Enhanced bone ingrowth onto rhBMP-2 coated implant in the bone marrow of rabbits.  
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INTRODUCTION:  
Implants with various surface structures are currently used without bone cement to obtain secure fixation to bone. Initial fixation of the implants to bone is achieved within several weeks via new bone formation at the bone/implant interface. Therefore, faster and more extensive growth of new bone with use of bone-inducing agents will promote tight implant/bone integration. In this study, we attempted to use recombinant bone morphogenetic protein-2 (rhBMP-2) and a Graph, for local delivery of it in combination with porous surfaced implants to examine the usefulness of BMP-2 for rapid and secure fixation of the implant placed in distal medullary canal of the rabbit femur, and evaluated the tightness of fixation of the rhBMP-2-coated porous titanium implant in the bone marrow.

METHODS:  
Ti6Al4V cylindrical test pieces (ø6x10mm) were prepared and the surface of the implants was plasma sprayed coating. Average porosity and average pore size of the pores were 50% and 380µm respectively. In the experimental groups, the surfaces of the implant were covered with paste (120mg) retaining various dose of rhBMP-2 (15µg, 30µg or 60µg of rhBMP-2) prepared as described below. The surfaces of the implant for two control groups were coated with either 120mg paste without rhBMP-2 or no paste (CP and C groups, respectively) (Table 1). A synthetic biodegradable polymer (polylactic acid/polyethylene glycol block co-polymer, PLA-PEG) with a molecular weight of 9200 was produced and donated to us by Taki Chemicals Co Ltd, (Kakogawa, Japan). The physicochemical characteristics and efficacy of the block copolymer as carrier material for rhBMP-2 has been reported previously by us [1]. Beta-tricalcium phosphate (β-TCP) powder with a particle size less than 100µm in diameter was obtained from Olympus Optics Co, (Tokyo, Japan). Eighty female New Zealand white rabbits (18-30 months of age, 3.0-4.0kg body weight) were randomly divided into five groups (3 experimental groups and 2 control groups, 16 per group). To the experimental and control group of animals were implanted with each group test pieces. The 8 rabbits from each group were sacrificed 3 and 6 weeks after surgery by injecting overdose anesthetics and distal femurs with implants were harvested and processed for further examination. This protocol was approved by the Institutional Committee for Animal Care and Experiments of Osaka City University Graduate School of Medicine. All distal femur with implant from each group animals were radiographed with a soft x-ray apparatus and then bone mineral density of bone areas adjacent to the implants were measured by dual-energy X-ray absorptiometry (DXA). The shear strength of the bone-implant interface was measured using a mechanical apparatus (Fig.1; Graph, Shimazu Co., Kyoto, Japan). The peak load was obtained before failure to provide a measurement of the bone-implant attachment strength. A distal femur from each rabbit group was removed and stained by Villanueva bone staining method.

Table.1: Implant assignment.  
<table>
<thead>
<tr>
<th>Group</th>
<th>BMP-2(µg)</th>
<th>PLA-PEG(mg)</th>
<th>β-TCP(mg)</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>BMP30</td>
<td>30</td>
<td>60</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>BMP15</td>
<td>15</td>
<td>60</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>CP</td>
<td>0</td>
<td>60</td>
<td>60</td>
<td>16</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

PLA/PEG: polylactic acid/polyethylene glycol block co-polymer  
β-TCP: Beta-tricalcium phosphate  

RESULTS:  
On soft Xray images, significant amount of radiopaque image around the implant was observed on the surface of implant of the BMP15 and 30 groups. The BMD values of the peri-implant regions of the distal femur from BMP15, BMP30, BMP60 group and CP group was significantly higher than that of control (C) group (p < 0.01). BMD of BMP30 group was also significantly higher than that of CP group (p < 0.01). At 6 weeks BMD values in BMP30 group tended to be higher than that of other groups. However, the difference was not significant (Fig.1). The peak shear strength of CP, BMP15, BMP30 and BMP60 group was significantly larger than that of C group (p < 0.01). The peak shear strength of BMP30 group was significantly higher than that of CP group (p < 0.01). However, the difference of the peak shear strength between CP and BMP60 group was not significant (p = 0.81). At 6 weeks after implantation, the difference of the peak shear strength values between each group was not significant (Fig.2).

In BMP groups at 3 weeks after implantation, histological analyses demonstrated more intense new bone formation on implant surfaces in BMP groups than in CP or C group. At interfaces of C section, a layer of fibrous tissue without bone was noted.

DISCUSSION:  
Previous reports described that rhBMP-2-induced new bone formation around the implant could increase mechanical strength between host bone and implant [2]. But no previous report described shortened time period for biological fixation of implant with use of BMP. The shortening of time to obtain solid biological fixation of prosthesis will be beneficial to better clinical results of cementless arthroplasty through avoiding sinking or loosening in the early postoperative stage. β-TCP/PLA-PEG absorbable paste used in this study as carrier material for rhBMP-2 was an original one. The results in this study indicated that addition of β-TCP/PLA-PEG paste with rhBMP-2 to the implant surface accelerated the stable fixation of implant by enhanced peri-implant bone formation. The effect of rhBMP-2 was not correlate to the dose of rhBMP-2 and the peri-implant new bone formation as well as fixation strength of the implant was increased by increased dose of rhBMP-2 from 15µg to 60µg, but decreased by increasing dose of rhBMP-2 from 30µg to 60µg. Such dissociated correlation between rhBMP-2 dose in high dose range and rhBMP-2-induced bone yield was indicated before [3]. With regard to effect of excessive dose of rhBMP-2 on local bone metabolism, McClellan et al. reported clinical cases of lumbar inter-body fusion with use of high dose rhBMP-2 that showed intensive bone resorption in early post-operative phase which was repaired by subsequent new bone formation [4]. Although exact mechanism of the rhBMP-2-induced bone resorption was not elucidated, significantly enhanced bone ingrowth to implant surface was noted in rhBMP-2 groups at 3 weeks, the bone ingrowth and peri-implant bone formation was overtaken by controls until 6 weeks and no significant differences of mechanical strength among groups was noted. Based on these results, we concluded that addition of the rhBMP-2 to the implant surface might hasten fixation of implants to bone.

REFERENCES:  