Introduction: Much experimental evidence confirms that fracture healing is strongly influenced by the local mechanical environment. However, relatively little attention has been paid to the effects of mechanical forces on the healing of large segmental defects. Previous investigations have shown that there is not one set of mechanical circumstances that suits all stages of fracture healing, and that healing might be improved by changing the stiffness of the fixator as healing progresses. Only a few clinical studies have attempted to determine the effect of dynamization at the various stages of fracture repair, and it is unclear whether this accelerates bone repair. However, there are no studies that have attempted to determine the effects of changing the mechanical environment during the healing of critical size segmental defects (CSDs). Therefore, the specific aim for this study was to determine whether the mechanical environment influences the healing of CSDs in response to rhBMP-2 in a rat, femoral, segmental defect model, and to determine whether healing could be improved by increasing the stiffness of fixation during the healing process.

Materials and Methods: The degree of early callus formation in response to external fixators with low (ExFixLow 114 M/mm), medium (ExFixMed 185 N/mm) and high (ExFixHigh 254 N/mm) stiffnesses was first determined in a pilot study. A 5 mm, CSD was created in the right hind limb of each of 27 male Sprague-Dawley rats (weight 325-360 g), treated with 11μg rhBMP-2 and stabilized by one of the external fixators. The rats were assigned to one of 3 groups with 3 animals per group, sacrificed 6, 9 and 14 days post surgery, and analyzed by histology. Once the histological data had shown which fixator provided the best callus formation and early mineralization, as well as an appropriate time to change to a stiffer fixator, a full healing study was performed. For the second part of the study, a 5 mm critical sized defect was created in the right femur of each of 12 male Sprague-Dawley rats treated with rhBMP-2, as before, and stabilized with ExFixLow. After 2 weeks, the external fixator was replaced by ExFixHigh for the remaining 6 weeks of treatment; this group will be referred to as Modulated. Animals were x-rayed weekly for 8 weeks to monitor bone healing; μCT, mechanical testing and histological analyses were performed at the end of treatment to evaluate the quality of healed defects. The animal protocol was approved by the local IACUC.

Results: Early callus formation and mineralization were seen with the 2 lower stiffness fixators, but not with ExFixHigh. Two weeks after surgery, the defects that had been treated with lower stiffness fixators were filled with extensive new woven bone around the residual sponge scaffold; this was not present in the group with ExFixHigh. Surprisingly, no cartilage was seen at any time with any fixator (data not shown). Based upon these data, it was decided to modulate the stiffness of the fixator 2 weeks post-surgery.

In the second part of the experiment, weekly X-rays revealed that the Modulated group had advanced defect healing compared to unmodulated low, medium and high stiffness groups. A week after the fixator was changed, 3 weeks after the initial surgery, x-rays revealed complete callus bridging with bony tissue and no evidence of radiolucent lines in the defect. In contrast, soft tissue persisted in those defects stabilized with continuous ExFixLow and ExFixMed until 4 weeks after the surgery, and with ExFixHigh for at least 6 weeks. μCT analyses, mechanical testing and histology were also in agreement with the X-ray data. μCT (Fig.1) and histological images (Fig.2) revealed that formation of new cortical bone had even circumferential distribution of the healed callus over the entire length of the segmental defect, which was not observed in the other groups. Bone area (BA, mm²) in the Modulated groups was significantly lower than in all other groups, and closer to the intact femur callus size. Total area (TA, mm²) was significantly smaller in the Modulated group as compared to the ExFixLow, but smaller than other groups (Fig.3). Torsional strength and stiffness was also significantly higher in the Modulated groups (data not shown).

Discussion: This study demonstrated for the first time that healing of CSDs in response to BMP-2 is highly responsive to the ambient mechanical environment. Moreover, the results of this study confirm that initial stabilization with ExFixLow followed at two weeks by modulation to ExFixHigh indeed improves defect healing both in terms of the speed of healing and the quality of the healed bone. The premise for the change to a much stiffer fixator at 2 weeks (fixator “Modulation”) was to accelerate the process of endochondral ossification. However, our preliminary histological examination provides no evidence of an endochondral process; this matter requires further investigation. Further investigation is needed to determine the pathway of bone healing in CSDs and how this is modulated by the ambient mechanical environment.

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