Development of chelate-setting hydroxyapatite cement with enhanced mechanical property and its biocompatibility in vivo using rabbit model

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ABSTRACT INTRODUCTION:
Hydroxyapatite (Ca10(PO4)6(OH)2; HAp) is an inorganic component of bone and teeth, and has excellent biocompatibility. We have developed novel HAp cements with good biocompatibility for application in orthopedic surgery as paste-like artificial bone [1, 2]. The novel cement is hardened by binding the individual HAp particles due to chelating-ability of inositol phosphate (IP6) when the HAp particles surface-modified with IP6 were mixed with pure water; thus, the cement maintains the phase composition of the starting materials without accompanying a chemical reaction. However, the cement had only about 3 MPa of initial compressive strength [1].

In order to enhance the enough compressive strength for clinical uses, we have prepared the HAp particle with smaller size to fabricate the IP6-HAp cement with enhanced mechanical property. This is based on idea that the strength increase with a number of chelating-site of IP6. Actually, we have tried to prepare the smaller HAp particles by two approaches: i) preparation of fine particles by mechanical milling [3] and ii) synthesis of HAp nano-particles by conventional wet process.

In the present work, we synthesized the HAp powder via conventional wet process, and fabricated the HAp cement using the HAp nano-particles. This paper will be described the mechanical property of the resulting cement specimen and its biocompatibility in vivo using a rabbit model.

METHODS:
HAp powder was synthesized via conventional wet process. A suspension of 0.5 mol dm⁻³ Ca(OH)₂ (Wako; Japan) in 500 cm³ distilled water was stirred at the rate of 200 rpm at 37 °C, and solution of 0.3 mol dm⁻³ H₂PO₄ (Wako; Japan) in 500 cm³ distilled water was dropped into the suspension. The pH of the mixed suspension was maintained at 10 by the addition of 25 % NH₄OH solution. The mixture was stirred for 1 h and aged at 37 °C for 24 h. The resulting slurry was filtrated. The cake was washed with pure water and freeze-dried for 24 h to prepare the as-synthesized HAp powder.

The IP6 solution with concentration of 1000 ppm was separately prepared, adjusting to pH 7.3 using aqueous NaOH and HCl solutions (0.1 mol dm⁻³). Ten grams of as-synthesized HAp powders were added into the IP6 solution (200 cm³), and then stirred at the rate of 400 rpm at 37 °C for 5 h. After that, the mixture slurry was filtrated. The filter cake was washed with pure water and freeze-dried for 24 h to prepare the HAp particle surface-modified with IP6, which will be hereafter referred to as ‘as-synthesized IP6-HAp’ powder. The synthesized HAp powder was ground by ball-milling using a pot and balls of zirconia ceramic for 5 min at 300 rpm under wet / dry conditions. After that, the powder was collected by filtration and the filter cake were added into the 1000 ppm IP6 solution (200 cm³), and then stirred at the rate of 400 rpm at 37 °C for 5 h. The mixture slurry was filtrated. The filter cake was washed with pure water and freeze-dried for 24 h to prepare the HAp particle surface-modified with IP6, which will be hereafter referred to as ‘milled IP6-HAp’ powder.

Each 0.2 g of synthesized IP6-HAp powder was mixed with a suitable amount of the pure water to fabricate the apatite cements having cylindrical shape of 5 mm in diameter and of 6-8 mm in height. The ratios of powder/liquid [P/L] were in the ranges of 1:0.35 to 1:0.60 [w/w]. The resulting apatite cements were kept under room temperature in air for about 24 h. The apatite cements fabricated as referred above were used for a compressive strength testing (SIMAZU AUTO GRAPH; Japan). The compressive strength of the cement specimens was determined according to the JIS R 1068 standard.

To investigate the biocompatibility of the IP6-HAp cements, we implanted cement specimen to white house rabbit tibia for 8 and 24 weeks. Rabbis used in this study was 16 weeks old and their weight was ~3 kg. Rabbit was anesthetized with Nembutal, diluted with saline solution, by intravenous injection. After shaving the hair inside of hind leg, incising for about 3 cm and exfoliating the periostea, we drilled hole (diameter, 4.4 mm) and implanted the cement specimen into it. A commercially-available HAp cement, Biopex-R (Pentax, Japan), was used as a positive control. After 8 and 24 weeks, we retrieved cement specimen with surrounding tibia for fabricating undecalcification section. We evaluated histologically the section after Hematoxylin-Eosin or Toluidine blue stains.

RESULTS:
We fabricated apatite cements using three kinds of sample powders: i) as-synthesized IP6- HAp powder, ii) dry-milled IP6-HAp powder and iii) wet-milled IP6-HAp powder. Compressive strength of the resulting cement specimen is given in Table 1. The compressive strength of the resulting IP6-HAp cement was 19 MPa (P/L ratio = 1.0.35 [w/w], n=6) in the case using as-synthesized IP6-HAp powder as a starting powder. As the P/L ratio was higher, the compressive strength of cements was higher. Moreover, we fabricated the cements using wet-milled IP6-HAp powders as starting powder; the compressive strength of the cement attained maximum 23 MPa (P/L ratio = 1.0.35 [w/w], n=6). However, the compressive strength of the dry-milled IP6-HAp cement was 12 MPa (P/L ratio = 1.0.35 [w/w], n=6).

The results of histological observation indicated that osteoblast and newly-formed bone was present around cement specimen as well as the case of BIOPLEX®-R one.

Table 1 Compressive strengths of typical IP6-HAp cements (n=6)

<table>
<thead>
<tr>
<th>Compressive strength [MPa]</th>
<th>1.0.35</th>
<th>1.0.4</th>
<th>1.0.45</th>
<th>1.0.5</th>
<th>1.0.55</th>
<th>1.0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-synthesized IP6-HAp powder</td>
<td>19.1</td>
<td>15.6</td>
<td>12.5</td>
<td>11.8</td>
<td>10.9</td>
<td>12.3</td>
</tr>
<tr>
<td>Dry-milled IP6-HAp powder (5 min)</td>
<td>19.3</td>
<td>15.6</td>
<td>12.5</td>
<td>11.8</td>
<td>10.9</td>
<td>12.3</td>
</tr>
<tr>
<td>Wet-milled IP6-HAp powder (5 min)</td>
<td>12.3</td>
<td>9.5</td>
<td>8.4</td>
<td>7.3</td>
<td>6.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

DISCUSSION:
We succeeded to develop novel HAp cement that has higher compressive strength (23 MPa) than that of human spine (15 MPa) and that good biocompatibility as much as BIOPLEX®-R. The present cement may be available for clinical application.

REFERENCES:

Fig. 1 Histological evaluations of the IP6-HAp cement by hematoxylin-eosin and toluidine blue stains.