

# Healing of rat femoral segmental defect with bone morphogenetic protein-2: A dose response study

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## Abstract

**Objective:** Use of recombinant human bone morphogenetic protein-2 (rhBMP-2) is becoming a common clinical approach to enhance bone repair. There is little or no information in the literature on the dose of rhBMP-2 required for effective healing of critical-sized defects such as those associated with trauma. In this study, we used a segmental defect model to assess the dose response of rhBMP-2 using quantitative and qualitative endpoints. **Methods:** Femoral defects in rats were replaced with absorbable collagen sponges carrying rhBMP-2 (0, 1, 5, 10 or 20 µg; N=5). At 4-weeks new bone formation was assessed using quantitative (radiography and microcomputed tomography) and qualitative (histology and backscattered-SEM) endpoints statistically compared. **Results:** rhBMP-2 showed increased bridging in the gap. Quantitative evaluation presented a bi-phasic dose response curve. Histological assessment revealed that with rhBMP-2 the defect showed the presence of spongy bone with the trabeculae layered with active osteoblasts and osteoclasts. The density and compactness of the bone varied with the dose of rhBMP-2. **Conclusions:** Our findings revealed that all doses of rhBMP-2 result in new bone formation. However, there is an optimum dose of 12 µg of rhBMP-2 for bone repair in this model, above which and below which less stimulation of bone occurs.

**Keywords:** Fracture, Growth Factors, Critical Size Defect

## Introduction

In 2004, about 12.36 million limb fractures occurred in United States alone accounting for ~25% of all musculoskeletal injuries<sup>1</sup>. A significant percentage (5-10%) of fractured bones experience delayed healing or result in a non-union<sup>2</sup>. Impaired healing is also not uncommon in failed arthroplasty, spinal arthrodesis and bone tumor resection. The conventional method of using autologous iliac crest bone grafts (ICBG) could increase complications such as deep infections, neurologic injuries, and iliac wing fractures at the donor side<sup>3</sup>. At the host side there are concerns about mechanical strength, rapid resorption and stress fractures<sup>4,5</sup>. The alternative

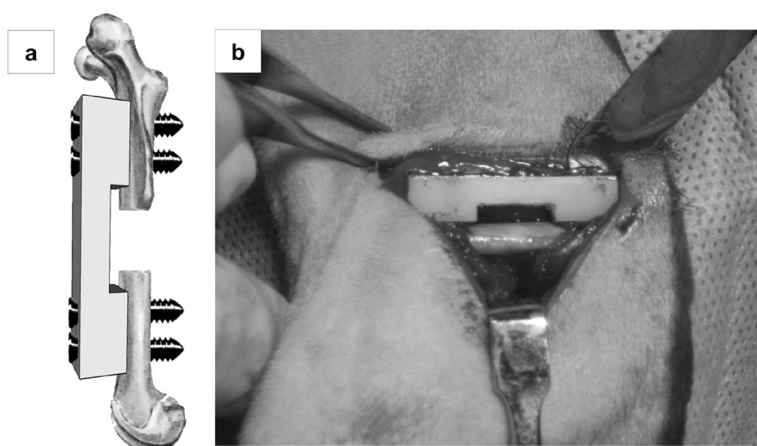
of using allografts could increase complications such as immunorejection and transmission of pathogens<sup>6,7</sup>. Local delivery of growth factors for bone repair is now becoming a common clinical approach<sup>8,9</sup>. Many growth factors such as transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and insulin-like growth factor-1 (IGF-1) play important roles in the cascade of fracture healing and have shown satisfactory results in healing fractures<sup>10-14</sup>.

Among all the factors identified in bone, BMPs are significant players in skeletal development and repair<sup>12,15,16</sup>. First identified because of their ability to induce ectopic bone formation *in vivo*<sup>17-19</sup>, they are now known to be multifunctional cytokines to play important role in skeletal tissue formation and healing<sup>18</sup>. BMPs regulate both intramembranous and endochondral ossification. Studies have shown BMP-2 can induce osteogenic differentiation in multipotent cells, progenitor cells and osteoblasts<sup>20-23</sup>. Recombinant human BMP-2 (rhBMP-2) has been most successful for bone repair in animal models<sup>24-28</sup> and clinically<sup>29</sup>. The concentrations of endogenous BMPs are much lower than the amount used clinically; nanograms compared to milligrams, respectively. Although recombinant proteins are free from adventitious matter, have consistent activity and reproducibility, they are considered less potent than native BMPs<sup>30</sup>. This makes high doses of

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**Figure 1.** a) Sketch of the internal fixator mounted on a femur with a 5 mm segmental defect (adapted(62)). b) Intraoperative view of the positioning of the internal fixator on the anterior surface of the left femur.

rhBMP-2 a requirement to produce an adequate biological response in humans<sup>16,31</sup>. Doses as low as 0.93  $\mu\text{g}$  in the presence of poly-lactic-co-glycolic acid (PLGA) have effectively aided the healing of segmental defects in rat femurs<sup>32</sup>. Doses of around 10  $\mu\text{g}$  have been shown to heal the defect in 42 days<sup>25,33</sup> while doses as low as 3.1  $\mu\text{g}$  have shown to cause a union at about 63 days<sup>32</sup>. So far the highest reported dose to show successful union in segmental defects is 11  $\mu\text{g}$  with demineralized bone matrix (DBM) as the carrier<sup>25</sup>. However, as these studies have used a variety of different carriers and time points, it is not possible to consolidate the information to deduce an optimum dose.

