

INTRODUCTION OF A THREE-DIMENSIONAL CO-CULTURE MODEL FOR IN VITRO ANGIOGENESIS IN BONE TISSUE ENGINEERING

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INTRODUCTION: One of the major limitations to the clinical application of tissue engineered bone substitutes remains vascularization of the transplant. The challenge of integrating an ordered capillary tree into tissue engineered constructs *ex vivo* still has to be solved [1]. We have developed a three-dimensional collagen-based co-culture system to assess interactions between human endothelial cells (hECs) and human osteoblasts (hOBs) *in vitro*. In fracture healing as well as in bone tissue engineering angiogenesis represents a crucial step in the formation of new bone. Our model serves as a tool for investigating the effect of heterotypic cell-cell interactions and angiogenic stimulation upon the formation of tube like structures by hECs in an osteoblast environment.

METHODS: Human umbilical vein endothelial cells (HUVECs) were grown as three-dimensional multicellular spheroids and seeded in a collagen matrix to assess sprouting of the spheroids. Sprouts emerging from endothelial cell spheroids form tube like structures resembling early capillaries [2]. Cell contact between hOBs and HUVEC spheroids was established by either adding hOBs into the collagen gel as a single cell suspension or through direct incorporation of hOBs into the EC spheroids thus forming heterogenous co-spheroids. Spatial organization of co-spheroids and sprout configuration was assessed using in gel immunohistochemical wholemount staining techniques and confocal laser microscopy. Cumulative sprout length of spheroids was quantitatively analyzed using digital imaging planimetry.

RESULTS: We were able to demonstrate that HUVECs and hOBs form heterogenous co-spheroids with distinct spatial organization. HUVEC sprouting upon angiogenic stimulation with VEGF and bFGF is suppressed in heterogenous HUVEC/hOB co-spheroids. In our model direct contact with hOBs inhibits

formation of tube like structures by hECs, whereas co-culture arrangement of hOBs and hECs without direct contact does not affect formation of lumenized structures.

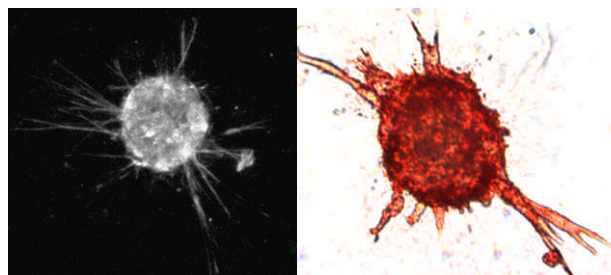


Fig. 1: Confocal laser microscopy image of HUVEC / hOB co-spheroid showing osteoblast filopodia protrusions from the co-spheroid (left) and immunohistochemical wholemount staining for CD31-positive tube like sprouts emerging from HUVEC spheroid in a collagen matrix (right)

Fig. 2: HUVEC spheroid sprouting in direct contact to hOBs (blue) compared to HUVEC spheroid sprouting in absence of hOBs (red)

DISCUSSION & CONCLUSIONS: This study introduces a novel model for investigating *in vitro* angiogenesis in an osteoblast environment. We have demonstrated that contact or proximity between hOBs and hECs modulates capillary formation in a three-dimensional collagen-based spheroid sprouting assay. The model systems introduced in this study will be useful in elucidating the mechanisms involved in regulating angiogenesis in bone formation and will allow further investigations into the character of heterotypic cell-cell interactions for applications in bone tissue engineering.

REFERENCES:¹G.B. Stark (2002) *Min Invas Ther & Allied Technol* **11(3)**: 85-86. ² T. Korff, H.G. Augustin (1999) *J Cell Sci* **112**: 3249-3258.

