

Generating Vascular Organoids

This is intended as a guide only; for full experimental details please read the reference provided.

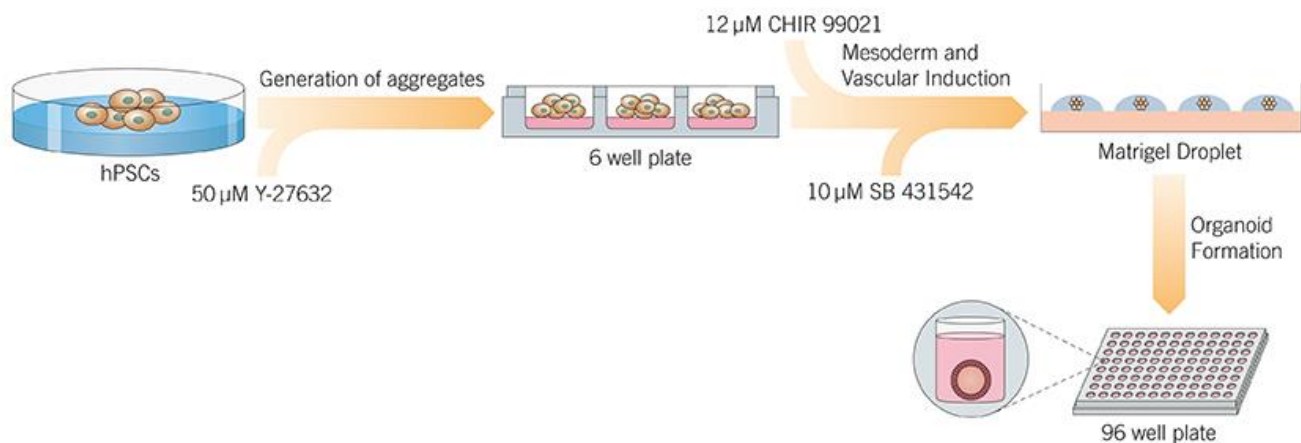
In Brief

Wimmer *et al.* describe a protocol to generate human blood vessel organoids from H9 ES cells or iPS cells.

Human H9 ES cells or iPS cells were disaggregated then resuspended in differentiation medium 1 containing Y-27632 (50 μ M) and plated into one well of an ultra-low attachment six-well plate for cell aggregation. On day 3 cell aggregates were treated with 12 μ M CHIR 99021, then on days 5, 7 and 9 BMP4, VEGF-A and FGF-2 were added.

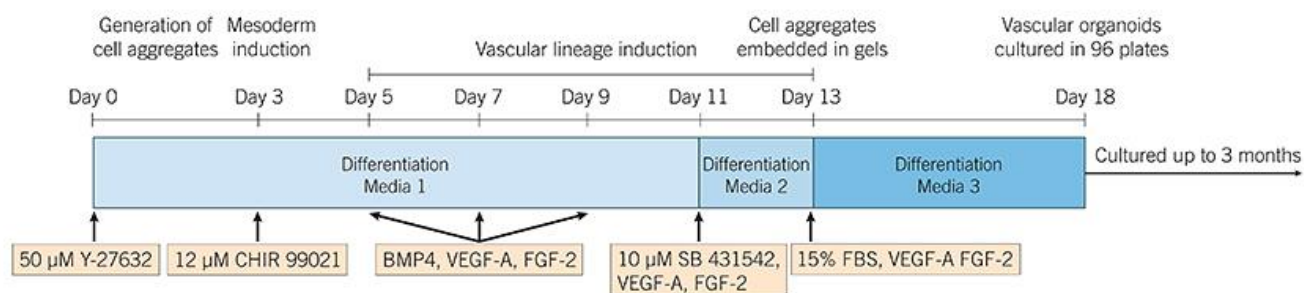
On day 11, differentiation media 2 containing VEGF-A, FGF-2 and SB 431542 (10 μ M) was added to increase the yield of endothelial cells and suppress pericyte formation.

On day 13 cell aggregates were embedded in Matrigel:collagen I (1:1) gels and differentiation media 3 was added. Media was changed every second to third day. Approximately day 18, vascular networks were established, extracted from the gels and further cultured in 96-well low-attachment plates. These vascular networks self-assembled into vascular organoids and could be cultured for up to 3 months.



Cocktails

Differentiation Media 1		Differentiation Media 2		Differentiation Media 3	
DMEM:F12 medium		DMEM:F12 medium		DMEM:F12 medium	
20% KOSR		20% KOSR		20% KOSR	
Glutamax		Glutamax		Glutamax	
NEAA		NEAA		NEAA	
Y-27632 (Cat.No. 1254)	50 µM	VEGF-A	30 ng ml ⁻¹	15% FBS	
CHIR 99021 (added day 3) (Cat.No. 4423)	20 µM	FGF-2	30 ng ml ⁻¹	VEGF-A	100 ng ml ⁻¹
BMP4 (added days 5, 7 and 9)	30 ng ml ⁻¹	SB 431542 (Cat.No. 1614)	10 µM	FGF-2	100 ng ml ⁻¹
VEGF-A (added days 5, 7 and 9)	30 ng ml ⁻¹				
FGF-2 (added days 5, 7 and 9)	30 ng ml ⁻¹				



Reference

Wimmer *et al.* (2019) Human blood vessel organoids as a model of diabetic vasculopathy. *Nature* **565** 505
PMID: [30651639](#)