Higher doses of rhBMP-2 can have adverse effects such as local soft tissue inflammation, postoperative radiculitis and neurocompressive ectopic bone formation in humans<sup>34-36</sup>. There is no information in the literature on the most effective dose of rhBMP-2 required to heal segmental defect in the presence of an absorbable collagen sponge (ACS), the most common carrier used clinically. To determine the effect of the dose of rhBMP-2 in the presence of ACS on bone formation we carried out a dose response study using a range (1-20  $\mu\text{g}$ ) of rhBMP-2 doses. In this study, we quantitatively and qualitatively assess the outcome of rhBMP-2 with ACS for bone repair in a rat critical-sized femoral segmental defect. The results from this study forms the basis for ongoing work in which other osteotropic factors (biological and biomechanical) are being combined with rhBMP-2 to provide a safe and effective stimulus to the healing process.

## Materials and Methods

### *Surgical Model*

In an IACUC approved study, 25 male Sprague-Dawley rats (400-450 g, Charles River Laboratories, Wilmington, MA) were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg). A 4-hole high-density polyethylene (Small Parts, Inc, Miami Lakes, FL) custom fabricated internal fixator measuring 23x4x5 mm with a 9 mm (2 mm deep) central notch was used for fixation of the left

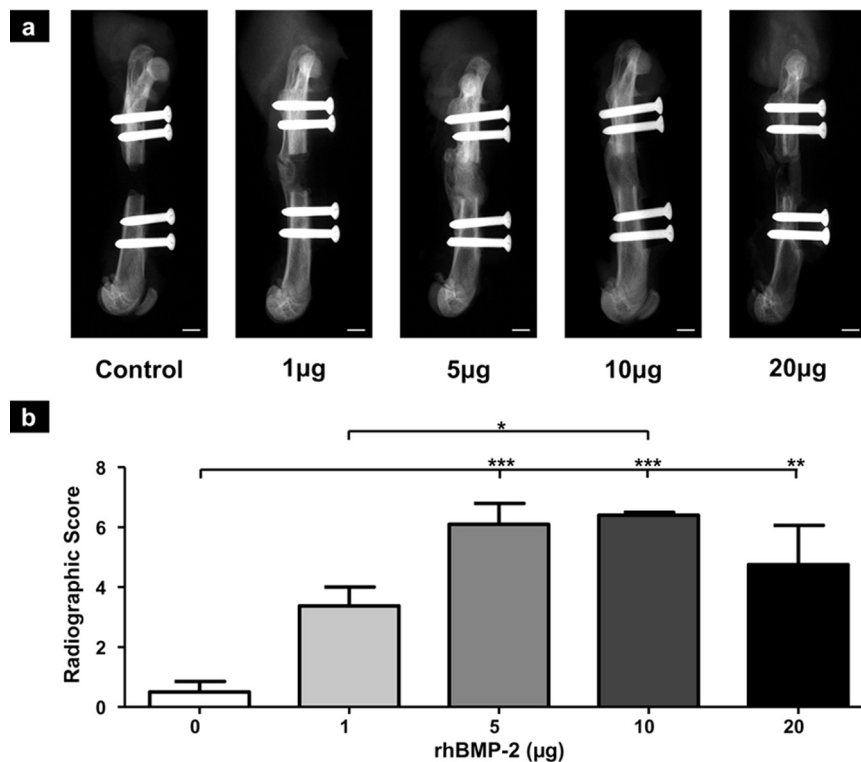
femur (Figure 1a). Two #0 stainless steel screws (Small Parts, Inc, Miami Lakes, FL) were used on each side of the defect. A 5-mm mid-diaphyseal defect was created in the femur using an oscillating saw (Stryker, Kalamazoo, MI) (Figure 1b). Subsequently, ACS (Helistat, Integra Lifesciences Co., Plainsboro, NJ) dry state size 12 X 6 X 7 mm was implanted into the defect. Prior to implantation the sponges were loaded under sterile conditions with 125  $\mu\text{L}$  buffer<sup>37</sup> containing 0 (control), 1, 5, 10 or 20  $\mu\text{g}$  ( $n=5$  per group) rhBMP-2 (Medtronic Sofamor Danek USA, Inc., Memphis, TN). Finally, the wound was closed in layers with sutures and surgical staples. Three rats were excluded due to screw loosening after surgery. All animals were sacrificed at 4 weeks. Extracted femurs were immediately fixed in 10% neutral buffered formalin for 48 hours and subsequently stored at 4°C in 70% ethanol.

