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The induction of bone formation in rat calvarial defects and subcutaneous tissues by recombinant human BMP-2, produced in *Escherichia coli*

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ABSTRACT

We investigated the ability of recombinant human bone morphogenetic protein-2, produced from *Escherichia coli* (ErhBMP-2), to form orthotopic and ectopic bone in rat models. BMP-2 was expressed in *E. coli* and extracted from the inclusion bodies. Critical-sized calvarial defects and subcutaneous pouches were created in rats, and an absorbable collagen sponge (ACS) was loaded with different doses of ErhBMP-2 for implantation. ACS alone and sham surgery controls were also included. Implant sites were evaluated by histological and/or histometric analyses following a 2- or 8-week healing interval.

In the calvarial defect model, enhanced bone formation was observed with all doses of ErhBMP-2, while only limited amounts of new bone were found in controls. In the ectopic subcutaneous implant model, bone formation was clearly observed in all animals treated with ErhBMP-2 at 2 weeks. However, at 8 weeks, less new bone formation was detected than at 2 weeks. Nevertheless, the remaining new bone showed an advanced degree of bone remodeling and more maturity than that observed at 2 weeks. These results showed that ErhBMP-2 was osteoinductive under controlled in vivo conditions. Thus, ErhBMP-2 has definite potential as an alternative to rhBMP-2 produced in a eukaryotic system for clinical use.

1. Introduction

Bone morphogenetic protein (BMP) has come into the spotlight in the area of orthopedics and dentistry due to its capacity for autoinduction [1]. In 1965, Urist found that nonviable bone extracts could induce bone formation. He named the factor responsible for bone induction BMP [2]. The development of recombinant technology made it possible to clone the DNA sequence (cDNA) and to synthesize recombinant human BMP protein (rhBMP). This, in turn, provided an opportunity for the production of a highly purified BMP protein preparation [3]. Subsequently, a number of studies regarding rhBMP have demonstrated the potential of rhBMP-2 as a safe and effective alternative to autogenous bone grafts [4–8].

Previously, most rhBMPs were produced in mammalian cells, like Chinese hamster ovary (CHO) cells [9]. However, the low yield (ng/ml) and high cost of rhBMP production in this well-established eukaryotic protein expression system has been considered problematic for clinical applications. In order to develop a viable commercial product, the cost of acquisition must also be considered. Many attempts have been made to produce and evaluate biologically active rhBMPs in *E. coli*, as an alternative to mammalian cells [10–14]. Despite the fact that prokaryotic expression systems lack the capacity to execute the correct posttranslational modifications inherent in mammalian cells, it has been shown that biologic activity can be restored by in vitro purification and refolding of expressed proteins [10,11,14]. However, the efficacy of rhBMPs produced in *E. coli* was found to be inferior to that of rhBMP produced in mammalian cells [15]. In addition, studies with rhBMP-2 produced in *E. coli* are currently in the initial stages of development; almost all of the studies have been performed in vitro. Only a few studies have been performed in ectopic sites in small animals; however, these are thought to be insufficient for a quantitative prediction of the expected efficacy at an orthotopic site. A demonstration that rhBMPs produced in *E. coli* had comparable biologic activity to that of rhBMP produced in a eukaryotic system would enable the production of a large quantity of rhBMPs at low cost.

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The aim of present study was to quantitatively evaluate bone formation induced by *E. coli*-produced rhBMP-2 (ErhBMP-2) in a rat model of calvarial defect and in an ectopic subcutaneous implant model.

2. Materials and methods

2.1. Animals

One hundred and forty-four male Sprague-Dawley rats (body weight 200–300 g) were included in the study. Animals were maintained in plastic cages in a room with 21-h day/night cycles at an ambient temperature of 21 °C with ad libitum access to water and a standard laboratory pellet diet. Animal selection and management, surgical protocols, and preparation procedures were approved by the Institutional Animal Care and Use committee, Yonsei Medical Center, Seoul, Korea.

2.2. Expression of rhBMP-2 in E. coli

The expression of rhBMP-2 in *E. coli* was performed at the research institute of Cowellmedi Co. Ltd, Pusan, Korea, as described previously [16]. Briefly, the total RNA of human osteosarcoma cells (U2OS) was reverse transcribed with reverse transcriptase (Gibco BRL, NY, USA). The cDNA encoding the mature form of the BMP-2 protein was amplified by polymerase chain reaction (PCR). This hBMP-2 cDNA was then subcloned into a pRSET(A) vector (Invitrogen, UK) to produce the pRSET(A)/hBMP-2 expression vector. Then, pRSET(A)/hBMP-2 was used to transform the *E. coli* BL21(DE3) strain. High cell-density cultivation of *E. coli* was accomplished with a bioreactor (KoBioTec, Incheon, Korea) as described by Tabandeh [17]. Briefly, transformed *E. coli* were cultured in a 5L fermenter (KoBioTec, Incheon, Korea), with 3L of the defined medium (batch culture; yeast extract 1 g/L, peptone 2 g/L) inoculated with the pre-culture (10% of the batch culture volume). The cultivation was performed at 30 °C, with stirring at 250 rpm for 48 h, and the addition of a pre-autoclaved nutrient medium (glucose 33.3 g/L, peptone 10 g/L, yeast extract 5 g/L, $MgSO_4$ 1 g/L, $FeSO_4$ 8.0 g/L, $CaCl_2$ 0.048 g/L, $ZnSO_4$ 0.0176 g/L, $CuSO_4$ 0.008 g/L).

