Introduction: Despite the intrinsic ability of bone to heal, there are still numerous clinical circumstances where bone healing is defective and demands the attention of the physician. Common examples include the delayed and non-union of fractures, and the loss of large segments of bone after traumatic injury, tumor resection and failed arthroplasty. There are several surgical approaches to enhance the healing of human bones, and these have been supplemented in recent years by the introduction of recombinant, human bone morphogenetic proteins -2 and -7 (BMP-2 and BMP-7) into clinical practice, in combination with different fixation devices. Despite many advances in technology, which help to improve the fixation of critical sized defects their clinical management remains problematic because it is unclear how stiff or flexible the fixation device should be. Our project is based on the hypothesis that the healing of critical-sized defects in response to BMP-2 can be dramatically improved by manipulating the mechanical environment within the defect. Accordingly, this project aims to investigate the influence of the mechanical environment on bone healing in response to BMP-2 in a rat, femoral, critical-sized defect model. The mechanical environment of the defect is altered with a custom made external fixator whose stiffness can be changed in a reliable, quantitative fashion.

Materials and Methods: External fixators that can be adjusted to provide three different stiffnesses were designed specifically for the rat femur. All materials in the fixator are the same as used clinically for human implants. The screws are made from titanium. The stability bars are made from polyetheretherketone (PEEK). The diameter of the screws is 1mm and their length is 12.75mm. The distance between the screws is 4mm and the distance between the middle screws is 11mm. All holes are predrilled using a 0.79mm drill bit. The screws are locked in the corresponding holes of the fixator, which is parallel to the bone surface and set at a distance of 5mm. The stability bars can be changed to provide stiffnesses of 40, 70 or 100%. To create reproducible 5mm defects in all rats the guide was designed for a 0.22mm wire saw, which clips on the external fixator in between the two middle screws. A rat, femoral, critical-sized defect model was used to test this fixator in-vivo. A 5mm defect was created in 6 Sprague-Dawley rats and treated with rhBMP-2 applied on a collagen sponge with external fixators providing 40, 70, 100% stiffness. Animals were x-rayed weekly for 8 weeks to monitor bone healing and µCT analysis was performed at the end of treatment to evaluate bone architecture in the healed defect. The animal protocol was approved by the local IACUC.

Results: The external fixator prototype was successfully created and manufactured for testing in-vivo (Figure 1). Weekly X-rays revealed that bone callus size was biggest in the group with the lowest stiffness (40%) fixator. Furthermore, early callus formation was seen in this group and 70% stiffness group after 9 days of treatment. However, the group with 100% stiffness external fixator had no callus formation after 9 days of treatment; callus formation was delayed until after two weeks of treatment. By the third week defects were bridged with all fixation methods with the biggest callus in 40% and 70% stiffness fixators and smaller callus with 100% stiffness (Figure 2). The same pattern of healing was seen at the end of 8 weeks treatment, as shown by X-ray and µCT analysis (Figure 2, 3). The biggest callus and bone areas were formed at the defect with the lowest stiffness fixator. There was a gradual decrease in the callus size as the fixator stiffness decreased, but the bone area was lowest with the 70% stiffness fixator (Figure 3).

Discussion: Loss of large segments of bone leads to critical-sized defects that fail to heal spontaneously. Although healing can be induced by recombinant, human bone morphogenetic protein-2 (rhBMP-2), the clinical response is modest. Our data suggest that fixator stiffness can strongly influence the biologic process of healing. Radiologically, we observed that with 40 and 70% stiffness, callus formation was already visible after 9 days of treatment, whereas defects subjected to 100% stiffness had no visible callus at this time. After two weeks bone regeneration was seen in all groups, but the two lower stiffness groups had bigger calluses; this pattern was also observed at the end of 8 weeks treatment by X-ray and µCT. This is consistent with literature reports that micro motion during bone healing produces bigger callus. A bigger study with 10 animals per group is underway to confirm the results from this pilot study. Furthermore, material, structural and mechanical testing will be performed to determine how important early callus formation and size are to the physical properties of the healed bone. With this information, it should prove possible to manipulate fixator stiffness through the course of healing to get rapid bone regeneration and optimize the quality of healed bone. The findings will be highly relevant to the surgical management of patients with segmental bone defects and, possibly, delayed and non-union fractures.

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