### *Radiographs*

Dorsoventral and lateral contact radiographs were obtained of the post sacrifice extracted femora for all the animals at 70 kVp for 12 seconds. The defect region was qualitatively analyzed by 2 independent observers who were blinded to the groups. The radiographs were scored for callus formation, quality of union, and bone remodeling according to a scoring system developed by Sarban et al<sup>38</sup>.

### *Microcomputed Tomography*

Specimens were held securely in a 20.7-mm specimen holder, which was filled with 70% ethanol. A 10 mm mid-shaft region of the bones spanning the defect was scanned using 30  $\mu\text{m}$  isotropic voxels at 55 kVp, 145  $\mu\text{A}$ , and 300 ms integration time ( $\mu\text{CT}40$ , Scanco Medical, Wayne, PA). The manufacturer's software was then used to filter noise (Sigma=0.8, Support=1) and segment the data for the newly formed bone at a threshold of 1432 Hounsfield Units in the created defect. The bone volume (BV,  $\text{mm}^3$ ) and tissue mineral density (mg/cc of hydroxyapatite, based on a standard calibration phantom provided by the manufacturer) were determined.



**Figure 2.** Representative (defect with the median value) mediolateral radiographs of the segmental defects after 4 weeks of implantation (Bar: 5 mm). Radiographic score, with 12 µg as the calculated optimum dose of rhBMP-2 for maximum radiographic healing for this model. All data represented as mean ± SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Total bone mineral content (BMC, mg of HA) was calculated by multiplying bone volume with the mineral density.

### Histology

For undecalcified histology, the extracted femurs (N=4, per group) were embedded in polymethylmethacrylate (PMMA). Once embedded in PMMA, the samples were longitudinally prepared to yield a thickness of approximately 100 µm<sup>39</sup> and stained with basic fuchsin-toluidine blue. For decalcified histology, one representative sample from each group was decalcified and embedded in paraffin. Serial sections (6 µm thick) were stained with Fast Green-Safranin-O or Tartrate Resistant Acid Phosphatase (TRAP)-Fast Green-Hematoxylin. All sections were analyzed under light microscope (Eclipse 80i; Nikon, Tokyo, Japan).

### Backscattered Imaging

Images of the newly formed bone were made in the center of the defect using the scanning electron microscopy (SEM) in the backscattered mode. Prior to the measurements, 2 representative sections previously used for undecalcified histology, were polished to a mirror finish and coated with a thin carbon layer<sup>40</sup>. qBEI was then performed using a digital SEM with a backscattered electron detector (Hitachi S-3000N, Pleasanton, CA) at a working distance of 14.6 mm, with probe current of 98 µA. The samples were imaged at 100X magnifi-

cation. To convert gray-level values into proportionate atomic number (Z-score) values, all images were calibrated using reference gray-values of methylmethacrylate (Z=6.23) and aluminium (Z=13). To observe the variations in the mineral content within the ROIs, the frequency distributions (histograms), depicting the spread of the gray values were plotted.

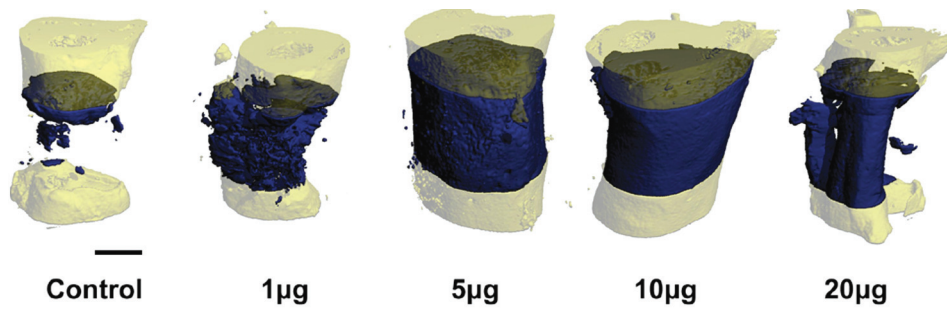
### Statistical analyses

The average radiographic score, BV and BMC data were expressed as mean ± standard error of the mean (SEM). Sample size for each group was at least 4. The significance of the group comparison was determined by one-way analysis of variance (ANOVA) test with Tukey's multiple comparison tests. For all tests, a confidence level of 95% ( $P < 0.05$ ) was chosen for statistical significance.