The cultured biomass was harvested and suspended in a sodium phosphate buffer (50 mM, pH7.0) at 220 mg wet weight/L. The harvested cells were crushed twice in a French press and centrifuged. The pellet was resuspended at 25 mg wet weight/ml in a suspension buffer (20 mM Tris–HCl [pH 8.5], 0.5 mM EDTA, 2% [v/v] Triton X-100) and was centrifuged again. The inclusion bodies (pellets) were resuspended in a solubilization buffer (6 M guanidine-HCl, 0.1 M Tris–HCl [pH 8.5], 0.1 M DTT, 1 mM EDTA) and incubated overnight at room temperature with constant stirring; the suspension was then centrifuged in order to remove any insoluble particles.

For *in vitro* dimerization, the solubilized rhBMP-2 was incubated in a renaturation buffer (0.5 M guanidine-HCl, 50 mM Tris–HCl [pH 8.5], 0.75 M CHES, 1 M NaCl, 5 mM EDTA, 3 mM total glutathione) for 72 h. Purification of the active rhBMP-2 (dimer) was performed with a Heparin Sepharose 6 Fast Flow column (GE healthcare, USA). The bound protein was eluted with a continuous NaCl gradient (0.1–1.5 M). After the elution profile was confirmed, the active rhBMP-2 protein was eluted and separated by a stepped NaCl gradient (0.15 M, 0.3 M, and 0.5 M).

2.3. ErhBMP-2 implant

ErhBMP-2 was reconstituted and diluted in a buffer to obtain concentrations of 0.025, 0.05, 0.1, and 0.2 mg/ml. For the calvarial defect model, sterile, 8 mm diameter, absorbable collagen sponges (ACS; Collatape, Calcitek, Carlsbad. CA, USA) were then loaded with 0.1 ml of ErhBMP-2 solution to produce implanted concentrations of 2.5, 5, 10, and 20 μg, respectively. For control experiments, the ACS was loaded with buffer alone. The ErhBMP-2 (and control implants) were allowed to bind for 5 min, and then were placed into the calvarial defects.

For the ectopic subcutaneous implant model, sterile ACS straps of 2.5 × 1.0 cm were loaded with 0.5 ml of one of the four different doses of ErhBMP-2, allowed to bind for 5 min, and then were inserted into the prepared subcutaneous pouch.

2.4. Surgical procedures

The animals were anesthetized by an intramuscular injection (5 mg/kg body wt) of a 4:1 solution of ketamine hydrochloride (Ketalar®, Yuhan Co., Seoul, Korea) and Xylazine (Rompun®, Bayer Korea, Seoul, Korea). The surgical site was shaved and scrubbed with iodine. For the calvarial defect model, an incision was made in the sagittal plane across the cranium, and a full thickness flap was reflected to expose the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium with a trephine drill (3i Implant Innovation, Palm Beach Gardens, FL, USA) and was copiously irrigated with saline. After removal of the trephined calvarial disk, ErhBMP-2 and control ACS treatments were applied to the defect sites. For the ectopic subcutaneous model, a vertical incision was made in the skin of the back; then, with blunt dissection, a subcutaneous pocket was prepared.

According to different ErhBMP-2 concentrations, the animals were divided into 6 groups of 24 animals each and allowed to heal for 2 or 8 weeks. For the calvarial defect model, each group received one of 6 experimental conditions: (1) a sham-surgery control, (2) ACS control, (3) 2.5 μg ErhBMP-2/ACS, (4) 5 μg ErhBMP-2/ACS, (5) 10 μg ErhBMP-2/ACS, or (6) 20 μg ErhBMP-2/ACS. For the ectopic subcutaneous model, only the four rhMBP-2 treatment conditions were tested. All surgical sites were sutured for primary closure with 4-0 Monosyn® (Glyconate absorbable monofilament, B-Braun, Aesculap, INC., PA, USA).

2.5. Histological processing and histometric measurements

Block sections of the surgical sites were removed at sacrifice. The sections were rinsed in sterile saline and fixed in 10% buffered formalin for 10 days. After rinsing in water, the sections were decalcified in 5% formic acid for 14 days and embedded in paraffin. Serial sections, 5 μm thick, were cut through the center of the circular calvarial defects and through the subcutaneous implants. From each block, the two most central sections were selected for staining with hematoxylin and eosin (H&E), and examined with a binocular microscope (Leica DM LB, Leica Microsystems Ltd., Wetzlar, Germany) equipped with a camera (Leica DC300F, Leica Microsystems Ltd., Heerburgg, Switzerland). Images of the slides were taken and saved as figure files.

For the calvarial defect model, after conventional microscopic examination, computer-assisted histometric measurements of the newly formed bone were obtained with an automated image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). Three parameters were measured: defect closure, new bone area, and bone density. Defect closure (%) was determined by measuring the distance between the defect margin and the new bone margin and was expressed as a percentage of the total defect width. New bone area (mm^2) was measured as newly-formed mineralized bone, excluding marrow and fibrovascular tissue. Bone density (%) was determined by the percentage of new bone area in the total augmented area, including all tissues within the boundaries of the newly formed bone (i.e., mineralized bone, fibrovascular tissue, bone marrow, and residual biomaterial used as carrier). Calculations are shown in Fig. 1.

2.6. Statistical analysis

Histomorphometric measurements of the samples in calvarial defects were used to calculate group means (±SD). A two-way analysis of variance (ANOVA) was used to analyze the effect of time and experimental conditions. The post-hoc Scheffe test was employed to analyze the differences between groups. $P < 0.01$ was taken to indicate significance.

3. Results

The postoperative period was generally uneventful for all animals except one that belonged to the group of 5 μg ErhBMP-2/ACS. This animal healed for period of 2 weeks after surgery, and died for unknown reasons. Thirteen specimens of the calvarial defect model were excluded due to technical complications in the histological processing, including: three sham surgery controls, two at 2 weeks and one at 8 weeks; four ACS controls, two each at 2 and 8 weeks; three 2.5 μg ErhBMP-2/ACS treatments, two at 2 weeks and one at 8 weeks; one 5 μg ErhBMP-2/ACS treatment at 8 weeks; one 10 μg ErhBMP-2/ACS treatment at 8 weeks; and one 20 μg ErhBMP-2/ACS treatment at 2 weeks. Ultimately, a total of 130 specimens from the calvarial defect model were investigated histomorphometrically.

3.1. Calvarial defect model

In the sham surgery and the ACS control groups, only a limited amount of new bone could be detected at the defect margins, regardless of healing times. The defect was filled with thin, loose connective tissue. In the ACS control, some of the collagen of the carrier could be detected (Fig. 2).

In the ErhBMP-2 treated groups, all the defects exhibited remarkable bone bridging, irrespective of the dose of ErhBMP-2 used; moreover, maturity and defect closure were more advanced at 8 weeks than at 2 weeks. At 2 weeks, the ACS carrier was embedded in newly formed bone, detectable in the center of the defect. Chondrocytic cells were associated with newly formed immature bone; this suggested that endochondral ossification was involved in bone formation (Fig. 3). At 8 weeks, the defect was completely filled with the newly formed bone and the original

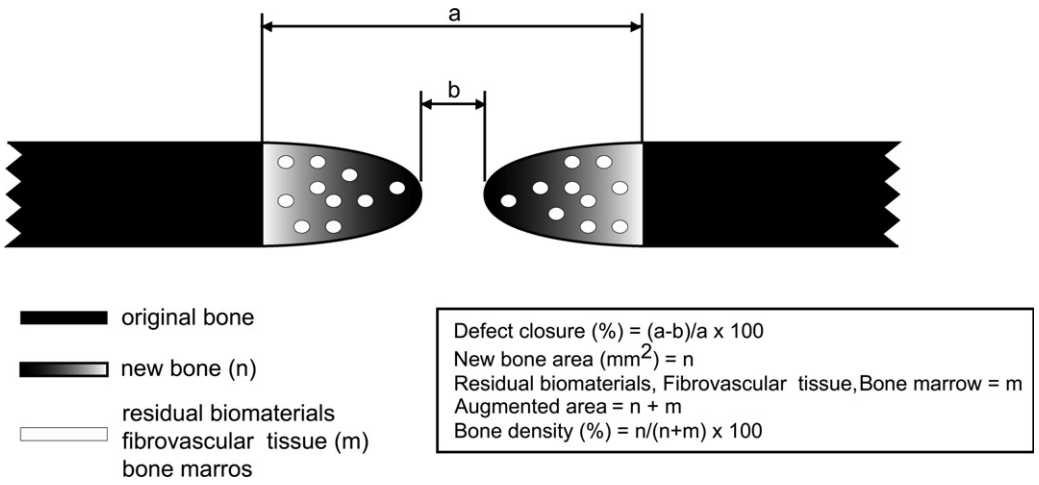

Fig. 1. Schematic drawing shows the histometric analysis of the calvarial defect model. The calculations for various measurements are shown.

defect margin was barely distinguishable from the new bone. In addition, the new bone had matured compared to that observed at 2 weeks; we detected a few osteocytes, a cemental line, and mature marrow space. No remnants of ACS could be detected (Fig. 4).

In quantitative terms, the ErhBMP-2 implantation resulted in the bony restoration of 80 to 100% of the defect, while values for control groups were in the range of 10–20% (Table 1). There was no significant difference in bone restoration at 2 and 8 weeks. However, new bone area was significantly greater at all doses in the ErhBMP-2 treated groups than in the untreated controls. Within the ErhBMP-2 treated groups, new bone area was significantly greater at 8 weeks than at 2 weeks for each concentration of ErhBMP-2 (Table 2). Bone density showed similar outcomes; the new bone area at 2 weeks in the ErhBMP-2 treated groups showed significantly greater bone density than the ACS controls, and bone density was significantly greater at 8 weeks than at 2 weeks (Table 3). These results demonstrated that, irrespective of dose, implantation of ErhBMP-2 produced in *E. coli* resulted in significantly more bone formation than the untreated controls.

