(OP 53) Co-Culture System of Osteoblasts and Endothelial Cells, an *In vitro* Strategy to Enhance Vascularization in Bone Regeneration

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For bone TE applications the formation of a prevascularized structure is not only required for the survival of seeded cells on scaffolds, but is also a fundamental process in bone formation and repair. In this work we describe an *in vitro* strategy consisting in the simultaneous culture of osteoblasts and endothelial cells (ECs) onto a starch-based fiber-mesh scaffold, aiming to accelerate the establishment of a vascular bed in the implanted construct.

In order to distinguish between cell populations, samples were stained after several time points for nuclei (both osteoblasts and ECs), PECAM-1 (endothelial specific) and visualized by confocal microscopy. After 21 days of culture the formation of tube-like structures with PECAM-1 expression at the cell-cell junctions was observed. The structural complexity was even higher after 35 days with ramified tube-like structures. Immunohistochemistry of co-culture/scaffold cross-sections revealed that the scaffold's void spaces were filled with a dense ECM positively stained for type I collagen. Inserted in this matrix were PECAM-stained ECs surrounding a patent lumen. Furthermore, these tube-like structures were stained for type IV collagen, a component of the vascular basal lamina. Cross-talk communication between the two cell types was another aspect under analysis. Osteoblasts in co-culture were producing higher amounts of VEGF than in monoculture. In addition, the expression of gap junction connexin43 in the co-culture demonstrates the heterotypic communication established between cells.

In short, the use of this co-culture system in conjunction with a starch-based 3D scaffold holds great potential for the formation of a prevascular network *in vitro*.