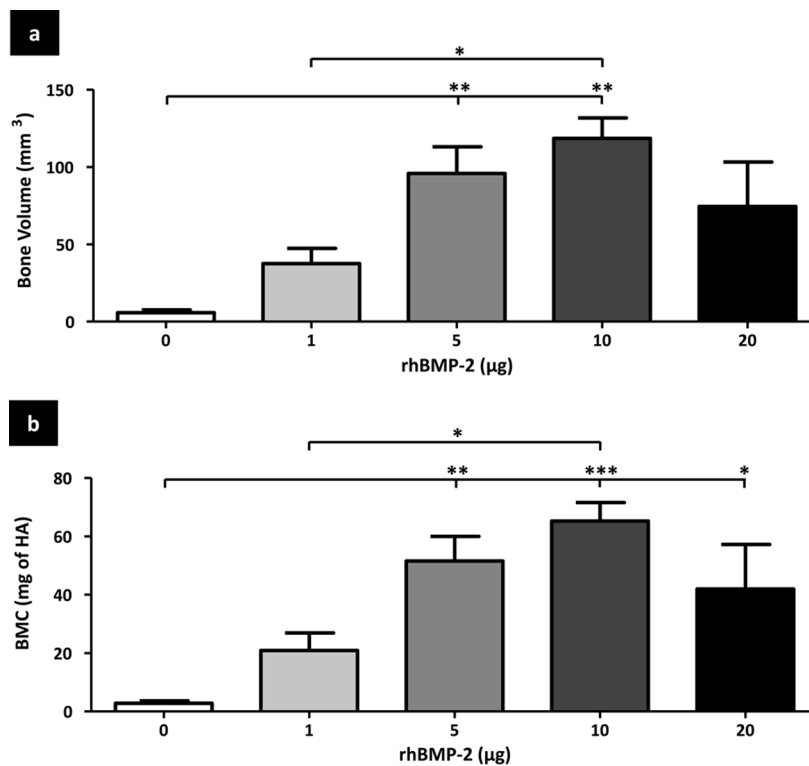
## Results

### Radiographic Evaluation

The newly formed bone was analyzed with radiographs taken after sacrifice by 2 independent observers (pearson coefficient for inter-observer reliability,  $r=0.97$  with  $P < 0.0001$ ). Radiographs showed that while control defects continued to be non-unions, all rhBMP-2 treated defects showed increased bridging with varied levels of callus formation (Figure 2a). Defects with higher doses of



**Figure 3.** Three dimensional reconstructions of the microCT slices. Representative (defect with the median value) microCT images of the segmental defect with varying rhBMP-2 doses loaded on ACS after four weeks of treatment. (Blue: newly formed bone in the 5 mm critical sized defect. Yellow: native cortical bone with periosteal reaction. Bar=2 mm).

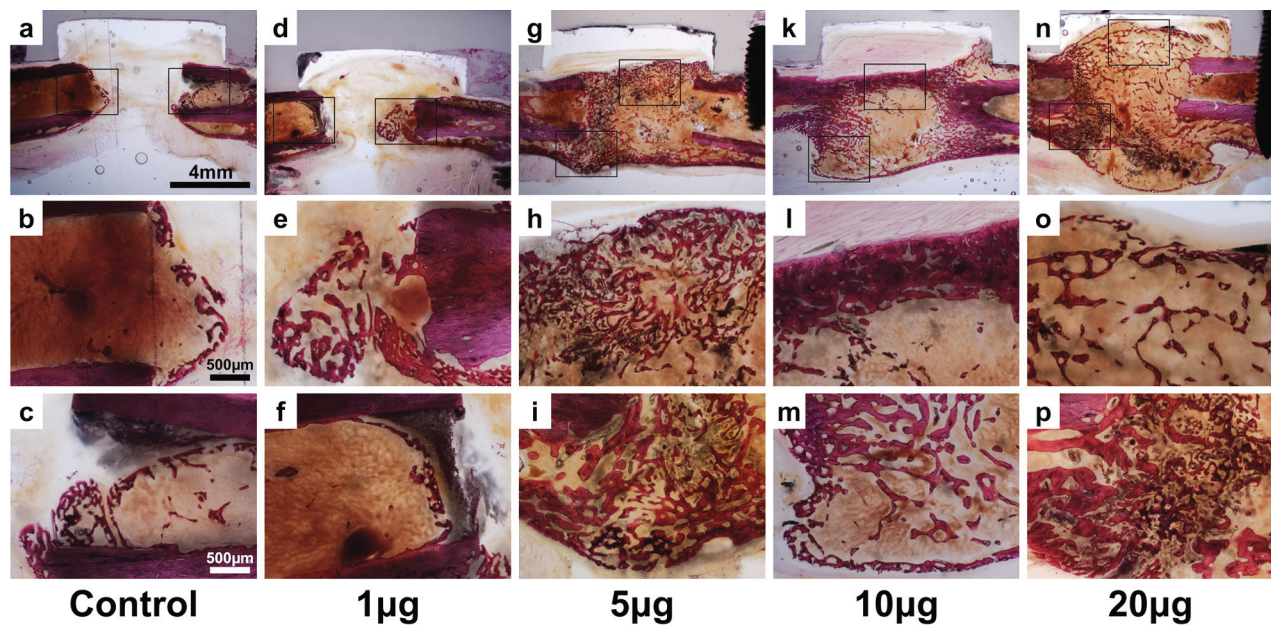


**Figure 4.** MicroCT evaluations after 4 weeks of rhBMP-2/ACS implantation. Bone volume (mm<sup>3</sup>) of the newly formed bone in the created defect (a). Bone mineral content (mg of hydroxyapatite) of the newly formed bone in the created defect (b). All data represented as mean  $\pm$  SEM. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001.

rhBMP-2 showed increased bridging in the gap with some levels of remodeling observed with the 10  $\mu$ g and 20  $\mu$ g of rhBMP-2. The radiographic score significantly increased with 5, 10 and 20  $\mu$ g rhBMP-2. The 10  $\mu$ g rhBMP-2 group also had a significantly higher radiographic score compared to the 1  $\mu$ g (P<0.05). Altogether, a biphasic dose response curve was observed as the dose increased with 20  $\mu$ g showing lesser healing than the 10  $\mu$ g (Figure 2b). A best-fit algorithm, second degree polynomial ( $R^2=0.87$ ), for the curve suggested 12  $\mu$ g being the optimum dose of rhBMP-2 for this model.