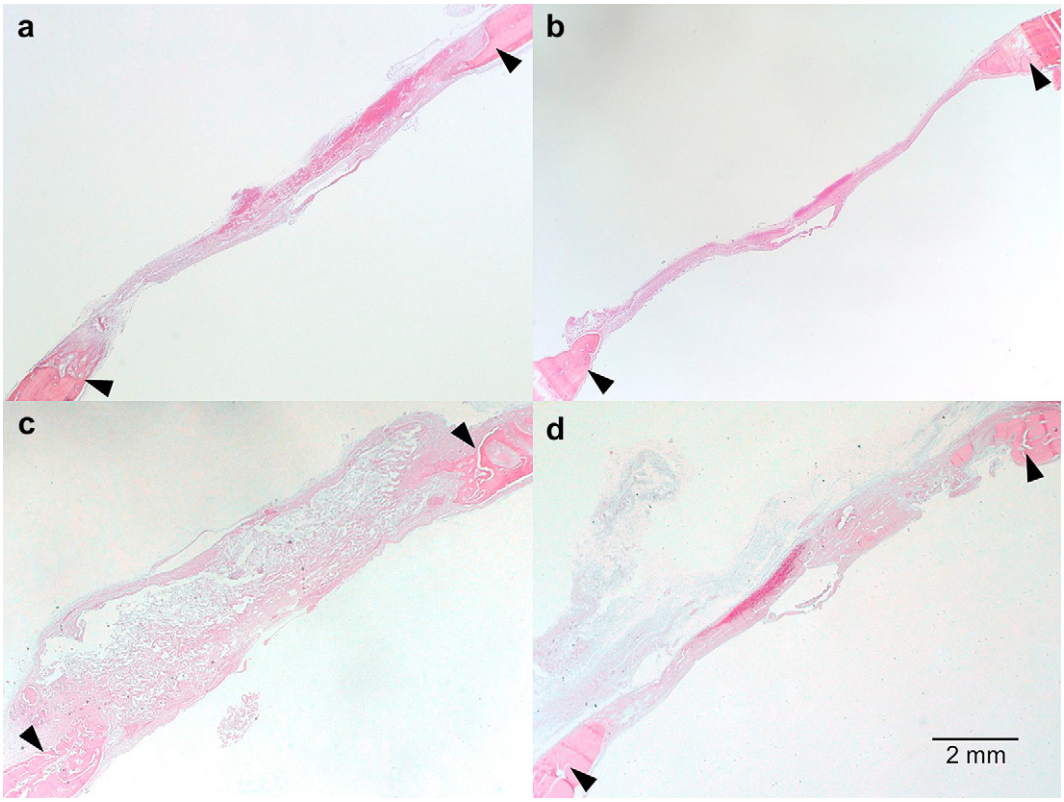

Fig. 2. Representative photomicrographs of the defect sites. Examples are shown of the sham surgery at 2 weeks (a), and 8 weeks (b); and the ACS carrier control at 2 weeks (c), and 8 weeks (d). In all cases, thin, loose connective tissue was observed between the defect margins. The ACS remnants were barely visible in the ACS control. (arrow heads = defect margin; H&E stain; original magnification ×10).

