Effects of the local mechanical environment on vertebrate tissue differentiation during repair: does repair recapitulate development?

The local mechanical environment is a crucial factor in determining cell and tissue differentiation during vertebrate skeletal development and repair. Unlike the basic response of bone to mechanical load, as described in Wolff's law, the mechanobiological relationship between the local mechanical environment and tissue differentiation influences everything from tissue type and molecular architecture to the formation of complex joints. This study tests the hypothesis that precisely controlled mechanical loading can regulate gene expression, tissue differentiation and tissue architecture in the adult skeleton and that precise manipulation of the defect's local mechanical environment can initiate a limited recapitulation of joint tissue development. We generated tissue type predictions using finite element models (FEMs) interpreted by published mechanobiological fate maps of tissue differentiation. The experiment included a custom-designed external fixator capable of introducing daily bending, shear or a combination of bending and shear load regimens to induce precisely controlled mechanical conditions within healing femoral defects. Tissue types and ratios were characterized using histomorphometrics and molecular markers. Tissue molecular architecture was quantified using polarized light and Fourier transforms, while immunological staining and in situ hybridization were used to characterize gene expression. The finite element models predicted the differentiation of cartilage within the defects and that substantial fibrous tissues would develop along the extreme excursion peripheries in the bending group. The three experimentally induced loading regimens produced contiguous cartilage bands across all experimental defects, inhibiting bony healing. Histomorphometric analysis of the ratios of cartilage to bone in the experimental groups were not significantly different from those for the knee joint, and Fourier transform analysis determined significantly different collagen fibril angle specializations within superficial, intermediate and deep layers of all experimental cartilages ($P<0.0001$), approximating those for articular cartilage. All stimulations resulted in the expression of collagen type II, while the bending stimulation also resulted in the expression of the joint-determining gene GDF-5. These findings indicate that the local mechanical environment is an important regulator of gene expression, tissue differentiation and tissue architecture.