In vitro-modified human peripheral blood monocytes for bone healing

INTRODUCTION:
Bone is one of the few adult tissues with the capacity to regenerate. However, large, unstable, or infected bone defects remain challenging clinical problems. Tissue engineering strategies that deliver cells, growth factors and genetic material on scaffolds have demonstrated considerable potential to develop bone graft substitutes. Delivery of exogenous cells capable of forming bony tissue may be especially important to repair bone defects in patients with a limited endogenous progenitor cell supply, such as older patients, smokers, or patients with certain diseases (e.g., osteoporosis). The success of cell-based therapies for bone regeneration has been limited, in part, by the inadequate availability of large quantities of osteogenic cells and an effective cell delivery system. Bone marrow derived mesenchymal stem cells (MSCs) have demonstrated a strong potential for differentiation into bone forming cells, and have been shown to promote repair of critically-sized bone defects in pre-clinical animal studies. However, the regenerative capacities of MSCs are limited.

A promising alternative might be programmable cells of monocytic origin (PCMO), which are monocyte derived cells isolated from peripheral blood. The group of Professor Fandrich in Kiel established a protocol to induce from these cells by in vitro culture an apparently more plastic derivative, which they can further differentiate into cells with certain hematopoietic or endothelial characteristics. Others were able to induce chondrogenic markers in these cells. Although not considered a classical adult stem cell, the peripheral blood monocyte is an extraordinarily versatile progenitor cell, giving rise to very diverse cell types. PCMO represent a promising tool in autologous stem cell therapies, combining lack of immunogenic issue, easy harvesting process and high tissue specific regenerative potential. We assume, healing of large bone defects or osteoporotic fractured bones can be improved by insertion of Scaffolds, filled with PCMO-derived osteoblastic cells, in bone defects. For this purpose we induce bone specific markers in PCMOs after incubation with adequate osteoblastic differentiation medium in cell culture experiments. Our goal is the transplantation of these PCMO-filled scaffolds into a rat femoral fracture model and subsequent testing of stability and consistency of the new bone in relation to healing by fixation with classic autologous cell delivery.

METHODS:
PCMOs were isolated by density gradient centrifugation from peripheral blood according to the patented standard protocol. Afterwards the cells were seeded on tissue culture plates or Polyurethane scaffolds, using thrombin-fibrinogen as a carrier, and cultured for 21 days with osteogenic medium. Subsequently scaffolds were destructed mechanically, cells were lysed and RNA was isolated following standard protocols. Specific gene expression of osteogenic markers (osteocalcin, Runx2, collagen I) was analyzed by quantitative real time PCR. Graphs show representative results from 3 different donors.

For histologic analyzes, scaffolds were fixed in ethanol and embedded in polymethylmethacrylate. Sections were cut and stained with hematoxylin-eosin and immunofluorescence staining was performed to detect osteocalcin. Cells seeded on chamber slides were incubated for 21 days in differentiation medium, fixed with acetone and also stained for osteocalcin expression.

RESULTS:
In monolayer cell cultures osteoblast marker genes were detectable after 21 days (Figure 1) of incubation in specific osteogenic medium (d1, d2 & d3). Similar results were found for 3D-cultures with 3-fold increased expression of runx2, osteocalcin and collagen I compared to controls. (Figure 2) Immunofluorescence staining showed strong signals for osteocalcin after 21 days cultured in medium d3 (Figure 3). Notably in monolayer cultures cells change their morphology from stem cell-like to a more osteoblast-like phenotype.

REFERENCES: