IN-VITRO 3D ANGIOGENESIS MODELS IN 3DPROSEED™ HYDROGEL WELL PLATE

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Summary

This application note demonstrates the use of 3DProSeed™ well plate for the simple and automation-compatible establishment of 3D angiogenesis models by co-culture of endothelial cells and mesenchymal support cells. Seeded on the gradient gels, endothelial cells together with support cells migrated inside the gel and assembled in 3D tube-like structures. These structures are networks of endothelial cells forming hollow structures surrounded by support mesenchymal cells, and can be used to screen for the pro- or anti- angiogenic potential of compounds. This platform offers a simple way to create 3D angiogenic models with the highest level of automation compatibility and enabling screening of compounds on these highly relevant systems.

Introduction

Regulating angiogenesis is critical for tumor growth and metastasis and represents an important strategy to fight tumor progression [1]. Consequently, a number of *in vitro* angiogenesis models have been developed for screening applications [2]. Endothelial cells (ECs), the cells lining the inner surface of all blood vessels, have been shown to be centrally involved in the new formation of vessels by processes such as sprouting and intussusception [3]. 3D hydrogel cultures of endothelial cells and support cells in hydrogels have been shown to support the formation of 3D networks resembling closely to native capillaries, even becoming functional and connecting to host vasculature when implanted in mice [4].

Here we demonstrate the simple formation of similar 3D vasculatures using 3DProSeed™. Co-seeding of endothelial cells (EC) with mesenchymal stromal cells (MSC) on 3DProSeed led spontaneous formation of 3D microvascular network. Using GFP-labelled endothelial cells allowed for an effortless monitoring of the microvascular network.

The combination of the simple readout and with the 96 well plate format enabled a dose-response assessment of a pro-angiogenic compound (FGF) in combination with an anti-angiogenic molecule (Suramin).

These data show the facile establishment of 3D micro-vascular networks as a relevant 3D model for compound testing.

Materials and methods

3DProSeed™ hydrogel well plate. The 3DProSeed™ hydrogel plate consists of a 96-well black microtiter plate with 180 micron glass bottom, containing a fully synthetic pre-assembled and hydrated hydrogel for ready-to-use and automation-compatible cell-based assays. The key innovation is the hydrogel surface featuring a so-called "in depth-density gradient" which enables the penetration into the hydrogel bulk of cells deposited on it. The hydrogel is a poly(ethylene glycol)-based formulation containing cell adhesion (RGD sequences) and degradation (MMP-cleavable sequences) motives.

Cells and media. Green fluorescent protein-labeled Human umbilical vein endothelial cells (GFP-HUVEC, PELO Biotech) cultured in EGM-2 (Lonza). Human bone-marrow derived mesenchymal stem cells (MSC, Lonza) were cultured in MEM-a supplemented with 10% fetal calf serum and 1 % penicillin/streptomycin (Gibco Life Technologies)

Cell seeding procedure. 1.0×10^4 GFP-HUVECs were co-seeded with 1.0×10^4 MSCs in each well and co-cultured for 7 days in a 75% MSC-medium, 25% EC-medium mixture.

Characterization of the 3D cultures. At the endpoint, samples were fixed and stained for f-actin. Cell invasion and hollow tube-like structure formation were monitored by CLSM.

Compound testing. The effect of pro- and anti-angiogenic compounds on the establishment of microvascular structure was assessed. Do to so, combinations of FGF and Suramin at different concentrations were added to the co-culture for 7 days. 7 days. After 7 days the vascular network were imaged using the GFP-signal of the endothelial cells and the tube length quantified using imageJ.

Results and discussion

Formation of 3D vascular networks. Seeded on the gradient gels, endothelial cells co-seeded with support cells migrated inside the gel and assembled in 3D tube-like structures (Figure 1 A-C). These structures are networks of endothelial cells forming hollow structures surrounded by support mesenchymal cells. Without these support cells, endothelial cells alone also migrated into the gels and started elongating but failed to establish stable structures, as described when encapsulated in similar gels.

