but trabeculae bone were not observed.

Group G showed that newly formed endochondral bone united each particles and osteoblast was sited along with trabeculae bone. Chondrocyte-like cell existed around particle and blood vessels were formed through ossified particle (Fig. 2b). There was no evidence of intense inflammation or foreign body reaction in the host tissue in all groups.

The ectopic bone area of group G was largest in all groups, and significantly large compared to group A, B and C (Table 1).

Discussion
The result of this study showed HAp-ChS is useful drug delivery system to release BMP-2 and to form ectopic new bone.

The HAp-ChS samples of group D are considered to disappear by degradation, and such degradation might have effect of sustained release of rhBMP-2 in group E, F, and G. The newly formed endochondral bone is remodeled and is the site for hematopoiesis. The size of 20μm diameter and the microsphere shape of HAp-ChS may have advantage of helping vascular invasion which is important route for incoming osteoblast. Osteocompatibility of HAp-ChS was proved by union to trabecular bone in group G and those particles are expected to be part of new bone matrix.

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Table 1. The number of implants has relevance to each treatments significantly (p < 0.01).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of remained samples</th>
<th>Ectopic bone area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>0 / 6</td>
<td>0</td>
</tr>
<tr>
<td>Group B</td>
<td>0 / 6</td>
<td>0</td>
</tr>
<tr>
<td>Group C</td>
<td>0 / 6</td>
<td>0</td>
</tr>
<tr>
<td>Group D</td>
<td>2 / 6</td>
<td>18.3 ± 24.3</td>
</tr>
<tr>
<td>Group E</td>
<td>5 / 6</td>
<td>24.6 ± 32.3</td>
</tr>
<tr>
<td>Group F</td>
<td>6 / 6</td>
<td>24.3 ± 18.8</td>
</tr>
<tr>
<td>Group G</td>
<td>5 / 6</td>
<td>45.5 ± 29.0 *</td>
</tr>
</tbody>
</table>

*p < 0.05; significant compared to group A, B, and C.

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