Stem Cell-Mediated Regenerated Bone Remodels and Regains Biomechanical Competence Based on Micro Finite Element Analysis

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INTRODUCTION:
Biomechanical evaluation of a regenerated tissue is an important application in skeletal regenerative medicine. Microcomputed tomography (microCT) is an imaging method that allows access to the fine three-dimensional (3D) microarchitecture of bone. Using microstructural finite element (microFE) models it is possible to simulate a mechanical test in great detail and with high precision[1]. We have previously shown that BMP-2 engineered Mesenchymal Stem Cells (MSCs) can induce non-union fracture repair in a radius bone defect model, showing prominent new bone formation[2]. Using nanobiomechanical tools we showed that the intrinsic properties of the newly formed bone mass were similar to the contralateral, intact, bone tissue[3]. However, the structural and biomechanical properties of the regenerated bone after a prolonged period of time remained unknown. In this study we hypothesized that the new bone tissue formed by BMP-engineered MSCs in a critical-size bone defect, remodels and provides biomechanical competence over time. In order to investigate this hypothesis we utilized a novel image-based system for the biomechanical analysis of murine radii.

METHODS:
22 C3H/HeN female mice were used for this study. The experimental protocol was evaluated and approved by the Animal Ethics Committee of the Hebrew University in Jerusalem. Segmental defects (2.5 mm long) were created in the mice radii. Cultured rhBMP-2–expressing MSCs (derived from the C3H10T1/2 MSC line)[3] were loaded onto a collagen sponge (Duragen, Integra LifeSciences Co., NJ, USA). The cell-loaded scaffold was placed in the space created by the microsurgery. The intact contralateral limbs of the same mice were used as controls. The mice forelimbs were scanned using a microCT scanner (µCT 40, Scanco) 10 and 35 weeks post implantation. Morphometric indices were determined for the newly formed bone in the nonunion fracture site, by evaluating the central 2 mm of the bone defect. Micro- FE models of the middle 8mm of the radius and ipsilateral ulna were created. Bone tissue was assumed to be isotropic and constant (E=10 GPa). The distal part of each model was fully fixed, whereas the proximal part underwent an axial displacement, resulting in 1% overall strain. The long axis of the bones was carefully aligned to the loading direction. The models were used to calculate the force needed to obtain the prescribed displacement, from which apparent stiffness was derived.

RESULTS:
Prominent formation of new bone tissue was evident in the fracture site (Fig.1). The regenerated bone tissue (Fig.1 b-c) demonstrated distinct morphology compared to the intact controls (Fig.1 a). Quantitative analysis of the newly formed bone in the nonunion fracture site, at 10 vs. 35 weeks after implantation, showed a decrease in bone volume and connectivity density (Fig.2 a-b) and an increase in bone mineral density (Fig.2 c). The structural indices of the regenerated bones, at 35 weeks, were closer to the indices of the intact untreated bones, than the indices at 10 weeks. These changes indicate a remodeling process of the regenerated bone. MicroFE analysis demonstrated that the regenerated bone was structurally intact. The regenerated bones were substantially stronger than the control ones, having a higher axial stiffness compared to the native bones (Fig.2 d).

DISCUSSION:
This study has shown that a long bone non-union fracture, regenerated by the implantation of MSCs engineered to express the BMP-2 gene, remodels, gains and maintains biomechanical functionality over a prolonged period of time. Using microCT imaging we were able to show changes in the structural parameters of the newly formed bone indicating tissue maturation and remodeling. Mineral density, geometry, microarchitecture, and the material characteristics of the bone are all components that determine the bone strength as defined by its ability to withstand loading. Although we have seen an increase in the stiffness of the regenerated bones, no clear correlation was found between the microCT bone volume and the bone axial stiffness. This might be due to partial fusion of the radius and ulna in the regenerated bones, which caused an elevation of the stiffness values. The assumption of constant bone tissue properties may provide a second explanation to this finding.

REFERENCES: