"Early" endothelial progenitor cells (EPC) on ß-Tricalcium Phosphate scaffold under osteogenic conditions are useful for vascularisation in Bone tissue engineering

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Background and Introduction:

Early vascularisation 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. For bone tissue engineering endothelial progenitor cells (EPC) may provide a powerful cellular therapeutic strategy for vascularisation of a bone matrix (1). This investigation tests in vitro the ability of two different types of human EPC seeded on suitable scaffold like ß-tricalcium phosphate (TCP). At least two types of EPC can be cultured from peripheral blood mononuclear cells: "Early" EPC which were derived from myeloide or dendritic cell precursors, they still express leukocyte markers such as CD45 and differentiate within 5 days; secondly, "late" EPC which were derived from bone marrow stem cells and express stem cell markers such as CD133 (2,3). Cell adhesion, survival and differentiation on TCP scaffold were evaluated in order to develop an efficient cell seeding protocol to improve the vascularization and therefore accelerate the healing process of large bone defects in vivo.

Rational:

Which type of EPC can be cultivated on a ß-TCP scaffold (cell adhesion, survival, differentiation)? Which kind of precoating (collagen, fibronectin) is more effective on TCP? Do EPC remain their endothelial cell specific gene expression also under osteogenic culture conditions?

Methods:

For the in vitro study the cells were first characterized for their expression of typical EPC markers, followed by expansion in culture and loading onto a TCP scaffold. "Early" EPC were isolated from a buffy coat by density gradient centrifugation. Cells were cultivated on a fibronectin coated 24-well culture dish in endothelial basal medium (EBM) supplemented with endothelial growth medium (EGM) for 3 days. "Late" EPC were isolated from a punction of bone marrow using CD133 microbeads. The CD 133+ cells were cultured in presence of vascular endothelial growth factor (VEGF) and SCF approximately for three weeks until a sufficient number of cells was reached (4,5).

To examine the seeding efficacy and the necessity of a fibronectin or collagen G pre-coating of the scaffold, β -TCP scaffolds were precoated with fibronectin or collagen G in a well of a 24-well culture dish. EPC were loaded onto β-TCP scaffold (6,7) *in vitro* and seeded in a density of 2,5 x 10^5 cells per well and subsequentely the seeding efficacy was determined. EPC were evaluated for cell adhesion and cell viability. RT-PCR quantification of important genes involved in metabolism, endothelial and osteogenic differentiation was used to monitor the survival and differentiation process of cells seeded on ß-TCP. After 24 h, 48 h, 72 h TCP-granula were taken and adherent cells were lysed, RNA of the adherent cells was isolated, reverse transcribed and subjected to RT-PCR using specific primers for GAPDH (control), vWF(von Willebrandt factor), VEGF, Osteonectin, CBFA-1, Osteocalcin and Collagen-1.

To evaluate the influence of osteogenic differentiation, cells were cultivated for 4, 8, 12 days with osteogenic medium (Ascorbic acid, beta-glycerolphosphat, dexamethason in standard concentration), afterwards measurements of gene expression using RT-PCR was performed.

Results:

Early EPC were cultivated successfully for over 72 h on ß-TCP (seeding efficacy 93%+/-5%) if they were differentiated over a period of 3 days after isolation from buffy coat. These "early" EPC expressed over the whole period on β -TCP vWF and in part VEGF. Even under osteogenic culture conditions they still show vWF gene expression. A weak gene expression of CBFA-1 and osteonectin was also detectable. In comparison, late EPC demonstrated a weaker adhesion on ß-TCP (seeding efficacy 65%+/-5%). A significant GAPDH gene expression was detectable only at 24h after seeding only on collagen precoated

TCP-Granula. Moreover, under osteogenic culture conditions late EPC lost vWF gene expression and started to express the genes for collagen-1, osteonectin and osteocalcin.

Discussion and Conclusion:
Our data suggest that "early" EPC could successfully cultivated on ß-TCP-scaffold, they did not lose their endothelial characteristics and remained, at least in part, in a functional state since VEGF gene expression was still detectable in a number of samples even if these cells were cultured under osteogenic conditions. "Late" EPC showed only a weak adherence to coated ß-TCP and adopt osteogenic marker if they were cultivated under osteogenic conditions *in vitro*. In conclusion, "early" EPC, even they are not stem cells per definition, are an interesting candidate for improvement of vascularisation in bone tissue engineering.

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ACKNOWLEDGEMENTS: This research work is supported by a grant from the AO Research Fund (Davos, Switzerland).