

INTEGRATION OF SHEEP PERIPHERAL VEIN ENDOTHELIAL CELLS INTO POLYURETHANE SCAFFOLDS- DELIVERING ANGIOGENIC POTENTIAL INTO AUTOLOGOUS GRAFT CONSTRUCTS

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INTRODUCTION: Current concepts in tissue engineering advocate the composition of bone grafts out of bioresorbable scaffolds and different stem- and progenitor cells¹. The particular importance of ingrowing vessels for the support of any bone-regenerating cell has been highlighted by many studies². In contrast, very few attempts to overcome this problem by the seeding of angiogenic cells into a transplant have been described. In principle angiogenic cell types have been grown from peripheral blood, bone marrow, adipose tissue and many other sources. Still, complex selection techniques and time consuming expansion periods remain an experimental (and future clinical) obstacle. Therefore we have developed a technique to harvest, expand and seed endothelial cells from sheep peripheral vein explants into a large polyurethane scaffold within less than a month after initial surgery. This scaffold can be implanted into a critical sized defect of the ovine tibia.

METHODS: Peripheral vein explants (*V. mediana antebrachii*/ *V. basilica*) were harvested from adult swiss mountain sheep. Sheep Endothelial Cells (SEC) were obtained by internal collagenase (0.2%) digestion from 5 cm Vein specimen. SEC were seeded in IMDM, 10% FBS and 10% sheep Platelet Released Growth Factors (PRGF). Matrigel® (BD Biosci)-coated well plates were used for demonstration of tube forming capacity. 40×10^6 cells were seeded into a 2.3 x 2.8 cm polyurethane scaffold (produced by our own group) in a 10 ml Platelet-Rich Plasma (PRP) suspension after activation with 50 U/ml bovine thrombin. Cryosections were stained with HE and Toluidine. Live and dead discrimination was performed by LDH staining.

RESULTS: Three weeks of cell culture were necessary to obtain a minimum of 40×10^6 SEC from two vein explants. SEC preserved endothelial lineage markers during culture as shown by RT-PCR (data not shown). All cultured cells proved functional endothelial capacity in a Matrigel® assay (Fig. 1).

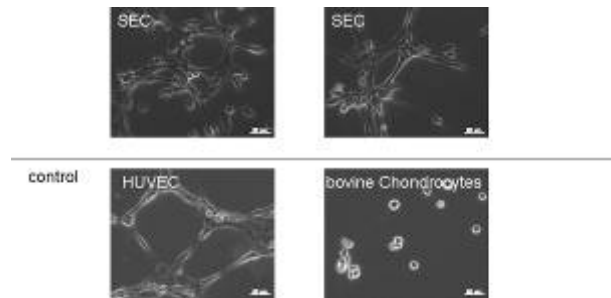


Fig. 1: Matrigel® tube formation assay, bovine chondrocytes as negative control

Histology revealed viability and even distribution of SEC seeded into the scaffold. After 7 days of culture in PRGF the majority of SEC had survived (Fig. 2).

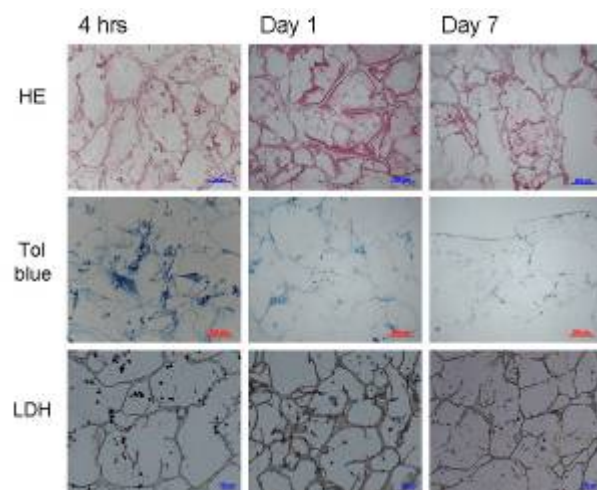


Fig. 2: Staining of Cryosectioned scaffold

DISCUSSION & CONCLUSIONS: Here we report on a successful method for harvesting, expansion and seeding of SEC into a bioresorbable scaffold. By this technique a tissue engineered autologous graft can be prepared for the bridging of critical sized defects. We envisage an animal study investigating SEC in combination with other cell types to prove our concept in vivo.

REFERENCES: ¹AI. Caplan (2005) Review: Mesenchymal stem cells: Cell based reconstructive therapy in orthopaedics. *Tissue Engineering* 11 (7/8), pp1198 ²J. Glowacki. (1998) Angiogenesis in fracture repair. *Clin Orthop Relat Res.*S82-S89