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# The influence of recombinant human BMP-2 on bone–implant osseointegration: biomechanical testing and histomorphometric analysis

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*Abstract.* The healing period for bone–implant osseointegration lasts 3–6 months or even longer. The aim of this study was to investigate whether osseointegration can be enhanced by the use of bone morphogenetic protein-2 (BMP-2). In the femurs of 8 Japanese white rabbits, 16 implants were applied with 1.0 mg recombinant human BMP-2 (rhBMP-2) as group A, and the other 16 implants without rhBMP-2 as group B. Calcein green 20 mg/kg and alizarin red 20 mg/kg were injected 4 and 8 weeks after implantation, respectively. At 12 weeks, the animals were killed. In 16 implant–bone blocks, binding strength was measured by pull-out test, and the extracted implants were observed under a scanning electronic microscope. The other blocks were analysed for percentage of marked bone adjacent to the implant surface by confocal laser scanning microscope. The pull-out strengths of group A were greater than that of group B ($P < 0.05$). Scanning electronic microscopy (SEM) showed more calcified substances on the surface of the implants of group A than B. There was more marked bone around group A than B implants at 4 weeks ($P < 0.05$) and 8 weeks ($P < 0.05$). rhBMP-2 improves the quantity and quality of implant–bone osseointegration. Biomechanical testing and histomorphometric analysis are reliable methods to use in researching the implant–bone interface.

Key words: implant; osseointegration; rhBMP-2; CLSM; pull-out test; SEM.

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Dental implant-supported prostheses have become a widely used treatment in dentistry. Many factors determine and influence implant survival and function. How to accelerate and enforce osseointegration of implant–bone is very important in shortening the period of second-stage surgery, protection from failure and enhancing prosthesis function. Many attempts have been made to improve the quality and the quantity of implant–bone osseointegration, such as grafting allo- or auto-bone[5,13], improving implant biocompatibility, changing surface characteristics[10] and modifying surgical technique[18,3,2]. Several growth

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factors have been shown to improve osteoblast differentiation and matrix mineralization, such as bone morphogenetic proteins (BMPs), insulin-like growth factor-I and basic fibroblast growth factor. Among them, BMPs play the most important role.

The aim of this study was to investigate the influence of recombination human BMP-2 (rhBMP-2) on implant–bone osseointegration using biomechanical and histomorphometric methods. A pull-out test was used to measure the binding strength of the implant–bone interface and the surface of the extracted implant was observed by scanning electronic microscopy (SEM). Confocal laser scanning microscopy (CLSM) was used to observe the quantity of new bone formation around the implant at different time points.

## Materials and methods

### Implant preparation

Thirty-two 5.5-mm-long, commercially pure, titanium-roughened, screw-type implants with diameter of 3.3 mm (Northwest Institute for Non-ferrous Metal Research, Baoji, China) were used. The implants were coated with polylactic acid membrane (L/DL 70/30, inherent viscosity 3.3 dl/g, provided by Wuhan University of Technology, China) and divided into two equal groups. Group A was soaked in 2 ml sterile water containing 1.0 mg rhBMP-2 (kindly provided by the Forth Military Medical University); group B was soaked in 2 ml sterile water without rhBMP-2. All implants were soaked for 30 min, then lyophilized and stored at −20 °C.

### Surgical procedures

Eight adult male, Japanese white rabbits (8 months old and 3–4 kg weight) were anesthetized by intravenous injection of pentobarbital at a dose of 30 mg/kg body weight. Surgery was performed under sterile conditions. The femurs were prepared for implant osteotomy using a series of dental burs (diameters: 2.3-mm round bur, 2.5-mm pilot drill, 2.8–3.2-mm implant bur), under copious external sterile saline irrigation to avoid overheating. Each rabbit received one group A and one group B implant, chosen randomly, in each femur. Postoperatively, the animals were given intra-muscular injections of penicillin (400,000 IU/ml, 0.1 ml/kg body weight).

