

## Title: Hydrogel Screening for 3D Vascularization Model on-Chip

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### Abstract

Hydrogels are biomaterials consisting of crosslinked polymer networks. They allow perfusion and give structural support to the cells. Hydrogels are crucial element for 3D cell models since they mimic the cells' natural 3D environment, extracellular matrix. Vasculature is responsible for transport of oxygen, nutrients and metabolites and is therefore essential for all living tissues in human body.

This study focused on analyzing hydrogel size change, testing protease inhibitors' effects on hydrogel's size change, and using these hydrogels ( $\pm$ inhibitors) to support 3D vascularization-on-chip. The tested hydrogels included fibrin, collagen I, gelatin-gellan gum and VitroGel®. Human lung fibroblasts were cultured embedded in hydrogels. Every day, the samples' area was measured with tile scan imaging, the thickness was measured by imaging fluorescent beads embedded in the hydrogels, and volume was calculated based on these measured values. Cell viability and morphology were analyzed with Live/Dead and cytochemical staining. Human bone marrow stem/stromal cells, and green fluorescent protein (GFP) tagged human umbilical vein endothelial cells were cultured inside a hydrogel in dynamic conditions. The cell morphology was analyzed with GFP signal and immunocytochemical staining.

Hydrogels showed varying characteristics in sample size changes and cell morphology. Functional inhibitors preventing the decrease in size of the tested hydrogels were discovered. The inhibitors supported vascularization, and the most mature vascular network appeared to be formed in fibrin. In conclusion, a method to analyze hydrogel size change was created, the hydrogel degradation was decreased and fibrin showed to be the most promising hydrogel to support 3D vasculature maintenance on-chip.