#### Microcomputed Tomography ( $\mu$ CT)

The mean measured defect size in all the animals was 4.82 mm (SEM=0.08, n=22) with no significant differences between groups. 3D reconstruction showed bridging with the use of rhBMP-2 while the control group showed no bridging (Figure 3). The new bone formation is not only in the defect but is also evident on the periosteal and endosteal surfaces of the native bone. In the 5 and 10  $\mu$ g dose groups, the mineralization of the fracture callus on the periosteal surface led to an appar-



**Figure 5.** Representative histological tissue mineralization in sections of the defect stained with basic fuschin after 4 weeks of rhBMP-2/ACS treatment. The control group and 1  $\mu\text{g}$  of rhBMP-2 showed mineralized bone at the ends of the defect site (a-c & d-f). With 10  $\mu\text{g}$  of rhBMP-2 the defect was bridged with a thickened anterior callus suggesting lamellar bone organization (k-m). 5  $\mu\text{g}$  and 20  $\mu\text{g}$  appeared to showed early architecture (g-i & n-p).

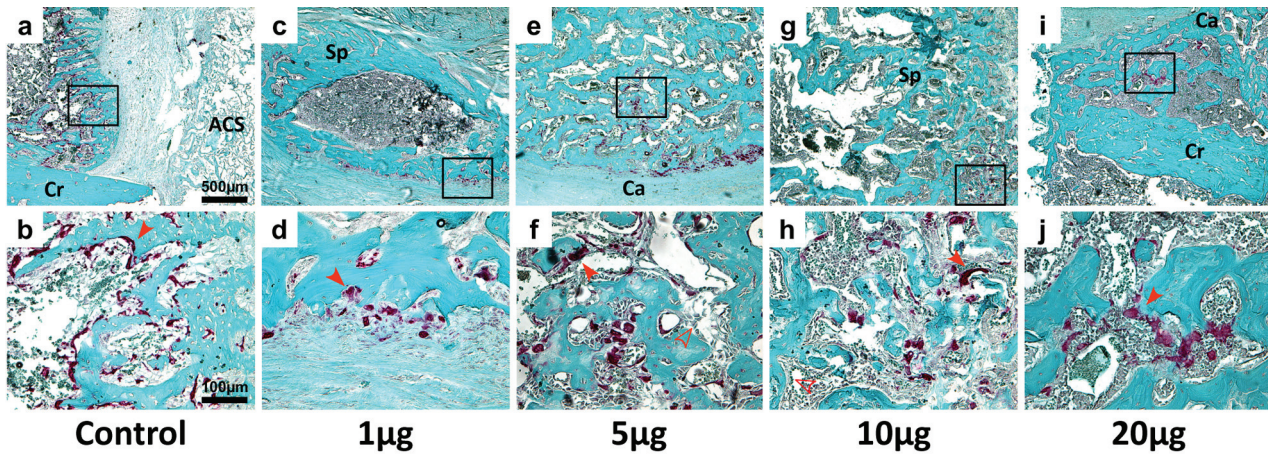
ent thickening of the cortex to about twice its original thickness. However, this thickening was not seen in the 1 and 20  $\mu\text{g}$  dose groups.

BV is the calculated total volume of newly formed bone-like tissue in the defect region. All doses of rhBMP-2 increased the BV in the defect region as compared to control (Figure 4a). At the end of 4 weeks, BV increased significantly with 5  $\mu\text{g}$  and 10  $\mu\text{g}$  as compared to the control group ( $P < 0.01$ ). The 10  $\mu\text{g}$  dose group also showed a significant increase in BV as compared to 1  $\mu\text{g}$  ( $P < 0.05$ ) group. However, BV in the 20  $\mu\text{g}$  dose group was not significantly different from any of the other groups. A bi-phasic dose response curve was observed as the dose increased with 20  $\mu\text{g}$  showing lesser BV than 10  $\mu\text{g}$  (Figure 4a). BMC calculated from the density of the mineral gives an estimate of the total amount of mineral deposited in the defect region in milligrams of hydroxyapatite (mgHA) equivalent. After four weeks, BMC was significantly higher with 5, 10 and 20  $\mu\text{g}$  doses, as compared to the control group (Figure 4b). The 10  $\mu\text{g}$  dose group also had higher BMC compared to 1  $\mu\text{g}$  ( $P < 0.05$ ) dose group. Again, a bi-phasic dose response curve was observed as BMC in the 20  $\mu\text{g}$  dose group was less than in the 10  $\mu\text{g}$  group (Figure 4b). A best-fit algorithm, second degree polynomial ( $R^2 = 0.98$  and  $R = 0.98$ ), for the curve suggested 12  $\mu\text{g}$  being the optimum dose of rhBMP-2 for this model.