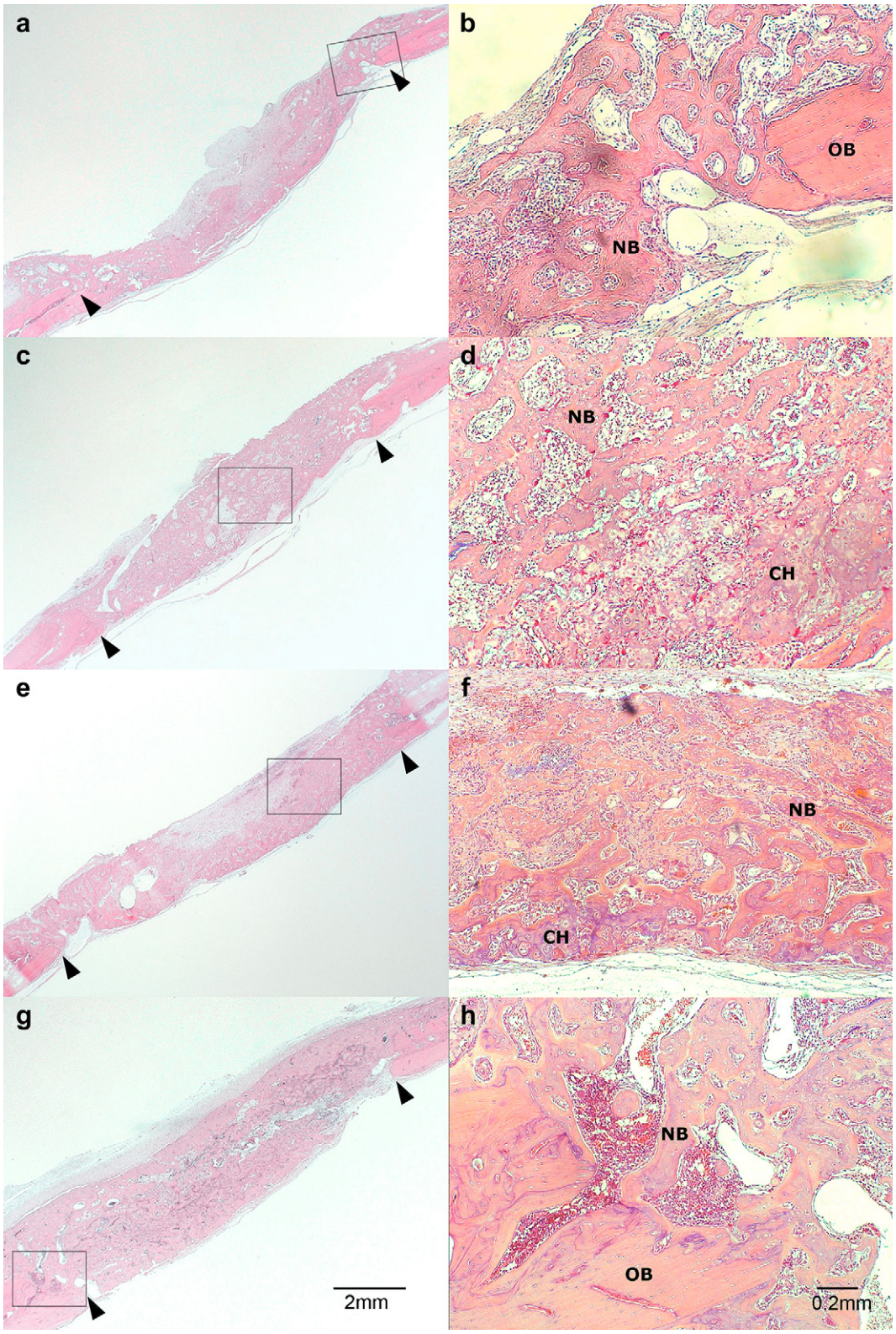

Fig. 3. Representative photomicrographs of calvarial defect sites that were treated with rhBMP-2/ACS implants for 2 weeks. Bone formation was observed with rhBMP-2/ACS doses of (a, b) 2.5 μg; (c, d) 5 μg; (e, f) 10 μg; and (g, h) 20 μg. Irrespective of the dose of rhBMP-2 loaded, immature, newly formed bone was observed with the embedded ACS remnants. In the center of some specimens (d, f), chondrocytic cells were associated with the new bone area. (arrow heads, defect margin; NB, new bone, OB, original bone, CH, chondrocytic cell; H&E stain; original magnification a, c, e, and g ×10; b, d, f, and h ×100).

