

FIBRIN MATRICES FOR CELL TYPE SPECIFIC DIFFERENTIATIONH.Hall & [J.A.Hubbell](#)*Institute for Biomedical Engineering and Department of Material Sciences,
ETH and University of Zurich, Zurich, Switzerland.*

INTRODUCTION: Fibrin clots get assembled at the surface of injured blood vessels and provide a provisional matrix for invading cells that induce the wound healing process. Finally they get proteolytically degraded and disappear. Our approach is to use modified fibrin matrices as 3D-scaffolds that induce cell type specific differentiation such as angiogenesis and nerve regeneration. Fibrin matrices become covalently modified by L1Ig6, a specific receptor for $\alpha v\beta 3$ -integrins that is expressed on human umbilical vein endothelial cells (HUVECs) and on chicken DRG- neurons. Both cell types are able to interact specifically with L1Ig6 (Hall et al., 2001; Yip and Siu, 2001) and form processes and myelinated axons, respectively. Modified fibrin matrices provide an interesting way to design a material that can be adsorbed to any surface shape, polymerize and provide a provisional guidance cue for specific tissue regeneration.

METHODS: HUVECs were purchased from PromoCell, Heidelberg, Germany and maintained under low serum conditions (2%) in the absence of additional growth factors. DRG neurons were dissected from E10 chicken embryos and cultured in Dulbecco's modified Eagle's medium containing glutamax-1, 10% fetal calf serum, 5% chick serum and 100 ng/ml 2.5S NGF (Sigma). DRG-neurons were induced to form myelin by 50 μ g/ml ascorbic acid. Both cell types were cultured in covalently L1Ig6-modified fibrin matrices (Hall et al., 2001), matrices filled with laminin-1 (Sigma) or native fibrin matrices. Process extension was determined after 8 days for HUVECs, and neurite length and myelination of DRG-neurons after 14 days. Living cells were fluorescently labeled with fluorescein diacetate and the morphology of the cells was analyzed by confocal or phase contrast microscopy.

RESULTS: Cell-matrix interactions are responsible for cell-type specific morphology and/or differentiation. Both cell types extend cell type specific processes in L1Ig6-modified fibrin hydrogels. HUVECs form multi-cellular extensions that become interconnected. These processes have been described as first indications of angiogenic differentiation *in vitro* (Pepper et al., 1996).

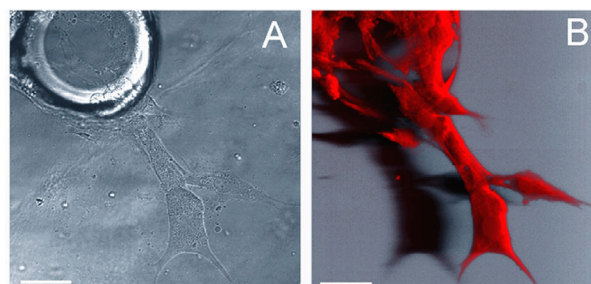


Fig. 1: HUVECs cultured on microcarrier beads in L1Ig6-modified fibrin hydrogels migrate and extend processes into the matrix. Processes were analyzed by phase contrast (A) or confocal microscopy (B). The scale bar represents 50 μ m.

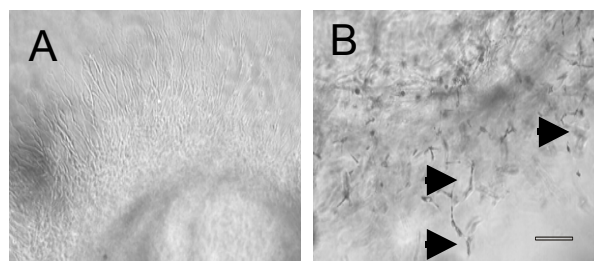


Fig. 2: Chick-DRG neurons in L1Ig6-modified fibrin matrices (A) and matrices containing soluble laminin-1 (B) extend neurites into the matrix and get myelinated (arrow heads). The scale bar represents 200 μ m.

DRG-neurons extend neurites and form networks in L1Ig6-modified or laminin-1 containing fibrin matrices that eventually get myelinated *in vitro*.

DISCUSSION & CONCLUSIONS: The fibrin matrix designed to interact specifically with $\alpha v\beta 3$ -integrins on angiogenic HUVEC and on neurite extending DRG-neurons is able to induce communication between the cell and the extracellular matrix that results in cell type specific differentiation. Therefore we think, that by using artificially designed matrices we are able to place a specific stimulus at the site of injury to induce specific tissue regeneration. Furthermore, it will be possible to use these matrices as guidance cues to direct cells towards their target organs.

REFERENCES: Hall et al, 2001, *Microvas. Res.* **62**, 315-326; Yip and Siu, 2001, *J. Neurochem.* **76**, 1552-1564. Pepper et al., 1996, *Enzyme Protein.* **49**, 138-162.