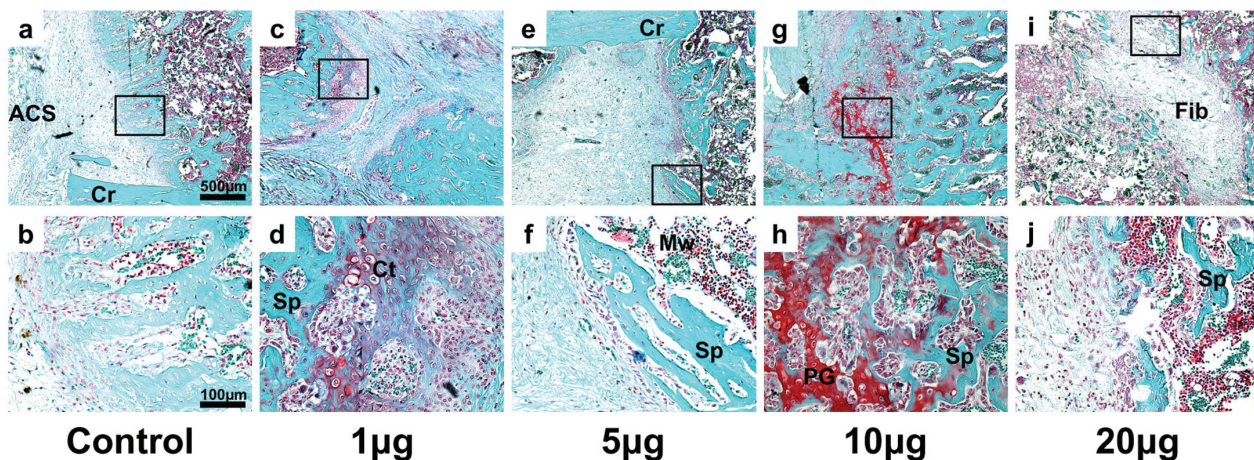
### Histology

Histology was used to confirm the bone formed at the defect site as demonstrated by  $\mu\text{CT}$ . All groups showed forma-

tion of callus and some showed cortical remodeling. A varied level of tissue mineralization and organization was observed depending on the dose of the rhBMP-2. The control group showed mineralized bone at the ends of the defect site (Figure 5a) surrounded with TRAP positive osteoclasts through the intra-medullary space (Figure 6b). By decalcified histology, remnants of the ACS were noted in the defect region in the control group (Figure 6a and 7a), while the ACS was absent in the rhBMP-2 treated defects. Treatment of 1  $\mu\text{g}$  showed mineralized bone at the ends (Figure 5d) of the defect with new trabecular bone islands in the defect region (Figure 6c) surrounded with active TRAP positive osteoclasts (Figure 6d). The defect region also showed the presence of calcified cartilage (Figure 7d). However, with 5 and 10  $\mu\text{g}$  dosing the cortical bone adjacent to fracture site was circumferentially thickened with cancellous bone (Figure 5g & 5k). Both groups qualitatively appeared to provide better union and cortical remodeling when compared to the other groups. The defect region consisted of spongy bone (Figure 6e & 6g) with the trabeculae layered with active osteoblasts and osteoclasts (Figure 6f & 6h). The hard callus showed presence of TRAP positive osteoclasts in the endocortical and periosteal regions. Pockets of bone marrow were visible in between the trabeculae (Figure 7f). The 10  $\mu\text{g}$  showed a thickened anterior callus with lamellar bone organization (Figure 5l). It also showed the presence of chondrocytes in the defect region which marks early stages of endochondral ossification (Figure 7h). The 20  $\mu\text{g}$  qualitatively showed a bigger callus (Figure 5n) with spongy bone and many trabeculae but low levels of lamellar organi-



**Figure 6.** Representative histological remodeling of the tissue with TRAP, fast green and hematoxylin stained sections of the defect after 4 weeks of rhBMP-2/ACS treatment. (Dark arrowheads: Osteoclasts, Open arrowheads: Osteoblasts, Sp: Spongy bone, Ca: Mineralized Callus, Cr: Cortical Bone).



**Figure 7.** Representative histological progression of the tissue with fast green and safranin-o stained sections of the defect after 4 weeks of rhBMP-2/ACS treatment. (Sp: Spongy bone, Ca: Mineralized Callus, Cr: Cortical Bone, Mw: Bone marrow, PG: Proteoglycans, Ct: Calcified Cartilage, Fib: Firous connective tissue).

zation (Figure 5o & 5p). Although the amount of osteoclastic resorption at the periosteal surface and hard callus was comparable to the other groups (Figure 6j) we observed some levels of fibrous connective tissue formed in the 20 µg groups (Figure 7i).

