

# USING 3D CULTURE SYSTEMS IN BIOMATERIAL RESEARCH FOR TISSUE ENGINEERING & REGENERATIVE MEDICINE

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In Regenerative Medicine (RegMed) strategies have been developed to use autologous cells, one well-established approach being Tissue Engineering (TE). This generally involves using a suitable biomaterial, preferably biodegradable, along with a bioactive signal molecule to initiate essential processes of cell proliferation and/or differentiation. In most cases tissue regeneration requires rapid and functional vascularization in order to supply oxygen and nutrients to metabolically active cells. Our principal focus in the past years has been on bone regeneration, using assays involving human endothelial cells (EC) and osteogenic cells, cultured alone on a suitable 3D scaffold or in co-culture in 2D and 3D systems with or without a biomaterial. The predominant EC used were human dermal microvascular endothelial cells (HDMEC) and endothelial progenitor cells (EPC) isolated from human peripheral blood and cultivated to yield so-called outgrowth EC (OEC), which possess a very stable phenotype [1]. In co-cultures of HDMEC and primary osteoblasts (pOB) on a 3D micromesh of silk fibroin an extensive network of capillary-like structures was formed in the absence of exogenous pro-angiogenic growth factors (VEGF, bFGF)[2]. It appears that during the heterotypic cell interactions on the scaffolds a tissue-like “self-assembly” occurs, with the osteoblasts acting as the “drug-delivery system” to drive vasculogenesis. A similar type of self-assembly occurs if outgrowth EC (OEC), derived from adult endothelial progenitor cells, are used as endothelial source, this even occurring in the absence of the biomaterial three-dimensionality [3].

More detailed co-culture studies on a microfibre scaffold of a blend of starch and poly(caprolactone)(SPCL) showed that the osteoblasts serve a dual function in the vascularization process. First, they provide the necessary pro-angiogenic growth factors (GFs), such as VEGF, in this *in vitro* situation, thus obviating the need for exogenous GFs. Second, in the co-culture setting the EC upregulate synthesis and secretion of osteoblastic collagen I, which in turn promotes matrix-guided vasculogenesis [4]. Currently, we are studying the effects of other GFs, such as IGF-1 & -2, and early embryonic signals, such as sonic hedgehog, on

vasculogenesis and osteogenesis. From our co-culture data these genes appear to play an important role in cellular crosstalk between these cell types. In particular, sonic hedgehog exerts a positive effect both on osteogenesis and angiogenesis, making it an important factor for bone regeneration strategies [5].

It is evident that culture models require validation *in vivo*, as TE & RegMed are translational in nature. Studies in immunodeficient (*scid*) mice show that the *in vitro* formed microvessel-like structures gain rapid connection to a pre-existing microcirculation following subcutaneous implantation [6].

**FUTURE DIRECTIONS:** Current experimental studies both *in vitro* and *in vivo* are investigating the role of the endothelial component in a preceeding approach in the final vascularization outcome *in vivo* [7]. Most recently, similar co-cultures of osteoblasts and EC revealed that PCL incorporated into calcium-deficient hydroxyapatite also promoted microvessel-like structure formation [8].

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