

REGENERATIVE EFFECT OF TRANSPLANTED AUTOLOGOUS BONE MARROW DERIVED MESENCHYMAL STROMAL CELLS IN HEALING THE INJURY OF RADIUS BONE IN RABBITS

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Introduction Reconstruction of bone requires repairing cells (i.e. bone marrow mesenchymal stromal cells /MSC/) and adequate scaffolds. The induction of appropriate differentiation could enhance specific tissue formation. The aim of the study was to investigate the efficiency of bone formation by MSCs and MSCs induced to osteogenic differentiation in culture before implantation into the injury site.

Methods Bone marrow was aspirated from femur of rabbits under light anesthesia and approximately 10^5 nucleated cells were seeded per cm^2 in α MEM media with 10% Fetal Bovine Serum. In 12-15 days confluent monolayer was treated with 0.05% trypsin+0.2 mM EDTA and cells were reseeded at a density of 4500 per cm^2 . MSCs were induced for osteogenic differentiation (osteo-MSC) for 4 days before implantation. One tenth part of implanted MSCs were transduced with concentrated (10^8 viral particles/ml) LeGO vector encoding EGFP and 1/10 of osteo-MSCs with LeGO vector encoding mCherry. Approximately 1 cm of radius bone was removed surgically. Implant for bone reconstitution consists of autologous MSCs or combination of MSCs with osteo-MSCs, INDOST (POLYSTOM) sponge and granules of PRODENCE[®] (WMT). INDOST sponge with adsorbed MSCs was transplanted into the injury site; fascias were taken in and than granules of PRODENCE were placed under it. Regeneration was monitored every 4 weeks for 18 weeks by radiographic imaging. In 18-22 weeks the newly formed bones were processed for decalcified histology and tested for marked cells by PCR.

Results MSCs with scaffolds implanted in the site of extracted bone displayed evidence of osteogenesis by 4 weeks, whereas there was no bone healing without special repair procedure up to 7 months after injury. The implantation of INDOST sponge and granules of PRODENCE[®] without MSCs did not contribute for bone formation up to 18 weeks. In MSCs loaded samples the process of new bone formation demonstrated increasing bone formation through 18 weeks. No difference was revealed in the dynamic of bone repair in animals reconstituted with MSCs or combination of MSCs with osteo-MSCs. The marked with EGFP MSCs were revealed in new developed bone, but mCherry marker was not detected. Moreover EGFP marker was observed in connective tissue surrounding new bone formed. This data suggest the development of bone and connective tissue from mesenchymal stem cells, capable to produce different lineages of differentiation. The induction of osteogenic differentiation before transplantation prevents MSCs marked with mCherry for long-term bone maintenance.

Conclusion These studies demonstrate that rabbit MSCs with appropriate scaffolds can regenerate bone. The regenerative potential in particular of MSCs through their differentiation into bone was proven. The induction of osteogenic differentiation before transplantation prevents MSCs marked with mCherry for long-term bone maintenance.