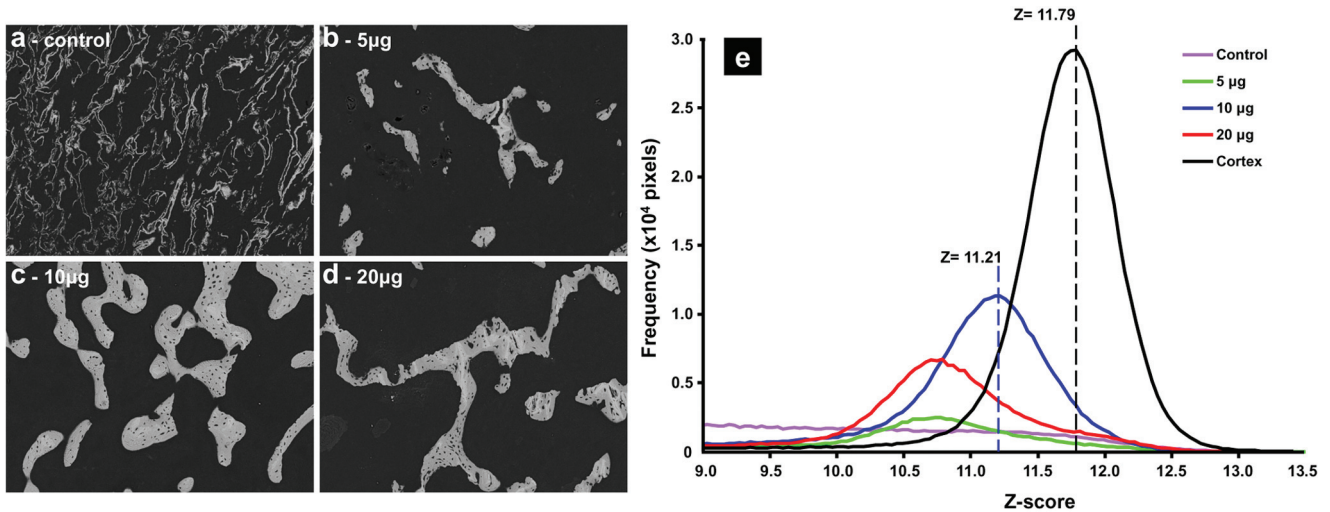
#### *Quantitative Backscattered Imaging*

qBEI images were obtained from the center of the defect. The control group showed deposits of mineral on the ACS (Figure 8a). The use of 5, 10 and 20 µg showed trabecular of newly formed bone (Figure 8b-8d). The density and compactness of the bone varied with the dose of the rhBMP-2. The frequency distribution of the mineral content is shown in (Figure 8e). The

mineral content in the defect with 10 µg of rhBMP-2 was closest to the mineral content of the cortical bone. The use of 5 and 20 µg showed similar frequency distribution but 20 µg showed higher absolute values than 5 µg group.

#### **Discussion**

rhBMP-2 is perhaps the most studied osteoinductive molecule and is the only one that is currently being used in the clinics for bone healing with FDA approval (Infuse<sup>®</sup>, Medtronic Inc.). However, the amount of rhBMP-2 delivered for such purposes is extremely high and poses risk of side-effects. Previously, investigations in the canine segmental defect models



**Figure 8.** Quantitative backscattered electron imaging – scanning electron microscopy (qBEI-SEM) images obtained from the center of the defect. The control group showed deposits of mineral on the ACS (a). The use of rhBMP-2 showed trabecular bone formation (b-d). The graph represents the histogram of the mineral content represented by proportionate atomic number (Z-score) values (e).

have been conducted but their primary focus has been to study the usage of bulking agents or bone void fillers along with rhBMP-2/ACS<sup>41,42</sup>. Boyce et al. employed two separate doses of rhBMP-2 in their study but did not observe any difference in the degree of healing between them<sup>41</sup>. Jones et al. observed a possible decrease or plateauing in the histological evaluation of bone bridging with higher dose but it was not statistically significant compared to the effective dose<sup>42</sup>. Sciadini et al. have reported a dose dependent effect on the healing process. They also observed a dose dependent increase in bone voids<sup>43</sup>. These findings support our hypothesis that higher doses of rhBMP-2 could have detrimental effects on the healing. However, these studies were performed in dogs and are not directly translatable to rats due to differences in their anatomy and physiology. Sciadini et al. also suggest that a species and application-specific dosing of rhBMP-2 is required. Experimental model of critical sized segmental defect in the rat femur is an established method to study large bone defects and compound fractures seen clinically. Yasko et al. have previously reported the use of 11 µg rhBMP-2 in a model similar to ours with similar findings<sup>25</sup>. However, that study was performed with demineralized bone matrix (DBM) as a carrier and not ACS. DBM by itself has osteoinductive properties and is known to contain a multitude of growth factors including BMPs. The model system of ACS/rhBMP-2 used in our study is identical to the current clinical practice of spinal fusion.

