

PLATELET RELEASED GROWTH FACTORS BOOST EXPANSION OF ENDOTHELIAL PROGENITOR CELLS

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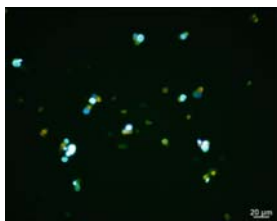
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INTRODUCTION: Cell based strategies using autologous bone marrow stromal cell (BMSC) transplants provide a promising approach for the treatment of critical sized bone defects. Although the basic concept is proven (see 1 for review), the ultimate efficiency of an artificial bone construct depends on the presence of viable cells presenting an osteogenic potential, meaning it depends on delivery and exchange of oxygen and nutrients form surrounding blood vessels to the BMSC. Thus, one of the most limiting aspects in obtaining tissue-engineered bone suitable for repairing large bone defects is the inadequate bone vascularisation. In our study we therefore address the enhancement of endothelial progenitor cells as one of the key mechanisms in autologous bone grafting. The means by which these progenitors for neovascularisation can be isolated and characterized have recently been described². However, one of the major obstacles preventing the clinical application, is the time needed to expand the EPC in vitro in order to obtain the required cell numbers.

METHODS: Bone marrow stromal cells were isolated by Ficoll-Paque density-gradient centrifugation. CD34+ and CD133+ cells were isolated using magnetically labelled antibodies on a miniMACS system (Miltenyi Biotec). PRGF was prepared from Thrombocyte concentrates. Three different media were used 1. IMDM medium (Gibco®, Invitrogen) containing 10 % fetal calf serum (FCS) and 1ng/ml basic-fibroblast growth factor (bFGF) 2. M200 angiogenic medium plus Low Serum Growth Supplement (LSGS) (Cascade Biologics) 3. IMDM medium supplemented with 10 % FCS and 10 % PRGF. Isolated cells were further characterized by immunolabelling using FITC- and PE- coupled antibodies against CD34 and CD133 (Miltenyi Biotech) (Figure 1). Cells were seeded at a density of 30.000 per cm² onto Matrigel®-thin layer coated well plates (BD Biosci.). Real-time RT PCR of angiogenic markers was performed.

Fig. 1 Immediate staining for CD34+ and CD133+ cells

RESULTS: After cell culture for 30 days



overall superiority of the PRGF supplemented medium was demonstrated (Figure 2).

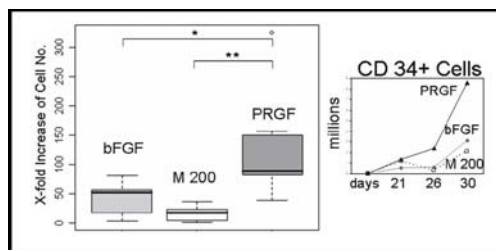


Fig. 2 Expansion of CD 34+ and CD 133+ Cells

All EPC showed tube formation when grown on Matrigel (Figure 3). Remarkably the mesh-like appearance of the separated and expanded EPC differed in a more pronounced knot-formation compared to standard HUVEC – hinting for a lesser homogeneity of the bone-marrow-derived cultures.

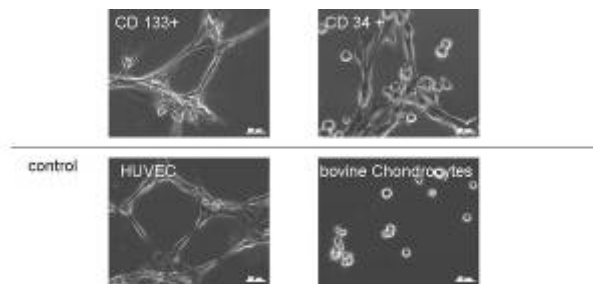


Fig. 3 Tubular Structure Formation of EPC

Gene expression analysis proved maintained angiogenic marker-expression in all cultures.

DISCUSSION & CONCLUSIONS: Here we show that PRGF constitutes a highly effective growth medium supplement that boosts the expansion of cultured endothelial progenitor cells. These cells may serve as an MSC-augmenting transplant population.

Further studies will show to which extent the PRGF cultured cell populations will be an effective inducer for vascularisation of stem cells loaded scaffolds.

REFERENCES:

¹T. Asahara and JM Isner Endothelial progenitor cells for vascular regeneration. *J Hematother Stem Cell Res* 11: 171-178, 2002. ²AI. Caplan. Review: mesenchymal stem cells: cell-based reconstructive therapy in orthopaedics. *Tissue Engineering* 11(7/8), 1198–1211, 2005