### Labeling of fluorescent dye

During the healing period, fluorescent bone markers were injected. Four weeks after implant placement, calcein green (20 mg/kg body weight) was injected i.v., and at 8 weeks alizarin red (20 mg/kg body weight) was injected i.v. All dyes were prepared immediately before use with sodium bicarbonate. The solution was filtered through a 0.45 μm filter and pH was adjusted to 7.4.

### Histological preparation

At 12 weeks after implantation, the animals were killed with air injected i.v. Implants and surrounding tissues were retrieved, amounting to 32 implant–bone blocks. Sixteen implant–bone blocks, eight each of group A and group B, had their binding strength measured using a pull-out testing machine. The extracted implants were observed under SEM. The other 16 implants were immediately fixed in 10% buffered formalin at 4 °C for 7 days. The specimens were dehydrated in ascending concentrations of alcohols from 70% to 100%, infiltrated and embedded in methylmethacrylate resin. After polymerization, the specimens were sectioned with the diamond disc at a thickness of 50 μm along the longitudinal axis of the implant, and the sections observed under CLSM.

### Mechanical testing and SEM observation

The fixation strength was measured by an Instron Universal Testing machine (model: A591-4, Dynamight 8841 testing system) within 2 h of animal death, and the loading fixture was used to attach the implant–bone block via the implant's internal region. Mechanical testing was accomplished at the rate of 1 mm/min and the force direction was parallel to the implant's longitudinal axis. The force–deflection curves were measured which determined the force of pull-out of the implant–bone interface[4]. Following pull-out testing, the extracted implants were stored in osmic acid, and then dehydrated, critical-point dried and coated with a thin platinum layer for SEM observation.

### CLSM observation

The sections were observed under CLSM (TCS-SP, Leica) equipped with software to measure the percent of marked bone. The barrier filters were BP530/30 nm and LP 590 nm combined with DD488/568 activation, and the photomultiplier for the fluorescence markers was 534 nm (calcein) and 357 nm (alizarin red)[12].

### Statistical analysis

The mean values and standard errors for binding strength of the two groups were analysed. Incorporation of the dyes was also analysed to determine the mean differences in bone formation between the two groups at different time points using the non-parametric Mann–Whitney *U*-test ($P < 0.05$). Because the data presented normal distribution, the independent-samples *t*-test was used to determine the differences in fixation strength and bone growth of the groups. $P < 0.05$ was considered statistically significant.

## Results

### Mechanical pull-out testing and SEM examination

No animal died during the postoperative period. Pull-out testing demonstrated that the binding strength of group A was $36.5 \pm 2.02N$, which was statistically greater ($P < 0.05$) than that of group B, $27.63 \pm 1.31N$ (Table 1). SEM images of the extracted implants showed extensive mineralized matrixes on the surface of the group A implants (Fig. 1), compared with the group B implants (Fig. 2).

### Confocal laser scanning microscopy

New bone formation was determined histomorphometrically by bone marker quantification, which represented the different healing periods in the two groups (Table 2). The mean percentage of marked bone in group A implants at 4 weeks was 7% (range 4–8) (Fig. 3a) and at 8 weeks 8% (range 6–11) (Fig. 3b), and the total marked bone was 15% (Fig. 3c). For the group B implants, the values were 4 weeks 5% (range 3–6) (Fig. 4a), 8 weeks 6% (range 4–7) (Fig. 4b) and total 11% (Fig. 4c). The percentage of marked bone in group A was significantly greater than in group B not only at 4 weeks ($P = 0.019$) but also at 8 weeks ($P = 0.001$) (Fig. 5). From Fig. 6 it can also be observed that the

*Table 1.* The pull-out strength (*N*) of group A and group B implants

| | Pull-out strength (*N*) | | | | | | | | $X \pm S_X$ |
|---|---|---|---|---|---|---|---|---|---|
| Group A | 36 | 45 | 31 | 33 | 28 | 41 | 37 | 41 | 36.50 ± 2.02 |
| Group B | 21 | 30 | 33 | 27 | 29 | 26 | 30 | 25 | 27.63 ± 1.31 |

The strength of group A was statistically greater than that of group B implants.