Exogenous rhBMP-2 accentuates the healing process by initiating chemotaxis, proliferation and osteoblast or chondrocyte differentiation<sup>44</sup>. In our study, the absence of rhBMP-2 continued to show relative radiolucency in the defect region which was consistent to the minimal or no bridging seen in µCT analysis. The use of ACS mimics the clinical approach of delivery of rhBMP-2. ACS provides a framework for cellular in-

filtration and electrostatic binding of rhBMP-2; and degrades into physiological end-products as bone formation progresses<sup>45</sup>. Histologically observed remnants of the ACS in the defect region warrant the necessity to recruit the appropriate cells in response to the use of rhBMP-2 to initiate the bone formation. As a result, at 4 weeks, an empty (ACS only) defect appears to develop into a critical sized non-union model. Use of rhBMP-2 increased the radio-opacity in the defect region and a hard callus was seen to be formed. µCT further supported our radiographic finding by showing enhanced bridging with the use of rhBMP-2. Use of 5 and 10 µg rhBMP-2 significantly increased the BV and BMC in the defect region as compared to control. Histologically, the newly formed bone, evident on the periosteal and endosteal surfaces, showed varied levels of tissue organization and the cortical bone adjacent to fracture site was circumferentially thickened with cancellous bone. The spongy bone presented trabeculae layered with active osteoblast and resorptive osteoclasts. The retained rhBMP-2 has the ability to stimulate endochondral ossification. The presence of cartilaginous tissue in the defect region, at 4 weeks could be indication of early stages of endochondral ossification, which might result into higher levels of BV if observed at a later time point.

The stiffness and the modulus of the bone is not only dependent on the mineral content<sup>46</sup> but also on the organic composition<sup>47</sup>. qBSEM evaluation showed 10 µg to stimulate the bone formation with mineral content close to that of the cortical bone. The similarity in the mineral properties 5 and 20 µg, suggests a comparable progression in the mineralization. However, at 4 weeks, 20 µg showed a reduction in the radiographic healing and bone formation as compared to the use of 5 and 10 µg of rhBMP-2. Some levels of fibrous connective tissue formation, mimics end-stage fracture nonunion<sup>48,49</sup>. But the

presence of some spongy bone with low levels of matured bone, as seen in qBSEM, suggests the lack of remodeling or slow progression of mineralization. High doses of rhBMP-2 have been associated with a significant risk of postoperative osteolysis<sup>50</sup>. In fact, Sumner et al., in the past have reported high doses of rhBMP-2 to initially stimulate net bone resorption in the adjacent host bone followed by bone formation<sup>51</sup>. In addition, Toth et al., have reported that overfilling or hyperconcentrating the rhBMP-2/ACS can lead to osteoclastic resorption of peri-implant cancellous bone<sup>52</sup>. However, the osteoclastic activity is transient and not observable at 4 weeks after surgery but replaced with osteoblastic activity and intramembranous bone formation. The histological findings qualitatively support our data that higher doses of rhBMP-2 could suppress bone formation. This could be a result of the net bone resorption initially stimulated by a high dose of rhBMP-2.

In summary, a biphasic dose dependant response was observed with a calculated optimum dose of 12 µg of rhBMP-2, above which and below which less stimulation of bone occurs. Studies in the past have demonstrated biphasic response on osteoinductive activity<sup>53</sup> and proliferation and/or migration of osteogenic cells and bone formation<sup>54</sup> with growth factors such as recombinant human basic fibroblast growth factor (rhFGF-2). TGF-β is also known to have a biphasic dose effect, *in vitro* on bone cell replication<sup>55</sup> and proliferation and osteoblastic differentiation of mesenchymal cells<sup>56</sup>. In fact Bostrom et al. have shown a dose dependent response to rhBMP-2 in the healing of a rabbit ulnar non-union<sup>57</sup>. In our study, we have shown a biphasic healing response to the use of rhBMP-2 for the first time in a segmental defect model. The reduction in bone content could be attributed to the activation of osteoclasts by the higher doses of rhBMP-2. Signaling pathways involved in this erroneous activation of osteoclasts have been reported previously<sup>58-60</sup>. The lack of the initial resorptive phase with the lower doses examined in the present study could explain why it is associated with more bone formation. The current study lack observations made at time points other than at 4 weeks. Although additional healing time may account for remodeling of the callus, most of the endochondral ossification occurring adjacent to the fracture site spans a period of up to 28 days<sup>61</sup>. However, at later time points, the characterization of the tissue mineralization might not be feasible due to the subtle variations. A more detailed time course study would be needed to confirm the currently observed dose response at later time points, or the ability of higher doses to rescue the bone formation at a later time point. The exact mechanism of the dose response is unclear and demands further study. It depends on several factors including the site and concomitant soft tissue injury. We plan to use the information from this study for determining (with qualitative, quantitative and functional end points) if lower doses of rhBMP-2 can be used in combination with other treatments and for investigating mechanisms of bone healing in this model.

## Conclusion

In conclusion, rhBMP-2 was effective in bridging and increasing the bone volume in the critical sized defect. The dose response curve exhibits a bi-phasic mode with 20 µg of rhBMP-2 showing lesser healing than the 10 µg. Although the calculated values from this study cannot be directly translated clinically, it is clear that there is an optimum dose of rhBMP-2, and less stimulation of bone occurs when the dose is either too high or too low.

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