Early vascularization induced by EPC in a critical bone defect enhances bone healing after eight weeks in rats

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**Introduction:**

Early vascularization of bone defects is a prerequisite for ingrowth of osteogenic reparative cells to regenerate bone in vivo. The size of the bone defect may limit the ingrowth of bone forming cells, since lack of vessels does not ensure a sufficient nutritional support of the bone graft. Endothelial progenitor cells (EPC) participate in neovascularization, thus playing an important role in the process of vascular repair (1,2,3,4). These cells may provide a powerful cellular therapeutic strategy for vascularization of a bone matrix.

This investigation tested the ability of human early endothelial progenitor cells (EPC, 5) in coculture with mesenchymal stem cells (MSC) seeded on suitable scaffold (β-tricalcium phosphate (β-TCP)) and placed into a critical-sized segmental defect in the femur of adult athymic rats. We hypothesized that coculture of EPC and MSC improve the vascularization and therefore accelerate the bone healing process of large bone defects in vivo.

**Material and Methods:**

For this in vivo study, human early EPC were isolated from buffy coat and human MSC were isolated from bone marrow aspirate by density gradient centrifugation. Cultivated EPC and MSC were loaded onto β-TCP in vitro. 128 critical-sized segmental bone defects (5mm) were created surgically in the femoral diaphysis of adult athymic rats and stabilized with an external fixateur. 8 empty defects served as control. The remaining defects were filled with β-TCP granules alone (n=24), EPC seeded on β-TCP (n=24), MSC seeded on β-TCP (n=24), coculture of EPC and MSC seeded on β-TCP (n=24) or autologe bone (n=24). Athymic rats were used to avoid graft-versus-host reactions. After 1, 4 and 8 weeks eight rats of each experimental group were sacrificed. All pins were checked (blindly) for pin loosening. If any of the pins was loose, this was regarded as pintract infection, and the rat was excluded. Histomorphometric examination was done for the critical bone defects. Histology and Immunohistology for qualitative determination of ingrowth-behavior in decalcified serial sections (staining of HE, VEGF-R2, PECAM, vWF, anti-CD34+) as well as quantitative analysis of vascularization and new bone formation in an image-analysis-system were performed. Radiological analyses were performed by CT. 4 point-bending test was done to evaluate the strength of bone healing. All specimens were blinded and examined in random order. For statistical analysis Kruskal-Wallis-test was used to test the hypothesis that cell transplantation (EPC, MSC) increase the bone healing of critical bone defect.

**Results:**

Formation of a primitive vascular plexus was also detectable, when TCP alone or MSC on TCP were implanted, but on a significant higher level, when EPC, autologe bone or coculture of EPC and MSC were implanted in the critical size bone defect. One week after implantation, gross morphology revealed an instable critical bone defect. Histological inspections showed newly formed bone structures (osteoid) in all specimens, but none in the control groups when no β-TCP were implanted. Compared to the TCP group there was significant more new bone formation in all other experimental groups observed. But no differences between EPC and MSC group after one week was detectable. Furthermore, the coculture group of EPC and MSC showed significant more bone formation than treatment with MSC alone (p<0,03). This implies that EPC has a nutrition support in a critical bone defect.

Overall, after eight weeks after transplantation a totally bony bridging over the critical bone defect took place only when coculture of EPC and MSC were transplanted in the critical size bone defect (71% of treatment were successful). All other experimental groups did not show bony bridging in the critical size bone defect.

**Discussion:**

This study shows that cell transplantation of human EPC in coculture with human MSC enhances bone healing in a critical size bone defect after eight weeks in rats. It appears that bony bridging is stimulated by early vascularization. This investigation displays a promising approach in developing capillary networks inside of bone constructs in vivo. Cell-seeded β-TCP with EPC and MSC seems to be a potential osteogenic construct for in vivo application. Moreover, beside MSC also EPC showed enhanced new bone formation. Potentially EPC have a strong chemotaxis effect for regenerative cells, so further studies are needed.

**Conclusions:**

This study suggests that EPC might be useful as a stimulator of early vascularization, which improves also the ingrowth behaviour of MSC in order to shorten the bone healing period of a critical bone size defect.

**References:**


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