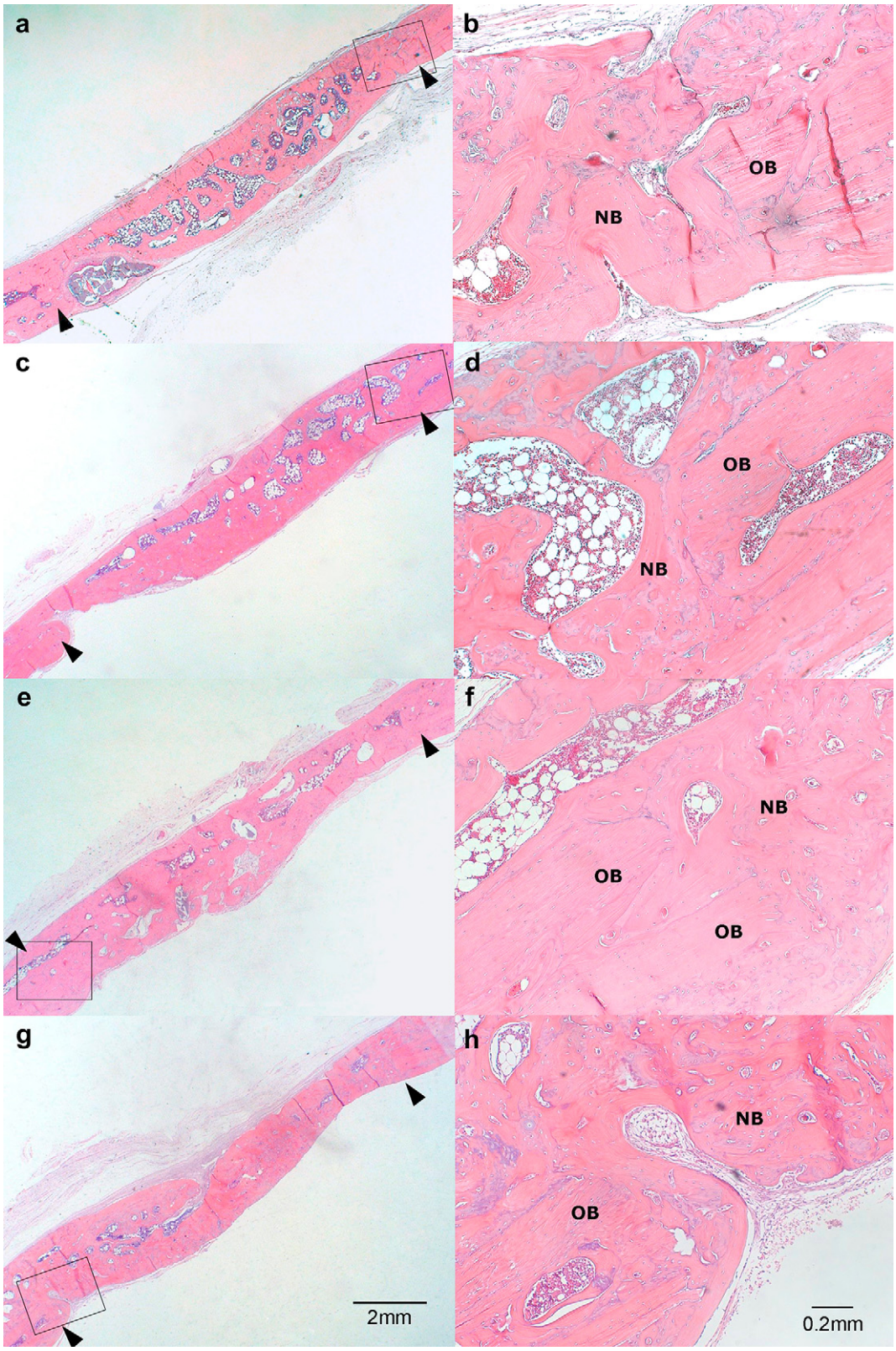

Fig. 4. Representative photomicrographs of calvarial defect sites that were treated with rhBMP-2/ACS implants for 8 weeks. Bone formation was observed with rhBMP-2/ACS doses of (a, b) 2.5 μg; (c, d) 5 μg; (e, f) 10 μg; and (g, h) 20 μg. Newly formed bone was integrated into the original bone; thus, it was difficult to distinguish the location of the original defect margin. (arrow heads, defect margin; NB, new bone, OB, original bone; H&E stain, original magnification a, c, e, and g ×10; b, d, f, and h ×100).

Table 1
Defect closure with implantation of recombinant human bone morphogenetic protein.

Group	%Closure after 2 weeks (N)	%Closure after 8 weeks (N)
Sham surgery control	13.5 ± 3.5 (10)	18.3 ± 8.7 (11)
ACS control	18.0 ± 7.7 (10)	21.9 ± 8.3 (10)
2.5 μg rhBMP-2/ACS	84.2 ± 24.8*,** (10)	98.4 ± 3.1*,** (11)
5 μg rhBMP-2/ACS	100.0 ± 0.0*,** (11)	100.0 ± 0.0*,** (11)
10 μg rhBMP-2/ACS	85.9 ± 16.3*,** (12)	100.0 ± 0.0*,** (11)
20 μg rhBMP-2/ACS	92.2 ± 11.3*,** (11)	100.0 ± 0.0*,** (12)

Values represent the group means ± SD; *N*, number of specimens.
* Statistically significant difference compared to sham surgery control group ($P < 0.05$); ** statistically significant difference compared to ACS control group ($P < 0.05$).

Table 3
Bone density after implantation of recombinant human bone morphogenetic protein.

Group	% Density after 2 weeks (*N*)	% Density after 8 weeks (*N*)
Sham surgery control	83.4 ± 10.3** (10)	83.4 ± 10.3 (11)
ACS control	13.6 ± 3.1* (10)	89.5 ± 7.0 (10)
2.5 μg rhBMP-2/ACS	46.1 ± 10.4*,** (10)	73.3 ± 7.0** (11)
5 μg rhBMP-2/ACS	43.1 ± 5.9*,** (11)	70.6 ± 10.8** (11)
10 μg rhBMP-2/ACS	36.9 ± 13.0*,** (12)	74.8 ± 6.7** (11)
20 μg rhBMP-2/ACS	37.3 ± 13.5*,** (11)	73.1 ± 7.4** (12)

Values represent the group means ± SD; *N*, number of specimens.
* Statistically significant difference compared to sham surgery control group ($P < 0.05$); ** statistically significant difference compared to ACS control group ($P < 0.05$).

3.2. Ectopic subcutaneous implant model

At 2 weeks after ErhBMP-2 implantation, all animals in the four ErhBMP-2 treated groups showed new bone formation, regardless of the dose. However, in this model, only very small amounts of newly formed bone were detected. Histologically, newly induced woven bone was observed at the periphery of the implant. The core of the implant consisted of ACS remnants and abundant loose connective tissue. The new bone or bone matrix was immature and lined with cells that were expected to become osteoblastic (Fig. 5). There was no evidence of significant adverse reactions.

At 8 weeks, the number of animals that showed new bone formation was smaller than the number at 2 weeks. The reduced number of observations was positively proportional to the dose of ErhBMP-2 (Table 4). At that time, ACS was completely resorbed and marrow tissue occupied most of the implant. The quantity of new bone was greater than that observed at 2 weeks, though it could not be measured histomorphometrically. Histological observations revealed that the newly formed bone included a few osteocytes and a cement line that separated the previously formed bone from the more recently deposited bone; this was a manifestation of advanced remodeling.

4. Discussion

The objective of the present study was to investigate the osteogenic efficacy of the *E. coli* – produced ErhBMP-2 protein. The efficacy was evaluated in two well-established experimental bone healing rat models. These rat models were also employed for the evaluation of rhBMP-2 produced in mammalian cell systems [4,6,18,19]. In the present study, the osteogenic efficacy of ErhBMP-2 in both orthotopic and ectopic sites was quantitatively assessed. Histologically, the ErhBMP-2 loading did not result in any obvious adverse tissue reactions, except a mild inflammation associated with the ACS carrier that presumably could be attributed to the nature of the collagen sponge. We observed definite, remarkable, new bone formation in the ErhBMP-2 treated groups, especially those with implants at the orthotopic site. In the groups with implants at the ectopic site, new bone could be observed in all rats at 2 weeks. Thus, when loaded on the ACS carrier, ErhBMP-2 could initially induce ossification in rat subcutaneous tissue. However, some of these bony formations disappeared at 8 weeks, despite the maturation and advanced nature of the remaining new bone. This result could be ascribed to the lack of a capacity of the ACS to maintain space, and the characteristics of the ectopic anatomic site. Our results are consistent with those of several studies that tested rhBMP-2 derived from CHO cells [4,12]. However, in those studies, higher doses were used and a greater number of animals showed evidence of new bone at 8 weeks. This could indicate that a high dose might overcome or compensate for the lack of capacity of the ACS carrier to maintain space.

In order to investigate the dose dependency of ErhBMP-2, we used four different doses that increased by a factor of 2 to treat rat calvarial defects. We observed that ErhBMP-2 clearly induced similar amounts of new bone and degrees of defect closure at each dose. Thus, under the conditions of this study, the doses were not sufficiently different to show distinct effects. Dose dependency can be affected by various factors, including the function of the carrier, the species, the experimental site, and the observation interval [20]. Many studies have attempted to determine factors that influence dose dependency, but this is still a controversial issue. In another study with ErhBMP-2, although no direct comparison to this study was possible, Kimura et al. showed that doses of ErhBMP-2 that increased by a factor of 10 (i.e. 0.1, 1.0, and 10 μg) resulted in defect closures of 48–64% on the rat mandible; but they also failed to show a dose response. On the other hand, Kubler et al. used 0.4, 4, and 40 μg doses of ErhBMP-2 and found that higher concentrations of rhBMPs could induce larger amounts of new bone formation in ectopic implants. These conflicting results might be due primarily to the effects of different experimental sites (i.e., orthotopic versus ectopic). We failed to show dose dependency in the calvarial defect model, because 2.5 μg or higher doses of ErhBMP-2 showed similar bone formation in calvarial defects. On the other hand, dose dependency was observed at 8 weeks in the ectopic site, but that site lacked inherent osteogenic potential. In the future, it may be possible to determine a threshold dose by using doses lower than 2.5 μg with the same study design.

The efficacy of ErhBMP-2 was estimated to be about one order of magnitude lower than the efficacy of rhBMP-2 produced in CHO cells or animal cells [21]. This finding is consistent with results from in vitro experiments [15], where the activity of ErhBMP-2 was estimated to be about five to ten times less active than rhBMP-2 produced in CHO cells. However, the study by Bessho et al. compared ErhBMP-2 activity to CHO cell-derived rhBMP-2 activity

Table 2
New bone area after implantation of recombinant human bone morphogenetic protein.

Group	Area (mm^2) after 2 weeks (*N*)	Area (mm^2) after 8 weeks (*N*)
Sham surgery control	0.18 ± 0.05 (10)	0.80 ± 0.27 (11)
ACS control	0.47 ± 0.14 (10)	0.80 ± 0.37 (10)
2.5 μg rhBMP-2/ACS	1.55 ± 0.72* (10)	5.13 ± 0.91*,** (11)
5 μg rhBMP-2/ACS	2.65 ± 0.46*,** (11)	6.18 ± 1.29*,** (11)
10 μg rhBMP-2/ACS	1.74 ± 0.65*,** (12)	6.28 ± 1.09*,** (11)
20 μg rhBMP-2/ACS	1.92 ± 1.26*,** (11)	5.75 ± 1.11*,** (12)

Values represent the group means ± SD; *N*, number of specimens.
* Statistically significant difference compared to sham surgery control group ($P < 0.05$); ** statistically significant difference compared to ACS control group ($P < 0.05$).