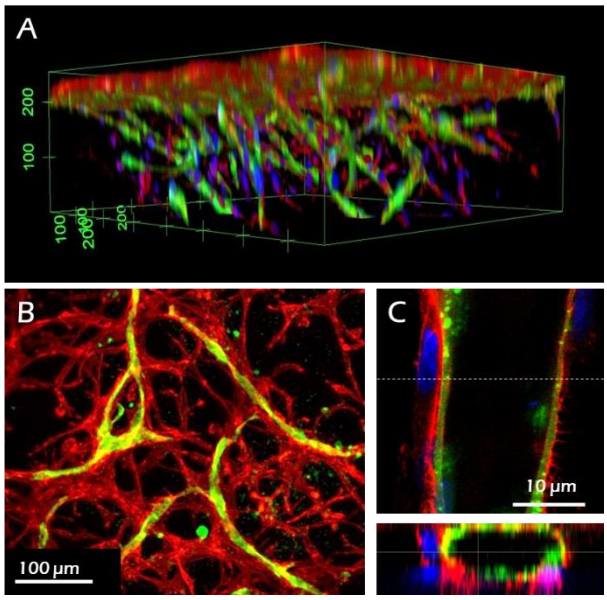

Figure 1, Spontaneous formation of 3D microvasculature: GFP-HUVECs (green) and cytoskeleton staining of MSCs (red) and nuclei (blue) indicate the presence of vascular networks in 3D. A represents a 3D reconstruction, while B is a top projection and C a close up of a hollow vascular tube.

Compounds testing. Figure 2 shows the 96 images taken from a single wellplate at the end point (7 days). These images were used to quantify the tube length in each condition and determine the effect of the compound combination on the vascular network. Interestingly this assay confirms the pro-angiogenic potential of FGF and anti-angiogenic potential of Suramin, and provide indications on their combined action. For instance, 240ng/mL FGF combined can counteract the effect of 100 uM Suramin, leading to an equivalent microvasculature comparable to when no compound was added.

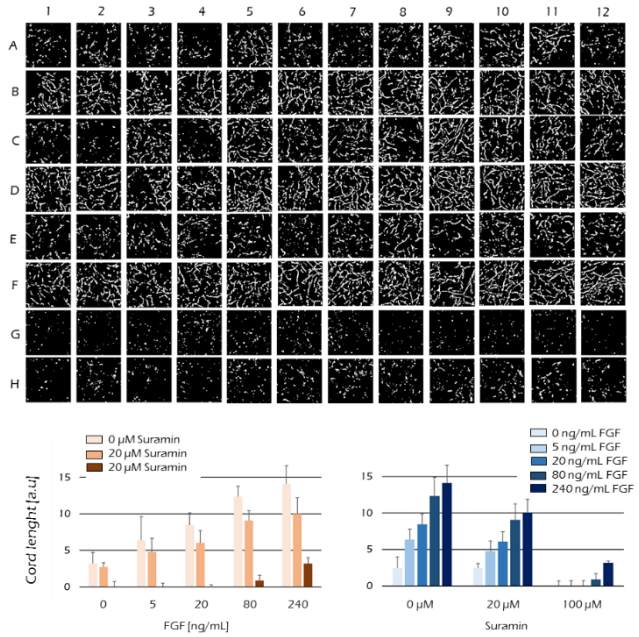

Figure 2 Screening for the angiogenic potential of compounds in 3D. Using a 96-well plate to test the dose response of combinations of FGF and Suramin, The quantification of the tube length of each condition gives an indication of the effect of the angiogenic effect of the compound combination.

Conclusion

This preliminary study demonstrates the use of 3DProSeed™ well plate for the simple and automation-compatible establishment of a 3D angiogenesis model.

The spontaneous formation of a vascular networks combined with simple microscopic readout in a 96 well plate format, offers a simple way for screening for the angiogenic potential of molecules.

Literature

[1] R.S. Samant, L.A. Shevde, Recent advances in anti-angiogenic therapy of cancer, Oncotarget 2(3) (2011) 122-34.
[2] L.C. Roudsari, J.L. West, Studying the influence of angiogenesis in in vitro cancer model systems, Adv Drug Deliv Rev 97 (2016) 250-9.
[3] P. Carmeliet, Angiogenesis in life, disease and medicine, Nature 438(7070) (2005) 932-936.
[4] U. Blache, S. Metzger, Q. Vallmajo-Martin, I. Martin, V. Djonov, M. Ehrbar, Dual Role of Mesenchymal Stem Cells Allows for Microvascularized Bone Tissue-Like Environments in PEG Hydrogels, Advanced healthcare materials (2015)