INTRODUCTION: Impaired osteogenic healing is not uncommon in orthopedics and includes fractures, distraction osteogenesis, arthroplasty, spinal arthrodesis and bone tumor resection. Using autologous and allogeneic bone grafts to aid repair increases the risk of donor site morbidity, immunorejection and transmission of pathogens. rhBMP-2, due to its pronounced effects in skeletal development and repair is becoming a common clinical approach to enhance bone repair [1,2]. Clinically, a large amount of rhBMP-2 is used to ensure healing but 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. Furthermore, high dose of rhBMP-2 carries the risk of adverse effects locally and remotely. In this study, we used a rat femoral segmental defect model to assess the dose response of rhBMP-2 using quantitative and qualitative endpoints.

METHODS: Animal model: In an IACUC approved study, a 5-mm mid-diaphyseal segment of bone was removed from the left femur of 25 male SD rats and internally stabilized with HDPE fixator and SS screws. The resulting gap was filled with absorbable collagen sponges (ACS) carrying rhBMP-2 (0, 1, 5, 10 or 20 µg; N=5). All animals were sacrificed at 4 weeks, femur excised and fixed in 10% formalin.  

Radiographs: Contact radiographs of the extracted femora were obtained for all the animals and scored by two independent blinded observers according to a previously developed scoring system [3]. Microcomputed Tomography (µCT): 10mm mid-shaft region spanning across the defect were scanned (55 kVp, 145 µA, 30µm element size). Bone volume (BV) and bone mineral content (BMC) were measured. Histology: Undecalcified PMMA embedded sagittal sections (~500 µm) were stained with basic fuchsin-toluidine blue. Decalcified samples were sectioned longitudinally and stained with Fast Green-Safranin-O or Tartarate Resistant Acid Phosphatase (TRAP) with a counter stain of Fast Green. Sections were viewed under an optical microscope and viewed under fluorescent light. qBEI: qBEI was performed using a SEM with a backscattered electron detector. Images were calibrated for gray-values and the frequency distributions (histograms) were plotted. Statistics: One way ANOVA with Tukey’s multiple comparison test.

RESULTS: Radiographs: ACS without rhBMP-2 exhibited non-unions while rhBMP-2 showed increased bridging in the gap (Fig 1). A bi-phasic second degree polynomial dose response curve (R²=0.87), revealed 12µg being the optimum dose of rhBMP-2 in this model.

DISCUSSION: Our findings revealed that all doses of rhBMP-2 enhanced bone formation compared to no rhBMP-2 when assessed by radiographic, µCT and histology criteria. The presence of rhBMP-2 resulted in hard callus and the mineralization occurred not only on the endosteal surface but also on the peristeal surface thus enhancing the bridging. The tissue architecture and progression of healing is dependent on the dose of BMP-2 delivered in the defect area. It appears, BMP-2 can be retained at the site of placement and stimulate endochondral ossification (marked by the presence of chondrocytic cells in the defect region) up to 4 weeks thereafter. Simultaneously, it also drives early mineralized tissue to remodel and form matured bone. However, higher amount of rhBMP-2 inhibited bone formation and/or maturation. The exact mechanism for this action is not clear. In summary, rhBMP-2 is effective in bridging and healing a critical sized defect. The dose response curve exhibits a bi-phasic mode, with 12µg as the calculated optimum dose of rhBMP-2 delivered on ACS for bone repair in a rat critical-sized femoral segmental defect.

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