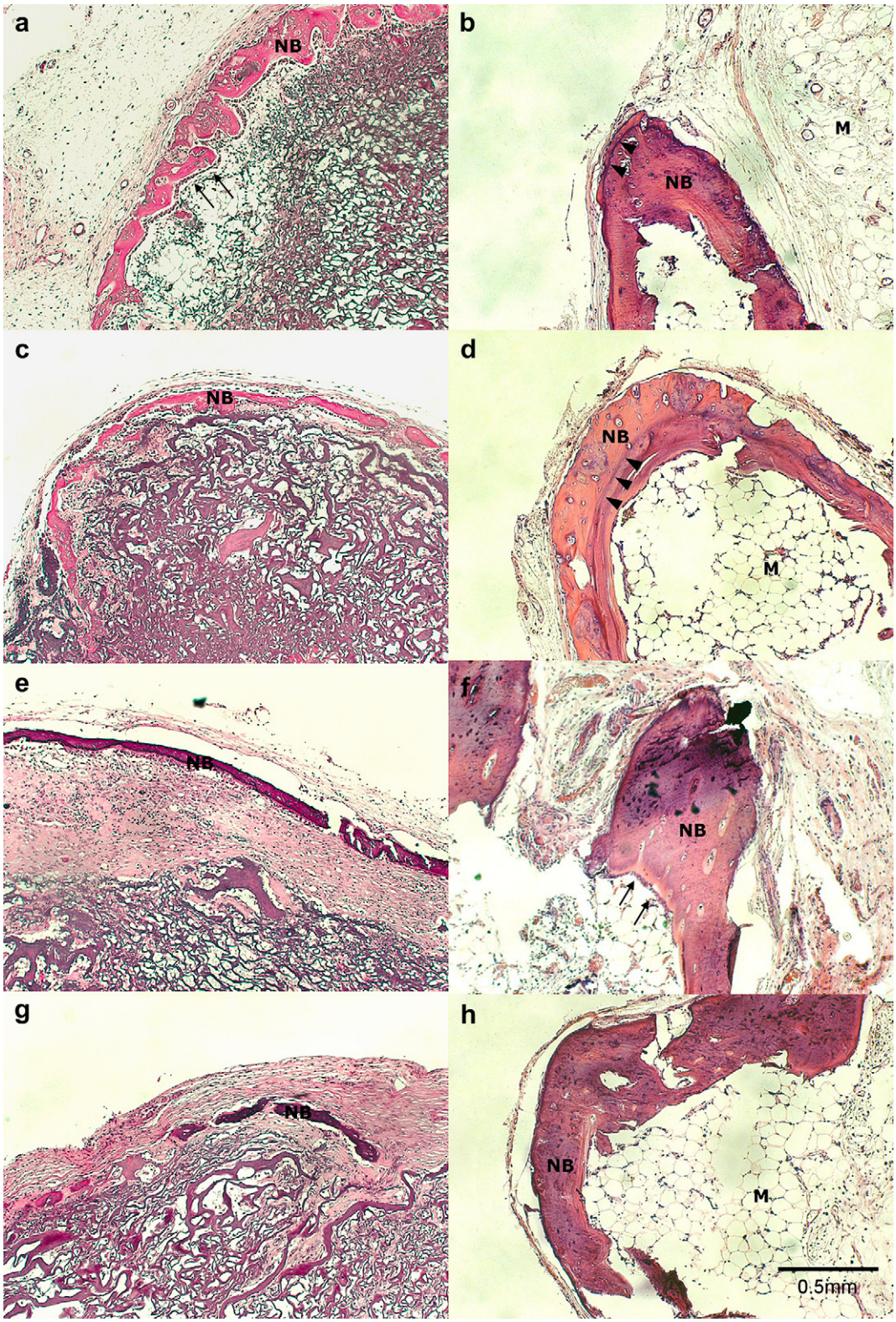

Fig. 5. Representative photomicrographs of ectopic sites that were treated with rhBMP-2/ACS implants for 2 and 8 weeks. The rhBMP-2 doses were: (a, b) 0.025 mg/ml; (c, d) 0.05 mg/ml; (e, f) 0.1 mg/ml; and (g, h) 0.2 mg/ml. At 2 weeks (a, c, e, and g), newly formed bone was observed at the periphery of the implant. These cells were assumed to be osteoblastic. The core of the implant consisted of abundant ACS remnants. At 8 weeks (b, d, f, and h), the ACS remnants completely disappeared and fatty marrow tissue occupied the core of the implant. Osteocytes, a reversal line, and osteoblastic lining also were observed. (arrow heads, cemental line; arrows, osteoblastic cell lining; NB, new bone; M, marrow tissue; H&E stain; original magnification ×100).

Table 4
Bone formation after implantation of recombinant human bone morphogenetic protein.

Group	Bone induction at 2 weeks	Bone induction at 8 weeks
2.5 μg rhBMP-2/ACS	12/12	1/12
5 μg rhBMP-2/ACS	11/11	6/12
10 μg rhBMP-2/ACS	12/12	8/12
20 μg rhBMP-2/ACS	12/12	9/12

Values represent the number of animals that showed bone induction/total number of animals involved in the histological observation.

in vitro and in vivo and concluded that they showed similar bone-inducing activities under some conditions [13]. The results of the present study were consistent with a study of rhBMP-4 in the rat calvarial model [18], which showed similar degrees of defect closure and bone density. Inherently, *E. coli* does not perform the post-translational modifications of BMP-2 that are presumably essential for a fully functional BMP-2 protein. These post-translational modifications include glycosylation, dimerization, and cleavage of the precursor protein into the mature form [14,22,23]. In early studies, the poor efficacy of ErhBMP-2 was thought to be due to the inefficient refolding process of the BMP-2 protein after expression in *E. coli* [15]. In recent years, successful methods for restoring the biologic activity after rhBMP-2 expression in *E. coli* have made it possible to produce fully functional BMP-2 from *E. coli* [10,24]. In addition, it was shown that post-translational glycosylation was not critical for a fully functional ErhBMP-2, because the basic glycosylated N-terminal domains of rhBMP-2 were not obligatory for receptor activation [25]. In this study, a non-glycosylated rhBMP-2 was extracted from inclusion bodies and refolded to its biologically active, dimer form in vitro. This study showed that implantations of ErhBMP-2 proved to be biologically active, osteoinductive, and quantitatively efficient at inducing bone formation.

5. Conclusion

In rat calvarial defects, ErhBMP-2 induced a significantly superior bone-fill compared with control, irrespective of its dose. Dose dependency was observed only in the ectopic sites. Time factor did not influence on the degree of restoration, but on the bone density and the amount of new bone significantly. It can be inferred that ErhBMP-2 might be a feasible alternative to rhBMP-2 produced in mammalian CHO cells for clinical use.

Acknowledgements

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Appendix

Most of the figures in this article have parts that are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2010.01.075.

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